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RESEARCH ARTICLE

NeuroArray: A Customized aCGH for the Analysis of Copy Number Variations in Neurological Disorders

Valentina La Cognata[1], Giovanna Morello[1], Giulia Gentile[1], Francesca Cavalcanti[2], Rita Cittadella[2], Francesca Luisa Conforti[2], Elvira Valeria De Marco[2], Angela Magariello[2], Maria Muglia[2], Alessandra Patitucci[2], Patrizia Spadafora[2], Velia D'Agata[3], Martino Ruggieri[4] and Sebastiano Cavallaro[1,2,*]

[1]*Institute of Neurological Sciences, National Research Council, Via Paolo Gaifami 18, 95125, Catania, Italy;* [2]*Institute of Neurological Sciences, National Research Council, 87050, Mangone, Cosenza, Italy;* [3]*Department of Biomedical and Biotechnological Sciences, Section of Human Anatomy and Histology, University of Catania, Catania, Italy;* [4]*Unit of Rare Diseases of the Nervous System in Childhood, Department of Clinical and Experimental Medicine, Section of Pediatrics and Child Neuropsychiatry, University of Catania, AOU "Policlinico-Vittorio Emanuele", Catania, Italy*

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Abstract: ***Background*:** Neurological disorders are a highly heterogeneous group of pathological conditions that affect both the peripheral and the central nervous system. These pathologies are characterized by a complex and multifactorial etiology involving numerous environmental agents and genetic susceptibility factors. For this reason, the investigation of their pathogenetic basis by means of traditional methodological approaches is rather arduous. High-throughput genotyping technologies, including the microarray-based comparative genomic hybridization (aCGH), are currently replacing classical detection methods, providing powerful molecular tools to identify genomic unbalanced structural rearrangements and explore their role in the pathogenesis of many complex human diseases.

***Methods*:** In this report, we comprehensively describe the design method, the procedures, validation, and implementation of an exon-centric customized aCGH (*NeuroArray* 1.0), tailored to detect both single and multi-exon deletions or duplications in a large set of multi- and monogenic neurological diseases. This focused platform enables a targeted measurement of structural imbalances across the human genome, targeting the clinically relevant genes at exon-level resolution.

***Conclusion*:** An increasing use of the *NeuroArray* platform may offer new insights in investigating potential overlapping gene signatures among neurological conditions and defining genotype-phenotype relationships.

Keywords: Methods, aCGH, CNVs, Neurological diseases, Genes, Custom array.

1. INTRODUCTION

Neurological disorders are multifactorial debilitating pathologies of the nervous system affecting hundreds of millions of people worldwide. The etiology of this heterogeneous group of diseases relies on a large spectrum of elements including genetic, epigenetic, and environmental contributions that are still far from being fully encoded. Despite the complex molecular mechanisms underlying such kind of diseases, the intensive efforts of the scientific community and the significant and rapid advancement of biotechnologies are fueling several steps towards the elucidation of the genetic components.

While Single Nucleotide Changes (SNPs) were considered for a long time the major disease-driving mutations, more recently Copy Number Variations (CNVs) and gene dosage alterations have emerged as critical elements for the development and maintenance of the nervous system [1]. CNVs (gain or loss of genomic material larger than 1 Kb) are very common across the human genome, constitute a prevalent source of genomic variations [2], and are known to contribute to familiar or sporadic neurological diseases, such as neuropathies, epilepsy forms, autistic syndromes, psychiatric illnesses and neurodegenerative diseases [3-8]. Just to mention a few examples, frequent duplications of the amyloid precursor protein gene (*APP*) or CNVs involving genes tightly related to amyloid-β peptide pathways have been detected in patients with Alzheimer's Disease (AD) [9-12]. In Parkinson's Disease (PD), a single *PARK2* gene loss seems to increase the risk of PD [13], while α-synuclein gene (*SNCA*) copy-number gains have proved to play a major role in the disease severity of PARK1 [3, 14]. Some rare CNVs have been also associated with Amyotrophic Lateral Sclerosis (ALS) susceptibility, such as the *DPP6* locus and the

*Address correspondence to this author at the Institute of Neurological Sciences, National Research Council, *Via* Paolo Gaifami 18, 95125, Catania, Italy; Tel: +39-095-7338111; E-mail: sebastiano.cavallaro@cnr.it

15q11.2 locus containing the *NIPA1* gene [15]. Moreover, different type of phenotypes can arise from dosage variations of the same gene: for example, two clinically distinct disorders can be caused by dosage variations of *PMP22* gene, like the Hereditary Neuropathy with liability to Pressure Palsies (HNPP) which is caused by a 1.5-Mb deletion spanning the same region tandem duplicated in most Charcot-Marie-Tooth (CMT) 1A disease cases [16].

In the past decades, the detection of CNVs and abnormal gene dosage has been based on traditional methodologies such as karyotyping, quantitative PCR, Multiple Ligation Probe Analysis or Southern blot. These approaches had some limits: they were time-consuming, labor-intensive, required multiple phase steps and severe equipment costs, and above all, did not offer a complete genomic overview of structural imbalances at sufficiently high resolution. Since a few years now, the employment of the whole-genome high-resolution aCGH platforms for detecting deletions or duplications has extensively grown. The application of the aCGH technology has dramatically enhanced and refined the detection of multiple CNVs of variable size, offering at the same time high resolution, high reproducibility, and scalability for an extensive genome-wide mapping of DNA alterations [17-21]. This biotechnology is now recognized as the first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies [22]. In addition, several customized high-density aCGH, suitably designed to focus on specific clinically relevant chromosomal locations have been developed [21, 23-28]. This approach has been already applied to different human diseases including neuromuscular diseases, cancer, autism, epilepsy, multiple sclerosis, mitochondrial and metabolic disorders [20, 29-38].

In this report, we describe the design and development of a customized exon-centric aCGH (hereafter called *NeuroArray 1.0*), tailored to detect exonic deletions and duplications in a large panel of genes involved in the most common molecularly diagnosed neuromuscolar diseases: AD and other dementias, PD, ALS, Epilepsies, Rett Syndrome (RTT), Autosomal dominant and recessive Limb-Girdle Muscular Dystrophy (LGMD), Muscular Duchenne (DMD)/ Becker Dystrophy (BMD), Hereditary Spastic Paraplegia (HSP), Spinocerebellar Ataxia (SCA), Neurofibromatosis (NF), Tuberous sclerosis (TSC), Peripheral Neuropathy (PN) and Stroke. Our report shows the advantages of a customized platform in terms of findings, time, and costs compared to other commercial approaches, as well as for the detection of new potential genetic biomarkers that may play roles in understanding the common linking mechanisms underlying neurological disorders.

2. MATERIALS AND METHODS

2.1. Gene Selection and aCGH Design Strategy

Our customized *NeuroArray* aCGH platform was built to permit a high-density probe coverage in the coding region of clinically relevant genes associated with major and frequently observed neurological disorders (AD and other dementias, PD, ALS, Epilepsies, RTT, LGMD, DMD/BMD, HSP, SCA, NF, TSC, PN and Stroke).

The selection of genes included in the array, relied on our extensive expertise in clinics and genetics of neurological diseases and on updated literature data, has been extended to the entire currently known sets of disease-linked genes collected in specific public databases available online, such as ALZgene (http://www.alzgene.org/), PDgene (http://www.pdgene.org/) and ALSgene (http://www.alsgene.org/). The set of selected genes embraces known and putative risk factors, disease-causing genes, and other related genetic regions affected by different types of mutations have been described in our previous work [39].

The array design was carried out by using the web-based Agilent SureDesign Software (Advanced Design Wizard option), version 1.2.1.15 (Agilent Technologies, Santa Clara, CA). This web application allows to define regions of interest and select the "best-performing" probes from the High-Density (HD) Agilent probe library. Chromosomal coordinates of all RefSeq genes were extrapolated from open-source databases, Biomart (http://www.biomart.org/) and UCSC Genome Browser according to Human Feb. 2009 Assembly (GRCh37/hg19) (http://genome.ucsc.edu). Exon coordinates of neuro-related genes were selected and formatted using a homemade R script [40] and then uploaded on SureDesign. Candidate probes were scored and filtered using bioinformatics prediction criteria for probe sensitivity, specificity, and responsiveness under appropriate conditions. We also selected a limited number of probes (2337) with the SureDesign Genomic Tiling option to cover regions inadequately represented in the Agilent database. A total amount of 40,973 probes with a median probe spacing of 355 bp were enriched in the coding regions of 1,632 genes. All probes were chosen with similar characteristics: isothermal probes, with melting temperature (Tm) of 80°C and probe length of about 60-mers. Further details about the number of total exon/targets and probes from HD library or Genomic Tiling and other characteristics of *NeuroArray* (*e.g.,* median/ average probes spacing, target coverage percentage, *etc.*) have been described in our previous work [39].

Biological probes were randomly distributed in the 8x60K array format that allows to contemporary process eight samples in a single experiment. For the purposes of raw data quality control and normalization processes, a set of 1262 normalization or 'backbone' probes and a set of 5000 (5 x 1000) replicate probes were also added to the array design, following manufacturer's instructions. Microarray slides were produced using Agilent's Sure-Print Inkjet technology (Agilent Technologies, Santa Clara, CA).

2.2. Clinical Samples Selection

To validate the *NeuroArray*, we selected DNA samples from individuals previously subjected to gene dosage or CNVs detection through Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR or other commercially available whole-genome aCGH. In addition, DNA of patients with neurological phenotypes and an incomplete molecular diagnosis were referred for *NeuroArray* molecular cytogenetic testing. In total, 40 samples were tested on *NeuroArray* platform.

2.3. Microarray Experiment

Genomic DNA was extracted from peripheral blood lymphocytes using the EZ1 DNA Blood extraction kit (Qiagen,

Hilden, Germany) by Biorobot EZ1 following manufacturer's recommendations (Qiagen, Hilden, Germany). Highly concentrated DNA was checked for quality using the NanoDrop spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA). Array experiments were performed as recommended by the manufacturer (Agilent Technologies, Santa Clara, CA). DNA test and a reference of the same sex (Euro Reference, Agilent Technologies, Santa Clara, CA), both at the concentration of 500 ng, were double digested with RsaI and AluI for 2 hours at 37°C. After heat inactivation of the enzymes at 65°C for 20 min, each digested sample was labeled by random priming by using the genomic DNA Enzymatic Labelling Kit (Agilent Technologies, Santa Clara, CA) for 2 hours using Cy5-dUTP for patient DNAs and Cy3-dUTP for reference DNAs. Labeled products were column purified by using the SureTag DNA Labeling Kit Purification Columns (Agilent Technologies, Santa Clara, CA). After probe denaturation and pre-annealing with Cot-1 DNA, hybridization was performed at 65°C with rotation for 24 hr. After two washing steps, the array was scanned by SureScan scanner (Agilent Technologies, Santa Clara, CA) at 3 microns. Array data were extracted from scanned images using Feature Extraction software (Agilent Technologies, Santa Clara, CA) and underwent a quality control step in order to check signal intensities and background noise. In particular, the following evaluation metrics were used to pass our quality control test: Derivative Log Ratio Spread $\leq$0.30, signal intensity $\geq$200, background noise of both channels $\leq$ 25, and signal to noise $\geq$30.

2.4. Data Analysis

The analysis and visualization of array data were performed using CytoGenomics software v.2.9.2.4. (Agilent Technologies, Santa Clara, CA). We used the Aberration Detection Method 1 (ADM-1) algorithm to identify all aberrant intervals in a given sample with consistently high or low log ratios based on a statistical score. The statistical score was calculated on the average log ratios of the probes and the number of probes for region. To make a positive call, our threshold settings for the aCGH analysis method were 6.0 for sensitivity, 0.25 for minimum absolute average log ratio and three as a minimum number of probes in the region.

2.5. Validation

Ad hoc quantitative real-time polymerase chain reaction (qPCR) assays were performed to validate some genomic imbalances detected by the *NeuroArray*. Primers flanking the putative exonic imbalances were designed using the Primer-Blast tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) as described [41]. A reference gene was used for normalization. Each qPCR assay was performed in triplicate using the LightCycler 1.5 (Roche Diagnostics, Germany). The relative quantification was measured using the $\Delta\Delta$Ct method that requires a healthy control sample (diploid) as a calibrator in all amplifications [42]. In particular, as calibrator control, we used the same DNA reference hybridized in the *NeuroArray* experiments. PCR products were visualized by agarose gel electrophoresis.

3. RESULTS

3.1. Disease Related Gene-panels Selection

To comprehensively analyze CNVs embracing genes linked to neurological diseases, we designed a customized oligonucleotide array here called *NeuroArray*. The group of diseases chosen for the *NeuroArray* design encompasses heterogeneous clinically neurological disorders, requiring genetic tests for clinical diagnosis. Specifically, we chose to focus on AD and other dementias, PD, ALS, Epilepsies, RTT, LGMD, DMD/BMD, HSP, SCA, NF, TSC, PN, and Stroke. Genomic DNA regions selected for the array design overlap with both causative and/or susceptibility genes previously linked to one or more neurological diseases. In the end, the *NeuroArray* design included a total amount of 1,632 human genes with a total coverage of exonic targets of 94%, as reported in our previous work [39]. In the next sections, we will summarize the major features of the disease-related targeted genetic-panels. It should, however, be kept in mind that the following disease-related groups are made only for simplicity purposes, considering that neurological disorders are often characterized by a continuum spectrum of genetic and phenotypic variations. A graphical representation of selected pathologies and the number of disease-linked genes used for *NeuroArray* customization is showed in Fig. (**1**).

3.1.1. Dementias

AD is the most common irreversible, progressive cause of dementia in the elderly, characterized by a gradual loss of memory and cognitive decline. Genetic variability is a key factor in the development and progression of AD, accounting for approximately 58-79% of phenotypic variation [43]. While mutations in *APP*, *PSEN1,* and *PSEN2* are known to primarily cause early-onset AD (age at onset<60 years), the leading genetic risk factor for the more common late-onset AD (age at onset>60 years) is the APOE ε4 allele [43]. In the last years, the advancement of genome-wide scanning methodologies has enabled the identification of a number of previously uncharacterized CNVs that might play a role in AD [44]. We selected AD-related genes from ALZGene database (release April 2011), including at the end a total amount of 641 genes and 9118 exonic regions.

Frontotemporal Dementia (FTD) is much less common than AD, but it is particularly frequent in patients younger than 65 years [45]. FTD term encompasses a group of neurological diseases characterized by progressive deficits in behavior, executive function, or language. Genetics represents an important risk factor for FTD: family histories of dementia are reported in up to 40% of cases of frontotemporal lobar degeneration, although a clear autosomal dominant inheritance accounts for only 10% of cases [45]. Interestingly, there is growing evidence of overlapping clinical, neuropathological and genetic features between FTD and other neurological diseases, including ALS. Indeed, it has been demonstrated that mutations in some ALS-related genes, such as *C9ORF72*, *MAPT*, and *GRN*, account for about 60% of all cases of inherited frontotemporal lobar degeneration. Further rare forms of FTD are also caused by mutations in other ALS-related genes, including *TARDBP*, *FUS*, *VCP*, or *CHMP2B* genes, emphasizing the close relationship between these two pathologies [45]. All common and ALS-related

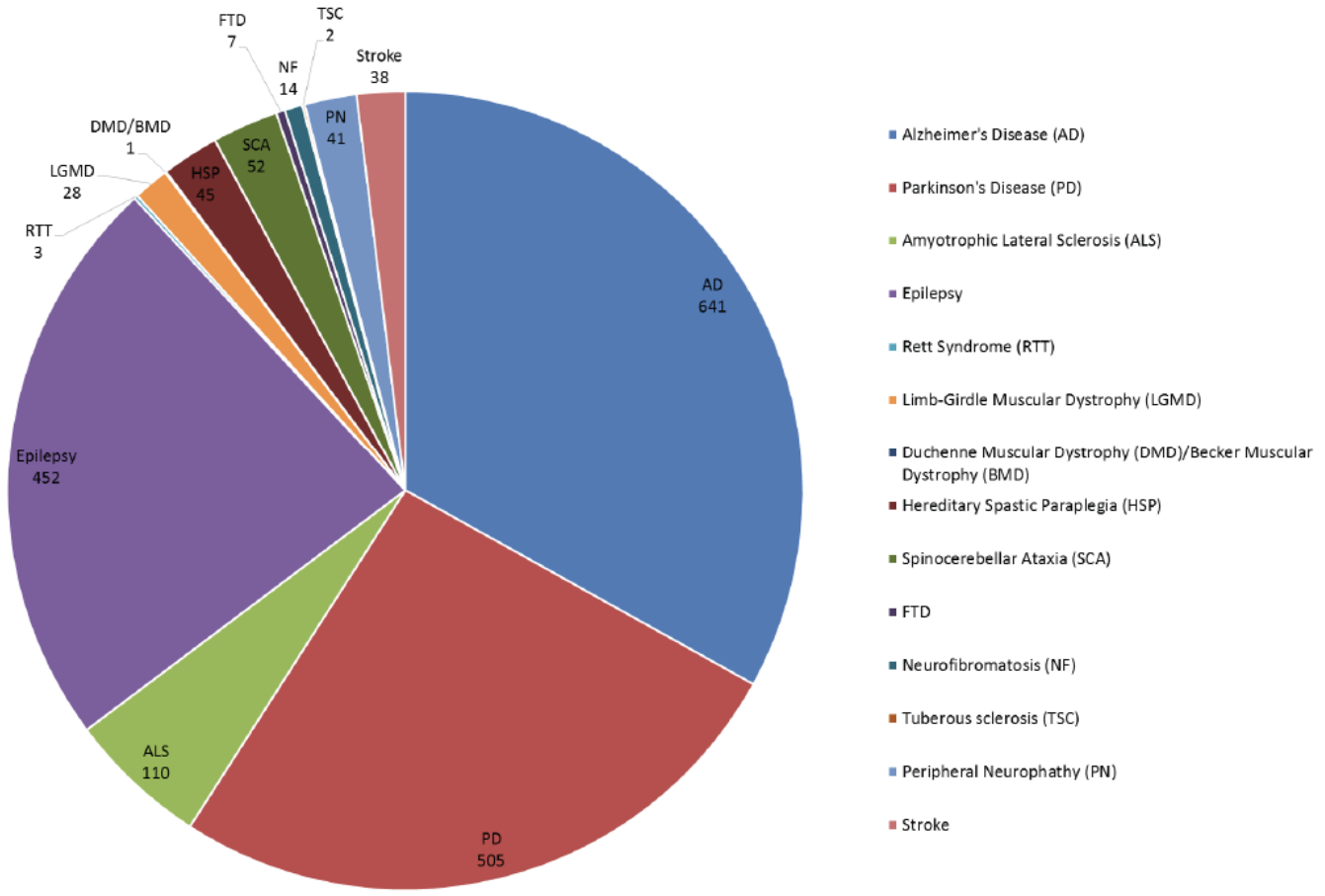

Fig. (1). Clinically relevant genes selected for the *NeuroArray* customization. Graphical representation showing the number of clinically relevant genes involved in neurological diseases and included in the aCGH *NeuroArray*. The largest number of genes belongs to Alzheimer's disease's panel, followed by Parkinson's disease and Epilepsy forms.

FTD-causing genes (including 7 genes and 82 exons) were covered in the *NeuroArray* design.

3.1.2. Movement Disorders

A set of neurological conditions selected for *NeuroArray* customization belongs to the class of movement disorders. PD is the most common, affecting approximately 1% of the population older than 65 years of age worldwide [46]. Clinically, PD patients present the classic tetrad of motor symptoms: low-frequency resting tremor, bradykinesia, rigidity of the skeletal muscles and postural instability. These major symptoms derive from the profound and selective loss of dopaminergic neurons in the *substantia nigra pars compacta*, coupled with the accumulation of eosinophilic intracytoplasmic aggregates termed Lewy bodies [46]. Although mutations in five main genes (*SNCA*, *LRRK2*, *PARK2*, *PINK1*, *DJ1*) have been identified as responsible for the dominant or recessive form of the disease, in the last years, thanks to genome-wide linkage scans and exome sequencing, researchers have discovered dozens of further loci, genes and risk factors that seem to contribute to PD [47-49]. By using PDgene database (Release February 2013), we selected for the PD-panel a total amount of 505 genes, including 6826 exons. The application of this specific panel has been deeply discussed in a previous paper [39].

ALS is a genetically heterogeneous disorder that shows a characteristic dichotomy of familial forms (10%), typically displaying Mendelian inheritance patterns, and isolated or sporadic ALS (90%) characterized by a multifactorial nature in which multiple genetic variants, each of small effect, combine with environmental triggers and risk factors [50]. Although identification of disease-linked mutations has played an important role in our understanding of ALS pathophysiology, the molecular mechanisms and precise genetic causes of ALS remain to be clarified. For the *NeuroArray* design, we included 110 genes in the ALS-panel, globally selected from ALSgene database, encompassing the major disease-causing genes (*e.g., SOD1, ALS2, SETX, SPG11, FUS, VAPB, ANG, TARDBP, FIG4, OPTN, ATXN2, UBQLN2, PGRN, PFN1, DCTN1* and *C9ORF72*) as well as several potential genetic risk factors [51].

SCA comprises a large group of heterogeneous neurodegenerative disorders inherited in an autosomal dominant, recessive or X-linked fashion. SCA conditions are characterized by progressive cerebellar ataxia with oculomotor dysfunction, dysarthria, pyramidal signs, extrapyramidal signs, pigmentary retinopathy, peripheral neuropathy, cognitive impairment and other symptoms [52]. Several forms of SCA are currently known and are classified according to the clinical manifestations or genetic nosology. Moreover, thanks to

the widespread clinical use of NGS, a large number of pathogenic genes underlying SCAs have been characterized [53]; among them, a small number contains pathogenic mutations that can be detected by the aCGH technology. We included in the *NeuroArray* design a panel of 52 genes related to both dominant and recessive forms of SCAs, covering a total amount of 1012 exonic regions.

HSP is a syndromic designation for a clinically and genetically heterogeneous group of inherited neurodegenerative or neurodevelopmental disorders in which the main neurological symptoms and signs are lower limb spasticity and weakness [54]. HSPs are classified according to the clinical phenotype, pattern of inheritance, or pathophysiological molecular mechanism. The genetic basis of HSPs is complex with more than 70 genetic subtypes involving all patterns of Mendelian inheritance (autosomal dominant, autosomal recessive, X-linked) and maternal mitochondrial transmission [54]. Among the HSP-related genes, we included in *NeuroArray* a list of 45 targets.

3.1.3. Episodic and Paroxysmal Disorders

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition [55]. Different families of disorders, having in common an abnormally increased predisposition to seizures, belong to epilepsy [55, 56]. The importance of genetics in the etiology of epilepsy has been widely confirmed in the last decades, involving both CNV and *de novo* mutations [56-61]. For the *NeuroArray* design, we have selected 452 genes with 7089 exonic targets involved in different epileptic forms. In particular, it allows the detection of CNVs of a large set of autosomal and X-linked genes involved in epilepsy.

3.1.4. Myopathies

Muscular dystrophy is a group of inherited diseases characterized by a primary structural or functional impairment of skeletal muscle. The most well-known are DMD and BMD, both caused by mutations in the dystrophin-gene [62]. The human *DMD* gene, the largest of our genome, is located in chromosome X and contains 79 exons spanning 2.2 Mb. Currently, the molecular diagnostic of DMD/BMD relies on the analysis of deletions/duplications performed by commercial aCGH, MLPA, and, more recently, by NGS panels [63]. Although these methods have provided relevant technical and diagnostic implications, these often fail to detect and characterize precisely the nature of rare, complex rearrangements associating duplications and triplications of several, non-consecutive exons. In this regard, our *NeuroArray* platform allows an exon-focused evaluation of structural imbalances at a higher resolution than whole-genome commercially available platforms, and lowers the costs of an "exon by exon" analysis through PCR-based approaches, representing a valuable, cost-effective tool for high throughput *DMD* molecular diagnosis as well as for definition of elusive *DMD* gene mutations.

LGMD encompasses a highly heterogeneous group of muscle disorders, affecting voluntary muscles of the hip and shoulder areas [64]. The genetic classification of LGMD is becoming increasingly complex since this acronym has also been used for a number of other myopathic disorders with overlapping phenotypes [64]. Today, the list of genes to be screened is too large for the gene-by-gene approach and it is well suited for targeted NGS or array panels including any gene strictly associated with the clinical picture of LGMD. Here, we selected 28 LGMD-related genes associated with both autosomal dominant and recessive forms of diseases.

3.1.5. Peripheral Neuropathies

Inherited peripheral neuropathies are among the most common genetic neuromuscular disorders worldwide. Their diagnosis is challenging due to their genotypic and phenotypic variability, but while different mutations in *PMP22* are the cause of HNPP [65], CMT disease (the most common PN form) is associated with mutations or CNVs in numerous genes, mainly encoding proteins involved in the development and functionality of Schwann cells and peripheral axons [66]. Although measuring the *PMP22* duplication in CMT patients is still a reasonable diagnostic test, the increasing availability and affordability of genome-wide technologies has ramped up gene discovery and drastically changed genetic screening strategies [67]. In *NeuroArray*, we included a PN-related genes set counting a total amount of 41 genetic targets.

3.1.6. Neurocutaneous Syndromes

NF and TS, both type 1 and type 2, are genetic phakomatoses syndromes, also known as neuro-genodermatoses, mainly characterized by cutaneous lesions, as well as peripheral or central nervous system neoplasms [68]. Like the majority of hereditary cancer syndromes, NF and TS are both autosomal dominant in inheritance [69]. NF affects one in 3,000 persons while TS occurs in one in every 30,000 to 50,000 births. Although the penetrance is high for both these diseases, new cases of NF and TS have frequently been associated with *de novo* mutations [70, 71]. In order to better investigate the role of CNVs in these two diseases, we included in the *NeuroArray* platform in addition to the two main genes associated with TS (*TSC1* and *TSC2*) those surrounding and including *NF1* and *NF2* loci [72]; in particular for NF1 they seem to be responsible for more severe clinical phenotypes [73, 74].

3.1.7. Other Neurological Diseases

In addition to above discussed disease-related gene panels, the *NeuroArray* platform includes a number of genes related to other neurological disease characterized by well-characterized genetic backgrounds (*i.e.,* stroke and RTT).

Stroke is the third leading cause of death worldwide, after heart disease and all forms of cancers, and represents an important cause of age-related cognitive decline and dementia [75]. Both genetic and environmental risk factors contribute to damaging cerebral blood vessels and, consequently, cause stroke [75]. Approximately 20% of strokes are hemorrhagic, while the remaining 80% are classified as ischemic strokes. Several CNVs seem to contribute to both hemorrhagic and ischemic stroke [75]. Here, we included 38 genes overall related to both types of stroke.

RTT is a rare genetic postnatal neurological disorder of the gray matter of the brain that almost exclusively affects females (it rarely affects male patients). The clinical features

include small hands and feet and a deceleration of the rate of head growth. Repetitive stereotyped hand movements, such as wringing and/or repeatedly putting hands into the mouth are also noted. Genetically, RTT is caused by mutations in the gene *MECP2* located on the X chromosome (which is involved in transcriptional silencing and epigenetic regulation of methylated DNA), and can arise sporadically or from germline mutations [76]. Atypical RTT variants involve mutations in *CDKL5* or *FOXG1* genes [76]. These three genes were included in *NeuroArray* design.

3.2. Comparison Between *NeuroArray* and Other CNV Detection Methodologies

Our comparative validation process has demonstrated that *NeuroArray* was able to detect both micro and macro genomic imbalances previously characterized by MLPA, qPCR or other commercially available whole-genome aCGH platforms. The array was also able to refine better the genomic intervals involved in the imbalances (inner start-stop coordinates). In the following sections, we will show some representative examples of *NeuroArray* tests compared to other CNV detection techniques.

3.2.1. NeuroArray vs. Commercial Whole-genome aCGH

The DNA sample of patient #1 was referred to our laboratory for molecular testing of *SPG7*, a gene localized on chromosome 16q24.3, and whose mutations are responsible for the onset of the autosomal recessive spastic paraplegia 7. We firstly sequenced the DNA sample for the entire genetic region of *SPG7*, detecting a heterozygous single point mutation in the exon 11, inherited from the mother's side. Since the recessive nature of this neurological disease, we also performed a double CNVs analysis by using both the commercially available whole-genome Agilent SurePrint G3 Human CGH Microarray 8x60K slide and the *NeuroArray* platform. The first test on the commercial whole-genome aCGH did not show any structural imbalance in the *SPG7* gene, because of the few probes covering this region. As shown in Fig. (**2**), in the 8x60K slide format, there are just three probes covering SPG7 gene (the exon 10 of the shorter isoform and two additional intronic regions respectively). Our customized *NeuroArray* design was able to detect a heterozygous deletion of the exons 2 and 3 of *SPG7* (Fig. **2**), because of the higher density probes targeting *SPG7* exons. This deletion was subsequently confirmed in both proband's and father's genomes by MLPA test with a concordance of 94% with *NeuroArray* (Kit P213-A2).

3.2.2. NeuroArray vs. MLPA Assay

The DNA sample of patient #2 was referred to our laboratory to confirm the clinical diagnosis of Neurofibromatosis type 1, a disorder caused by inactivating mutations in the 17q11.2-located Neurofibromin 1 (*NF1)* gene. This gene contains 58 exons spanning approximately 282 kb of genomic DNA and encodes neurofibromin protein. The point mutations analysis of *NF1* in the DNA sample resulted negative. To detect the deletion/duplication in the *NF*1 gene, two different MLPA kits (SALSA MLPA P081/082 and P122-C1 by MRC-Holland, Amsterdam, The Netherlands) were used. A whole-gene heterozygous deletion comprising the deletion

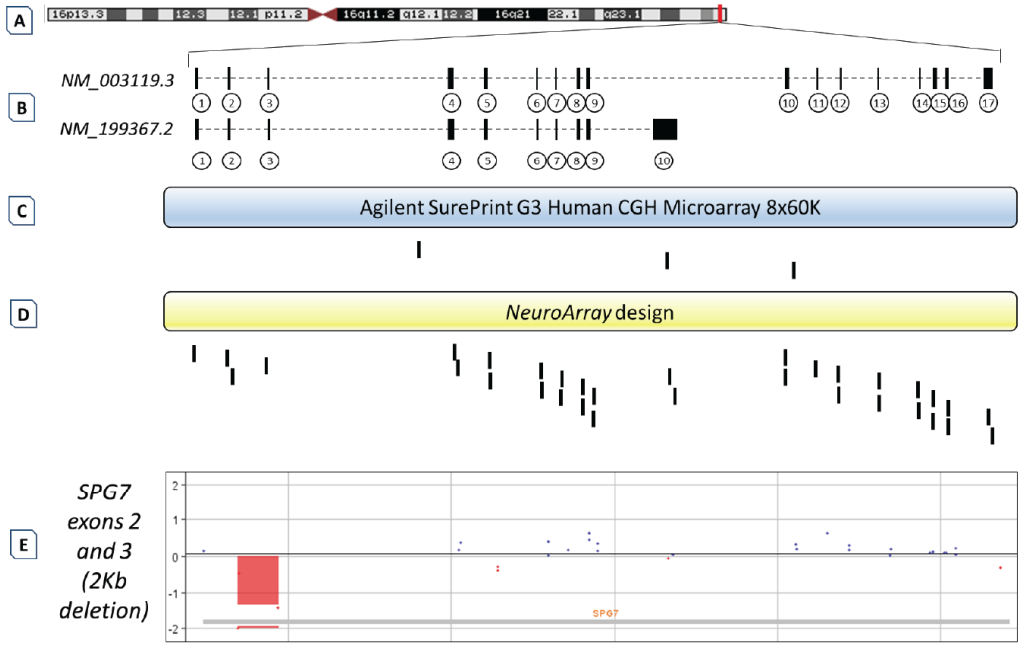

Fig. (2). ***NeuroArray vs.*** **commercial platforms. A:** The human *SPG7* gene is located on chromosome 16q24.3, spanning 49.3 Kb of genomic DNA. **B:** This gene produces two different transcripts, the longest of which encompasses 17 exonic regions. Both transcripts are illustrated in the figure and are indicated by the NCBI Accession Number on the right. *SPG7* exons are represented in the figure by black boxes and are numbered consecutively. The dashed line represents intronic regions. **C:** Distribution of oligonucleotide probes on the commercially available whole-genome Agilent SurePrint G3 Human CGH Microarray 8x60K. **D:** Distribution of oligonucleotide probes in the entire exonic regions of *SPG7* gene in the customized *NeuroArray* design. **E:** Detection of heterozygous exonic deletion in *SPG7* gene in a patient with Hereditary Spastic Paraplegia 7. *NeuroArray* showed the deletion of the exon 2 and 3 of *SPG7* gene, later confirmed by MLPA assay.

of *UTP6* and *SUZ12*, two genes telomeric to the *NF1* gene, was detected. The *NeuroArray* test has confirmed the heterozygous deletion (844 kb, Chr.17 from 29,483,057 to 30,327,458), encompassing *NF1* and further neighboring genes (Fig. **3**), with a concordance of 98% with MLPA assay. Deletions >700kb in this region have been described in patients with atypical *NF1* microdeletion and are often characterized by a more severe phenotype compared to patients with intragenic *NF1* mutations [73].

3.2.3. NeuroArray vs. Real-time PCR

The DNA sample from patient #3 was referred to our laboratory for molecular testing of sporadic ALS. The genetic screening of major ALS known genes *SOD1, C9ORF72, FUS, TARDBP,* and *ANG* was negative. The test on *NeuroArray* has revealed heterozygous deletions within the following three regions: i) 64,184 Kb deleted on Chr.2 (cytoband 2p14) embracing *VPS54* gene (Fig. **4, panel A**); ii) 5,1 Kb in the cytoband 2q24 including the *SCN7A* gene (Fig. **4, panel B**) and iii) a region on Chr.3 (2.5 Mb from cytoband 3p11.2 to 3p11.1) embracing *CHMP2B* and further overlapping and neighboring genes (Fig. **4, panel C**). The qPCR assay performed on exon 4 of *VPS54*, exon 3 of *SCN7A* and exon 5 *CHMP2B* was able to confirm the deletion with 100% of concordance. It is interesting to note that genetic/genomic aberrations of these genes have been already associated with ALS pathogenesis [77-80]. In particular, a heterozygous deletion in the *CHMP2B* gene has already been implicated in ALS pathogenesis [15], as well as in other related neurodegenerative disorders, including FTD [81, 82]. However, in contrast to the previously reported data [79], our ALS case showed weakness and muscular atrophy in both upper limbs with onset in the upper limbs and was without features of FTD. The oligo-genic nature of the disease leads us to interpret these imbalances carefully as causative of the patient's phenotype and further studies are needed to explore their role in the pathogenesis of ALS.

4. DISCUSSION

The aCGH biotechnology is widely used to detect unbalanced chromosomal changes, including both large and

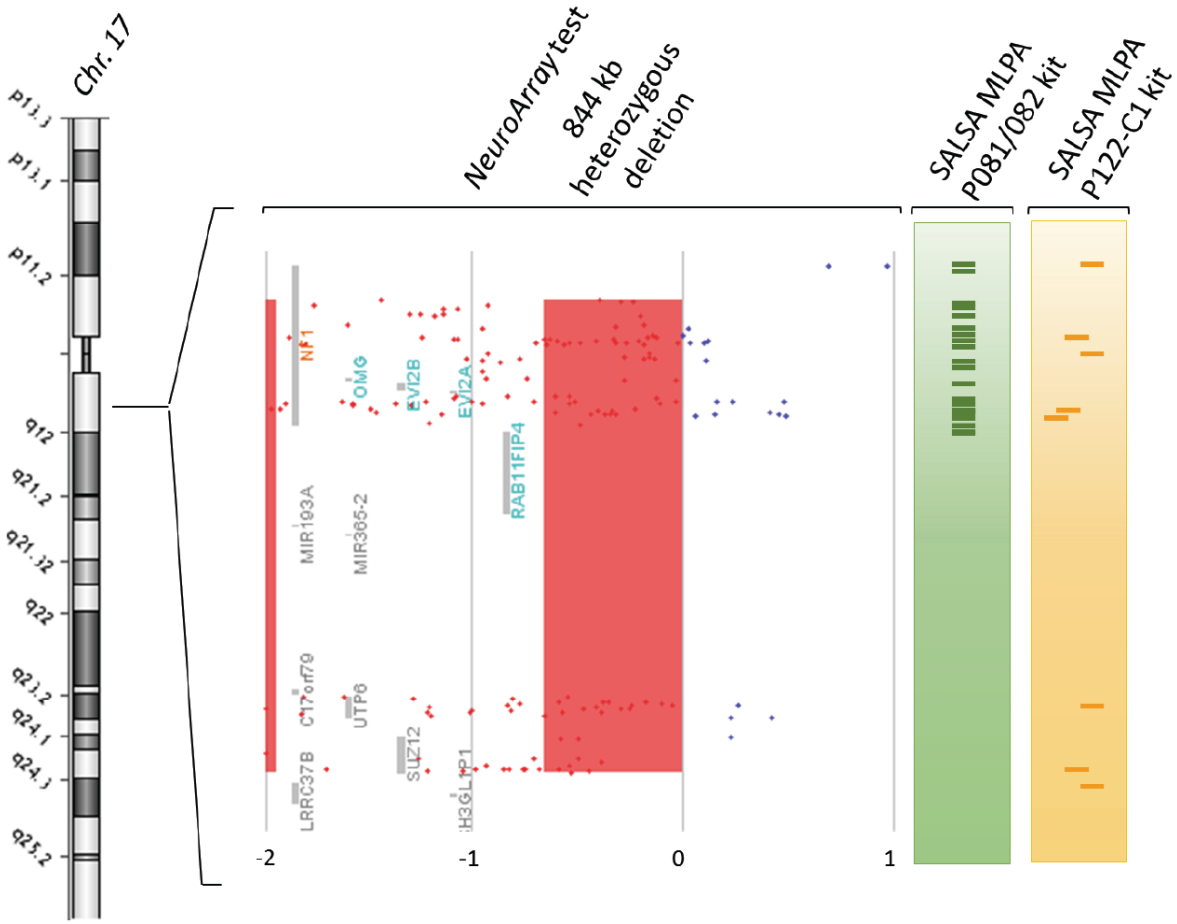

Fig. (3). Detection of *NF1* gene and neighboring genes deletion in a patient with Neurofibromatosis type I. *NeuroArray* confirmed the deletion of the tumor-suppressive *NF1* gene, previously detected by two MLPA kits *(SALSA MLPA P081/082 and P122-C1 by MRC-Holland, Amsterdam, The Netherlands)*. Furthermore, it revealed a larger deletion including further overlapping or neighboring genes. On the left is represented chromosome 17. *NeuroArray* aCGH data visualization and analysis obtained from CytoGenomics software are shown in the middle panel. The red area represents the deleted region. On top of the panel, the size of the deletion and the chromosomal locus are indicated. Red and blue dots represent the log2 ratios for the relative hybridization intensities of each spotted probe. The dots with an average log2 ratio around -1 indicate a heterozygous deletion. On the right, probe coverage of MLPA kits is graphically represented. (*For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.*)

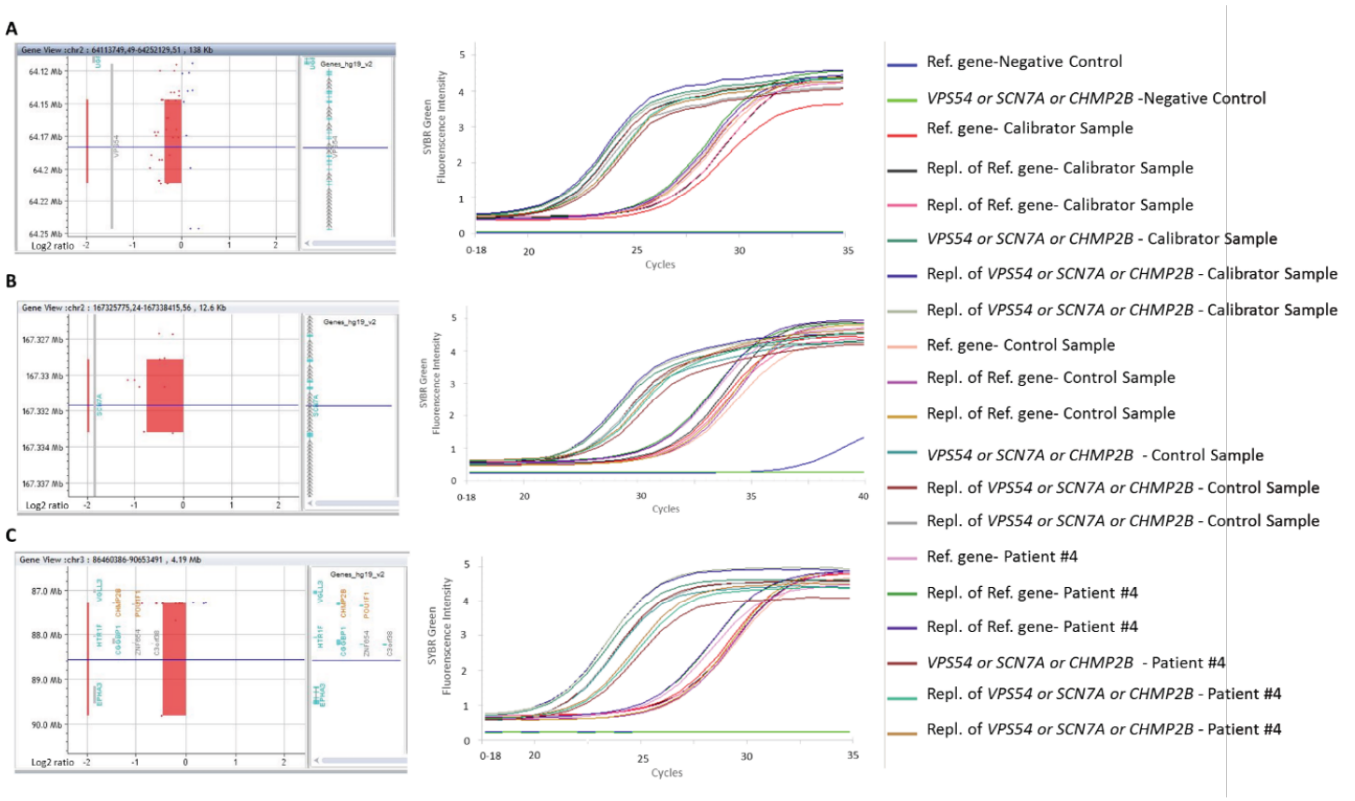

Fig. (4). Detection of three single-gene deletions (*VPS54*, *SNC7A*, and *CHMP2B*) in a patient with ALS. *NeuroArray* has revealed the heterozygous deletion of three genomic regions encompassing three known ALS-related genes in an ALS patient. In particular, the regions included: i) 64,184 Kb deleted on Chr.2 (cytoband 2p14) embracing *VPS54* gene (**Panel A**); ii) 5,1 Kb in the cytoband 2q24 including the *SCN7A* gene (**Panel B**) and iii) a region on Chr.3 (2.5 Mb from cytoband 3p11.2 to 3p11.1) embracing *CHMP2B* and further overlapping and neighboring genes (**Panel C**). The qPCR assay, performed on exon 4 of *VPS54*, exon 3 of *SCN7A* and exon 5 *CHMP2B*, has confirmed *NeuroArray* findings. For each gene is reported the comparison between *NeuroArray* aCGH results (left) and the corresponding measurement by real-time quantitative qPCR (right). *NeuroArray* aCGH data visualization and analysis were performed by CytoGenomics software. Resultant Real-Time qPCR standard curves for detection of each gene and related calibrator controls are shown; cycle number (axis X) is blotted against fluorescent signal (axis Y) obtained in every cycle at the end of the annealing step.

submicroscopic deletions and duplications across the human genome, and aCGH applications to screen common benign and rare pathogenetic CNVs are extensively growing [20, 29-33].

Targeted aCGH present several advantages over other available molecular methods, such as Southern blotting, MLPA, qPCR and Sanger sequencing [83]. Each of these methods is a gold-standard test when applied to monogenic disorders. However, when applied to multigenic pathologies (such as neurologic disorders), these methods require higher equipment costs, time, steps and personnel [33]. In contrast, the targeted aCGH is a rapid, comprehensive, relatively inexpensive, highly sensitive and accurate method for simultaneously detecting single- and multiexon deletions and duplications in numerous genes on a unique common platform. For this reason, several aCGH have been already implemented for the detection of intragenic copy number changes involved in several human diseases including neuromuscular diseases (NMDs), cancer, autism, epilepsy, multiple sclerosis and mitochondrial and metabolic disorders [20, 29-35]. In addition, the utility of a custom-designed exon-targeted oligonucleotide array to detect intragenic copy number changes has been demonstrated in patients with various clinical complex phenotypes, among which neurological ones [24], and has been commercialized for post-natal diagnosis (Agilent Technologies, Santa Clara, CA).

In this study, we have designed, validated, and implemented a focused exon-centric aCGH array as a molecular tool to detect CNVs and abnormal single-gene dosage in a large set of clinically relevant chromosomal locations linked to multiple neurological diseases. The customized *NeuroArray* offers some considerable advantages: it allows an exon-focused evaluation of structural imbalances in clinically relevant genes at a higher resolution than whole-genome commercially available platforms, and lowers the costs of an "exon by exon" analysis through PCR-based approaches, providing at the same time an extensive window of further potentially involved genetic alterations. Although the use of a 180k format would have guaranteed the interrogation of thousands of CNV regions simultaneously in a single experiment at higher level of resolution and coverage, the 8×60K platform has proven to be suitable for high-resolution analysis and it is also reasonable from an economic point of view, offering the possibility to hybridize eight samples simultaneously.

The routinely use of dedicated high-throughput genotyping platforms as our *NeuroArray* could offer new opportuni-

ties for both research and clinics: i) the large-scale screening of genes involved in neurological diseases could become a way to explore the potential genetic overlapping among different neurological conditions [84]; ii) the simultaneous detection of multiple genes involved in the nervous system diseases could aid to refine the genotype-phenotype correlations, offering a method to cluster genetic alterations commonly responsible for a specific phenotypic characters (such as dementia, motor impairment, cognition, *etc.*).

Despite the ultimate goal of the *NeuroArray* platform design is to provide a comprehensive coverage of neurologically relevant genes at exon-level resolution, the low density of normalized or "backbone" probes in intergenic regions might make breakpoint boundary characterization more difficult. Moreover, as others aCGH-based platform, *NeuroArray* is not able to detect nucleotide repeat expansions (*e.g., C9ORF72* or *ATXN2* genes), which are known to be genetic determinants of SCA or ALS, as well as balanced structural chromosomal abnormalities. Another potential limitation of the platform refers to the mosaicism conditions. As previously reported, the aCGH technology allows detecting low-grade as well as submicroscopic mosaics (<10%) [85, 86]. Anyway, the possibility to reach a correct identification of a low-represented mosaic depends on several factors (*i.e.,* the DNA quality, the algorithm used for analysis, the platform design itself). Exon-targeted high-resolution oligonucleotide arrays (such as our *NeuroArray*) have been used to detect low represented mosaicism conditions, but the good success could be related to the size of the region involved [87]. To overcome all these limitations, we are planning to design *NeuroArray* 2.0 in order to refine the probe coverage in skipped genomic regions, including additional widely-spaced backbone probes providing coverage across the whole genome and (where necessary) the intronic flanking regions and the alternatively spliced cassette exons [88-91].

During the last few years, the scientific community is having a debate on the overcoming of aCGH for CNVs detection using Whole-Exome Sequencing (WES) and powerful tools for data analysis, showing that WES seems to have reached good results compared with some whole-genome aCGH formats but not yet with exon-targeted aCGH [37, 38, 92-96]. According to this, custom-designed exon-focused aCGH can be used alone or complement next-generation sequencing data, either in detection or in validation.

Taking all these considerations, we believe that our *NeuroArray* will serve as a powerful analytic tool for the investigation of genetic factors associated with neurological diseases, overcoming some limits of commercially available whole-genome aCGH platforms and PCR-based methodologies, complementing or validating also next-generation sequencing results. In particular, the *NeuroArray* platform could be helpful in enhancing molecular diagnosis of neurological disorders, mainly for familial or sporadic cases with uncertain or not completely conclusive diagnostic outcome, elucidating inherited potential or *de novo* structural alterations that may constitute candidate drivers, therapeutic targets, and prognostic biomarkers for these multifactorial, and often incurable, diseases. We fully expect this array to become a starting point for the genetic analysis of neurologic disorders, given its relevant and up-to-date genotyping content as well as its low cost.

CONCLUSION

The custom designed *NeuroArray* 1.0 represents a robust molecular testing tool for the detection of genomic abnormalities linked to neurological diseases. Compared to commercially available tests (such as PCR-based assays or whole-genome commercial platforms), it offers at lower costs a targeted resolution, and allows to measure in more detail structural or dosage alterations in clinically relevant exonic-regions., The use of customized platform such as our *NeuroArray* 1.0 may offer, in future years, further information about the genetic overlapping of different neurological conditions and could aid to refine the genotype-phenotype correlations.

LIST OF ABBREVIATIONS

aCGH	=	Comparative Genomic Hybridization Array
AD	=	Alzheimer's Disease
ALS	=	Amyotrophic Lateral Sclerosis
BMD	=	Becker Muscular Dystrophy
CNVs	=	Copy Number Variations
DMD	=	Duchenne Muscular Dystrophy
FTD	=	Frontotemporal Dementia
GWAS	=	Genome-Wide Associations Studies
HNPP	=	Hereditary Neuropathy with Liability to Pressure Palsies
HSP	=	Hereditary Spastic Paraplegia
LGMD	=	Limb-Girdle Muscular Dystrophy
MLPA	=	Multiplex Ligation-dependent Probe Amplification
NF	=	Neurofibromatosis
NGS	=	Next Generation Sequencing
PD	=	Parkinson's Disease
PN	=	Peripheral Neuropathy
qPCR	=	Quantitative Polymerase Chain Reaction
RTT	=	Rett Syndrome
SCA	=	Spinocerebellar Ataxia
SNP	=	Single Nucleotide Polymorphism
TSC	=	Tuberous Sclerosis
WES	=	Whole-Exome Sequencing

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Study involving human participants was approved by the Ethical Committee of the "Vittorio Emanuele" University Hospital in Catania (Approval No. 17239).

HUMAN AND ANIMAL RIGHTS

No animals were involved in this study. All human research procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2008.

CONSENT FOR PUBLICATION

Informed consent was obtained for the use of DNA samples and for the access to medical records for research purposes and data publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Gu, W.; Lupski, J.R. CNV and nervous system diseases - What's new? *Cytogenet. Genome Res.,* **2008**, *123*(1-4), 54-64.

[2] Zarrei, M.; MacDonald, J.R.; Merico, D.; Scherer, S.W. A copy number variation map of the human genome. *Nat. Rev. Genet*., **2015**, *16*(3), 172-183.

[3] Lee, J.A.; Lupski, J.R. Genomic rearrangements and gene copy-number alterations as a cause of nervous system disorders. *Neuron,* **2006**, *52*(1), 103-121.

[4] Kalman, B.; Vitale, E. Structural chromosomal variations in neurological diseases. *Neurologist,* **2009**, *15*(5), 245-253.

[5] Hoyer, H.; Braathen, G.J.; Eek, A.K.; Nordang, G.B.; Skjelbred, C.F.; Russell, M.B. Copy number variations in a population-based study of Charcot-Marie-Tooth disease. *BioMed Res. Int.,* **2015**, *2015*, 960404. Available from: https://www.hindawi.com/journals/bmri/2015/960404/

[6] Olson, H.; Shen, Y.; Avallone, J.; Sheidley, B.R.; Pinsky, R.; Bergin, A.M.; Berry, G.T.; Duffy, F.H.; Eksioglu, Y.; Harris, D.J.; Hisama, F.M.; Ho, E.; Irons, M.; Jacobsen, C.M.; James, P.; Kothare, S.; Khwaja, O.; Lipton, J.; Loddenkemper, T.; Markowitz, J.; Maski, K.; Megerian, J.T.; Neilan, E.; Raffalli, P.C.; Robbins, M.; Roberts, A.; Roe, E.; Rollins, C.; Sahin, M.; Sarco, D.; Schonwald, A.; Smith, S.E.; Soul, J.; Stoler, J.M.; Takeoka, M.; Tan, W.H.; Torres, A.R.; Tsai, P.; Urion, D.K.; Weissman, L.; Wolff, R.; Wu, B.L.; Miller, D.T.; Poduri, A. Copy number variation plays an important role in clinical epilepsy. *Ann. Neurol.,* **2014**, *75*(6), 943-958.

[7] Grayton, H.M.; Fernandes, C.; Rujescu, D.; Collier, D.A. Copy number variations in neurodevelopmental disorders. *Prog. Neurobiol.,* **2012**, *99*(1), 81-91.

[8] Wang, L.; Nuytemans, K.; Bademci, G.; Jauregui, C.; Martin, E.R.; Scott, W.K.; Vance, J.M.; Zuchner, S. High-resolution survey in familial Parkinson disease genes reveals multiple independent copy number variation events in PARK2. *Hum. Mutat*., **2013**, *34*(8), 1071-1074.

[9] Rovelet-Lecrux, A.; Legallic, S.; Wallon, D.; Flaman, J.M.; Martinaud, O.; Bombois, S.; Rollin-Sillaire, A.; Michon, A.; Le Ber, I.; Pariente, J.; Puel, M.; Paquet, C.; Croisile, B.; Thomas-Anterion, C.; Vercelletto, M.; Levy, R.; Frebourg, T.; Hannequin, D.; Campion, D.; Investigators of the GMAJ project. A genome-wide study reveals rare CNVs exclusive to extreme phenotypes of Alzheimer disease. *Eur. J. Hum. Genet*., **2012**, *20*(6), 613-617.

[10] Kasuga, K.; Shimohata, T.; Nishimura, A.; Shiga, A.; Mizuguchi, T.; Tokunaga, J.; Ohno, T.; Miyashita, A.; Kuwano, R.; Matsumoto, N.; Onodera, O.; Nishizawa, M.; Ikeuchi, T. Identification of independent APP locus duplication in Japanese patients with early-onset Alzheimer disease. *J. Neurol. Neurosurg. Psychiatry,* **2009**, *80*(9), 1050-1052.

[11] Swaminathan, S.; Huentelman, M.J.; Corneveaux, J.J.; Myers, A.J.; Faber, K.M.; Foroud, T.; Mayeux, R.; Shen, L.; Kim, S.; Turk, M.; Hardy, J.; Reiman, E.M.; Saykin, A.J.; Alzheimer's Disease Neuroimaging, I.; Group, N.-L.N.F.S. Analysis of copy number variation in Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. *PLoS One,* **2012**, *7*(12), e50640. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0050640

[12] Rovelet-Lecrux, A.; Charbonnier, C.; Wallon, D.; Nicolas, G.; Seaman, M.N.; Pottier, C.; Breusegem, S.Y.; Mathur, P.P.; Jenardhanan, P.; Le Guennec, K.; Mukadam, A.S.; Quenez, O.; Coutant, S.; Rousseau, S.; Richard, A.C.; Boland, A.; Deleuze, J.F.; Frebourg, T.; Hannequin, D.; Campion, D. *De novo* deleterious genetic variations target a biological network centered on Abeta peptide in early-onset Alzheimer disease. *Mol. Psychiatry,* **2015**, *20*(9), 1046-1056.

[13] Pankratz, N.; Dumitriu, A.; Hetrick, K.N.; Sun, M.; Latourelle, J.C.; Wilk, J.B.; Halter, C.; Doheny, K.F.; Gusella, J.F.; Nichols, W.C.; Myers, R.H.; Foroud, T.; DeStefano, A.L.; Psg, P.; GenePd Investigators, C.; Molecular Genetic, L. Copy number variation in familial Parkinson disease. *PLoS One,* **2011**, *6*(8), e20988. Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0020988

[14] Kara, E.; Kiely, A.P.; Proukakis, C.; Giffin, N.; Love, S.; Hehir, J.; Rantell, K.; Pandraud, A.; Hernandez, D.G.; Nacheva, E.; Pittman, A.M.; Nalls, M.A.; Singleton, A.B.; Revesz, T.; Bhatia, K.P.; Quinn, N.; Hardy, J.; Holton, J.L.; Houlden, H. A 6.4 Mb duplication of the alpha-synuclein locus causing frontotemporal dementia and Parkinsonism: Phenotype-genotype correlations. *JAMA Neurol.,* **2014**, *71*(9), 1162-1171.

[15] Blauw, H.M.; Al-Chalabi, A.; Andersen, P.M.; van Vught, P.W.; Diekstra, F.P.; van Es, M.A.; Saris, C.G.; Groen, E.J.; van Rheenen, W.; Koppers, M.; Van't Slot, R.; Strengman, E.; Estrada, K.; Rivadeneira, F.; Hofman, A.; Uitterlinden, A.G.; Kiemeney, L.A.; Vermeulen, S.H.; Birve, A.; Waibel, S.; Meyer, T.; Cronin, S.; McLaughlin, R.L.; Hardiman, O.; Sapp, P.C.; Tobin, M.D.; Wain, L.V.; Tomik, B.; Slowik, A.; Lemmens, R.; Rujescu, D.; Schulte, C.; Gasser, T.; Brown, R.H., Jr.; Landers, J.E.; Robberecht, W.; Ludolph, A.C.; Ophoff, R.A.; Veldink, J.H.; van den Berg, L.H. A large genome scan for rare CNVs in amyotrophic lateral sclerosis. *Hum. Mol. Genet*., **2010**, *19*(20), 4091-4099.

[16] Murakami, T.; Garcia, C.A.; Reiter, L.T.; Lupski, J.R. Charcot-Marie-Tooth disease and related inherited neuropathies. *Medicine,* **1996**, *75*(5), 233-250.

[17] Carter, N.P.; Fiegler, H.; Piper, J. Comparative analysis of comparative genomic hybridization microarray technologies: Report of a workshop sponsored by the Wellcome Trust. *Cytometry,* **2002**, *49*(2), 43-48.

[18] Shaw-Smith, C.; Redon, R.; Rickman, L.; Rio, M.; Willatt, L.; Fiegler, H.; Firth, H.; Sanlaville, D.; Winter, R.; Colleaux, L.; Bobrow, M.; Carter, N.P. Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J. Med. Genet*., **2004**, *41*(4), 241-248.

[19] Iafrate, A.J.; Feuk, L.; Rivera, M.N.; Listewnik, M.L.; Donahoe, P.K.; Qi, Y.; Scherer, S.W.; Lee, C. Detection of large-scale variation in the human genome. *Nat. Genet*., **2004**, *36*(9), 949-951.

[20] Inazawa, J.; Inoue, J.; Imoto, I. Comparative genomic hybridization (CGH)-arrays pave the way for identification of novel cancer-related genes. *Cancer Sci.,* **2004**, *95*(7), 559-563.

[21] Ishkanian, A.S.; Malloff, C.A.; Watson, S.K.; DeLeeuw, R.J.; Chi, B.; Coe, B.P.; Snijders, A.; Albertson, D.G.; Pinkel, D.; Marra, M.A.; Ling, V.; MacAulay, C.; Lam, W.L. A tiling resolution DNA microarray with complete coverage of the human genome. *Nat. Genet*., **2004**, *36*(3), 299-303.

[22] Miller, D.T.; Adam, M.P.; Aradhya, S.; Biesecker, L.G.; Brothman, A.R.; Carter, N.P.; Church, D.M.; Crolla, J.A.; Eichler, E.E.; Epstein, C.J.; Faucett, W.A.; Feuk, L.; Friedman, J.M.; Hamosh, A.; Jackson, L.; Kaminsky, E.B.; Kok, K.; Krantz, I.D.; Kuhn, R.M.; Lee, C.; Ostell, J.M.; Rosenberg, C.; Scherer, S.W.; Spinner, N.B.; Stavropoulos, D.J.; Tepperberg, J.H.; Thorland, E.C.; Vermeesch, J.R.; Waggoner, D.J.; Watson, M.S.; Martin, C.L.; Ledbetter, D.H. Consensus statement: Chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am. J. Hum. Genet*., **2010**, *86*(5), 749-764.

[23] Vasson, A.; Leroux, C.; Orhant, L.; Boimard, M.; Toussaint, A.; Leroy, C.; Commere, V.; Ghiotti, T.; Deburgrave, N.; Saillour, Y.; Atlan, I.; Fouveaut, C.; Beldjord, C.; Valleix, S.; Leturcq, F.; Dode, C.; Bienvenu, T.; Chelly, J.; Cossee, M. Custom oligonucleotide array-based CGH: A reliable diagnostic tool for detection of exonic copy-number changes in multiple targeted genes. *Eur. J. Hum. Genet*., **2013**, *21*(9), 977-987.

[24] Boone, P.M.; Bacino, C.A.; Shaw, C.A.; Eng, P.A.; Hixson, P.M.; Pursley, A.N.; Kang, S.H.; Yang, Y.; Wiszniewska, J.; Nowakowska, B.A.; del Gaudio, D.; Xia, Z.; Simpson-Patel, G.; Immken, L.L.; Gibson, J.B.; Tsai, A.C.; Bowers, J.A.; Reimschisel, T.E.; Schaaf, C.P.; Potocki, L.; Scaglia, F.; Gambin, T.; Sykulski, M.; Bartnik, M.; Derwinska, K.; Wisniowiecka-Kowalnik, B.; Lalani, S.R.; Probst, F.J.; Bi, W.; Beaudet, A.L.; Patel, A.; Lupski, J.R.; Cheung, S.W.; Stankiewicz, P. Detection of clinically relevant ex-

onic copy-number changes by array CGH. *Hum. Mutat*., **2010**, *31*(12), 1326-1342.

[25] Pasmant, E.; Sabbagh, A.; Masliah-Planchon, J.; Haddad, V.; Hamel, M.-J.; Laurendeau, I.; Soulier, J.; Parfait, B.; Wolkenstein, P.; Bièche, I.; Vidaud, M.; Vidaud, D. Detection and characterization of NF1 microdeletions by custom high resolution array CGH. *J. Mol. Diagn.,* **2009**, *11*(6), 524-529.

[26] Vallespin, E.; Palomares Bralo, M.; Mori, M.A.; Martin, R.; Garcia-Minaur, S.; Fernandez, L.; de Torres, M.L.; Garcia-Santiago, F.; Mansilla, E.; Santos, F.; VE, M.M.; Crespo, M.C.; Martin, S.; Martinez-Glez, V.; Delicado, A.; Lapunzina, P.; Nevado, J. Customized high resolution CGH-array for clinical diagnosis reveals additional genomic imbalances in previous well-defined pathological samples. *Am. J. Med. Genetics Part A,* **2013**, *161A*(8), 1950-1960.

[27] Tucker, T.; Zahir, F.R.; Griffith, M.; Delaney, A.; Chai, D.; Tsang, E.; Lemyre, E.; Dobrzeniecka, S.; Marra, M.; Eydoux, P.; Langlois, S.; Hamdan, F.F.; Michaud, J.L.; Friedman, J.M. Single exon-resolution targeted chromosomal microarray analysis of known and candidate intellectual disability genes. *Eur. J. Hum. Genet*., **2014**, *22*(6), 792-800.

[28] Bruno, D.L.; Stark, Z.; Amor, D.J.; Burgess, T.; Butler, K.; Corrie, S.; Francis, D.; Ganesamoorthy, D.; Hills, L.; James, P.A.; O'Rielly, D.; Oertel, R.; Savarirayan, R.; Prabhakara, K.; Salce, N.; Slater, H.R. Extending the scope of diagnostic chromosome analysis: Detection of single gene defects using high-resolution SNP microarrays. *Hum. Mutat.,* **2011**, *32*(12), 1500-1506.

[29] Ferlini, A.; Bovolenta, M.; Neri, M.; Gualandi, F.; Balboni, A.; Yuryev, A.; Salvi, F.; Gemmati, D.; Liboni, A.; Zamboni, P. Custom CGH array profiling of copy number variations (CNVs) on chromosome 6p21.32 (HLA locus) in patients with venous malformations associated with multiple sclerosis. *BMC Med. Genet*., **2010**, *11*, 64. Available from: https://bmcmedgenet.biomedcentral.com/articles/10.1186/1471-2350-11-64/open-peer-review

[30] Piluso, G.; Dionisi, M.; Del Vecchio Blanco, F.; Torella, A.; Aurino, S.; Savarese, M.; Giugliano, T.; Bertini, E.; Terracciano, A.; Vainzof, M.; Criscuolo, C.; Politano, L.; Casali, C.; Santorelli, F.M.; Nigro, V. Motor chip: A comparative genomic hybridization microarray for copy-number mutations in 245 neuromuscular disorders. *Clin. Chem.,* **2011**, *57*(11), 1584-1596.

[31] Wisniowiecka-Kowalnik, B.; Kastory-Bronowska, M.; Bartnik, M.; Derwinska, K.; Dymczak-Domini, W.; Szumbarska, D.; Ziemka, E.; Szczaluba, K.; Sykulski, M.; Gambin, T.; Gambin, A.; Shaw, C.A.; Mazurczak, T.; Obersztyn, E.; Bocian, E.; Stankiewicz, P. Application of custom-designed oligonucleotide array CGH in 145 patients with autistic spectrum disorders. *Eur. J. Hum. Genet*., **2013**, *21*(6), 620-625.

[32] Mefford, H.C.; Muhle, H.; Ostertag, P.; von Spiczak, S.; Buysse, K.; Baker, C.; Franke, A.; Malafosse, A.; Genton, P.; Thomas, P.; Gurnett, C.A.; Schreiber, S.; Bassuk, A.G.; Guipponi, M.; Stephani, U.; Helbig, I.; Eichler, E.E. Genome-wide copy number variation in epilepsy: Novel susceptibility loci in idiopathic generalized and focal epilepsies. *PLoS Genet*., **2010**, *6*(5), e1000962. Available from: http://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1000962

[33] Tayeh, M.K.; Chin, E.L.; Miller, V.R.; Bean, L.J.; Coffee, B.; Hegde, M. Targeted comparative genomic hybridization array for the detection of single- and multiexon gene deletions and duplications. *Genet. Med.,* **2009**, *11*(4), 232-240.

[34] Wang, J.; Zhan, H.; Li, F.Y.; Pursley, A.N.; Schmitt, E.S.; Wong, L.J. Targeted array CGH as a valuable molecular diagnostic approach: experience in the diagnosis of mitochondrial and metabolic disorders. *Mol. Genet. Metab.,* **2012**, *106*(2), 221-230.

[35] Wang, J.; Rakhade, M. Utility of array CGH in molecular diagnosis of mitochondrial disorders. *Meth. Mol. Biol.,* **2012**, *837*, 301-312.

[36] Aradhya, S.; Lewis, R.; Bonaga, T.; Nwokekeh, N.; Stafford, A.; Boggs, B.; Hruska, K.; Smaoui, N.; Compton, J.G.; Richard, G.; Suchy, S. Exon-level array CGH in a large clinical cohort demonstrates increased sensitivity of diagnostic testing for Mendelian disorders. *Genet. Med.,* **2012**, *14*(6), 594-603.

[37] Retterer, K.; Scuffins, J.; Schmidt, D.; Lewis, R.; Pineda-Alvarez, D.; Stafford, A.; Schmidt, L.; Warren, S.; Gibellini, F.; Kondakova, A.; Blair, A.; Bale, S.; Matyakhina, L.; Meck, J.; Aradhya, S.; Haverfield, E. Assessing copy number from exome sequencing and exome array CGH based on CNV spectrum in a large clinical cohort. *Genet. Med.,* **2015**, *17*(8), 623-629.

[38] Gambin, T.; Yuan, B.; Bi, W.; Liu, P.; Rosenfeld, J.A.; Coban-Akdemir, Z.; Pursley, A.N.; Nagamani, S.C.S.; Marom, R.; Golla, S.; Dengle, L.; Petrie, H.G.; Matalon, R.; Emrick, L.; Proud, M.B.; Treadwell-Deering, D.; Chao, H.T.; Koillinen, H.; Brown, C.; Urraca, N.; Mostafavi, R.; Bernes, S.; Roeder, E.R.; Nugent, K.M.; Bader, P.I.; Bellus, G.; Cummings, M.; Northrup, H.; Ashfaq, M.; Westman, R.; Wildin, R.; Beck, A.E.; Immken, L.; Elton, L.; Varghese, S.; Buchanan, E.; Faivre, L.; Lefebvre, M.; Schaaf, C.P.; Walkiewicz, M.; Yang, Y.; Kang, S.L.; Lalani, S.R.; Bacino, C.A.; Beaudet, A.L.; Breman, A.M.; Smith, J.L.; Cheung, S.W.; Lupski, J.R.; Patel, A.; Shaw, C.A.; Stankiewicz, P. Identification of novel candidate disease genes from *de novo* exonic copy number variants. *Genom. Med.,* **2017**, *9*(1), 83. Available from: https:// genomemedicine.biomedcentral.com/articles/10.1186/s13073-017-0472-7

[39] La Cognata, V.; Morello, G.; Gentile, G.; D'Agata, V.; Criscuolo, C.; Cavalcanti, F.; Cavallaro, S. A customized high-resolution array-comparative genomic hybridization to explore copy number variations in Parkinson's disease. *Neurogenetics,* **2016**, *17*(4), 233-244.

[40] Team, R.C. R: A language and environment for statistical computing. R Foundation for Statistical Computing: Vienna, Austria; **2012**.

[41] D'haene, B.; Vandesompele, J.; Hellemans, J. Accurate and objective copy number profiling using real-time quantitative PCR. *Methods,* **2010**, *50*(4), 262-270.

[42] Patitucci, A.; Muglia, M.; Magariello, A.; Gabriele, A.L.; Peluso, G.; Sprovieri, T.; Conforti, F.L.; Mazzei, R.; Ungaro, C.; Condino, F.; Valentino, P.; Bono, F.; Rodolico, C.; Mazzeo, A.; Toscano, A.; Vita, G.; Quattrone, A. Comparison of different techniques for detecting 17p12 duplication in CMT1A. *Neuromusc.Disord.,* **2005**, *15*(7), 488-492.

[43] Swaminathan, S.; Shen, L.; Kim, S.; Inlow, M.; West, J.D.; Faber, K.M.; Foroud, T.; Mayeux, R.; Saykin, A.J.; Alzheimer's Disease Neuroimaging, I.; Group, N.-L.N.F.S. Analysis of copy number variation in Alzheimer's disease: The NIALOAD/ NCRAD family study. *Curr. Alzheimer Res.,* **2012**, *9*(7), 801-814.

[44] Cuccaro, D.; De Marco, E.V.; Cittadella, R.; Cavallaro, S. Copy number variants in Alzheimer's disease. *J. Alzheimer Dis.,* **2017**, *55*(1), 37-52.

[45] Bang, J.; Spina, S.; Miller, B.L. Frontotemporal dementia. *Lancet,* **2015**, *386*(10004), 1672-1682.

[46] Moore, D.J.; West, A.B.; Dawson, V.L.; Dawson, T.M. Molecular pathophysiology of Parkinson's disease. *Ann. Rev. Neurosci.,* **2005**, *28*, 57-87. Available from: https://www.annualreviews.org/doi/10.1146/annurev.neuro.28.061604.135718

[47] Klein, C.; Westenberger, A. Genetics of Parkinson's disease. *Cold Spring Harb. Perspect. Med.,* **2012**, *2*(1), a008888. Available from: http://perspectivesinmedicine.cshlp.org/content/2/1/a008888

[48] Lesage, S.; Brice, A. Role of Mendelian genes in "sporadic" Parkinson's disease. *Parkinsonism Relat. Disord.,* **2012**, *18*(Supp 1), S66-S70.

[49] La Cognata, V.; Morello, G.; D'Agata, V.; Cavallaro, S. Copy number variability in Parkinson's disease: Assembling the puzzle through a systems biology approach. *Hum. Genet*., **2017**, *136*(1), 13-37.

[50] Morello, G.; Guarnaccia, M.; Spampinato, A.G.; La Cognata, V.; D'Agata, V.; Cavallaro, S. Copy number variations in amyotrophic lateral sclerosis: Piecing the mosaic tiles together through a systems biology approach. *Mol. Neurobiol.,* **2018**, *55*(2), 1299-1322.

[51] Aronica, E.; Baas, F.; Iyer, A.; ten Asbroek, A.L.; Morello, G.; Cavallaro, S. Molecular classification of amyotrophic lateral sclerosis by unsupervised clustering of gene expression in motor cortex. *Neurobiol. Dis.,* **2015**, *74*, 359-376.

[52] Sun, Y.M.; Lu, C.; Wu, Z.Y. Spinocerebellar ataxia: Relationship between phenotype and genotype - a review. *Clin. Genet*., **2016**, *90*(4), 305-314.

[53] Geschwind, D.H.; Perlman, S.; Figueroa, K.P.; Karrim, J.; Baloh, R.W.; Pulst, S.M. Spinocerebellar ataxia type 6. Frequency of the mutation and genotype-phenotype correlations. *Neurology,* **1997**, *49*(5), 1247-1251.

[54] de Souza, P.V.; de Rezende Pinto, W.B.; de Rezende Batistella, G.N.; Bortholin, T.; Oliveira, A.S. Hereditary spastic paraplegia: Clinical and genetic hallmarks. *Cerebellum,* **2017**, *16*(2), 525-551.

[55] Fisher, R.S.; van Emde Boas, W.; Blume, W.; Elger, C.; Genton, P.; Lee, P.; Engel, J. Jr. Epileptic seizures and epilepsy: Definitions

proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia,* **2005**, *46*(4), 470-472.

[56] Garofalo, S.; Cornacchione, M.; Di Costanzo, A. From genetics to genomics of epilepsy. *Neurol. Res Int.,* **2012**, *2012*, 876234. Available from: https://www.hindawi.com/journals/nri/2012/876234/

[57] Thomas, R.H.; Berkovic, S.F. The hidden genetics of epilepsy - a clinically important new paradigm. *Nat. Rev. Neurol.,* **2014**, *10*(5), 283-292.

[58] Poduri, A.; Sheidley, B.R.; Shostak, S.; Ottman, R. Genetic testing in the epilepsies-developments and dilemmas. *Nat. Rev. Neurol.,* **2014**, *10*(5), 293-299.

[59] Kearney, J.A. Advances in epilepsy genetics and genomics. *Epilepsy Curr.,* **2012**, *12*(4), 143-146.

[60] Ottman, R.; Hirose, S.; Jain, S.; Lerche, H.; Lopes-Cendes, I.; Noebels, J.L.; Serratosa, J.; Zara, F.; Scheffer, I.E. Genetic testing in the epilepsies--report of the ILAE Genetics Commission. *Epilepsia,* **2010**, *51*(4), 655-670.

[61] Lemke, J.R.; Riesch, E.; Scheurenbrand, T.; Schubach, M.; Wilhelm, C.; Steiner, I.; Hansen, J.; Courage, C.; Gallati, S.; Burki, S.; Strozzi, S.; Simonetti, B.G.; Grunt, S.; Steinlin, M.; Alber, M.; Wolff, M.; Klopstock, T.; Prott, E.C.; Lorenz, R.; Spaich, C.; Rona, S.; Lakshminarasimhan, M.; Kroll, J.; Dorn, T.; Kramer, G.; Synofzik, M.; Becker, F.; Weber, Y.G.; Lerche, H.; Bohm, D.; Biskup, S. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia,* **2012**, *53*(8), 1387-1398.

[62] Aartsma-Rus, A.; Ginjaar, I.B.; Bushby, K. The importance of genetic diagnosis for Duchenne muscular dystrophy. *J. Med. Genet.*, **2016**, *53*(3), 145-151.

[63] Falzarano, M.S.; Scotton, C.; Passarelli, C.; Ferlini, A. Duchenne muscular dystrophy: From diagnosis to therapy. *Molecules,* **2015**, *20*(10), 18168-18184.

[64] Nigro, V.; Savarese, M. Genetic basis of limb-girdle muscular dystrophies: The 2014 update. *Acta Myol.,* **2014**, *33*(1), 1-12.

[65] Li, J.; Parker, B.; Martyn, C.; Natarajan, C.; Guo, J. The PMP22 gene and its related diseases. *Mol. Neurobiol.,* **2013**, *47*(2), 673-698.

[66] Saporta, M.A. Charcot-Marie-Tooth disease and other inherited neuropathies. *Continuum,* **2014**, *20*(5 Peripheral Nervous System Disorders), 1208-1225.

[67] Baets, J.; De Jonghe, P.; Timmerman, V. Recent advances in Charcot-Marie-Tooth disease. *Curr. Opin. Neurol.,* **2014**, *27*(5), 532-540.

[68] Strowd, R.E.; Strowd, L.C.; Blakeley, J.O. Cutaneous manifestations in neuro-oncology: Clinically relevant tumor and treatment associated dermatologic findings. *Sem. Oncology,* **2016**, *43*(3), 401-407.

[69] Nguyen, K.T.; Chiu, M. Segmental neurofibromatosis associated with renal angiomyolipomas. *Cutis,* **2008**, *82*(1), 65-68.

[70] Santoro, C.; Malan, V.; Bertoli, M.; Boddaert, N.; Vidaud, D.; Lyonnet, S. Sporadic NF1 mutation associated with a *de-novo* 20q11.3 deletion explains the association of unusual facies, Moyamoya vasculopathy, and developmental delay, reported by Bertoli *et al.* in 2009. *Clin. Dysmorphol.,* **2013**, *22*(1), 42-43.

[71] Au, K.S.; Williams, A.T.; Roach, E.S.; Batchelor, L.; Sparagana, S.P.; Delgado, M.R.; Wheless, J.W.; Baumgartner, J.E.; Roa, B.B.; Wilson, C.M.; Smith-Knuppel, T.K.; Cheung, M.Y.; Whittemore, V.H.; King, T.M.; Northrup, H. Genotype/phenotype correlation in 325 individuals referred for a diagnosis of tuberous sclerosis complex in the United States. *Genet. Med.,* **2007**, *9*(2), 88-100.

[72] Diaz de Stahl, T.; Hansson, C.M.; de Bustos, C.; Mantripragada, K.K.; Piotrowski, A.; Benetkiewicz, M.; Jarbo, C.; Wiklund, L.; Mathiesen, T.; Nyberg, G.; Collins, V.P.; Evans, D.G.; Ichimura, K.; Dumanski, J.P. High-resolution array-CGH profiling of germline and tumor-specific copy number alterations on chromosome 22 in patients affected with schwannomas. *Hum. Genetics,* **2005**, *118*(1), 35-44.

[73] Pasmant, E.; Sabbagh, A.; Spurlock, G.; Laurendeau, I.; Grillo, E.; Hamel, M.J.; Martin, L.; Barbarot, S.; Leheup, B.; Rodriguez, D.; Lacombe, D.; Dollfus, H.; Pasquier, L.; Isidor, B.; Ferkal, S.; Soulier, J.; Sanson, M.; Dieux-Coeslier, A.; Bieche, I.; Parfait, B.; Vidaud, M.; Wolkenstein, P.; Upadhyaya, M.; Vidaud, D.; Members of the, N.F.F.N. NF1 microdeletions in neurofibromatosis type 1: from genotype to phenotype. *Hum. Mut.,* **2010**, *31*(6), E1506-E1518. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/humu.21271

[74] Vogt, J.; Mussotter, T.; Bengesser, K.; Claes, K.; Hogel, J.; Chuzhanova, N.; Fu, C.; van den Ende, J.; Mautner, V.F.; Cooper, D.N.; Messiaen, L.; Kehrer-Sawatzki, H. Identification of recurrent type-2 NF1 microdeletions reveals a mitotic nonallelic homologous recombination hotspot underlying a human genomic disorder. *Hum. Mutat.*, **2012**, *33*(11), 1599-1609.

[75] Colaianni, V.; Mazzei, R.; Cavallaro, S. Copy number variations and stroke. *Neurolog. Sci.,* **2016**, *37*(12), 1895-1904.

[76] Lucariello, M.; Vidal, E.; Vidal, S.; Saez, M.; Roa, L.; Huertas, D.; Pineda, M.; Dalfo, E.; Dopazo, J.; Jurado, P.; Armstrong, J.; Esteller, M. Whole exome sequencing of Rett syndrome-like patients reveals the mutational diversity of the clinical phenotype. *Hum. Genet.*, **2016**, *35*(12), 1343-1354.

[77] Meisler, M.H.; Russ, C.; Montgomery, K.T.; Greenway, M.; Ennis, S.; Hardiman, O.; Figlewicz, D.A.; Quenneville, N.R.; Conibear, E.; Brown, R.H., Jr. Evaluation of the Golgi trafficking protein VPS54 (wobbler) as a candidate for ALS. *Amyotroph. Lateral Scler.,* **2008**, *9*(3), 141-148.

[78] Parkinson, N.; Ince, P.G.; Smith, M.O.; Highley, R.; Skibinski, G.; Andersen, P.M.; Morrison, K.E.; Pall, H.S.; Hardiman, O.; Collinge, J.; Shaw, P.J.; Fisher, E.M.; Study, M.R.C.P.I.A.; Consortium, F.R. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology,* **2006**, *67*(6), 1074-1077.

[79] Cox, L.E.; Ferraiuolo, L.; Goodall, E.F.; Heath, P.R.; Higginbottom, A.; Mortiboys, H.; Hollinger, H.C.; Hartley, J.A.; Brockington, A.; Burness, C.E.; Morrison, K.E.; Wharton, S.B.; Grierson, A.J.; Ince, P.G.; Kirby, J.; Shaw, P.J. Mutations in CHMP2B in lower motor neuron predominant amyotrophic lateral sclerosis (ALS). *PLoS One,* **2010**, *5*(3), e9872. Available from: journals.plos.org/plosone/article?id=10.1371/journal.pone.0009872

[80] Boutahar, N.; Wierinckx, A.; Camdessanche, J.P.; Antoine, J.C.; Reynaud, E.; Lassabliere, F.; Lachuer, J.; Borg, J. Differential effect of oxidative or excitotoxic stress on the transcriptional profile of amyotrophic lateral sclerosis-linked mutant SOD1 cultured neurons. *J. Neurosci. Res.,* **2011**, *89*(9), 1439-1450.

[81] Hooli, B.V.; Kovacs-Vajna, Z.M.; Mullin, K.; Blumenthal, M.A.; Mattheisen, M.; Zhang, C.; Lange, C.; Mohapatra, G.; Bertram, L.; Tanzi, R.E. Rare autosomal copy number variations in early-onset familial Alzheimer's disease. *Mol. Psychiatry,* **2014**, *19*(6), 676-681.

[82] Koyama, S.; Sato, H.; Kato, T. Copy number variations in sporadic amyotrophic lateral sclerosis. *J. Neurol. Sci.,* **2017**, *381*, 563.

[83] Li, W.; Olivier, M. Current analysis platforms and methods for detecting copy number variation. *Physiol. Genomics,* **2013**, *45*(1), 1-16.

[84] Desikan, R.S.; Schork, A.J.; Wang, Y.; Witoelar, A.; Sharma, M.; McEvoy, L.K.; Holland, D.; Brewer, J.B.; Chen, C.H.; Thompson, W.K.; Harold, D.; Williams, J.; Owen, M.J.; O'Donovan, M.C.; Pericak-Vance, M.A.; Mayeux, R.; Haines, J.L.; Farrer, L.A.; Schellenberg, G.D.; Heutink, P.; Singleton, A.B.; Brice, A.; Wood, N.W.; Hardy, J.; Martinez, M.; Choi, S.H.; DeStefano, A.; Ikram, M.A.; Bis, J.C.; Smith, A.; Fitzpatrick, A.L.; Launer, L.; van Duijn, C.; Seshadri, S.; Ulstein, I.D.; Aarsland, D.; Fladby, T.; Djurovic, S.; Hyman, B.T.; Snaedal, J.; Stefansson, H.; Stefansson, K.; Gasser, T.; Andreassen, O.A.; Dale, A.M. Genetic overlap between Alzheimer's disease and Parkinson's disease at the MAPT locus. *Mol. Psychiatry,* **2015**, *20*(12), 1588-1595. Available from: https://www.nature.com/articles/mp20156

[85] Hoang, S.; Ahn, J.; Mann, K.; Bint, S.; Mansour, S.; Homfray, T.; Mohammed, S.; Ogilvie, C.M. Detection of mosaicism for genome imbalance in a cohort of 3,042 clinical cases using an oligonucleotide array CGH platform. *Eur. J. Med. Genet.*, **2011**, *54*(2), 121-129.

[86] Valli, R.; Marletta, C.; Pressato, B.; Montalbano, G.; Lo Curto, F.; Pasquali, F.; Maserati, E. Comparative genomic hybridization on microarray (a-CGH) in constitutional and acquired mosaicism may detect as low as 8% abnormal cells. *Mol. Cytogenet.*, **2011**, *4*, 13. Available from: https://molecularcytogenetics.biomedcentral.com/articles/10.1186/1755-8166-4-13

[87] Pham, J.; Shaw, C.; Pursley, A.; Hixson, P.; Sampath, S.; Roney, E.; Gambin, T.; Kang, S.H.; Bi, W.; Lalani, S.; Bacino, C.; Lupski, J.R.; Stankiewicz, P.; Patel, A.; Cheung, S.W. Somatic mosaicism detected by exon-targeted, high-resolution aCGH in 10,362 consecutive cases. *Eur. J. Hum. Genet.*, **2014**, *22*(8), 969-978.

[88] La Cognata, V.; D'Agata, V.; Cavalcanti, F.; Cavallaro, S. Splicing:

Is there an alternative contribution to Parkinson's disease? *Neurogenetics,* **2015**, *16*(4), 245-263.

[89] La Cognata, V.; Iemmolo, R.; D'Agata, V.; Scuderi, S.; Drago, F.; Zappia, M.; Cavallaro, S. Increasing the coding potential of genomes through alternative splicing: The case of PARK2 gene. *Curr. Genomics,* **2014**, *15*(3), 203-216.

[90] Scuderi, S.; La Cognata, V.; Drago, F.; Cavallaro, S.; D'Agata, V. Alternative splicing generates different parkin protein isoforms: Evidences in human, rat, and mouse brain. *BioMed Res. Int.,* **2014**, *2014*, 690796. Available from: https://www.hindawi.com/journals/bmri/2014/690796/ref/

[91] Dagata, V.; Cavallaro, S. Parkin transcript variants in rat and human brain. *Neurochem. Res.,* **2004**, *29*(9), 1715-1724.

[92] Epilepsy Phenome/Genome Project Epi4K Consortium. Copy number variant analysis from exome data in 349 patients with epileptic encephalopathy. *Ann. Neurol.,* **2015**, *78*(2), 323-328.

[93] Pfundt, R.; del Rosario, M.; Vissers, L.E.L.M.; Kwint, M.P.; Janssen, I.M.; de Leeuw, N.; Yntema, H.G.; Nelen, M.R.; Lugtenberg, D.; Kamsteeg, E.-J.; Wieskamp, N.; Stegmann, A.P.A.; Stevens, S.J.C.; Rodenburg, R.J.T.; Simons, A.; Mensenkamp, A.R.; Rinne, T.; Gilissen, C.; Scheffer, H.; Veltman, J.A.; Hehir-Kwa, J.Y. Detection of clinically relevant copy-number variants by exome sequencing in a large cohort of genetic disorders. *Genet. Med.,* **2017**, *19*(6), 667-675.

[94] Jo, H.Y.; Park, M.H.; Woo, H.M.; Han, M.H.; Kim, B.Y.; Choi, B.O.; Chung, K.W.; Koo, S.K. Application of whole-exome sequencing for detecting copy number variants in CMT1A/HNPP. *Clin. Genet.,* **2016**, *90*(2), 177-181.

[95] D'Aurizio, R.; Pippucci, T.; Tattini, L.; Giusti, B.; Pellegrini, M.; Magi, A. Enhanced copy number variants detection from whole-exome sequencing data using EXCAVATOR2. *Nuc. Acids Res.,* **2016**, *44*(20), e154-e154.

[96] Fogel, B.L.; Lee, H.; Strom, S.P.; Deignan, J.L.; Nelson, S.F. Clinical exome sequencing in neurogenetic and neuropsychiatric disorders. *Ann. N.Y. Acad. Sci.,* **2016**, *1366*(1), 49-60.