Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2023 20(1): 193-207. **OPEN ACCESS**

NGS in Cardiology, neurology, and pediatrics: Its pathway to precision medicine

Siraj B. Al Harthi [1,3] Ahmed Elkady [2], Ijtihed Kilani [2] Rezkallah Douna [2] , Naif Alosaimi[3] Ameen Nadheef[4] Mohammad AL-Abdalli[3] and Romar Pascual [2]

[1]Department of Biological Sciences, King Abdulaziz University, Jeddah**, Kingdom of Saudi Arabia**
[2]Science Department, Shorouq Al Mamlakah International, Taif, **Kingdom of Saudi Arabia**
[3]Molecular Diagnostic Unit, Alhada Armed Forces Hospital, Taif, **Kingdom of Saudi Arabia**
[4]Department of Laboratory and Blood Bank, Abdul-Aziz Specialist Hospital, Taif, **Kingdom of Saudi Arabia**

***Correspondence: sirajj1411@gmail.com** **Received: 22-02-2023, Revised: 22-03-2023, Accepted: 25-03-2023 e-Published: 31-03-2023**

The advancement of next-generation sequencing (NGS) technology has considerably expanded our knowledge of the genesis of Mendelian a nd complex disorders. However, the complicated genomic nature of these diseases has slowed the discovery of their mutational causes. Diagnosis, treatment, and prevention can be aided by selecting the most appropriate genetic test for the task due to cost, capacity, and sequencing range. Whole-exome and whole- genome sequencing uncovers new mutations, and gene panels can be used to investigate the role of specific genes in various disorders. We explore the uses, advantages, and restrictions of NGS in neurology, cardiology, and pediatrics in this paper. Likewise, the view on the diagnosis of disorders and the application of precision medicine in their treatment was investigated. Examples of NGS- based research were presented in numerous groups of illnesses, including epilepsy, hypertrophic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, LVNCs, and pediatric brain cancers.

Keywords: Next generation sequencing; sanger sequencing; cardiology; neurology; pediatrics; Whole Exome sequencing; Whole Genome Sequencing; cardiomyopathy; epilepsy; pediatric brain tumors

INTRODUCTION

The reading and identification of nucleotide patterns in organisms' DNA, also known as genome sequencing, has been used for the past decades to decipher and delve deeper into organisms' composition (Marian, 2014). Whole-genome or Sanger sequencing, which incorporates dideoxynucleotides at random into the DNA chain and uses electrophoresis to interpret the DNA, is the most well-known and common method of genome analysis. As a result, disease research may now be conducted in considerably greater depth, notably in the departments of cardiology, neurology, and pediatrics (Ravin , 2010).

Over the past few years, a strong connection between hereditary variables and neurological illnesses has been revealed (Németh et al. 2013). Next-generation sequencing has greatly improved molecular genetic technology, allowing for the identification of numerous new diseases and the mutations responsible for them (Németh et al. 2013). The revolutionary technique has solved the problem of the diagnostic odyssey in neurological genetic conditions (Salunkhe et al. 2022).

High throughput genomics has immense promise for the detection and treatment of patients suffering from both unusual and common forms of cardiovascular disease (Ware et al. 2011). For illnesses like cardiomyopathies, arrhythmic syndromes, acropathies, and other Mendelian-inherited cardiovascular diseases, most guidelines recommend DNA testing (Charron et al. 2010; Grupa Robocza Europejskiego Towarzystwa Kardiologicznego 2011), and using NGS for their diagnosis would ensure an improved and cost-effective outcome (Ware et al. 2011).

NGS as a revolution in molecular biology has made personal genomes affordable. There is a significant optimism that NGS technologies will be successfully used to understand the biology of diseases, allowing for the development of precision medicine that would allow for treatment decisions based on a patient's unique genetic background (Marian et al. 2014).

The utilization of next-generation sequencing in the pediatric field is increasing, allowing researchers to identify the underlying mutations of an increasing number of hereditary diseases and develop new, more effective therapies for treating pediatric brain tumors (Németh et al. 2013; Salunkhe et al. 2022).

Since gene sequencing is widely used in medicine, particularly in clinical practice, a new approach was developed. First generation sequencing was the technique initially used to record mutations and variations in the human genome, but it had a number of drawbacks and inefficiencies, including time and the type of mutation.

NGS was developed as a more effective method that requires minimal time and finds many more sorts of variants. NGS, for example, could sequence the entire human genome in about a day. The previous Sanger sequencing technology, on the other hand, took more than ten years to fully decipher the human genome (Ravin 2010). In this paper, we note the application and effectiveness of the recent method.

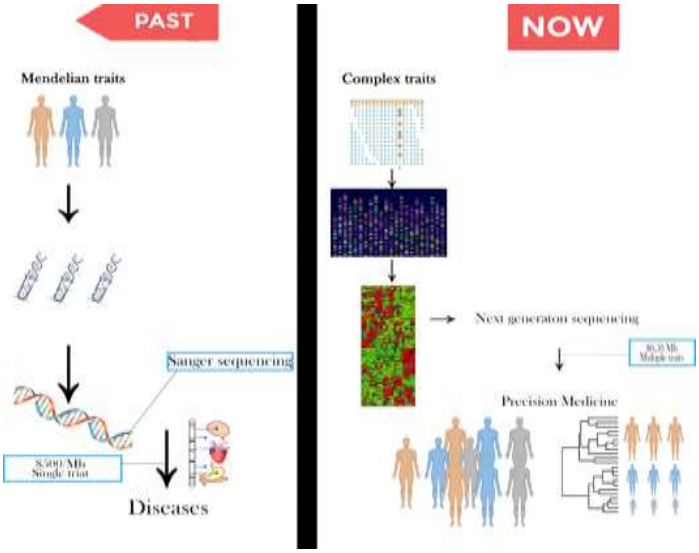

Figure 1: Illustration of the temporal evolution of genetic sequencing technologies: from monogenic to complex genetic research analysis.

(A) The process of Sanger Sequencing: deoxynucleotides are randomly incorporated into the DNA chain and is put through a process called electrophoresis to decipher the DNA. A time-consuming, limited, less efficient alternative to NGS; it takes almost a decade to reach the final draft. (B) The integration of genetic technologies (NGS and CGH) with computational strategies enables the identification of casual disease systems and patient categorization towards personalized medicine. Because it is massively parallel, NGS takes significantly less time than its counterpart (24 hours), while being up to 6000 times cheaper.

2. Types of NGS

2.1. Whole Genome Sequencing

A rigorous method for investigating complete genomes is whole-genome sequencing (WGS). Mendelian diseases, inherited diseases, and personalized medicine have all benefited from genomic information. Due to constantly falling sequencing prices and the ability to generate massive volumes of data with today's sequencers, whole-genome sequencing is a potent tool for genomics research. To be sequenced, DNA can be extracted from cell sources such as leukocytes in peripheral circulation and split into many pieces. These sequencing findings are subjected to advanced automated examination and cautious contrast with genomic reference sequences (in related databases) in order to obtain further annotated information (Biesecker et al. 2012; Westerink et al. 2014). WGS examines the entire genome for new gene variants, denovo mutations, and loci related to particular traits (Biesecker et al. 2012; Westerink et al. 2014), including but not limited to coding and noncoding regions. Exonic SNVs (Single Nucleotide Variants) and repeat expansions can be discovered using WGS. WGS fastened the discovery of three novel mitochondrial disease-causing genes such as COX6A1, TIMMDC1, and COQ5 (Malicdan et al. 2018; Tamiya et al. 2014). Furthermore, WGS includes more genes and exons than WES in the American College of Medical Genetics and Genomics (ACMG) and RefSeq databases (Meienberg et al. 2016). As a result, WGS is now regarded to be more effective than WES at detecting CNVs and SNVs. The complicated basis of polyneuropathies such as ALS was revealed by familial WGS (Gilissen et al. 2014) with a much greater rate of genomic coverage than WES. Notably, the vast number of WGS-identified variations can limit their proper prioritizing as well as WGS's capacity to provide a clear explanation or interpretation of their relative significance (Koboldt et al. 2013; Altshuler et al. 2015). However, as more non-coding variants and SVs are assessed, the diagnosis rate of WGS is projected to rise (Koboldt et al. 2013; Altshuler et al. 2015). A couple of studies have discovered that the pricing of WGS has begun to reduce, whereas WES has not (Meienberg et al. 2016). As a result, it is anticipated that WGS will soon be a diagnostic test that is widely accessible and that, in the long run, it will be more effective at finding harmful mutations than gene panels or WES.

2.2. Whole Exome Sequencing

WES uses NGS technologies like Illumina to sequence the genome's protein-coding sections. Exons make up less than 2% of the human genome, according to initial sequencing and analysis, but they are responsible for 85% of the DNA changes that lead to highly penetrant genetic disorders (Winer et al. 2013; Wang et al. 2019). WES has been the most widely utilized standard sequencing technique in clinical applications because of its reduced cost and processing time as compared to WGS. For instance, WES has helped further understand the genetics of ALS, also known as amyotrophic lateral sclerosis, a disease marked by the decline of neurogenic function. The first genetic (Chia et al. 2018). The mutation SOD1 protein develops a lethal role independent of its normal enzyme activity, and SOD1 RNA expression in cerebrospinal fluid acts as an indication of disease intensity in ALS patients (Winer et al. 2013; Schoch and Miller 2017)

Table 1: DNA Sequencing methods used by NGS systems

	Pyrosequencing	Sequencing by synthesis	Sequencing by ligation	Ion semiconductorsequencing	SMRT sequencing	Nanopore Sequencing
Read length	700bp	50–600 bp	35–60 bp	Up to 400 bp	10–15 kb maximum >40 kb	5.4 kb average (Up to 300 kb reported)
Reads per run	1 million	200–500 million Up to 2.5–3 billion	1.2–1.4 billion	Up to 80 million	500–1000 mega	4.4 million
Time per run	24 h	1–11 days	1–2 weeks	2 h	0.5–4 h	0.17–48 h
Accuracy[A]	99.99%	98% 99.9% (Phred30)	99.9%	98%	87%	~90% (up to 99% consensus)
Cost per one million bases	$10	$0.05–$0.15	$0.13	$1	$0.13–$0.60	$0.11–$0.50
Advantages	High throughput Large read length generation	Overcomes homopolymer issue due to terminated nucleotides Extremely efficient	Low cost per base	Time-efficient Cost-effective	Single molecule templates Lower cost per base Easy sample preparation Faster runtime Simplified primary data analysis	Very long reads Affordable equipment (MinION starter kit is only 1000 USD) Portable (Palm sized)
Disadvantages	Homopolymer errors Signal amplification step is needed High reagent cost Expensive as compared to its competitors	Need for signal amplification step Increase in error rate of machine with increasing read length Equipment can be very expensive Requires high concentrations of DNA	Slower than other competitors Issues in sequencing palindromic sequences	Homopolymer errors Still a "wash-and-scan" system Requiring PCR amplification Overall read length is limited	Moderate throughput. Equipment can be very expensive	Lower throughput than other competitors Only 90% single read accuracy
Platform example	454 GS FLX (Roche)	HiSeq 2000 (Illumina)	SOLiDv4 (Applied Biosystems[B])	Ion torrent (LifeTechnologies)	PacBio (Pacific Biosciences)	MinION (Oxford Nanopore)

"NGS: next-generation sequencing; SMRT: Single-molecule real-time.
A. Single read not consensus.
B. Was later acquired by Life Technologies, now part of Thermo Fisher Scientific."

WES has identified seven new genes associated with ALS since 2014: MATR3, CHCHD10, TBK1, TUBA4A, NEK1, C21orf2, and CCNF (Smith et al. 2014). The cytoskeleton's ability to generate microtubules is inhibited by mutated TUBA4A, suggesting that treatments that strengthen the cytoskeleton may stop or even reverse the progression of disease (Smith et al. 2014). Takayasu arteritis (TA) management and treatment have undergone historical modifications as a result of the identification of underlying genetic variables. The diagnosis rate in various clinical laboratories increased to 65.52% attributed to advances in sequencing technology (Wang et al. 2019; Stenton and Prokisch 2020). The overall diagnostic rate is still low, however, as a result of challenges in identifying harmful mutations that can be labeled as VUS (Variant of Unknown Significances) or exist in the non-coding area, evading WES detection. Meanwhile, utilizing WES data enables mitochondrial DNA (mt-DNA) examination in holistic level (Stenton and Prokisch 2020). However, an inconsistent discovery within one tissue type does not rule out the potential of mt-DNA variants in other tissues, as mt-DNA may vary between tissue types, due to variations in energy requirements. As a result, when employing WES to determine the molecular causes of neurogenetic illnesses, other genetic variables ought not be discounted too quickly. In essence, by integrating WES results with patient clinical data and, if necessary, with other genetic sequencing techniques, useful genetic information can be retrieved (Skinner et al. 2016).

3. Applications of NGS in Cardiology

3.1. General Applications of NGS in cardiological diseases

The etiologies of the majority of CVDs include a genetic component. It is considered that a high percentage of patients have polygenic/multifactorial diseases, in which illnesses are brought on by two or more genetic errors in the same or different genes, as well as environmental factors. These cases are believed to be far more common than the exceedingly uncommon Mendelian Cardiovascular instances with completely monogenic dominant inheritance. In addition, common polymorphisms can alter the prognosis of the symptoms of a monogenic illness (Faita et al. 2012). Comprehensive genetic molecular diagnosis methods are needed since such diseases are so complex. Thanks to NGS, we can now evaluate a huge number of genes simultaneously, which will likely help us better understand the pathology of complex diseases like CVD. Additionally, It might be beneficial for locating rare mutations in small families. The most frequent CVDs in clinical practice are complex illnesses like CAD (Coronary Artery Disease) and stroke. which are caused by complex gene-gene and gene-environment interactions (Faita et al. 2012; Hirschhorn and Daly 2005). Molecular DNA testing has recently been employed in clinical diagnostic settings, not only as a tool for research but also because it promises to give families

more tailored and intelligent counseling (Jongbloed et al. 2011).

3.2. Hereditary Cardiomyopathies

Restrictive cardiomyopathy (RCM), hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right-ventricular cardiomyopathy (ARVC)/ Unclassified cardiomyopathies with left ventricular noncompaction and arrhythmogenic ventricular cardiomyopathy (LVNC) are the most common morphological and functional phenotypes of cardiomyopathies based on changes in the structure and function of the heart muscle; each trait is a single rare mutation that accounts for the vast majority of familial cardiomyopathies (Elliott et al. 2008; Maron et al. 2006). The most prevalent inherited cardiomyopathies are HCM and DCM, which represent a diverse collection of illnesses mainly inherited through families (20% to 35%) caused by mutations in at least 30 genes (Dellefave-Castillo and McNally 2010; Kimura , 2011; Elliott et al. 2008). AARS2, MRPL3, and MRPL44 mutations in mitochondrial cardiomyopathies and the GATAD1 mutation in DCM are only a few of the uncommon genes that have been linked to cardiomyopathy thanks to the potent approach WES. Despite the limited resources, determining the responsible gene and the underlying mutation for cardiomyopathies has prognostic implications. For instance, several gene variations associated with cardiomyopathy have been associated with an early onset of the illness, a poor prognosis overall, or a high incidence of sudden cardiac death (Faita et al. 2012; Elliott et al. 2008; Maron et al. 2006). Additionally, patients with HCM who have a genetic cause are more likely to have a mild phenotype (Ingles et al. 2013) than those who have several sarcomeric gene mutations, which result in a more severe phenotype and a bad prognosis (Olivotto et al. 2008; Ho et al. 2015). Common polymorphisms may change the HCM phenotype, according to the link between a mutation in the ACE gene, which codes for angiotensin-converting enzyme, and a faster progression of hypertrophy and a higher incidence of sudden cardiac death in HCM (Marian et al. 1993; Doolan et al. 2004). Due to a multitude of factors, including the vast range of reported mutation frequencies and the widely variable nature of clinical manifestations, the use of genetic information in medical therapy for hereditary cardiomyopathies has been restricted (Bos et al. 2009).
The most effective strategy for research and the creation of new diagnostic tests seems to be targeted resequencing of “many causative genes” (Vecoli 2015). In recent years, NGS technologies have greatly improved our comprehension of the underlying genetic reasons. Although environmental factors including age, gender, and lifestyle may affect clinical symptoms, HCM, the most prevalent inherited heart disease, has been related to 20 genes and roughly 1400 different mutations (Ho et al. 2015). The majority of the causative mutations are missense variants, which result in structurally abnormal polypeptides that compromise conventional sarcomere function. About 80% of HCM cases are caused by mutations in the cardiac myosin-binding protein C (MYH7 and MYBPC3) (Ho et al. 2015).
In 2011, Meder et al. made history by being the first to screen patients for hereditary cardiomyopathy using a targeted NGS technique. Ten HCM and DCM patients had mutations identified using a 47-gene panel, and 27 additional potentially harmful variants were also found (Meder et al. 2011). To detect cardiomyopathies "time and cost-efficiently," the author created a microarray based on target enrichment, followed by SOLiD NGS (Meder et al. 2011). In relation to that, the clinical diagnosis of cardiomyopathies used the NGS approach (Soor et al. 2009). A significant NGS study examined the coding, intronic, and regulatory regions of 41 cardiovascular genes in 223 unrelated HCM patients using massive parallel resequencing on the Illumina GAIIx (Taylor et al. 2004).With titin excluded, 152 potentially harmful mutations in sarcomeric or related genes were present in 64 percent of patients (89 novel). Uncommon non-synonymous single-nucleotide polymorphisms increased in four sarcomeric genes (MYH7, MYBPC3, TNNI3, and TNNT2) (nsSNPs) when cases and controls were compared (Watkins et al. 2011), with 34% of patients possibly having changes in desmosomal and ion channel proteins. The discovery of a truncating mutation in the TTN gene as the main cause of DCM is another noteworthy instance of how NGS has assisted in illuminating the genomic spectrum of cardiomyopathies (Gerull et al. 2002). The big muscle filament titin-encoding gene TTN has been linked to family DCM since 1999 (Siu et al. 1999; Gerull et al. 2002), but because of its vast size (363 exons), standard Sanger sequencing has had difficulty analyzing this gene. As a result, the magnitude of this causation has been miscalculated for a long time. In order to analyze the deleterious modifications for co-segregation in the studied families, the full coding TTN sequence was evaluated in patients with DCM, those with HCM, and the control subjects, primarily utilizing an NGS technique (Herman et al. 2012).
About 18% of sporadic idiopathic DCM cases and 25% of familial DCM cases had TTN truncating mutations. The researchers discovered that including TTN sequencing analysis in genetic testing may increase its sensitivity by about 50%, enabling earlier identification and treatment of DCM patients to halt the progression of the disease (Herman et al. 2012). This finding was supported in 2014 by another investigation (Pugh et al. 2014). One of the largest studies to date on the use of broad genome panels in cardiomyopathy had 766 DCM patients from the US who received genetic testing at a molecular diagnostics lab over a 5-year period (Hershberger et al. 2018). Starting with a 5-gene Sanger panel and moving up to an NGS 46-gene panel, the patients were examined utilizing gene panels with previously identified involvement in DCM

that ranged in size from 5 to 46 genes. By increasing the size of gene panels, from 7.7-10% to 27-37%, the clinical sensitivity for DCM diagnosis more than tripled, mostly due to the TTN gene's inclusion. However, this improvement in sensitivity was countered by a rise in the proportion of patients whose test results were unclear increased from 4.6 to 6.5% to 51-61% (Hershberger et al. 2018). A single exon resolution NGS-based copy number analysis for up to 46 cardiomyopathy genes was performed in a large study with over 1400 patients who had cardiomyopathies like HCM, DCM, ARVC, RCM, and LVNC (Ceyhan-Birsoy et al. 2016). These researchers came to the conclusion that the improved advantage of exon level deletion/duplication analysis had not been cost-effective in routine diagnostic testing while finding clinically relevant deletions and duplications in less than 1% of individuals (Ceyhan-Birsoy et al. 2016).

Recent studies with fewer HCM patients confirmed the targeted NGS methodology's value for clinical objectives and the necessity of choosing the right patient for more efficient molecular genetic testing performance (Rubattu et al. 2016; Ackerman et al. 2011). The uncommon hereditary cardiomyopathy ARVC, which causes sudden death in children and athletes, seldom manifests in childhood and is challenging to diagnose at an early stage (Gandjbakhch et al. 2018). This heart-muscle condition can be hard to identify from DCM in its severe stages (Medeiros-Domingo et al. 2017). In a study, 14 ARVC cases were subjected to targeted sequencing using an "Illumina HighSeq 2000," with an emphasis on 96 known cardiomyopathy and channelopathy genes for filtering. According to the 2010 task force criteria, patients with a "possible" phenotype had changes in DCM-related genes, while 75% of persons with a confirmed diagnosis of ARVC had desmosomal mutations. Because they enable simultaneous molecular analysis of all disease-related genes, NGS-based panels are especially attractive for the diagnosis of ARVC and are suitable for better defining the phenotype (Gandjbakhch et al. 2018). Recent research using an NGS approach, and a panel of many genes linked to cardiomyopathy and arrhythmias has successfully discovered the variety of genetic causes in RCM patients (Kostareva et al. 2016).

3.3. Precision Medicine for Hypertrophic Cardiomyopathy

HCM patients who undergo genetic testing may give their families a diagnostic reference. A positive DNA test would enable systematic analysis of an HCM-affected proband's relatives to identify those who are mutation positive regardless of their current clinical phenotypic manifestation, leading to early identification and proper selection of relatives for ongoing medical monitoring while exempting those who are mutation negative/phenotype negative from standard cardiac exams and echocardiograms. General or specific (MYBPC3, MYH7, TNNI3, TNNT2, TPM1) HCM genetic testing is useful for any patient in whom a cardiologist has established a medical assessment of HCM based on an examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic phenotype, and mutation-specific genetic testing is advised for family members and individuals with known genetic mutations (Ackerman et al. 2011).

4. Applications of NGS in Pediatrics

The utilization of next-generation sequencing in the pediatric profession is advancing, which is making it possible to identify the causes of many more genetic illnesses and to create new, more effective treatments for those diseases. This is exemplified in disorders like pediatric brain tumors, where NGS has developed a way to quickly identify mutations and their hosts (Shirian et al. 2019). Diffuse Gliomas (DGs), for example, are a common central nervous system malignancy that typically affect the cerebral hemisphere (Carter et al. 2017). A more inexpensive and effective method of identifying these low-grade mutations for therapy is the NGS-based 1p/19q co-deletion status procedure (Dubbink et al. 2016). TP53, ATRX, CIC, IDH1, IDH2, FUBP1, PI3KC, EGFR, H3F3A, TERT, BRAF, PTEN, and NOTCH gene mutations as well as copy number variations of chromosomes 1p, 19q, 10q, and 7 were all searched for using targeted NGS to detect DG (Johnson et al. 2017).

Gliomas with the histone H3 K27M mutation, which were once classified as invasive brainstem or pontine gliomas, are now referred to as "diffuse midline glioma, H3 K27M-mutation" in the most recent WHO classification (Kallappagoudar et al. 2015). Through WES, the Histone “H3 K27M” gene mutation was discovered to be a driver mutation in 2014 (Khuong Quang et al. 2012; Wu et al. 2014; Schwartzentruber et al. 2012). Tumors of similar classification are believed to be deadlier without the H3 K27M. When using current medicines, the two-year survival rate is typically less than 10% (Shirian et al. 2019). Genetic sequencing is needed to identify pilocytic astrocytomas (PAs), WHO grade I CNS tumors that are challenging to diagnose based on anaplastic morphology (Porkholm et al. 2018). Sanger sequencing methods have shown that almost all PA cases are one-pathway diseases, only mutating the MAPK pathway through a single alteration (Johnson et al. 2017). NGS-based pediatric analysis has identified KIAA1549:BRAF fusion variants, FAM131B:BRAF fusion, four BRAFV600E mutations, and one BRAF599T mutation in pediatric astrocytomas (Johnson et al. 2017). This fusion probably functions as a tumor driver by turning on the MAPK signaling pathway (Johnson et al. 2017).

MB is the most common type of CNS embryonal tumor and mainly affects children’s cerebellums, though they could form in the dorsal brainstem (Pietsch and Haberler 2016). In accordance with the known inheritable disorders and physical features, it is divided into two classification

systems: genetically determined and morphologically determined categorization. The genetic MB classification is made up of four groups: group 3, group 4, Sonic Hedgehog (SHH)-activated, and WNT-activated. These MBs have had better prognosis with the introduction of NGS (Zhukova et al. 2013; Cavalli et al. 2017; Northcott et al. 2017), although it is still required to look at the mutation of multiple genes and chromosome duplication number variation in order to genetically categorize MBs. The inheritable groups of MB can now be further differentiated, according to a 2017 study. In other words, it is classified into four groups—WNT to to, SHH to to, group 3 to to, and group 4 to to—that more accurately reflect its prognostic and clinical characteristics (Northcott et al. 2017; Jennings et al. 2017).

Genome-wide research techniques like whole exome sequencing (WES) and whole genome sequencing (WGS) have made tremendous progress in our understanding of the molecular characteristics of brain malignancies (Myers et al. 2015). However, for the everyday clinical practice of brain tumor diagnosis and therapy, tailored NGS panels made up of a small number of genes are required. For the clinical NGS test of brain tumors, it is important to consider the gene contents, target enrichment system, type of tissues such as “fresh frozen (FF) or formalin-fixed paraffin-embedded (FFPE) tissues” and “pan-cancer panel or organ-specific panel differences” (Shirian et al. In Press).

Pan-cancer, brain tumor-specific, and glioma-specific panels are the three different types of panels. Pan-cancer panels often contain more than 300 key oncogenes, excrescence suppressor genes, and druggable genes that are often changed in different types of malignancies. Although pan-cancer panels have superior performance in copy number modifications because of the large target region (CNA), they take longer and are costlier. Additionally, pan-cancer panels do not cover genes that are only altered in a specific form of cancer with a limited frequency, such as HIST1H3B or HIST1H3C. Organ-specific panels, although cheaper and have more limited gene coverage, contain genes with unusual mutation rates that are particular to various cancer types. Clinical applications of the organ-specific panel are constrained in clinical trials and CNA analysis (Carter et al. 2017; Porkholm et al. 2018; Shirian et al. In Press)

5. Applications of NGS in Neurology

5.1.Epilepsy

Epilepsy affects over 65 million people worldwide, with genetic factors accounting for 70-80% of cases (Demarest et al. 2018). Whole-exome sequencing (WES) or targeted panels can provide a genetic diagnosis for up to 30% of early-onset epileptic patients and approximately 25% of patients with de novo mutations (Dunn et al. 2018). Complex genotype-phenotype correlations, on the other hand, make epilepsy etiology and treatment difficult (Hardies et al. 2016; Jiang et al. 2020). The fact that favorable diagnostic results are closely correlated with age is an important discovery. The diagnostic yield for early-onset epilepsy, commonly known as childhood epilepsy, is greater than that for adult-onset epilepsy (Stödberg et al. 2020; Amadori et al. 2020), suggesting that the molecular pathways are different. Several voltage-gated ion channel genes have been linked to "developmental epileptic encephalopathies (DEE)" using next-generation sequencing technology (Masnada et al. 2017; Ambrosino et al. 2020). A patient with "an uncommon sort of hyperkinetic focal motor seizure in EE" was described in a recent study. This patient had a newly identified KCNT2 mutation that altered the protein's putative pore-forming region (Ambrosino et al. 2020).

A class of diseases known as DEE that are characterized by monogenic inheritance and developmental abnormalities are mainly benefited by the use of NGS (Masnada et al. 2017). The voltage-gated K+ channel KV1.2 is encoded by the DEE-associated gene KCNA2 (Masnada et al. 2017). The first patient with KCNA2 mosaicism was described in a paper that was published in 2020. This patient had two distinct mosaic mutant alleles in KCNA2 at the same nucleotide: "c.1225A>T and c.1225A>C" (Ambrosino et al. 2020). Researchers have suggested that genetic abnormalities may occasionally be the cause for EE as the etiology of the majority of EE is unknown. Several genes linked to EE have also been discovered (Masnada et al. 2017). This newly discovered variant is similar to p.P302L, another de novo GABRG2 variant discovered in a Dravet syndrome patient (Masnada et al. 2017). The M2 transmembrane segment is affected by both variants. Furthermore, p.P302L has been shown to impair GABAA gating, cause hyper-excitability, desensitization of GABAA receptors, and, ultimately, the epilepsy phenotype (Masnada et al. 2017; Ambrosino et al. 2020). In a study of 205 cases of DEE with unknown causes, three people with refractory epilepsy and abnormal MRI results were found to have four distinct SZT2 mutations. In a study of 205 cases of DEE with unknown causes, three people with refractory epilepsy and abnormal MRI results were found to have four distinct SZT2 mutations (Chan et al. 2020). In two unrelated individuals, Japanese researchers discovered a de novo mutation in NUS1 (c.691+1C>A). Both individuals developed scoliosis, in contrast to other NUS1 mutations linked to DEE, ataxia, intellectual impairment, and developmental delays. Because of this, there is significant evidence from this work that NUS1 loss-of-function mutations, which cause the removal of the cis-PTase domain in the NgBR C-terminus, may be associated to scoliosis and represent a new phenotype (Bonzanni et al. 2018). These discoveries increase the NUS1 gene’s phenotypic spectrum. Seizures are the most common symptom of hereditary epilepsy. Numerous genetic explanations for hereditary epilepsy have been proposed, but the pathogenic genes in

the vast majority of cases remain unknown. The SCN9A gene, which has been linked to febrile seizures and hereditary epilepsy, encodes the Nav1.7 sodium channel protein. In a case study of a family with a male proband who had genetic epilepsy, a gene panel assay was used (Ittiwut et al. 2021). In a Malaysian-Chinese family with a range of epilepsy symptoms, WES revealed a novel nonsynonymous mutation in SCN1A (c.5753C>T, p.S1918F), and it was present in all family members with genetic generalized epilepsy (Wu et al. 2020). A de novo variant (c.467A>T) in ATP6V0C, a conserved termination codon mutation, was discovered using next-generation sequencing in a patient with severe epilepsy and intellectual disability (Ittiwut et al. 2021). A study suggests that NEXMIF with X-inactivation patterns may have contributed to minor intellectual impairment (Wu et al. 2020).

The atypical etiology of a few neurological diseases that are accompanied by epileptic seizures has been successfully identified using NGS. For instance, cerebral folate deficiency (CFD), a neuropsychiatric disorder, is characterized by low levels of 5-methyltetrahydrofolate in the cerebral spinal fluid (MTHF). The majority of patients with FOLR1 mutations, a rare cause of CFD, have clinical characteristics comparable to other more common causes, such as frequent epileptic seizures (Ramaekers et al. 2013; Steinfeld et al. 2009). A study linked the observed myoclonic seizures to a novel variant in FOLR1 (c.197 G>A) identified by WES (Cario et al. 2009).

Table 2: A sample of research studies that employ next-generation sequencing (NGS) as a strategy to find uncommon variants are shown in the table below

Disease	Ref	Country	Gene	Variant	NGS	Inheritance
Epilepsy	Inuzuka et al.[109]	Brazil	KCNT2	c.725C>A	WES	
	Gong et al.[111]	China	KCNA2	c.1225A>T, c.1225A>C	WES	
	Komulainen et al.[112]	Finland	GABRG2	c.917C >T	Panel	
	Sun et al.[114]	China	SZT2	c.1626+1G>A, c.5772dupA, c.4209C>A, c.7307_7308insG	Panel	
	Den et al.[115]	Japan	NUS1	c.691+1C>A	WES	
	Banfi et al.[116]	Italy	SCN9A	c.319 T>C	Panel	
	Chan et al.[117]	Malaysia	SCN1A	c.5753C>T	WES	
	Bonzanni et al.[118]	Italy	HCN1	c.469C>G	Panel	
	Ittiwut et al.[119]	Thailand	ATP6V0C	c.467A>T	WES	
	Wu et al.[120]	China	NEXMIF	c.1063delC	WES	
	Mafi et al.[124]	France	FOLR1	c.197G>A	NGS	

5.2. Precision Medicine for Epilepsy

Many harmful situations, such as unnecessary or worsening treatments, can be avoided with early diagnosis. Paroxysmal non-epileptic episodes occur in 20% to 30% of patients undergoing EEG testing for intractable epilepsy (Kotagal et al. 2002; Boesebeck et al. 2010; Chaves and Sander , 2005) and differentiating these events from epileptic seizures can be difficult. In one study, for example, anti-seizure medications were given to 14% of patients admitted to the intensive care unit after receiving an incorrect diagnosis of seizures (Boesebeck et al. 2010).

Certain anti-seizure drugs have been associated with poorer long-term epilepsy and cognitive outcomes, as well as increased seizure frequency and duration, depending on the epileptic syndrome (Parker et al. 1998; Pawluski et al. 2018; Guerrini et al. 1998; de Lange et al. 2018). When given unsuccessful carbamazepine medication for two weeks before getting effective treatment for six weeks, a group of mice in a research using a mouse model of absence epilepsy experienced more seizures at the end of the eight-week period than a control group that simply got saline (Hauser et al. 2018).

Similarly, lamotrigine treatment was linked to an increase in seizure frequency and duration in people with Dravet syndrome (Hauser et al. 1991). Lamotrigine and other sodium channel blockers may have a negative impact on Dravet syndrome patients' cognitive outcomes in the first five years after their seizures begin (Mohanraj and Brodie , 2013). To the best of our knowledge, these two investigations (Hauser et al. 1991; Mohanraj and Brodie 2013) are among the first to reveal a detrimental disease-modifying impact linked to inadequate epilepsy therapy.

There should be no delay in making the epilepsy diagnosis or starting the right course of therapy. In order to meet the clinical criteria for epilepsy in 1991, two unprovoked seizures had to take place within a 24-hour period (Fisher et al. 2014). This criteria was revised in 2014 to take into consideration how many seizures might affect a patient's prognosis (Kim et al. 2006). In fact, risk factors for seizure recurrence have been found as epilepsy duration and number of seizures before therapy (Hauser et al. 1998; O'Callaghan et al. 2011; Auvin et al. 2012; Eisermann et al. 2003). One research found that individuals who had previously had two symptomatic seizures were more likely to develop another seizure than those who had only had one (Bok et al. 2012).

The new clinical definition of epilepsy is based on the 1991 definition but adds two conditions: "one unprovoked (or reflex) seizure and a probability of further seizures comparable to the general recurrence risk (at least 60%) after two unprovoked seizures occurring within the next ten years" and "diagnosis of an epilepsy syndrome." If either of these conditions is met, an individual is diagnosed with epilepsy. Delayed treatment has been linked to poorer patient in a variety of epilepsies and epilepsy syndromes, including epileptic spasms (O'Callaghan et al. 2011; Eisermann et al. 2003), localized epilepsies (Malmgren and Edelvik , 2017; Skirrow et al. 2019), pyridoxine-responsive epilepsy (Bok et al. 2012; Al Teneiji et al. 2017), autoimmune epilepsy (not specified), and epileptic spasm (Delalande et al. 2007).

CONCLUSIONS

The most accurate method of identifying genetic diseases is through identifying a causative mutation. However, whether the disease is highly genetically diverse or the causative gene(s) has not been explored, this conventional strategy is inadequate for detecting many single-gene disorders. Sanger sequencing may be able to identify the causal mutation in diseases brought on by a limited number of gene mutations. NGS has shown that re-sequencing the patient's full exome (or genome) can be an effective method for studying Mendelian diseases. Additionally, the implementation of these systems to complex disorders such as CAD and other CVDs may result in the examination of the genetic basis of these illnesses. Despite the fact that GWAS have considerably enhanced our knowledge of the genetic etiology of complicated CVDs, genetic variations account for only a minor fraction of heritable CVD risk. In light of this, the question of whether rare mutations, or variants with an allelic frequency of less than 1% that are not reflected on standard SNP arrays, can account for at least some of the reported missing heritability has been raised. By applying cutting-edge technologies like NGS, it is possible to detect structural and uncommon variants, as well as the difficulties posed by various types of variation and phenotypic. WGS is now a practical way for acquiring global genomic data thanks to NGS technologies. Cardiovascular genetics currently uses a number of NGS systems that have similar fundamental processing steps but differ in certain technical facets, enabling us to weigh the benefits and drawbacks of each platform. Recently, it has been demonstrated that NGS has a tremendous potential for finding novel causal variants in a variety of Mendelian variations. Another area where NGS is projected to play a rising role is the research of multifactorial features like CVDs, where risk assessment through the discovery and identification of causative genes remains a significant barrier to advancement in treatment and prevention. Although their utility for regular genetic test may be constrained by technical concerns, the fundamental limitation of WGS and WES applications for diagnosis is the analysis of genetic data

ACKOWLEDGMENT

We appreciate Shorouq Al-Mamlakah International School, Alhada Armed Forces Hospital, and King Abdulaziz University's cooperation and input on this review.

AUTHOR CONTRIBUTIONS

All authors contributed equally and have been involved in the writing of the manuscript at draft, any revision stages, and have read and approved the final version.

REFERENCES

Aaberg, K.M.; Gunnes, N.; Bakken, I.J.; Lund Søraas, C.; Berntsen, A.; Magnus, P.; Lossius, M.I.; Stoltenberg, C.; Chin, R.; Surén, P. Incidence and Prevalence of Childhood Epilepsy: A Nationwide Cohort Study. *Pediatrics* 2017, *139* [https://publications.aap.org/pediatrics/article-pdf/139/5/e20163908/1062871/peds_20163908.pdf]. e20163908, https://doi.org/10.1542/peds.2016-3908.

Abel, T.J.; Losito, E.; Ibrahim, G.M.; Asano, E.; Rutka, J.T. Multimodal localization and surgery for epileptic spasms of focal origin: a review. *Neurosurg Focus* 2018, *45*, E4.

Ackerman, M.; Priori, S.; Willems, S.; Berul, C.; Brugada, R.; Calkins, H.; Camm, J.; Ellinor, P.; Gollob, M.; Hamilton, R.; et al. HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies: This document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Europace : European pacing, arrhythmias, and cardiac electrophysiology : journal of the working groups on cardiac pacing, arrhythmias, and cardiac cellular electrophysiology of the European Society of Cardiology* 2011, *13*, 1077–109. https: //doi.org/10.1093/europace/eur245.

Ackerman, M.J.; Priori, S.G.; Willems, S.; Berul, C.; Brugada, R.; Calkins, H.; Camm, A.J.; Ellinor, P.T.; Gollob, M.; Hamilton, R.; et al. HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm* 2011, *8*, 1308–1339.

Ackerman, M.J.; VanDriest, S.L.; Ommen, S.R.; Will, M.L.; Nishimura, R.A.; Tajik, A.; Gersh, B.J. Prevalence and age-dependence of malignant mutations in the beta-myosin heavy chain and troponin t genes in hypertrophic cardiomyopathy: A comprehensive outpatient perspective. *Journal of the American College of Cardiology* 2002, *39*, 2042–2048. https://doi.org/https://doi.org/10.1016/ S0735-1097(02)01900-9.

Al Teneiji, A.; Bruun, T.U.J.; Cordeiro, D.; Patel, J.; Inbar-

Feigenberg, M.; Weiss, S.; Struys, E.; Mercimek-Mahmutoglu, S. Phenotype, biochemical features, genotype and treatment outcome of pyridoxine-dependent epilepsy. *Metab. Brain Dis.* 2017, *32*, 443–451.

Altshuler, D.; Durbin, R.; Abecasis, G.; Bentley, D.; Chakravarti, A.; Clark, A.; Donnelly, P.; Eichler, E.; Flicek, P.; Gabriel, S.; et al. A global reference for human genetic variation. *NATURE* 2015, *526*.

Amadori, E.; Scala, M.; Cereda, G.; Vari, M.; Marchese, F.; Pisa, V.; Mancardi, M.; Giacomini, T.; Siri, L.; Vercellino, F.; et al. Targeted re-sequencing for early diagnosis of genetic causes of childhood epilepsy: The Italian experience from the 'beyond epilepsy' project. *Italian Journal of Pediatrics* 2020, *46*. https://doi.org/10.1186/s13052-020-00860-1.

Ambrosino, P.; Soldovieri, M.; Bast, T.; Turnpenny, P.; Uhrig, S.; Biskup, S.; Döcker, M.; Fleck, T.; Mosca, I.; Manocchio, L.; et al. De novo gain-of-function variants in KCNT2 as a novel cause of developmental and epileptic encephalopathy. *Annals of Neurology* 2018, *83*. https://doi.org/10.1002/ana.25248.

Auvin, S.; Hartman, A.L.; Desnous, B.; Moreau, A.C.; Alberti, C.; Delanoe, C.; Romano, A.; Terrone, G.; Kossoff, E.H.; Del Giudice, E.; et al. Diagnosis delay in West syndrome: misdiagnosis and consequences. *Eur J Pediatr* 2012, *171*, 1695–1701.

Bae, J.M.; Won, J.K.; Park, S.H. Recent advancement of the molecular diagnosis in pediatric brain tumor. *J. Korean Neurosurg. Soc.*2018, *61*, 376–385.

Banfi, P.; Coll, M.; Oliva, A.; Alcalde, M.; Striano, P.; Mauri, M.; Princiotta, L.; Campuzano, O.; Versino, M.; Brugada, R. Lamotrigine induced Brugada-pattern in a patient with genetic epilepsy associated with a novel variant in SCN9A. *Gene* 2020, *754*, 144847. https://doi.org/10.1016/j.gene.2020.144847.

Bayart, C.B.; Ishak, G.E.; Finn, L.S.; Lee, A.; Baran, F.; Sun, A.; Gupta, D.; Vitanza, N.A. Pilocytic astrocytoma with leptomeningeal spread in a patient with incontinentia pigmenti presenting with unilateral nystagmus. *Pediatr. Blood Cancer* 2018, *65*.

Behjati, S.; Tarpey, P.S. What is next generation sequencing? *Arch. Dis. Child. Educ. Pract. Ed.* 2013, *98*, 236–238.

Biesecker, L. Opportunities and challenges for the integration of massively parallel genomic sequencing into clinical practice: Lessons from the ClinSeq project. *Genetics in medicine : official journal of the American College of Medical Genetics* 2012, *14*, 393–8. https://doi.org/10.1038/gim.2011.78.

Boesebeck, F.; Freermann, S.; Kellinghaus, C.; Evers, S. Misdiagnosis of epileptic and non-epileptic seizures in a neurological intensive care unit. *Acta Neurol. Scand.* 2010, *122*, 189–195.

Bok, L.A.; Halbertsma, F.J.; Houterman, S.; Wevers, R.A.; Vreeswijk, C.; Jakobs, C.; Struys, E.; Van Der Hoeven, J.H.; Sival, D.A.; Willemsen, M.A. Long-term outcome in pyridoxine-dependent epilepsy. *Dev. Med. Child Neurol.* 2012, *54*, 849–854.

Bonzanni, M.; DiFrancesco, J.; Milanesi, R.; Campostrini, G.; Castellotti, B.; Bucchi, A.; Baruscotti, M.; Ferrarese, C.; Silvana, F.; Canafoglia, L.; et al. A novel de novo HCN1 loss-of-function mutation in genetic generalized epilepsy causing increased neuronal excitability. *Neurobiology of Disease* 2018, *118*. https://doi.org/10.1016/j.nbd.2018.06.012.

Bos, J.M.; Towbin, J.A.; Ackerman, M.J. Diagnostic, prognostic, and therapeutic implications of genetic testing for hypertrophic cardiomyopathy. *J. Am. Coll. Cardiol.* 2009, *54*, 201–211.

Brown, R.J.; Reuber, M. Psychological and psychiatric aspects of psychogenic non-epileptic seizures (PNES): A systematic review.

Cario, H.; Bode, H.; Debatin, K.; Opladen, T.; Schwarz, K. Congenital null mutations of the FOLR1 gene: a progressive neurologic disease and its treatment. *Neurology* 2009, *73*, 2127–9. https://doi.org/10.1212/WNL.0b013e3181c679df.

Carroll, C.J.; Isohanni, P.; Pöyhönen, R.; Euro, L.; Richter, U.; Brilhante, V.; Götz, A.; Lahtinen, T.; Paetau, A.; Pihko, H.; et al. Whole-exome sequencing identifies a mutation in the mitochondrial ribosome protein MRPL44 to underlie mitochondrial infantile cardiomyopathy. *J. Med. Genet.* 2013, *50*, 151–159.

Carter, J.H.; McNulty, S.N.; Cimino, P.J.; Cottrell, C.E.; Heusel, J.W.; Vigh-Conrad, K.A.; Duncavage, E.J. Targeted next-generation sequencing in molecular subtyping of lower-grade diffuse gliomas: Application of the World Health Organization's 2016 revised criteria for Central Nervous System tumors. *J. Mol. Diagn.* 2017, *19*, 328–337.

Cavalli, F.; Remke, M.; Rampasek, L.; Peacock, J.; Shih, D.; Luu, B.; Garzia, L.; Torchia, J.; Nor, C.; Morrissy, A.; et al. Intertumoral Heterogeneity within Medulloblastoma Subgroups. *Cancer Cell* 2017, *31*, 737–754.e6. https://doi.org/10.1016/j.ccell.2017.05.005.

Cecconi, M.; Parodi, M.I.; Formisano, F.; Spirito, P.; Autore, C.; Musumeci, M.B.; Favale, S.; Forleo, C.; Rapezzi, C.; Biagini, E.; et al. Targeted next-generation sequencing helps to decipher the genetic and phenotypic heterogeneity of hypertrophic cardiomyopathy. *Int. J. Mol. Med.* 2016, *38*, 1111–1124.

Ceyhan-Birsoy, O.; Pugh, T.J.; Bowser, M.J.; Hynes, E.; Frisella, A.L.; Mahanta, L.M.; Lebo, M.S.; Amr, S.S.; Funke, B.H. Next generation sequencing-based copy number analysis reveals low prevalence of deletions and duplications in 46 genes associated with genetic cardiomyopathies. *Mol. Genet. Genomic Med.* 2016, *4*, 143–151.

Chan, C.K.; Low, J.S.Y.; Lim, K.S.; Low, S.K.; Tan, C.T.; Ng, C.C. Whole exome sequencing identifies a novel

SCN1A mutation in genetic (idiopathic) generalized epilepsy and juvenile myoclonic epilepsy subtypes. *Neurol. Sci.* 2020, *41*, 591–598.

Charron, P.; Arad, M.; Arbustini, E.; Basso, C.; Bilinska, Z.; Elliott, P.; Helio, T.; Keren, A.; McKenna, W.J.; Monserrat, L.; et al. Genetic counselling and testing in cardiomyopathies: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *European Heart Journal* 2010, *31*, 2715–2726, [https://academic.oup.com/eurheartj/article-pdf/31/22/2715/1326005/ehq271.pdf]. https://doi.org/10.1093/eurheartj/ehq271.

Chaves, J.; Sander, J.W. Seizure aggravation in idiopathic generalized epilepsies. *Epilepsia* 2005, *46 Suppl 9*, 133–139.

Chia, R.; Chiò, A.; Traynor, B.J. Novel genes associated with amyotrophic lateral sclerosis: diagnostic and clinical implications.*Lancet Neurol.* 2018, *17*, 94–102.

Clin Psychol Rev 2016, *45*, 157–182.

Collins, V.; Jones, D.; Giannini, C. Pilocytic astrocytoma: pathology, molecular mechanisms and markers. *Acta neuropathological* 2015, *129*. https://doi.org/10.1007/s00401-015-1410-7.

Colombo, M.; Botto, N.; Vittorini, S.; Paradossi, U.; Andreassi, M.G. Clinical utility of genetic tests for inherited hypertrophic and dilated cardiomyopathies. *Cardiovascular ultrasound* 2009, *6*, 62. https://doi.org/10.1186/1476-7120-6-62.

de Lange, I.M.; Gunning, B.; Sonsma, A.C.M.; van Gemert, L.; van Kempen, M.; Verbeek, N.E.; Nicolai, J.; Knoers, N.V.A.M.; Koeleman, B.P.C.; Brilstra, E.H. Influence of contraindicated medication use on cognitive outcome in Dravet syndrome and age at first afebrile seizure as a clinical predictor in SCN1A-related seizure phenotypes. *Epilepsia* 2018, *59*, 1154–1165.

Delalande, O.; Bulteau, C.; Dellatolas, G.; Fohlen, M.; Jalin, C.; Buret, V.; Viguier, D.; Dorfmüller, G.; Jambaqué, I. Vertical parasagittal hemispherotomy: surgical procedures and clinical long-term outcomes in a population of 83 children. *Neurosurgery* 2007, *60*, ONS19–32; discussion ONS32.

Dellefave-Castillo, L.; Mcnally, E. The genetics of dilated cardiomyopathy. *Current opinion in cardiology* 2010, *25*, 198–204.https://doi.org/10.1097/HCO.0b013e328337ba52.

Demarest, S.; Brooks-Kayal, A. From molecules to medicines: the dawn of targeted therapies for genetic epilepsies. *Nature Reviews Neurology* 2018, *14*, 1. https://doi.org/10.1038/s41582-018-0099-3.

Den, K.; Kudo, Y.; Kato, M.; Watanabe, K.; Doi, H.; Tanaka, F.; Oguni, H.; Miyatake, S.; Mizuguchi, T.; Takata, A.; et al. Recurrent NUS1 canonical splice donor site mutation in two unrelated individuals with epilepsy, myoclonus, ataxia and scoliosis- A case report. *BMC Neurology* 2019, *19*. https://doi.org/10.1186/s12883-019-1489-x.

Doolan, G.; Nguyen, L.; Chung, J.; Ingles, J.; Semsarian, C. Progression of left ventricular hypertrophy and the angiotensin- converting enzyme gene polymorphism in hypertrophic cardiomyopathy. *Int. J. Cardiol.* 2004, *96*, 157–163.

Dubbink, H.J.; Atmodimedjo, P.N.; van Marion, R.; Krol, N.M.G.; Riegman, P.H.J.; Kros, J.M.; van den Bent, M.J.; Dinjens, W.N.M. Diagnostic detection of Allelic losses and imbalances by next-generation sequencing: 1p/19q co-deletion analysis of gliomas. *J. Mol. Diagn.* 2016, *18*, 775–786.

Dunn, P.; Albury, C.; Maksemous, N.; Benton, M.; Sutherland, H.; Smith, R.; Haupt, L.; Griffiths, L. Next Generation Sequencing Methods for Diagnosis of Epilepsy Syndromes. *Frontiers in Genetics* 2018, *9*. https://doi.org/10.3389/fgene.2018.00020.

Eisermann, M.M.; DeLaRaillère, A.; Dellatolas, G.; Tozzi, E.; Nabbout, R.; Dulac, O.; Chiron, C. Infantile spasms in Down syndrome–effects of delayed anticonvulsive treatment. *Epilepsy Res* 2003, *55*, 21–27.

Elliott, P.; Andersson, B.; Arbustini, E.; Bilinska, Z.; Cecchi, F.; Charron, P.; Dubourg, O.; Kühl, U.; Maisch, B.; McKenna, W.J.; et al. Classification of the cardiomyopathies: a position statement from the European Society Of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur. Heart J.* 2008, *29*, 270–276.

Faita, F.; Vecoli, C.; Foffa, I.; Andreassi, M.G. Next generation sequencing in cardiovascular diseases. *World journal of cardiology* 2012, *4*, 288–95. https://doi.org/10.4330/wjc.v4.i10.288.

Fisher, R.S.; Acevedo, C.; Arzimanoglou, A.; Bogacz, A.; Cross, J.H.; Elger, C.E.; Engel, J.; Forsgren, L.; French, J.A.; Glynn, M.; et al. ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 2014, *55*, 475–482.

Galmiche, L.; Serre, V.; Beinat, M.; Assouline, Z.; Lebre, A.S.; Chretien, D.; Nietschke, P.; Benes, V.; Boddaert, N.; Sidi, D.; et al. Exome sequencing identifies MRPL3 mutation in mitochondrial cardiomyopathy. *Hum. Mutat.* 2011, *32*, 1225–1231.

Gandjbakhch, E.; Redheuil, A.; Pousset, F.; Charron, P.; Frank, R. Clinical diagnosis, imaging, and genetics of arrhythmogenic right ventricular cardiomyopathy/dysplasia: JACC state-of-the-art review. *J. Am. Coll. Cardiol.* 2018, *72*, 784–804.

Gerull, B.; Gramlich, M.; Atherton, J.; McNabb, M.; Trombitás, K.; Sasse-Klaassen, S.; Seidman, J.G.; Seidman, C.; Granzier, H.; Labeit, S.; et al. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat. Genet.* 2002, *30*, 201–204.

Gibson, P.; Tong, Y.; Robinson, G.; Thompson, M.; Currle, D.; Eden, C.; Kranenburg, T.; Hogg, T.; Poppleton,

H.; Martin, J.; et al. Subtypes of medulloblastoma have distinct developmental origins. *Nature* 2010, *468*, 1095–9. https://doi.org/10.1038/nature095 87.

Gilissen, C.; Hehir-Kwa, J.; Thung, D.; Vorst, M.; van Bon, B.; Willemsen, M.; Kwint, M.; Janssen, I.; Hoischen, A.; Schenck, A.; et al. Genome sequencing identifies major causes of severe intellectual disability. *Nature* 2014, *511*. https://doi.org/10.1038/ nature13394.

Gong, P.; Jiao, X.; Zhang, Y.; Yang, Z. Complex Mosaicism of Two Distinct Mutations in a Female Patient With KCNA2-Related Encephalopathy: A Case Report. *Frontiers in Genetics* 2020, *11*, 911. https://doi.org/10.3389/fgene.2020.00911.

Götz, A.; Tyynismaa, H.; Euro, L.; Ellonen, P.; Hyötyläinen, T.; Ojala, T.; Hämäläinen, R.H.; Tommiska, J.; Raivio, T.; Oresic, M.; et al. Exome sequencing identifies mitochondrial alanyl-tRNA synthetase mutations in infantile mitochondrial cardiomyopathy. *Am. J. Hum. Genet.* 2011, *88*, 635–642.

Grupa Robocza Europejskiego Towarzystwa Kardiologicznego (ESC) do spraw rozpoznawania i postepowania w kardiomiopatii przerostowej.; Elliott, P.M.; Anastasakis, A.; Borger, M.A.; Borggrefe, M.; Cecchi, F.; Charron, P.; Hagege, A.A.; Lafont, A.; Limongelli, G.; et al. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy. *Kardiol. Pol.* 2014, *72*, 1054–1126.

Guerrini, R.; Dravet, C.; Genton, P.; Belmonte, A.; Kaminska, A.; Dulac, O. Lamotrigine and seizure aggravation in severe myoclonic epilepsy. *Epilepsia* 1998, *39*, 508–512.

Haldar, S.M.; Srivastava, D. Sarcomeres and cardiac growth: Tension in the relationship. *Trends Mol. Med.* 2016, *22*, 530–533.

Hancock, E.C.; Osborne, J.P.; Edwards, S.W. Treatment of infantile spasms. *Cochrane Database Syst Rev* 2013, p. CD001770.

Hardies, K.; Weckhuysen, S.; De Jonghe, P.; Suls, A. Lessons learned from gene identification studies in Mendelian epilepsy disorders. *European Journal of Human Genetics* 2016, *24*, 961–967.

Hauser, W.A.; Annegers, J.F.; Kurland, L.T. Prevalence of epilepsy in Rochester, Minnesota: 1940-1980. *Epilepsia* 1991, *32*, 429–445.

Hauser, W.A.; Rich, S.S.; Lee, J.R.; Annegers, J.F.; Anderson, V.E. Risk of recurrent seizures after two unprovoked seizures. *N Engl J Med* 1998, *338*, 429–434.

Herman, D.S.; Lam, L.; Taylor, M.R.G.; Wang, L.; Teekakirikul, P.; Christodoulou, D.; Conner, L.; DePalma, S.R.; McDonough, B.; Sparks, E.; et al. Truncations of titin causing dilated cardiomyopathy. *N. Engl. J. Med.* 2012, *366*, 619–628.

Hernandez, C.; Kong, W.; Hu, N.; Zhang, Y.; Shen, W.; Jackson, L.; Liu, X.; Jiang, Y.; Macdonald, R. Altered Channel Conductance States and Gating of GABAA Receptors by a Pore Mutation Linked to Dravet Syndrome. *eneuro* 2017, *4*, ENEURO.0251–16.2017. https://doi.org/10.1523/ENEURO.0251-16.2017.

Hirschhorn, J.N.; Daly, M.J. Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.* 2005,*6*, 95–108.

Ho, C.Y.; Charron, P.; Richard, P.; Girolami, F.; Van Spaendonck-Zwarts, K.Y.; Pinto, Y. Genetic advances in sarcomeric cardiomyopathies: state of the art. *Cardiovasc. Res.* 2015, *105*, 397–408. https://doi.org/10.1007/s00439-015-1631-9.

Hussain, S.A.; Lay, J.; Cheng, E.; Weng, J.; Sankar, R.; Baca, C.B. Recognition of Infantile Spasms Is Often Delayed: The ASSIST Study. *J Pediatr* 2017, *190*, 215–221.

Ingles, J.; Sarina, T.; Yeates, L.; Hunt, L.; Macciocca, I.; McCormack, L.; Winship, I.; McGaughran, J.; Atherton, J.; Semsarian, C. Clinical predictors of genetic testing outcomes in hypertrophic cardiomyopathy. *Genet. Med.* 2013, *15*, 972–977.

Inuzuka, L.; Macedo-Souza, L.I.; Della-Ripa, B.; Monteiro, F.; Pires Ramos, L.L.; Kitajima, J.; Garzon, E.; Kok, F. Additional observation of a de novo pathogenic variant in KCNT2 leading to epileptic encephalopathy with clinical features of frontal lobe epilepsy. *Brain and Development* 2020, *42*. https://doi.org/10.1016/j.braindev.2020.05.003.

Ittiwut, C.; Poonmaksatit, S.; Boonsimma, P.; Desudchit, T.; Suphapeetiporn, K.; Ittiwut, R.; Shotelersuk, V. Novel de novo mutation substantiates ATP6V0C as a gene causing epilepsy with intellectual disability. *Brain Dev.* 2021, *43*, 490–494.

Jansweijer, J.A.; Nieuwhof, K.; Russo, F.; Hoorntje, E.T.; Jongbloed, J.D.H.; Lekanne Deprez, R.H.; Postma, A.V.; Bronk, M.; van Rijsingen, I.A.W.; de Haij, S.; et al. Truncating titin mutations are associated with a mild and treatable form of dilated cardiomyopathy. *Eur. J. Heart Fail.* 2017, *19*, 512–521.

Jennings, L.; Arcila, M.; Corless, C.; Kamel-Reid, S.; Lubin, I.; Pfeifer, J.; Temple-Smolkin, R.; Voelkerding, K.; Nikiforova, M. Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels. *The Journal of Molecular Diagnostics* 2017, *19*. https://doi.org/10.1016/j.jmoldx.2017.01.011.

Jiang, Y.l.; Song, C.; Wang, Y.; Zhao, J.; Yang, F.; Gao, Q.; Leng, X.; Man, Y.; Jiang, W. Clinical Utility of Exome Sequencing and Reinterpreting Genetic Test Results in Children and Adults With Epilepsy. *Frontiers in Genetics* 2020, *11*. https://doi.org/10.3389/ fgene.2020.591434.

Johnson, A.; Severson, E.; Gay, L.; Vergilio, J.A.; Elvin, J.; Suh, J.; Daniel, S.; Covert, M.; Frampton, G.; Hsu, S.; et al. Comprehensive Genomic Profiling of 282 Pediatric Low- and High-Grade Gliomas Reveals Genomic Drivers, Tumor Mutational Burden, and Hypermutation Signatures. *The oncologist* 2017, *22*.

https://doi.org/10.1634/theoncologist.2017-0242.

Jones, D.; Hutter, B.; Jäger, N.; Korshunov, A.; Kool, M.; Warnatz, H.J.; Zichner, T.; Lambert, S.; Ryzhova, M.; Khuong Quang, D.A.; et al. Recurrent somatic alterations of FGFR1 and NTRK2 in pilocytic astrocytoma. *Nature Genetics* 2013, *Epub ahead of print*. https://doi.org/10.1038/ng.2682.

Jongbloed, J.D.; Pósafalvi, A.; Kerstjens-Frederikse, W.S.; Sinke, R.J.; van Tintelen, J.P. New clinical molecular diagnostic methods for congenital and inherited heart disease. *Expert Opin. Med. Diagn.* 2011, *5*, 9–24.

Kallappagoudar, S.; Yadav, R.; Lowe, B.; Partridge, J. Histone H3 mutations—A special role for H3.3 in tumorigenesis? *Chromosoma* 2015, *124*. https://doi.org/10.1007/s00412-015-0510-4.

Kanemoto, K.; LaFrance, W.C.; Duncan, R.; Gigineishvili, D.; Park, S.P.; Tadokoro, Y.; Ikeda, H.; Paul, R.; Zhou, D.; Taniguchi, G.; et al. PNES around the world: Where we are now and how we can close the diagnosis and treatment gaps-an ILAE PNES Task Force report. *Epilepsia Open* 2017, *2*, 307–316.

Karahoca, A.; Bayraktar, E.; Tatoglu, E.; Karahoca, D. Information system design for a hospital emergency department: a usability analysis of software prototypes. *J. Biomed. Inform.* 2010, *43*, 224–232.

Khuong Quang, D.A.; Buczkowicz, P.; Rakopoulos, P.; Liu, X.; Fontebasso, A.; Bouffet, E.; Bartels, U.; Albrecht, S.; Schwartzentru- ber, J.; Letourneau, L.; et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathologica* 2012, *124*, 439–47. https://doi.org/10.1007/s00401-012-0998-0.

Kim, L.G.; Johnson, T.L.; Marson, A.G.; Chadwick, D.W. Prediction of risk of seizure recurrence after a single seizure and early epilepsy: further results from the MESS trial. *Lancet Neurol* 2006, *5*, 317–322.

Kimura, A. Contribution of Genetic Factors to the Pathogenesis of Dilated Cardiomyopathy - The Cause of Dilated Cardiomyopa- thy: Genetic or Acquired? (Genetic-Side). *Circulation journal : official journal of the Japanese Circulation Society* 2011, *75*, 1756–65; discussion 1765. https://doi.org/10.1253/circj.CJ-11-0368.

Koboldt, D.; Steinberg, K.; Larson, D.; Wilson, R.; Mardis, E. The Next-Generation Sequencing Revolution and Its Impact on Genomics. *Cell* 2013, *155*, 27–38. https://doi.org/10.1016/j.cell.2013.09.006.

Komulainen-Ebrahim, J.; Schreiber, J.; Kangas, S.; Pylkäs, K.; Suo-Palosaari, M.; Elisa, R.; Liinamaa, M.; Immonen, E.V.; Hassinen, I.; Myllynen, P.; et al. Novel variants and phenotypes widen the phenotypic spectrum of GABRG2-related disorders. *Seizure* 2019,*69*.https://doi.org/10.1016/j.seizure.2019.03.010.

Kostareva, A.; Kiselev, A.; Gudkova, A.; Frishman, G.; Ruepp, A.; Frishman, D.; Smolina, N.; Tarnovskaya, S.; Nilsson, D.; Zlotina, A.; et al. Genetic spectrum of idiopathic restrictive cardiomyopathy uncovered by next-generation sequencing. *PLoS One* 2016, *11*, e0163362.

Kotagal, P.; Costa, M.; Wyllie, E.; Wolgamuth, B. Paroxysmal nonepileptic events in children and adolescents. *Pediatrics* 2002, *110*, e46.

Lalonde, E.; Rentas, S.; Lin, F.; Dulik, M.C.; Skraban, C.M.; Spinner, N.B. Genomic Diagnosis for Pediatric Disorders: Revolution and Evolution. *Frontiers in Pediatrics* 2020, *8*. https://doi.org/10.3389/fped.2020.00373.

Lopes, L.R.; Zekavati, A.; Syrris, P.; Hubank, M.; Giambartolomei, C.; Dalageorgou, C.; Jenkins, S.; McKenna, W.; Uk10k Consortium.; Plagnol, V.; et al. Genetic complexity in hypertrophic cardiomyopathy revealed by high-throughput sequencing. *J. Med. Genet.* 2013, *50*, 228–239.

Mafi, S.; Laroche-Raynaud, C.; Chazelas, P.; Lia-Baldini, A.S.; Derouault, P.; Sturtz, F.; Baaj, Y.; Froget, R.; Rio, M.; Benoist, J.F.; et al. Pharmacoresistant Epilepsy in Childhood: Think of the Cerebral Folate Deficiency, a Treatable Disease. *Brain Sciences* 2020. https://doi.org/10.3390/brainsci10110762.

Malicdan, M.C.V.; Vilboux, T.; Ben-Zeev, B.; Guo, J.; Eliyahu, A.; Pode-Shakked, B.; Dori, A.; Kakani, S.; Chandrasekharappa, S.C.; Ferreira, C.R.; et al. A novel inborn error of the coenzyme Q10 biosynthesis pathway: cerebellar ataxia and static encephalomyopathy due to COQ5 C-methyltransferase deficiency. *Hum. Mutat.* 2018, *39*, 69–79.

Malmgren, K.; Edelvik, A. Long-term outcomes of surgical treatment for epilepsy in adults with regard to seizures, antiepileptic drug treatment and employment. *Seizure* 2017, *44*, 217–224.

Marian, A. Sequencing your Genome: What Does It Mean? *Methodist DeBakey cardiovascular journal* 2014, *10*, 3–6. https://doi.org/10.14797/mdcj-10-1-3.

Marian, A.J.; Yu, Q.T.; Workman, R.; Greve, G.; Roberts, R. Angiotensin-converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. *Lancet* 1993, *342*, 1085–1086.

Maron, B.J.; Towbin, J.A.; Thiene, G.; Antzelevitch, C.; Corrado, D.; Arnett, D.; Moss, A.J.; Seidman, C.E.; Young, J.B.; American Heart Association.; et al. Contemporary definitions and classification of the cardiomyopathies: An American heart associa-tion scientific statement from the council on clinical cardiology, heart failure and transplantation committee; Quality of care and outcomes research and functional genomics and translational biology interdisciplinary working groups; And council on epidemiology and prevention. *Circulation* 2006, *113*, 1807–1816.

Masnada, S.; Hedrich, U.; Gardella, E.; Schubert, J.; Kaiwar, C.; Klee, E.; Lanpher, B.; Gavrilova, R.;

Synofzik, M.; Bast, T.; et al. Clinical spectrum and genotype–phenotype associations of KCNA2-related encephalopathies. *Brain* 2017, *doi:10.1093/brain/awx184*. https://doi.org/10.1093/brain/awx184.

Medeiros-Domingo, A.; Saguner, A.M.; Magyar, I.; Bahr, A.; Akdis, D.; Brunckhorst, C.; Duru, F.; Berger, W. Arrhythmogenic right ventricular cardiomyopathy: implications of next-generation sequencing in appropriate diagnosis. *Europace* 2017, *19*, 1063–1069.

Meder, B.; Haas, J.; Keller, A.; Heid, C.; Just, S.; Borries, A.; Boisguerin, V.; Scharfenberger-Schmeer, M.; Stähler, P.; Beier, M.; et al. Targeted next-generation sequencing for the molecular genetic diagnostics of cardiomyopathies. *Circ. Cardiovasc. Genet.* 2011, *4*, 110–122.

Meienberg, J.; Bruggmann, R.; Oexle, K.; Matyas, G. Clinical sequencing: is WGS the better WES? *Human Genetics* 2016, *135*.

Mohanraj, R.; Brodie, M.J. Early predictors of outcome in newly diagnosed epilepsy. *Seizure* 2013, *22*, 333–344.

Møller, R.; Hammer, T.; Rubboli, G.; Lemke, J.; Johannesen, K. From next generation sequencing to targeted treatment of non-acquired epilepsies. *Expert Review of Molecular Diagnostics* 2019, *19*. https://doi.org/10.1080/14737159.2019.1573144.

Myers, C.T.; Mefford, H.C. Advancing epilepsy genetics in the genomic era. *Genome Med.* 2015, *7*, 91.

Németh, A.H.; Kwasniewska, A.C.; Lise, S.; Parolin Schnekenberg, R.; Becker, E.B.E.; Bera, K.D.; Shanks, M.E.; Gregory, L.; Buck, D.; Zameel Cader, M.; et al. Next generation sequencing for molecular diagnosis of neurological disorders using ataxias as a model. *Brain* 2013, *136*, 3106–3118, [https://academic.oup.com/brain/article-pdf/136/10/3106/13796047/awt236.pdf]. https://doi.org/10.1093/brain/awt236.

Niimura, H.; Bachinski, L.L.; Sangwatanaroj, S.; Watkins, H.; Chudley, A.E.; McKenna, W.; Kristinsson, A.; Roberts, R.; Sole, M.; Maron, B.J.; et al. Mutations in the gene for cardiac myosin-binding protein C and late-onset familial hypertrophic cardiomyopathy.*N. Engl. J. Med.* 1998, *338*, 1248–1257.

Nikbakht, H.; Panditharatna, E.; Mikael, L.; Li, R.; Gayden, T.; Osmond, M.; Ho, C.Y.; Kambhampati, M.; Hwang, E.; Faury, D.; et al. Spatial and temporal homogeneity of driver mutations in diffuse intrinsic pontine glioma. *Nature Communications* 2016, *7*, 11185. https://doi.org/10.1038/ncomms11185.

Nikiforova, M.; Wald, A.; Melan, M.; Roy, S.; Zhong, S.; Hamilton, R.; Lieberman, F.; Drappatz, J.; Amankulor, N.; Pollack, I.; et al. Targeted next-generation sequencing panel (GlioSeq) provides comprehensive genetic profiling of central nervous system tumors. *Neuro-oncology* 2015, *18*. https://doi.org/10.1093/neuonc/nov289.

Northcott, P.; Buchhalter, I.; Morrissy, A.; Hovestadt, V.; Weischenfeldt, J.; Ehrenberger, T.; Gröbner, S.; Segura-Wang, M.; Zichner, T.; Rudneva, V.; et al. The whole-genome landscape of medulloblastoma subtypes. *Nature* 2017, *547*, 311–317. https://doi.org/10.1038/nature22973.

O'Callaghan, F.J.; Lux, A.L.; Darke, K.; Edwards, S.W.; Hancock, E.; Johnson, A.L.; Kennedy, C.R.; Newton, R.W.; Verity, C.M.; Osborne, J.P. The effect of lead time to treatment and of age of onset on developmental outcome at 4 years in infantile spasms: evidence from the United Kingdom Infantile Spasms Study. *Epilepsia* 2011, *52*, 1359–1364.

O'Callaghan, F.J.K.; Edwards, S.W.; Alber, F.D.; Cortina Borja, M.; Hancock, E.; Johnson, A.L.; Kennedy, C.R.; Likeman, M.; Lux, A.L.; Mackay, M.T.; et al. Vigabatrin with hormonal treatment versus hormonal treatment alone (ICISS) for infantile spasms: 18-month outcomes of an open-label, randomised controlled trial. *Lancet Child Adolesc Health* 2018, *2*, 715–725.

Olivotto, I.; Girolami, F.; Ackerman, M.J.; Nistri, S.; Bos, J.M.; Zachara, E.; Ommen, S.R.; Theis, J.L.; Vaubel, R.A.; Re, F.; et al. Myofilament protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin. Proc.* 2008, *83*, 630–638.

Palmquist, R.; Jenkins, S.M.; Bentley, D.; Miller, C.; Mao, R.; Meibos, B.; Bayrak-Toydemir, P.; Tvrdik, T.; Nadauld, L.D.; Bleyl, S.B.; et al. Evaluating use of changing technologies for rapid next-generation sequencing in pediatrics. *Pediatr. Res.* 2022.

Parker, A.P.; Agathonikou, A.; Robinson, R.O.; Panayiotopoulos, C.P. Inappropriate use of carbamazepine and vigabatrin in typical absence seizures. *Dev Med Child Neurol* 1998, *40*, 517–519.

Pawluski, J.L.; Kuchenbuch, M.; Hadjadj, S.; Dieuset, G.; Costet, N.; Vercueil, L.; Biraben, A.; Martin, B. Long-term negative impact of an inappropriate first antiepileptic medication on the efficacy of a second antiepileptic medication in mice. *Epilepsia* 2018, *59*, e109–e113.

Pietsch, T.; Haberler, C. Update on the integrated histopathological and genetic classification of medulloblastoma - a practical diagnostic guideline. *Clin. Neuropathol.* 2016, *35*, 344–352.

Porkholm, M.; Raunio, A.; Vainionpää, R.; Salonen, T.; Hernesniemi, J.; Valanne, L.; Satopää, J.; Karppinen, A.; Oinas, M.; Tynninen, O.; et al. Molecular alterations in pediatric brainstem gliomas. *Pediatr. Blood Cancer* 2018, *65*.

Pugh, T.J.; Kelly, M.A.; Gowrisankar, S.; Hynes, E.; Seidman, M.A.; Baxter, S.M.; Bowser, M.; Harrison, B.; Aaron, D.; Mahanta, L.M.; et al. The landscape of genetic variation in dilated cardiomyopathy as surveyed by clinical DNA sequencing. *Genet. Med.* 2014, *16*, 601–608.

Punetha, J.; Hoffman, E.P. Short read (next-generation) sequencing: a tutorial with cardiomyopathy diagnostics as an exemplar.*Circ. Cardiovasc. Genet.* 2013, *6*, 427–434.

Rabbani, B.; Nakaoka, H.; Akhondzadeh, S.; Tekin, M.; Mahdieh, N. Next Generation Sequencing: Implications in Personalized Medicine and Pharmacogenomics. *Mol. BioSyst.* 2016, *12*. https://doi.org/10.1039/C6MB00115G.

Ramaekers, V.; Sequeira, J.M.; Quadros, E.V. Clinical recognition and aspects of the cerebral folate deficiency syndromes. *Clin. Chem. Lab. Med.* 2013, *51*, 497–511.

Ravin, N. Modern Methods of Genome Sequencing and Their Application for Deciphering Genomes of Microorganisms. *Applied Biochemistry and Microbiology* 2010, *46*, 663–670. https://doi.org/10.1134/S000368381007001X.

Richard, P.; Charron, P.; Carrier, L.; Ledeuil, C.; Cheav, T.; Pichereau, C.; Benaiche, A.; Isnard, R.; Dubourg, O.; Burban, M.; et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003, *107*, 2227–2232.

Rodríguez, J.E.; McCudden, C.R.; Willis, M.S. Familial hypertrophic cardiomyopathy: basic concepts and future molecular diagnostics. *Clin. Biochem.* 2009, *42*, 755–765.

Rubattu, S.; Bozzao, C.; Pennacchini, E.; Pagannone, E.; Musumeci, B.M.; Piane, M.; Germani, A.; Savio, C.; Francia, P.; Volpe, M.; et al. A Next-Generation Sequencing approach to identify gene mutations in early- and late-onset hypertrophic cardiomyopathy patients of an Italian cohort. *Int. J. Mol. Sci.* 2016, *17*, 1239.

Salunkhe, M.; Agarwal, A.; Faruq, M.; Srivastava, A.K. Genetic testing in neurology: What every neurologist must know. *Ann. Indian Acad. Neurol.* 2022, *25*, 350–353.

Scheffer, I.; Berkovic, S.; Capovilla, G.; Connolly, M.; French, J.; Guilhoto, L.; Hirsch, E.; Jain, S.; Mathern, G.; Moshe, S.; et al. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017,*58*. https://doi.org/10.1111/epi.13709.

Schoch, K.; Miller, T. Antisense Oligonucleotides: Translation from Mouse Models to Human Neurodegenerative Diseases.*Neuron* 2017, *94*, 1056–1070. https://doi.org/10.1016/j.neuron.2017.04.010.

Schwarze, K.; Buchanan, J.; Taylor, J.C.; Wordsworth, S. Are whole-exome and whole-genome sequencing approaches cost- effective? A systematic review of the literature. *Genet. Med.* 2018, *20*, 1122–1130.

Seidman, J.G.; Seidman, C. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. *Cell*2001, *104*, 557–567.

Shinnar, S.; Berg, A.; O'Dell, C.; Newstein, D.; Moshe, S.; Hauser, W. Predictors of multiple seizures in a cohort of children prospectively followed from the time of their first seizure. *Annals of neurology* 2000, *48*, 140–7. https://doi.org/10.1002/1531-8249(200008)48:2<140::AID-ANA2>3.0.CO;2-Y.

Shirian, S.; Daneshbod, Y.; Jangjoo, S.; Ghaemi, A.; Goodarzi, A.; Ghavideldarestani, M.; Emadi, S.A.; Ai, A.; Ahmadi, A.; Ai, J. The Role of Next Generation Sequencing in Diagnosis of Brain Tumors: A Review Study. *Archives of Neuroscience* 2019, *In Press*. https://doi.org/10.5812/ans.68874.

Sikkema-Raddatz, B.; Johansson, L.F.; de Boer, E.N.; Almomani, R.; Boven, L.G.; van den Berg, M.P.; van Spaendonck-Zwarts, K.Y.; van Tintelen, J.P.; Sijmons, R.H.; Jongbloed, J.D.H.; et al. Targeted Next-Generation Sequencing can Replace Sanger Sequencing in Clinical Diagnostics. *Human Mutation* 2013, *34*, 1035–1042, [https://onlinelibrary.wiley.com/doi/pdf/10.1002/humu.22332]. https://doi.org/https://doi.org/10.1002/humu.22332.

Siu, B.L.; Niimura, H.; Osborne, J.A.; Fatkin, D.; MacRae, C.; Solomon, S.; Benson, D.W.; Seidman, J.G.; Seidman, C.E. Familial dilated cardiomyopathy locus maps to chromosome 2q31. *Circulation* 1999, *99*, 1022–1026.

Skinner, D.; Raspberry, K.; King, M. The nuanced negative: Meanings of a negative diagnostic result in clinical exome sequencing.*Sociology of health & illness* 2016, *38*. https://doi.org/10.1111/1467-9566.12460.

Skirrow, C.; Cross, J.H.; Owens, R.; Weiss-Croft, L.; Martin-Sanfilippo, P.; Banks, T.; Shah, E.; Harkness, W.; Vargha-Khadem, F.; Baldeweg, T. Determinants of IQ outcome after focal epilepsy surgery in childhood: A longitudinal case-control neuroimaging study. *Epilepsia* 2019, *60*, 872–884.

Smith, B.; Ticozzi, N.; Fallini, C.; Gkazi, A.S.; Topp, S.; Kenna, K.; Scotter, E.; Kost, J.; Keagle, P.; Miller, J.; et al. Exome- wide Rare Variant Analysis Identifies TUBA4A Mutations Associated with Familial ALS. *Neuron* 2014, *84*, 324–31. https://doi.org/10.1016/j.neuron.2014.09.027.

Smith, E.D.; Lakdawala, N.K.; Papoutsidakis, N.; Aubert, G.; Mazzanti, A.; McCanta, A.C.; Agarwal, P.P.; Arscott, P.; Dellefave- Castillo, L.M.; Vorovich, E.E.; et al. Desmoplakin cardiomyopathy, a fibrotic and inflammatory form of cardiomyopathy distinct from typical dilated or arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2020, *141*, 1872–1884.

Soor, G.S.; Luk, A.; Ahn, E.; Abraham, J.R.; Woo, A.; Ralph-Edwards, A.; Butany, J. Hypertrophic cardiomyopathy: current understanding and treatment objectives. *J. Clin. Pathol.* 2009, *62*, 226–235.

Spence, T.; Perotti, C.; Sin-Chan, P.; Picard, D.; Wu, W.;

Singh, A.; Anderson, C.; Blough, M.D.; Cairncross, J.G.; Lafay-Cousin, L.; et al. A novel C19MC amplified cell line links Lin28/let-7 to mTOR signaling in embryonal tumor with multilayered rosettes. *Neuro. Oncol.* 2014, *16*, 62–71.

Steinfeld, R.; Grapp, M.; Krätzner, R.; Dreha-Kulaczewski, S.; Helms, G.; Dechent, P.; Wevers, R.; Grosso, S.; Gaertner, J. Folate Receptor Alpha Defect Causes Cerebral Folate Transport Deficiency: A Treatable Neurodegenerative Disorder Associated with Disturbed Myelin Metabolism. *American journal of human genetics* 2009, *85*, 354–63. https://doi.org/10.1016/j.ajhg.2009.08.005.

Stenton, S.; Prokisch, H. Genetics of mitochondrial diseases: Identifying mutations to help diagnosis. *EBioMedicine* 2020,*56*, 102784. https://doi.org/10.1016/j.ebiom.2020.102784.

Stödberg, T.; Tomson, T.; Barbaro, M.; Stranneheim, H.; Anderlid, B.M.; Carlsson, S.; Amark, P.; Wedell, A. Epilepsy syndromes, etiologies, and the use of next-generation sequencing in epilepsy presenting in the first 2 years of life: A population-based study. *Epilepsia* 2020, *61*. https://doi.org/10.1111/epi.16701.

Sudmant, P.; Rausch, T.; Gardner, E.; RE, H.; Abyzov, A.; Huddleston, J.; Zhang, Y.; Ye, K.; Jun, G.; Fritz, M.; et al. An integrated map of structural variation in 2,504 human genomes. *Nature* 2015, *526*, 75–81.

Sun, X.; Zhong, X.; Li, T. Novel SZT2 mutations in three patients with developmental and epileptic encephalopathies. *Molecular Genetics & Genomic Medicine* 2019, *7*. https://doi.org/10.1002/mgg3.926.

T.M. Homozygosity mapping and exome sequencing reveal GATAD1 mutation in autosomal recessive dilated cardiomyopathy.*Circ. Cardiovasc. Genet.* 2011, *4*, 585–594.

Tamiya, G.; Makino, S.; Hayashi, M.; Abe, A.; Numakura, C.; Ueki, M.; Tanaka, A.; Ito, C.; Toshimori, K.; Ogawa, N.; et al. A Mutation of COX6A1 Causes a Recessive Axonal or Mixed Form of Charcot-Marie-Tooth Disease. *American journal of human genetics* 2014, *95*. https://doi.org/10.1016/j.ajhg.2014.07.013.

Taylor, J.; Martin, H.; Lise, S.; Broxholme, J.; Cazier, J.B.; Rimmer, A.; Kanapin, A.; Lunter, G.; Fiddy, S.; Allan, C.; et al. Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nature genetics* 2015, *47*. https://doi.org/10.1038/ng.3304.

Taylor, M.R.G.; Carniel, E.; Mestroni, L. Familial hypertrophic cardiomyopathy: clinical features, molecular genetics and molecular genetic testing. *Expert Rev. Mol. Diagn.* 2004, *4*, 99–113.

Theis, J.L.; Sharpe, K.M.; Matsumoto, M.E.; Chai, H.S.; Nair, A.A.; Theis, J.D.; de Andrade, M.; Wieben, E.D.; Michels, V.V.; Olson,

Vecoli, C. Next-generation sequencing technology in the genetics of cardiovascular disease. *Methods Mol. Biol.* 2015, *1299*, 51–64.

Voelkerding, K.V.; Dames, S.; Durtschi, J.D. Next generation sequencing for clinical diagnostics-principles and application to targeted resequencing for hypertrophic cardiomyopathy: a paper from the 2009 William Beaumont Hospital Symposium on Molecular Pathology. *J. Mol. Diagn.* 2010, *12*, 539–551.

Wang, X.; Shen, X.; Fang, F.; Ding, C.H.; Zhang, H.; Cao, Z.H.; An, D.Y. Phenotype-Driven Virtual Panel Is an Effective Method to Analyze WES Data of Neurological Disease. *Frontiers in Pharmacology* 2019, *9*. https://doi.org/10.3389/fphar.2018.01529.

Ware, J.; Roberts, A.; Cook, S. Next generation sequencing for clinical diagnostics and personalised medicine: Implications for the next generation cardiologist. *Heart (British Cardiac Society)* 2011, *98*, 276–81. https://doi.org/10.1136/heartjnl-2011-300742.

Watkins, H.; McKenna, W.J.; Thierfelder, L.; Suk, H.J.; Anan, R.; O'Donoghue, A.; Spirito, P.; Matsumori, A.; Moravec, C.S.; Seidman, J.G. Mutations in the genes for cardiac troponin T and alpha-tropomyosin in hypertrophic cardiomyopathy. *N. Engl. J. Med.* 1995, *332*, 1058–1064.

Westerink, J.; Visseren, F.; Spiering, W. Diagnostic Clinical Genome and Exome Sequencing. *The New England journal of medicine* 2014, *371*, 1169–1170. https://doi.org/10.1056/NEJMc1408914#SA1.

Winer, L.; Srinivasan, D.; Chun, S.; Lacomis, D.; Jaffa, M.; Fagan, A.; Holtzman, D.M.; Wancewicz, E.; Bennett, C.F.; Bowser, R.; et al. SOD1 in cerebral spinal fluid as a pharmacodynamic marker for antisense oligonucleotide therapy. *JAMA Neurol.* 2013, *70*, 201–207.

Wu, D.; Ji, C.; Chen, Z.; Wang, K. Novel NEXMIF gene pathogenic variant in a female patient with refractory epilepsy and intellectual disability. *American journal of medical genetics. Part A* 2020, *182*. https://doi.org/10.1002/ajmg.a.61848.

Wu, G.; Broniscer, A.; Mceachron, T.; Lu, C.; Paugh, B.; Becksfort, J.; Qu, C.; Ding, L.; Huether, R.; Parker, M.; et al. Somatic Histone H3 Alterations in Paediatric Diffuse Intrinsic Pontine Gliomas and Non-Brainstem Glioblastomas. *Nature genetics* 2012, *44*, 251–3. https://doi.org/10.1038/ng.1102.

Zhukova, N.; Ramaswamy, V.; Remke, M.; Pfaff, E.; Shih, D.; Martin, D.; Castelo-Branco, P.; Baskin, B.; Ray, P.; Bouffet, E.; et al. Subgroup-Specific Prognostic Implications of TP53 Mutation in Medulloblastoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2013, *31*. https://doi.org/10.1200/JCO.2012.48.5052.