

REVIEW ARTICLES

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Applicability of next generation genetic testing in epilepsy through whole exome sequencing

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Abstract

Background: Epilepsy affects around 1% of the general population. With already acknowledged strong genetic contributions, >50% of epilepsy cases still remain undiagnosed. This is primordially due to the multifactorial condition of epilepsy that makes it a challenge to select the optimal genetic test for each specific case. Recently, next-generation sequencing (NGS) led to massive gene discovery, including epilepsy that also imposed serious financial burdens on healthcare systems. This study review highlights the progress in the field of epilepsy genetics and argues on how the genetic architecture of common epilepsies is progressively being unraveled. Since the 1995 finding of *CHRNA4* mutation, more than 500 genes were estimated to play a significant role in epilepsy. To date, the majority of diagnostic genetic testing is conducted in the pediatric population, while the utility of such testing is less well understood in adults with epilepsy. A broad range in the diagnostic rate of NGS, especially of the Whole Exome Sequencing (WES), in epilepsy has been described. However, NGS introduces new challenges, yet to be resolved.

Conclusions: Epilepsy's genetic background is nowadays undeniable; however, the complexity of this condition makes it difficult to be solved. WES has increasingly been used to uncover the role of the coding genetic material in the human genome and is nowadays considered one of the most cost-effective genetic tests for epilepsy, being a prerequisite for personalized treatment approaches and for reducing the epilepsy patient's "diagnostic odyssey".

Key words: epilepsy genetics, next-generation sequencing, whole exome sequencing.

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Introduction

Epilepsy affects approximately 0.6% to 0.8% of the general population and it is a disorder with strong genetic contributions [1]. Globally, the idiopathic epilepsy, a term introduced in 1985, within the International League Against Epilepsy (ILAE)'s proposal for classification of epilepsies and epileptic syndrome [2], means epilepsy of genetic origin or without a definite structural, metabolic, infective, or immune cause / or when diagnostic assessment did not reveal a causative factor) – ranked the 5th among neurological disorders after stroke, migraine, dementia, and meningitis and even the 2nd in some particular areas (southern sub-Saharan Africa) [3].

The incidence of epilepsy is nearly 70 per 100000 children younger than 2 years and genetic epilepsies account for more than 0.4% of the general population, constituting 30% of all epilepsies [4]. A study on a larger group of severe epilepsy cases starting before the 18-month-age found an incidence of one in 2000 births [5-7].

Globally, in 2016, there were 45.9 million patients with all-active epilepsy (both idiopathic and secondary epilepsy globally). Of these patients, 24 million had active idiopathic epilepsy (prevalence 326.7 per 100000 population) [8].

Idiopathic epilepsy accounted for 0.23% of deaths and 0.56% of disability-adjusted life-years (DALYs) from all causes. Global age-standardized mortality rates of idiopathic epilepsy were 1.74 per 100 000 population (1.40 per 100000 population for women and 2.09 per 100000 population for men) [8]. A decrease in death and DALYs rates in patients with epilepsy between 1990 and 2016 was recorded, however the changes varied across geographical areas and based on the available data within countries. Furthermore, changes were linked to the socio-demographic development status, which should prompt more action in economically deprived areas. The success of reducing the burden of idiopathic epilepsy relies mostly on access to treatment and diagnostic techniques [3, 8].

Several diseases and injuries are involved in the origin of

epileptic seizures, showing a variable distribution worldwide [9]. Meanwhile, 4% to 78% of selected patients with initially unknown epilepsy etiology have genetic variants of probable or definitive etiologic significance [10]. The estimated proportion of individuals who carry a pathogenic variant that contributes substantially or causes epilepsy is approximately 17% of patients for epileptic encephalopathies, 5% of patients with genetic generalized epilepsies, and 2% for non-lesional focal epilepsies [11]. However, more than 50% of patients with developmental and epileptic encephalopathies (DEEs) cannot be genetically diagnosed despite state-of-the-art genetic testing techniques [9, 12].

In 2019, more than 140 epilepsy-associated genes or loci have been listed within the Online Mendelian Inheritance in Man database [13].

Familial analysis in epilepsy

Human genetics research has established that a genetic basis contributes to the susceptibility to epilepsy in most cases. However, the multifactorial condition of epilepsy that subsumes a variety of epilepsy types, seizures, levels of severity, and comorbidity has made it a core challenge to disentangle the genetic architecture for different types of epilepsy and to determine the specific genetic risks for each individual with epilepsy [14].

Early epilepsy gene discoveries used the strategy of ascertaining very large families, typically with 10 or more affected individuals, where the family history supported the presence of simple inheritance, and success utilizing parametric linkage analysis was likely [15]. This approach led to the recognition of a number of familial epilepsies and some of their genetic determinants.

The epilepsy diathesis hypothesis suggested that a familial predisposition for epilepsy exists due to the inheritance of susceptibility variants. In support of this was the discovery that rare inherited copy number variants can increase risk for different epilepsy syndromes [16].

Since the historical finding of a *CHRNA4* mutation causing autosomal dominant sleep-related hypermotor epilepsy (formerly known as autosomal dominant nocturnal frontal lobe epilepsy) in 1995 [17], discoveries of epilepsy genes have advanced greatly and accelerated further with the advent of next generation sequencing [10, 18].

Most genes identified to date come from monogenic families of focal epilepsies, and attempts to identify risk genes associated with genetic generalized epilepsies (GGE) have been largely unsuccessful [19]. Besides that, to date, reports from largescale Whole Exome Sequencing (WES) projects in epilepsy have focused mainly on cohorts with severe epilepsies of infancy and childhood, particularly the epileptic encephalopathies [20, 21]. These studies have reported diagnostic, monogenic causes in almost 27% of cases, identifiable via exome sequencing [22-24].

Fakhro et al. recently confirmed the benefit of working with families whose large sizes facilitate the assessment of multiple siblings [25]. The effect of adding siblings to the analysis of recessive variants was even more drastic than

for *de novo* variation. Between 12 to 42% of recessive variants discovered in an index case were shared by a single sibling, and only 1.3 to 11% were shared by two siblings. For families where there were 3 affected siblings, for example, GD001, the only variant remaining after filtration was the disease-causing variant. Conversely, in settings where siblings do not share the phenotype, the additional siblings can help sort benign family-specific polymorphisms from *bona fide* disease variants [25]. At the same time, index cases may appear to have as many as 10 *de novo* protein-altering variants when compared only with their parents, requiring significant time and resource investment for experimental validation. Therefore, introduction of a single sibling will reduce that number by more than half, while introduction of two siblings reduced the mean number of high quality protein-altering *de novo* variants to 0.5 per individual, consistent with previous reports [26].

Relatives of people with epilepsy have shown an increased incidence of epilepsy, even in families without Mendelian (monogenic) patterns of inheritance [27]. Moreover, studies on twins and families have shown that specific features of epilepsy are themselves heritable traits, including specific epilepsy syndromes [28], seizure types and symptoms [29], and EEG patterns [30]. Furthermore, the risk of epilepsy appears to be higher in the relatives of probands with generalized epilepsy than in the relatives of probands with focal epilepsy [27].

A lot of other, still incompletely studied family features may have genetic determinants that are distinct from the genetic determinants of epilepsy per se, just as in a recent study that proved the age at seizure onset to be an independent familial trait, with possible genetic determinants distinct from the determinants of particular epilepsy syndromes [13].

Several novel genes and disorders associated with DEE have been identified in the last few years [31-33]. Many of the genes causing epilepsy encode components of neuronal ion channels leading to neuronal hyperexcitability or depletion of inhibitory mechanisms [34, 35]. However, recently, several new genes coding for proteins other than ion channels have been identified, such as chromatin remodelers, intracellular signaling molecules, metabolic enzymes, transcription factors, and mitochondrial complex genes [6, 36].

Genetic testing in epilepsy

Clinical features often drive the choice of a particular genetic test or testing strategy, but in many patients, their presentation is not suggestive of a specific gene, or set of genes. WES and epilepsy panels (EP) are nowadays considered the most cost-effective genetic tests for epilepsy [37].

Gene panels provide a higher sequencing depth and lower cost when compared to the exome or genome sequencing, but restrict the diagnosis to specific genes in the panel, commercially available EPs typically targeting from 70 to 465 genes [38].

Importantly, some large panels are now based on WES, with restricted analysis of only the “panel” genes, so the benefit of higher depth of coverage is lost, but this opens

up the possibility of future reanalysis to include the whole exome [12].

Considering the fact that copy-number variants (CNVs) contribute significantly to variation in the human genome and estimating that they cause 1.2% difference for every reference genome [39], previous recommendations used the stepwise chromosomal microarrays method (CMA) \pm EP \pm WES testing strategy in epilepsy. CNVs can be detected by several genomic methods including conventional karyotype (deletions/duplications >5 Mb), CMA (~ 100 kb–5 Mb) and/or other methods, such as quantitative PCR and multiplex ligation-dependent probe amplification that target to detect smaller variations (<1 kb) [12].

Although less expensive, CMA has a lower diagnostic yield in epilepsy, and its use as the first-tier test is thus not anymore supported from a cost-effectiveness perspective [37]. However, in specific scenarios like epilepsy plus intellectual disability, epilepsy plus autism spectrum disorder, epilepsy with dysmorphic features – CMA is still considered be the most cost-effective and clinically useful test [37]. Studies using CMA have shown that pathogenic CNVs account for 5–10% of childhood epilepsies including DEE [40, 41]. Besides that, the most common types of genetic causes of DEE are sequence changes, responsible for 30–40% of cases, and chromosomal deletions or duplications, responsible for 5–10% of cases [10, 42]. Thus, an individualized evaluation of cost-effectiveness based on prior diagnostic yields for each of the targeted populations and costs for each test should be considered that is expected to optimize the diagnostic yield and use of resource. It is worth mentioning that the diagnostic yield of copy number variants (CNVs) is better understood in paediatric epilepsy compared to adult patients with epilepsy [43, 44].

More recently, *de novo* mutagenesis has emerged as the major genetic mechanism in epileptic encephalopathies and rapid progress in identifying them has been facilitated by WES [45, 46].

An increasingly appreciated and clinically important subtlety for the *de novo* paradigm is the role of mosaicism – post-zygotic mutations not present in every cell in the body. This kind of somatic mosaicism might contribute to the phenotypic heterogeneity seen with many epilepsy genes [13]. This new genetic mechanism has been recently identified as playing a larger role in focal epilepsies than it was previously thought. The repeated expansions in intronic regions – identified as the cause of a familial epilepsy syndrome associated with myoclonus [47] and tremor [48] suggest the role of these type of variants in epilepsies, an important aspect that is not easily detected by current sequencing technologies, the vast non-coding portion of the genome (including intronic and intergenic regions) that are currently explored in neurodevelopmental disorders and the analysis of the regulatory regions (e.g., promoters and enhancers) in patients with autism and developmental delay [49]. Another aspect is represented by the genes, the mutations in which they evoke a range of different phenotypes, yet to be described, starting with complex, neonatal onset diseases at the severe end and a childhood onset at the milder end

of the spectrum, including or excluding epilepsy from the picture, depending on the type of the mutation [50].

Another issue to be discussed is the use of Next Generation Sequencing (NGS) methods to identify disease-causing variants in poorly characterized populations that presents several challenges. For example, it was recently discovered that up to 15% of “variants” detected in >1000 Arabian people when aligned to reference genome GRCh37/hg19 had a minor allele frequency (MAF) $>50\%$ in the same cohort and therefore should to be considered reference alleles for this population [51].

Despite all the previously mentioned challenges, the need to identify causative genes for genetic disorders is an urgent issue, given that Mendelian diseases on aggregate affect $\sim 8\%$ of live births and are the leading cause of morbidity and mortality in children worldwide [52]. This also poses serious financial burdens on healthcare systems – in the cases where healthcare intervention is available, the total cost of care over an individual’s lifetime may exceed \$5 000 000 [53].

Whole Exome Sequencing in epilepsy

Over the last decade, NGS has significantly advanced the field of human genetics and genomics [54], leading to an explosion of gene discovery across many human disorders. The number of disease-associated genes has grown to 4132, and over 50 genes have been newly associated with epilepsy in the last three years alone [55].

It was previously established that WES, in combination with array-comparative genomic hybridization (aCGH), provides a diagnostic rate of 27% in unrelated adult epilepsy patients, 42% in unrelated paediatric patients, and 31% in a combined adult and paediatric cohort of unrelated patients with medically refractory epilepsy and co-morbid intellectual disability, that indicates that WES has similar utility in both adult and paediatric cohorts and is appropriate for diagnostic testing in both epilepsy patient groups [56]. To date, the majority of diagnostic genetic testing is conducted in the paediatric population, while the utility of such testing is less well understood in adults with epilepsy.

Another recent meta-analysis comprising more than 20000 children proved the diagnostic and clinical utility of whole exome/genome sequencing to be greater than chromosomal microarray alone, and that it should be considered as the first-line genomic test for children with suspected genetic diseases [57]. WES alone, judging on the previous studies, in mixed-age populations with multiple seizure types, has a diagnostic yield of 33–38% [10, 24, 58].

WES is not yet a match for CMA for CNV detection, as it can provide data about only the protein coding or exonic regions, but it is an increasingly powerful diagnostic tool, since a growing number of algorithms are being developed to aid the detection of CNVs by NGS and it is now possible to detect both single nucleotide variations (SNVs) and CNVs using an exome – or genome-wide approach with a single test [59].

A broad range in the diagnostic rate of WES in epilepsy has been described, the result of the variable definition of

each cohort depending on factors, such as type of epilepsy, phenotypic features, disease severity or prior genetic screening. In focal epilepsy, genetic diagnostic rate varies between 12.5% of cases [60] to 43% of cases with epileptic encephalopathy (EE) and in 33% of epilepsy cohort overall [10].

In 2011, the International League Against Epilepsy (ILAE) launched the Consortium on Complex Epilepsies, to facilitate meta-analysis in epilepsy genomics. In 2014, the first such meta-analysis was reported comprising 8696 cases and 26157 controls. This led to the identification of 2q24.3, 4p15.1, and 2p16.1 as epilepsy loci [61].

A recent analysis of exome sequencing in unrelated individuals with a family history of epilepsy shows an increased burden of ultra-rare variants among the currently known epilepsy genes [62]. However, the relevance of variants in these genes to common epilepsies, where inheritance is complex, remains uncertain, and molecular genetics advances have been modest [63].

In 2016, Afawi Z. et al. published their results on 211 families ascertained over an 11-year period in Israel, and pathogenic variants were identified in 49/211 families (23%). The majority were found in established epilepsy genes (e.g., SCN1A, KCNQ2, CSTB), however in 11 families, this cohort contributed to the initial discovery (e.g., KCNT1, PCDH19, TBC1D24) [63].

In 2017, the Epi4K Consortium, assembled and analyzed a cohort of 303 families. These findings suggested that specific patterns of syndromic familial aggregation occur, including newly recognized forms of familial focal epilepsy; although syndrome-specificity usually occurs in multiplex families, the one-third of families with features of both focal and generalized epilepsy is suggestive of shared genetic determinants; and that patterns of features observed across families including pedigree structure, sex, and age of onset may hold clues for future gene identification [64].

Recently, International League Against Epilepsy Consortium on Complex Epilepsies, performed a Genome-wide mega-analysis, and identified new 16 epilepsy loci. Importantly, 11 of these loci are associated with the genetic generalized epilepsies; the group of epilepsies where despite having the highest heritability there were made the least genetic progress to date [65].

The largest exome study of epilepsies to date showed that deleterious ultra-rare variants (URVs) – variation absent in a large population-based exome database – is enriched across the severity spectrum for epilepsy syndromes, when individuals with these syndromes are compared to ancestrally matched controls. Specifically, they observed a significant excess of deleterious URVs in constrained genes, established epilepsy-associated genes, and GABAA receptor subunit genes, a larger group of genes delineating the GABAergic pathway, and also in all cation-channel-encoding genes. The evidence that URVs contribute partially to genetic generalized epilepsies and non-acquired focal epilepsies is clear, but what remains unclear is the extent to which the excess rate of URVs observed in individuals with epilepsy that is a consequence of a small subset of affected individuals carrying highly penetrant mutations or a result of URVs that confer

risk, yet instead of rising to the level of Mendelian acting mutations, simply contribute to an overall polygenic risk for these syndromes [14].

Single gene causes of the more common forms of epilepsy appear to be relatively rare [64]. These common forms are likely multifactorial, with a significant and complex genetic architecture [66]. Solving the genetic architecture of common complex diseases remains a major challenge in the genetics field, since these findings might highlight that genes commonly involved in epilepsy span a wider range of epilepsy phenotypes than previously assumed [67].

Despite recent molecular advances in epilepsy, genetic investigation is often overlooked in adult practice. Diagnostic yields of different genetic testing methods have not yet been established for adult epilepsy patients. Further studies including larger population samples could be aimed to assess more prevalent genes related to epilepsy in adulthood, and whether these are similar to or different from those previously reported in paediatric cohorts [68]. Less is known about the diagnostic yield of WES in adult epilepsy populations, and it is unknown if adult patients with epileptic encephalopathy who survive into adulthood have a different genetic etiology compared to a paediatric patient cohort [56].

The reanalysis can increase the diagnostic yield in larger cohorts. Re-analysis and diagnosis are particularly important in epilepsy due to the rapid rate of gene discovery and potential for treatment implications [10]. For example, recently, a study identified intragenic, multi-exon deletions in TANGO2 by reanalysis of ES data [69, 70].

The Epilepsy Genetics Initiative (EGI) was formed in 2014 to create a centrally managed database of clinically generated exome sequence data. EGI performs systematic research-based reanalysis to identify new molecular diagnoses that were not possible at the time of initial sequencing and to aid in novel gene discovery. They recently showed a diagnostic rate of 5.8% in previously negative cases – a considerable increase in diagnostic yield demonstrating the value of periodic reinterrogation of whole exome data [8].

Whole Genome Sequencing (WGS) is increasingly being used to uncover the role of non-coding genetic material in the human genome [71, 72].

Several studies have proposed a genetic testing strategy to achieve the highest clinical utility, cost-effectiveness, and diagnostic yield for individuals with epilepsy [24, 37, 73], but specific testing algorithms are likely to change over time as new tests are introduced and the costs of existing tests decrease. New assays may be required to detect lesser-known but important molecular mechanisms [12].

Risk prediction in epilepsy

For most common epilepsies not caused by a single gene mutation, the relative risk to first-degree family members is 6–8 times greater for generalized epilepsy and 2–3 times greater for focal epilepsy, relative to a baseline cumulative incidence around 1% by age 20 years [27].

If a dominant monogenic cause is identified by genetic

testing, or strongly suspected from the family history, then a recurrence risk approaching 50% is expected (slightly reduced by incomplete penetrance, which is approximately 60–80% for most dominant Mendelian epilepsies) [74]. For children with *de novo* mutations, the recurrence risk in siblings should theoretically be zero. However, parental mosaicism elevates that risk and might be more common than previously suspected [7].

Among relatives of all probands (patient zero with epilepsy), cumulative incidence of epilepsy up to the age of 40 is 4.7%, and the risk shows a 3.3-fold increase compared with population incidence. The risk is largely higher in relatives of probands with idiopathic generalized epilepsies and epilepsies associated with intellectual or motor disability presumably present since birth ('prenatal/developmental cause'). Among relatives of probands with epilepsy without an identified cause (including epilepsies classified as 'idiopathic' or 'unknown cause'), the risk was significantly higher for epilepsy of prenatal/developmental cause. In relatives of probands with generalized epilepsy, standardized incidence ratios were 8.3 for generalized epilepsy and 2.5 for focal epilepsy. In relatives of probands with focal epilepsy, standardized incidence ratios were 1.0 for generalized epilepsy and 2.6 for focal epilepsy [27].

Gender analysis showed that epilepsy incidence was greater in offspring of female probands than in offspring of male probands, and this "maternal effect" was restricted to offspring of probands with focal epilepsy [75].

The results suggest that risks for epilepsies of unknown and prenatal/developmental cause may be influenced by shared genetic mechanisms. They also suggest that some of the genetic influences on generalized and focal epilepsies are distinct. However, a similar increase in risk for focal epilepsy among relatives of probands with either generalized (2.5-fold) or focal epilepsy (2.6-fold) may reflect some coexisting shared genetic influences [27].

In addition to single-gene Mendelian inheritance, there is an ample evidence for gene variants conferring risk of disease due to variable alterations in cellular function, sometimes modulated by other genes or epigenetic and environmental cues [76]. Consequently, many variants occur among population with minor degrees of potential influence on disease. Separately, they might not be enough to cause the disease in most circumstances. They would rather probably affect health by altering the risk of sporadic disease, in combination with other factors. Additional research is needed to realize the potential of linking strategies for genetic risk assessment to disease prevention and therapy.

Limitations, such as referral and reporting biases, small sample size, ambiguous disease definitions in probands and relatives, lack of controls, and failure to control adequately for age in the relatives should be considered when interpreting historical genetic studies in epilepsy.

Precision medicine in epilepsy

There are ample data to support the use of next-generation sequencing in reducing the patient's time to diagnosis, often referred to as the "diagnostic odyssey". Precision

health encompasses the use of patient-specific data to tailor patient-specific care [77].

We are now entering the era of genomics-driven personalized medicine, whereby novel treatments can be designed which are not solely symptomatic, but address the underlying cause of the epilepsy in the individual person and offer opportunities for truly disease modifying effects [78].

An increasing body of evidence indicates that identifying the pathogenic variant in individual patients with genetic epilepsies is relevant not only for diagnosis and prognosis, but also for treatment selection [79, 80]. This finding is not surprising, because responses to specific treatments can vary depending on the disease's underlying mechanisms which, in turn, may differ even across individuals sharing the same phenotype [6].

Precision approaches have also helped progress in the diagnosis and treatment of epilepsy syndromes. For example, in genetic epilepsy syndromes due to single-gene Mendelian mutations (about 1% of paediatric epilepsies), the efficacy of specific anti-epileptic drugs can be directly related to the underlying mutation, as is the case in Dravet syndrome, for which treating patients with sodium channel blockers is contraindicated [81]. Also, a more recent report of Kim et al. [32] described the discovery, development, and administration of an antisense oligonucleotide (ASO) therapy specifically designed for a single patient with CLN7 neuronal ceroid lipofuscinosis (a form of Batten's disease), a fatal genetic neurodegenerative disorder. The most remarkable is the fact that some neurological diseases, previously of unknown etiology, are nowadays proved as being treatable, without too much effort, as in case of vitamin B6 utility in neuropathies characterized by reduced PLP levels [82].

Reaching a genetic diagnosis in epilepsy may modify treatment, although this occurs in a minority of cases. The most frequent benefits of a genetic diagnosis of epilepsy are difficult to quantify, though this might include the answer to what is causing the disease, the ability to search for other symptoms associated with the gene variant, additional prognostic information, a sense of belonging to a specific support group for the families, informed reproductive choices, and possibly enrollment in clinical trials that are genotype specific [41].

Common problems in refractory epilepsy include the challenges of trial-and-error drug selection that can result in undesirable polytherapy, seizure-related injury, side effects, cost and even the development of some structural changes under the influence of the medication in some patients [60, 83-85].

The advanced knowledge of the molecular mechanisms leading to the development of epilepsy and its comorbidities might facilitate the patients' management by applying truly personalized therapies. Rather than relying on empirical observations relating genotypes to response to specific drugs, further prevailing paradigms will involve characterization of the functional consequences of the pathogenic gene variant and thus searching for available treatments that could correct the specific dysfunction responsible for the manifestations of the disease in each individual patient [86].

If no available treatment is identified, then new treatments may be designed and developed to address the pathogenic defect or the resulting functional abnormalities [6, 78, 87]. The alternative to drug repurposing consists in developing totally novel treatments, which can be designed once the mechanisms of the disease have been sufficiently characterized. The development of effective therapies for genetic CNS disorders is facilitated by advances in gene therapies, sense and anti-sense oligonucleotides, and other innovative therapeutics [6, 88, 89]. Applied research in this area also benefits from improved understanding of structure-activity relationships, and from access to 3D structural information on thousands of protein molecules through the Protein Data Bank [90].

The availability of animal models, which reproduce the targeted genetic defect is especially highly valuable to streamline preclinical development [91, 92].

Finally yet importantly, the application of pharmacogenetics to treatment and diagnosis extends beyond epilepsy and is a clinical area that is still under development. Over time, the use of patient's genetic data to predict drug efficacy and minimize side-effects will probably expand as research into these areas progresses. With unprecedented amounts of human data being generated from patients and healthy individuals, coupled with major developments in technology and large-scale data analysis, advances in genomics and precision health are creating new opportunities for evidence-based and patient-centered care. The next decade provides major shifts in the translation of these technologies into the clinical setting that will certainly benefit patients with neurological diseases.

Conclusions

Epilepsy's genetic background is nowadays undeniable; more than 140 genes or loci being already associated with this worldwide spread disease. However, the complexity of this health burden makes it a challenge to rapidly determine the cause and to pursue the best treatment management.

It is already proved that relatives of people with epilepsy have an increased risk to develop epilepsy, even in families without Mendelian inheritance.

Whole Exome Sequencing (WES) and epilepsy panels (EP) are nowadays considered the most cost-effective genetic tests for epilepsy, though the familial genetic analysis is an approach that could furthermore reduce the epilepsy patient's "diagnostic odyssey", by increasing the chances of identifying the truly disease-causing variant after filtration.

Despite recent molecular advances in epilepsy, genetic investigation is often overlooked in adult practice and much more details should be considered when interpreting historical genetic studies in epilepsy.

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DC and VC conceptualized the project and designed the research; DC drafted the first manuscript; VC and SG revised the manuscript critically. All the authors revised and approved the final version of the manuscript.

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