뇌전증의 유전적 진단

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Molecular Diagnosis of Epilepsy in Clinical Practice

Major advances in genetics allow us to uncover genetic causes of epilepsy and influence therapeutic decisions. As precision medicine becomes a focus for bio-health industry, laws related to next generation sequencing are improving and its application will continue to expand. Although genetic testing can be considered for anyone who is suspected to have a genetic cause, the most suitable indication is drug-resistant epilepsy. Epileptic patients who have genetic testing can be classified into three groups: (1) patients with defined phenotypes suggesting monogenic epilepsy syndrome; (2) patients with neuropsychiatric comorbidities or dysmorphic features; and (3) patients with undefined phenotypes with multiple candidate genes. Applicable genetic testing methods for epilepsy are as follows: Sanger sequencing, chromosome microarray, targeted gene panels, whole exome sequencing, and whole genome sequencing. Sanger sequencing is indicated for patients with defined phenotypes by a single gene mutation. Chromosome microarray is recommended as part of the initial evaluation of unexplained epilepsy, particularly among patients with associated neuropsychiatric comorbidities or dysmorphic features. Next generation sequencing can be informative in patients with undefined phenotypes with multiple candidate genes. Targeted gene panels are a cost-effective alternative to both Sanger sequencing and whole exome sequencing for the genetic diagnosis of epilepsy. There are, however, social, ethical and even financial hurdles to overcome in the transition of next generation sequencing technology into the clinic. This review focuses on recent transformation of epilepsy genetics and suggests a strategy for actual clinical practice.

Key Words: Genetics, Epilepsy, Counseling, Array comparative genomic hybridization, High-throughput DNA sequencing

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Introduction

With recent advances in genetic testing, the utility of the designation “idiopathic epilepsy” has dwindled and most of the cases are now thought to have genetic causes¹⁾. Genetic epilepsy comprises over 70% of cases and identification of the molecular basis is now a practical clinical task²⁾. Genetic testing may end the diagnostic odyssey, and also provide better counseling about prognosis and recurrence risk within the family. Certain genetic findings influence therapeutic decisions and this precision medicine will be applicable more in the near future³⁾.

Human genetic variations are classified roughly into single nucleotide variant

(SNV) or copy number variation (CNV) of a deoxyribonucleic acid (DNA) sequence. Deletions and duplications of one kilobase or larger DNA segment in comparison with a reference genome are defined as CNV. Genome-wide CNVs can be detected using array comparative genomic hybridization (CGH) with high resolution, and has now replaced karyotype analysis in most clinical and research applications. Sanger sequencing is still the gold standard for detecting small sequence variations, while next generation sequencing (NGS) allows for the analysis of thousands of genes simultaneously. CMA and NGS have become widespread diagnostic tools in neurology. They have been increasingly used for their high-throughput abilities and cost-efficiency during the past decade. Despite the wide availability of CMA and NGS, phenotypic heterogeneity, clinical overlap between different epilepsy syndromes, and lack of consensus make epilepsy genetics more complicated.

South Korea is focusing on fostering research and development (R&D) in genetic medicine as a part of “Post-genome Multi-ministry Gene Project” (from 2014 to 2021) supported by the Ministry of Welfare, the Ministry of Future Planning and the Ministry of Industry (<http://www.mohw.go.kr/eng/>). Laws and regulations related to NGS are improving and its application may be covered by national health insurance service before long. There are, however, social, ethical and even financial hurdles to

overcome in the transition of NGS into the clinic. This review focuses on recent transformation of epilepsy genetics and suggests a strategy for actual clinical practice.

Who should have genetic testing for epilepsy?

The indications for genetic testing continue to be transformed as more genes and their mechanisms are identified. Although genetic testing can be considered for anyone who is suspected to have a genetic cause, the most suitable indication is drug-resistant epilepsy. Genetic testing is generally not recommended in drug-responsive epilepsy or at epilepsy onset⁴. Recently, the International League Against Epilepsy (ILAE) also recommended that genetic evaluation should be performed at a tertiary level of epilepsy care where patients of any age after failure of one anti-epileptic drug should be referred⁵. Epileptic patients who have genetic testing can be classified into three groups: (1) patients with defined phenotypes suggesting monogenic epilepsy syndrome; (2) patients with neuropsychiatric comorbidities or dysmorphic features; and (3) patients with undefined phenotypes with multiple candidate genes. A molecular diagnostic strategy for epilepsy is in Fig. 1.

Fig. 1. A molecular diagnostic strategy for epilepsy.

Genetic testing methods

Applicable genetic testing methods for epilepsy are as follows: Sanger sequencing, CMA, targeted gene panels, whole exome sequencing (WES), and whole genome sequencing (WGS). SNVs and small insertions/deletions are covered by sequencing, WES or WGS; CNVs are covered by CMA or WGS. Genetic testing can follow an orderly progression from CMA or gene panels to WES or WGS if diagnosis is still unclear.

1. Sanger sequencing

Sanger sequencing is indicated for patients with defined phenotypes by a single gene mutation. There are well-known monogenic familial epilepsy syndromes, and the examples are benign familial neonatal convulsions (BFNC) and autosomal dominant familial focal seizures, including lateral temporal lobe epilepsy and nocturnal frontal lobe epilepsy. *KCNQ2* or *KCNQ3* mutations are responsible for 70% of families with BFNC⁽⁶⁾, with *KCNQ2* responsible for 90% of genetically identified cases⁽⁷⁾. *LGII* mutations account for 50% of patients with lateral temporal lobe epilepsy^(8,9). Even if clinical phenotype and family history is distinctive, fewer than 20% of families with nocturnal frontal lobe epilepsy have their molecular basis identified⁽¹⁰⁾. The order in which pathogenic variants most commonly occur is *CHRNA4*, *CHRNA2*, *KCNT1*, *DEPDC5*, *CHRNA2*, and *CRH*, with *CHRNA4* responsible for 10–20% of the cases⁽¹¹⁾. Serial single gene sequencing based on the order is possible but not recommended because of costs, time and the low mutation detection rate⁽¹²⁾. Gene panel including the candidate genes, WES or WGS can be considered instead of serial single gene sequencing.

Molecular testing is essential for severe monogenic epilepsy syndromes such as Dravet syndrome and Epilepsy limited to Females with Mental Retardation (EFMR). About 80% of patients with Dravet syndrome have genetic variations in the *SCN1A* gene, mostly due to single nucleotide mutations or small indels, and less than 5% have pathogenic CNVs⁽¹³⁾. More than 90% of cases have a de novo mutation, however, parental germ line and somatic mosaicism are need to be considered in estimating recurrence rates⁽¹⁴⁾. EFMR is a well-known monogenic epilepsy syndrome, which overlap with symptoms seen in Dravet syndrome. Affected girls slightly differed from Dravet syndrome due to later mean onset (9 months compared with 6 months), fewer absence and myoclonic seizures, and better developmental outcome⁽¹²⁾. Over 10% of girls with onset of seizures under 5 years of age have *PCDH19* mutations; many are de novo mutations and do not have the characteristic family history^(15–17). With or without intellectual disability, *PCDH19* sequencing should be considered in

girls who present with clusters of febrile seizures from infancy⁽¹²⁾.

There are monogenic epilepsy syndromes which have significant implications for therapeutic decision and genetic counseling. Glucose transporter deficiency syndrome type 1 (GLUT1 deficiency) is caused by heterozygous mutations in the *SLC2A1* gene encoding the glucose transporter GLUT1, which limit glucose transport across the blood-brain barrier. *SLC2A1* mutations occur mostly de novo, but may rarely be seen in family members with autosomal dominant pattern. GLUT1 deficiency is likely under diagnosed due to variable clinical phenotypes and severities, such as mental retardation, acquired microcephaly, complex motor disorders, epilepsies and non-epileptic paroxysmal episodes. In particular, the diagnosis should be considered in patients with paroxysmal exercise-induced dyskinesia or with early-onset absence epilepsy⁽¹⁸⁾. Low cerebrospinal fluid (CSF) level of glucose, relative to blood level is the best biochemical clue to the diagnosis although not constantly found. Molecular analysis of the *SLC2A1* gene confirms the diagnosis. Ketogenic diet improves seizures, movement disorders, and cognitive outcome. Steroids and carbonic anhydrase inhibitors can also be helpful⁽¹⁹⁾.

2. Chromosomal microarrays

Many CNVs have no or minor influence on phenotype, but some act as risk factors or causes of disease⁽²⁾. The development of CMA technology facilitated high throughput screening and has confirmed that CNVs play an important role in epilepsy^(14,20). CMA is recommended as part of the initial evaluation of unexplained epilepsy, particularly among patients with associated neuropsychiatric comorbidities or dysmorphic features. Recent studies found that 5–10% of patients with childhood epilepsies had a pathogenic or potentially pathogenic CNV^(21,22). CMA is especially recommended as a first step for patients with neuropsychiatric comorbidities or dysmorphic features because of the diagnostic yield as high as 15% to 20%⁽²³⁾. Studies of patients with genetic generalized epilepsy, idiopathic focal epilepsies or epileptic encephalopathies have provided similar yields, and there is evidence that up to 10% of patients with epilepsy and intellectual delay have disease associated-CNVS^(24–28). Genomic hotspots of idiopathic epilepsy include 1q21.1, 15q11.2, 15q13.3, 15q11–q13, 16p11.2, 16p13.11, and Xp22.31^(21,25–28), while these CNVs are rarely identified in epileptic encephalopathies.

3. Next generation sequencing

NGS technologies have achieved great success for de novo mutation detection in neurodevelopmental disorders. NGS can be informative in undefined phenotypes with multiple candidate genes. Several tests can be performed using NGS: targeted gene

panels, WES and WGS. While WES and WGS sequences focuses on many epilepsy-unrelated variants, targeted gene panels focus on genes in which epilepsy is a major phenotypic feature according to the Online Mendelian Inheritance in Man (OMIM) database. This leads to a significant increase in coverage for the target genes and simplifies bioinformatics analysis. Yield for targeted panels or whole exome sequencing is from 10% to over 50%, and a high yield among patients ascertained through tertiary care epilepsy programs²⁹⁾. There are several commercial epilepsy panels and the number of genes included is 70–465 genes³⁰⁾. Despite the inclusion of numerous genes in sequencing panels, only 38-gene panel was sufficient for 93% of the patients³⁰⁾. The more genes included on a panel, the lower diagnostic yield was resulted in due to increased cost and variants of uncertain clinical significance (VUS)²⁹⁾. It is important to select an appropriate panel depending on patient's age and seizure types for high diagnostic yield. The diagnostic yield of targeted gene panels for epilepsy is similar to WES but achieved at a lower cost per base than WES or Sanger sequencing. WES or WGS can be considered when targeted sequencing results were negative. Unlike focused approaches such as exome sequencing or targeted resequencing, which analyze a limited portion of the genome, WGS delivers a comprehensive view of the entire genome, which can detect SNVs, insertions/deletions, CNVs and large structural variants.

Genetic counseling for epilepsy patients

Epidemiological studies of familial aggregation provide empirical risk for genetic counseling, and play an important role in genetic contributions to complex disorders such as the epilepsies³¹⁾. Analyses of familial risk in regard to proband and relative phenotypes can help to identify the genetic influences on different clinical features³²⁾. The complexities of phenotype-genotype relationships, genetic heterogeneity, pleiotropy, number of genes involved, and the emerging significance of somatic mutations contribute to uncertain genetic architecture and make genetic counseling more challenging in epilepsy^{33–35)}. Inheritance pattern of most epilepsies is non-Mendelian and risks to relatives are considerably lower. The clinician must explain the possibility of relatives with mild epilepsies that they were unaware of and the complex inheritance³⁾. A recent study estimated the frequency of epilepsy in relatives of individuals with epilepsy. By the age of 40, the overall risk was increased 3.3-fold (4.7% in incidence) above the general population, with a higher increase in risk for idiopathic generalized epilepsies (7.3-fold; 6.0% in incidence) compared to idiopathic focal epilepsies (2.0-fold; 2.7% in inci-

dence)³⁶⁾. We must also consider the ethical issues that arise from new technologies and explore the risks and benefits of new approaches to testing and to talking about test results³⁷⁾.

Therapeutic decisions based on genetic findings

Genetic testing can be more useful when it can help assist in treatment of the patient. The success of any diagnostic testing strategy should be measured in terms of changes in clinical management, and ultimately in patient outcomes²⁹⁾. Therapeutic benefits based on molecular diagnosis of epilepsy are listed in Table 1. For Dravet syndrome with *SCN1A* mutations, sodium channel blockers such as lamotrigine and carbamazepine should be avoided, but valproic acid, topiramate, clobazam, and stiripentol appear to be beneficial^{38,39)}. *PCDH19* related epilepsy showed good response to stiripentol⁴⁰⁾. For *KCNQ2* encephalopathy, although ezogabine targets these channels, toxicity to the retina, skin, and nails may limit its use. Recent study suggests that sodium channel blockers, carbamazepine and phenytoin are effective in *KCNQ2* encephalopathy⁴¹⁾. *KCNQ3* encephalopathy is generally controlled with phenobarbital, phenytoin, carbamazepine, and valproate⁴²⁾. For *SCN8A* encephalopathy, sodium channel blockers may be effective in some cases⁴³⁾. Levetiracetam showed dramatic effect in *STXBP1* encephalopathy⁴⁴⁾. For *GLUT1* deficiency with *SLC2A1* mutations, many patients with mild phenotype respond to conventional antiepileptic drugs, but ketogenic diet therapy is essential for refractory cases³⁾. For vitamin responsive epilepsies, pyridoxine-dependent epilepsy due to *ALDH7A1* mutations responds to pyridoxine⁴⁵⁾, pyridoxamine 5'-phosphate

Table 1. Therapeutic Benefits Based on Molecular Diagnosis of Epilepsy

Gene	Therapeutic benefits
<i>SCN1A</i> ^{38,39)}	Valproic acid, topiramate, clobazam, stiripentol Avoid: Lamotrigine, carbamazepine
<i>PCDH19</i> ⁴⁰⁾	Stiripentol
<i>KCNQ2</i> ⁴¹⁾	Carbamazepine, phenytoin, ezogabine
<i>KCNQ3</i> ⁴²⁾	Phenobarbital, phenytoin, carbamazepine, valproic acid
<i>SCN8A</i> ⁴³⁾	Sodium channel blockers
<i>STXBP1</i> ⁴⁴⁾	Levetiracetam
<i>SLC2A1</i> ¹³⁾	Ketogenic diet, steroids, carbonic anhydrase inhibitors
<i>ALDH7A1</i> ⁴⁵⁾	Pyridoxine
<i>PNPO</i> ⁴⁶⁾	Pyridoxal 5'-phosphate
<i>KCNT1</i> ^{47,48)}	Quinidine
<i>GRIN2A</i> ⁴⁹⁾	Memantine
<i>DEPDC5</i> ^{50–52)}	Rapamycin analogues
<i>CHRNA4</i> Ser284Leu ^{53–55)}	Zonisamide

oxidase deficiency owing to *PNPO* mutations responds to pyridoxal 5'-phosphate⁴⁶⁾. *KCNT1* mutations in epilepsy with migrating focal seizures of infancy respond to quinidine^{47,48)}, *GRIN2A* mutations in epilepsy respond to memantine⁴⁹⁾, and *DEPDC5* mutations are known to be a regulator of mTOR, suggesting the possibility of treatment with rapamycin analogues⁵⁰⁻⁵²⁾. *CHRNA4* Ser284Leu mutation responds well to zonisamide⁵³⁻⁵⁵⁾.

Conclusions

Genetic testing not only provides diagnostic confirmation but also influences therapeutic decisions. Targeted gene panels are a cost-effective alternative to both Sanger sequencing and WES for the genetic diagnosis of epilepsy⁵⁶⁾. Early adoption and implementation of NGS raised issues of moral norm, patient selection, analysis technique and communication of results. Accurate WGS with cheaper prices will be available in the near future and the availability of this technology will challenge ethical, legal and economic regulation in clinical genetics³⁷⁾.

요약

최근 유전자 검사 기법의 급속한 발전으로 인해 이전까지 특발성 뇌전증으로 분류되었던 환자들에서 많은 유전적 원인이 밝혀지게 되었고, 맞춤 치료로 연결되는 경우도 늘었다. 바이오헬스 산업이 정밀의료에 초점을 맞추게 되면서 차세대 유전자 검사와 관련된 법과 규제들도 많이 완화가 되었고 보험급여가 시행될 날이 머지않은 시점에 있다. 유전자 검사는 유전적 원인을 가진 것으로 의심되는 뇌전증 환자 누구에게나 고려될 수 있지만, 항경련제에 잘 반응하지 않는 난치성 뇌전증 환자들에게 시행하는 편이 적합하고 적용될 수 있는 검사 방법에 따라 (1) 단일 유전자 변이에 의한 질환이 의심되는 경우, (2) 다른 신경/정신학적인 질환을 동반하거나 기형이 있는 경우, (3) 임상양상이 한정적이지 않고 관련될 수 있는 유전자가 여러 개인 경우로 환자를 분류할 수 있다. 적용할 수 있는 유전자 검사로는 Sanger sequencing, chromosome microarray, targeted gene panels, whole exome sequencing, whole genome sequencing이 있다. 단일 유전자 변이에 의한 질환이 의심되는 경우는 Sanger sequencing, 다른 신경/정신학적인 질환을 동반하거나 기형이 있는 경우는 chromosome microarray, 임상양상이 한정적이지 않고 관련될 수 있는 유전자가 여러 개인 경우는 targeted gene panels을 우선 고려할 수 있으며, 검사 결과가 음성인 경우는 whole exome sequencing이나 whole genome sequencing에 이르기까지 순차적으로 검사할 수 있다. Targeted gene panels는 Sanger sequencing이나 whole exome sequencing 등에 비해 염기서열 당 분석 비용이 싼 편이며 진단율이 높고 시간을 절약할 수 있는 장점이 있다. 차세대 유전자 검사의 장점에도 불구하고 아직까지는 임상에서 적극적으로

활용하기 위해 넘어야 할 사회적, 윤리적, 경제적 장벽들이 있다. 본 중설에서는 최근 뇌전증 유전학의 변화와 임상 실재에서 활용할 수 있는 유전자 검사 방법을 제시한다.

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