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HIROSNAKI UNIVERSITY
EPILEPSY GENES: EXCITEMENT TRACED TO ION CHANNELS

Sunao Kaneko, Kazumaru Wada, Motohiro Okada and Yuko Kawata

Abstract Epilepsy is a neurological disorder characterized by recurring seizures. It is physiologically characterized by abnormal, excessive and self-terminating discharges from neurons. Epilepsy affects more than 0.5% of the world's population and has a large genetic component. The most common human genetic epilepsies display a complex pattern of inheritance and the identity of the susceptibility genes is largely unknown. This article reviews recent progress made in molecular genetics of epilepsy, including our own discovery of two novel mutations in the genes of autosomal dominant nocturnal frontal lobe epilepsy and benign familial neonatal convulsions, and our mapping of the genetic locus of benign adult familial myoclonic epilepsy. Pathogenesis of epilepsy as a channelopathy and perspectives of molecular genetic study of epilepsy are also discussed.

I. Introduction

Our department has the longest history of epilepsy research in Japan. In the past two decades, therapeutic drug monitoring of antiepileptic drugs (AEDs), mechanisms of action of AEDs, prognosis of epilepsy, discontinuation of AED, malformations and psychomotor development of offspring of epileptic mothers, and clinical genetics of epilepsy were the main epilepsy research subjects of our department. Recently, with the most active institutions of epilepsy research and treatment in Japan, we started a collaborative study on the molecular genetic factors associated with epilepsy.

Epilepsy is a neurological disorder characterized by recurring seizures. It is physiologically characterized by abnormal, excessive and self-terminating discharges from neurons.

Epilepsy affects more than 0.5% of the world's population and has a large genetic component. The most common human genetic epilepsies display a complex pattern of inheritance and the identity of the susceptibility genes is largely unknown. The focus of research on the genetics of the epilepsies is the identification of mutations causing epilepsies, and the abnormal properties of the neuron glia system through which the mutations are expressed and result in clinical epilepsy. Such research seeks to explain the entire series of events by which an epilepsy genotype is converted into a clinical seizure phenotype. Recent rapid progress in mapping human epilepsy genes has provided some glimmer of hope to answer these questions. This article reviews recent progress made in molecular genetics of epilepsy, including our own discovery of two novel mutations in the genes of autosomal dominant nocturnal frontal lobe epilepsy (ADNFL) and benign familial neonatal convulsions (BFNC), and our mapping of the genetic locus of benign adult familial myoclonic epilepsy (BAFME).

II. Why map epilepsy genes?

Once the mutated code scripts are identified in the epilepsies, we can investigate the mechanisms on a molecular, cellular, and systemic level, which are the means by which such inheritance leads to a specific epilepsy syndrome. This will lead to an understanding of how neurons are regulated in the face of such abnormal code scripts, and how development is affected during embryogenesis and the immediate postnatal period. Improving the classification of epilepsy genotypes will undoubtedly improve calculations of sibling risk for epilepsy, and this, in turn, improves the accuracy of risk assess-
III. Chromosomal localization of epilepsy genes

Twenty two separate epilepsy genes have been chromosomally localized, and identifications of mutations in some types of epilepsy genes have been reported (Table 1).^{41}

III-1) Localization related epilepsies

Autosomal dominant nocturnal frontal lobe epilepsy

- Benign Rolandic epilepsy
  - Localization-related epilepsies
    - Autosomal dominant nocturnal frontal lobe epilepsy
      - Chromosome: 20q13.2-13.3
    - Familial partial epilepsy (temporal lobe)
      - Chromosome: 10q?
    - Benign Rolandic epilepsy
      - Chromosome: 1q

Idiopathic generalized epilepsies

- Juvenile myoclonic epilepsy
  - Chromosome: 6p11-12
- Benign familial neonatal convulsions (EBN1)
  - Chromosome: 20q13
- Benign familial neonatal convulsions (EBN2)
  - Chromosome: 8q
- Benign infantile familial convulsions
  - Chromosome: 19q
- Childhood absence epilepsy (possible ECA1)
  - Chromosome: 20q

Specific syndromes

- Progressive myoclonus epilepsies
  - Unverricht-Lundborg type (EPN1)
    - Chromosome: 21q23
  - Infantile type of neuronal ceroid lipofuscinosis (CLN1)
    - Chromosome: 1p
  - Juvenile type of neuronal ceroid lipofuscinosis (CLN3)
    - Chromosome: 16p
- Finish-variant late-infantile type of neuronal ceroid lipofuscinosis (CLN5)
  - Chromosome: 13q
- Juvenile Gaucher's disease
  - Chromosome: 1q21-31
- Lafora's disease [Autosomal recessive]
  - Chromosome: 6q24
- Myoclonus epilepsy and ragged-red fibers
  - Chromosome: 8p
- Northern epilepsy
  - Chromosome: 12p
- Dentatorubral pallidoluysian atrophy
  - Chromosome: 8q23.3-24.1

Other diseases which cause seizures

- Febrile convulsions
  - Chromosome: 8q13-21?
- Generalized epilepsy with febrile seizures
  - Chromosome: 19q13.1
- Angelman syndrome
  - Chromosome: 15q11-13
- Prader-Willi syndrome
  - Chromosome: 15q11-13

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epilepsy (ADNFLE) was first reported in 1994, and has since been recognized as a new clinical entity of epilepsy\(^5\). This partial epilepsy is characterized by a brief seizure during light sleep, and is often misdiagnosed as nightmare or parasomnia. ADNFLE is a monogenic disorder inherited as an autosomal dominant trait with high penetrance. The responsible gene has been mapped to chromosome 20q13.2\(^6\), and a missense mutation in the neuronal nicotinic acetylcholine receptor \(\alpha_4\) subunit (CHRNA4) gene has been reported in an Australian family with ADNFLE\(^7\) followed by another report of an insertional mutation of the CHRNA4 gene\(^8\).

Recently, Hirose \textit{et al.} reported another point mutation in a Japanese family with ADNFLE. A ‘‘C’’ to ‘‘T’’ exchange (C755T) was found in exon 5 of the CHRNA4 gene on one allele of affected individuals. C755T replaced Ser 252 in the second membrane spanning domain (M2) of CHRNA4 with a leucine. Ser 252 is conserved characteristically in the \(\alpha_4\) subunit acetylcholine receptor, a \(\alpha_4\) subunit acetylcholine receptor that is considered to play an important role in the channel function\(^9\).

Vaughn \textit{et al.} reported (1996) two children with centrotemporal spike discharges and with seminological similarities to benign rolandic epilepsy. Although the two children also expressed many manifestations that are not detected in benign rolandic epilepsy, which might reflect extensive deletion of chromosome 1, they suggested that the distal portion of the long arm of chromosome 1 is a potential site for a candidate gene for benign rolandic epilepsy\(^10\). The responsible gene for familial partial epilepsy (temporal lobe) has been mapped to 10q\(^11\), but this locus has not been confirmed\(^12\).

There have been no other reports on other types of localization-related epilepsies to date.

**III-2) Generalized epilepsies**

The most common forms of idiopathic generalized epilepsies (IGE) are juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), and epilepsy with grand mal seizures on awakening.

JME is a commonly occurring form of IGE, and is widely accepted to be genetically determined, although its mode of inheritance remains controversial. Studies so far reported have provided evidence both for and against the existence of locus on chromosome 6p\(^13\) or 15q\(^14\). Recently, Le Hellard \textit{et al.} (1999) found no evidence that susceptibility to JME was associated with HLA-DR13 (6p) in a French population\(^15\).

Benign familial neonatal convulsions (BFNC) is an autosomal dominantly inherited disorder of newborns. BFNC has been linked to mutations in two \(K\)-channel genes, KCNQ2 on chromosome 20 and KCNQ3 on chromosome 8. Singh \textit{et al.} (1998) found that there are mutations in KCNQ2, including a sub-microscopic deletion, two transmembrane missense mutations, two frameshifts and one splice-site mutation\(^16\). At present, only one mutation of KCNQ3 has been identified, which is a missense mutation in the critical pore of KCNQ3 (G to T at nucleotide 263 : replaced Gly with Val)\(^17\). Hirose \textit{et al.} (1999) screened six Japanese families with BFNC for mutations of KCNQ3, and found a T to C exchange (cDNA925T>C) on one allele in affected individuals but not on 200 alleles of healthy volunteers. cDNA925T>C replaced Try262, a conserved residue within P-loop of the KCNQ family, with an Arg (W262R)\(^18\).

Benign infantile familial convulsions (BIFC) are an autosomal-dominant epileptic syndrome characterized by an age of onset within the first year of age. Guipponi \textit{et al.} (1998) mapped the BIFC gene to chromosome 19 in five Italian families with a maximum two-point lod score of 6.36 at D19S114.

CAE is one of most common epilepsies, and has been mapped to chromosome 8q24.3\(^19\). Recently, a candidate gene named JH8 (jerky homologue of Human on chromosome 8) has been suggested\(^20\) for CAE, and this is supported by the high homology of the JH8 gene to the mouse jerky gene, by the location of the gene in the CAE candidate region on chromosome band 8q24, and by the phenotypic similarities between jerky mice and human CAE.

Regarding studies of mapping of genes predisposing to IGE, possible association of a silent polymorphism in CHRNA4 with IGE has been suggested by Steinlein \textit{et al.} (1997)\(^21\), but the gene encoding the \(\alpha_1A\)-voltage-dependent calcium channel (CACN1A4) has been related neither to IGE, CAE, juvenile absence epilepsy nor JME\(^22\).

**III-3) Specific syndrome**

Significant progress has recently been made in the mapping and isolation of genes for symptomatic Mendelian epilepsies such as Unverricht-Lundborg disease, the neuronal ceroid lipofuscinoses, Lafora body disease, sialidosis, dentatorubral-pallidolu-
sian atrophy and myoclonic epilepsy with ragged red fibers (Table 1). Progressive myoclonic epilepsies (PMEs) are heterogeneous group of debilitating, often fatal epileptic encephalopathies characterized by segmental arrhythmic myoclonus, massive myoclonias, GTCs or clonic seizures, with or without absence seizures, dementia, and other progressive neurologic manifestations, especially cerebellar\(^5\). There is ethnic and geographic variation in the frequency of these disease syndromes, and most of the PMEs are autosomal recessive in inheritance. Details of molecular biological findings in these specific syndromes have been reviewed elsewhere\(^4\).

Benign adult familial myoclonic epilepsy (BAFME) is an autosomal dominant-inherited idiopathic epileptic syndrome characterized by adult-onset tremulous finger movement, myoclonus, epileptic seizure, and a non-progressive course. The gene for BAFME was recently assigned to chromosome 8q23.3-q24.1 in a Japanese family by our collaborative study group\(^2\). Lewis \textit{et al.} reported linkage of benign familial neonatal convulsions (BFNC2) with markers D8S284 and D8S256 localized at 8q24.13-qter\(^2\), and familial febrile convulsions has been mapped to 8q13-21\(^2\). However, we obtained significantly negative lod score of these loci, indicating that BAFME is distinct from these PMEs.

III-4) Other diseases which cause seizures

Febrile convulsions affect approximately 3% of all children under 6 years of age and are far the most common seizure disorders. Two putative loci have been mapped (FEB1 : 8q; FEB2 : 19p)\(^2\), but specific genes have not been reported yet. A clinical subset, termed generalized epilepsy with febrile seizures plus (GEFS+), in which many family members have seizures with fever that may persist beyond six years of age or be associated with afebrile generalized seizures has been mapped to 8q13-21\(^2\). However, we obtained significantly negative lod score of these loci, indicating that BAFME is distinct from these PMEs.

Four. Epilepsy genes which lead to the dysfunction of ion channels or receptors

Abnormalities of molecular mechanisms responsible for regulating neuronal excitability should be associated with the pathogenesis of epilepsy. The \(\alpha4\) subunit gene of the neuronal nicotinic acetylcholine receptor (CHRNA4) has been identified as the first gene underlying an idiopathic partial epilepsy syndrome in humans, ADNFLE\(^7,28\). The properties of the wild type receptor composed of \(\alpha4\) and \(\beta2\) subunits and the mutant receptor where \(\alpha4\) subunits carry the mutation at Ser 248 were studied by Weiland \textit{et al.}(1996)\(^2\), and they reported that the mutant receptor exhibited faster desensitization upon activation by acetylcholine and that recovery from the desensitized state was much slower than in the wild type receptor. Based upon these results, they suggested that the reported mutation caused seizures via a diminution of the activity of the \(\alpha4\beta2\) neuronal nicotinic acetylcholine receptor. Thus, the mutation in CHRNA4 may cause receptor hyperactivity that can lead to neuronal epileptic activity. The gene for the electroencephalographic variant pattern 1 (EEGV1) also maps to the same region, suggesting that the gene CHRNA4 may possibly be involved in several epileptic disorders, such as CAE, juvenile absence epilepsy and JME.

BFNC (EBN1 and EBN2) is caused by mutations in the KCNQ2 or the KCNQ3 potassium channel genes. Both potassium channels are distributed widely in brain with large overlapping. The formation of heteromeric KCNQ2/KCNQ3 potassium channels have been found with currents that are at least tenfold larger than those of the respective homomeric channels, and KCNQ2/KCNQ3 currents can be increased by intracellular cyclic AMP, an effect that depends on an intact phosphorylation site in the KCNQ2 amino terminus. KCNQ2/KCNQ3 mutations identified in BFNC pedigrees compromise the function of the respective subunits, but exert no dominant-negative effect on KCNQ2/ KCNQ3 heteromeric channels. Schroeder \textit{et al.} predicted that a 25% loss of heteromeric KCNQ2/ KCNQ3 channel function is sufficient to cause the
electrical hyperexcitability in BFNC\textsuperscript{29}, indicating that BFNC is due to a loss of KCNQ2/KCNQ3 heteromeric channels which may lead to impairment of repolarization. Amino acid sequence comparison revealed that both genes shared strong homology to KvLQT1, which is responsible over 50\% of inherited long QT syndrome. Individually, expression of KCNQ2 or KCNQ3 in Xenopus oocytes elicits voltage gated, rapidly activating $K^+$-selective currents similar to KCNQ1, but, unlike KCNQ1, KCNQ2 and KCNQ3 currents are not augmented by co-expression with the KCNQ1\beta subunit, KCNE1 (min K, IsK). Co-expression of KCNE1 with the two channels strongly suppressed current amplitude and slowed kinetics of activation\textsuperscript{30}. The functional interaction between KCNQ2 and KCNQ3 provides a framework for understanding how mutations in either channel can cause a form of idiopathic generalized epilepsy\textsuperscript{30}.

V. Characterization of epilepsy as channelopathies

SCN1B was identified as a responsible gene underlying febrile convulsion plus generalized epilepsy. EBN1 and EBN2 result from mutations in genes encoding KCNQ2 and KCNQ3, respectively. Animal studies also suggest that genes encoding $K^+$, $Na^+$ and $Ca^{2+}$ channels are at least partly responsible for convulsions, and most AEDs bind to or affect various ion channels\textsuperscript{31}. These reports support a hypothesis that some types of idiopathic epilepsy are a form of channelopathy. Understanding gained from work in this areas of epilepsy research is not only allowing characterization of the molecular and physiologic basis of these epilepsies, but also ultimately sheds light on our understanding of pathophysiology of more common epilepsies\textsuperscript{32}, and promises new vistas of AED and may benefit large numbers of affected individuals.

VI. Perspectives of molecular study of epilepsy

Future studies will ascertain whether IGEs and other forms of epilepsies, including those that are less genetically traceable, involve mutations in various ion channel genes. Such studies may lead to new strategies for treatment such as gene therapy as well as development of new types of AEDs. In particular, new AEDs will be developed based upon the data of molecular genetics of epilepsy research, such as drugs which raise intracellular cAMP may prove beneficial in BFNC\textsuperscript{29} and drugs which bind to site two of $Na^+$ channel may benefit large number of patients with eilepsy\textsuperscript{33}.

Identifying the gene defects in the epilepsies will brighten prospects for gene therapy. Somatic or germ cell gene replacement will have a decided advantage over present AED therapy by requiring fewer treatment deliveries, if not one curative treatment delivery. Delivery through progenitor stem cells can have a long-lived effect. Should untoward effects occur, a “suicide gene” can be used to kill the delivery construct\textsuperscript{3}.

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てんかんの遺伝子：イオンチャネルへと向かう興奮

兼子 直 和 田 一 丸 岡 田 元 宏

抄録 てんかんは人口の0.5％に認められる頻度の高い中枢性疾患であり、原因は神経細胞の異常発射であるが、その背景に遺伝的関与があることに異論はない。しかし、てんかんの遺伝形式が複雑であるため、一部のてんかんを除いて、多くのてんかんの遺伝子は未だ不明である。本稿では、新たに我々が発見した常染色体優性夜間性前頭葉てんかんと良性家族性新生児型てんかんの遺伝子異常、良性成人型ミオクロニーテンかんの遺伝子解析結果を含めて、これまでに判明したてんかんに関わる遺伝子あるいはその座位、てんかんの遺伝子解析の意義、てんかん発現に関連するイオンチャネル、チャネル病としてのてんかん、今後の研究の展望などについて概観する。

キーワード：てんかん；遺伝子；常染色体優性夜間性前頭葉てんかん；良性家族性新生児型てんかん；良性成人型ミオクロニーテンかん。

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