Validation criteria for genetic animal models of epilepsy

Motohiro Okada¹, Gan Zhu², Shukuko Yoshida³ and Sunao Kaneko³

¹Department of Psychiatry, Division of Neuroscience, Graduate School of Medicine, Mie University, Tsu, Mie, Japan
²Department of Psychiatry, the First Hospital of China Medical University, Shenyang110001, China
³Department of Neuropsychiatry, Graduate School of Medicine, Hirosaki University, Hirosaki, Japan

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Summary

In the past decades, several mutant genes that encode ion channel subunit proteins or their functionally-related proteins have been identified in pedigrees of idiopathic epilepsy. To explore the pathogenesis and pathophysicsiology of epilepsy syndrome, the functional abnormalities of transmission in genetic animal models bearing the mutant genes identified in pedigrees of idiopathic epilepsy should be analyzed. In spite of these efforts, it is important to identify the most suitable genetic animal models for exploration of epileptogenesis and ictogenesis, as well as the development of novel antiepileptic drugs. In the literature on genetic epileptic animal models, there is no systematic discussion on how to assess the validation criteria for such models. In this review, we describe the validation criteria for genetic animal models of epilepsy.
Introduction

Epileptic seizures are clinical manifestations of excessive, synchronous, abnormal firing patterns of neurons located predominantly in the brain [1]. Epilepsy is defined by WHO as “a chronic brain disorder of various etiologies characterized by recurrent seizures due to excessive discharge of cerebral neurons (epileptic seizures), associated with a variety of clinical and laboratory manifestations” [2]. Epilepsy is a common neurological disorder afflicting 1–2% of the general population worldwide, and approximately 5% of the people experience at least one seizure in their lifetime [3]. Epilepsy is a heterogeneous group of neurological conditions with a common feature of recurrent, usually unprovoked epileptic seizure, defined as a sudden stereotyped episode of motor activity, sensation, behavior, emotion, memory or consciousness [1].

Evidence of a genetic basis in idiopathic epilepsies, which are currently considered to represent 39 to 59% of all epilepsies [4], came from studies on twins showing rates consistently higher in monozygotic than in dizygotic twins [5, 6]. Several efforts have been made during the past decade to study the genetic bases of common idiopathic epilepsies. Two different approaches have been used to link defects in single genes to an epileptic phenotype. The conventional forward approach is based on the positional cloning: a family history has first to be collected, a linkage analysis must then be performed with the purpose of narrowing down the location of the disease gene to a small chromosome region. The alternative approach starts with the selection of candidate genes. To investigate the candidate gene, the establishment of appropriate animal models bearing the candidate mutations is necessary. Success of generation of genetic animal models of idiopathic epilepsies can be useful also in the evaluation of the neurological phenotypes and in comparisons of the epileptic phenotypes with those underlying human epilepsies.

Several genetic animal models bearing mutant genes identified in pedigrees of idiopathic epilepsy have been described recently. They include autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [7-9], benign familial neonatal convulsion (BFNC) [10, 11], childhood absence epilepsy (CAE) [12, 13], severe myoclonic epilepsy in infancy (SMEI) [14] and febrile seizures (FS) [15]. However, their phenotypic features have not been determined fully. Furthermore, in the literature on epileptic animal models, there is a lack of systematic discussion on how to assess the validation criteria. To answer this question, we propose a set of validation criteria for genetic animal models of epilepsy, composed of face, construct and predictive validities. The first genetic abnormality of idiopathic epilepsy was documented for ADNFLE in 1994 [16]. We recently generated four genetic animal models of ADNFLE, and evaluated their validities according to the above validation criteria.

Validation criteria for a suitable genetic animal model of epilepsy

The optimal animal model should mimic
human cases in terms of etiology, biochemistry, symptomatology, and treatment [17]. In 1992, Sarter et al [18, 19] developed validation criteria for animal models of human disorders. According to their studies, the model must conform to three validation criteria: face validity, construct validity, and predictive validity. Face validity is the ability to fundamentally mimic the behavioral clinical characteristics of the disorder. Construct validity is the conformity to a theoretical rationale for the disorder. Predictive validity is the ability to predict previously unknown aspects of behavior, genetics, and neurobiology of the disorder from the model. Based on the validation criteria of Sarter et al., [18, 19], we propose the validation criteria for genetic animal models of epilepsy syndrome as shown in Table 1.

Specific criteria for genetic animal models of ADNFLE

Criteria for face validity (Table 3)

The clinical features of the main parasomnias (sleepwalking, night terrors and nocturnal enuresis) are well known from the early 1960s [20, 21]. Numerous studies have been performed over the last 30 years on patients with abnormal nocturnal motor and behavioral phenomena; however, these studies have not yet clarified the epileptic or non-epileptic origin of the nocturnal phenomena. In recent years, studies involving video-polysomnographic monitoring have identified a distinct form of clear-cut attacks originating from epileptic foci located in the frontal lobe and emerging almost exclusively during sleep (mainly non-rapid eye movement, NREM sleep II/III) [22-26]. The seizures are characterized by a wide spectrum of clinical features: assumption of postures, rhythmic and repetitive movements of arms and legs, rapid uncoordinated movements with dystonic or dyskinetic components, complex motor activities (deambulation, wandering, and pelvic thrusting), sudden elevation of the trunk and head associated with expression of fear, and vocalization. In a series of 100 consecutive patients with ADNFLE and its sporadic forms

<table>
<thead>
<tr>
<th>Table 1. Validation Criteria for genetic animal model of epilepsy</th>
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<tbody>
<tr>
<td>Face validity: Ability to mimic the characteristics seizure behavioral, electro-clinical (EEG) features, age at onset of epilepsy syndrome (equal to diagnostic criteria)</td>
</tr>
<tr>
<td>Construct validity: Conforms to a theoretical rationale for the epilepsy syndrome, mutant gene expression pattern, promoter function</td>
</tr>
<tr>
<td>Predictive validity: Ability to predict previously unknown aspects of neurobiology, responses to antiepileptic drugs, responses to proconvulsants, complications and/or comorbidity of epilepsy syndrome</td>
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</table>
(NFLE), Provini et al. [27] emphasized the usefulness of clinical distinction of motor manifestations of ADNFLE/NFLE seizures into three subgroups (Table 2). However, all the three types of seizures frequently occur in the same patient [27]. Age at onset of ADNFLE/NFLE varies from 1 to 64 years (mean 14±10 years), although it centers during infancy and adolescence [27].

During wakefulness, the electroencephalograms (EEG) of almost all patients with ADNFLE/NFLE are within the normal limits [27, 28]. Surprisingly, the interictal EEG during sleep is normal [27, 28]. A few ictal EEG recordings display clear-cut epileptic activities (spikes and spikes & waves), but in a few subjects, diffuse (background flattening) or focal EEG activity may be recorded (rhythmic theta or delta activity prominent over the anterior quadrants) [27-29]. Therefore, EEG recording alone is not sufficient to establish a diagnosis of ADNFLE/NFLE; since without simultaneous video and polygraphic recordings of seizures, the lack of EEG epileptic abnormalities does not exclude ADNFLE/NFLE. Taken together with the clinical features of ADNFLE/NFLE, we propose the face validity for genetic animal models of ADNFLE as shown in Table 3.

<table>
<thead>
<tr>
<th>Table 2. Components of ADNFLE seizures.</th>
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<tbody>
<tr>
<td>Nocturnal paroxysmal arousals</td>
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<tr>
<td>Nocturnal paroxysmal dystonia</td>
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<tr>
<td>Episodic nocturnal wandering</td>
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<tr>
<th>Table 3. Face validity for genetic animal model of ADNFLE.</th>
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<tbody>
<tr>
<td>1. The ADNFLE model must exhibit spontaneous epileptic seizures resembling nocturnal paroxysmal arousals, nocturnal paroxysmal dystonia and episodic nocturnal wandering, during NREM sleep.</td>
</tr>
<tr>
<td>2. The interictal EEG is normal.</td>
</tr>
<tr>
<td>3. The foci of ictal or interictal discharge are localized in frontal or over the anterior quadrants.</td>
</tr>
<tr>
<td>4. The age of onset of ADNFLE seizures is around puberty.</td>
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</table>
Criteria for predictive validity (Table 4)

In spite of the diagnostic difficulties, the seizures of a large proportion of ADNFLE/NFLE patients are usually well controlled by carbamazepine (CBZ) [28, 30, 31]. CBZ reduces both the frequency and complexity of nocturnal seizures in more than 60% of patients with ADNFLE/NFLE, including those with S280F and insL mutations [28, 30, 31]. However, some cases (especially, individuals with S284L [32-34], T293I [35], V287M [36] and I312M [37]) are resistant to CBZ but respond to other antiepileptic drugs such as acetazolamide, benzodiazepine (BZP), topiramate and zonisamide (ZNS), or antiepileptic drugs-resistant [28, 30, 32, 33, 36, 38, 39]. (The amino acid numbering of CHRNA4 mutations used here is based on the deduced amino acid sequence of human α4 subunit and accordingly differs from those in the original articles that were based on the Torpedo sequence and nomenclature system).

Comorbidities of autism and mental retardation have been reported in ADNFLE patients with S284L-mutation [33, 38]. Furthermore, comorbidity of schizophrenia-like psychosis has been reported in ADNFLE patients with insL-mutation [40].

Criteria for construct validity (Table 5)

In general, knock-out models are inappropriate genetic animal models of the epilepsy syndrome. However, genetic animal models of ADNFLE bearing mutant genes using knock-in technique are probably appropriate, although the expression pattern and the transgene expression levels in genetic animal models using transgenic technique must be analyzed, since the promoter is not natural.

Table 4. Face validity for genetic animal model of ADNFLE.

1. The genetic animal models with S280F and insL mutations are controlled by CBZ.
2. The genetic animal models with S284L, T293I, V287M and I312M mutations respond only partially to CBZ, but are more susceptible to other antiepileptic drugs (ZNS and BZP).
3. The comorbidity (autism, mental retardation or psychosis) is identified in models with S284L and insL mutations (not indispensability).

Table 5. Construct validity for genetic animal model of ADNFLE

1: The knock-in model is usually appropriate; however, the expression of mutant gene should be determined.
2: The expression pattern and levels of trans-gene of transgenic model is determined
Validities of genetic animal models of ADNFLE

The validities of four genetic animal models of ADNFLE are summarized in Table 6.

**Face validity of ADNFLE models**

The phenotypes of two strains of knock-in mice with S280 mutation are different: one strain exhibits spontaneous epileptic seizures (pS280F-KM), while the other does not exhibit spontaneous epileptic seizures (nS280F-KM) [7, 8]. The spontaneous epileptic seizure of pS280F-KM consists of epileptic wandering like seizure movement observed in wakefulness but not during sleep [8]. The epileptic wandering like seizure is characterized by paroxysmal onset and sudden termination. Although the ictal discharge of pS280F-KM shows complex patterns of spike and wave activity with a high-amplitude and low-frequency power spectrum, the background activities in the EEG of the pS280F-KM show abnormal patterns characterized by marked increase in d-wave activity (0.5–4 Hz) [8]. Contrary to pS280F-KM, no abnormalities are detected in the EEG activities of nS280F-KM [7].

### Table 6. Validity of ADNFLE models

<table>
<thead>
<tr>
<th>Mutant gene</th>
<th>insL</th>
<th>S280F</th>
<th>S280F</th>
<th>S284L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus (variety)</td>
<td>Mouse (C57BL/6J)</td>
<td>Mouse (C57BL/6J)</td>
<td>Mouse (C57BL/6J)</td>
<td>Rat (SD)</td>
</tr>
<tr>
<td>Technology</td>
<td>Knock-in</td>
<td>Knock-in</td>
<td>Knock-in</td>
<td>Transgenic</td>
</tr>
<tr>
<td><strong>Face validity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous seizure</td>
<td>Epileptic wandering during W</td>
<td>Epileptic wandering during W</td>
<td>No seizure</td>
<td>ADNFLE seizure during SWS</td>
</tr>
<tr>
<td>Background EEG</td>
<td>slow wave increase</td>
<td>slow wave increase</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td><strong>Construct validity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRNA</td>
<td>Equal expression (whole brain)</td>
<td>Equal expression (Cor, Hip, Th)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein</td>
<td></td>
<td></td>
<td>Equal expression (Cor, Hip, Th)</td>
<td></td>
</tr>
<tr>
<td><strong>Predictive validity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AED response</td>
<td></td>
<td>Suppressed by CBZ</td>
<td>Suppressed by ZNS</td>
<td></td>
</tr>
<tr>
<td>Nicotine-induced seizure</td>
<td>Potentiation generalized seizures</td>
<td>Potentiation generalized seizures</td>
<td>No difference</td>
<td>Potentiation partial seizures</td>
</tr>
<tr>
<td>W: wakefulness</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
The phenotypic features of knock-in mouse with insL (insL-KM) have been analyzed. The insL-KM exhibits epileptic wandering like spontaneous epileptic discharges with paroxysmal onset and sudden termination during wakefulness [8]. The EEG features of insL-KM are similar to pS280F-KM. The ictal discharge of insL-KM shows complex patterns of spike and wave activity with high-amplitude and low-frequency power spectrum. The EEG of insL-KM during spontaneous seizure shows a more asymmetric and diffuse pattern than that of pS280F-KM. The background activities in EEG of insL-KM show also marked increase in d-wave activity [8].

The transgenic rat with S284L mutation (S284L-TG) exhibits the three distinct ADNFLE seizures during NREM sleep: paroxysmal arousals (brief episodes characterized by sudden frightened expression), paroxysmal dystonia (brief episodes of dystonic posturing), and epileptic wandering (episodes of longer duration ranging from 1 to 3 min with head shaking accompanied by stereotyped paroxysmal ambulation and bizarre movements) [9]. The onset of ictal discharges is synchronized with seizure behaviors. No abnormalities of background activities are observed in EEG of S284L-TG. The epileptogenic focus of both ictal and interictal discharges is the frontal sensori-motor cortex region. In general, the onset of interictal discharges is 6 weeks of age; however, the onset of spontaneous ADNFLE seizure is preceded by onset of interictal discharge in the same transgenic rat. At 8 weeks of age, 90% of S284L-TG exhibit spontaneous seizures during NREM sleep. These characteristics of epileptic seizures of S284L transgenic rats are quite similar to those of ADNFLE patients with S284L mutation [9].

The published phenotypic features of the four genetic animal models of ADNFLE show that the face validity for S284L-TG is adequate, whereas the pS280F-KM, nS280F-KM and insL-KM are unsuitable genetic animal models of ADNFLE.

**Predictive validity of ADNFLE models**

Although the functional abnormalities of both mutant nAChR with S280F and insL are loss-of-function (enhanced steady-state desensitization) with gain-of-function (enhanced ACh sensitivity and use-dependent potentiation) [41-43], both knock-in mice; pS280F-KM and insL-KM, are considerably more sensitive to nicotine-induced seizures than wild-type mice. Indeed, in nicotine-induced seizure tests, these two types of knock-in mice show a lower threshold dose of nicotine for generalized seizures, shorter latencies to seizure onset, and longer seizure durations compared with their wild-type littermates [8].

The sensitivity to nicotine-induced seizures has also been studied in another knock-in mouse model with S280F (nS280F-KM), which does not exhibit spontaneous epileptic seizures. The nS280F-KM does not display particularly different response in nicotine-induced seizure test compared with wild-type. However, they develop ADNFLE-like epileptic wandering following administration of 1 mg/kg nicotine [7], and these seizures are prevented by supratherapeutic doses of CBZ.
The sensitivity of S284L-TG to nicotine-induced seizures has also been studied. Surprisingly, there is no difference in latency of nicotine-induced seizure between S284L-TG and non-TG littermate. Nicotine-induced seizures in S284L-TG are mainly partial seizures, whereas those in non-TG littermates are generalized seizures. In S284L-TG, sub-chronic administration (two weeks) of diazepam and ZNS at therapeutically relevant doses reduces the frequency of interictal discharge by 43 and 48%, respectively, whereas CBZ at therapeutically relevant doses has no effect on the frequency of interictal discharge [9].

The results of the nicotine-induced seizure test show different responses among the four genetic animal models of ADNFLE. Some develop ADNFLE-like seizures (nS280F-KM and S284L-TG), epileptic wandering (nS280F-KM and S284L-TG), paroxysmal arousal (S284L-TG) and paroxysmal dystonia (S284L-TG) in response to nicotine administration. Thus, the predictive validity is proven for S284L-TG, but not established for nS280F-KM, pS280F-KM or insL-KM.

Construct validity of ADNFLE models

The promoter of S284L-TG is not a natural promoter but a PDGF-b promoter [9]. The expression of Chrna4 in S284L-TG has been determined using in situ hybridization, real-time PCR and immunohistochemistry. In situ hybridization using a nonselective probe (sensitive to both wild-type and S284L mutant Chrna4 mRNA) showed no differences in the cerebral expression of Chrna4 mRNA between non-TG and S284L-TG. The total amount of Chrna4 mRNA (wild-type plus S284L Chrna4) in the frontal cortex of S284L-TG was almost equal to that in non-TG. The expression of wild-type versus S284L Chrna4 was 45% versus 55%. In the focus region, there was no significant difference in the number of nAChR a4-immunopositive neurons between non-TG and S284L-TG. No distorted expression of wild-type or S284L Chrna4 was observed in the cell populations of neurons, astrocytes and oligodendrocytes. In spite of these findings, the wild-type Chrna4 mRNA is predominantly expressed in the thalamus and cortex, whereas the S284L mutant Chrna4 mRNA is mainly expressed in the cortex and thalamus. Laser-capture microdissection with single-cell reverse-transcription quantitative PCR has demonstrated the lack of ectopic expression of the transgene in neurons and glial cells.

There is little or no information on the expression of mutant Chrna4 gene or nAChR a4-subunit protein in pS280F-KM, nS280F-KM and insL-KM [7, 8]. Although the promoter in knock-in technique is a natural promoter, the mRNA and protein expression levels should be determined. Why does the pS280F-KM but not the nS280F-KM exhibit spontaneous epileptic seizure? To answer this important question, the expression of S280F mutant Chrna4 should be determined.

Thus, we can conclude that there are no suitable ADNFLE genetic animal models according to the construct validity. To study the pathogenesis and pathophysiology of ADNFLE, knock-in mice with established
construct validity or transgenic animal model with a natural promoter should be generated.

**Conclusion**

During this decade, various mutant genes that encode ion channels have been identified in pedigrees of idiopathic epilepsy. Several genetic animal models bearing mutant genes identified in the pedigrees of idiopathic epilepsy have also been generated. However, such genetic animal models are not adequate. Therefore, there is a need to generate new genetic animal models for epilepsy that are based on our proposed validation criteria for epilepsy.

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