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**—Review—**

# Advances on genetic rat models of epilepsy

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**Abstract:** Considering the suitability of laboratory rats in epilepsy research, we and other groups have been developing genetic models of epilepsy in this species. After epileptic rats or seizure-susceptible rats were sporadically found in outbred stocks, the epileptic traits were usually genetically-fixed by selective breeding. So far, the absence seizure models GAERS and WAG/Rij, audiogenic seizure models GEPR-3 and GEPR-9, generalized tonic-clonic seizure models IER, NER and WER, and Canavan-disease related epileptic models TRM and SER have been established. Dissection of the genetic bases including causative genes in these epileptic rat models would be a significant step toward understanding epileptogenesis. *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis provides a systematic approach which allowed us to develop two novel epileptic rat models: heat-induced seizure susceptible (Hiss) rats with an *Scn1a* missense mutation and autosomal dominant lateral temporal epilepsy (ADLTE) model rats with an *Lgi1* missense mutation. In addition, we have established episodic ataxia type 1 (EA1) model rats with a *Kcna1* missense mutation derived from the ENU-induced rat mutant stock, and identified a *Cacna1a* missense mutation in a *N*-Methyl-*N*-nitrosourea (MNU)-induced mutant rat strain GRY, resulting in the discovery of episodic ataxia type 2 (EA2) model rats. Thus, epileptic rat models have been established on the two paths: 'phenotype to gene' and 'gene to phenotype'. In the near future, development of novel epileptic rat models will be extensively promoted by the use of sophisticated genome editing technologies.

**Key words:** antiepileptic drug (AED), ENU mutagenesis, epileptic rat model, epileptogenesis, spontaneous mutation

## Introduction

Epilepsy is a brain disorder affecting approximately 1% of the worldwide population. The etiology of epilepsy would be fundamentally divided into two categories: idiopathic and symptomatic. Pre-clinical studies using animal models play a very important role in the evaluation of new antiepileptic drugs (AEDs). Simple models of acute seizures, such as the maximal electroshock seizure test and the subcutaneous pentylenetetrazole seizure test in mice and rats, were regarded to be the standard procedures for predicting the clinical anticonvulsant activity of drug candidates in former years. Next, kindling models and genetic models in the species were added to overcome the serious problems such as false-positive and false-negative evaluations of the drug candidates. Certainly, with the appropriate medication, seizures in the majority of patients can be well controlled. However, current medications still fail to control seizures in 20–30% of patients [63]. Uncontrollable seizures have a significant negative impact on patients' quality of life, and can also interfere with memory and

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cognitive function. Positional cloning strategies in multigenerational families of Mendelian idiopathic epilepsies have successfully revealed gene mutations [15]. Since the introduction of the next generation sequencing, identification of causal gene mutations in epileptic patients has been speedily processed by using exome sequencing and dense microarray based comparative genomic hybridization (array CGH). Eighty-one representative epileptic syndromes and familial seizures, in which causal mutations were confirmed, are listed in the Online Mendelian Inheritance in Man (OMIM) (see Table 1).

Two keywords, epileptogenesis and epilepsy biomarkers, are regarded as the essential clues for overcoming epilepsy. Epileptogenesis is defined as the gradual process by which epilepsy develops in normal brain following initial brain insults or gene mutations. Numerous clinical and preclinical studies demonstrate that therapeutic intervention to suppress epileptogenesis is an attractive new strategy for prevention and treatment of epilepsy. The biomarker subgroup for the London Workshop of the International League Against Epilepsy (ILAE) defined 'epilepsy biomarker' as summarized in Table 2 [22].

Genetic epileptic models have been reported in mice, gerbils, rats, dogs, baboons and chickens [61]. If epileptic animals or seizure-susceptible animals were found in stocks, the epileptic traits were usually genetically-fixed by selective breeding. So far, genetically established mice and rat strains have been preferentially used for epilepsy research [24].

The laboratory rats (*Rattus norvegicus*) has a suitable size for behavior studies, surgical manipulations, multichannel electroencephalographic recordings, separate brain tissue sampling, continuous blood sampling or body fluid monitoring. With efforts of the rat genetic and genomic research community [1], highly polymorphic genetic markers, microsatellite markers [97], and single nucleotide polymorphism (SNP) markers [91], genetic linkage maps [97, 122], and genome sequences of more than 27 inbred rat strains have been prepared [11]. More than 650 genetically defined rat strains, including epileptic rats, are now available from the National BioResource Project − Rat (NBRP-Rat) in Japan [98].

Once the causal gene mutations were identified in epileptic patients, experimental studies about functional changes were conducted *in vitro*, *ex vivo* or *in vivo* using mutated cells and/or mutant mice or rats. A zebra fish model for Dravet syndrome (or severe myoclonic epilepsy of infancy (SMEI)) was also developed for screening of novel AEDs [12]. Genome-editing technology has been rapidly improved in most laboratory animals including rats. Considering the future research aims, such as for understanding ictogenesis, epileptogenesis and discovery of epilepsy biomarkers, we expect that rats will become a more useful species in the epilepsy research. Existing epileptic rat models, which were developed by two paths 'phenotype to gene' and 'gene to phenotype' (Fig. 1), are summarized in Table 3.

In the present review, causal mutations and genetic bases of each epileptic rat strain are mainly described, and future prospects on rat models for epilepsy research are added in the last paragraph. A list of abbreviations of antiepileptic drugs and epilepsy related words in this review is presented in Table 4.

## Audiogenic Seizure Models: GEPR-3 and GEPR-9

According to the report of Griffiths, it was already known around 1940 that loud sounds of high frequency have a profound effect on the behavior of laboratory rats [29]. The sounds produce in the rats a high state of excitement characterized by wild undirected running and culminating in a convulsive seizure. In all instances the rats were derived from common strains which have been bred and raised under laboratory conditions for many generations

*GEPR-3 and GEPR-9*

Dailey and Jobe established two colonies of genetically epilepsy-prone rats (GEPR) [18]. Virtually all of the animals in the first colony experience a wild running fit that terminates in a generalized clonic convulsion when they are stimulated by sound. According to their convulsion intensity scoring system, these animals have audiogenic response score (ARS) of 3 and the colony was designated the GEPR-3 colony. In the second colony, more than 95% of the rats experience a wild running phase terminating in a tonic extensor convulsion by sound stimulation. The rats have an ARS of 9 and the colony was designated the GEPR-9 colony. The GEPR-3 and GEPR-9 rats both show incomplete penetrance and variable expressivity of the underlying genetic predisposition [60]. Therefore, genetic analysis of quantitative trait loci (QTL) for seizures has not been reported yet.

**Table 1.** A list of 81 human epilepsy syndromes and familiar febrile seizuers confirmed causal gene mutations

| OMIM-I.D. | Epielpsy syndrome or familial seizures | Human Cytoband | Gene symbol |
|---|---|---|---|
| # 609056 | AMISH INFANTILE EPILEPSY SYNDROME | 2p11.2 | *SIAT9* |
| # 610003 | CEROID LIPOFUSCINOSIS, NEURONAL, 8, NORTHERN EPILEPSY VARIANT | 8p23.3 | *CLN8* |
| # 610042 | CORTICAL DYSPLASIA-FOCAL EPILEPSY SYNDROME | 7q35–q36 | *CNTNAP2* |
| # 121200 | EPILEPSY, BENIGN NEONATAL, 1, AND/OR MYOKYMIA | 20q13.33 | *KCNQ2* |
| # 607681 | EPILEPSY, CHILDHOOD ABSENCE, SUSCEPTIBILITY TO, 2; ECA2 | 5q34 | *GABRG2* |
| # 612269 | EPILEPSY, CHILDHOOD ABSENCE, SUSCEPTIBILITY TO, 5; ECA5 | 15q12 | *GABRB3* |
| # 611942 | EPILEPSY, CHILDHOOD ABSENCE, SUSCEPTIBILITY TO, 6; ECA6 | 16p13.3 | *CACNA1H* |
| # 615400 | EPILEPSY, FAMILIAL ADULT MYOCLONIC, 5; FAME5 | 1q32.1 | *CNTN2* |
| # 604364 | EPILEPSY, FAMILIAL FOCAL, WITH VARIABLE FOCI; FFEVF | 22q12.2–q12.3 | *DEPDC5* |
| # 600512 | EPILEPSY, FAMILIAL TEMPORAL LOBE, 1; ETL1 | 10q23.33 | *LGI1* |
| # 614417 | EPILEPSY, FAMILIAL TEMPORAL LOBE, 5; ETL5 | 8q13.2 | *CPA6* |
| # 245570 | EPILEPSY, FOCAL, WITH SPEECH DISORDER AND WITH OR WITHOUT MENTAL RETARDATION; FESD | 16p13.2 | *GRIN2A* |
| # 613060 | EPILEPSY, IDIOPATHIC GENERALIZED, SUSCEPTIBILITY TO, 10; EIG10 | 1p36.33 | *GABRD* |
| # 607628 | EPILEPSY, IDIOPATHIC GENERALIZED, SUSCEPTIBILITY TO, 11; EIG11 | 3q27.1 | *CLCN2* |
| # 614847 | EPILEPSY, IDIOPATHIC GENERALIZED, SUSCEPTIBILITY TO, 12; EIG12 | 1p34.2 | *SLC2A1* |
| # 611136 | EPILEPSY, IDIOPATHIC GENERALIZED, SUSCEPTIBILITY TO, 13; EIG13 | 5q34 | *GABRA1* |
| # 612899 | EPILEPSY, IDIOPATHIC GENERALIZED, SUSCEPTIBILITY TO, 8; EIG8 | 3q21.1 | *CASR* |
| # 607682 | EPILEPSY, IDIOPATHIC GENERALIZED, SUSCEPTIBILITY TO, 9; EIG9 | 2q23.3 | *CACNB4* |
| # 607631 | EPILEPSY, JUVENILE ABSENCE, SUSCEPTIBILITY TO, 1; EJA1 | 6p12.2 | *EFHC1* |
| # 611136 | EPILEPSY, JUVENILE MYOCLONIC, SUSCEPTIBILITY TO, 5 | 5q34 | *GABRA1* |
| # 607682 | EPILEPSY, JUVENILE MYOCLONIC, SUSCEPTIBILITY TO, 6 | 2q23.3 | *CACNB4* |
| # 607628 | EPILEPSY, JUVENILE MYOCLONIC, SUSCEPTIBILITY TO, 8 | | *CLCN2* |
| # 254770 | EPILEPSY, MYOCLONIC JUVENILE; EJM | 6p12.2 | *EFHC1* |
| # 600513 | EPILEPSY, NOCTURNAL FRONTAL LOBE, 1; ENFL1 | 20q13.33 | *CHRNA4* |
| # 605375 | EPILEPSY, NOCTURNAL FRONTAL LOBE, 3; ENFL3 | 1q21.3 | *CHRNB2* |
| # 610353 | EPILEPSY, NOCTURNAL FRONTAL LOBE, 4; ENFL4 | 8p21.2 | *CHRNA2* |
| # 615005 | EPILEPSY, NOCTURNAL FRONTAL LOBE, 5; ENFL5 | 9q34.3 | *KCNT1* |
| # 612437 | EPILEPSY, PROGRESSIVE MYOCLONIC 1B; EPM1B | 12q12 | *PRICKLE1* |
| # 254780 | EPILEPSY, PROGRESSIVE MYOCLONIC 2A (LAFORA) | 6q24.3 | *EPM2A* |
| # 254780 | EPILEPSY, PROGRESSIVE MYOCLONIC 2B (LAFORA) | 6p22.3 | *NHLRC1* |
| # 611726 | EPILEPSY, PROGRESSIVE MYOCLONIC 3, WITH OR WITHOUT INTRACELLULAR INCLUSIONS; EPM3 | 7q11.21 | *KCTD7* |
| # 254900 | EPILEPSY, PROGRESSIVE MYOCLONIC 4, WITH OR WITHOUT RENAL FAILURE; EPM4 | 4q21.1 | *SCARB2* |
| # 613832 | EPILEPSY, PROGRESSIVE MYOCLONIC 5; EPM5 | 3p14.1 | *PRICKLE2* |
| # 614018 | EPILEPSY, PROGRESSIVE MYOCLONIC 6; EPM6 | 17q21.32 | *GOSR2* |
| # 266100 | EPILEPSY, PYRIDOXINE-DEPENDENT; EPD | 5q23.2 | *ALDH7A1* |
| # 300491 | EPILEPSY, X-LINKED, WITH VARIABLE LEARNING DISABILITIES AND BEHAVIOR DISORDERS | Xp11.23 | *SYN1* |
| # 615369 | EPILEPTIC ENCEPHALOPATHY, CHILDHOOD-ONSET; EEOC | 15q26.1 | *CHD2* |
| # 308350 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 1; EIEE1 | Xp21.3 | *ARX* |
| # 613402 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 10; EIEE10 | 19q13.33 | *PNKP* |
| # 613721 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 11; EIEE11 | 2q24.3 | *SCN2A* |
| # 613722 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 12; EIEE12 | 20p12.3 | *PLCB1* |
| # 614558 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 13; EIEE13 | 12q13.13 | *SCN8A* |
| # 614959 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 14; EIEE14 | 9q34.3 | *KCNT1* |
| # 615006 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 15; EIEE15 | 1p34.1 | *ST3GAL3* |
| # 615338 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 16; EIEE16 | 16p13.3 | *TBC1D24* |
| # 615473 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 17; EIEE17 | 16q12.2 | *GNAO1* |
| # 615476 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 18; EIEE18 | 1p34.2 | *SZT2* |
| # 615744 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 19; EIEE19 | 5q34 | *GABRA1* |
| # 300672 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 2; EIEE2 | Xp22.13 | *CDKL5* |
| # 615859 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 23; EIEE23 | 1p31.3 | *DOCK7* |
| # 609304 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 3; EIEE3 | 11p15.5 | *SLC25A22* |
| # 612164 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 4; EIEE4 | 9q34.11 | *STXBP1* |
| # 613477 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 5; EIEE5 | 9q34.11 | *SPTAN1* |
| # 607208 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 6; EIEE6 | 2q24.3 | *SCN1A* |
| # 607208 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 6; EIEE6 | 2q24.3 | *SCN9A* |
| # 613720 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 7; EIEE7 | 20q13.33 | *KCNQ2* |
| # 300607 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 8; EIEE8 | Xq11.1–q11.2 | *ARHGEF9* |
| # 300088 | EPILEPTIC ENCEPHALOPATHY, EARLY INFANTILE, 9; EIEE9 | Xq22.1 | *PCDH19* |
| # 614418 | FEBRILE SEIZURES, FAMILIAL, 11; FEB11 | 8q13.2 | *CPA6* |
| # 613863 | FEBRILE SEIZURES, FAMILIAL, 3B | 2q24.3 | *SCN9A* |
| # 604352 | FEBRILE SEIZURES, FAMILIAL, 4; FEB4 | 5q14.3 | *GPR98* |
| # 609446 | GENERALIZED EPILEPSY AND PAROXYSMAL DYSKINESIA; GEPD | 10q22.3 | *KCNMA1* |
| # 604233 | GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS, TYPE 1; GEFSP1 | 19q13.12 | *SCN1B* |
| # 604403 | GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS, TYPE 2; GEFSP2 | 2q24.3 | *SCN1A* |
| # 611277 | GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS, TYPE 3; GEFSP3 | 5q34 | *GABRG2* |
| # 613060 | GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS, TYPE 5, GEFSP5 | 1p36.33 | *GABRD* |
| # 613863 | GENERALIZED EPILEPSY WITH FEBRILE SEIZURES PLUS, TYPE 7; GEFSP7 | 2q24.3 | *SCN9A* |
| # 300419 | MENTAL RETARDATION, X-LINKED, WITH OR WITHOUT SEIZURES, ARX-RELATED; MRXARX | Xp21.3 | *ARX* |
| # 614231 | MICROCEPHALY, EPILEPSY, AND DIABETES SYNDROME; MEDS | 18q21.1 | *IER3IP1* |
| # 254800 | MYOCLONIC EPILEPSY OF UNVERRICHT AND LUNDBORG | 21q22.3 | *CSTB* |
| # 605021 | MYOCLONIC EPILEPSY, FAMILIAL INFANTILE; FIME | 16p13.3 | *TBC1D24* |
| # 254770 | MYOCLONIC EPILEPSY, JUVENILE, SUSCEPTIBILITY TO, 1, INCLUDED | 6p12.2 | *EFHC1* |
| # 611087 | POLYHYDRAMNIOS, MEGALENCEPHALY, AND SYMPTOMATIC EPILEPSY; PMSE | 17q23.3 | *STRADA* |
| # 614501 | PSYCHOMOTOR RETARDATION, EPILEPSY, AND CRANIOFACIAL DYSMORPHISM; PMRED | 1p34.3 | *SNIP1* |
| # 300643 | ROLANDIC EPILEPSY, MENTAL RETARDATION, AND SPEECH DYSPRAXIA, X-LINKED; RESDX | Xq22.1 | *SRPX2* |
| # 605751 | SEIZURES, BENIGN FAMILIAL INFANTILE, 2; BFIS2 | 16p11.2 | *PRRT2* |
| # 607745 | SEIZURES, BENIGN FAMILIAL INFANTILE, 3; BFIS3 | 2q24.3 | *SCN2A* |
| # 121200 | SEIZURES, BENIGN FAMILIAL NEONATAL, 1; BFNS1 | 20q13.33 | *KCNQ2* |
| # 121201 | SEIZURES, BENIGN FAMILIAL NEONATAL, 2; BFNS2 | 8q24.22 | *KCNQ3* |
| # 612780 | SEIZURES, SENSORINEURAL DEAFNESS, ATAXIA, MENTAL RETARDATION, AND ELECTROLYTE IMBALANCE; SESAMES | 1q23.2 | *KCNJ10* |
| # 159950 | SPINAL MUSCULAR ATROPHY WITH PROGRESSIVE MYOCLONIC EPILEPSY; SMAPME | 8p22 | *ASAH1* |

This list only displays the epileptic diseases that contain "epilepsy" or "febrile seizures" in its name. Data extaction from the online Mendelian inheritance in man (OMIM) was done on 8 July 2014.

**Table 2.** Values of epilepsy biomarkers in epileptogenesis and ictogenesis

1. to predict the development of an epilepsy condition
2. to identify the presence of tissue capable of generating spontaneous seizures
3. to measure progression after the condition is established
4. to be used to create animal models for more cost-efficient screening of potential antiepileptogenic and antiseizure drugs and devices
5. to reduce the cost of clinical trials of potential antieipleptogenic interventions by enriching the trial population with patients at high risk for developing epilepsy

Quotation from the report of the biomarker subgroup in ILAE London Workshop [22]; Ictogenesis, The process by which the brain develops seizures; Epileptogenesis, The process by which the brain develops epilepsy.

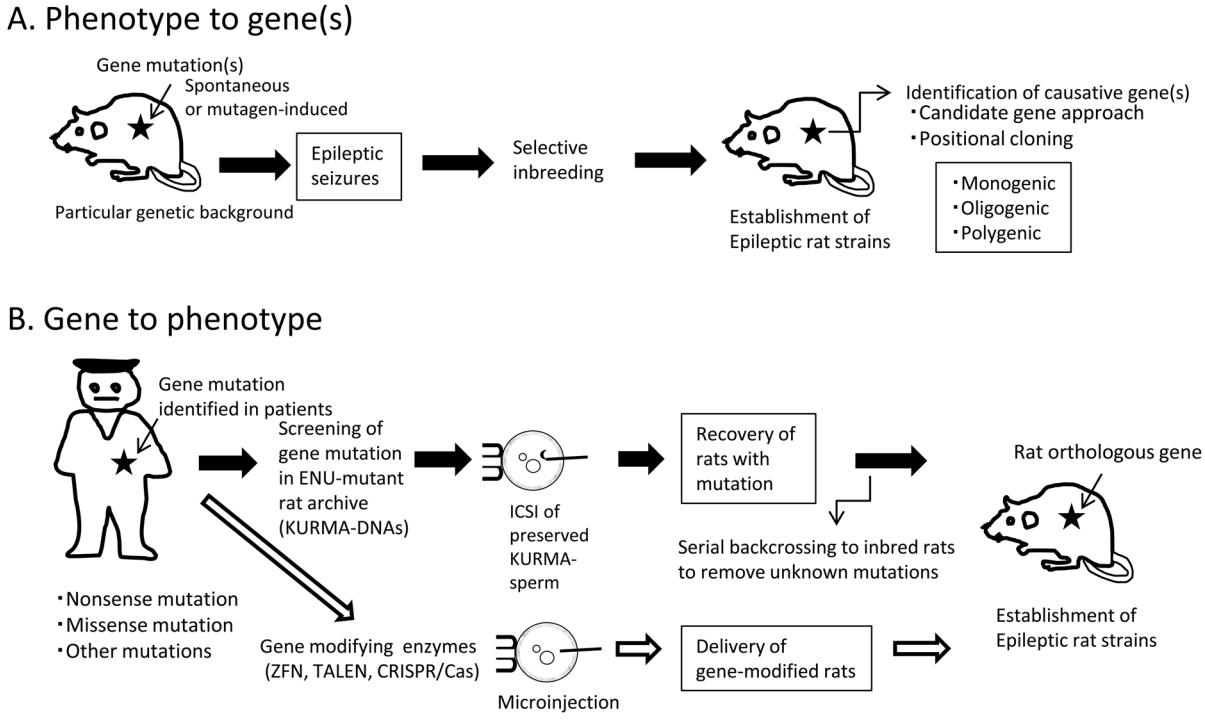


**Fig. 1.** Two paths used to develop epileptic rat strains. KURMA, Kyoto University Rat Mutant Archive; ICSI, intra cytoplasmic sperm injection; Black-filled arrows, showing the process used in the development of the epileptic rats listed in Table 3; opened arrows, showing the process which will be used in the near future.

Faingold *et al*. summarize that susceptibility to the audiogenic seizures (AGS) is associated with the "two hit hypothesis" of genetic predisposition plus environmental insult to the brain, as proposed for human epilepsy. In GEPR-9s the first hit is a genetic defect in GABA-mediated inhibition, and the second hit is an intense acoustic stimulus, which initiates AGS. The neuronal network for AGS in GEPR-9s is confined exclusively to the brain stem, including the lower brainstem primary auditory nuclei, but forebrain structures are not required for AGS [23].

## Absence Seizures Models: GAERS and WAG/Rij

In 1977, Robinson and Gilmore originally reported spontaneous occurrence of generalized epileptiform discharge in the electroencephalograms (EEG) of some male Charles River CD albino rats. These discharges, 6-10Hz spike-and-wave discharges (SWD), occurred during periods of quite wakefulness and were accompanied by locomotors arrest and often by mild clonic facial movement. Subsequently, it has become apparent that these phenomena are not restricted to the subpopulation.

**Table 3.** Genetic rat models of epilepsy

| Strains | Seizure types/models | Causative gene mutations | Spontaneous or induced mutations | Major pathological findings in CNS |
|---|---|---|---|---|
| (A) Established by inbreeding combined with selection | | | | |
| GAERS/Mave | Absense | Polygenes, *Cacna1h*, R1584P | Spontaneous | None |
| WAG/Rij | Absense | Polygenic | Spontaneous | None |
| WER | Absence, Tonic-clonic | Unknown | Spontaneous | None |
| IER/Ihr | Tonic-clonic | Chr8, Chr15 for cataract | Spontaneous | Neuronal microdysgenesis in hipocamopus |
| NER/Kyo | Tonic-clonic | Oligogenic | Spontaneous | None |
| SER/Kyo | Absense, Tonic, Tonic-clonic | *tm* (a genomic deletion*), *zi* (mutated *Atrn*) | Spontaneous crossing of two mutants | Spongiform degeneration |
| TRM/Kyo | Absense | *tm* | Spontaneous | Spongiform degeneration |
| GEPR-3, GEPR-9 | Audiogenic | Unknown | Spontaneous | None |
| (B) Generated by chemical mutagenesis | | | | |
| • Phenotype-driven | | | | |
| GRY/Idr | EA2 | *Cacna1a*, M251K | MNU | Reduction in size of the cerebellum |
| F344-*Adms*/Kyo | EA1 (ADMS rat) | *Kcna1*, T925A | ENU | None |
| • Gene-driven | | | | |
| F344-*Lgi1*$^{m1Kyo}$ | ADLTE | *Lgi1*, L385R | ENU | None |
| F344-*Scn1a*$^{m1Kyo}$ | Febrile seizures (Hiss rat) | *Scn1a*, N1417H | ENU | None |

*The mutant gene *tm* is a genomic deletion spanning approximately 240kb, including at least *Shpk* (partial), *Trpv1*, *Trpv3*, *Aspa*, *Spata3*, and 7 *Olr* genes.

**Table 4.** Abbreviations of antiepileptic drugs and epilepsy related words in this review

| Antiepileptic drugs | Epilepsy related words |
|---|---|
| CBZ, carbamazepin | ADLTE, autosomal dominant lateral temporal epilepsy |
| CLB, clobazam | AEDs, antiepileptic drugs |
| CZP, clonazepam | AGS, audiogenic seizures |
| DZP, diazepam | EA1, episodic ataxia type 1 |
| ESM, ethosuximide | EA2, episodic ataxia type 2 |
| LTG, lamotrigine | GTCS, generalized tonic clonic seizures |
| LEV, levetiracetam | ILAE, The International League Against Epilepsy |
| PB, phenobarbital | SMEI, severe myoclonic epilepsy of infancy |
| PHT, phenytoin | SWD, spike-and-wave discharge |
| TMO, trimethadione | TLE, human temporal lobe epilepsy |
| ZNS, zonisamide | |
| VPA, valproate | |

Identical abnormalities have been also found in tracings Wistar and Lewis rats [88].

*GAERS*

While working on pharmacological models of experimental epilepsy in rats, Vergnes *et al*. observed spontaneously occurring recurrent electroclinical paroxysmal phenomena in some naive animals. They conclude that the reported phenomenon demonstrates similarities with other experimental models of human petit mal epilepsy and could become valid for further research [119]. Marescaux *et al*. have selected a strain of rats and designated it the Genetic Absence Epilepsy Rat from Strasbourg (GAERS). In this strain, 100% of the animals present recurrent generalized non-convulsive seizures characterized by bilateral and synchronous SWD accompanied with behavioral arrest, staring and sometimes twitching of the vibrissae. Usually, spontaneous 7–11 Hz SWD start and end abruptly on a normal background EEG, during a state of quiet wakefulness [66]. The variable expression of SWD in offspring from GAERS × control reciprocal crosses may be due to the existence

of multiple genes. Using $F_2$ progenies derived from GAERS and BN rats, Rudolf *et al*. identified QTLs influencing SWD intermediate phenotypes in rat chromosome (Chr) 4, 7, and 8 [89]. Surprisingly, a homozygous, missense, single nucleotide (G to C) mutation in the $Ca_v3.2$ T-type $Ca^{2+}$ channel gene has been reported in GAERS, although the gene *Cacna1h* is located in rat Chr 10q12. The GAERS $Ca_v3.2$ mutation produces an arginine to proline (R1584P) substitution in exon 24 of *Cacna1h*, encoding a portion of the III–IV linker region in $Ca_v3.2$. This mutation segregates codominantly with the number of seizures and time in seizure activity in progeny of an $F_1$ intercross. Two major thalamic *Cacna1h* splice variants were also identified, either with or without exon 25. The mutation introduced into the splice variants acts "epistatically," requiring the presence of exon 25 to produce significantly faster recovery from channel inactivation and greater charge transference during high-frequency bursts [86].

*WAG/Rij*

While studying the sleep-wake characteristics of WAG/Rij, van Luijtelaar and Coenen discovered two types of aberrant discharges, SWD type-1 (7.5–9.5 Hz, polarity of spikes upward, duration of 3–4 sec) and SWD type-2 (8 Hz, polarity of spikes downward, duration of ~1 sec), spontaneously occurring in the cortical EEG [116]. In the parental strains, WAG/Rij and ACI (a control rat strain with no signs of epilepsy), reciprocal WAG/Rij × ACI $F_1$ hybrids, $F_2$, $B_1$, and $B_2$ generations, the number and duration of SWD were determined. One hundred percent of the $F_1$ animals showed SWD, while the percentages for the $F_2$, $B_1$, and $B_2$ generations were 79, 95, and 37%, respectively. The results suggest that one dominant gene determines the occurrence, while others manipulate the number and duration of epileptic phenomena [84]. Gauguier *et al*. studied the genetic basis of the EEG properties of these SWDs using (WAG/Rij × ACI) $F_2$ rats, and identified two QTLs, *T1swd/wag* for controlling the average duration of SWD type-1 in rat Chr 5 and *T2swd/wag* for the total duration of SWD type-2 in Chr 9, respectively [27].

Hyperpolarization-activated, cyclic nucleotide–gated (HCN) channels contribute to cationic $I_h$ current in neurons and regulate the excitability of neuronal networks. Nava *et al*. recently found *de novo HCN1* point mutations in individuals who had clinical features resembling those of the Dravet syndrome with progression toward atypical absences, intellectual disability and autistic traits [77]. In WAG/Rij, the HCN1 channel down-regulation occurred temporally before the development onset of SWD in the dendrites of cortical L5 neurons and at a cellular level, HCN1 played a direct role in promoting dendritic $Ca^{2+}$ electrogenesis and burst firing [57]. Genetic factors involved in HCN1 down-regulation in WAG/Rij have not been elucidated yet.

*GAERS and WAG/Rij*

Akman *et al.* reported that WAG/Rij has significant differences compared with GAERS in the number, mean duration, cumulative duration and frequency of SWD and in the spectral characteristics of the pre-SWD [3]. Increased $Ca_v3.2$ expression and increased T-type currents were detected in GAERS as well as WAG/Rij [86], although primal gene mutations in WAG/Rij have not been clarified yet.

Neurophysiologic and signal analytical studies in WAG/Rij and GAERS demonstrated that the SWD originated from the deep layers of the somatosensory cortex quickly spread over the cortex and invade the thalamus, and that the reticular thalamic nucleus and other thalamic nuclei provide a resonance circuitry for the amplification, spreading and entrainment of SWD [117].

Ethosuximide (ESM) is a first-line clinical symptomatic treatment for absence seizures. Russo *et al*. have demonstrated that chronic treatment with ESM as well as levetiracetam (LEV), a broad-spectrum AED with antiabsence and antiepileptogenic properties, when initiated prior to the onset of the epilepsy, has antiepileptogenic effects in WAG/Rij [90], and Dezsi *et al*. also reported that ESM reduced epileptogenesis and behavioral comorbidity, such as anxiety-like behavior, in GAERS [19]. Recently, van Luijtelaar and Zobeiri review the progress and outlooks in WAG/Rij as well as GAERS, as the two best characterized genetic animal models for absence epilepsy [118].

## Generalized Tonic-Clonic Seizure Models: IER, NER and WER

*IER*

Ihara found that some of Ihara's cataract rats showed GTCS in the form of major attacks. From the original mating between a male rat with severe seizures and its female littermates, selective inbreeding was repeated for

more than 30 generations. Spontaneous epileptic seizures, including generalized convulsions, occur in almost all of the male rats, without any external stimuli, by approximately 5 months after birth [5]. Linkage analysis indicated that *Catil* on Chr 8 would be involved in the occurrence of cataract, and *Cati2* on Chr 15 in the timing of onset of the cataract, respectively [126]. In every IER examined, there were invariable and fundamental neuropathological findings consisting of abnormal neuronal clusters in the CA1 of the hippocampal formation. Moreover, disarrangement of neuronal cells, such as dispersion and gaps in lamination of pyramidal neurons, were observed. These changes were thought to represent genetically programmed lesions, neuronal microdysgenesis, because they were common findings in 2-month-old with neither abnormal behaviors nor any seizure activity. The neuronal microdysgenesis in the hippocampal formation of IER was considered to have an intimate relationship with epileptogenesis and/or an enhancement of seizure susceptibility [113]. Arai *et al*. reported that the pattern of up- and down regulation of a variety of genes in the hippocampus of IER in comparison to control Wistar rats [9]. However, the gene mutation which is involved to the genetically programmed lesions has not been elucidated yet.

Miura *et al*. reported the results of chronic 5-day treatment with clobazam (CLB), clonazepam (CZP), and zonisamide (ZNS) in IERs. Although both CLB and ZNS reduced the daily and total incidence of GTCS during treatment, CZP could not prevent the appearance of GTCS at all. Clinical features of the AEDs against human temporal lobe epilepsy (TLE) were fitted to their efficacies against GTCS in IERs, supporting the predictability of IERs for the novel drugs against refractory human limbic seizures in TLE [72].

*NER*

Noda *et al.* found 4 rats (3 females and one male) that spontaneously showed generalized tonic clonic seizures (GTCS) in a group of Crj:Wistar rats purchased from Charles River Japan in 1985. By brother-sister mating of rats with frequent seizures, Noda epileptic rat (NER) was established as an inbred strain ($F_{29}$, 1977) [78]. Although NERs usually exhibit spontaneous GTCS from about 14 weeks of age with a frequency about one per 30h, Iida *et al*. have found that NERs given a modified acoustic priming, that is, repeated weekly sound stimulation from 3 weeks of age, had a high incidence (100%) of AGS from 9 weeks of age [44]. Under tossing-stimulating conditions, GTCS were provoked in 90% of NER and 66% of ($F_1$ × NER) backcross animals, but no seizures occurred in the F344, $F_1$, or ($F_1$ × F344) backcross animals. Routine monitoring of nonstimulated animals revealed spontaneous GTCS in 100% of NER and 64.2% of ($F_1$ × NER) backcross animals, but no seizures in F344 or $F_1$ animals. Gender effect on seizure susceptibility was negligible in ($F_1$ × NER) backcross for both conditions. Seizure susceptibility loci, designated *Ner1* and *Ner2*, have been mapped on rat Chr 1 and Chr 3, respectively. It is speculated that *Ner1* controls the inheritance of spontaneous tonic-clonic seizures in an autosomal recessive mode, whereas *Ner2* affects the occurrence of tossing-induced seizures [65].

Ohno *et al.* suggested that GTCS in NER are of forebrain origin and are evoked primarily by activation of the limbic and/or cortical seizure circuits [80]. Many researches support that hippocampus plays an important role in epileptogenicity in NER [34, 54, 83, 106]. On the other hand, Harada *et al*. reported that astrocytic Kir4.1 expression of NERs was markedly reduced in the amygdala in a subunit- and region-specific manner, specifically in astrocytic processes. Although it remains to be determined if the Kir4.1 down-regulation in NERs could be a primary cause or a consequence of seizure activity, their results suggest that reduced activity of Kir4.1 channels in the amygdala is involved in limbic hyperexcitability in NER [39]. Ishimaru *et al*. reported the antiepileptic effects of LEV on hippocampal kindling in NERs [49], and Inoue *et al*. also showed inhibition effects of traditional AEDs, phenobarbital (PB) and valproate (VPA), against seizures evoked by strong environmental stimuli in mature NERs [45].

*WER*

Tsubota *et al*. found a new epileptic rat showing absence-like immobile behavior and head droop implying neck flaccidity in a Kwl:Wistar colony purchased from Kiwa Laboratory Animals Co., Ltd. (Wakayama, Japan) in 1993. They started inbreeding between the mutant male and normal females of the same colony to develop a new strain. After the $F_2$ generation, affected rats spontaneously exhibited both absence-like immobile behavior with SWD in cortical EEG and GTCS. The GTCS in WER strain established was confirmed to be inherited as an autosomal recessive trait with 86% incidence [112].

## Canavan-Disease Related Epileptic Models: TRM and SER

According to the report of Traeger and Rabin, some 38 of 60 children of Canavan disease, a white matter disorder with aspartoacylase (ASPA) deficiency, had epilepsy and the age of onset was quite variable, from birth to 15 years. Thirty children were treated with AEDs, and good control was attained in 18 children [111].

*TRM*

Serikawa *et al*. found mutant rats that exhibit body tremor, curled whiskers and hair in a colony of Kyo:Wistar in 1985. The rats aged 2–3 months showed paroxysmal and synchronized 5–7 Hz SWD in both cortical and hippocampal EEG during absence-like seizures [99]. The tremor rats were established as a segregating inbred strain TRM, in which the causative mutation tremor *(tm*) is maintained in heterozygous state by brother × sister matings. TRM *(tm/tm*) exhibited also spongiform degeneration in the central nervous system (CNS) and sterility with gonadal hypoplasia in both sexes. Although genetic analysis suggested that absence-like seizures are semidominant traits [43], the other anomalies were regarded to be a set of inherited autosomal recessive traits. By positional cloning, *tm* has been identified as a genomic deletion in Chr 10q24. The centromere-proximal end of the deleted region was mapped inside the 7th exon of sedoheptulokinase (*Shpk*), and the centromere-distal end was located within a LINE1 element downstream of *Olr1472*. Within the determined 240-kb deleted region, entire transcribed regions of 12 genes besides part of the 7th exon of *Shpk* were localized: transient receptor potential vanilloid 1 and 3 (*Trpv1/3*); *Aspa*; the *Spata22* ortholog; seven olfactory receptor genes; and one hypothetical gene, *LOC100359760* [50]. Accordingly, no ASPA expression was detected in any of the tissues examined, and abnormal accumulation of *N*-acetyl-L-aspartate (NAA) was shown in the mutant brain, in correlation with the severity of the vacuole formation [53]. Elevated urine NAA, a biomarker, was also detected in tremor rats, although the details were not described in the publication. Since then, tremor rats have been used as a valuable rat model of the Canavan disease [13, 55, 120]. In addition, effects of ASPA gene transfer to the brain of tremor rats were pathophysiologically evaluated [56, 70].

Interestingly, direct injection of NAA into normal rat cerebroventricle induced 4- to 10-Hz polyspikes or spike-wave-like complexes in cortical and hippocampal EEG, concomitantly with behavior characterized by sudden immobility and staring [2]. Actually NAA produced an activation of acutely dissociated hippocampal neurons via metabotropic glutamate receptors [123]. NAA has been also found to activate hippocampal CA3 neurons in brain slice preparations of Wistar rats [31]. Furthermore, adenovirus gene transfer of ASPA into the cerebroventicle of tremor rats inhibited absence-like seizures [95]. These results suggested that accumulated NAA in the CNS would induce neuroexcitation and neurodegeneration directly or indirectly.

Hanaya *et al.* examined the effects of conventional AEDs on absence-like seizures in tremor rats. Trimethadione (TMO), ESM, and PB effectively inhibited the seizures, whereas phenytoin (PHT) was ineffective. The profiles on effects of conventional AEDs were similar to those observed in human absence seizures [37].

*SER*

The Zitter rat, which is characterized by a hair anomaly, generalized body tremor, progressive flaccid paresis, hypomyelination and vacuole formation in the CNS, was found in a colony of SD rats in Hannover, Germany. By positional cloning, Kuramoto *et al*. found a marked decrease in Attractin *(Atrn*) mRNA in the brain of the rats and identified the zitter mutation *(zi)* as an 8-bp deletion at a splice donor site of *Atrn* [59]. The double mutant, termed spontaneously epileptic rat (SER; *zi/zi, tm/tm*), was obtained in an $F_2$ progeny between the two mutant strains, zitter rats and tremor heterozygous rats, and has been maintained by intercrossing between individuals with *zi/zi, tm/+* genotype among the litter mates of SER [100]. SER exhibited spontaneously and frequently both absence-like seizures and tonic convulsive seizures, and running and/or jumping episodes after the termination of tonic convulsion were also observed without any stimuli. Double mutant mice carrying both homozygous *Aspa*-knockout and $Atrn^{mg\text{-}3J}$ mutant alleles exhibited both absence-like and tonic seizures [28], supporting that both *Aspa* and *Atrn* deficiencies would be responsible for epileptic seizures in SER. Actually adenovirus gene transfer of ASPA into the cerebroventricle of SER results in an inhibition of tonic convulsions [96].

The seizure inhibition effects of various existing AEDs to the two types of seizures of SER suggested that absence seizures and tonic seizures are independently able

to be evaluated with specificity. The absence-like seizures were inhibited by TMO and ESM, whereas the tonic convulsion was not affected by these AEDs. In contrast, PHT inhibited the tonic seizures without affecting the absence-like seizures. PB and VPA inhibited both seizures. SER is therefore regarded to be a powerful tool for evaluating AEDs [94]. Since then, SERs have been used for evaluating other AEDs and candidates; TRH [114], CNK-602A (TRH-related analogue) [73, 87], topiramate [38, 52, 76], 20-hydroxyecdysone (neurosteroid) [33], vigabatrin [35], S-312-d (novel calcium channel antagonist) [6]. Significantly longer inhibition of seizures after prolonged treatment with LEV compared with that of PHT, PB, VPA, and carbamazepin (CBZ) suggested that LEV possesses not only antiseizure effects but also antiepileptogenic properties [17, 32, 124, 125].

SER and the parental mutant strains, TRM (*tm/tm*) and ZI (Zitter inbred strain, *zi/zi*), have morphological changes in the CNS [46, 58] which would be mostly caused by primal gene mutations. In addition, acquired morphological changes would be added in SER, since the rats exhibit frequently both absence and convulsive seizures. Hippocampal CA3 neurons in SER display a long-lasting depolarization shift accompanied by repetitive firing, which was attributed to abnormalities of the L-type $Ca^{2+}$ channels, with a single stimulation of the mossy fibers [4, 7, 48, 74]. Sprouting of mossy fibers was observed in the dentate of mature SER, and expression of the brain-derived neurotrophic factor (BDNF) was higher in the hilus, CA3, and in the granular cell layer of the dentate gyrus in SER than in normal Wistar rats. Repetitive tonic seizures and vulnerable CA3 neurons of SER could be involved in the induction of sclerosis-like changes in the hippocampus [36]. The hippocampal sclerosis-like neuronal degeneration and/or regeneration was inhibited by prophylactic treatment with LEV [104].

In addition to abnormalities of the $Ca^{2+}$ channels described above, functional alterations induced by repeated seizures in SER were also studied in $GABA_A$ receptor [25], benzodiazepine receptor [101], NMDA receptor [71], muscarinic cholinergic receptor [75], and voltage-gated sodium channels subtypes [30], and for contents of neuropeptides [92, 105]. Aberrant reduction in inhibitory protein factor (IPF), which potently inhibits glutamate uptake into synaptic vesicles, was found in hippocampus of aged SER, suggesting the seizures are involved in excessive glutamate transmission [8]. Although SER exhibit retardation in learning ability, their poor operant performance was improved by continual intake of PB in the rats [10], suggesting the importance of seizure control.

Comparative studies on the morphological and functional changes with or without the continuous suppression of seizures in SER might give new clues for suppression of epileptogenesis and prevention of pathophysiological changes.

## Chemical Mutagenesis Derived Models: Febrile Seizure, ADLTE, EA1, and EA2 Models

Chemical mutagenesis is a powerful strategy to produce genetically modified mutations in many species. We have generated a large repository of *N*-ethyl-*N*-nitrosourea (ENU)-induced mutations, called the Kyoto University Rat Mutant Archive (KURMA). DNA mutations in the repository can be efficiently screened with a high-throughput and low-cost assay based on the Mu-transposition reaction (MuT-POWER). Animals carrying any mutations can be recovered from frozen sperm by intracytoplasmic sperm injection (ICSI) [69]. There are estimated to be 2.5–3.5 million mutations in the KURMA, which results in a genome-wide average of at least two mutations per gene within 10,000 stored DNA/sperm samples. In addition, 250 sets of sperm and DNA from Hubrecht ENU Rat Archive (HERA) which was derived from *Msh6* KO rats (HsdCpb:WU) [115], are integrated into the NBRP-Rat repository [98].

### *Febrile seizure model: Hiss rat, missense mutant of Scn1a gene, F344-Scn1a$^{m1Kyo}$*

Although febrile seizures (FS) are the most common convulsive syndrome in infants and childhood, the etiology of FS has remained unclarified. Various missense mutations of the $Na_v1.1$ channel (SCN1A), which alter channel properties, have been reported in a familial syndrome of generalized epilepsy with febrile seizures plus (GEFS+). *Scn1a*-targeted rats carrying a missense mutation (N1417H) in the third pore region of the sodium channel were developed by gene-driven ENU mutagenesis. Despite their normal appearance under ordinary circumstances, *Scn1a* mutant rats exhibited remarkably high susceptibility to hyperthermia-induced seizures. Experimental studies with the mutant rats showed that the missense mutation (N1417H) confers susceptibility to FS, suggesting that the impaired physiological func-

tions of inhibitory GABAergic neurons underlie one of the mechanisms of FS [68, 79, 82]. The *Scn1a* homozygous mutant rats are named as hyperthermia-induced seizure susceptible (Hiss) rats. Hiss rats were used for evaluating FS-specific AEDs. Diazepam (DZP) and potassium bromide (Kbr) showed potent inhibitory effects whereas CBZ exhibited adverse effects [41].

*Autosomal-dominant lateral temporal lobe epilepsy (ADLTE) model: Missense mutant of Lgi1 gene, F344-Lgi1$^{m1Kyo}$*

Mutations of the leucine-rich glioma-inactivated 1 (*LGI1*) gene cause an autosomal dominant partial epilepsy with auditory features also known as autosomal-dominant lateral temporal lobe epilepsy (ADLTE). *LGI1* encodes a neuronal secreted protein, whose brain function is still poorly understood. *Lgi1*-mutant rats carrying a missense mutation (L385R) were generated by gene-driven ENU mutagenesis. Experimental studies with the mutant rats showed that the L385R mutation prevents the secretion of LGI1 protein and the mutant protein may be destabilized *in vivo*. Homozygous mutant rats *Lgi1*$^{L385R/L385R}$ exhibited early-onset spontaneous epileptic seizures from P10 and died prematurely, and heterozygous *Lgi1*$^{+/L385R}$ rats were more susceptible to sound-induced, generalized tonic-clonic seizures than control rats. The AGS were suppressed by AEDs such as CBZ, PHT and LEV, which are commonly used to treat partial seizures, but not by the specific absence seizure drug, ESM [16]. To acoustic stimuli in heterozygous *Lgi1*$^{+/L385R}$ rats, an elevated level of Fos expression, a biomarker of neural excitation, was found in the CNS, especially in the temporal lobe, thalamus, and subthalamic nucleus. In addition, a number of differentially expressed genes that may be involved in epileptogenesis were found by microarray analysis [26].

*Episodic ataxia type 1 (EA1) model: ADMS rats, missense mutant of Kcna1 gene, F344-Adms/Kyo*

Mutations in the KCNA1 gene, which encodes for the α subunit of the voltage-gated potassium channel Kv1.1, cause episodic ataxia type 1 (EA1). EA1 is a dominant human neurological disorder characterized by variable phenotypes of brief episodes of ataxia, myokymia, neuromyotonia, and associated epilepsy. In a stock of ENU-mutated G1 rats, some rats, which dominantly exhibit myokymia, neuromyotonia and generalized tonic-clonic seizures, were found and maintained by selective breeding. Ishida *et al*. identified a missense mutation (S309T) in the voltage-sensor domain, S4, of the *Kcna1* gene as a causal mutation by positional cloning. Heterozygous *Kcna1* $^{S309T/+}$ mutant rats as named autosomal dominant myokymia and seizures (ADMS) rats showed cold stress-induced tremor, neuromyotonia, and motor incoordination. Expression studies of homomeric and heteromeric Kv1.1 channels suggested a dominant-negative effect of the S309T mutation on potassium channel function. Spontaneous convulsive seizures and twitching phenotypes in ADMS rats were significantly prevented after the administration of CBZ. ADMS rats would be a unique model for studying the diverse functions of Kv1.1 *in vivo*, as well as for understanding the pathology of EA1. Homozygous *Kcna1* $^{S309T / S309T}$ mutant rats showed tremor, motor-incoordination, mainly caused by the extension of hind limbs, and spontaneous convulsive seizures from postnatal two weeks, and died until three weeks of age [47].

*Episodic ataxia type 2 (EA2) model: GRY rats, missense mutant of Cacna1a gene*

Takeuchi *et al.* found a new mutant rat displaying abnormal movement in the progeny of a female Wistar rat which had been given 10 mg/kg MNU at an early stage of the gestational period [107]. Genetic studies revealed that the character is inherited by an autosomal single recessive gene and named groggy rat. The abnormal movement of the groggy rats was first apparent around postnatal day 15, while the histological studies revealed the appearance of numerous necrotic neurons in the striatum of the groggy rat on postnatal days 60 and 120.

Tokuda *et al*. confirmed that the established GRY inbred strain exhibit ataxia, an unstable gait, and paroxysmal severe extension of the entire body. Adult rats show a reduction in size of the cerebellum and presynaptic and axon terminal abnormalities of Purkinje cells. These neurological abnormalities are inherited in an autosomal recessive manner, and a missense (M251K) mutation in the alpha (1A) subunit of the P/Q-type voltage-gated $Ca^{2+}$ channel gene *Cacna1a* was identified by positional cloning. This mutation was located at a highly conserved site close to the ion-selective pore and led to the shortening of the inactivation phase of the $Ca^{2+}$ channel current without a change of peak current density or current-voltage relationship in whole cell patch recordings of the recombinant $Ca^{2+}$channel expressed in HEK cells. GRY rats exhibited absence-like seizures from 6

to 8 weeks of age, which were characterized by bilateral and synchronous 7–8 Hz SWD concomitant with sudden immobility and staring, on cortical and hippocampal EEGs. The pharmacological profile of the seizures was similar to that of human absence epilepsy: the seizures were inhibited by ESM, VPA and LEV but not PHT [109, 110]. Tanaka *et al*. suggest that increased high-voltage-activated (HVA) $Ca^{2+}$ channel function underlies the cerebellar dysfunction and ataxic phenotype of GRY rats [108]. Ohno *et al*. also suggest that the serotonergic system negatively regulates the incidence of absence seizures in GRY rats by stimulation of 5-HT (1A) and 5-HT (2) receptor [81].

Although *CACN1A* mutations are associated with three neurological disorders in humans, familial hemiplegic migraine type 1 (FHM1), episodic ataxia type 2 (EA2), and spinocerebellar ataxia type 6 (SCA6), the missense mutations found in familial EA2 patients affected are located at S5-S6 linker in domain I, very close to the position of the mutation in GRY rats. Therefore, GRY rats would be used as a model specialized for the investigation of EA2 [109].

## Future Prospects on Development of Rat Models for Epilepsy Research

To defeat drug-resistant epilepsy, development of drug resistant epilepsy models has been considered. Originally, both amygdala and hippocampal kindling models are proposed as models of TLE in AED screening [121]. Löscher *et al*. reported the differences in kindling development in outbred and inbred rats strains [62], and they showed that PHT-resistant kindled rats were possible to be divided from the population of kindled Wistar rats [21, 51, 64]. As another models of drug-resistant epilepsy, lamotrigine (LTG)-resistant kindled Sprague-Dawley rats were also reported [85, 102]. These results suggest that drug-resistant kindled inbred strains would be possible to develop from selective inbreeding.

The recombinant inbred (RI) strains would be also a useful model system to find seizure-susceptible genes which are involved in epileptogenesis. Although it was a preliminary trial, we succeeded to identify two QTLs, which are involved in the development of amygdala kindling, on rat Chr 2 by using a set of LE (LE/Stm) X F (F344/Stm)/FXL-RI strains, and several positional candidate genes, such as *Hspb3, Itgal, Itgae,* and *Plk2*, were raised from the restricted regions [40]. To identify genes involved in the development/expression of anxiety/fear, Díaz-Morán *et al*. analyzed the gene expression profile in the hippocampus of genetically heterogeneous NIH-HS rats [20]. The strategy, combined sequence-based and genetic mapping analysis of the complex traits in outbred rats [14], might be suitable for the identification of genes involved in the development of kindling.

There is a report that EEGs are non-sufficient biomarkers for the prediction of pharmacoresistant epilepsy. Additional factors such as etiology and pathophysiology should also be taken into consideration [103]. Since neurophysiological and imaging tools have been rapidly improved, new epilepsy biomarkers employing such tools may be developed in the coming years and become clinical routine [42]. Studies focused on the discovery of epilepsy biomarkers except for the EEG monitoring were not evident on the epileptic rat models yet. However, established epileptic rat models would be appropriate tools in identifying biomarkers that may be worth for clinical use.

In the present review, we introduced the ENU-induced mutant rat archive (KURMA), especially gene-driven mutagenesis, with two epileptic rat models as the outcome. Recently, advanced gene editing technologies, such as zinc finger nuclease (ZFN) [67], transcription activator-like effector nucleases (TALEN) [93], and the clustered interspaced short palindromic repeats (CRISPR)/Cas system, an RNA-based genome engineering technique [127], have become available in the rat. Therefore, a variety of novel rat models with similar mutations as those in epileptic patients is likely to be developed in the near future. We expect that the research activities with epileptic rat models will be successfully processed toward better treatment and prevention of this disease.

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