Commentary

Neuronal survival in epilepsy: to die or not to die?

Epilepsy is a chronic neurological condition characterized by recurrent and unprovoked seizures, affecting 1% of people worldwide. Historically, epilepsy was called the "sacred disease" because people thought that epileptic seizures were a form of attack by demons, and that the visions epileptics experienced were sent by the Gods (Riggs and Riggs 2005). Epilepsy can have many causes, including brain injury, poisoning, head trauma, or stroke; and these factors are not restricted to any age group, sex, or race and neither is epilepsy. Moreover, genetic factors are known to play a major role in many epileptic forms, accounting for 40% of all epilepsies (Annegers *et al* 1996). A small proportion of epilepsy subtypes are inherited as single-gene ('Mendelian') traits. In the remaining cases, the etiology is complex, arising from the contribution of multiple genetic and non-genetic factors. The rapidly expanding and exciting discoveries in epilepsy genetics in the last ten years are the key for unraveling the basic mechanisms of seizure disorders and predicting what lies ahead for patients. Currently, more than twenty five genes have been identified to cause epilepsy in human, primarily the monogenic forms (tables 1 and 2). However, the genetic architecture of some of these Mendelian forms can explain the complexities of the common epilepsies (Delgado-Escueta *et al* 2003). Beyond genetics, we are finally looking at what these mutations do in cellular, animal and human models by developing sophisticated *in vitro* and *in vivo* functional validation assays. Such approaches link epilepsy genes to both anti- and pro-apoptotic functions. This note introduces the cellular functions of a few epilepsy genes and discusses a unifying model for epileptogenesis.

Progressive myoclonus epilepsy (PME) is a subsyndrome of epilepsies involving the central nervous system (Delgado-Escueta *et al* 2001). The PME include: Unverricht–Lundborg disease (ULD), Lafora disease (LD), myoclonic epilepsy with ragged-red fibre syndrome (MERRF), the neuronalceroid lipofuscinoses (NCL), dentadorubropallidoluysian atrophy (DRPLA) and sialadosis (table 1). A hallmark of PME is the progressive neurologic deterioration and neurodegeneration (Delgado-Escueta *et al* 2001). While the genetic cause of each of these PMEs is identified, defects in at least four genes are proven to be directly linked to neuronal cell death. In ULD, the causative gene, *CSTB*, encodes a cysteine proteases inhibitor named as cystatin B (Pennacchio *et al* 1996). Mice deficient for cystatin B, produced by targeted disruption of the mouse *Cstb* gene, display a phenotype similar to the human disease, with progressive ataxia and myoclonic seizures. The mice show neuronal atrophy, apoptosis and gliosis as well as increased expression of apoptosis and glial activation genes (Pennacchio *et al* 1998; Lieuallen *et al* 2001). Under normal circumstances, the action of caspases, a family of cysteine proteases involved in the initiation of apoptosis, is directly inhibited by cystatin B. In the absence of functional cystatin B, however, caspases would trigger the apoptotic process leading to cell death (Delgado-Escueta *et al* 2001). The second player in PME involved in neuronal cell death is the *EPM2A* gene encoded laforin phosphatase, the protein defective in LD (Ganesh *et al* 2000). Targeted disruption of *Epm2a* gene in mice led to the development of widespread neurodegeneration, inclusion bodies, ataxia, and myoclonus epilepsy, the defining phenotypes of LD in humans (Ganesh *et al* 2002). Notably, dying neurons in the mutant mice exhibited swelling in the endoplasmic reticulum, Golgi networks and mitochondria in the absence of apoptotic bodies or fragmentation of DNA, suggesting the involvement of a novel form of cell-death in LD and that laforin is critical for neuronal survival (Ganesh *et al* 2002). The third player is the product of the gene involved in juvenile form of NCL, also known as Batten disease (BD) (International Batten Disease Consortium 1995) (table 1). BD is associated with accelerated apoptotic death of photoreceptors and neurons resulting from defects in the *CLN3* gene. Intriguingly, CLN3 protein is anti-apoptotic when overexpressed in neuronal precursor cells and therefore has been suggested as a novel molecular target for cancer drug

discovery (Dhar *et al* 2002; Rylova *et al* 2002). And the fourth player directly involved in neuronal survival is atrophin-1 involved in DRPLA. While loss-of-function mutations result in the development of UCL, LD and BD, a gain-of-function mutation leads to the development of DRPLA; the expansions of a CAG trinucleotide repeat encoding polyglutamine in the atrophin-1 gene (Nagafuchi *et al* 1994). Both patients and DRPLA transgenic mice have nuclear accumulation of polyglutamine containing atrophin-1 and the cleavage of atrophin-1 by caspases enhances the cytotoxicity leading to cell death (Sato *et al* 1999; Nucifora *et al* 2003). Thus, combined degeneration of the dentatorubral tract and pallidoluysian system is thought to underlie the neuropathology in DRPLA (Delgado-Escueta *et al* 2001).

Contrary to the pathological features seen in PMEs, the epileptic forms that lack 'detectable' brain lesions and/or other neurological defects are called as idiopathic epilepsies (IE). The latter ones are presumed to be genetic and are generally age-dependent. Thus, the most common forms of IEs are epilepsies of infancy, childhood, and adolescence, namely febrile convulsions, juvenile myoclonus epilepsy and childhood absence epilepsy (Delgado-Escueta *et al* 2003; Mulley *et al* 2003). Progress has also been made with respect to identifying genes involved in several IEs – although with limited success. As suspected on the basis of physiological evidence, a majority of these genes encode subunits of voltage-gated or neurotransmitter-gated ion channels, leading to the concept that a majority of the IEs are 'channelopathies', underscoring abnormal channel functions in neuronal excitability and that they provide important targets for anticonvulsant drugs (Mulley *et al* 2003; Kass 2005) (table 2). Nonetheless, success with finding mutations in ion channels genes has not been encouraging as only a very few families screened showed defective genes (less than 2%; table 2) as against hundreds of families for PMEs. These observations provide further evidence for the high level of genetic heterogeneity associated with IEs (table 2). This would as well mean that the Mendelian mutations

Table 1. Genes implicated in progressive myoclonus epilepsies.

Disease	Inheritance*	Chromosomal locus	Gene	Protein	Function	MIM ID†
Unverricht-Lundborg disease (ULD)	AR	21q22·3	*CSTB*	Cystatin B	Lysosmal cysteine protease inhibitor	254800
Lafora's disease (LD)	AR	6q24	*EPM2A*	Laforin	Dual specificity protein phosphatase	254780
		6p22	*NHLRC1* (*EPM2B*)	Malin	E3 ubiquitin liagse	
Myoclonic epilepsy with ragged red fibres (MERRF)	M	Mitochondrial DNA	*MTTK*	–	Encodes tRNA for lysine amino acid residue	545000
Neuronal ceroid lipofuscinoses (NCLs)						
Classic late infantile NCL (type 2)	AR	11p15·5	*TPP1* (*CLN2*)	Tripeptidyl peptidase 1	Lysosomal serine protease	204500
Juvenile NCL (type 3); Batten disease	AR	16p12·1	*CLN3*	CLN3 protein	Membrane protein with an anti apoptotic function	607042
Late infantile Finnish variant NCL (type 5)	AR	13q21·1-q32	*CLN5*	CLN5 protein	Lysosomal protein with unknown functions	608102
Sialidoses	AR	6p21·3	*NEU1*	Sialidase 1	Removal of sialic acid from glycolipids and glycoproteins	608272
Dentatorubral-pallidoluysian atrophy (DRPLA)	AD	12p13·31	*DRPLA* (*ATN1*)	Atrophin 1	Phosphoprotein with unknown functions	607462

*AR, autosomal recessive; AD, autosomal dominant; M, maternal.
†Further details on the gene, mutations, and disease can be obtained from the OMIM link (http://www.ncbi.nlm.nih.gov/omim), using the MIM number provided.

associated with IEs are perhaps unique to specific families because mutant alleles are rare even in the same ethnic population they were discovered (Delgado-Escueta *et al* 2003).

One of the few exceptions to the notion of 'channelopathies' is the recent discovery of *EFHC1* gene, implicated in juvenile myoclonus epilepsy (JME) (Suzuki *et al* 2004). Mutations in *EFHC1* were identified in Mexican families with JME and all mutations were single amino acid substitutions. The gene encodes a 640-amino acid long protein, named myoclonin, and contains an EF hand calcium-binding motif. In mice, myoclonin is localized on neuronal dendrites and soma, but were not

Table 2. Genes implicated in idiopathic epilepsies.

Gene	Chromosome locus	Type of epilepsy[†]	Number of families with mutations	MIM ID*	Major function
Sodium channel genes					
SCN2A	2q23–q24·3	BFNIS	8	607745	Generation and propagation of
		GEFS	1	604233	action potentials in striated
SCN1B	19q13·1	GEFS	3	604233	muscle and neuronal tissues
SCN1A	2q24	GEFS	10	604233	
		SMEI	54	607208	
Calcium channel genes					
CACNB4	2q22–23	IGE	1	600669	Mediate the influx of calcium
		JME	1	606904	ions into the cell upon mem-
CACNA1A	19p13	EA	2	108500	brane polarization
Nicotinic acetylcholine receptor genes					
CHRNA4	20q13·2–q13·3	ADNFLE	4	6000513	Mediate fast transmission at synapses
CHRNB2	1p21	ADNFLE	2	605375	
Potassium channel genes					
KCNQ2	20q13·3	BFNC/	7	121200	Regulate neuronal excitability
		myokymia	1	606437	
KCNQ3	8q24	BFNC	1	121201	
KCNA1	12p13	EA	1	160120	
Gamma-amino butyric acid (GABA) receptor genes					
GABRA1	5q34	JME	1	606904	Increases the permeability to
GABRG2	5q31·1-q33·1	GEFS	2	604233	chloride ions, leading to
		CAE	2	607681	hyperpolarization of the neuron or inhibition
Chloride channel genes					
CLCN2	3q26	CAE	1	607682	Regulates the electric excit-
	3q26-qter	EGMA	1	607628	ability of plasma membrane
Non ion channel genes					
LGI1	10q24	ADPEAF	10	600512	Transmembrane protein involved in signal transduction pathway
EFHC1	6p12–p11	JME	6	254770	Enhances calcium ion influx
BRD2	6p21·3	JME	CC	608816	Mitogen-activated kinase
ME2	18q21	IGE	CC	600669	NAD-dependent malic enzyme localized in mitochondrial

[†]BFNIS, benign familial neonatal and infantile seizures; GEFS, generalized epilepsy with febrile seizures; SMEI, severe myoclonic epilepsy in inafancy; IGE, idiopathic generalized epilepsy; JME, juvenile myoclonus epilepsy; EA, episodic ataxia; CAE, childhood absence epilepsy; ADPEAF, autosomaldominant partial epilepsy with auditory features; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; BFNC, benign familial neonatal convulsions; EGMA, epilepsy with grand mal upon awakening; CC, case-control analysis.
[*]Further details on the gene, mutations, and disease can be obtained from the OMIM link (http://www.ncbi.nlm.nih.gov/omim), using the MIM number provided.

observed at axons. Curiously, expression of wild type myoclonin in the same system resulted in shorter neuritic processes with fewer branches, subsequently leading to cell death through the apoptotic process. Expression of *EFHC1* mutants, on the other hand, substantially reduced the cell-death effect, suggesting that wild-type myoclonin is involved in triggering apoptosis and that the JME mutations might disrupt this complex process of programmed cell death. Intriguingly, usage of calcium channel blockers increased the survival rate of neurons expressing wild-type myoclonin. The induction of apoptosis should therefore be through the activation of calcium channels. Patch-clamp experiments did demonstrate that expression of myoclonin increased the activity of $Ca_V2{\cdot}3$ voltage-gated calcium channels. Thus, apoptosis is mostly likely to be triggered by the increased calcium influx as a result of myoclonin mediated $Ca_V2{\cdot}3$ activation. Indeed, the immunosignals of the myoclonin and $Ca_V2{\cdot}3$ proteins show overlapping localization patterns in neurons and these two proteins do interact physically. Taken together, this study reveals a molecular basis for JME and suggests that mutations in *EFHC1* might abolish the apoptotic process in the brain by failing to activate the voltage gated calcium channels (Suzuki *et al* 2004).

This conclusion brings us to an intriguing question as to how a failure to initiate the programmed cell death (PCD) would lead to the development epileptic symptoms. One answer to this question lies in the developmental mechanisms. PCD is known to be an important event in the normal development of the mammalian nervous system, where it regulates the final number of neuronal and glial cell populations. During embryonic development up to 70% of the neural cells are eliminated through PCD (De Zio *et al* 2005). This elimination is very much required for the morphogenetic changes occurring in the developing brain, for example the neural tube closure. The delicate balance between proliferation and death of these cells thus underlie the critical developmental process of the nervous system. The *EFHC1* gene product myoclonin could be one of such critical players, and mutations therein might cause the unwanted neurons to survive, resulting in an increased density of a subset of neurons in the JME patients. A rigorous quantitative magnetic resonance imaging analysis indeed detect an increase in cortical gray matter of JME patients, suggesting a pathological mechanism resulting in subtle cerebral structural abnormality (Woermann *et al* 1999). These changes may be associated with abnormalities in functional connectivity leading to the production of hyperexcitable circuits and epileptic seizures. It is of interest to note that myoclonin is not the only player that is involved in both epileptogenesis and pro-apoptotic function. *BRD2* gene showed a significant association for a genetic polymorphism and susceptibility for developing JME (Pal *et al* 2003). The BRD2 protein is a nuclear transcriptional regulator that is found in the human brain and plays an important function in the development of the central nervous system (Guo *et al* 2000; Crowley *et al* 2004). BRD2 may also be a pro-apoptotic protein because of its proposed function as a mitogen-activated protein kinase (MAPK). MAPK constitute one of the critical components of a signal transduction pathway that regulates a variety of cellular process including cell growth and apoptosis (Liou *et al* 2003). LGI1 protein is yet another factor that modulates the MAPK pathway (Kunapuli *et al* 2004). *LGI1* was first identified as a candidate tumour suppressor gene for glioma (Chernova *et al* 1998). Surprisingly, mutations in *LGI1* were also associated with an IE subtype, the autosomal dominant lateral temporal lobe epilepsy (Kalachikov *et al* 2002).

The recent advances in the understanding of cellular functions of epilepsy genes, such as *CSTB*, *EPM2A*, *CLN3*, *EFHC1*, and *LGI1*, unravel several hitherto unknown pathways in the genesis of epilepsy. They also identify the molecular players that control the development and maintenance of normal brain structure and functions, which are regulated by the cell survival as well as cell-death pathways. Whereas an extensive neuronal degeneration is associated with the PME, slight changes in neuronal density might underlie the pathophysiology of IEs. Thus either an increase or decrease in the neuronal population would results in disorganized neuronal connectivity leading to epileptic seizures. These studies propose structural changes in the brain as the underlying cause in both PME and IE, and might prove useful to future medicine and surgery.

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