# R.C.P.U. NEWSLETTER

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## Molecular Biology of Epilepsy Genes


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### Introduction

Epilepsy is one of the most common brain disorders with one in 10 individuals having at least one seizure during his/her lifespan, and about 30% of these developing epilepsy. Epilepsy occurs for a variety of reasons, and can occur due to an environmental event alone without any genetic involvement. Other seizures occur without apparent cause and have a chronic course with recurrent unprovoked episodes, and these seizures are classified in the group of common epilepsies. The common epilepsies can be distinguished from epilepsy disorders that have single gene causation or have a syndromic component associated with brain or other clinical anomalies. The epilepsies have a population prevalence of 0.5 to 1% (Hauser et al., 1996).

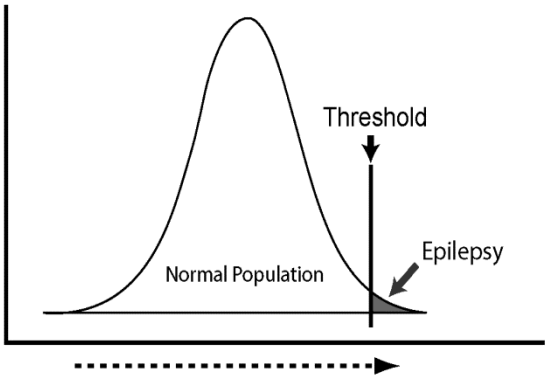
The epilepsies can be classified in many different ways, but the most common classification comes from the International League Against Epilepsy (ILAE). The most recent report classifies epilepsies into three broad groups: 1. Genetic (includes single gene causes without structural brain anomalies or syndromic associations). 2. Structural-metabolic (applied to syndromic disorders such as Tuberous Sclerosis or Rett syndrome in which there are syndromic features or pervasive brain anomalies. 3. Unknown cause (Berg et al., 2010).

The biological underpinnings of epilepsy are extremely heterogeneous. The common thread is the presumption that each of these mechanisms disturbs the balance between excitation and inhibition of neuronal circuits. Historically, epilepsy was believed to be due to dysfunction of ion channels, but over the past decade new genes have emerged which indicate many different mechanisms for epilepsy (Poduri and Lowenstein, 2011). In the first category is the "major" epilepsy gene *SCN1A*, the most well described channelopathy gene. In the second category, we discuss the *ARX* disorders which include seizure phenotypes as well as intellectual impairment and/or severe developmental brain malformations, and the chromosome abnormalities associated with seizures such as the classical Wolf-Hirschhorn syndrome (Battaglia et al., 2009), the 1p36 deletion syndrome (Bahi-Buisson et al., 2008b), variants within 15q11.2-q13 (Battaglia, 2008; Battaglia and Guerrini, 2005) and the more subtle or more newly discovered copy number variants which confer varying degrees of epilepsy risk.

Although we will discuss discrete genes associated with epilepsies, the most compelling issue in understanding the genetic etiology for the common epilepsies involves multifactorial inheritance. This concept is crucial in thinking about the polygenic origin of the common epilepsies and also for our understanding of how single gene defects operate in our genomic constitution and in the midst of environmental exposures.

### Multifactorial Inheritance

For many decades it was known that the common epilepsies demonstrated multifactorial inheritance, meaning the involvement of both genetic and environmental factors. This inheritance pattern results in higher recurrence risk among siblings ( 3-5%), compared to the <1% risk for the normal population (Anderson et al., 1991; Hemminki et al., 2006), and a higher degree of concordance of seizures in monozygotic compared to dizygotic twins (Berkovic et al., 1998; Kjeldsen et al., 2001). There is a strong genetic influence, and heritability is estimated to be approximately 70% (Hemminki et al., 2006). A crucial part of multifactorial inheritance model is the threshold effect (Fig 1). In this model, a population has a distribution of risk genes that in combination with environmental factors lead to a threshold effect whereby the disease phenotype becomes expressed (Lobo, 2008; Turnpenny and Ellard, 2004).




### Polygenic Effects

New molecular tools are now helping to better appreciate how multiple genes contribute to the epilepsy disorders. These tools include genome wide association studies (GWASs) which detects subtle gene variations or polymorphisms that may associate with the disease under study.

GWAS studies looking for gene risks with >1.3 odds ratio have not identified

any gene with this degree of risk effect (Kasperaviticiute et al., 2010). One interpretation of these data is that the contribution of risk genes may be more subtle, conferring risks <1.3. The study of small deletions/duplications known as copy number variants (CNVs) has been more productive in identifying genomic changes that are clearly associated with epilepsy and other developmental conditions. These studies have identified deletion or duplication regions particularly involving chromosome regions 16p13.11, 15q13.3 and 15q11.2 (de Kovel et al., 2010; Dibbens et al., 2009; Heinzen et al., 2010; Masurel-Paulet et al., 2010; Mefford et al., 2010; van Bon et al., 2009).

Evaluation of family histories indicate that often a parent inherits one of the same CNVs but does not demonstrate any seizure phenotype, indicating that these CNV's serve as only risk factors but the disease expression is modulated presumably by other aspects of one's genetic constitution and/or yet to be identified environmental effects. It is also significant that most CNV's that are enriched in epilepsy cohorts have failed to find any significant risk genes. It could be that the epilepsy-linked CNVs confer risk by haploinsufficiency of single or multiple genes or by relative large increases in gene dosage through the deletion or duplication event, each mechanism leading to large differences in the amount of expressed protein.

## Environmental effects

Little progress has been made in identifying specific environmental factors that modulate or interact with these risk genes, so this area remains largely a "black box" problem (Berg et al., 2010). Environmental effects are well known to induce seizures and include the effects of infectious diseases, tumors, autoimmune disorders, toxic exposures and traumas. Although many of these events are associated with abnormalities in certain genetic or molecular pathways, it has not been possible to implicate these related environmental factors as contributors to the general heritable problem of idiopathic epilepsy in the population.

Taken together the molecular studies illustrate how multiplex the problem of epilepsy is likely to be. These studies stand in contrast to the "single gene" epilepsy's in which established mutations lead predictably to disease or lead to an established high risk for the seizure phenotype.

## Single Gene Disorders

### *SCN1A*

The *SCN1A* gene is located at chromosome 2q24 and encodes a component of a sodium channel gene which is widely expressed in the brain. This component consists of a voltage sensor and a sodium conducting pore. The 2q24 region contains a cluster of sodium channel genes within a 1.4 Mb area and includes *SCN3A*, *SCN2A*, *SCN 9A* and *SCN7A* (Meisler et al., 2010). The intron and exon structure of *SCN1A* is identical to *SCN8A* on 12q13.13.

Mutations that disrupt function of *SCN1A* play a major role in single gene related epilepsy and it is the most clinically relevant epilepsy gene (Mulley et al., 2005). *SCN1A* mutations were initially identified in individuals having genetic (generalized) epilepsy with febrile seizures plus (GEFS$^+$) (Escayg et al., 2000; Scheffer et al., 2009). GEFS+ is a familial epilepsy syndrome that expresses various different seizure phenotypes from classical febrile seizures, febrile seizures plus, followed by a chronic course of generalized and sometimes focal seizures. Shortly after this discovery, *SCN1A* mutations were identified in individuals with Dravet Syndrome or severe myoclonic epilepsy of infancy (SMEI) (Claes et al., 2001). Dravet Syndrome is a severe condition having seizure onset before the first year of life in an otherwise normal infant. Between one and four years, myoclonic, atypical absences, focal and other types of seizures appear. There is psychomotor delay and often an ataxic or clumsy-like movement problem. Dravet Syndrome can be associated with frequent status epilepticus and resistance to anticonvulsant treatment (Dravet, 1978; Dravet, 2011). As experience increased with mutation analysis of *SCN1A*, additional *SCN1A*-associated epilepsy phenotypes were identified including borderline cases of SMEI (SMEB) and intractable childhood epilepsy with generalized tonic clonic seizures (ICEGTC). *SCN1A* mutations have not been described as associated with infantile spasms.

### *ARX*

The *ARX* (*Aristaless*-related homeobox) gene is one of the more frequently mutated genes associated with brain malformations, seizures and/or intellectual deficiency. *ARX* is relevant to epilepsy since its mutations can cause the EEG findings of hypsarrhythmia and burst suppression seen in the West (Kato et al., 2003; Stromme et al., 2002) and Ohtahara syndromes(Giordano et al., 2010; Kato et al., 2010) .

*ARX* maps to region Xp22.13, and it is known to be a regulator of multiple genes. Since *ARX* is on the X chromosome, those identified with mutations are typically males although females may be less affected. Familial seizure disorders with X-linked inheritance and onset in infancy are thus prime candidates for consideration for *ARX* mutation testing. The clinical spectrum of *ARX* mutations is immense, ranging from X-linked lissencephaly with ambiguous genitalia (XLAG) to nonspecific intellectual deficiency; ten different clinical disorders have been associated with *ARX* mutations (Kato et al., 2004; Shoubridge et al., 2010). Although mutations have been described in various regions of *ARX*, seven recurrent mutations account for about 70% of the approximate 100 families reported.

Table 1: Major Epilepsy Genes

| Syndrome/ Condition | Location | Gene | Protein Function | Inherit. |
|---|---|---|---|---|
| BFNS1<br>BFNS2 | 20q13.3<br>8q24 | *KCNQ2*<br>*KCNQ3* | K channel<br>K channel | AD |
| BFNIS3 | 2q23-q24.3 | *SCN2A* | Na α-subunit | |
| GEFS+ | 2q23-q24.3<br>2q23-q24.3<br>19q13.1<br>5q31.1-q33.1 | *SCN1A*<br>*SCN2A*<br>*SCN1B*<br>*GABRG2* | Na α-subunit<br>Na α-subunit<br>Na β-subunit<br>GABA A-type subunit | AD |
| Dravet/SMEI | 2q23-q24.3 | *SCN1A* | Na α-subunit | AD |
| Ohtahara/EIEE syndrome; | 9q34.11<br>Xp22.13 | *STXBP1*<br>*ARX* | Involved in synaptic vesicle release<br>Homeobox gene | AD |
| EFMR | Xq22 | *PCDH19* | Member of protocadherin family, cell-cell adhesion | XLD |
| ADNFLE | 20q13.3<br>1q21<br>8p21 | *CHRNA4*<br>*CHRNB2*<br>*CHRNA2* | Acetylcholine receptor subunit $\alpha$4-subunit<br>β2-subunit<br>$\alpha$2-subunit | AD |
| EJM1<br>EJM5<br>EJM6<br>EJM7<br>EJM8 | 6p12-6 p11<br>5q33<br>2q22-23<br>1p36.3<br>3q26 | *EFHC1*<br>*GABRA1*<br>*CACNB4*<br>*GABRD*<br>*CLCN2* | Calcium homeostasis<br>GABA A-type subunit<br>Calcium channel subunit<br>GABA subunit<br>Chloride channel | AD |
| ADLTEAF | 10q24 | *LGI1* | Secreted protein with a G- protein component interaction domain | AD |

Table 2: Seizure Disorders Associated with Clinical Syndromes

| Syndrome | Location | Gene | Protein Function |
|---|---|---|---|
| Rett | xq28 | *MeCP2* | Methylation binding protein |
| Angelman | 15q11.2-q13 | *UBE3A* | Ubiquitin ligase enzyme |
| Fragile X | Xq27.3 | *FMR1* | Translation repressor |
| ARX disorder (Infantile spasm; lissencephaly; variable phenotype) | Xp22.13 | *ARX* | Homeobox |
| CDKL5 disorder (Rett-like phenotype) | Xp22 | *STK9 (CDKL5)* | Serine-Threonine Protein Kinase |
| Cortical dysplasia-focal epilepsy syndrome (CDFE) | 7q35-36 | *CNTNAP2 (CASPR2)* | Neurexin super family, cell-cell adhesion |
| EAST syndrome: epilepsy, ataxia, sensorineural hearing loss, tubulopathy | 1q23.2 | *KCNJ10* | Potassium channel protein |

Table 3: Chromosome Disorders Associated with Epilepsy

| Chromosome region | Syndrome name | Gene | Percent with Seizures |
|---|---|---|---|
| Del 16p13.11 | CNV | - | ?<50% |
| Del 15q13.3 | CNV | ?*CHRN7* | 50% |
| Del 15q11.2 | CNV | - | ?20% |
| Del 1p36 | 1p36 microdeletion syndrome | - | >50 |
| Del 4p16.3 | Wolf-Hirschhorn syndrome | - | >50 |
| Trisomy 12p | Trisomy 12p syndrome | - | ? |
| Ring 14 | Ring 14 syndrome | - | ? |
| Del 15q11.2-q13 | Angelman syndrome | *UBE3A* | >50 |
| Dup 15q11.2-q13<br>Idic(15) | Autism/PDD<br>Severe DD/ID, epilepsy, autism | -<br>- | 10-30<br>>50 |
| Del 17p13.3 | Lissencephaly, Type 1 | *LIS1* | >50 |
| Ring 20(p13;q13.3) | Ring 20 syndrome | - | >50 |
| Trisomy 21 | Down syndrome | - | <10 |
| Del 22q11.2 | Velo-cardio-facial, DiGeorge | - | <10 |
| XXY | Klinefelter syndrome | - | 10-20% |

## Other genes of varied function

Another X-linked gene, *PCDH19*, cause a disorder of epilepsy and mental retardation limited to females (EFMR) (Dibbens et al., 2008) with a clinical presentation that mimics Dravet syndrome (Depienne et al., 2011). Disruption of *UBE3A*, is responsible for Angelman syndrome. 90% of AS patients have epilepsy (Zori et al., 1992), with atypical absences, myoclonic seizures, GTC, and unilateral seizures. Mutations in *STXBP1* have been linked to Ohtahara syndrome or early infantile epileptic encephalopathy with suppression burst (EIEE) disorder, and the encoded protein appears to be involved in synaptic vesicle release (Saitsu et al., 2008). Disruption of *MECP2* causes Rett syndrome. *MECP2,* like *UBE3A* appears crucial to the process of neuronal plasticity. *STK9* (*CDKL5*) mutations impair a serine/threonine protein kinase, an enzyme involved in intracellular signaling (Tao et al., 2004; Weaving et al., 2004). Such mutations have been reported in patients with severe neurodevelopmental disorders characterized by early-onset seizures, infantile spasms, some Rett syndrome features (deceleration of head growth, stereotypies, and hand apraxia), and refractory epilepsy (Bahi-Buisson et al., 2008c). Mutations in *CNTNAP2* (Neurexin IV) disrupt a protein that is a member of the Neurexin super family and cause the cortical dysplasia focal epilepsy syndrome (CDFE) (Strauss et al., 2006). Neurexin IV resides in the synaptic cleft in the nodes of Ranvier. Identification of these and other genes illustrate the enormous cell complexity and varied pathophysiology that leads to epilepsy, including defects in cell membrane function, synaptic development and maintenance, intracellular signaling and transcriptional regulation.

## Conclusion

The common epilepsies arise from a complex mixture of multiple genes and environmental effects and conform to the theoretical model of multifactorial inheritance. Although many epilepsy syndromes are attributed to mutations in single genes, the common epilepsies have remained elusive regarding identifying individual risk genes that have even a moderate effect. It is thus likely that a large repertoire of genes confer only mild risk effects for epilepsy. Availability of more refined genetic and biomolecular analyses will further delineate the genetic structure of the human genome and proteonome. Hopefully this refinement will help identify epilepsy risk genes and help clarify how certain CNVs contribute to epilepsy risk. As more knowledge about the polygenic aspects of epilepsy emerges, multiplex gene sequencing testing might then be used for diagnosis and early identification of many genetic-based epilepsy disorders.

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## *About the RCPU*

The Raymond C. Philips Research and Education Unit began in 1978 when the legislature established section 393.20, F.S., of what is now known as the "prevention" legislation. It is named after Raymond C. Philips, who was the Superintendent of Gainesville's Tacachale (formerly Sunland) Center for 38 years, and was an acknowledged state and national leader in services for mentally retarded persons. The Unit is located on the Tacachale campus and is funded through a contract with the Department of Children and Families and the Department of Health.

The purpose of the R.C.P.U. is to treat, prevent, and/or ameliorate mental retardation through medical evaluations, education and research. The unit provides direct evaluations and counseling to families and promotes service, education, and prevention projects.
Some of the conditions currently under study at the RCPU involve Angelman, Velo-Cardio-Facial, Prader-Willi, Fragile X, Williams and Smith-Lemli-Opitz syndromes.

The R.C. Philips Unit is a resource for all Floridians interested in the diagnosis, treatment and prevention of mental retardation. Staff members are available for consultation and for educational programs for health professionals and for the community at large.

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