Genetics of epilepsy: Epilepsy Research Foundation workshop report

Sanjay Sisodiya, J. Helen Cross, Ingmar Blümcke, David Chadwick, John Craig, Peter B. Crino, Paul Debenham, Norman Delanty, Frances Elmslie, Mark Gardiner, Jeffrey Golden, David Goldstein, David A. Greenberg, Renzo Guerrini, Michael Hanna, John Harris, Paul Harrison, Michael R. Johnson, George Kirov, Dimitri M. Kullman, Andrew Makoff, Carla Marini, Rima Nabbout, Lina Nashef, Jeffrey L. Noebels, Ruth Ottman, Munir Pirmohamed, Asla Pitkänen, Ingrid Scheffer, Simon Shorvon, Graeme Sills, Nicholas Wood, Sameer Zuberi

Epilepsy Research Foundation, United Kingdom

ABSTRACT – The Sixth Epilepsy Research Foundation workshop, held in Oxford in March 2006, brought together basic scientists, geneticists, epidemiologists, statisticians, pharmacologists and clinicians to consider progress, issues and strategies for harnessing genetics to improve the understanding and treatment of the epilepsies. General principles were considered, including the fundamental importance of clear study design, adequate patient numbers, defined phenotypes, robust statistical data handling, and follow-up of genetic discoveries. Topics where some progress had been made were considered including chromosomal abnormalities, neurodevelopment, hippocampal sclerosis, juvenile myoclonic epilepsy, focal cortical dysplasia and pharmacogenetics. The ethical aspects of epilepsy genetics were reviewed. Principles and limitations of collaboration were discussed. Presentations and their matched discussions are produced here. There was optimism that further genetic research in epilepsy was not only feasible, but might lead to improvements in the lives of people with epilepsy.

Key words: epilepsy, genetics, pharmacogenetics, anti-epileptic drug, chromosome
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What is the role of genetics in epilepsy?

Ingrid E. Scheffer1,2, Leanne Dibbens34, Samuel F. Berkovic1, John C. Mulley35

1Department of Medicine, The University of Melbourne, Austin Health, Heidelberg, Victoria 3084, Australia. <scheffer@unimelb.edu.au>
2Department of Paediatrics, The University of Melbourne, Royal Children's Hospital, Parkville, Victoria 3052, Australia
3Department of Genetic Medicine, Women's and Children's Hospital, North Adelaide, South Australia 5006, Australia.
Departments of 4Paediatrics and 5Molecular and Biomedical Sciences, The University of Adelaide, South Australia 5005, Australia.

Over the past two decades molecular genetics has increasingly found application in diagnosis, understanding of biological mechanisms and treatment in virtually all fields of medicine. Epilepsy is no exception and over the past decade, significant insights have been gained into the role that genes play.

Clinical genetics has long acknowledged the key role of genetics in epilepsy: as long ago as 400 BC, Hippocrates recognised that epilepsy had a hereditary component. Twin studies have highlighted the key role of genetics in the idiopathic generalised epilepsies where monozygotic twins have case-wise concordance rates of 0.81, with even higher concordance rates when epileptiform abnormalities on EEG are taken into account (Berkovic et al. 1998). Importantly, twin studies show that focal epilepsies also have a genetic component with case-wise concordance rates of 0.36 in monozygotic twins compared with 0.05 in dizygotic twins.

Febrile seizures, an example of gene-environment interaction whereby a fever is an essential trigger for a seizure to occur, have an intermediate concordance of 0.58 in monozygotic twins. These findings were first determined by Lennox who encapsulated the concept of a spectrum of genetic and environmental factors underlying an individual's seizure disorder. At one end, a patient may have epilepsy entirely due to genetic factors while at the other end, environmentally acquired factors are responsible, with many individuals falling along the spectrum with the aetiology of their epilepsy including both genetic and acquired factors. Complicating the picture is the realisation that most febrile and afebrile seizures seen in the clinics are multifactorial with the underlying genetic basis being polygenic.

The framework of the International League Against Epilepsy classification of the epilepsies divides epilepsies broadly into the generalised and focal epilepsies, which are then further broken down into idiopathic and symptomatic categories (Commission on Classification and Terminology 1989). The idiopathic generalised and focal categories have been defined as having no known cause apart from hereditary factors. Whilst this is well accepted for the idiopathic generalised epilepsies, it has recently been drawn into question for the archetypal form of idiopathic focal epilepsy, benign childhood epilepsy with centrotoral spikes. Twin studies of 18 monozygotic pairs with this syndrome showed zero concordance, challenging the dogma that this is primarily a genetic disorder (Vadlamudi et al. 2006), a finding that requires replication with larger studies.

In contrast, there is increasing evidence of the role of genes in the symptomatic/cryptogenic epilepsies. Amongst the generalised epilepsies these broadly include syndromes such as Lennox-Gastaut syndrome and the “genetic” form of myoclonic-astatic epilepsy or Doose syndrome. The latter disorder usually is assumed to have a polygenic basis. In rare cases, mutations in sodium channel subunit genes SCN1A, SCN1B, or a GABA receptor subunit gene GABRG2, have been reported where familial mutations are presumably one of a number of genes contributing to a more severe phenotype (Scheffer et al. 2005). Similarly, genes have a role in the symptomatic focal epilepsies. The most straightforward example is where a malformation, such as subcortical band heterotopia, is associated with a known gene mutation (Guerrini and Marini 2006).

Genetics has already had a practical impact on clinical practice. The most salient example is severe myoclonic epilepsy of infancy (SMEI) or Dravet syndrome. In this devastating epileptic encephalopathy, around 70% individuals have a mutation of SCN1A, the gene encoding the α1 subunit of the sodium channel with most mutations arising de novo (Mulley et al. 2005b). Finding a SCN1A mutation obviates the need for further investigation. The diagnosis guides choice of AED therapy, as lamotrigine may exacerbate seizures in Dravet syndrome whereas topiramate and stiripentol are often effective (Chiron et al. 2000). It is likely that seizure control will improve developmental outcome so earlier precise diagnosis is critical.

Currently about 14 genes are confirmed for idiopathic epilepsy but these have largely been found in rare Mendelian disorders, often in large families with multiple affected members. The majority encode ion channel subunits with mutations discovered in both voltage-gated and ligand-gated channels. Mutations in voltage-gated subunits include sodium, calcium, potassium and chloride channels and are associated with various forms of generalised epilepsies. Mutations in ligand-gated ion channel subunits involve GABAergic receptors and nicotinic acetylcholine receptors;
the former are associated with generalised epilepsies, the latter with autosomal dominant nocturnal frontal lobe epilepsy (Gourfinkel-An et al. 2004). Of the non-ion channel subunits, LGII mutations are associated with a familial form of lateral temporal lobe epilepsy; however, LGII has recently been shown to have a critical role in potassium channel function, reinforcing the central role of ion channels in the epilepsies (Schulte et al. 2006). This alerts us to a whole new class of candidate genes, apart from ion channel genes, that are associated with or interact with ion channels.

The greatest challenge is to solve the complex genetics underlying the common forms of idiopathic epilepsy where a number of genes and possibly environmental factors contribute to the aetiology of an epilepsy syndrome (Mulley et al. 2005a). Common idiopathic epilepsies are likely to be oligogenic although the requisite number of susceptibility alleles to produce a phenotype is not known. Each susceptibility allele is likely to have a small functional effect and it will be the cumulative effect of a number of alleles with epistatic (gene-gene) and epigenetic effects adding further to the equation. Functional effects consistent with predisposition to epilepsy have been found experimentally for naturally occurring genetic variation found in a calcium channel gene and a GABA receptor subunit gene. Models for the genetic architecture underlying the complex epilepsies have been suggested (Mulley et al. 2005a) but as yet there is little data upon which such models can be refined. It is likely that there will be a pool of susceptibility genes in the population from which polymorphisms and rare variants will contribute to an individual’s risk. Various combinations and permutations will then produce a specific sub-syndrome or phenotype.

The first substantive step toward assessing the extent of the genetic load attributable to ion channel variation (channelopathy load) is now underway with the advent of technology and expertise to support large scale resequencing of many ion channels in patients with complex idiopathic epilepsies. Running in parallel are applications to complex epilepsies of statistical approaches now applied to complex disorders in general. This approach is potentially the best non-candidate gene approach and perhaps the only hope at present for an assessment of the non-channelopathy component of the susceptibility load for the epilepsies. However, the power of this approach may be limited for the epilepsies depending upon the extent of genetic heterogeneity and effect sizes of susceptibility genes underlying genetic predisposition (Tan et al. 2006).

How then can one determine the significance of additive or interactive acquired environmental factors on the genetic susceptibility load of a person to render them prone to seizures? There is increasing evidence for acquired channelopathies where environmentally acquired insults such as trauma, hypoxia or vascular lesions result in changes in transcription, assembly or function of ion channels (Berkovic et al. 2006). It may be that acquired genetic or epigenetic lesions augment inherited predisposing genes to result in a specific phenotype at a given age. There is thus increasing evidence of the importance of genetics in epilepsy in both the inherited and acquired domains.

 Whilst during the past decade we have advanced to an established appreciation of the role of genetics in epilepsy, we are currently at the tip of the iceberg in terms of truly characterising the full extent of genomic variation related to multifactorial or complex epilepsy.

References
Modern molecular genetics has great potential power to identify underlying mechanisms of seizures and epilepsies, but its ultimate impact will be judged by its ability to deliver improved patient care across the whole range of human epilepsy. To do this it will have to address successfully a whole range of complexities resulting from the extreme heterogeneity of epilepsy as a human disorder.

Currently, to the outside observer, genetics research concentrates on a search for susceptibility genes, where the main success has been in identifying single gene disorders in unusual pedigrees. The relevance of the identified genes to the greater volume of genetically determined epilepsy remains uncertain. There have to be concerns that the current approach may lack efficiency in delivering real clinical advances. It is unlikely that effective interventions will be developed for the potentially large number of primary genetic abnormalities. It is more likely that we will need to understand down-stream effects and later common pathways in order to develop new targets for intervention that will be of interest to pharmaceutical companies in order to deliver accessible treatments.

It seems more likely that modern genetics might impact first on everyday clinical care through pharmacogenetics. Here there may be more readily answerable questions. The lowest hanging fruit is likely to be identifying genes which increase the risk of serious adverse events, something that could increase usage of effective drugs currently avoided such as vigabatrin and felbamate. This seems to be an area of lesser interest to researchers than the search for genes that could influence efficacy of drugs. Here we see a rush of investigations which target transporter systems as potential agents of drug resistance. These give us an indication of how difficult it will be to reach firm conclusions in an area where the very concept of drug resistance is controversial. We surely need some common definition of resistance and large scale collaborative studies with adequate power to identify genes of importance or more challengingly exclude other genes’ contributions. We will also need to ensure that genetic information adds greater precision to prediction of prognosis over and above that provided by clinical prediction alone, another considerable hurdle.

Currently, we are at a point where there seems to be unrestrained enthusiasm for genetic approaches to medicine in general and epilepsy in particular. The pendulum will inevitably swing back from this point as we try to tease out real advances from within the complexity that is epilepsy.

John Harris
CSEP/IMLAB, School of Law, University of Manchester, Williamson Building, Oxford Road, Manchester M13 9PL, UK
<john.harris@manchester.ac.uk>

Increasing understanding of the genetics of any condition, whether that condition is part of normal functioning or species-typical functioning or whether it is thought of as a disease or disability, raises acute problems concerning the privacy of genetic information, data security of that information and issues of potential discrimination.

I describe an attempt by John Sulston and myself to address some of these concerns by proposing a “principle of genetic equity” (Harris and Sulston 2004). There is a strong presumption in our society, and indeed in most others, that individuals’ moral claims derive from their dignity and standing as human persons. This dignity and standing might be explained or justified in a number of ways, although usually they are neither explained not justified but simply taken as given, as a sort of axiom in the system or as a familiar and accepted principle of a shared morality.

When we appeal for example to “human rights” we are appealing to and drawing upon what we believe to be a shared morality, a set of agreed long standing norms that are so generally accepted that we can rely on others both understanding and responding to our appeal.

A principle of genetic equity

We therefore proposed a Principle of Genetic Equity: “Humans are born equal; they are entitled to freedom from discrimination and equality of opportunity to flourish; genetic information may not be used to limit that equality.”

It follows that neither genetic constitution nor genetic information should be the basis of discrimination or stigmatisation of an individual, family or group. No one’s genes, or genetic information about them, can or should derogate from their equal standing and dignity in the human community and their equal entitlement to the concern, respect and protection of others or of society.

Gene-relative moral claims

This Principle of Genetic Equity is, as we have suggested, the application of a more general principle. That, more general principle, might be taken as asserting: that each person is entitled to the same concern, respect and protection of society as is accorded to any other person. This principle of equality has the advantage of...
very wide appeal and acceptance, and versions of it are enshrined in many national constitutions throughout the world, for example those of the United States of America and France, and in various declarations of human rights (Universal Declaration of Human Rights 1948, European Convention for the Protection of Human Rights and Fundamental Freedoms 1950). The Principle of Genetic Equity reminds us that the principle of equality applies as much in the face of discrimination on the basis of genome as it does to discrimination on the basis of gender, race and other arbitrary features. The Principle of Genetic Equity derives from strong moral arguments; it sums up those arguments and presents their conclusions in a form which also acquires an independent resonance and appeal. The strength of a clearly articulated principle which is resonant enough to be inspiring and at the same time firmly grounded in established moral theory, custom and practice, is that it can inform and guide a community’s approach to a broad range of legislative, policy and funding initiatives.

Conclusion

Genetic equity is already well established for certain traits, notably gender and colour. With the rapidly increasing ability of science to analyse genetic information, the time has come to extend the concept of genetic equity so as to guarantee non-discrimination to everyone.

References


Table 1. Two scenarios for the impact of the discovery of a genetic predictive test on the care of patients with epilepsy.

<table>
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<tr>
<th>Scenario</th>
<th>Description</th>
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<tr>
<td>(1)</td>
<td>The test brings disorder definition, but no advance or advantage in care or treatment</td>
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<tr>
<td></td>
<td>- It has value to confirm or exclude the disorder</td>
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<tr>
<td></td>
<td>- It could enable pre-conception, or pre-natal, decisions but with no insight into care improvements</td>
</tr>
<tr>
<td></td>
<td>- It may enable future treatment research</td>
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<tr>
<td></td>
<td>- It may, or may not help with current education, employment and insurance considerations</td>
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<tr>
<td></td>
<td>- It might raise issues with relatives of the patient and whether they want to know their status or not.</td>
</tr>
<tr>
<td>(2)</td>
<td>The test brings clinical definition, informs drug management, advances quality of life</td>
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<tr>
<td></td>
<td>- It has value beyond confirming or excluding a definition of epilepsy</td>
</tr>
<tr>
<td></td>
<td>- It is concordant with tailored medication for the disorder</td>
</tr>
<tr>
<td></td>
<td>- Education, employment and insurance issues can be realistically considered</td>
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<tr>
<td></td>
<td>- It will aid the avoidance of adverse reactions</td>
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<tr>
<td></td>
<td>- It will encourage new treatment research and regimes.</td>
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Discussion by Paul Debenham

LGC, Queens Rd, Teddington TW11 0LY, UK <paul.debenham@lgc.co.uk>

International scientific effort is focused on investigating the genetic contribution to epilepsy. It is to be hoped that such studies will identify meaningful associations that in effect create a DNA diagnostic test linked to the definition of a subtype of the disorder, or a predictor of response to treatment. In planning for the application of such a test there are a number of social perspectives that arise, particularly for a predictive test for epilepsy. The impact of the actual discovery in practice will depend on the level of definition it provides to the care of patients with epilepsy. Table 1 details some considerations in this respect. Ideally the test would be of the second class with no obvious negative social consequences. In practice the current state of the science is more to do with genetic associations that adjust the risk of epilepsy for a patient; thus tests are still some way away from even attaining the capabilities of the first class.

The introduction of any such test is a radical development that needs to find its place within our healthcare system. The psychiatric care community is perhaps most forward in its thinking with respect to the application of DNA information. However the greater healthcare community that manages the testing process may not be so ready to implement any such test. Table 2 reflects on some early experience with a predictive test in the related field of clozapine response in patients with schizophrenia.
Table 2. Early experience with a predictive test for clozapine response in patients with schizophrenia.

| Pharmacogenetics is understood amongst psychiatric medicine professionals. |
| Current medication practice for patients with schizophrenia involves a progression of different drug treatments over many years. |
| The use of a genetic test to aid medication care was well received, particularly supporting the use of clozapine. |
| The actual decision to apply the test seems to depend on differing hierarchies of patient and budget management in different mental health care organisations. |
| Achieving clarity with respect to what the test result means to clinicians (and patients) is most important. |
| Matters of patient consent, a central issue for DNA tests, can be complicated with the patient base for this test. |
| Although a DNA test service, there is a requirement for compliance with European Union In Vitro Diagnostics Directive (European Parliament and Council 1998). This is not a straightforward matter. |

Should epidemiology guide genetics or vice-versa?

Ruth Ottman

Department of Epidemiology, Mailman School of Public Health, and Department of Neurology and GH Sergievsky Center, Columbia University, New York, NY 10032, USA
<ro6@columbia.edu>

Epidemiology plays an extremely important role in guiding genetic research on the epilepsies. This role includes guidance in data collection methodology, study design, and selecting clinically defined subgroups likely to be most informative for genetic studies. Epidemiology is the study of the distribution and determinants of health-related states in populations (Rothman and Greenland 1998). It includes both descriptive studies designed to evaluate disease prevalence or incidence, and analytical studies designed to evaluate the effects of exposures on disease risk. For all of these study designs, measurement plays a crucial role: the validity of study results often rests on accurate and unbiased measurement of disease and exposures. Consequently epidemiologists devote considerable efforts to ensuring reliable and accurate measurement, including development of data collection strategies that can be used consistently on a large scale (such as standardised questionnaires and instruments for abstracting information from medical records), and development of operational criteria for classification that can be applied consistently across studies, or across sites within a multi-site study.

Standardised measurement is also extremely important in genetic research. The clinical and genetic heterogeneity of the epilepsies presents formidable challenges for genetic analysis, making it essential to divide patients and families into subgroups likely to share susceptibility genes. The clinical features relevant for classification in genetic studies are not restricted to the epilepsy syndromes as currently defined, but include characteristics such as seizure types (Winawer et al. 2003, Winawer et al. 2005, Durner et al. 2001) and photosensitivity (Tauer et al. 2005) within the idiopathic generalised epilepsies, seizure semiology within the localisation-related epilepsies (Ottman et al. 2004), and AED resistance (Tate et al. 2005, Soranzo et al. 2005). The International League Against Epilepsy has provided systems for classification of seizures (Commission on Classification and Terminology 1981) and syndromes (Commission on Classification and Terminology 1989), and a system based exclusively on seizure semiology has also been developed (Luders et al. 1998). However, these systems do not encompass all of the features relevant for classification in genetic studies. Moreover, they have not been accompanied by operational criteria to ensure that different diagnosticians interpret patients’ signs and symptoms in the same way, or standardised methods for data collection to ensure that the same questions are asked of each patient and no essential information is omitted. Questionnaires for this purpose have been developed and validated in epidemiologic studies (Ottman et al. 1990, Ottman et al. 1993, Reutens et al. 1992).

Analytic epidemiologic studies have been used to evaluate the impact of family history on disease risk (Annegers 1991), providing important information to guide the selection of patients for molecular studies. These studies have addressed the magnitude of increased risk of epilepsy in specific relative types (Annegers et al. 1982, Ottman et al. 1988, Ottman et al. 1996b), which clinically defined subgroups are likely to have the greatest genetic contribution (Ottman et al. 1996b, Ottman et al. 1996a), and whether genetic effects are likely to be shared or distinct across epilepsy syndromes (Ottman et al. 1989, Ottman et al. 1998).

Allelic association studies are being used increasingly in efforts to identify genes in the genetically complex epilepsies. These studies are essentially epidemiologic designs in which the outcome is epilepsy (or a subtype such as pharmacoresistance) and the “risk factor” is an allelic variant, and hence epidemiologic
principles are very important for guiding research design. This includes identification of unbiased case ascertainment sources, selection of appropriate controls, and evaluation of potential confounding. Also, in these studies, very large sample sizes are needed for sufficient statistical power to detect genes with a small effect on epilepsy risk, and multi-site studies are essential to obtain these samples. The importance of using standardised measurements is accentuated in such studies, to assure consistency across sites.

In summary, epidemiology is the scientific foundation of clinical research, and its role is crucial to clinical genetic research on the epilepsies. The role of genetics in shaping epidemiologic research is less clear, although certainly the results of genetic studies should become the focus of descriptive epidemiologic studies of the prevalence of genetic variants and genetically-defined syndromes (Berkovic and Ottman 2000).

References


Discussion by Lisa Nashef

Department of Neurology, King’s College Hospital, London SE5 9RS, UK

L.Nashef@kingsch.nhs.uk

Before considering whether epidemiology should guide genetics or vice-versa, we need to reflect on our aims, namely to address strategies for elucidating the genetic basis of epilepsy. We may consider three categories for study: individuals with de novo mutations; those with Mendelian pedigrees, where the inherited genetic mutation has a strong effect, modified by genetic and environmental factors; and those with complex inheritance. The relative importance of these categories and their relationship are still to be defined. There is overlap between them, with the same genes likely to be implicated in all three. Linkage strategies, which lead to identifying causative mutations in Mendelian pedigrees, are well known, with many identified and, no doubt, many more to be found. Modifier genes and other factors influencing phenotype are yet to be understood. Sequencing is needed to identify de novo mutations, and careful phenotype assessment can help make this better targeted and therefore more efficient, as has been shown in severe myoclonic
epilepsy and related disorders. Shortcuts are unlikely, except if and where specific haplotypes in certain genes are shown to predispose to mutations. Strategies are harder when we address the (presumed) complexly inherited, a category likely to encompass true complex inheritance, de novo mutations and phenocopies. Do we go for large scale sequencing of candidate genes or for association studies, which utilise an epidemiological approach, using the most efficient methods at our disposal? If the latter, which phenotypes should be studied? Which clinical features can reasonably be grouped together? Which have the highest heritability? And consequently, what cohort size is needed to identify genes of small, moderate or large effect? By demonstrating and defining the heritability of epilepsy, epidemiology currently justifies genetic studies, but does it guide them in relation to the questions above?

The overlap of idiopathic generalised epilepsy (IGE) subtypes described in affected relatives of probands with IGE, in our view, justified considering IGE as a broad phenotype in our cohort (Chioza et al. 2002) with a minimum requirement of generalised spike wave on EEG and a compatible clinical presentation. This, of course, does not preclude, when numbers allow, analysing for subtype or subgroup specific association. Others may have used epidemiological data to guide their own work. It is difficult, however, with so many published underpowered association studies, in phenotypes with perhaps lower heritability, not to conclude that this is often not the case. Hope and expediency triumph over reason; cohorts are frequently too small and largely unsuccessful, despite a valid approach (see What is the role of replication? below).

Another important area relates to how clinical epidemiological data are collected and presented. Yet, despite, as clearly presented by Professor Ottman, “distinct genetic influences on myoclonic versus absence seizures, generalised tonic-clonic seizures within IGEs, juvenile myoclonic epilepsy versus juvenile absence epilepsy-childhood absence epilepsy combined and generalised versus focal epilepsies” (to which one may wish to add other categories with clear heritability, such as photoparoxysmal response on EEG, generalised spike wave on EEG or febrile seizures), data are often reduced to International League Against Epilepsy subtypes, both a blessing and a hindrance.

Accuracy and detail suffer where large numbers are required, and to improve consistency and reliability of data, standardisation is unavoidable, although this can result in the interesting, the unusual, the unrecognised or simply the unfashionable to be missed. More worrying is the reduction of a range of clinical information to a syndrome label, which although useful, has limitations. There are many examples of heterogeneity within syndromes, even where tightly defined. There are also examples of the same genetic defect resulting in different syndromes/phenotypes. Within the framework of the broad phenotype or syndrome under study, we advocate maintaining raw data, as this allows for analysis of cases with shared characteristics. Corrections for multiple testing are essential, and only subgroups with sufficient power should be tested, with an a priori hypothesis guided by epidemiological data.

The interaction between genetics and epidemiology is two-way. The available epidemiology should guide the design of genetic studies, and perhaps could do so better than it does now. The results of genetic studies are then followed by genotype/phenotype correlation which, as already stated, depends on quality of clinical data and consistency across studies. Classifications emerging from these correlations can then provide a new basis for new clinical epidemiological studies.

**Reference**


**What do syndromes mean in the genetic world?**

Rima Nabbout

Neuropediatrics Department, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France <rima.nabbout@nck.ap-hop-paris.fr>

Recent advances in clinical epileptology have been essential to progress in epilepsy genetics. The first step in any genetic study is a strict phenotype definition and recognition of each specific epileptic syndrome. The homogeneity of inherited epilepsy syndromes was essential in the discovery of several genes, including autosomal dominant nocturnal frontal lobe epilepsy, familial temporal lobe epilepsy, and familial partial epilepsy with variable foci (Gourinckel-An et al. 2004). The description of families with generalised epilepsy with febrile seizures plus (GEFS+) (Scheffer and Berkovic 1997) has been a major advance in epilepsy genetics. In these families the heterogeneous epilepsy phenotypes were considered to be due to the same genetic defect with an autosomal dominant transmission. Severe myoclonic epilepsy in infancy (SMEI) and myoclonic-astatic epilepsy (MAE) were reported among the various epilepsy phenotypes. This finding led to the discovery that SCN1A, the major gene reported in GEFS+ families, was involved in the pathogenesis of SMEI (Claes et al. 2001). However, in MAE, no causal mutations were found in all reported GEFS+
genes (Nabbout et al. 2003b). The genetic findings in these two syndromes confirmed the previously suspected common genetic predisposition of SMEI and febrile seizures, which is not the case in MAE. Despite some homogeneity in SMEI patients’ profiles (clinical characteristics and AED response), not all patients presented the genetic defect (Nabbout et al. 2003a). This genetic heterogeneity is common to all epilepsy syndromes. Genetic findings in epilepsy syndromes will help understand the physiopathology although a “genetic” classification is not feasible to date. The major challenge is to resolve the genetics of the common forms of epilepsy where a polygenic or even a multifactorial inheritance is suspected (Ottman 2005).

References

The genetics of schizophrenia: recent progress and lessons for epilepsy

Paul J. Harrison
University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK
<paul.harrison@psych.ox.ac.uk>

Twin studies show that schizophrenia has a high heritability, with a recent estimate of 81% (95% CI, 73-90%; Sullivan et al. 2003). Finding the chromosomal loci and genes underlying this heritability has proved difficult, almost certainly because schizophrenia is a complex genetic disorder, lacking a Mendelian form and without any genes of major effect (Owen et al. 2004). However, considerable progress has been made in the past four years or so. Two meta-analyses of the genome scans of schizophrenia found evidence for several loci that either meet, or come close to, criteria for genome-wide significance (Badner and Gershon 2002, Lewis et al. 2003) summarised in table 3. Moreover, under several of these linkage peaks – and elsewhere – genes have been identified for which case control and family association studies show associations with schizophrenia (table 4; Harrison and Weinberger 2005, Owen et al. 2005a).

For two of the genes, neuregulin 1 and dysbindin, the evidence is now strong. For example, association between neuregulin and schizophrenia has been found in 16 out of the 20 populations studied (Harrison and Lewis 2006). On the other hand, no single polymorphism nor haplotype has been shown to explain the association for any of the genes, and no coding mutations have been found — the only partial exception is the functional Val149Met polymorphism in catechol-o-methyltransferase (Tunbridge et al. 2006). Moreover, the effect size, even for the relatively robust genes, is small, typically with odds ratios of less than 1.5. The field is considering what constitutes proof for association of a small effect gene in a complex disorder and to what extent other forms of evidence, particularly biological plausibility and a molecular mechanism, should be taken into account when reviewing the candidacy of a gene (Harrison and Weinberger 2005). With regard to the latter point, two features of the current list of susceptibility genes are notable. First, as mentioned, virtually all the schizophrenia-associated polymorphisms are conservative or in non-coding regions. This suggests that, unless they are markers of an as yet unidentified coding mutation, any effect they have upon disease risk is mediated via their effect on expression of the gene (Harrison and Weinberger 2005). Empirical evidence to support this possibility has now accumulated for several of the genes. For example, a change in the relative expression of neuregulin isoforms has been found in patients with the illness, and in association with a core risk

<table>
<thead>
<tr>
<th>Table 3. Chromosomal loci linked with schizophrenia identified by meta-analysis.</th>
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<tr>
<td><strong>Strongest evidence</strong></td>
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<td>------------------------</td>
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<tr>
<td>Badner and Gershon 2002</td>
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<tr>
<td>Lewis et al. 2003</td>
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</table>

The meta-analyses differed methodologically and in their criteria for significance. The Table is a brief summary of the findings. For discussion, see Harrison and Weinberger 2005.
The genetic advances on disease classification, pathogenesis, are both going to be daunting tasks. The first issue of identifying a risk gene, and then working out in what way it contributes to disease pathogenesis, are both going to be daunting tasks. The apparent that confirming the identity of a risk gene, and then working out in what way it contributes to disease pathogenesis, are both going to be daunting tasks. The impact of the genetic advances on disease classification (Cavalleri et al. 2003, Owen et al. 2005b) and the NMDA receptor (Olney et al. 1999). Whether this attractive convergence is real or apparent remains to be seen.

In summary, schizophrenia research has reached a tantalising point. At least two probable susceptibility genes have been identified, and the first clues have been revealed as to the pathophysiological pathways that are involved. However, none of the genes can be considered proven beyond any doubt, and it is already becoming apparent that confirming the identity of a risk gene, and then working out in what way it contributes to disease pathogenesis, are both going to be daunting tasks. The impact of the genetic advances on disease classification (Cavalleri et al. 2005) and on therapeutics are also matters of active debate. With similar issues affecting the study of epilepsy (Cavalleri et al. 2005) and genetically complex disorders in general (Page et al. 2003), sharing of experiences and ideas between researchers in different disease areas may be mutually beneficial.

Acknowledgements

My group’s work is supported by the UK Medical Research Council, Stanley Medical Research Institute, Wellcome Trust, and the National Alliance for Research into Schizophrenia and Depression.

References


Table 4. Schizophrenia susceptibility genes.

<table>
<thead>
<tr>
<th>Gene abbreviation</th>
<th>Name</th>
<th>Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRG1</td>
<td>Neuregulin 1</td>
<td>8p</td>
</tr>
<tr>
<td>DTNBPI</td>
<td>Dysbindin</td>
<td>6p</td>
</tr>
<tr>
<td>DISC1</td>
<td>Disrupted in schizophrenia 1</td>
<td>1q</td>
</tr>
<tr>
<td>G72/DAOA</td>
<td>D-amino acid oxidase activator</td>
<td>13q</td>
</tr>
<tr>
<td>DAAO</td>
<td>D-amino acid oxidase</td>
<td>12q</td>
</tr>
<tr>
<td>RGS4</td>
<td>Regulator of G protein signalling-4</td>
<td>1q</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-o-methyltransferase</td>
<td>22q</td>
</tr>
</tbody>
</table>

haplotype (Law et al. 2006). Second, what is known of the function of the genes suggests that most converge upon NMDA receptor-mediated synaptic transmission (Harrison and Weinberger 2005, Harrison and Owen 2003, Moghaddam 2003). This is of interest, given that the “pre-genetic” hypotheses about the nature of schizophrenia centred upon synaptic functioning (Frankle et al. 2003, Owen et al. 2005b) and the NMDA receptor (Olney et al. 1999). Whether this attractive convergence is real or apparent remains to be seen.

In summary, schizophrenia research has reached a tantalising point. At least two probable susceptibility genes have been identified, and the first clues have been revealed as to the pathophysiological pathways that are involved. However, none of the genes can be considered proven beyond any doubt, and it is already becoming apparent that confirming the identity of a risk gene, and then working out in what way it contributes to disease pathogenesis, are both going to be daunting tasks. The impact of the genetic advances on disease classification (Cavalleri et al. 2005) and on therapeutics are also matters of active debate. With similar issues affecting the study of epilepsy (Cavalleri et al. 2005) and genetically complex disorders in general (Page et al. 2003), sharing of experiences and ideas between researchers in different disease areas may be mutually beneficial.

Acknowledgements

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References


The power of association: lessons from SCN1A and beyond

David Goldstein

Center for Population Genomics and Pharmacogenetics, Duke University, 103 Research Drive, Box 3471 DUMC, Durham, NC 27710, USA
<dgoldstein@duke.edu>

We showed that the SCN1A IVS5N+5G→A polymorphism is significantly associated with maximum dose of both phenytoin and carbamazepine in a cohort of patients with various forms of epilepsy. Dosing in epilepsy, and in many other therapeutic areas, often involves long periods of trial and error and could benefit from genetic predictors of dosing requirements. In our first study we showed that drug dose is lowest in patients with the GG genotype, intermediate in those with GA, and highest in those with AA (Tate et al. 2005). We followed up this study in an independent cohort of patients of Chinese ancestry and found a correlation between SCN1A IVS5N+5G→A and phenytoin serum concentrations at maintenance dose, again in the direction AA>GA>GG (Tate et al. 2006).

The phenotype of serum concentration at maintenance dose was chosen in order to eliminate the variation between drug doses and serum levels (likely to be caused by pharmacokinetic factors), therefore increasing the chance of detecting the effect of pharmacodynamic variation in the drug target. Interestingly, the SCN1A IVS5N+5G→A is located in the consensus site sequence located after the neonatal form of exon 5 (exon 5N) of the SCN1A gene. Preliminary data reported by Tate et al. (2005) suggest that the SCN1A IVS5N+5G→A polymorphism may control the proportion of the two transcript forms in adults. This work shows that dosing can be a valuable pharmacogenetic phenotype in epilepsy, both allowing the identification of previously unknown functional variation relevant to drug response and in suggesting that it may be possible to identify a panel of gene variants that collectively explain enough of the variation among patients in dose requirements to achieve clinical value.

Discussion by Mark Gardiner

Department of Paediatrics and Child Health, Royal Free and University College Medical School, University College London, London WC1E 6JJ, UK
<mark.gardiner@ucl.ac.uk>

A number of well-established AEDs act at least in part on neuronal sodium channels. Genes encoding such drug targets are clearly candidates for evaluation in any attempt to correlate efficacy with genetic variation. A recent report (Tate et al. 2005) documented an association between a putative “functional” variant (IVS5-91G>A) in SCN1A and the maximum dose of carbamazepine prescribed in 425 patients.

These data emphasise some of the challenges confronting investigators in the field of epilepsy pharmacogenetics. Clearly many variables intervene between the dose of a drug prescribed and the exposure of neuronal sodium channels to that drug, and it is debatable whether dosage can be used as a proxy for “sensitivity” in the absence of information on response. It is argued however that all potential confounders would act to obscure rather than drive an association, thereby enhancing the likely validity of any association which did emerge.

The definition of “functionality” and whether or not “functionality” equates with causality is a further challenge. The variant analysed appears to influence the dose of a drug prescribed and the exposure of neuronal sodium channels to that drug, and it is debatable whether dosage can be used as a proxy for “sensitivity” in the absence of information on response. It is argued however that all potential confounders would act to obscure rather than drive an association, thereby enhancing the likely validity of any association which did emerge.

The power of association: lessons from SCN1A and beyond

References


Epileptic Disord Vol. 9, No. 2, June 2007

Reference

Optimising associations: chips and pooling

George Kirov

Department of Psychological Medicine, Cardiff University, Henry Wellcome Building, Heath Park, Cardiff CF14 4XN, UK <kirov@cardiff.ac.uk>

DNA pooling with Affymetrix microarrays works! In our previous work (Kirov et al. 2006) we prepared DNA pools from the parents and offspring of the 30 Centre d’Etude du Polymorphisme Humain trios used by the HapMap project. We analysed them with Affymetrix 10K Xba 142 2.0 Arrays. The availability of the HapMap data allowed us to validate the performance of 6843 single nucleotide polymorphisms (SNPs) for which we had both complete individual and pooled genotyping data. Pooled analyses averaged over five to six micro-arrays resulted in highly reproducible results. The average error for the predicted allelic frequencies differences between the two pools, compared with the real differences, was 1.37%, and 95% of SNPs showed an error of < 3.2%. Figure 1 presents the fit between the real and predicted allele frequency differences between the two pools.

Following this we started working on DNA pooling with 500K arrays that can genotype over half a million SNPs. The 250K Nsp array performed worse than the 10k. We could not use relative allele signal scores as they were too variable, but we derived a different measure for the signal intensity, that ignores mismatch intensities. We obtained a mean error of predictions of 2.2%, translating into a higher false-positive and negative rate. This is demonstrated in figure 2 which shows a much worse fit between predicted and true frequency differences. We believe that this problem can be overcome by averaging the results of a higher number of arrays and by discarding the results of poorly performing arrays, improving a lot on the fit in figure 2. We are now working on DNA pooling for schizophrenia and using up to 12 replicate arrays.

Figure 1. Real and predicted allele frequency differences between parents and offspring: 6843 SNPs on the 10K array.

Figure 2. Approximately 2% of the ~250,000 SNPs on the array.

What is the role of replication?

Andrew Makoff

Department of Psychological Medicine, Institute of Psychiatry, Denmark Hill, London SE5 8AF, UK <a.makoff@iop.kcl.ac.uk>

Many rarer forms of epilepsy are caused by single gene defects and several have been detected using linkage studies. Common forms appear to be caused by the action of many genes but are very difficult to detect by linkage. Association studies are generally seen to provide a more powerful approach.

Table 5 shows the results of several epilepsy association studies where multiple replications of an original significant study have been attempted. These were mainly reviewed in Tan et al. 2004, but include more recent studies (Cavalleri et al. 2005, Kira et al. 2005, Tan et al. 2005, Ma et al. 2005, Stogmann et al. 2006, Ma et al. 2006, Buono et al. 2004, Lenzen et al. 2005). The interleukin $\beta C511T$ studies for temporal lobe epilepsy with hippocampal sclerosis (TLE/HS) all failed to replicate the original study and its more recent update. A subset of that updated sample with febrile convulsions (FC) does replicate an earlier...
Table 5. Epilepsy association studies with at least one significant association.

<table>
<thead>
<tr>
<th>Numbers Allele frequency</th>
<th>Cases</th>
<th>Controls</th>
<th>Cases</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Interleukin 1β C511T rs16944 (T allele)</strong></td>
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<tr>
<td>Kanemoto et al. 2000 (TLE/HS)*</td>
<td>50</td>
<td>112</td>
<td>0.63</td>
<td>0.46</td>
<td>p=0.006</td>
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<tr>
<td>Heils et al. 2000 (TLE/HS)*</td>
<td>86</td>
<td>133</td>
<td>0.37</td>
<td>0.35</td>
<td>p=0.6</td>
</tr>
<tr>
<td>Buono et al. 2001 (TLE/HS)*</td>
<td>61</td>
<td>119</td>
<td>0.3</td>
<td>0.34</td>
<td>p=0.3</td>
</tr>
<tr>
<td>Jin et al. 2003 (TLE/HS)*</td>
<td>67</td>
<td>115</td>
<td>0.52</td>
<td>0.5</td>
<td>p=0.7</td>
</tr>
<tr>
<td>Cavalleri et al. 2005 (TLE/HS)</td>
<td>131</td>
<td>364</td>
<td>0.33</td>
<td>0.34</td>
<td>p=0.9</td>
</tr>
<tr>
<td>Kanemoto et al. 2003 (TLE/HS)*</td>
<td>66</td>
<td>166</td>
<td>0.64</td>
<td>0.48</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Tilgen et al. 2002 (TLE/HS)*</td>
<td>43</td>
<td>126</td>
<td>0.29</td>
<td>0.35</td>
<td>p=0.4</td>
</tr>
<tr>
<td>Tilgen et al. 2002 (FC)*</td>
<td>99</td>
<td>126</td>
<td>0.37</td>
<td>0.35</td>
<td>p=0.8</td>
</tr>
<tr>
<td>Virta et al. 2002 (FC)*</td>
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<td>0.41</td>
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<tr>
<td>Kanemoto et al. 2003 (FC)*</td>
<td>35</td>
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<td>0.69</td>
<td>0.48</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Kira et al. 2005 (FC)*</td>
<td>168</td>
<td>158</td>
<td>0.48</td>
<td>0.43</td>
<td>p=0.2</td>
</tr>
<tr>
<td><strong>GABBR1 G1465A rs1805057 (A allele)</strong></td>
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<tr>
<td>Gambardella et al. 2003 (TLE)*</td>
<td>141</td>
<td>372</td>
<td>0.085</td>
<td>0.003</td>
<td>p=10^{-13}</td>
</tr>
<tr>
<td>Tan et al. 2005 (TLE)</td>
<td>234</td>
<td>164</td>
<td>0.002</td>
<td>0.003</td>
<td>p=0.6</td>
</tr>
<tr>
<td>Ma et al. 2005 (TLE)</td>
<td>120</td>
<td>118</td>
<td>0.005</td>
<td>0.009</td>
<td>p=0.5</td>
</tr>
<tr>
<td>Cavalleri et al. 2005 (TLE)</td>
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<td>1089</td>
<td>0.002</td>
<td>0.004</td>
<td>p=0.68</td>
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<tr>
<td>Stogmann et al. 2006 (TLE)</td>
<td>188</td>
<td>259</td>
<td>0.005</td>
<td>0</td>
<td>p=0.1</td>
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<tr>
<td>Sander et al. 1999 (IGE)*</td>
<td>113</td>
<td>130</td>
<td>0.035</td>
<td>0.054</td>
<td>p=0.4</td>
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<td>Cavalleri et al. 2005 (IGE)*</td>
<td>96</td>
<td>1089</td>
<td>0.006</td>
<td>0.004</td>
<td>p=0.7</td>
</tr>
<tr>
<td><strong>GABRG2 rs211037 (T allele)</strong></td>
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<td></td>
</tr>
<tr>
<td>Lu et al. 2002 (CAE)* (informative trios only)</td>
<td>58 trios</td>
<td>22 transmitted</td>
<td>0.57</td>
<td>0.7</td>
<td>p=0.01</td>
</tr>
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<td>Chou et al. 2003 (FC)*</td>
<td>104</td>
<td>83</td>
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<td>0.51</td>
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</tr>
<tr>
<td>Nakayama et al. 2003 (FC)*</td>
<td>94</td>
<td>106</td>
<td>0.25</td>
<td>0.21</td>
<td>p=0.2</td>
</tr>
<tr>
<td>Cavalleri et al. 2005 (FC)</td>
<td>107</td>
<td>384</td>
<td>0.007</td>
<td>0.02</td>
<td>p=0.8</td>
</tr>
<tr>
<td>Ma et al. 2006 (FC)</td>
<td>74</td>
<td>118</td>
<td>0.21</td>
<td>0.19</td>
<td>p=0.5</td>
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<tr>
<td>Kanamura et al. 2002 (IAE)*</td>
<td>135</td>
<td>154</td>
<td>0.23</td>
<td>0.21</td>
<td>p=0.6</td>
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<tr>
<td>Cavalleri et al. 2005 (IGE)*</td>
<td>96</td>
<td>384</td>
<td>0.23</td>
<td>0.21</td>
<td>p=0.6</td>
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<tr>
<td><strong>CHRNA4 C1545T Ser rs1044396 (T allele)</strong></td>
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<td>Chou et al. 2003 (FC)*</td>
<td>102</td>
<td>80</td>
<td>0.39</td>
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<tr>
<td>Mulley et al. 2004 (FC)*</td>
<td>49</td>
<td>93</td>
<td>0.5</td>
<td>0.58</td>
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<tr>
<td>Cavalleri et al. 2005 (FC)*</td>
<td>107</td>
<td>384</td>
<td>0.39</td>
<td>0.42</td>
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<tr>
<td>Steinlein et al. 1997 (IGE)*</td>
<td>194</td>
<td>162</td>
<td>0.49</td>
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<td>Chioza et al. 2000 (IGE)*</td>
<td>144</td>
<td>174</td>
<td>0.56</td>
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<td>Cavalleri et al. 2005 (IGE)</td>
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<td>384</td>
<td>0.46</td>
<td>0.42</td>
<td>p=0.5</td>
</tr>
<tr>
<td><strong>Prodynorphin 68bp repeat (L allele)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Stogmann et al. 2002 (TLE/FH)*</td>
<td>43</td>
<td>202</td>
<td>0.5</td>
<td>0.31</td>
<td>p=0.0006</td>
</tr>
<tr>
<td>Tilgen et al. 2003 (TLE/FH)*</td>
<td>46</td>
<td>205</td>
<td>0.29</td>
<td>0.31</td>
<td>p=0.7</td>
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<tr>
<td>Gambardella et al. 2003 (TLE/FH)*</td>
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<tr>
<td>Cavalleri et al. 2005 (TLE/FH)</td>
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<td>365</td>
<td>0.4</td>
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<td>p=0.04</td>
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<tr>
<td>Stratified Mantel-Haenszel analysis</td>
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<td></td>
<td></td>
<td></td>
<td>p=0.002</td>
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### Table 5

<table>
<thead>
<tr>
<th>Allele frequency</th>
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</thead>
<tbody>
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<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>KCNJ10 rs1130183 (T allele)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buono et al. 2004 German sample (IGE)</td>
<td>138</td>
<td>132</td>
<td>0.06</td>
<td>0.1</td>
<td>p=0.06</td>
</tr>
<tr>
<td>Buono et al. 2004 US sample (IGE)</td>
<td>116</td>
<td>152</td>
<td>0.04</td>
<td>0.06</td>
<td>p=0.3</td>
</tr>
<tr>
<td>Lenzen et al. 2005 (IGE)</td>
<td>446</td>
<td>660</td>
<td>0.07</td>
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<td>p=0.07</td>
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<td>Stratified Mantel-Haenszel analysis</td>
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<td></td>
<td></td>
<td></td>
<td><strong>p=0.01</strong></td>
</tr>
</tbody>
</table>

Allele frequencies refer to allele indicated.

TLE = temporal lobe epilepsy; HS = hippocampal sclerosis; FC = febrile convulsions; IGE = idiopathic generalised epilepsy; CAE = childhood absence epilepsy; IAE = idiopathic absence epilepsy; FH = familial history.

*Study reported in Tan et al. 2004.
† Studies where some of the data are included in other studies for same polymorphism.
‡ L allele = 1 or 2 repeats.

### Table 6. Other significant epilepsy association studies.

<table>
<thead>
<tr>
<th>Allele frequency</th>
<th>Numbers</th>
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<tbody>
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<td>Cases</td>
<td>Controls</td>
<td>Cases</td>
<td>Controls</td>
<td>p-value</td>
</tr>
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<td><strong>BDNF C240T rs7124442 (T allele)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanemoto et al. 2003 (PE)*</td>
<td>219</td>
<td>325</td>
<td>0.055</td>
<td>0.022</td>
<td>p=0.003</td>
</tr>
<tr>
<td>Kanemoto et al. 2003 (TLE)*</td>
<td>127</td>
<td>325</td>
<td>0.051</td>
<td>0.022</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Lohoff et al. 2005 (TLE)</td>
<td>136</td>
<td>181</td>
<td>0.051</td>
<td>0.066</td>
<td>p=0.4</td>
</tr>
<tr>
<td><strong>CHRNA4 C594T Cys rs1044394 (T allele)</strong></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Steinlein et al. 1997 (IGE)*</td>
<td>188</td>
<td>184</td>
<td>0.09</td>
<td>0.03</td>
<td>p=0.02</td>
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<tr>
<td>Chioza et al. 2000 (IGE)*</td>
<td>182</td>
<td>178</td>
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<tr>
<td><strong>CACNA1A Exon 8 SNP rs2248069 (G allele)</strong></td>
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<td>Chioza et al. 2001 (IGE)*</td>
<td>191</td>
<td>200</td>
<td>0.25</td>
<td>0.37</td>
<td>p=0.0003</td>
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<td>TDT analysis (informative trios only)*</td>
<td>65 trios</td>
<td>23 transmitted</td>
<td>42 untransmitted</td>
<td></td>
<td>p=0.02</td>
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<tr>
<td>Sander et al. 2002 (IGE)*</td>
<td>354</td>
<td>186</td>
<td>0.33</td>
<td>0.32</td>
<td>p=0.7</td>
</tr>
<tr>
<td>TDT analysis (informative trios only)*</td>
<td>97 trios</td>
<td>47 transmitted</td>
<td>50 untransmitted</td>
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<td>p=0.8</td>
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<td><strong>OPRM1 A118G rs1799971 (G allele)</strong></td>
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<td>Sander et al. 2000 (IAE)*</td>
<td>72</td>
<td>340</td>
<td>0.14</td>
<td>0.08</td>
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<td>Barratt et al. 2006 (IGE)</td>
<td>230</td>
<td>246</td>
<td>0.15</td>
<td>0.15</td>
<td>p=0.8</td>
</tr>
<tr>
<td>Barratt et al. 2006 (IAE)†</td>
<td>105</td>
<td>246</td>
<td>0.14</td>
<td>0.15</td>
<td>p=0.7</td>
</tr>
<tr>
<td><strong>AE3 Ala867Asp rs635311 (A allele)</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Sander et al. 2002 (IGE)*</td>
<td>366</td>
<td>183</td>
<td>0.27</td>
<td>0.21</td>
<td>p=0.02</td>
</tr>
<tr>
<td><strong>DAT 3' 40bp repeat (9 repeats allele)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sander et al. 2000 (IGE)*</td>
<td>130</td>
<td>223</td>
<td>0.3</td>
<td>0.23</td>
<td>p=0.04</td>
</tr>
</tbody>
</table>

Allele frequencies refer to allele indicated.

PE = partial epilepsy; TLE = temporal lobe epilepsy; IGE = idiopathic generalised epilepsy; IAE = idiopathic absence epilepsy; TDT = transmission desequilibrium test.

*Study reported in Tan et al. 2004.
† Studies where some of the data are included in other studies for same polymorphism.
significant association with FC, but both have a small number of cases and two other studies failed to replicate. Several GABBR1 G1465A studies all failed to replicate an apparently strongly significant initial study, but the very low minor allele frequency makes this single nucleotide polymorphism particularly vulnerable to errors. Only two polymorphisms (prodynorphin 68bp repeat and KCNJ10 rs1130183) show any evidence for agreement between studies and both are significant in stratified analyses. Table 6 shows significant associations where no or limited replication has been attempted (Tan et al. 2004, Lohoff et al. 2005).

Despite the many reported significant associations, none have been convincingly replicated in other laboratories. Conversely, most studies (including many negative studies, not shown) have examined only one or a few polymorphisms per gene, so that a negative result can only exclude the polymorphism(s) under study, but not the gene.

References
Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: “the truth is out there”. Epilepsia 2004; 45: 1429-42.

From monogenic to polygenic: insights or distractions?
Renzo Guerrini and Carla Marini
Neurogenetics Laboratory, Department of Child Neurology and Psychiatry, IRCCS Fondazione Stella Maris, Via dei Giacinti 2, Calambrone, 56018 Pisa, Italy
<renzo.guerrini@inpe.unipi.it>

Linkage analysis of extended pedigrees has successfully identified 15 genes causing several monogenic epilepsies (table 7) (Guerrini 2006, Aridon et al. 2006). Most identified genes encode components of ion channels or ligand-gated receptors (Gargus 2003). Impaired function of ion channels is almost certainly a main pathophysiological mechanism for idiopathic epilepsies (Gargus 2003). In addition, numerous families with rare or more common forms of epilepsy have been assigned to about 30 loci (table 8), providing an indication that further genes may potentially be implicated in epilepsy (Scheffer and Berkovic 2003).

Methods for identifying genes for monogenic epilepsies are well known. The discovery of these genes is facilitated by the availability of single large families with many affected members with clear Mendelian inheritance. However, we still do not know why sporadic patients with similar phenotypes do not carry the mutations identified in the families. Not many studies have specifically addressed this problem. Briellmann et al. (2001) found no differences between familial and sporadic patients with idiopathic generalised epilepsies (IGEs) in a series of 98 patients. However, familial cases included in this study were not associated to specific gene mutations. Phenotypes of patients with IGE and mutations of the GABRG2, SCN1A, GABRA1 and EFHC1 appear to overlap with those of the common IGE syndromes not associated with mutations in these genes. However, greater numbers would be required to verify the full phenotypic similarities. Bisulli et al. (2004) reported that patients with autosomal dominant lateral temporal lobe epilepsy harbouring LGI1 gene mutations were phenotypically similar to sporadic patients carrying no LGI1 mutations.

The common epilepsies have complex inheritance and their genetic characterisation has been difficult. IGEs have been a main target of genetic studies because epidemiological, family and twin studies strongly support a primary role for genetic factors. However, their genetic architecture remains elusive. Family studies have estimated a 4-10% risk of developing
epilepsy in close relatives of IGE probands (Marini et al. 2004). Higher risk is seen in siblings and offspring and is lower in second degree relatives. Polygenic inheritance, compared to monogenic inheritance, leads to a more rapid decrease in risk for relatives as the distance from affected individuals increases (Risch 1990). Therefore, current data suggest that oligogenic inheritance is more likely, with two or more genes interacting to produce the epilepsy phenotype.

There are several problems with studying the genetics of common polygenic IGEs:

- the epilepsy phenotypes within a single family vary;
- the mode of inheritance is uncertain;
- the methods for defining the genetic contribution to genetically complex conditions such IGEs, multiple sclerosis, schizophrenia or diabetes (to name only a few) have not been fully developed.

IGEs include sub-syndromes with age-related expression of seizure types for which an overlapping genetic predisposition has been shown in some families. However, recent epidemiological studies suggest distinct genetic effects in IGEs with myoclonic seizures compared with IGEs with predominant absence seizures (Winawer et al. 2005). The seizure type in the family, as opposed to the epilepsy syndrome of the proband, might be the critical phenotype for genetic studies, with the implicit assumption that loci might interact in various ways to produce the different IGE sub-syndromes. Using a similar strategy, loci predisposing to absence seizures and other loci predisposing to myoclonic seizures have been identified (Durner et al. 2001). This study suggested the existence of a locus common to most IGEs that could decrease seizure threshold but that by itself was not sufficient to produce seizures. The simultaneous presence of specific genotypes at other loci leads to seizure expression but seizure type is influenced by the identity of the other loci present. This could explain why the seizure types of family members of probands with IGEs is that of IGE but with a variable sub-syndrome.

This oligogenic model of inheritance (Durner et al. 2001), possibly comprising genes of major effect, for example ion channel subunits conferring non-specific seizure predisposition, is a very plausible model. It could also account for the presence of IGE patients without a family history of epilepsy: candidate genes may be common but clinically silent in the general population, unless the “right” combination with other loci occurs. This model is yet to be proved but rare families have been described with mutations in a single major epilepsy gene and the suggestion of a second locus (Baulac et al. 2001; Marini et al. 2003); families with common haplotypes in two loci are also known (Baulac et al. 2001). A two-gene model, one dominant and the other recessive, or both recessively inherited, has also been suggested as a model of inheritance for IGEs (Greenberg 1981).

An animal model of “digenic” inheritance carrying simultaneously mutations in *scn2a* and *kcnq2* has been reported showing a severe epilepsy phenotype (Keary et al. 2006). This observation indicates that the interaction between (these) two genes might contribute to the variable expressivity observed in human epilepsies with sodium channel mutations. However, it also indicates that the phenotype to be expected with mutations of two genes is much more severe than that seen with a single gene mutation. However, there may be some methodological drawbacks with the oligogenic model of inheritance. Genetic differences might be related to ethnic variability and this could partially explain why many studies have not been replicated. In addition, assuming that constant relationship exists between genotype and phenotype may be an oversimplification.

As noted above, the methods for defining the genetic contribution to genetically complex conditions remain a major challenge. Several authors have attempted to collect large samples of clinically well characterised multiplex IGE families for a genome-wide linkage analysis (Durner et al. 2001, Sander et al. 2000). The latter study identified several loci with putative genes, and mutations of the CLC-2 voltage-gated chloride channel (*CLCN2*) gene have been found in three families (Haug et al. 2003).

Association studies are considered to be a better tool to elucidate the genetic basis of diseases that show a high familial recurrence rate but are probably not caused by a single gene (Risch and Merikangas 1996). In the last decade more than 50 association studies have been performed but gene defects for common polygenic epilepsies have not yet been identified, leading to some scepticism about the association-study approach (reviewed by Tan et al. 2004).

Allelic association studies have identified possible risk-conferring polymorphisms but their role in IGE aetiology has yet to be confirmed (Gu et al. 2004, Heron et al. 2004, Pal et al. 2003, Greenberg et al. 2005).

None of the gene mutations identified to date in monogenic epilepsies appear to have any substantial effects on the genetic variance of common syndromes. Future strategies will need to consider the possible role of genes causing disorders that co-occur with epilepsy, such as migraine (Vanmolkot et al. 2003) or paroxysmal dyskinesia (Du et al. 2005) and of developmental genes (Guerrini 2006). However, available evidence suggests that it is not likely that findings from large pedigrees will apply to the general population.
Table 7. Genes known to cause several monogenic epilepsies identified by linkage analysis of extended pedigrees.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Locus</th>
<th>Epilepsy syndrome</th>
<th>Seizure types</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN1A</td>
<td>Sodium channel α1 subunit</td>
<td>2q24</td>
<td>GEFS+; SMEI</td>
<td>Febrile, absence, myoclonic, tonic-clonic, partial</td>
</tr>
<tr>
<td>SCN2A</td>
<td>Sodium channel α2 subunit</td>
<td>2q24</td>
<td>GEFS+; BFNC</td>
<td>Febrile, afebrile generalised tonic and tonic-clonic</td>
</tr>
<tr>
<td>SCN1B</td>
<td>Sodium channel β1 subunit</td>
<td>19q13</td>
<td>GEFS+</td>
<td>Febrile, absence, tonic-clonic, myoclonic</td>
</tr>
<tr>
<td>GABRA1</td>
<td>GABA_α1 receptor α1 subunit</td>
<td>5q34</td>
<td>AD JME</td>
<td>Tonic-clonic, myoclonic, absence</td>
</tr>
<tr>
<td>GABRG2</td>
<td>GABA_α2 receptor γ2 subunit</td>
<td>5q31</td>
<td>FS; CAE; GEFS+</td>
<td>Febrile, absence, tonic-clonic, myoclonic, clonic, partial</td>
</tr>
<tr>
<td>GABRD</td>
<td>GABA_β2 receptor β subunit</td>
<td>1p36</td>
<td>GEFS+</td>
<td>Febrile and afebrile seizures</td>
</tr>
<tr>
<td>EFHC1</td>
<td>Protein with an EF-hand motif</td>
<td>6p12-p11</td>
<td>JME</td>
<td>Tonic-clonic, myoclonic</td>
</tr>
<tr>
<td>BRD2 (RING3)</td>
<td>Nuclear transcriptional regulator</td>
<td>?</td>
<td>6p21</td>
<td>Tonic-clonic, myoclonic</td>
</tr>
<tr>
<td>KCNQ2</td>
<td>Potassium channel M current</td>
<td>20q13</td>
<td>BFNC</td>
<td>Neonatal convulsions</td>
</tr>
<tr>
<td>KCNQ3</td>
<td>Potassium channel M current</td>
<td>8q24</td>
<td>BFNC</td>
<td>Neonatal convulsions</td>
</tr>
<tr>
<td>ATP1A2</td>
<td>Na+, K^-ATPase pump</td>
<td>1q23</td>
<td>BFNC and familial hemiplegic migraine</td>
<td>Infantile convulsions</td>
</tr>
<tr>
<td>CLCN2</td>
<td>Voltage gated-chloride channel</td>
<td>3q26</td>
<td>IGEs</td>
<td>Tonic-clonic, myoclonic, absence</td>
</tr>
<tr>
<td>CHRNA4</td>
<td>Acetylcholine receptor α4 subunit</td>
<td>20q13</td>
<td>ADNFLE</td>
<td>Sleep-related focal seizures</td>
</tr>
<tr>
<td>CHRNA2</td>
<td>Acetylcholine receptor α2 subunit</td>
<td>8q</td>
<td>ADNFLE</td>
<td>Sleep-related focal seizures</td>
</tr>
<tr>
<td>CHRNB2</td>
<td>Acetylcholine receptor β2 subunit</td>
<td>1p21</td>
<td>ADNFLE</td>
<td>Sleep-related focal seizures</td>
</tr>
<tr>
<td>LGII</td>
<td>Leucine-rich, glioma-inactivated</td>
<td>10q24</td>
<td>ADPEAF</td>
<td>Partial seizures with auditory or visual hallucinations</td>
</tr>
<tr>
<td>KCNMA1</td>
<td>Calcium-sensitive potassium (BK) channel</td>
<td>10q22</td>
<td>Generalised epilepsy and paroxysmal dyskinesia</td>
<td></td>
</tr>
</tbody>
</table>

AD = autosomal dominant; ADNFLE = autosomal dominant nocturnal frontal lobe epilepsy; ADPEAF = autosomal dominant partial epilepsy with auditory features; BFNC = benign familial neonatal convulsions; BFNIC = benign familial neonatal-infantile convulsions; CAE = childhood absence epilepsy; FS = febrile seizures; GABA = gamma-aminobutyric acid; GEFS+ = generalised epilepsy with febrile seizures plus; IGE = idiopathic generalised epilepsy; JME = juvenile myoclonic epilepsy; SMEI = severe myoclonic epilepsy of infancy.
Conference report

Table 8. The published loci for idiopathic generalised epilepsies.

<table>
<thead>
<tr>
<th>Epilepsy syndrome</th>
<th>Locus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Idiopathic generalised epilepsy</td>
<td>3q26; 14q23; 2q36</td>
</tr>
<tr>
<td>Adolescent onset idiopathic generalised epilepsy</td>
<td>8p12; 18q12; 5p</td>
</tr>
<tr>
<td>Childhood absence epilepsy</td>
<td>1p; 8q24</td>
</tr>
<tr>
<td>Juvenile myoclonic epilepsy</td>
<td>6p21; 15q14</td>
</tr>
<tr>
<td>Autosomal dominant cortical myoclonus and epilepsy</td>
<td>2p11.1</td>
</tr>
<tr>
<td>Familial adult myoclonic epilepsy</td>
<td>8q23.3</td>
</tr>
<tr>
<td>Familial idiopathic myoclonic epilepsy</td>
<td>16p13</td>
</tr>
<tr>
<td>Infantile convulsions and paroxysmal choreothetosis</td>
<td>16p12</td>
</tr>
<tr>
<td>Benign familial infantile convulsions</td>
<td>19q</td>
</tr>
<tr>
<td>Benign rolandic epilepsy</td>
<td>15q24</td>
</tr>
<tr>
<td>Partial epilepsy with pericentral spikes</td>
<td>4p15</td>
</tr>
<tr>
<td>Rolandic epilepsy/exercise-induced dystonia/writer’s cramp</td>
<td>16p12</td>
</tr>
<tr>
<td>Autosomal dominant nocturnal frontal lobe epilepsy</td>
<td>15q24; 3p22; 8q11.2</td>
</tr>
<tr>
<td>Familial partial epilepsy with variable foci</td>
<td>22q11</td>
</tr>
<tr>
<td>Febrile seizures</td>
<td>2q; 5q14; 8q13; 19p; 6q22</td>
</tr>
<tr>
<td>Generalised tonic-clonic seizures</td>
<td>10q25</td>
</tr>
</tbody>
</table>

References


Tan NC, Mulley JC, Berkovic SF. Genetic association studies in epilepsy: “the truth is out there”. *Epilepsia* 2004; 45: 1429-42.


Discussion by Nicholas Wood

Department of Molecular Neuroscience, Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK <N.Wood@ion.ucl.ac.uk>

There is little doubt that genetics has contributed to our understanding of epilepsy. The successes, however, have come almost entirely from the study of monogenic, highly penetrant disorders. In this situation the genetic mutation plays a major role in determining the phenotype. Generally they are very rare but have provided useful insights for further pathophysiological investigations.

The next major genetic challenge for epilepsy is understanding the more common, or polygenic, forms of epilepsy. These are common, complex and almost certainly have a multi-factorial causation which involves both genes and environment. Not surprisingly these are much more difficult to crack. A major thrust of research in the last five or so years has been to take lessons from the genes identified in monogenic studies and ask whether these play a role in the common or-garden forms of epilepsy. Initially such studies predominately focussed on using linkage techniques but this has turned out to be a rather blunt tool, although when evidence of linkage is found this is robust. Association studies provide a much more powerful and slightly more risky approach. The risks involve problems with false positives and false negatives. They have a poor history of replication and until recently there have been some technical limitations in terms of the robustness and accuracy of large-scale genotyping platforms.

However the future for association studies is looking rosier than it has ever before, partly because the technology platforms are now robust and becoming increasingly affordable. The international HapMap project has produced a list of polymorphisms which can capture a very high percentage of the variation in the genome. It is therefore a relatively simple matter to design a genome-wide association if one has sufficient money and samples. The onus is now on the biomedical scientists to ask the “right” phenotype question. This may involve a rethinking of seizure or syndromic classifications.

On balance however the role of families should not be ignored and there is still much to be learnt and understood from the Mendelian forms of epilepsy. These two approaches are complementary rather than competitive.

Do mice tell us more about mechanisms than men?

Jeffrey L. Noebels

Developmental Neurogenetics Laboratory, Department of Neurology, Baylor College of Medicine, Houston, Texas, USA <jnoebels@bcm.tmc.edu>

Thanks to recent gene discoveries based on the study of human families with monogenic epilepsy, we can now envision the creation of biologically faithful experimental models by insertion of the identical mutated DNA sequence in genetically engineered mice. These “humanised” mouse models will soon permit precise and reproducible analysis of the effects of the mutation on the excitability of neural networks, and are necessary to understand why, where, and when abnormal synchronisation arises in the developing brain, and how best to reverse or prevent it.

While this strategy of “man to mouse” genetic replication will play a decisive role in improving the care of patients with inherited epilepsy, there is much to be learned from currently available gene models that are similar but not identical to any known human condition. Many mice bearing such epilepsy mutations, often occurring within the same gene as those identified in human or in a novel gene yet to be discovered in the clinic, have been isolated by screening spontaneous and gene-targeted mutant mice, or found among those arising from random mutagenesis programmes, and have already proven to be of exceptional research value (Noebels 2006, Burgess 2006).

Over 75 genes for epilepsy have now been reported, nearly one half of these in mice. The genes point to many different underlying biological pathways, and functional annotation identifies a clear convergence of many of these aetiological gene lesions on mechanisms that may impair synaptic inhibition (figure 3) (Noebels 2003). While many such pathways can be readily predicted based on bioinformatic data alone, only developmental analysis of the nervous system in each mouse model can pinpoint the actual intervening lesion.

As one example, studies of mice with voltage-gated ion channelopathies are providing new insight into our understanding of how downstream molecular plasticity and the microanatomy of gene expression combine to regulate the clinical phenotypes of absence and temporal lobe seizures, two prevalent forms of human epilepsy. In the first case, we are learning that calcium ion channel defects are likely to
initiate spike-wave epileptogenesis through a series of steps that begin with synapse-specific decreases in neurotransmitter release, followed by cell-specific enhancement of low voltage-activated pacemaker currents in thalamocortical circuitry (Qian and Noebels 2000, Zhang et al. 2002, Zhang et al. 2004) (figure 4). The generalised absence seizures in these mice depend upon an unstable oscillating circuit that emerges in the second postnatal week according to a normal developmental programme of molecular plasticity that shifts neurotransmitter release dependence from one pore-forming calcium channel subunit (N-type) to another (P/Q type) in selected circuits (Iwasaki et al. 2000). This model provides a likely mechanism for the age-dependent onset of thalamocortical seizures described in humans with certain mutations in (P/Q type) calcium channels (Brenner et al. 2005).

In contrast, mutation of another type of ion channel, the calcium-activated potassium channel (BK), produces a partial onset epilepsy arising from the temporal lobe. In this example, the seizures originate in the temporal lobe since the anatomical expression of the

**Figure 3.** Genes linked with human and murine epileptic phenotypes impair synaptic inhibition in the CNS via several mechanisms, including migration of interneurons, synthesis and reuptake of GABA, interneuronal excitability, neurotransmitter release, and responses of pre- and post-synaptic receptors (Noebels 2003). Reprinted, with permission, from the Annual Review of Neuroscience, Volume 26 © 2003 by Annual Reviews http://www.annualreviews.org.

**Figure 4.** Model of step-wise, developmental cascade of CNS plasticity mediating the expression of inherited spike-wave seizures. Primary inherited defects in genes controlling neurotransmitter release (mutations of voltage-gated ion channel subunits and the SNAP-25 gene for exocytosis) progress through a period of subunit switching (altered dependence of release from N type to P/Q type channels), leading to enhanced expression of low-voltage activated (T-type) calcium currents in thalamic neurons. These pacemaker currents mediate the hypersynchronisation of spike-wave seizures.
mutant regulatory subunit (B4) is restricted to dentate granule cells and adjacent neurons in entorhinal cortex (figure 5). Loss of the B4 regulatory subunit in these cells impairs the critical gating properties of the dentate network on the synaptic input to the hippocampal formation (Brenner et al. 2005). The difference in seizure type (hippocampal versus generalised absence) seen between this mouse and a human family with absence epilepsy bearing a mutation in a related alpha subunit of this BK channel (Du et al. 2005) can be explained by the more widely distributed expression pattern of the latter gene in the brain.

In summary, developmental analyses of epilepsy genes in mouse models constitute an essential step in the journey from gene errors to future therapeutic strategies involving the clinical management of inherited epilepsy.

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References


Discussion by Michael Hanna, Tracey Graves and Dimitri Kullmann

Departments of Molecular Neuroscience and Experimental Epilepsy, Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK
<mhanna@ion.ucl.ac.uk>

Ataxia and epilepsy in mice and men

In recent years we have studied the clinical and molecular genetic features of families with episodic ataxia. We have described families who exhibit epilepsy as part of the phenotype and obtained evidence that suggests dysfunction of the voltage gated calcium channel Cav2.1 can associate with human epilepsy. These findings may indicate a link with observations made by Noebels and colleagues in spontaneously occurring calcium channel mutant mice.

Figure 5. Selective expression of the B4 subunit of the calcium-activated potassium BK ion channel in entorhinal cortex and dentate gyrus regulates high frequency burst firing properties in dentate granule cells (left). Loss of the subunit in a homozygous knockout mouse impairs filtering of cortical inputs into the hippocampal network, leading to onset of spontaneous partial onset hippocampal seizures with generalisation (right). Modified from Brenner et al. 2005.
Since identification of the causative calcium channel gene CACNA1A in episodic ataxia type 2 (EA2) we and others have identified families who manifest seizures as well as ataxic episodes. The first case we identified exhibited a severe phenotype including EA2, generalised and absence seizures and mental retardation with a primary generalised EEG pattern. The truncation mutation we identified caused a severe impairment of calcium channel function and our expression data suggested that the mutant channel exhibited a dominant negative effect on wild type. This single case, in combination with the mouse observations, prompted us to assess whether the phenotypic spectrum of disease associated with CACNA1A mutations may extend to include epilepsy. It is notable that EEG abnormalities ranging from non-specific changes through to clear epileptiform changes have been widely reported in the EA2 literature both prior and subsequent to the identification of CACNA1A as the causative gene.

We were able to identify a cohort of 18 patients with a clinical phenotype of absence epilepsy and EA2. To date, novel CACNA1A mutations in two of these families have been identified. In one large North American family it was notable that in some individuals the phenotype was difficult to distinguish from pure absence epilepsy. In this family molecular expression studies provided evidence that the missense mutation is pathogenic but did not indicate a dominant negative effect.

The data obtained to date suggest that there may be a human "equivalent" of the well described calcium channel mouse mutants that exhibit epilepsy. It is suggested that the phenotypic spectrum of disease associated with mutations in CACNA1A includes epilepsy. However, there is no obvious difference between mutations in CACNA1A associated with pure EA2 and those which associated with epilepsy. It also remains to be determined if genetic variation in CACNA1A has any role in common forms of human absence epilepsy.

I have found altered gene expression during epileptogenesis: what next?

Asla Pitkänen1,2, Terhi Pirttilä1, Cagri Yalgin1, Katarzyna Lukasiuk3

1A.I. Virtanen Institute, University of Kuopio, PO Box 1627, FIN-70 211 Kuopio, Finland
<asla.pitkanen@uku.fi>

2Department of Neurology, Kuopio University Hospital, PO Box 1777, FIN-70 211 Kuopio, Finland

3Epileptogenesis Laboratory, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteur 3, 02-093 Warsaw, Poland

The reorganisation of neuronal circuits after acquired epileptogenic brain insults (e.g. traumatic brain injury [TBI], status epilepticus [SE], stroke) consists of several neurobiological alterations that are orchestrated in parallel and serial fashion. These include cellular death, axonal and dendritic plasticity, neurogenesis and gliogenesis, vascular alterations, axonal damage, and remodelling of the extracellular matrix and cellular membranes.

The application of molecular biology to study epilepsy can be divided into two broad categories: a) studies of the genetic or inherited basis of epilepsies (which will not be discussed here) and b) studies of the role of alterations in the expression levels of specific genes or ensembles of genes during epileptogenesis and in epilepsy. Recent large-scale molecular profiling studies have revealed alterations in the expression of up to 700 genes during acquired epileptogenesis (for a review, see Lukasiuk et al. 2006). These data provide a starting point for the formulation of hypotheses of the molecular mechanisms of neuronal reorganisation leading to symptomatic epilepsies. One of the altered genes is cystatin C that we and two other groups have demonstrated to be up-regulated during SE-induced epileptogenesis (Aronica et al. 2001, Elliott et al. 2003).

What is cystatin C?

Cystatin C is the most abundant extracellular inhibitor of lysosomal cysteine proteinases, namely cathepsins B, H, and L. The data on its localisation, biochemistry, and function come largely from cancer literature, and only recently have there been studies investigating cystatin C in the CNS. Therefore, many of the findings, such as the stimuli leading to increased cystatin C expression, need to be confirmed in preparations derived from the CNS. The data from other organs, however, provides an exciting starting point for these studies (for a complete list of references for this paragraph, see Pirttilä 2006).

Cystatin C (formerly known as γ-trace) is a 120-amino acid alkaline protein with a molecular weight of about 13.5 kDa. It belongs to family two of the cystatin superfamily. In the CNS, cystatin C is synthesised in neurons, astrocytes, microglia, neuronal progenitor cells, leptomeningeal cells, and choroid plexus epithelial cells. It is present both in glycosylated and non-glycosylated forms, the latter functioning as a cofactor for bFGF (basic fibroblast growth factor) and facilitating neurogenesis (Taupin et al. 2000). Interestingly, adult-derived hippocampal nestin-positive progenitor cells (many of which are radial glia) secrete five forms of glycosylated cystatin C, suggesting posttranslational modifications of cystatin C in these cells (Dahl et al. 2003, Dahl et al. 2004).

Cystatin C is present in monomeric form or as an inactive dimer in the compartments from which it is secreted as an active monomer to the extracellular space.
and body fluids, including CSF. Regulation of secretion is not well understood. A single point mutation in the cystatin C gene, however, leads to the formation of stable dimers, which causes impaired secretion and the formation of intracellular protein aggregates. Studies of growth factors have shown that bFGF has no effect on cystatin C secretion in podocytes whereas TGF (transforming growth factor)-β1 increased secretion. Secreted cystatin C is proposed to bind to other molecules on the extracellular plasma membrane of neurons and hippocampal progenitor cells (Taupin et al. 2000). In the eye, there is an active, temperature-independent uptake system for cystatin C into several cell types of the cornea, ciliary body, and retina. The cell types that take up cystatin C are generally the same that contain endogenous cystatin C. It is also possible that this uptake system may regulate extracellular levels of cystatin C (Wassellius et al. 2004).

In the normal brain, biological cystatin C activity is inhibited by dimerisation. This biophysical property makes cystatin C an amyloidogenic protein. Dimerisation is accelerated by mutations. What little is known about the degradation of cystatin C suggests that neutrophil elastase cleaves cystatin C in neutral pH (Abrahamson et al. 1991).

Regulation of cystatin C expression

Pathologic conditions. Expression of cystatin C is altered in a variety of human CNS diseases (table 9). Anoxia induced in organotypic cultures leads to activated microglia that overexpress cystatin C (Engelberg et al. 2004). A similar effect occurs with perforant pathway transection-induced cystatin C expression in the glial cells in deafferented areas of the outer molecular layer of the dentate gyrus and stratum lacunsum moleculare (Ying et al. 2002). Also, chronic pain up-regulates cystatin C in the dorsal horn (Yang et al. 2001). Hypophysectomy triggers expression of cystatin C in hypothalamic magnocellular neurons (Katakai et al. 1997). Bunina bodies, a specific hallmark of amyotrophic lateral sclerosis, are neuronal intracytoplasmic eosinophilic inclusions that immunostain intensely for cystatin C (Wada et al. 1999). Brown et al. (2004) showed increased neuronal expression in the hippocampus of ME7/CV mice, a mouse model of scrapie, that preceded the clinical disease by 30 days. Lesions induced by 6-hydroxy-dopamine (6-OHDA) in the medial forebrain bundle in rats caused long-lasting over-expression of cystatin C in neurons, astrocytes and microglia in the striatum. Interestingly, administration of a single dose of cystatin C into the substantia nigra pars compacta of these animals substantially reduced neurodegeneration when assessed four weeks after injection (Xu et al. 2005).

The increased expression of cystatin C and its secretion to extracellular space and CSF have recently stimulated studies investigating its use as a biomarker for CNS diseases, including pain, multiple sclerosis, amyotrophic lateral sclerosis, subclinical brain infarcts, frontotemporal dementia, and Alzheimer’s disease.

Molecular pathways. Little is known about the stimuli that modulate transcription of the cystatin C gene (table 10). In macrophages, cystatin C secretion is stimulated by polysaccharides, and even more potently, by human plasma low- and high-density lipoproteins. TGF-β, a multifunctional cytokine that is released by injury and governs cell growth and motility, up-regulates cystatin C mRNA and protein in murine fibroblasts and astrocytes. Otherwise, cystatin C inhibits TGF-β binding to TGF-β type II receptors, and blocks TGF-β-induced gene expression, signalling, and induced cellular invasion (Sokol and Schiemann 2004, Sokol et al. 2005). This was proposed to occur via inhibition of cathepsin B by cystatin C which upregulates TGF-β. It is of interest that in cancer cells TGF-β also coordinates the expression of metalloproteinases (MMP-2, MMP-9) and uPA (uPAR/plasmin up-regulation by cathepsin B), all of which are also elevated in the epileptogenic tissue. Also, TGF-β expression is increased after various epileptogenic brain insults. Dexamethasone treatment dose-dependently increases cystatin C production in HeLa cells. This is proposed to occur via promoter-mediated increased transcription of the cystatin C gene. Transcription factor Zif268 was recently shown to down-regulate cystatin C expression by 24% in PC12 neurons and it was speculated that suppression of expression could occur via the Zif response element located in the promoter region of the cystatin C gene (James et al. 2005). Recent data suggest that chronic treatment of embryonic spinal cord motoneurons with NMDA triggers long-term accumulation of cystatin C in motoneurons (Tarabal et al. 2001). Otherwise, exposure of hippocampal primary cell cultures to NMDA did not affect cystatin C expression (James et al. 2005). A high oxygen level causing oxidative stress can trigger increased cystatin C mRNA and protein expression in cultured neurons. The time-lag between the up-regulation of mRNA and protein suggests that there are post-transcriptional regulation and accumulation mechanisms (Nishio et al. 2000).

Candidate functions for cystatin C in the CNS

Cystatin C has several functions in the normal CNS and other tissues. It contributes to neuronal survival (neuroprotection), migration, and remodelling of the extracellular matrix via its cysteine proteinase inhibitory action, and to neuronal cell proliferation as a required cofactor for the mitogenic activity of...
bFGF. In glial cells TGF-β-induced cystatin C expression precedes glial fibrillary acidic protein expression (Kumada et al. 2004) suggesting a role for cystatin C in astrocyte differentiation. It may also have an effect on inflammatory response by modulating the migration of inflammatory cells. Cystatin C also prevented the degradation of substance P by soluble fraction and lysosomal extract obtained from synaptosomes (Aghajanyan et al. 1988). The antibacterial effect of the enzymatically active cystatin C amino acid sequence has been proposed to be independent of the proteinase inhibitory effect in the regulation of inflammatory response (data from peripheral tissues). Thus, it is likely that cystatin C targets both protease-dependent and -independent pathways.

Cystatin C also has a role in several human neurodegenerative diseases. A point mutation or polymorphism in the gene encoding the signal peptide of cystatin C (a G/A transition causes an Ala/Thr polymorphism) results in a cystatin C protein that has altered binding properties to other proteins like β-amyloid. Deposition of altered cystatin C protein with β-amyloid is supposed to underlie the co-localisation of cystatin C and β-amyloid precursor protein in human amyloidoses like Alzheimer’s disease: 83% of Alzheimer’s disease cases have cerebral amyloid angiopathy. Mutations in the cystatin C gene may also lead to reduced synthesis and secretion of cystatin C. Also, dimers formed by the mutated protein are more stable. Overexpression of cystatin C does not affect the basic

Table 9. Diseases and conditions of the nervous system in which cystatin C levels are altered.

<table>
<thead>
<tr>
<th>Disease/condition</th>
<th>Change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Lower concentrations in CSF</td>
<td>Pasinetti et al. 2006</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Immunoreactivity in Bunina bodies in motor neurons</td>
<td>Okamoto et al. 1993, Wada et al. 1999</td>
</tr>
<tr>
<td>Amyotrophic lateral sclerosis</td>
<td>Increased expression in skin</td>
<td>Ono et al. 2000</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Codeposition with amyloid-beta protein in parenchymal and vascular deposits</td>
<td>Levy et al. 2001</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Association with CST3 B haplotype</td>
<td>Finckh et al. 2001</td>
</tr>
<tr>
<td>Axotomy</td>
<td>Increased in microglia</td>
<td>Miyake et al. 1996</td>
</tr>
<tr>
<td>Chronic inflammatory demyelinating polyneuropathy</td>
<td>Decrease in CSF</td>
<td>Nagai et al. 2000</td>
</tr>
<tr>
<td>Creutzfeld-Jakob disease</td>
<td>Increased level in CSF</td>
<td>Sanchez et al. 2004, Piubelli et al. 2006</td>
</tr>
<tr>
<td>Frontotemporal dementia</td>
<td>Truncated form in CSF</td>
<td>Ruetschi et al. 2006</td>
</tr>
<tr>
<td>Glioma</td>
<td>Decreased expression in high grade tumors</td>
<td>Nakabayashi et al. 2005</td>
</tr>
<tr>
<td>Guillain-Barré syndrome</td>
<td>Decrease in CSF</td>
<td>Nagai et al. 2000</td>
</tr>
<tr>
<td>Hereditary cystatin C amyloid angiopathy</td>
<td>Deposition of L68Q mutant</td>
<td>Palsdottir et al. 1988</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>Increased expression in hypothalamus</td>
<td>Katakai et al. 1997</td>
</tr>
<tr>
<td>Leptomeningeal metastasis</td>
<td>Decreased concentration in CSF</td>
<td>Nagai et al. 2003</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Decrease in CSF</td>
<td>Bollengier 1987</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Cleaved form in CSF</td>
<td>Irani et al. 2006</td>
</tr>
<tr>
<td>Pain</td>
<td>Increase in CSF</td>
<td>Mannes et al. 2003</td>
</tr>
<tr>
<td>Perforant path transection</td>
<td>Increased expression in hippocampus</td>
<td>Ying et al. 2002</td>
</tr>
<tr>
<td>Sciatica</td>
<td>Decreased in CSF</td>
<td>Liu et al. 2005</td>
</tr>
<tr>
<td>Scapie</td>
<td>Increased mRNA expression in hippocampus</td>
<td>Brown et al. 2004</td>
</tr>
<tr>
<td>Spinal nerve root injury following lumbar disk herniation</td>
<td>Increased in CSF</td>
<td>Liu et al. 2006</td>
</tr>
<tr>
<td>Sporadic amyloid angiopathy</td>
<td>Deposition of L68Q mutant</td>
<td>Graffagnino et al. 1995</td>
</tr>
</tbody>
</table>
levels of β-amyloid. Direct application of cystatin C to the normal hippocampus induced loss of dentate granule and hilar cells, and was accompanied by astrocyte and microglia activation (Nagai et al. 2002). On the other hand, lack of cystatin C in cystatin C-deficient mice has been shown to aggravate brain damage following focal ischaemia but decrease neuronal injury after global ischaemia (Olsson et al. 2004).

Expression and candidate functions of cystatin C in the epileptogenic brain

After initial demonstration of the increased expression of cystatin C during epileptogenesis by complementary DNA microarray and RT-PCR (Lukasiuk et al. 2003), we next investigated the cellular and regional expression of cystatin C in normal, epileptogenic, and epileptic brain tissue. In normal rat, mouse, and human hippocampi, cystatin C is present at low levels, mainly in astrocytes and microglia (figure 6). Following epileptogenic brain insults such as SE or photothrombotic stroke, cystatin C expression showed a substantial increase in activated glial cells (Lukasiuk et al. 2003, Pirttila and Pitkanen 2006). This up-regulation occurred in a delayed manner, peaking four to seven days after the insult. Interestingly, cystatin C up-regulation in the hippocampus persisted in the chronic phase of epilepsy both in the rat model of temporal lobe epilepsy (TLE) and in human TLE (Pirttila et al. 2005a). By using cystatin C−/− mice, we demonstrated that cystatin C deficiency is associated with reduced neurodegeneration after SE and also impaired proliferation and migration of newborn neurons in the dentate gyrus (Pirttila et al. 2005b). These data suggest that cystatin C functions to promote neuronal death and/or as a migratory cue for newborn neurons. Also, in human patients with TLE, increased cystatin C expression is associated with the abnormal migration of newborn neurons to the molecular layer and granule cell dispersion, suggesting that cystatin C participates in the development of granule cell dispersion (Pirttila et al. 2005a). The next step will be to investigate the molecular cascades that trigger and mediate these functions of cystatin C in the epileptogenic and epileptic brain.

The complicated and variable pattern of cystatin C expression in the CNS in different conditions; the variety of candidate functions, ranging from the control of the proteolytic activity of other proteins to the regulation of neuronal differentiation and migration; and the variable effects manipulating brain cystatin C levels has on neurodegeneration, make it difficult to draw a final conclusion about the role of cystatin C in the diseased brain. On the other hand, these observations, together with the fact that up-regulation of cystatin C expression generally accompanies neurodegeneration, make it a very exciting molecule for future functional studies.

To conclude, the detection of altered gene expression during epileptogenesis is only the beginning of a long journey which we hope will eventually lead to an understanding of the function of the protein product in epileptogenesis.

### Table 10: Stimuli that modulate the expression of cystatin C in vitro and in vivo.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Change</th>
<th>Tissue</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-hydroxypamine</td>
<td>Increased expression</td>
<td><em>in vitro</em>, PC12 cells</td>
<td>Lee et al. 2006</td>
</tr>
<tr>
<td>6-hydroxypamine</td>
<td>Increased expression</td>
<td><em>in vivo</em>, rat striatum</td>
<td>Xu et al. 2005</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Increased expression</td>
<td><em>in vitro</em>, HeLa cells</td>
<td>Bjarnadottir et al. 1995</td>
</tr>
<tr>
<td>Fibrillar collagen type I</td>
<td>Increased expression</td>
<td><em>in vitro</em>, human melanoma cell lines</td>
<td>Klose et al. 2006</td>
</tr>
<tr>
<td>IL-6-STAT-3</td>
<td>Decreased expression</td>
<td><em>in vitro</em>, bone marrow dendritic cells</td>
<td>Kitamura et al. 2005</td>
</tr>
<tr>
<td>Induction of Zif268 transcription factor</td>
<td>Down regulation</td>
<td><em>in vitro</em>, PC12 cells</td>
<td>James et al. 2005</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Down regulation</td>
<td><em>in vitro</em>, oral carcinoma cell line</td>
<td>Wickramasinghe et al. 2005</td>
</tr>
<tr>
<td>Interferon regulatory factor-8</td>
<td>Promotor activation</td>
<td><em>in vitro</em>, macrophages</td>
<td>Tamura et al. 2005</td>
</tr>
<tr>
<td>-82G/−5G/+4A haplotype of Cys C gene</td>
<td>Higher plasma levels</td>
<td><em>in vivo</em>, plasma</td>
<td>Loew et al. 2005</td>
</tr>
<tr>
<td>Transforming growth factor beta</td>
<td>Upregulated</td>
<td><em>in vitro</em>, astrocyte precursor (serum-free mouse embryo) cells</td>
<td>Solem et al. 1990</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Increased expression</td>
<td><em>in vivo</em>, vagina</td>
<td>Slayden et al. 2004</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>Increased expression</td>
<td><em>in vivo</em>, alveolar macrophages</td>
<td>Huaux et al. 1995</td>
</tr>
</tbody>
</table>
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References


Figure 6. Representative digital photomicrographs demonstrating expression of cystatin C protein in the mouse, rat and human dentate gyrus. In the normal mouse (A), rat (C) and human (E) dentate gyrus, light cystatin C expression was present particularly in the molecular layer of the dentate gyrus (m). Following an epileptogenic insult (kainate treatment in mice (B) or electrical stimulation of the amygdala in rat (D)) the expression cystatin C increased in the molecular layer. A similar increase in the cystatin C expression was evident in the human patients with TLE (F). The morphology of cystatin C immunoreactive cells resembled that of glia (see arrows in panels A-F). Scale bars, 100 µm (A-B, E-F), 50 µm (C-D). h = hilus; g = granule cell layer; m = molecular layer of the dentate gyrus.


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Discussion by Dimitri M. Kullmann

Institute of Neurology, University College London, Queen Square, London WC1N 3BG, UK
<dkullman@ion.ucl.ac.uk>

Recent advances in molecular biology offer the possibility of identifying and quantifying changes in gene expression in disease models and in human tissue samples. There are several pitfalls in inferring from changes in transcript levels that protein levels are altered. Moreover, changes in signalling proteins may be expected to have distinct consequences depending on whether they occur in principal neurons, interneurons or glia. These considerations call for hypothesis-driven manipulation of candidate signalling cascades mainly by genetic or pharmacological means. The goals of such studies are to determine whether enhancing or blocking the action of a candidate protein mimics, occludes or reverses an aspect of the disease model. The discovery process can be summarised as “See, Stop, Push”: “See” that expression of a particular gene is altered; “Stop” the consequence of altered expression for a signalling pathway to determine how it affects epileptogenesis or an associated phenomenon; “Push” the same signalling pathway in isolation to see if it can reproduce the pathological process. These represent a major challenge, which will need to be overcome to distinguish pathogenic processes from compensatory changes and epiphenomena.

What can the chromosomal basis of epilepsy tell us more broadly?

Sameer Zuberi

Fraser of Allander Neurosciences Unit, Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ, UK
<sameer.zuberi@yorkhill.scot.nhs.uk>

Epilepsy is a recognised association in more than 400 chromosomal abnormalities. About 10% of these anomalies have epilepsy as a common feature (Singh et al. 2002). In a series reported by Ieshima and Takeshita (1988), 6% of patients with learning disability and epilepsy had a chromosomal abnormality. However there are only a small number of chromosomal abnormalities, listed in table 11, where epilepsy is a consistent feature. Can we learn broader lessons about epilepsy, its co-morbidities and the genetic mechanisms underlying epileptogenesis by studying these disorders in detail? Such analyses are important for a number of reasons. Firstly, they can aid clinicians in the diagnosis of certain syndromes. If the epilepsies associated with certain chromosomal disorders are properly delineated, characteristic patterns can be recognised in the clinical presentation, EEG features, treatment response and prognosis. Secondly, semiology may help to define the neuronal networks involved in the epileptic seizures. Electroclinical descriptions allied to more sophisticated cytogenetic techniques may provide clues in the search for genes associated with epilepsy.

Singh et al. (2002) provide a simple way of conceptualising how chromosomal disorders may cause an epilepsy phenotype. Chromosomal anomalies will cause a
genotype-dosage effect; either haploinsufficiency (reduced gene dosage) or “triplo-excess” (increased gene dosage). These may result from deletion syndromes, breakpoint disruptions or duplication syndromes.

The chromosomal syndrome best studied in relation to epilepsy is Angelman Syndrome, in which a broadly similar phenotype may be produced by a variety of linked genetic lesions. There are at least five genetic classes: maternal 15q11-13 deletions, paternal uniparental disomy, UBE3A mutations, imprinting centre defects and unidentified lesions. Even with a classical phenotype it remains important to define the specific genetic aetiology both for genetic counseling and prognostic information. Epilepsy tends to be less severe (later age of onset and lower frequency of seizures) in the uniparental disomy group compared to the deletion group (Williams 2005). The genetic lesions must affect specific neuronal networks rather than simply give the individual a broad predisposition to generalised epileptic seizures. This is evidently the case as specific EEG patterns are associated with Angelman syndrome (Laan and Vein 2005). Indeed it is not unusual for the neurophysiologist to correctly suggest the diagnosis based simply on an EEG requested for a child with learning disability and epilepsy.

The likelihood of a child with epileptic seizures and more than three major dysmorphic features having a chromosomal anomaly was around 50% in one series (Ieshima and Takeshita 1988). Studying the epilepsy phenotype of Wolf-Hirschhorn Syndrome, in which there is a deletion of the tip of chromosome 4 (4p-), has revealed a specific pattern of generalised spike-wave abnormalities and a phenotype associated with febrile seizures in some ways akin to severe myoclonic epilepsy in infancy (SMEI; Dravet Syndrome). The epilepsy begins in early infancy with clusters of clonic seizures or status epilepticus, often fever-related (Battaglia and Guerrini 2005). Atypical absences may develop. These clinical observations raise the possibility that further febrile seizure or SMEI-related genes may be present on chromosome 4.

Chromosomal anomalies have also given significant insights into cortical malformations, epileptogenesis and normal brain development. The recognition that Miller-Dieker Syndrome (specific dysmorphic features and lissencephaly) was associated with a chromosome 17p13.3 deletion led to the discovery of the Lis1 gene within the deleted region (Dobyns et al. 1993). Lis1 mutations are responsible for one form of isolated lissencephaly without dysmorphism. The gene appears to be involved in controlling nuclear translocation, a process key to normal neuronal migration. Many other chromosomal anomalies are associated with malformations of cortical development and therefore provide a way into understanding the processes underlying normal brain development (Barth 2003).

Many of the chromosomal syndromes in table 11 are uncommon: one epileptologist will see perhaps only a few such patients. Collaborative international studies are key to providing new insights. In an ongoing study of children with ring chromosome 20 syndrome, more than 60 cases have been collected worldwide (Zuberi and Biraben 2004). Affected individuals are typically not dysmorphic and have normal development until the onset of epilepsy during primary school years (Macleod et al. 2005). Many suffer from refractory seizures and an epileptic encephalopathy but 50% of adults have IQs in the normal range and may have full time jobs and be educated to degree level. This is important information for families and for geneticists. We know that if ring 20 is detected on amniocentesis it frequently results in termination of the pregnancy.

Family support groups may hold databases of children with rare chromosomal anomalies. They not only give the family important educational and emotional support but may allow recognition of new syndromes. If these new syndromes are associated with small deletions then the potential to identify new genes is clear to see. We must work towards a model of even closer collaboration between different medical and scientific disciplines, patients and families.

References

Table 11. Chromosomal disorders strongly associated with epilepsy.

<table>
<thead>
<tr>
<th>Chromosomal Syndrome</th>
<th>Chromosome Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolf-Hirschhorn Syndrome</td>
<td>4p-</td>
</tr>
<tr>
<td>Miller-Dieker Syndrome</td>
<td>del 17p13.3</td>
</tr>
<tr>
<td>Angelman Syndrome</td>
<td></td>
</tr>
<tr>
<td>Inversion duplication 15 Syndrome</td>
<td></td>
</tr>
<tr>
<td>Terminal deletion chromosome 1q</td>
<td></td>
</tr>
<tr>
<td>Terminal deletion chromosome 1p</td>
<td></td>
</tr>
<tr>
<td>Ring 14 syndrome</td>
<td></td>
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<tr>
<td>Ring 20 syndrome</td>
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Discussion by Frances Elmslie

St George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK
<frances.elmslie@stgeorges.nhs.uk>

Many different chromosomal syndromes are associated with epilepsy (Gardiner et al. 2002). However, epilepsy is not always a consistent feature of a specific chromosomal abnormality, and the epilepsy phenotype varies in some chromosomal abnormalities the epilepsy occurs secondarily to an obvious developmental brain abnormality, while in others the brain appears structurally normal. In most instances, the genes responsible are unknown.

A number of mechanisms for the presence of epilepsy have been postulated (Schinzel and Niedrist 2001):

- A gene or genes when deleted or duplicated cause abnormal brain development or perturb neuronal function.
- The chromosome abnormality exerts a position effect on genes outside the deleted or duplicated segment.
- The combined action of many genes, together with environmental influences, results in epilepsy (the polygenic model).

It is likely that all three of these mechanisms provide an explanation for epilepsy in different situations.

The one chromosomal abnormality in which the mechanism is understood is that of Miller-Dieker syndrome. The Miller-Dieker region on chromosome 17p13.3 contains the gene encoding LIS1 [deletions and mutations of which cause isolated lissencephaly (Reiner et al. 1993)] and the gene encoding 14-3-3ε, which acts by maintaining phosphorylation of NUDEL (Toyo-oka et al. 2003). Deficiency of both LIS1 and 14-3-3ε accounts for the more severe phenotype seen in Miller-Dieker syndrome. These genes were identified by detailed mapping of the deletions in a large group of patients. Similar studies in individuals with distinctive epilepsy phenotypes, such as ring 20 mosaicism, may be possible. Clinicians need to be aware of the possibility that epilepsy may be related to a chromosome abnormality, and consider requesting higher resolution studies such as comparative genomic hybridisation, particularly when the epilepsy is difficult to treat. In addition, apparently balanced chromosomal rearrangements should not be dismissed as unrelated to the epilepsy but should be investigated further.

References


Does hippocampal sclerosis have a genetic basis (a developmental viewpoint)?

Jeffrey Golden

Department of Pathology, Children’s Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia PA 19104, USA
<goldenj@mail.med.upenn.edu>

Hippocampal sclerosis (HS), also known as mesial temporal sclerosis, is a common pathologic finding in medically intractable temporal lobe epilepsy (Cross and Harding 2004). Despite its frequency, the pathogenesis and contribution to epilepsy remains uncertain. More specifically, does HS beget seizures or are seizures the precipitating factor for the development of HS?

Hippocampal development

Development of the hippocampus is a multistep process that begins with specification in the dorsal-medial cerebral wall (Li and Pleasure 2005; Zhao et al. 1999). Once specified, a complex orchestration of differentiation, migration, process outgrowth, establishment of appropriate connections and solidification of circuiting ensues. The resulting hippocampus exhibits the classic cornu Ammonis morphology for the pyramidal cells along with dorsal and ventral arms to the fascia dentata. The molecular and cellular pathways leading to this development have begun to be elucidated and will briefly be reviewed.
Hippocampal sclerosis: pathology

The hallmark of HS is the loss of pyramidal neurons in CA1 and CA3, with more severe examples also involving CA2 and the dentate granular neurons (Cross and Harding 2004). Although these features define HS, HS is often seen with pathologic features generally considered malformations in origin. These include dysplasia of the hippocampus, as well as the adjacent temporal lobes, cortex and fascia dentata pathologies like granular cell dispersion and “tram” tracking (Harding and Thom 2001).

Hippocampal sclerosis: possible precipitating causes

Various factors have been implicated in predisposing an individual to the development of HS. Among the most common are febrile seizures (and significantly prolonged febrile seizures), head trauma, CNS infections, and birth trauma (French et al. 1993, Lewis 2005, Lewis et al. 2002). However, not all individuals, even with similar histories, will develop HS, implicating at least one other factor in the pathogenesis of HS. What are these other factors? One possibility is recurrent seizures (Parent et al. 1997). Recurrent seizures can lead to increased neuronal injury and cell death. This begs the question: what causes the seizures? Here we come back to the findings of dual pathology, HS with another abnormality that commonly appears to be developmental in origin and implicated in the pathogenesis of seizures:

- Granular cell dispersion: Although possibly having both a degenerative/regenerative and a developmental origin, the presence of this pathology in animal models and in some humans at autopsy suggest a primary origin in at least some cases;
- Cortical dysplasia: A common malformation of cortical development commonly found in association with HS.

Hippocampal sclerosis: genetics

Although limited, reports of familial HS are emerging. These are important families to study and to date the data are incomplete. However, there is a better association with familial forms of febrile seizures and as noted above this is a predisposing factor for HS (Fernandez et al. 1998). Equally important, familial forms of HS do not show a high association with febrile seizures indicating a separate and genetic aetiology in some cases (Kobayashi et al. 2003).

A unifying hypothesis

It is likely that the pathogenesis of HS is multifactorial. A genetic predisposition would increase an individual’s susceptibility to the development of HS, but only in the presence of a second factor. A variety of factors have been implicated and they may be different for different individuals. Unfortunately, to date, the gene or genes that confer susceptibility are unknown and will likely be difficult to uncover.

References


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Is focal cortical dysplasia genetic in origin?

Peter B. Crino

Department of Neurology, University of Pennsylvania Medical Center, Philadelphia PA 19104, USA
<crinop@mail.med.upenn.edu>

Molecular pathogenesis of focal cortical malformations: mTOR and b-catenin

Cortical dysplasia with balloon cells (CDBC) and hemimegalencephaly (HMEG) are developmental malformations of the cerebral cortex that are highly associ-
ated with medically intractable epilepsy. While CDBC affects a restricted cortical region, HMEG involves an entire hemisphere.

CDBC is characterised histologically by disorganised or absent cortical lamination and by the presence of enlarged cells known as balloon cells (BCs) that exhibit a large cell soma, short processes of indeterminate identity, a laterally displaced nucleus, and phenotypic features suggestive of a mixed astrocytic and neuronal lineage (Crino et al. 2002). For example, BCs have been shown to express vimentin, nestin, glial fibrillary acidic protein (GFAP), and neurofilament proteins. A subset of HMEG cases also exhibit BCs. Intermingled with BCs are large, dysmorphic neurons (cytomegalic neurons) that exhibit aberrant dendritic arbors and loss of radial orientation within the cortex in relation to the pial surface. CDBC and HMEG may also exhibit excess numbers of astrocytes.

There have been no identified family pedigrees with isolated CDBC or HMEG and we have hypothesised that CDBC and HMEG are part of a spectrum of sporadic cortical malformations that result from somatic gene mutations occurring in progenitor cells during cortical development. Thus, the search for causative gene mutations in CDBC and HMEG has proven to be a daunting challenge since traditional positional cloning strategies cannot be readily applied to sporadic and likely somatic mutational events.

The loss of lamination in CDBC and HMEG suggests a cortical developmental abnormality affecting select steps during cell proliferative and migratory phases of cortical development. However, based on evidence in a related malformation, the tuberous sclerosis complex (TSC), we propose that BCs and specifically mechanisms leading to cytomegaly, are central in the pathogenesis of CDBC and HMEG.

A recent insight into the pathogenesis of CDBC and HMEG was the identification of selective expression of phospho-ribosomal S6 protein in BCs in CDBC and HMEG (figure 7). Phospho-S6 protein resides downstream of the Akt/mTOR cascade and is activated by phosphorylation in response to growth factors such as IGF-1, energy or nutrient demands, and cell stress (figure 8). Constitutive negative modulators of the mTOR cascade are the TSC1- and TSC2-encoded proteins hamartin and tuberin. Mutations in TSC1 or TSC2 are the cause of TSC. Loss of TSC1 or TSC2 function leads to mTOR activation and resultant cytomegaly in the brain (Baybis et al. 2004). In both CDBC (Baybis et al. 2004) and HMEG (Crino, unpublished observations), there is high expression of phospho-S6 protein confined to BCs and cytomegalic neurons. Previous studies have identified sequence polymorphisms but not deleterious mutations in TSC1 and TSC2 in CDBC and thus it is unlikely that CDBC results from somatic TSC1/TSC2 gene mutations. There are numerous proteins that modulate this pathway and thus both loss of function and activating mutations in encoding genes could enhance phospho-S6 expression. Potential candidate genes include PTEN, notch, Akt, and Rheb. Sequence analysis of these and other genes is ongoing in our laboratory.

As a corollary strategy, we have implemented cDNA arrays in HMEG specimens to define possible candidate genes (Yu et al. 2005). We found increased expression of cyclin D1 and c-myc mRNAs. Expression of cyclin D1 and c-myc genes is transcriptionally activated by β-catenin. Western analysis demonstrated increased levels of non-phosphorylated β-catenin in HMEG cortex. Reduced levels of Ser33, Ser37, and Thr41 phospho-β-catenin, sites known to be phosphorylated by glycogen synthase kinase-3 (GSK-3) and to be essential for β-catenin inactivation, were detected in HMEG.

Figure 7. Phospho-S6 expression in cytomegalic cells in CDBC (left) and HMEG (right) (courtesy of E. Aronica).
Mutations in β-catenin and in PTEN, a negative modulator of this cascade, have not been identified in DNA isolated from HMEG specimens. Interestingly, the β-catenin and Akt/mTOR pathways intersect and both may be involved in CDBC and HMEG. We recently found that there was co-expression of cyclin D1 and phospho-S6 in HMEG specimens specifically within BCs and cytomegalic neurons.

While a gene mutation affecting the mTOR or β-catenin cascades could lead to CDBC or HMEG, other downstream changes in gene and protein expression are likely to contribute to the laminar disorganization, aberrant cellular morphologies, and seizure propensity of these malformations. For example, altered expression of select growth and transcription factors such as TGF-β, cyclic AMP response binding protein and IGF-1 (Baybis et al. 2004, Kim et al. 2003) could enhance cell proliferation whereas altered expression of neurotransmitter receptor subunits may lead to enhanced excitability and seizures (Moddel et al. 2005). Future studies will be necessary to define how changes in gene and protein expression in CDBC and HMEG lead to lesion formation and epilepsy.

These data suggest that there may be enhanced activation of the β-catenin cascade in HMEG which in turn may foster the activation of the Akt/mTOR pathway and culminate in hemispheric enlargement. Enhanced expression of phospho-S6 and activated β-catenin suggests two converging cell pathways that may be pivotal in the pathogenesis of CDBC and HMEG.

References
Circumscribed malformative lesions of the CNS comprise a wide spectrum of neuroradiological and histomorphological features (Becker et al. 2006). Recent progress in our understanding of brain development has identified signalling pathways that significantly contribute to epilepsy-associated focal lesions (Schwartzkroin and Walsh 2000). However, a plethora of different histopathological entities can be recognised in surgical specimens obtained from epilepsy patients and there is still no international classification system available to separate distinct entities (Palmini et al. 2004). Focal cortical dysplasia (FCD) presenting with dysplastic neurons and balloon cells (i.e. FCD type IIb) is a well-recognised exception from this limitation (figure 9, table 12). It shares morphological similarities with cortical tubers observed in patients with tuberous sclerosis. These features have focussed our interest to carefully examine the insulin growth factor receptor signalling pathway known to interact with hamartin (TSC1) and tuberin (TSC2) (Becker et al. 2002). Extensive analysis of additional molecular players involved in this pathway (see Crino above) is compatible with a distinct pathogenic involvement in FCD type IIb. However, other histopathological variants presenting with architectural disturbances of cortical organisation rather than with dysplastic cellular features, i.e. FCD type I, remain to be further characterised (Hildebrandt et al. 2005). Hence, we were not yet able to identify specific pathogens. Whether the broad spectrum of FCDs is always “genetic in origin” needs, therefore, further studies. It is tempting to speculate that additional mechanisms, such as seizure-associated progressive disturbances including dysregulation of transcriptional/translation control also contribute to cortical maldevelopment in epilepsy patients.

References

Figure 9. Focal cortical dysplasia presenting with dysplastic neurons (left) and type IIb balloon cells (right). Scale bars both 50 µm.

Table 12. Classification issues in focal cortical dysplasia, modified from Palmini et al. 2004 and Becker et al. 2006.

<table>
<thead>
<tr>
<th>ILAE classification</th>
<th>Molecular patho-mechanisms</th>
</tr>
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<tbody>
<tr>
<td>FCD type I</td>
<td>Architectural dysplasia (microcolumns, hypoplasia, immature neurons)</td>
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</tr>
<tr>
<td>FCD type IIa</td>
<td>Cytoarchitectural dysplasia (without balloon cells)</td>
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</tr>
<tr>
<td>FCD type IIb</td>
<td>Cytoarchitectural dysplasia (with balloon cells)</td>
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FCD = focal cortical dysplasia; MTS = mesial temporal sclerosis.


Juvenile Myoclonic Epilepsy – one syndrome or many?

David A. Greenberg

Department of Biostatistics and Psychiatry, Mailman School of Public Health, Columbia University and the New York State Psychiatric Institute, New York, NY 10032, USA <dag@shallot.cpmc.columbia.edu>

For genetic studies, two confounders always loom the largest: phenotype definition and heterogeneity. Idiopathic generalised epilepsy (IGE) has both problems. Our decision to study juvenile myoclonic epilepsy (JME; Greenberg et al. 1988) was made because myoclonus on awakening was a phenotype that easily distinguished JME from other IGEs. However, studies in the genetics of common diseases, even then, showed that while narrowing the phenotype helped reduce heterogeneity, the existence of heterogeneity within the data must always be assumed. Thus, restrictive phenotyping of subjects should be the norm in studying common disease, but we must also expect that the same phenotype may be caused by different genes, especially in different populations.

Careful symptom classification is critical

Our study of the genetics of JME began in 1981. We chose to focus on JME, rather than IGE, because JME appeared to be a narrow and restrictive diagnosis, suggesting reduced heterogeneity. But we quickly learned that simply a diagnosis of JME in the medical record was not necessarily the only criterion to use for acceptance of a family into the study.

Physicians are obligated to patients before everything else. Thus, the purpose of a medical diagnosis is to lead to a treatment. If the treatment is successful, the diagnosis was correct, from a medical point of view. But that criterion may be entirely inappropriate for genetic studies, because the same treatment can be effective for different aetiologies. In order to decrease heterogeneity, one must be suspicious of any variation from a narrow set of criteria. In the case of myoclonias, not everything that causes jerks is JME. Among the rejection criteria that we used were:

- Myoclonic jerks not on awakening but randomly during the day
- Tonic-clonic seizures in sleep or at random times during the day
- Photomyoclonia
- Myoclonic absence
- History of childhood onset epilepsy (including childhood absence epilepsy)
- Fewer than four witnessed seizures.

Different genes in different populations

Several studies have identified genes for JME, and it appears that ethnic background plays an important role in determining which genes will be susceptibility genes in different populations. There are at least five loci that have been suggested as leading to JME, loci which are (perhaps) mutually exclusive, but that, in any case, are seen in different populations:

- Chr. 6p21 (BRD2) linkage and association in New York data (Greenberg et al. 2000, Pal et al. 2003).
- Chr. 15q13 locus in British data (Elmslie et al. 1997).
- Chr. 6p11 (EFHC1) linkage in Mexican data (Suzuki et al. 2004).
- Chr. 5q34 (GABRA1) in a single family (Cossette et al. 2002).
- Chr. 10q25 locus in a population from India (Puranam et al. 2005).

In addition, in our New York study (Greenberg et al. 2000), we originally collected families irrespective of ethnic background. (Discrimination on the basis of ethnicity is forbidden unless there is a reason). When we did the analysis, we found that Caucasian families showed linkage to 6p21 whereas families with African American or Hispanic backgrounds did not. Recall that all the families in our study were enrolled using the same stringent criteria. Thus, all the evidence is that the aetiology of JME differs in different ethnicities. Thus, it is likely that all of the above findings are “correct” in that they are not false positives. If all of these genes are involved in JME expression, it suggests an underlying mechanism.

But we also know that JME is not a single gene disorder. We can expect different combinations of genes will produce the same phenotype, especially when ethnicity is a variable. Our evidence suggests that it is seizure type that may be genetically determined, rather than IGE syndrome. Under such a phenotypic model, we should expect that heterogeneity within IGE, and even within an apparently specific syndrome
like JME, will be the rule. However, once we have identified a gene and identified a mutation or population association, we can use the knowledge of the genotype to “reverse engineer” the phenotype and determine in which populations or in which kinds of phenotypes, that gene will be a susceptibility gene.

References


Discussion by Michael R. Johnson

Department of Clinical Neuroscience, Imperial College London, Charing Cross Hospital, London W6 8RF, UK

The problem of disease definition in juvenile myoclonic epilepsy (JME) is closely related to the troubling issue of heterogeneity of genetic aetiology. A disease definition is a statement of the criteria by which any given example falls into the category to which a name has been applied (Scadding 1959, Cossette et al. 2002). This is not the same as a disease description, which may include additional clinical or laboratory features that are not essential to the diagnosis (Flier and de Vries Robbe 1999).

In their work on the relationship between the definition of a disease and its necessary cause, Flier and de Vries Robbe (1999) draw distinctions between various levels of disease definition according to conventional notions of disease mechanism and cause. According-ly, at a pre-theoretical level, JME can be defined by a collection of clinical features thought to be associated in a non-fortuitous manner, which is the principle of the International League Against Epilepsy (ILAE) defi-

nition (Commission on Classification and Terminology 1981). This definition is inclusive, and uses multiple characteristics. Such multiple characteristics are usually determined by a committee of “wise-men and –women”: in the case of JME, the ILAE Commission. A pre-theoretical definition of JME however, tells us nothing about the relation between JME and its cause. A theoretical definition of JME emerges only after research into the underlying cause. For example, in one JME pedigree, JME might be defined as an A322D mutation in GABRA1 (Cossette et al. 2002). This theoretical definition of JME is monothetic, and tells us something about its cause.

This distinction between pre-theoretical and theoretical definitions of disease is rarely applied in the medical lexicon, but has a critical bearing on diagnostic accuracy. Under a pre-theoretical definition of JME, the gold standard for measuring diagnostic accuracy is the essential characteristics mentioned in the definition of the diagnostic term by the ILAE. Under a theoretical definition of JME, the gold standard might be a test for the A322D mutation in GABRA1.

So how many JME syndromes there are, depends on how it is defined. Under a pre-theoretical definition there is necessarily only one: the one defined by the ILAE. Under a theoretical definition, the question becomes invalid, since JME is necessarily re-defined!

References


What can we tell patients using genetic information?

John Craig

Department of Neurology, Royal Group of Hospitals, Grosvenor Road, Belfast BT12 6BA, UK

What role does genetics play in AED teratogenicity?

Exposure to AEDs during pregnancy may be associated with an increased risk of congenital malformations and have an adverse effect on foetal growth and
psychomotor development. With an estimated three to four pregnancies in every thousand occurring to women with active epilepsy (Dansky and Finnell 1991, Olafsson et al. 1998), this means between 1800 and 2400 children will be born to such women each year in the United Kingdom.

For most infants born to mothers taking AEDs, development is not affected. Recent reports show between 3.5 and 9.0% of all AED-exposed pregnancies will have a major congenital malformation, compared with a background risk of 1-2% (Samren et al. 1997, Canger et al. 1999, Kaneko et al. 1999, Samren et al. 1999, Holmes et al. 2001, Kaaja et al. 2003, Morrow et al. 2006). In contrast, recurrence risks for an adverse outcome among siblings of infants with AED embryopathy is thought to be much higher, with Moore et al. (2000) and Dean et al. (2002) reporting recurrence rates for some sort of abnormality in AED-exposed infants of 55% and 43% respectively. Such findings have also been reported in small case series for valproate, when changing to an alternative AED led to the birth of a healthy infant (Duncan et al. 2001, Malm et al. 2002). Along with the results from preclinical models, the above observations support the concept of an underlying genetic susceptibility to adverse foetal outcomes in pregnancies exposed to AEDs. Obviously understanding the genetic factors involved would greatly aid in the management of women who are required to use AEDs long-term.

Before using genetic information in the clinical situation we are faced with a number of difficulties:
– The molecular mechanisms underlying AED teratogenicity are largely unknown.
– The mechanisms are likely to be different for different drugs.
– The mechanisms are likely to be different for different malformations.
– Similar problems are also likely when considering the genetic basis of susceptibility to AED teratogenicity.
– There are problems using preclinical models to predict effects in human pregnancies.
– Accessing human material to study genetic associations for AED-induced teratogenicity has been difficult.

For phenytoin, an increased susceptibility to birth defects due to decreased function of epoxide hydrolase (EPHX1), an enzyme which metabolises oxidative intermediates (epoxides) of the parent compound, has been postulated to be the result of an autosomal dominant mutation in one study (Strickler et al. 1985) and an autosomal recessive gene in another (Buehler et al. 1990). Damage to cellular macromolecules (DNA, lipid, protein) and activation of signal transduction cascades from reactive oxygen species created through embryonic xenobiotic-enhanced oxidative stress may also contribute to birth defects in humans.

Preclinical models provide substantial evidence on the effects of altered folate metabolism on the risk of birth defects, particularly for valproate and neural tube defects. Mutation (677 C>T) in the methylenetetrahydrofolate reductase gene in a mother taking valproate, phenytoin or carbamazepine during pregnancy was shown to be associated with foetal anticonvulsant syndrome (OR 3.2, 95% CI 1.02–10.04; Dean et al. 1999). Association studies of other candidate genes, such as folate receptor alpha and beta, have not been reported for human pregnancies exposed to AEDs. While differential expression of their equivalents in preclinical models has been postulated to account for some of the varying susceptibility to valproate teratogenicity (Finnell et al. 1997), no evidence for an association with neural tube defects has been noted in non-AED-exposed populations (Trembath et al. 1999). Furthermore, periconceptional supplementation with folic acid for human pregnancies exposed to AEDs does not seem to reduce the risk of other birth defects associated with folate deficiency (Hernandez-Diaz et al. 2000).

Proposed modes of action, other than altered folate metabolism, for the developmental effects of valproate include altered Wnt-dependent gene expression activated through inhibition of histone deacetylase (Wiltse 2005), and induction of the anti-apoptotic gene Bcl-2 (Manji and Chen 2002). Disruption of developmental control genes including altered expression of Hox and Pax are other targets that have been studied (Faiella et al. 2000, Whitsel et al. 2002).

When counselling women with epilepsy who are either contemplating pregnancy or are already pregnant, what genetic information can we use in the clinic? For the most part, despite significant advances in basic research and available clinical information from human pregnancies, our knowledge is still very limited and largely based on the older AEDs. Except for lamotrigine (Morrow et al. 2006, Cunnington et al. 2005) there are little or no human data, with preclinical research on the newer AEDs essentially consisting of little more than that required for regulatory purposes. Likewise for the significant numbers of women exposed to polytherapy during pregnancy, data are limited. In considering the issues, however, clinicians need to counsel on the:
– Risks of overall malformation rates by AEDs, where known,
– Patterns of malformations by exposure,
– Recurrence risks of malformations in siblings of previously affected infants,
– Risks of seizures in pregnancy,
- The known and unknown benefits of folic acid,
- Need for prenatal screening,
- Unknowns, including psychomotor development and the need for much more research.

While pregnancy registries will hopefully provide some of the above, future study of the molecular basis of teratogenicity, in particular understanding how genetic variability influences the risk of exposure to individual AEDs, has the potential to prevent harmful exposures, while at the same time not denying women effective treatment with the AEDs that at present would seem to carry the highest risk of adverse outcomes.

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Discussion by J Helen Cross

Wolfson Centre, Mecklenburgh Square, London WC1N 2AP, UK <hcross@ich.ucl.ac.uk>

Prior to the development of the pregnancy registries, the literature provided very little consistent information about the effects of AED exposure to the foetus. Although many studies had been reported, these were on variable populations of small size with regard to individual drug exposure. The pregnancy registries are increasing the amount of data available on the risk of malformations, with prospective assessment of a large number of individuals using standardised methodology. Such registries are likely to provide answers to questions on the relative teratogenic risks of the common monotherapies, relative risks of the most common AED combinations, possible drug-specific malformations and the impact of dose or other major covariates. However only partial (if any) answers will be found for risks associated with the less commonly
used AEDs or most combinations, or the role of any genetic predisposition or other potential confounders. The role of genetic susceptibility in the occurrence of malformations will therefore remain unknown.

Increasing case reports highlight the existence of foetal anticonvulsant syndrome, in particular foetal valproate syndrome (DiLiberti et al. 1984). Some dysomorphic features, such as smooth or shallow philtrum or thin upper lip (Moore et al. 2000), described in individuals exposed to different AEDs appear to be common to all AEDs as well as to other toxin exposures such as alcohol. However most authors report specific features to valproate exposure, e.g. high broad forehead, infra-orbital grooves, and broad nasal root with short nose (Moore et al. 2000, Kini et al. 2006). Recent data have also emphasised the risk of developmental delay in children exposed to valproate in utero (Adab et al. 2004), in contrast to exposure to other anticonvulsants, particularly carbamazepine (Adab et al. 2004, Gaily et al. 2004). A consistent correlation with the degree of dysmorphism shown and likelihood of significant cognitive impairment in valproate-exposed children has also been documented (Gaily et al. 2004).

The fact that not all children exposed display such features, and the increasing number of descriptions of exposed siblings (Kozma 2001, Malm et al. 2002, Schorry et al. 2005) suggest a genetic susceptibility. Other facts also make this likely: why is there a moderately increased risk over the general population rather than 100%, whether for overt malformations or minor anomalies/learning difficulty? The degree to which genetic susceptibility is responsible, and through what mechanism, remains to date unknown, and therefore how we counsel mothers at risk, in question. Our duty remains to keep women informed of possible risks and from an early stage.

References


This is the drug for you, Madam!

Munir Pirmohamed

Department of Pharmacology and Therapeutics, University of Liverpool, Ashton Street, Liverpool L69 3GE, UK
<munirp@liv.ac.uk>

The aim of prescribing a drug for a patient should be to maximise benefits and minimise harms. In most instances, drug prescribing does involve some degree of personalisation. That is, the drug chosen for treatment will be based on various factors including age, gender, renal and hepatic function impairment, and avoidance of drug-drug interactions. Therefore, to suggest that doctors currently prescribe the same drugs for all patients with the same disease is an over-simplification. However, there is still a relatively unsophisticated approach, which leads to a large and unpredictable inter-individual variability in the response to drugs (efficacy and toxicity). Part of this variability is genetically determined, which has led to great interest, particularly since the completion of the human genome project, in the area of pharmacogenetics. This can be defined as the study of the genetic basis for the difference between individuals in response to drugs. The aim of pharmacogenetics is to identify, through predictive genetic testing, those individuals who are unlikely to respond to a drug and those who are likely to respond adversely to the same drug, and enrich the patient population receiving the drug, thereby maximising efficacy. Thus, pharmacogenetics is likely to lead to a higher degree of personalisation, much more than can be achieved through current best clinical practice.

The Royal Society has recently produced a report entitled “Pharmacogenetics: the hopes and realities of personalised medicines” which highlights the importance of pharmacogenetics, but also delineates the many hurdles that will need to be overcome for it to become entrenched in clinical practice (Royal Society 2005). Clearly, the presence of unequivocal evidence of clinical benefit is a necessary step in translating any laboratory findings into practice. However, many areas of pharmacogenetics are bedevilled by the lack of replication between different studies. In epilepsy, this is exemplified by the contradictory data on the role of MDR1 genetics in pharmacoresistant epilepsy (Leschziner et al. 2006).
Nevertheless, there have been some recent successes, which provide some hope for the future. For example, the identification of the HLA-B*5701 allele in predisposing to abacavir hypersensitivity in HIV-positive Caucasian patients has been shown in three studies (Hughes et al. 2004), and preliminary data suggest that its incorporation into HIV clinical practice has indeed led to a reduction in the frequency of abacavir hypersensitivity (Martin et al. 2004). Pre-prescription genotyping to prevent abacavir hypersensitivity may also be cost-effective given the high background prevalence of this reaction (5%; Hughes et al. 2004). A striking association has also been reported in Han Chinese patients between HLA-B*1502 and carbamazepine-induced Stevens-Johnson syndrome (Chung et al. 2004). However, to complicate matters further, this association may be specific to patients of Asian origin, since it has not been demonstrated in Caucasians (Alfirevic et al. 2006). Furthermore, given the rarity of the adverse reaction (one in 5,000-10,000 patients), it could be argued that it would not be cost-effective to genotype patients before administering despite the severity of the reaction. However, this requires formal analysis.

In the future, as whole genome scanning becomes cheaper, it may provide an unbiased assessment of the predisposition of an individual to beneficial and adverse drug responses. However, it is important to bear in mind that at the same time, we need to develop better informatics strategies that link health-related data to biological data, and incorporate these into decision tools that lead to readily understandable prescribing information for clinicians, most of whom will not be fully conversant in pharmacogenetics.

So are we currently in a position to say “This is the drug for you, Madam”? For most disease areas, including epilepsy, we are not there yet. We do try to personalise medicines in our current clinical practice, but this is still inadequate to predict individual responses with a high degree of accuracy. Pharmacogenetics offers the opportunity to refine our prescribing practices, and maximise the benefits from drugs, while at the same time minimising harms. However, as outlined by the Royal Society (2005), further research is needed, and it is important that funding be made available to tackle these issues.

**References**


**Discussion by Simon Shorvon**

Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK <cs.shorvon@ion.ucl.ac.uk>

Pharmacogenetics in epilepsy holds the promise for personalised medicine. There is a variation in the response to drugs and the side effects of drugs, and it is quite certain that at least partly, this response must be genetically determined. The human genome project and the advances in high throughput technology have improved our ability to detect genetic variation. However, there are formidable problems to overcome—scientific, practical and ethical. Some of the scientific hurdles include:

- **The confounding influences of environment**
  
  Including: age effects; upregulation and downregulation; change in gene expression; drug metabolising enzyme induction or de-induction; interaction with other drugs.

- **The confounding influences of disease variables**
  
  Including: the aetiology of the epilepsy; the biochemical mechanisms of the epilepsies; the extent and position of the epileptic lesion; the severity of seizures; the secondary consequences of seizures.

- **Issues in relation to drug action**
  
  Including: the fact that a drug may not have a single action; that multiple actions equal multiple genes; and that a single drug action may be determined by multiple genes.

- **Methodological problems relating to study design**
  
  Including: matching of patients/controls (and stratification of populations); the arbitrariness of phenotyping and its dependence on age, stage of disease, extent of investigation; genotyping issues; statistical issues (multiple testing/power) and the problems of replication.
Clinical relevance

Including: the fact that 70% of new patients are well controlled; most side effects are dose-related; severe idiosyncratic side effects are mercifully rare; it is easy to measure blood levels and the predictive value of test must be high.

In spite of these problems, and over-optimistic claims made in the past, clearly this is a field in which further study is needed. It is possible that toxicity prediction will prove easier than the prediction of responsiveness.

On collaboration: ways forward

Norman Delanty

Epilepsy Programme, Beaumont Hospital, Beaumont Road, Dublin 9, Ireland
<normandelanty@eircom.net>

There are over 15 academic epilepsy centres worldwide now collecting, storing, and analysing DNA from patients with epilepsy in the evolving field of epilepsy pharmacogenomics. In addition, a multi-centre pharmacogenetic collection is due to begin in the United States in the near future. Presently, most if not all collections involve hundreds of patient samples, with some centres having approximately one thousand samples. Analysis of at least several thousand well-phenotyped individuals with epilepsy will be necessary if pharmacogenomics is to answer questions of clinical relevance. Collaboration is now seen as imperative in clinical genomic research (Kaput et al. 2005).

Collaboration (from the Latin "collaborare", to work together) is believed to have an evolutionary basis in human society under the principle of indirect reciprocity (Bowles and Gintis 2002, Michor and Nowak 2002, Nowak and Sigmund 2005), i.e. if I scratch your back, someone else will scratch mine, and so on, so that the whole community benefits. Collaboration is essential if epilepsy pharmacogenomics is to advance our knowledge of the biology of epilepsy and help us begin to tailor optimum treatments for individual patients (Ferraro and Buono 2005, Tate and Goldstein 2004). Collaboration may be ongoing or once off, formal or informal, and may use shared or separate data sets and resources. Collaboration benefits through elaboration and sharing of research ideas and expertise, increasing the numbers of samples collected (especially for questions confined to specific phenotypes), encouraging more standardised phenotype definition, and optimising expertise in bioinformatics, genotyping, and functional genomics. Collaboration is vital to confirm initial positive associations before publication, to avoid the publication of unhelpful and misleading false-positive associations, and helps us address issues of fundamental biological importance across different populations.

Although having many benefits, collaboration itself also poses many challenges. These include, but are not limited to, initiating such collaborations in the first instance, with either implicit or explicit well-understood ground-rules for responsibility and reward; decisions with regard to authorship; the need for having a strategy with regard to intellectual property (Nunnally et al. 2005); and managing large amounts of data across multiple institutions.

Authorship issues can be a barrier to collaboration. Authorship conflict may be implicit (engendering silent bitterness) or explicit (engendering open hostility). It is easier to assign authorship when the original idea clearly comes from one of the collaborating centres, but is more difficult when there is approximately similar intellectual input between centres and when collaborating centres have “the same good idea at the same time”. Sharing of both first and last (senior) authorships may be a reasonable solution, but again, the situation becomes more complicated when there are more than two collaborating centres contributing to a completed manuscript.

Human factors may also impede collaboration. Ideally there should be free exchange of ideas, but in practice, this may not happen, leading to (over-) protection or the hoarding of ideas within groups. Academic jealousy; ego, a “history” between potential collaborators, and real or imagined issues of trust may all be formidable barriers to be overcome in initiating productive collaboration within the research community.

Development of fruitful collaboration in epilepsy pharmacogenomics will involve an explicit acknowledgement of the difficulties involved, frequent informal and formal meeting and dialogue, agreement of phenotypic definitions, and agreed authorship and intellectual property guidelines.

References


**Discussion by Graeme Sills**

Epilepsy Unit, Division of Cardiovascular and Medical Sciences, University of Glasgow, Western Infirmary, Glasgow G11 6NT, UK <G.J.Sills@clinmed.gla.ac.uk>

The nature of genetic and pharmacogenetic research in epilepsy is such that the number of patients required in any given study is often more than can be recruited at a single academic centre. Under such circumstances, collaboration may be the only means of arriving at a statistically and scientifically valid conclusion. This is not some alien concept. Collaborative efforts have been fundamental to the success of many epilepsy research projects in recent years. Effective collaboration in genetic studies may require specialist guidelines and specific agreements with regard to publication and intellectual property, but these should avoid excessive formality and prohibitive bureaucracy wherever possible. Issues of research direction, funding, and the division of labour can be readily addressed by an informal advisory board on which all interested parties are represented by invitation but without obligation. In an increasingly competitive research environment, genuine collaboration requires that the pursuit of scientific truth be placed above that of personal or institutional gain. We need to engender a culture of mutual cooperation, without compromising scientific integrity or promoting mediocrity. Simple models based on game theory, with direct applicability to the evolution of human behaviour, identify collective cooperation as the most profitable servant of the greater good (Axelrod 1985, Nowak et al. 1995). A culture of reciprocal censure in the peer-review process, particularly behind the veil of anonymity, serves only to divert attention and funding into other areas of medicine. Collaboration can succeed in the field of genetic research in epilepsy, but only if we learn to help each other to help ourselves.

**References**


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**Erratum**

In the review «Multimodality imaging for focus localization in pediatric pharmaco resistant epilepsy » by M. Kurian et al., published in the precedent issue of *Epileptic Disorders* (2007; 9(1): 20-31), an error has been introduced during printing in one author’s name: please read J.G. Villemure instead of J.G. Villeneuve.

All our excuses to this author.