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CRITICAL REVIEW AND INVITED COMMENTARY

Blinders, phenotype, and fashionable genetic analysis: A critical examination of the current state of epilepsy genetic studies

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Summary

Although it is accepted that idiopathic generalized epilepsy (IGE) is strongly, if not exclusively, influenced by genetic factors, there is little consensus on what those genetic influences may be, except for one point of agreement: epilepsy is a "channelopathy." This point of agreement has continued despite the failure of studies investigating channel genes to demonstrate the primacy of their influence on IGE expression. The belief is sufficiently entrenched that the more important issues involving phenotype definition, data collection, methods of analysis, and the interpretation of results have become subordinate to it. The goal of this article is to spark discussion of where the study of epilepsy genetics has been and where it is going, suggesting we may never get there if we continue on the current road. We use the long history of psychiatric genetic studies as a mirror and starting point to illustrate that only when we expand our outlook on how to study the genetics of the epilepsies, consider other mechanisms that could lead to epilepsy susceptibility, and, especially, focus on the critical problem of phenotype definition, will the major influences on common epilepsy begin to be understood.

KEY WORDS: Genetic analysis, Channelopathy, Phenotype, Genetic heterogeneity, Psychiatric genetics.

It is accepted that idiopathic generalized epilepsy (IGE) is mostly, if not entirely, caused by "genetic factors." In addition, it is virtually certain that two or more interacting genetic factors (let's call them genes) are responsible for IGE expression. Over the last 25 years, since the first strong evidence of a molecular genetic factor in an IGE was identified (Greenberg et al., 1988), there has been a plethora of papers reporting evidence that this or that gene predisposes to IGE, yet much of that evidence has not been replicated, has been observed in only one family, or remains equivocal. Genes have been discovered as causative for specific, very rare epilepsies or epilepsy-related syndromes [e.g., ragged red fiber disease (Shoffner et al., 1990), Baltic myoclonus (Pennacchio et al., 1996), Dravet syndrome (Claes et al., 2003), benign familial neonatal convulsions (BFNC) (Singh et al., 2003)], but there is no consistent evidence that any of these rare syndrome-related genes predispose to more common forms of inherited epilepsy or even provide much insight into causes of common epilepsy. Therefore, despite our acceptance of the role of genes in IGE susceptibility and despite the large number of purported findings, few of the genes involved in IGE susceptibility appear to have been identified.

We discuss herein major reasons that we believe identifying IGE-related genes has been problematic. Part of the answer lies in the weakness of genetic analysis methods, which we will discuss later. But a major factor over the last decade, one that looms large over the field, has been the assumption—or, rather, the dogma—that "epilepsy" is a "channelopathy." This belief, in our view, has so prejudiced the search for epilepsy genes that it has actively derailed wider scientific approaches to the problem. As we see it, once it was promulgated that channel gene dysfunction was the underlying mechanism that causes epilepsy, the search for IGE-related genes narrowed to that declared mechanism.

This sort of group behavior in a scientific discipline is not unusual. To reflect our points in another scientific mirror, we will start with a discussion of psychiatric genetics. Here, too, certain beliefs kept researchers from devising creative ways to search for genes for schizophrenia. These beliefs inhibited thoughtful data collection and experimental design, a foreshadowing of the epilepsy genetics discussion.

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A Brief History of Psychiatric Genetics

Psychiatrists have had problems with studies of schizophrenia even greater than those we have had in epilepsy studies. Psychiatric diseases—e.g., schizophrenia—also have evidence of strong genetic influences, just as are found in IGE family studies. However, unlike the case with epilepsy, the only phenotypes for psychiatric studies are behaviors. In epilepsy studies, we have other observations: seizure type, age of onset, seizure pattern, and so on, on which to base possible genetically related phenotypes; in schizophrenia, there is little comparable. To psychiatry's credit, it pioneered the approaches in trying to find disease genes for common diseases, but it also pioneered the mistakes (Greenberg, 1992).

Over the years there were three habits of thought that we think contributed to the failures to find schizophrenia-related loci. We label these problems Blinders, Phenotype Definition, and Methods of Genetic Analysis.

Blinders, or the dopamine hypothesis

For about 40 years, it was accepted as ''fact'' that disturbances in the dopamine system caused schizophrenia. As a result of this belief, if some molecule even came within hailing distance of dopamine or a dopamine receptor or could be tied in any way to that system, it was a candidate for a cause of schizophrenia. The belief that ''we know disruption of the dopamine system causes schizophrenia'' kept many investigators from stopping to consider whether they should be looking for anything different, or looking in a different way.

Phenotype definition

Second, in addition to believing that ''the'' cause of schizophrenia was known, investigators usually considered that they knew what schizophrenia was. Schizophrenia was what the Diagnostic and Statistical Manual (the DSM) said it was. Numerous articles on the search for schizophrenia genes started out, ''Schizophrenia is a complex, heterogeneous disorder with both genetic and environmental components, etc., etc.,''—but in the Methods section, the criteria used for defining schizophrenia were those arrived at by a committee that wasn't thinking about genetic studies but about treatment. This is critical because, as we discuss below, defining the phenotype is probably the most important step in trying to identify disease genes. Yet this step is simply glossed over in many studies of common disease. [This trend of muddying the phenotypic waters continues. Reports have it that the DSM-V will do away with the diagnoses of Asperger's Syndrome, Pervasive Developmental Delay (PPD), and autism, and instead everything will be lumped together as ''autism spectrum disorder'' (Grinker, 2010).]

Methods of genetic analysis

Third is the question of genetic analysis methods. Until about the early 1990s, it was imagined that the biggest problem in trying to identify common disease genes was so-called reduced penetrance. (''Penetrance'' is usually defined as the probability of expressing the disease, given that someone has the disease genotype.) The chosen path to overcome the penetrance problem was to look for the rare, large, dense pedigree in which all or most of the gene ''carriers'' had the disease, thereby eliminating the penetrance problem (Greenberg, 1992). However, this approach, first, created the problem of trying to find such rare pedigrees and, second, ignored a much greater confounder, the problem of heterogeneity. The situation got even worse in the mid-1990s when the scientific fashion changed: Suddenly, large dense pedigrees were ''out'' and studies of large numbers of affected sib pairs (ASPs) were ''in.'' (With ASPs, penetrance was not a problem because both sibs were affected.) In fact, the ASP approach made the heterogeneity situation much worse. At least with one large family, there is the hope that only one form of disease is segregating, something that cannot be expected when studying a dataset consisting of hundreds of sib pairs.

Following in the same misguided tradition of ''one method fits all,'' over the last 5 years, we have had genome-wide association studies (GWAS). Unfortunately, these did not yield the cornucopia of genetic solutions that were expected to solve the problematic issues of genetic analysis (Risch & Merikangas, 1996) have not justified the tremendous investment of resources that was made in them (Manolio et al., 2009; Mcclellan & King, 2010).

A recurring pathology in the sociology of genetic analysis is that the analysis approach ''du jour'' is the only method that seems to be acceptable, be it large pedigrees, ASPs, Transmission Disequilibrium Test (TDT), or genome wide association studies (GWAS), all of which were once viewed as *the* analysis method of choice (to be joined soon, perhaps, by ''deep sequencing''). And it is not that problems with the methods go unnoticed (Spence et al., 2003), but that the conventional wisdom is often so loud that dissenting voices go unheard.

Epilepsy Genetics—Blinders

There are strong parallels between the history of psychiatric genetics and what has happened in the field of epilepsy genetics. We have our own blinders, problems of phenotype definition, and beliefs about genetic analysis methods.

Blinders (''epilepsy is a channelopathy'')

By analogy to what happened in psychiatric genetics, the focus of much of epilepsy genetics on channel genes has narrowed thinking to the point where the suggestion that other systems could be causes of the common epilepsies is greeted with disbelief.

The narrative in an archetypal paper during the last few years in epilepsy genetics might be caricatured something like this: ''We found a large family with epilepsy. We performed a linkage analysis and found a region on some chromosome. There were dozens of identified genes in the region, among them a channel gene. Because epilepsy is a channelopathy, that gene must be the cause. So we sequenced the exons and we found a mutation. When we then put that mutated gene into a cultured cell, it changed the membrane potential (or response to neurotransmitters, or, and so on). It is true that some members of the family were carrying the mutation but did not have epilepsy, and other members of the family had epilepsy but were not carrying the mutation, but clearly that just means that members of the first group are not penetrant and members of the second group are phenocopies.''

The circular reasoning should be obvious.

Then there are the studies that pick a channel gene, study it in cases and controls, observe a statistically significant difference in the frequency of a polymorphism, and declare that gene to be a ''cause'' of epilepsy, even though the phenotypes used in the study often represent a mix of many different types of epilepsy. These studies will often report a difference in the frequency of a polymorphism between cases and controls that may be statistically significant but of questionable biologic significance (example below).

We do not yet know what causes IGE (among other epilepsies); we do not even know how many causes there are. Channel genes represent a reasonable hypothesis, one that is simple and intuitively appealing. However, given the number of channel genes that have been studied and the number that have been proposed but not replicated, or, even more confusing, the number that show a statistically significant but biologically meaningless increase in groups of patients with a variety of different epilepsy syndromes, maybe it is time to expand our thinking.

We turn to the issues of phenotype definition and genetic analysis methods and see how these issues play out in the case of epilepsy.

Phenotype definition

Phenotype definition and specificity should be a major issue in any study of common disease. Just as in psychiatric genetic studies, in which investigators use the DSM as their guide, in studies of epilepsy, investigators use the ILAE Commission on Classification. Yet this classification scheme, like the DSM, was not designed with genetic studies in mind. In fact, flying in the face of even the Commission's classification efforts, in many published epilepsy studies the criterion for including a patient is ''seizures,'' irrespective of type. In other words, phenotypes are defined in a rather cavalier manner, yet how subjects are chosen for genetic studies is absolutely critical.

Important point: Diagnosing for treatment is different from diagnosing for genetic studies. In diagnosing for treatment, one catalogs the symptoms, determines which diagnosis they fit, and determines a treatment appropriate to the diagnosis. If the treatment is successful, the diagnosis is considered ''correct.'' If the treatment is unsuccessful, one reconsiders the diagnosis and/or changes treatments. But diagnoses determined that way do not work for genetic studies. It is a weakness of genetic analysis methods that they assume a relatively pure genetic cause. This often has a devastating effect on finding evidence for the underlying genetic etiology(ies).

Example: Juvenile myoclonic epilepsy (JME)

Here is one example of how this ''diagnosis for treatment'' approach can lead to problems. This is based on our own experience collecting families for IGE studies.

One would think that juvenile myoclonic epilepsy (JME) would be straightforward to diagnose: presence of myoclonic jerks in the morning shortly after awakening; usually tonic–clonic seizures are present; 30% of patients have absence. But not everyone diagnoses JME the same way. We have seen so-called JME patients referred who were given their diagnoses of JME for the following reasons: patient responded to valproic acid; patient had 4–6 Hz spike and wave on electroencephalography (EEG); patient reports ''twitches''; jerks occurred only in response to flashing lights; jerks occurred any time of day (not only or mostly on awakening); and jerks occurred at night in sleep.

Obviously the first three symptoms on this list are insufficient for a diagnosis of JME. The others MIGHT be the same as JME, but might not. The danger is that, if they are not JME but are included, then one is introducing heterogeneity and potentially destroying the genetic signal in the data.

Issues when collaborating

For many genetic studies, it is almost impossible to recruit enough well-diagnosed patients and families from a single hospital, clinic, or facility. Therefore, collaboration is required. Because of the issues of diagnosis, investigators collecting genetic study data should not assume that a diagnosis for treatment yields patients with a sufficiently specific phenotype to include in the analysis. For example, an autism patient may have developed IGE in adolescence. Perhaps 20% of autism cases develop epilepsy and/or have epileptiform EEGs, but it would be a mistake to include autism patients, or a subject with any other developmental problem, in a study of epilepsy genetics. Including such a patient as having a ''diagnosis'' of IGE would be introducing likely heterogeneity into the data. This may seem self-evident, but the literature is full of studies of the genetics of ''epilepsy'' in which patients with broadly defined epilepsy and epilepsy as a comorbidity are included. It is difficult enough, even with rigid standards for acceptance, to reduce signal-degrading heterogeneity. Careful scrutiny and rediagnosis of all subjects

is essential. Such rediagnosis is done with an eye toward reducing inclusion of patients/families that give indications of not fitting criteria. Not "purifying" the data will lead to a failure to find biologically meaningful signals that can be followed up. The laboratory effort to turn a statistical indication into biologic proof of a gene's disease involvement can dwarf the effort to find the signal in the first place.

The message is this: Every case to be included in a study must be diagnosed to rigid standards applied to ensure narrowness and consistency in the phenotype definition. Collecting narrowly defined phenotypes is the best way to minimize the problem of phenotype specificity in the data.

Heterogeneity

Heterogeneity represents the single biggest contributor to phenotype-related problems in genetic analysis of *any* common disease. When clinically similar conditions have different etiologies, the genetic analysis methods fail. Subtly different phenotypes may be etiologically the same yet appear different because of variable expression, or they could represent etiologically different diseases. As a result, typical datasets contain mixtures of diseases with different etiologies that appear clinically similar. This is why good phenotype definition is so critical: The best way to resolve heterogeneity is by using solid clinical criteria—even *after* data have been collected. But this requires collecting detailed clinical data in the first place. The failure to collect detailed information can render a laboriously collected dataset useless in testing hypotheses that arise after initial data analysis, whereas the existence of sufficient information can lead to fundamental findings (see example in subsequent text of this article). This is one of the major reasons that virtually all GWAS yield relative risks little greater than 1, risks that may be statistically significant but are often biologically meaningless. If good phenotypic data were collected, one could then correlate which phenotypes or phenotypic configurations created the greatest risk. But the belief that "size is everything" when it comes to dataset collection dictates accumulating enormously large and expensive datasets (10,000 or more subjects), a strategy that precludes getting detailed phenotypic information on all participants.

An example of using phenotype to resolve heterogeneity

Here is an example of the effect of heterogeneity on an analysis and how such heterogeneity can be resolved by consideration of clinical data.

Figure 1 shows results of a linkage analysis of IGE, from a study showing linkage evidence on chromosome 8 (Durner et al., 1999). There are three different IGE classifications in the dataset: (a) All IGEs together, (b) one set of families in which the proband has JME, and (c) a set in which the proband does not have JME but some other, seemingly similar IGE. In the first graph, the "All IGE" classifi-

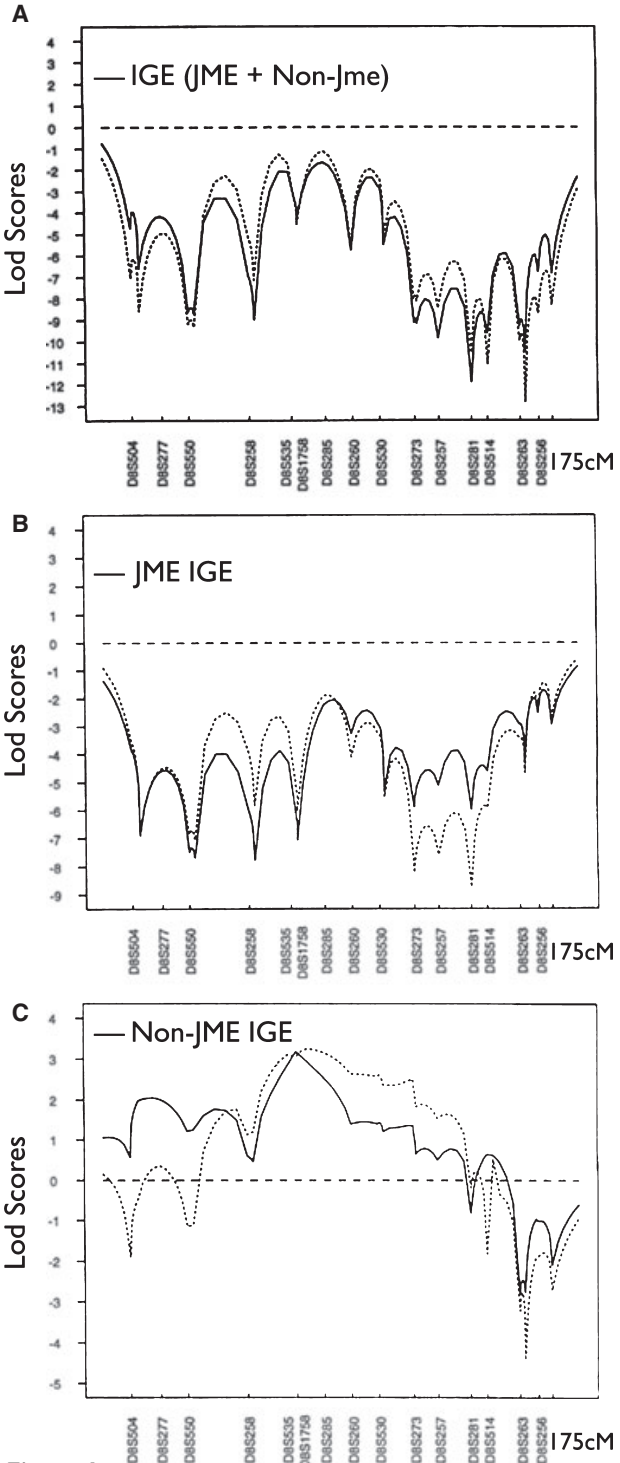

Figure 1.
Multipoint linkage analyses of chromosome 8 for three affectedness definitions (from Durner et al., 1999). See text for description. The solid line represents analysis defining only family members with seizures as affected. The dotted line indicates analysis that includes classifying spike-wave EEG in clinically unaffected family members as "affected."
Epilepsia © ILAE

cation shows strong evidence against linkage. The second graph shows the analysis with JME families only, and the third shows the non-JME families analyzed separately. The non-JME IGE families show significant evidence of linkage, evidence that was masked when all the IGE data were analyzed as a single phenotype.

Linkage analysis has a crude method of dealing with heterogeneity (Smith, 1963; Vieland & Logue, 2002), but in this case, it was insufficient to identify positive linkage evidence. (Note, in contrast, that association analysis has no such heterogeneity-distinguishing method.)

The most important point is that phenotypic information is critical even after initial data analysis. Careful examination of phenotypic differences can provide clues as to which genes affect what phenotypes, and can guide both later follow-up and later study design.

Epilepsy Genetics—Methods of Genetic Analysis

The approach to collecting the data cannot be completely divorced from the chosen analysis methodology. There are at least two common data collection approaches for studying epilepsy: (1) to collect many families identified through a proband with a specific form of epilepsy or (2) to find an atypical large pedigree that is greatly enriched for individuals with ''epilepsy'' (usually with various forms of epilepsy). We discussed the major problem of collecting many families: heterogeneity. What about the single-family approach?

Problems with the single-family approach

The approach of studying single dense pedigrees has revealed genes in some diseases [e.g., benign familial neonatal convulsions (BFNC) (Leppert et al., 1989), familial Parkinson's (Kitada et al., 1998)], but it is not without problems when applied to common disease.

When we discussed psychiatric genetics, we mentioned that the first favored method for schizophrenia-related gene identification was to identify the rare pedigree in which most gene carriers were penetrant, in the hope that ''*the*'' gene would be found. This has also been a frequent approach in epilepsy genetic studies, including the assumption that one gene (''*the* gene'') was what really mattered.

But the problems with this approach are several.

First, such pedigrees are rare. A consequence of this rarity is that genes that *are* discovered by this method are a cause of disease only in rare and/or often severe, cases: for example, *LGI1* in autosomal dominant partial epilepsy with auditory features (ADPEAF) (Kalachikov et al., 2002), Dravet syndrome, caused by *SCN1A* mutations are examples. But even in *SCN1A*, most cases are caused by new mutations and are, therefore, not inherited (Claes et al., 2003). The contribution of *SCN1A* to generalized epilepsy with febrile convulsions (plus) (GEFS+), another rare, family based syndrome, remains unclear (Selmer et al., 2008; Hahn & Neubauer, 2009; Scheffer et al., 2009). On the other hand, rare cases may be more likely caused by a single mutation, increasing the likelihood that the gene can be discovered by the newer sequencing techniques.

Second, when linkage analyses of such pedigrees yield no linked loci (a not uncommon phenomenon), the cause of the failure may be explained by the very reason the family has many affected members. It may be that there are multiple, independent causes of the disease that have come together in this one, unusual family. As more affected members are required for ascertaining a family, the greater the likelihood that there are two or more independent causes of the condition in the family (Durner et al., 1992), creating intrafamilial heterogeneity.

Third, because any disease-causing gene found in a single family will likely be uncommon, replication could be difficult.

Fourth, it may be difficult to prove that the gene you suspect to be related to epilepsy expression is really an epilepsy gene, because association analysis (which can be used to test the genetic contribution to a disease of an allele at a specified locus), especially family based association analysis, if based on a single, or small number of pedigrees, has limited power. Finding a major, easily identifiable mutation (e.g., a deletion) could be the only kind of genetic variant that is findable in a single or small group of families. More subtle causes (e.g., splicing variations) are not easily noticed.

Single-gene thinking

But most problematic to us is the notion that the disease is ''caused'' ''mostly'' by one gene, a notion that is common whether the single large pedigree approach or the multiple pedigree approach is adopted.

Indeed, the whole notion of ''penetrance'' was originally based on thinking about traits as ''caused'' by a single gene. Even today, the talk is often of so-called ''modifier'' genes, the assumption being that the disease is ''caused'' by a single gene but that ''modifiers'' suppress or change the expression of that ''major'' gene. Even though the notion that multiple interacting genes must be involved in genetic epilepsy expression is widely accepted, the way these multiple genes interact will be complex, and will probably not adhere to the notion of a single *major* gene. The simplistic idea that one gene is the ''real'' cause and that the ''modifiers'' are somehow ''adjuncts'' to the main gene can set misleading expectations. This oversimplification leads to an attitude that one is searching for *the* gene and that the modifiers are mere nuisances. If one thinks instead about other interacting loci as being ''causative,'' expectations take on a subtly different hue. If epilepsies require multiple genes for expression, such a model of inheritance has important consequences when attempting to understand the genetics.

What happens if the focus is on single genes?

If, let's say, disease alleles at two loci are *required* for disease expression, then both are equally important, and disease alleles must be present at both loci. A critical consequence of requiring two genes is that the disease allele frequency at both loci must be relatively high if the disease is to have an appreciable frequency in the population.

Here is a simple example: Suppose two genes interact to cause disease. In this example, there must be at least one disease allele at one of the loci and two disease alleles at the other, that is, a dominant-recessive two-locus epistatic mode of inheritance.

Figure 2 shows that if the population prevalence is going to be as high as 1%, then the frequencies of the disease allele(s) at the dominant disease locus can be found in perhaps 10% of the population (depending on the recessive allele frequency at the second locus). At the same time, at the recessively inherited locus, the risk allele(s) frequency in the general population must be about 25%, depending on the frequency at the dominant locus. Allele frequencies must be substantially higher than "rare" (e.g., 0.01) in order for the disease prevalence to be even 1/10th of 1%. Therefore, no matter what the particular allele frequencies, one or both disease risk alleles should appear with some frequency in controls. In fact, given our current evidence, it is highly likely that the genetic epilepsies result from an interaction among developmental variation, metabolism genes, channel gene polymorphisms, and perhaps other factors that we will learn about in the fullness of time. This dictates that notable allele frequencies should be found in the general population. The more interactions that are required, the higher the disease-related allele frequencies at the loci.

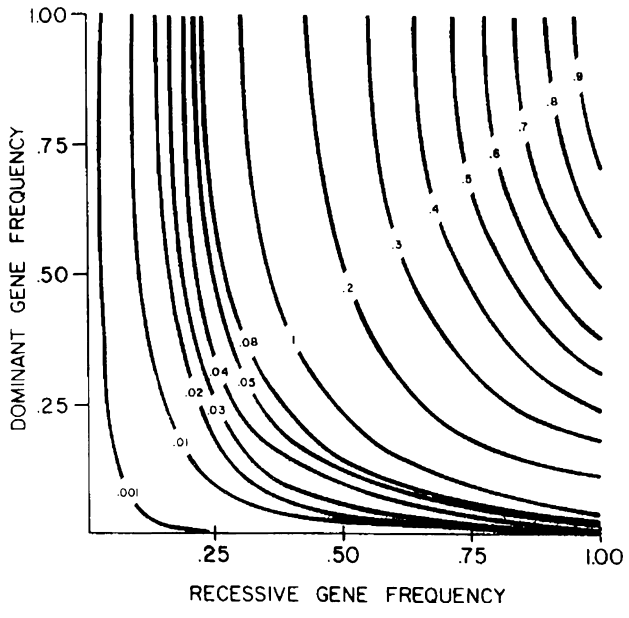

Figure 2.
Population prevalence dependence for a two-locus epistatic model with one locus showing dominant and one locus showing recessive inheritance. "Contour lines" show lines of constant population prevalence of the disease at a given combination of allele frequencies at the two loci (Greenberg, 1981).

Therefore, if one identifies a putative causative mutation for a relatively common condition but one finds that mutation occurs rarely or not at all among controls, this casts doubt as to whether one has discovered a gene for a common disease. If there is "reduced penetrance," then a lot of people who are not affected must still carry the disease allele(s). If a putative mutation is not seen, or is extremely rare, in controls, then one can easily reach statistical significance, but what does such a finding mean biologically?

Here is but one prominent example, but the literature has many findings of this type. In a study of IGE by (Helbig et al., 2009) the authors examined a single, specific locus. That locus, *CHRNA7* on chromosome 15q13, had different microdeletions. Among 1,223 patients with IGE, 12 had *some* microdeletion, but no microdeletions were found in 3,699 controls. (That is, only about 1% of the *cases* had some microdeletion.) The allele frequency difference between cases and controls is highly statistically significant, but statistical significance is not biologic significance. A finding of this kind begs the question: What is the next step? Is this gene worth pursuing in order to answer questions about IGE? A *rare* mutation, irrespective of its statistical significance, is unlikely to be a major contributor to a *common* disease. Moreover, pursuing any finding to prove that the gene found is the gene sought takes a major laboratory effort. Pursing a statistically significant, but etiologically weak, finding may well lead to little gain in understanding of disease mechanisms, especially in light of heterogeneity, since "IGE" is not a specific diagnosis.

Epilepsy Genetics—Nonchannelopathy Mechanisms

There has been relatively little work on nonchannel mechanisms in genetic epilepsies, despite solid evidence of the importance of these mechanisms in epilepsy susceptibility.

Unverricht-Lundborg

Although Unverricht-Lundborg is not a common form of epilepsy, it provides an illustration that nature can surprise us. This progressive myoclonus epilepsy is caused by cystatin B, an inhibitor of cysteine proteases (Pennacchio et al., 1996). Even so, before the cause was discovered, it was suggested that this recessively inherited disease was caused by a channel gene (Yamakawa et al., 1995).

Transcriptional machinery

The *BRD2* gene may be the only common epilepsy-related gene in which: (1) the locus was identified in

multiple linkage analyses (Greenberg et al., 1988, 2000; Weissbecker et al., 1991), (2) the association was replicated in independent studies (Pal et al., 2003; Lorenz et al., 2006; Cavalleri et al., 2007), (3) a mouse possessing only one functioning gene was shown to be more sensitive to seizures (Greenberg et al., 2007), and (4) the mouse was shown to have fewer than normal γ-aminobutyric acid (GABA)ergic neurons in relevant parts of the brain (Greenberg et al., 2007). But, a priori, *BRD2* does not provide an immediately intuitive mechanism for how it may lead to seizure susceptibility. We know it is an element in a transcription factor complex; that is, it does something during making RNA on a DNA template.

Adding to the interest in transcription is the finding that the *ELP4* (elongator protein complex 4) gene was recently identified as a cause of centrotemporal spikes in rolandic epilepsy (Strug et al., 2009). *ELP4* is involved in gene transcription and in tRNA modification. Other molecules involved in the elongator complex may be involved in cell migration of neurons particularly during cortical development (Creppe et al., 2009).

The mouse findings discussed previously, plus the finding that components of the transcription machinery are involved in two relatively common epilepsies that are of genetic origin, suggest that the bases of these IGEs are laid down during development. In the case of *BRD2*, the finding that there is a deficit of GABAergic neurons in haploinsufficient mice suggests that a subtle but fundamental alteration in brain structure may be responsible for part of the genetic susceptibility. This kind of change cannot be easily ''buffered'' by other systems or processes, whereas such buffering can be observed in processes that are embedded in metabolic pathways. A prominent example of such buffering can be seen most prominently in systems involving channels. Pathways involving channel genes are well known for displaying such buffering by altering levels of expression in a compensatory fashion in response to alterations in normal circuitry (Grimm et al., 2008; Black et al., 2010; Kreiner et al., 2010).

Brain energy

Brain energy metabolism is another area that could fruitfully be more widely explored. Defects in mitochondria lead to epilepsy (Fukuhara et al., 1980), that hypoglycemia can lead to seizures and neural damage (Arieff et al., 1974; Sapolsky & Stein, 1989; Velisek et al., 2008), that the ketogenic diet works for some patients with epilepsy, and that glucose metabolism syndromes can lead to seizures (Brockmann et al., 2001; Dufour et al., 2001; Yudkoff et al., 2001; Roll et al., 2002). We have found evidence that the *ME2* gene, a Krebs cycle–related gene involved in neurotransmitter synthesis, is linked and associated with IGE (Greenberg et al., 2005).

The question is: Why focus so exclusively on channel genes and ignore developmental genes, brain energy metabolism, or other mechanisms? We are not proposing that genes involved in transcription or energy metabolism should be the first pursued in studying the causes of inherited epilepsy, but the presence of such a gene in a linkage region or around an associated single nucleotide polymorphism (SNP) should not be ignored simply because it is not a channel gene.

Summary

Looking back on the common themes of the search for channel genes in epilepsy, three types of studies predominate: (1) studies in which a locus is identified by linkage analysis of one family with many affected members, and then a channel gene in the linkage region is chosen for further study *because* it is a channel gene; (2) studies reporting small groups of families (2–10), usually with several affected members, often with mixed diagnoses (which makes them atypical to begin with), that are used to investigate specific channel genes—finding any mutation in any one of those genes in any one of the families causes a jump to the conclusion that this mutation is related to all the epilepsies in the family(ies); (3) more recent approaches, which analyze an assembly of large numbers of patients of often undefined or poorly defined epilepsy syndromes in which specific genes or loci are studied. Then, mutations in the channel gene, or whatever the locus under study, in, say, 1% of patients and finding no mutations in controls, leads to statistical significance and a declaration that the gene in question is a susceptibility gene for epilepsy.

What is missing in these approaches is for investigators to question the relationship of proposed genes to epilepsy phenotypes. To our knowledge none of the genes identified by the first two approaches has been shown to increase epilepsy susceptibility in common epilepsies and it is difficult even to prove that the mutations are the actual ''cause'' of the epilepsy in the families in which they were discovered. Expressing the mutated gene in cultured cells is one experiment, but it tells us nothing about the gene's relationship to epilepsy susceptibility. A mutated channel gene is likely to cause a difference in membrane potential, neurotransmitter response, or some other measure in the right cell line. Creating a knockin (or knockout) mouse is more informative, but time-consuming and expensive, and there remains the question of whether other genes play a determining role in epilepsy expression. In the case of a finding based on the third approach discussed in the preceding, that is, looking at hundreds, or thousands, of patients with mixed diagnoses, the next question should be: Are there clinical features of carriers of the associated allele that would help us identify those patients influenced by the putative disease gene, which is presumably near the associated marker/SNP? Answering this question could potentially be the most useful aspect of these types of studies. Although a statistically significant association with a relative risk on the order of 1.2 is

not a biologically convincing finding, bringing clinical information to bear could help resolve the undoubted heterogeneity and refine which phenotypes are influenced. Unfortunately, clinical details that play such an important role in phenotype definition are usually not reported, or even collected.

Let us be clear on a critical point: We are not saying that channel genes are not involved in some types of epilepsy. We are not saying that channel genes should not be studied. We are arguing against the dogma that ''epilepsy'' equals ''channelopathy.'' Given that ''epilepsy'' is not a unitary disease, this dogma, on the face of it, has been uninformative at best and misleading at worst when it comes to IGE. The evidence that has accumulated since the channelopathy hypothesis was put forward is that the majority of common inherited epilepsies are not strongly influenced by any channel genes yet studied, but are influenced by genes of other types. Confining ourselves to studies of only one mechanism will not cure IGE.

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