The focus of the Area I Benchmarks is on understanding the etiologies of the epilepsies and related conditions. The greatest advances in the past 3 years have been made in our understanding of the genetic and immune causes of epilepsy. Novel gene and autoimmune discoveries have been facilitated by technologic advances and by large collaborative efforts to combine patients and streamline experimental studies. The underlying mechanisms of seizures as they present in the different epilepsy syndromes continue to be a main focus of funded studies. Many studies have examined the bidirectional relationship between epilepsy and psychiatric comorbidity; effects of seizures on both behavior and cognition in animal models; and the illness-related and psychosocial variables associated with the psychiatric, cognitive, linguistic, and social comorbidities of epilepsy. Yet, advances in “identifying the underlying mechanisms, interacting mechanisms, and consequences of the first condition (epilepsy) of these comorbidities” have been slow to emerge. We identify key advances and discuss the factors that have promoted or hindered progress in achieving these goals, and we consider the research that should be conducted to move the field forward.

Key Advances in Area I

Epilepsy Genetics

Remarkable progress has been made over the past several years owing to increased availability and decreased costs of genomic technologies. The largest slice of the etiology pie known as “idiopathic epilepsy” is being progressively shrunk by the discovery of new genetic causes of epilepsy. A major advance, facilitated by whole exome sequencing in “trios” of an affected child and both unaffected parents, is the discovery that epileptic encephalopathies are often caused by de novo mutations. The largest studies to date include sequence analysis of 356 trios in which the proband presented with infantile spasms (IS) or Lennox–Gastaut syndrome (LGS). The first study, carried out by the Epilepsy Phenome/Genome Project (EPGP), presented data from 264 trios and identified GABRB3 and ALG13 as novel genes in which de novo mutations cause IS or LGS (1). In a follow-up, collaborative study with the EuroEPINOMICS consortium, 92 additional trios were sequenced, and DNMT1 was confirmed as another causative gene (2). Mutations in GRIN2A were described by three groups as a cause of up to 20% of epilepsy aphasia syndromes (3–5). This confirms the importance of genetic factors in a class of epilepsies that were once thought to be acquired. A plethora of smaller studies using whole exome or targeted gene sequencing in probands and parents have identified de novo mutations causing epileptic encephalopathies in numerous other genes (6–16). Together, genetic advances in the epileptic encephalopathies highlight the importance of de novo mutation but also the genetic heterogeneity, which has implications for diagnostic testing of epileptic encephalopathies and, likely, their optimal treatment.

The International League Against Epilepsy (ILAE) Consortium on Complex Epilepsies recently performed a meta-analysis of genome-wide association studies (GWAS) in focal and generalized epilepsies (17). Analysis of data from >8,000 cases and >26,000 controls revealed genome-wide significant loci implicating SCN1A and PCDH7 in the combined focal and generalized cohorts and VRK2 or FANCL in the generalized epilepsy cohorts, though the clinical implications of these findings are unclear. In another GWAS that focused on patients with simple febrile seizures, Feenstra and colleagues identified...
several risk alleles for febrile seizures, as well as two loci that are specific for risk of febrile seizures related to the measles, mumps, rubella (MMR) vaccine (18).

Another recent development in the field of epilepsy genetics is the increasing recognition that somatic mutations play a role in focal epilepsy, particularly focal epilepsy associated with structural brain malformations. To date, several cases of focal cortical dysplasia (FCD) and hemimegalencephaly have been explained by somatic and germline point mutations as well as copy number abnormalities involving several genes, including AKT3, PI3KCA, PI3KR2, PTEN, DEPDC5, and MTOR (19–23). These findings represent the power of next-generation sequencing to identify somatic mosaic mutations present in a relatively low percentage of cells (<10% in some cases) in a tissue assayed. The convergence of these findings on the mTOR pathway genes suggests that precision medicine may one day be applicable to the treatment of epilepsy associated with rare malformations such as hemimegalencephaly as well as relatively common conditions such as FCD.

One of the major goals of gene discovery is to be able to provide a diagnosis that points to specific therapies depending on the underlying cause—an approach termed “precision medicine.” A few examples are emerging, though appropriate clinical trials are still necessary to confirm anecdotal findings. Examples include experimental treatment of patients with KCNT1 mutations with quinidine, which acts directly on the KCNT1 channel (24, 25); memantine for patients with GRIN2A mutations (26); and rapamycin for patients with mutations in mTOR pathway genes (27).

Autoimmune Epilepsies
Another area that has seen significant progress during this period is our understanding of epilepsy associated with autoimmune encephalitis syndromes. Several new autoimmune epileptic encephalitides associated with neuronal autoantibodies have been described in recent years, and there is growing recognition that these conditions represent an important and not uncommon cause of previously unexplained refractory epilepsy presentations. Recently, a novel syndrome has been described in six patients who had refractory status epilepticus or epilepsy partialis continua with extensive neuroimaging abnormalities and high titers of serum and CSF antibodies against the α-1 or β-3 subunits of the GABA<sub>A</sub> receptor. These antibodies were shown to cause a selective decrease in GABA<sub>A</sub> receptor clusters at synapses, a plausible biological mechanism for hyperexcitability. Some patients improved in response to immunotherapy (28).

In addition, several recent reports have identified herpes simplex virus (HSV) encephalitis as an antecedent infection to a classic anti-NMDA receptor encephalitis presentation, and cases of relapsing encephalitis after successful treatment for HSV have been associated with the new development of autoantibodies against the NMDA receptor and other antigens. Anti-NMDA receptor encephalitis is recognized as a distinct syndrome presenting with behavioral and psychiatric changes, seizures, dyskinesias, and autonomic lability, often progressing to prolonged coma and disability. Up to half of these cases are paraneoplastic and associated with ovarian teratoma, but the remainder are unexplained. While HSV-related limbic encephalitis is a well-known cause of epilepsy, the identification of postinfectious autoimmune encephalitis following successfully treated HSV infection and its potential link to known autoimmune-mediated syndromes is relatively new (29–31).

Epilepsy-Related Conditions
There is growing awareness that epilepsy is a multifaceted disorder in which seizures are accompanied by psychiatric, cognitive, and social comorbidities, all of which impact the long-term outcome and quality of life for patients and their families. There is evidence for a time-sensitive occurrence of suicide in patients during a window of ±3 years after onset of epilepsy (32) and of unprovoked seizures in the window of ±2 years of hospitalization for psychiatric disorders (33). Nevertheless, few advances have been made during the past 3 years in our understanding of the mechanisms underlying the psychosocial comorbidities of epilepsy and their demographic risk factors.

Pearson et al. (34) report that reactive oxygen species production may be a key driver in processes underlying cognitive dysfunction associated with epileptogenesis and is, therefore, a viable therapeutic target. The authors found pharmacologic removal of reactive oxygen species reverses the effects of oxidative stress secondary to status epilepticus on memory and cognition in rats. Most important, this effect was evident despite continued seizures.

Several studies have revealed a diversity of epilepsy-associated behavioral perturbations even under otherwise standard conditions (e.g., epilepsy model; strain, sex, and age), thus opening opportunities for exploring candidate endophenotypes that predispose to specific comorbidities of epilepsy. Becker et al. (35) found that predisposition of rats to epilepsy comorbidities depends on the reactivity of brain-derived neurotrophic factor (BDNF) signaling. Animals with a sustained decrease of serum BDNF level in response to social defeat developed depressive behavior and cognitive deficits after status epilepticus (SE). Conversely, rats in which BDNF levels promptly recovered after stress displayed no behavioral impairments despite developing chronic epilepsy (35). Pineda et al. (36) connected types of epilepsy comorbidities with specific perturbations in ascending monoamine pathways. Rats with post-SE epilepsy with suppressed serotonergic tone in the raphe nucleus–prefrontal cortex presented with depressive behavior, whereas dysfunctional locus coeruleus–prefrontal-cortex transmission correlated with attention deficit hyperactivity disorder (ADHD)-like behaviors (36).

Looking Forward: Challenges and Opportunities
Access to technology and “Team Science” are two major factors that have accelerated our understanding of epilepsy genetics. The introduction and accessibility of genomic technologies has allowed efficient and affordable interrogation of the entire genome (or exome) for copy number and sequence variants in individuals, families, and large cohorts. Studies to date make it increasingly clear that all types of epilepsy are genetically heterogeneous. That is, there are many genes in which mutations can cause disease, and even though gene discovery is proceeding at an unprecedented pace, many of the genes explain a very small fraction of cases. This, in turn, means that very
large cohorts must be studied to identify multiple patients with the same genetic cause and confirm novel findings. The development of large teams such as Epi4K, EuroEPINOMICS, the Pediatric Epilepsy Research Consortium, and others bring together scientists, clinicians, and patient cohorts to move the field forward.

Building on the success of the team science approach to genetics, it may be fruitful for the epilepsy community to develop multidisciplinary teams to conduct well-powered, collaborative projects to identify the underlying mechanisms, interacting mechanisms, and consequences of epilepsy comorbidities. Such studies should be designed around the clinical needs of individuals with epilepsy and involve close interactions between clinician scientists specializing in epilepsy and researchers outside of the field of epilepsy (especially in psychiatry) as well as with basic science researchers in different disciplines.

Currently, for example, integrated biological and psychosocial studies are not routinely conducted. Inclusion of heritability of psychiatric disorders and epilepsy, parent factors and the influence of any related parent psychopathology in pediatric epilepsy, exposure to educational instruction, demographic variables known to be risk factors for psychiatric disorders, and the social context of adult epilepsy (stigma, unemployment, seizure control, and antiepileptic drug [AED] use) are essential to delineate the underlying mechanisms of comorbid psychiatric disorders. Birth cohort studies on the development of seizures, psychopathology, and both seizures and psychopathology in the offspring of mothers with/without epilepsy and with/without depression and anxiety disorders that control for parenting and other psychosocial variables will shed light on the chronology and predictors of the two-way relationship between epilepsy and psychiatric disorders. Large-scale prospective studies in adults and children with new-onset seizures that screen for genetic abnormalities found in psychiatric disorders, saliva cortisol levels, stressors and adversity, and examine multimodal structural and functional imaging, as well as psychosocial environmental variables at baseline and over time can begin to delineate the mechanisms involved in the trajectory of psychopathology in patients with epilepsy.

In addition, developing a standardized approach to characterizing the psychiatric, cognitive, and social comorbidities of epilepsy will be important. The literature to date includes prospective short-, intermediate-, and long-term studies on psychopathology and cognition in epilepsy that use different methodologies (instruments, informants, interim intervention), contain heterogeneous patient samples, and lack power to examine how the interrelationship among seizure, cognitive, psychiatric, and demographic variables determine the functional long-term trajectory.

One key issue is that studies have not routinely included contrast groups of patients with psychiatric or cognitive disorders but without epilepsy to examine the specificity of the findings or lack thereof for epilepsy. In the areas of cognition and language function, the application of statistical tools that have causal inference, namely graph theory, to analyze studies conducted on large representative homogenous samples of epilepsy subjects controlled for illness severity, epilepsy syndrome, and AEDs could result in new insights. (37).

Standardization of preclinical assays, including behavior and other tests, could also accelerate progress in basic science research as well. Such comparative data could help distinguish the most useful models of neurodevelopmental disorders among the many available (e.g., transgenic animals, inbred strains, pharmacologic, surgical models) for studying epilepsy phenotypes and the two-way relationship between these disorders and epilepsy. It is worth highlighting the critical importance of carefully differentiating behaviors in animals (e.g., attention, hypo- and hyperactivity, cognitive deficits, fear and anxiety, pain) before attributing a specific behavioral result to a particular neuropsychiatric association. The ILAE/American Epilepsy Society (AES) Translational Task Force (TTF) is undertaking a systematic review of animal model data on epilepsy comorbidities and is developing preclinical Common Data Elements, standardized procedures and protocols for the examination of neurobehavioral comorbidities of epilepsy in the laboratory setting. It is also worth noting that the majority of animal studies have been performed in males despite female predominance in depression and anxiety disorders in the general population. Studies on the role of gender in the neuropsychiatric comorbidities of epilepsy will also bring research in line with recent NIH policies on balancing sex in cell and animal studies (33).

There has been significant progress in achieving the Area 1 Benchmarks, especially in our understanding of the genetic and immune etiologies of epilepsy. These advances pave the way for improved diagnosis and therapy, and future studies should focus on the development and implementation of disease-, antibody-, and gene-specific treatments. Progress toward understanding the etiology of epilepsy comorbidities has been slower. Addressing the challenges described above through well-designed, large, longitudinal cohort studies, more effective collaboration between clinical and basic science researchers, and the use of standardized models, assays, and batteries are an essential first step toward delineating the mechanisms underlying the psychiatric, cognitive, social, and vocational problems associated with epilepsy and identifying patients at highest risk for these comorbidities.

References
2. EuroEPINOMICS-RES Consortium; Epilepsy Phenome/Genome Project; Epi4K Consortium. De novo mutations in synaptic transmission...


