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Review

Unraveling the genetics of common epilepsies: Approaches, platforms, and caveats

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ABSTRACT

With no known intervention to prevent or cure epilepsy, treatment is primarily symptomatic and requires long-term administration of medications to suppress seizure occurrence. Current antiepileptic drugs (AEDs) are ineffective in one-third of patients (Kwan and Brodie, 2000, [1]). Such therapeutic inadequacy is largely due to our insufficient understanding of the basic molecular pathophysiological processes that underlie epileptogenesis. Breakthroughs are needed in the identification of new molecular targets that will translate to novel intervention approaches.

Discovering genetic variants that increase the susceptibility to disease is a promising avenue to identifying such targets. However, early candidate gene-based studies in epilepsy proved ineffective in identifying genetic risk factors for the non-Mendelian, complex epilepsies, which represent >95% of clinically encountered epilepsy. Furthermore, genome-wide association studies (GWAS) of epilepsy patients have been largely negative, with the exception of several putative susceptibility loci discovered in Han Chinese focal epilepsy and European Caucasian GGE patients (Kasperaviciute et al., 2010; Guo et al., 2012; Consortium et al., 2012, [2–4]). Results of these GWAS suggest that, similar to other common diseases, associations with common single nucleotide variants (SNV) appear likely to account for a small fraction of the heritability of epilepsy, thus fuelling the effort to also search for alternative genetic contributors, with a recent increased emphasis on rare variants with larger effects (Manolio et al., 2009, [5]).

It is possible that both common and rare variants contribute to an increased susceptibility to common epilepsy syndromes (Mulley et al., 2005, [6]). We review the approaches that have been taken to identify genetic risk markers of the common epilepsy syndromes, the experimental platforms, and their caveats. We discuss current technologies and analytical frameworks that might expedite the discovery of these variants by leveraging advances in microarray-based, high-throughput, genotyping technology, and complementary interdisciplinary expertise of study teams including the need for meta-analyses under global collaborative frameworks. We briefly discuss the analytical options made available through rapid advances in sequencing and other genomic technologies.

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1. Introduction

Instead of a single disease, epilepsy is considered to represent a collection of disorders of a variety of causes characterized by an enduring predisposition for recurrent, usually spontaneous, seizures [7] that in many patients do not respond to available antiepileptic pharmacotherapy [1]. Epilepsy is classified as symptomatic, "structural/metabolic", when its development can be related to a preceding brain insult/structural abnormality (e.g., stroke, head injury, and tumor) and idiopathic, "genetic", when a genetic basis is assumed [8]. Twin and family studies suggest that epilepsy, across the syndromes, is highly heritable. One of the most comprehensive investigations of heritability in epilepsy came from an Australian twin registry, which concluded that genetic factors are not only particularly important in the generalized epilepsies but also play a role in partial epilepsies, such as temporal lobe epilepsy (TLE) [9]. In that study, the high frequency of concordant monozygotic (MZ) pairs with epilepsy (44.4%, casewise concordance = 0.62) compared to dizygous (DZ) pairs (9.7%, casewise concordance = 0.18) indicated a strong genetic basis for the epilepsies. The casewise concordance appeared particularly strong not only in generalized epilepsies (MZ = 0.82; DZ = 0.26) but also in focal epilepsies (MZ = 0.36; DZ = 0.05). Other published estimates of heritability have provided further support that epilepsy has a strong genetic basis [10,11]. In an unselected sample of twins recruited from the population-based Danish

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Twin Registry, it was estimated that 70–88% of the liability to epilepsy could be attributed to genetic factors [12]. It is clear that if there are genetic risk factors for 'acquired' epilepsies, they do not have similar levels of genetic load compared to the genetic generalized epilepsies. There is, however, mounting evidence suggesting that even these structural/metabolic epilepsies might, in some cases, be influenced by a degree of underlying inherited susceptibility factors that interact with environmental factors [13–15]. Literature for this is often considered not definitive, but evidence accumulated from concordance rates in twin studies [9], cohort studies such as the recent Danish study on post-traumatic epilepsy [16], and from animal studies [17] support the possibility that the 'acquired' focal epilepsies could also be influenced by genetic susceptibility markers [13,18]. For instance, in a candidate gene study, *APOE* ε4 allele carriers were found to have a 2.4-fold increase in risk of epilepsy following traumatic brain injury [19].

2. Gene studies of common epilepsies based on candidate genes

One of the earliest ventures into understanding the genetic basis of the complex epilepsies was through investigating candidate genes with plausible biological mechanisms, or as identified in monogenic epilepsies from large pedigrees [20]. Fine linkage mapping in large pedigrees has successfully elucidated the causative genes in a number of monogenic familial epilepsies [21]. This approach leverages information from the meiotic crossover events to localize candidate genomic region(s) that appears shared amongst affected individuals of a monogenic epilepsy disorder. However, such monogenic epilepsy disorders tend to follow Mendelian inheritance and are not as individually prevalent in the more common epilepsies, which are believed to be a result of more complex inheritance patterns. Disappointing results from the candidate based studies [20] argued for a genome-wide approach without a priori assumptions that could discover previously unsuspected markers. This was made possible by the sequencing of the human genome and the International HapMap project together with developments in microarray-based high-throughput technology that genotype hundreds of thousands of single nucleotide polymorphisms (SNPs) across the genome to look for polymorphisms (regions) in the genome significantly associated with susceptibility.

3. Assessing the common disease common variant hypothesis

Based on the 'common disease, common variant' (CDCV) hypothesis, genome-wide association studies (GWAS) have been performed largely on common SNPs (minor allele frequency [MAF] >5%). In GWAS, the allele frequencies are compared in unrelated affected and unaffected individuals, with large sample sizes required to generate sufficient statistical power to detect true disease associations after correcting for multiple testing. Despite some success of GWAS in identifying variants associated with many common diseases, they are most often associated with small increments in risk (1.1–1.5-fold) and cumulatively still explain only a small fraction of known heritability, thus providing limited value in a clinical context.

The first reported GWAS of epilepsy included Caucasian subjects with partial (focal) epilepsy of known (symptomatic) and unknown (cryptogenic) causes [2]. Although the quantile–quantile plots showed a slight departure from normal expectation, none of the P-values reached genome-wide significance. However, because of differences in genetic structures between ethnic populations, it is possible that some genetic factors influencing susceptibility to epilepsy may differ. In the combined analysis of the 2-stage Han Chinese GWAS of symptomatic epilepsy, which was the second published GWAS of epilepsy and the first such study in Chinese [3], the strongest signals were observed with two highly correlated SNPs, rs2292096 [G] ($P = 1.0 \times 10^{-8}$, OR = 0.63) and rs6660197 [T] ($P = 9.9 \times 10^{-7}$, OR = 0.69), with the former reaching significance below the common threshold of 5.0×10^{-8}, on 1q32.1 in the *CAMSAP1L1* gene, which encodes a cytoskeletal protein. These findings, however, might not reflect the situation in other epilepsy syndromes. More recently, the EPICURE study published the first large GWAS in the GGEs. Consistent with the majority of reported GWAS association signals in complex diseases, neither of their two reported GGE GWAS 'significant' regions, 2p16.1 (rs13026414) and 17q21.32 (rs72823592), were within close proximity to one of the usual suspect genes — in the case of GGE, the ion channel coding genes [4]. Given that the signals from these regions were not observed in the other epilepsy GWAS, independent replication in external datasets of these intriguing findings will strengthen the certainty of an association.

Despite these examples of putative association to complex epilepsies, a large portion of heritable variance in common epilepsy remains unaccounted for, i.e., the "missing heritability" [5]. A more successful strategy within potentially heterogeneous disorders like epilepsy could be to split the large cohorts into well-characterized groups representing specific epilepsy sub-syndromes or endophenotypes. While such a 'lump-and-split' approach succumbs to further multiple-testing correction, it could represent a powerful opportunity to elucidate genetic markers associated with specific epilepsy characteristics that might not be powered enough to emerge in a "lump" investigation. Efforts of this nature are underway through the ILAE consortium on complex epilepsies. This consortium, of which both authors are members, is combining GWAS data from multiple studies using a uniformed imputation and analytical platform to both perform meta-analyses in the "lump" category for the genetic generalized epilepsies and the focal epilepsies, followed by subsequent "split" analyses, which with combined international cohorts, could provide a unique opportunity to have well-powered cohorts for specific epilepsy syndromes. Results of this consortium are anticipated to provide the best opportunity to elucidate what proportion of the epilepsy heritability is explained by the CDCV hypothesis. However, such studies are less applicable if the genomic variance underlying the epilepsies with complex genetics is a result of rare variants. Alternative strategies to CDCV are often classed into a "common disease, rare variants" hypothesis. This hypothesis is becoming increasingly attractive given not only the relatively sparse findings in currently published GWAS-based associations but also the increasing availability of assessing this hypothesis through improved sequencing technology, affordability, and analytical frameworks [22].

4. The promise of the common disease rare variants hypothesis

This "common disease, rare variants" hypothesis proposes that rare variants with higher penetrance may underlie common diseases as well as rare diseases, with a disease caused by different variants in different people [22]. This hypothesis has had support from the discovery that rare copy number variants (CNVs) contribute mutations associated with multiple common diseases, as observed by the rare 15q13.3, 16p13.11, and 15q11.2 microdeletions that increase risk across a range of neurological conditions, including neuropsychiatric illnesses [23–26]. Current gene-mapping study designs in common diseases have evolved to capture low frequency rare variants, potentially of higher individual risk [27]. *Whole-exome sequencing* (*WES*) provides an investigational platform when the goal is to examine the functional variants in the protein-coding sequence of the genome and to allow for a load-based assessment involving different qualifying variants, to identify if there are genes that contain multiple rare variants (both single nucleotide and structural) across 'affected' individuals. Genotyping chips are unlikely to be as effective as sequencing if rare, and in particular, private or de novo, mutations are responsible. However, unlike Mendelian disorders, the underlying genetic architecture of common epilepsies is largely unknown, and it is yet to be determined what role other features, such as regulatory factors not captured by exome sequencing, could play.

The recently available exome chip represents a cost-effective alternative to large-scale WES. This genotyping chip provides a unique