

Epilepsy Panel Testing Criteria: A Clinical Assessment

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Abstract

Epilepsy is a common, and often genetic, neurological disorder. The most cost-effective genetic tests for identifying an idiopathic epilepsy etiology are Next-Generation Sequencing (NGS) gene panels. Since 2017, the Genetic Testing Stewardship Program (GTSP) at UPMC Children's Hospital of Pittsburgh (CHP) has been utilizing a set of epilepsy panel (EP) testing criteria to facilitate appropriate EP ordering practices for patients with epilepsy. Outside of this setting, few guidelines exist to help medical providers decide when to order EPs or to help insurance companies decide on covering them. Following IRB approval, retrospective chart review of the electronic medical record (EMR) was performed for 1,242 patients that were evaluated in the CHP Neurology department for a primary diagnosis of epilepsy between 2016 and 2018. The goal of the study was to identify patients with positive and negative EPs that met criteria in order to calculate the sensitivities and positive predictive values (PPVs) of the criteria. Criteria were organized into four categories, with each successive category accounting for an increased proportion of criteria combinations. The highest respective sensitivity and PPV results in each category were as follows: Category 1 (64.7% and 60%); Category 2, (88% and 30.3%); Category 3, (94.1% and 27.1%); Category 4, (94.1% and 25.4%). Overall, the sensitivities increased and PPVs decreased but remained similar when more criteria were considered together. Family history played a key role in increasing sensitivity. Confidence intervals narrowed as category level increased, improving the reliability of the results. When applied to the untested population from

the study cohort, the PPV result from Category 4 predicted 121 patients with unidentified positive EP results. This study presents data supporting the reliability and predictive capabilities of the EP testing criteria and further suggests the addition of a family history criterion. This study impacts public health by opening the door for conversations about adopting evidence-driven policies and by suggesting vetted guidelines to ease EP ordering and coverage decisions and subsequently improve patient access to EP testing.

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Preface

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Abbreviations

Frequently Used Abbreviations

EP = Epilepsy Panel

AED = Anti-Epileptic Drug

IGE = Idiopathic Generalized Epilepsy

WES = Whole Exome Sequencing

CMA = Chromosomal Microarray Analysis

NGS = Next Generation Sequencing

SUDEP = Sudden Unexplained Death in Epilepsy

CHP = UPMC Children's Hospital of Pittsburgh

UM = Utilization Management

EMR = Electronic Medical Record

GTSP = Genetic Testing Stewardship Program

CPT® = Current Procedural Terminology

PPV = Positive Predictive Value

NCCN = National Comprehensive Cancer Network

1.0 Introduction

1.1 Background

Epilepsy is a common neurological problem and there is a genetic basis for approximately 40% of the epilepsy cases that can be attributed to a specific cause (Zhang, Liu, & Deng, 2017). Roughly 5% of the population will develop epilepsy in their lifetime, and new cases of epilepsy are most common in childhood, typically in the first year of life (Hesdorffer et al., 2011).

The clinical utility of genetic testing in epilepsy is well-established. Understanding the etiology of an individual's epilepsy can influence selection of an antiepileptic drug (AED). In patients with infantile spasms and pathogenic/likely pathogenic variants in *TSC1*, *TSC2*, *CDKL5*, or *STXBP1*, for example, vigabatrin is often prescribed. Patients with creatine deficiencies due to pathogenic/likely pathogenic variants in *SLC6A8*, *GAMT*, or *GATM* typically receive oral creatine as treatment (Pong, Pal, & Chung, 2011). Often the frontline treatment therapies for patients with *SCN1A* pathogenic/likely pathogenic variants are valproate, clobazam, stiripentol and levetiracetam (Pong et al., 2011). Additionally, some medications may be contraindicated for patients with a specific genetic diagnosis. Valproic acid, also known as Depakote, should be avoided for patients with *POLG* pathogenic/likely pathogenic variants (Stewart et al., 2010). Sodium channel blockers such as phenytoin, carbamazepine, and lamotrigine should not be given to patients with *SCN1A* pathogenic/likely pathogenic variants (Snoeijen-Schouwenaars et al., 2015). Also, for patients with *CSTB* pathogenic/likely pathogenic variants, channel blockers and GABAergic drugs should be avoided (Pong et al., 2011). A recent study suggests that epilepsy panels (EPs) are the most cost-effective genetic testing method when used to determine genetic

etiology in idiopathic epilepsy cases (Sanchez Fernandez, Loddenkemper, Gainza-Lein, Sheidley, & Poduri, 2019). An EP is a multigene genetic test that includes genes that are associated with epilepsy (Lee, Lee, & Lee, 2018).

EPs are the highest volume genetic testing panel ordered at UPMC Children's Hospital of Pittsburgh (CHP). At CHP, a group of genetic counselors constitute the Genetic Testing Stewardship Program (GTSP), which communicates with and educates providers in order to ensure that the most appropriate genetic tests are ordered in both an inpatient and outpatient setting. Part of this process involves reviewing genetic test orders, including those for EPs. To aid in the decision-making process for these reviews, the GTSP created a set of genetic testing criteria specific to EPs. The use of these criteria is in lieu of comprehensive genetic epilepsy policies. Additionally, the Patient Centered Laboratory Utilization Guidance Services (PLUGS) Insurance Alignment Committee has worked diligently to create a Current Procedure Terminology (CPT[®]) code specific to EPs that will be incorporated into the code book in the year 2021.

Given the frequency of EP orders, peer reviewed literature supporting the utility and cost effectiveness of EPs, and the creation of an EP CPT[®] code, this study will help identify important factors for the modification of existing test criteria and for application in future EP policy development. To date, there is no literature on a clinical-driven assessment of EP testing criteria.

This study will utilize the electronic medical record (EMR) to assess the effectiveness of the EP testing criteria at CHP. Since 2017, these criteria have been primarily utilized by the GTSP to determine if EP testing is appropriate for patients. The EMR review will involve pulling ICD-10 diagnosis codes to account for all outpatients with new epilepsy evaluations from 2016 to 2018. Genetic tests that were ordered up until June 30, 2019 will be reviewed. Patients will be categorized based on whether they met testing criteria and whether they had an EP as part of their evaluation.

Statistical analysis will assess the sensitivity of the criteria as well as their ability to predict positive results. The criteria will be set to overlay patients with no genetic testing as a means to capture the potential of their predictive capabilities. The conclusions drawn from this study will be used to modify the current criteria and lay a foundation for future EP policy development.

1.2 Specific Aims

1.2.1 Specific aim 1

Conduct a retrospective chart review using the EMR at CHP. The data to be collected includes demographic information, genetic test results, and clinical information pertaining to EP genetic testing criteria.

1.2.2 Specific aim 2

Use statistical analysis on retrospective chart review data to determine the sensitivity and positive predictive value (PPV) of EP genetic testing criteria.

1.2.3 Specific aim 3

Use EP testing criteria PPV to determine the potential number of unidentified genetic epilepsy diagnoses in the untested patient population.

1.2.4 Specific aim 4

Suggest modifications and future considerations for the EP genetic testing criteria based on the statistical analysis outcomes.

2.0 Literature Review

2.1 Epilepsy

Epilepsy is a disorder that results in recurrent, unprovoked seizures. Seizures are defined as sudden, disruptive discharges of electrical stimulation in the brain that inhibit normal neurological function (Gavvala & Schuele, 2016). Epilepsy encompasses a broad spectrum of syndromes and seizure types, which are generally characterized by the location of the initial activity in the brain and the size of the participating brain area. Generalized epilepsy may involve the entire brain or large brain areas at seizure onset, and loss of consciousness is common (Gavvala & Schuele, 2016). Focal epilepsy usually begins in a smaller brain area, and seizure onset may be accompanied by warning signs such as abnormal sensory experiences or involuntary movements (Gavvala & Schuele, 2016). Age of onset plays an important role in explaining the nature of specific epilepsy cases as well (Rácz et al., 2018). Seizures in infants and children, for instance, can be highly indicative of structural brain abnormalities, metabolic disorders, and/or genetic disease (Gavvala & Schuele, 2016).

In children, epileptic seizures and their percent distributions, which are represented here as ranges of reported values from various studies, can further be broken down into the following categories: generalized tonic-clonic (12-24%), complex partial (8-31%), simple partial (2-12%), other partial (7-29%), unclassified/mix (4-43%), other generalized (<1-3%), myoclonic (1-11%), infantile spasms (1-9%), and absence (5-22%) (Cowan, 2002). The specifics of each seizure varies per type, but repeated, jerking, or convulsive movements accompanied by loss of consciousness is a recurring theme.

Regardless of whether the aforementioned seizure types occur as isolated symptoms or are part of a syndrome or not, their etiologies remain heterogeneous. Etiology is undetermined in approximately 55% to 75% of all seizure types, with the remaining 25% to 45% being attributable to a specific cause (Cowan, 2002). Approximately 40% of these specific causes are genetic, though simple Mendelian genetics accounts for about 1% of these cases (Zhang et al., 2017). Indeed, much is still left to be discovered regarding the etiologic heterogeneity of epilepsy.

2.1.1 Diagnosis and natural history

An epilepsy diagnosis is made if the patient experienced any of the following: two or more unprovoked seizures that occurred more than 24 hours apart, one unprovoked seizure and increased risk for additional seizures, or diagnosis of an epilepsy syndrome, such as Dravet syndrome or West syndrome (Fisher et al., 2014; Scheffer et al., 2017). The use of brain imaging and human electroencephalogram after the diagnosis of epilepsy has been made can further aid in determining seizure type, epilepsy type, and if the patient is affected by an epilepsy syndrome (Smith, 2005). The International League Against Epilepsy (ILAE) has released several classification articles laying out their recommended process for classifying epilepsies. The ILAE classifies seizure types as Focal, Generalized, or Unknown (Scheffer et al., 2017). Epilepsy types are classified as Focal, Generalized, Unknown, or Combined Generalized and Focal. The addition of the combined category accounts for the fact that many cases of epilepsy involve multiple seizure types (Scheffer et al., 2017). Epilepsy syndromes are classified as a result of preceding seizure and epilepsy classifications (Scheffer et al., 2017).

Of note, there is a subgroup of syndromes derived from the Generalized epilepsy category that the ILAE classifies as Idiopathic Generalized Epilepsies (IGE), or Genetic Generalized

Epilepsies (GGE). This subgroup includes the following syndromes: Generalized Tonic–Clonic Seizures Alone, Juvenile Absence Epilepsy, Childhood Absence Epilepsy, and Juvenile Myoclonic Epilepsy (Scheffer et al., 2017). Whether the designation of IGE or GGE is more appropriate depends on the individual case and the medical provider’s discretion. Generally, IGE is considered appropriate, but the suspicion of a genetic etiology in the presence of one of the four aforementioned syndromes could make GGE a more descriptive choice of terminology (Scheffer et al., 2017). For the purposes of this manuscript, IGE is sufficient to suggest genetic etiology.

Defining an epilepsy prognosis is multi-faceted and depends on whether the epilepsy is treated or not, as well as what disease feature, such as seizure recurrence or mortality risk, is being investigated. Furthermore, actually determining the prognosis of epilepsy depends not only on the type of seizures and epilepsy involved, but also on the population that the reference data is collected from and the prognostic factors that are included (Beghi, Giussani, & Sander, 2015).

The prognosis for the majority of epilepsy patients is favorable, with the average length of active epilepsy being ten years and the post-unprovoked seizure relapse risk being between 23% and 71% (Beghi et al., 2015). However, mortality risk is increased above that of the general population in some epilepsy patients. One of the major causes of mortality is SUDEP, or Sudden Unexplained Death in Epilepsy, which is defined as, “sudden, unexpected, nontraumatic, non-drowning death in an individual with epilepsy, witnessed or unwitnessed, in which post-mortem examination does not reveal an anatomical or toxicological cause of death” (Shankar, Donner, McLean, Nashef, & Tomson, 2017). Like epilepsy prognosis, epilepsy mortality risk is highly dependent on seizure type and origin. In the IGE category, mortality risk has not been found to be increased. However, when mortality risk in non-IGE seizure categories is found to be increased, it is lowest in the unprovoked seizure category and highest in the central nervous system (CNS)

lesion category (Gaitatzis & Sander, 2004; Loiseau, Picot, & Loiseau, 1999). In addition to these innate risks, suicide rates have been found to be increased in individuals with epilepsy, which further compounds the issue of mortality (Bell & Sander, 2009).

Generally, 60% of children who are diagnosed with epilepsy and placed on standard anti-epileptic drugs (AEDs) complete their five-year remission and are subsequently removed from treatment (Sillanpää & Schmidt, 2015). Similarly, 61% of adults achieve remission and are removed from treatment ten years after seizure onset (Lindsten, Stenlund, & Forsgren, 2001). However, there are certain factors that are highly associated with refractory epilepsy, which represents a poor remission prognosis. One study lists these factors as: remote symptomatic seizures, which are defined as seizures occurring >1 week after diagnosis of an epilepsy-risk disorder (50% of refractory patients in this study had this factor), abnormal neurological development (74.4%), status epilepticus, which is defined as having a seizure that lasts >5 minutes or two or more continuous seizures within a 5 minute interval (47.2%), high initial seizure frequency (87.2%), and seizures present during sleep (66.7%) (Sillanpää, 1993). It can be said, then, that patients affected by any of these factors are at a higher risk of also having medically intractable epilepsy.

Moreover, epilepsy etiology is the predictive factor most closely associated with seizure remission. For instance, individuals with IGE have higher rates of five-year remission at 15 years from seizure onset (42%) than individuals with structural or metabolic epilepsy etiologies (30%) (Annegers, Hauser, & Elveback, 1979). This shows that genetic epilepsies typically have a better prognosis than structural or metabolic epilepsies. This is due in part to the treatment options available following a genetic diagnosis of epilepsy, which can lead to favorable patient outcomes.

2.1.2 Treatment of epilepsy

The goal of epilepsy treatment is for patients to achieve a therapeutic state free of both seizures and side effects. The treatment approach may differ depending on the epilepsy diagnosis or syndrome. Typically, antiepileptic drugs (AEDs) are the gold standard and first option when tasked with combating seizures. Other options exist, such as diet, surgery, and neurostimulation. There are about thirty AEDs on the market that can be combined in different ways to achieve therapy, the success of which depends on identifying optimal dosage and method of delivery (Novak, 2017).

During the process of coordinating initial treatment options, it is crucial that patients are given proper informed consent regarding any risks or side effects of prescribed drugs, which also applies for any procedures with inherent risk, such as surgery. There may also be a need to treat comorbidities of epilepsy such as depression, and patients should be educated properly regarding this correlation and its management (Vosburgh & Owens, 2018). Timeliness in treatment is a factor as well. A recent population study in the United States found that out of 59,970 epilepsy patients, 51.4% went untreated for 6 months following diagnosis and 36.7% remained untreated for 3 years. Those patients in the 3-year category experienced a higher rate of adverse events associated with epilepsy, such as falls, burns, and motor vehicle accidents as well as comorbidities such as suicidal tendencies. Consequently, health care utilization increased in this category as these patients experienced more hospitalizations and emergency department visits than their treated counterparts (Kalilani et al., 2019). This adverse outcomes data supports the need to understand the optimal treatment plan for patients in order to deliver appropriate and timely medical management. For some patients, genetic testing plays an integral role in this process.

2.2 Epilepsy Genetic Testing

For patients where a genetic etiology is suspected, techniques exist to interrogate their DNA to determine an explanation for their symptoms. These genetic techniques include chromosomal microarray analysis (CMA), targeted gene panel testing via Next Generation Sequencing (NGS), and whole exome sequencing (WES). Based on yield and cost data, these tests have historically been applied to epilepsy algorithmically in that order (Sanchez Fernandez et al., 2019). However, with each test having its strengths and weaknesses, the responsibility of selecting the most appropriate test for the situation falls on the provider when evaluating new patients. Providers must then sift through and interpret the genetic results, which may vary depending on the selected lab and testing modality.

2.2.1 Chromosomal microarray analysis

CMA is a genetic test that analyzes single nucleotide polymorphisms (SNPs) in a person's DNA and compares them to those known to be in the general population, to determine if there are any deviations from the norm. These deviations are either missing or extra DNA material, called deletions or duplications respectively. Deletions or duplications that are 1 kb (1 kilobase = 1,000 nucleotides) or larger are called copy number variants (CNVs) (Wapner et al., 2012). CMA has been clinically validated and is generally considered to be the “gold-standard” in detecting CNVs across the entire genome. Current microarray platforms allow for the detection of CNVs as small as 10-20 kb (D. T. Miller et al., 2010). Because of this, CMA data is often utilized to evaluate the CNV calling capabilities of other testing methods such as NGS-based targeted gene panels and WES (Yao et al., 2017). CMA is especially useful in detecting genetic etiologies in patients with

altered neurological development (e.g., developmental delay, autism) as well as patients with multiple congenital anomalies. In fact, CMA identifies a genetic explanation in around 12 to 15% of these cases, making it a preferred first-choice genetic testing option in this setting (D. T. Miller et al., 2010).

CMA does have some limitations, however. The first is that CMA cannot detect very small deletions or duplications (i.e., <10 kb) (D. T. Miller et al., 2010). It also cannot detect chromosome alterations in which there is no loss or gain of DNA material. Examples of this include balanced inversions and translocations, which involve swapping chromosome segments either within the same chromosome or between different chromosomes respectively. Typically, these rearrangements do not cause disease in the affected individual unless a particular gene is disrupted as a result. Additionally, CMA cannot detect the presence of low-level mosaicism (i.e., mosaicism below 20-25%) in body tissues, which has been shown to contribute to patient phenotypes in several cases involving intellectual and developmental disability, atresia of the auditory canal, and post-axial polydactyly (Oneda et al., 2017). Finally, unlike NGS technology, CMA cannot detect single nucleotide changes, or point mutations, in the DNA sequence (Levy & Wapner, 2018).

2.2.2 NGS targeted gene panels

Targeted gene panels are multigene tests designed to enrich and capture genetic variation at specific sites in the DNA. Targeted panels often utilize NGS technology, which allows for rapid and large-scale sequencing, or “reading,” of DNA segments. The enrichment process is achieved through polymerase chain reaction (PCR) amplification of the gene regions of interest, which results in the availability of many DNA segments for use with NGS. Generally, the more DNA segments that are available for sequencing, the better a test’s read depth will be. Read depth, or

coverage, describes the number of times a DNA base, or nucleotide, is represented among all available sequence reads. The higher the read depth, the more confident one can be about the detection of a gene variant on the test. With that being said, if a patient's phenotype can be narrowed down to a category of genetic disease, such as cardiomyopathy or epilepsy, a panel will typically provide better coverage and confidence than WES (Sims, Sudbery, Iltott, Heger, & Ponting, 2014).

On the current market, there are thousands of NGS gene panels available to choose from. This is a result of variation between genetic testing labs and a general lack of guidance regarding which genes to include on panels. Labs may choose to offer panels with different gene selections, different costs and turn-around-times, or different inherent technological methods. Some of these differences interact as trade-offs to one another.

A STAT gene panel, for instance, might be ordered on a patient for whom rapid turn-around-time is critically important for their health management. However, a STAT panel typically offers fewer genes than a comprehensive counterpart and incurs an upcharge for expediency. When time is not of the essence, a comprehensive targeted panel or expanded panel might be offered, with the latter including thousands more genes than the prior. The trade-off here is one of methods and interpretive quality. Expanded panels can be performed based on exome data, or an "exome backbone," as opposed to the standard NGS-paired PCR amplification of a comprehensive targeted panel (GeneDx, 2017). This provides a broader search for implicated genes, but a reduced quality in read depth, at a higher cost. As such, the decision as to which panel to select involves many considerations and is often done on a case-by-case basis for each patient.

2.2.3 Whole exome sequencing

The exome is the portion of DNA that contains exons, or the coding regions which produce functional proteins in the body. It makes up about 2% of the entire genome and accounts for roughly 85% of known pathogenic/likely pathogenic variants (van Dijk, Auger, Jaszczyszyn, & Thermes, 2014). Overall, the diagnostic yield, or probability of identifying a result explanatory for a patient's condition, for WES is around 25 to 30% (Chitty, Friedman, & Langlois, 2016). The strategy behind WES is to sequence all of the exons to discover genetic etiologies for disease. Often a provider or genetics team will provide the reference lab with a list detailing the patient's phenotypic description. The lab then uses non-PCR-paired NGS to read through all of the patient's exons, but only interprets and reports findings in the genes known to be associated with the patient's phenotype. This allows for a certain degree of specificity in an otherwise broad genetic test.

Because WES is a broad test, it is best suited to situations where a specific genetic etiology cannot be narrowed down. This means that patients whose features include multi-systemic anomalies or dysmorphisms that do not fit a known syndrome or disease pattern stand to benefit most from the wide-net approach that WES offers. The downside to being broad is that WES lacks the read-depth attainable by the PCR amplification of targeted panel testing and the CNV sensitivity attributed to CMA (Biesecker, Shianna, & Mullikin, 2011). It also cannot detect minute deletions and duplications, rearrangements (i.e., inversions), methylation, trinucleotide repeats, or changes in non-coding DNA (Sanchez Fernandez et al., 2019).

WES also has the potential to reveal unwanted or unexpected information. This means that while exploring possible epilepsy etiologies, for instance, WES could reveal variants associated with cancer predisposition or cardiomyopathy. The American College of Medical Genetics and

Genomics (ACMG) assembled a list of 59 genes associated with secondary findings that should be reported if found with WES (Kalia et al., 2017). In one study, 5.6% of patients that underwent WES were found to have a pathogenic/likely pathogenic variant in an ACMG secondary finding gene (Gambin et al., 2015). Patients are given the option to opt in or out of receiving these secondary findings during the informed consent process for testing. Additionally, variants of uncertain significance (VUS) are a possible result of any genetic test but occur more often with WES, which was reported in one study to have a VUS rate of 44% (Trujillano et al., 2017). VUS results offer little in regards to disease explanation or medical management. On top of this, WES can be costly, with each test costing \$6,750 on average (Sanchez Fernandez et al., 2019). As such, for WES, and indeed any genetic test, the benefits and limitations must be considered prior to being ordered for a patient.

2.2.4 Epilepsy testing utility

The decision to order any one of the aforementioned tests for a patient with epilepsy is dependent on the patient's overall phenotype, the testing criteria that they meet, and the yield and cost of the test. The goal for providers is to select a test that is likely to yield a positive result while remaining cost-effective for the patient and their insurance. In regards to IGE, a study conducted by Sanchez Fernandez et al. compared the diagnostic yield and cost-effectiveness of different testing strategies involving CMA, EPs, and WES in order to determine if any of the individual tests or testing combinations out-performed the others (2019).

Sanchez Fernandez et al. reviewed 23 studies that reported diagnostic yield information for CMA, EPs, and WES and collected pricing information from major genetic testing laboratories in the US. To account for publication bias, Sanchez et al. conducted sensitivity analyses and created

adjusted yields for each test. Each test was given an incremental cost-effectiveness ratio (ICER) that utilized “no genetic testing” as a baseline comparison point with no associated cost. They subsequently found that the most cost-effective individual test, with an ICER of \$15,848/diagnosis, was an EP. WES followed with an ICER of \$34,500/diagnosis. It was determined that CMA would only be cost-effective if its cost was lowered below \$1,267 or diagnostic yield improved to 0.1 or higher (Sanchez Fernandez et al., 2019). When the same process was applied to testing combination strategies, it was found that an EP, followed by CMA, followed by WES, was the most cost-effective iteration with an ICER of \$18,385/diagnosis (Sanchez Fernandez et al., 2019).

The data from this study does not support the historical CMA → EP → WES testing iteration and instead suggests that EPs are the most cost-effective first-tier test to offer patients with IGE (Sanchez Fernandez et al., 2019). However, it is always important to consider phenotype when deciding to order a test for a patient with epilepsy. As mentioned previously, CMA has been shown to have increased yield for patients affected by congenital malformations and developmental delay, and/or autism spectrum disorder. WES has a 25-30% yield overall and is useful when many different body systems are affected, or the patient’s phenotype is non-specific (Chitty et al., 2016). When patients present fitting these scenarios in addition to having epilepsy, more consideration should be given before automatically ordering an EP. That being said, the data provided by Sanchez Fernandez et al. suggests that patients who meet established EP testing criteria stand to benefit from a high-yield, cost-effective genetic test.

2.3 Epilepsy Testing Outcomes for Patients

For patients with epilepsy, often the driving goal of genetic testing is to provide an answer for their symptoms and ultimately guide management. This management can include a change in diet, a change or avoidance of certain medications, or surgical therapy (Ko et al., 2018; Lin & Wang, 2017; Stewart et al., 2010). Beyond the medical benefit, information can be gleaned from genetic testing that is useful for familial decision making. Family members of affected individuals may seek to pursue their own testing or may incorporate this information into their reproductive decision-making. These decisions, however, depend on the identified gene variant(s) and any associated medical management considerations. This section will first broadly review possible treatment outcomes following genetic testing for epilepsy, then will provide a review of well-described, actionable epilepsy genes and their respective outcomes.

2.3.1 The ketogenic diet

For some patients with epilepsy, diet changes can be extremely effective in maintaining health and seizure freedom. The ketogenic diet is the most well-studied and effective diet for patients with epilepsy and has been used since the 1920s (Neal et al., 2008). It can be particularly effective for patients with drug-resistant, or intractable, epilepsy and epilepsy caused by specific genetic mutations (Ko et al., 2018). As such, one benefit of finding a genetic etiology for epilepsy is the opportunity to manage seizure symptoms without depending on medications.

The ketogenic diet maximizes fat intake while minimizing carbohydrate and protein intake. This intake pattern mimics starvation conditions in the body and initiates large-scale mitochondrial fatty-acid oxidation and ketone production in the liver. Serum ketones are able to cross the blood-

brain barrier and function as a fuel source for the brain, which at other times is almost exclusively glucose (Hartman, Gasior, Vining, & Rogawski, 2007). This mechanism of action is well-known as being therapeutic for *SLC2A1*-related Glut1-DS, especially in children and infants (Cremer, 1982). In the majority of Glut1-DS patients who begin the ketogenic diet, seizures are controlled immediately and completely (Klepper, 2008). The ketogenic diet has also been shown to have response rates ranging from 77.8% to 100% in patients with *SCN1A*, *KCNQ2*, *STXBP1*, and *SCN2A* pathogenic/likely pathogenic variants (Ko *et al.*, 2018).

2.3.2 Medication guidance and contraindications

One of the most critical outcomes of epilepsy genetic testing is management of medications. Discovery of a genetic etiology for a patient's epilepsy may prompt a physician to prescribe a new drug, change current dosing or existing prescriptions entirely, and avoid any drugs that are contraindicated for the patient's diagnosis. It is for this reason that genetic testing should be done early during the evaluation process for new patients with epilepsy, as the wrong medication decision can have catastrophic effects (Stewart *et al.*, 2010). Proper epilepsy dosing and management is not only beneficial for patient health, but ultimately lowers overall healthcare costs and utilization (Rajagopalan, Candrilli, & Ajmera, 2018).

2.3.3 Neurostimulation

Neurostimulation is a current therapy option for some patients with intractable epilepsy. Vagus nerve stimulation (VNS) devices have been approved by the Food and Drug Administration in the United States for use in refractory focal seizures affecting patients 12 years or older, and

new devices are currently being investigated (Lin & Wang, 2017). VNS works as a surgical chest implant that continuously delivers signals to the vagus nerve via electrodes. The therapy is thought to work as this stimulation activates brain stem nuclei and norepinephrine release in epileptic brain regions (Lin & Wang, 2017). There is evidence supporting the efficacy of VNS for patients with *CDKL5* pathogenic/likely pathogenic variants, with over two-thirds of patients reportedly experiencing a reduction in seizure frequency and duration following treatment. Behavioral and mood improvements were also reported in some patients (Lim et al., 2018). Perhaps due to phenotypic overlap, VNS has also been seen to be effective for *MECP2*-related epilepsy, with seizure reduction rates of 50% or greater (Engineer, Hays, & Kilgard, 2017).

2.3.4 Information for family members

Once a genetic diagnosis has been made in a family, there is opportunity for myriad beneficial outcomes. Depending on the dynamics of the family in question and the level of information shared throughout, an inherited epilepsy can be informative in family planning and health management. For instance, it is known that for autosomal dominant conditions such as Dravet syndrome, any offspring of an affected individual will have a 50% chance of inheriting the condition. Also, for the 10% of cases where the condition is inherited from a less-affected parent, recurrence risk in siblings is likewise 50% (de Lange et al., 2018). Conversely, for the 90% of cases that are *de novo*, recurrence risks are <1% due to the possibility of gonadal mosaicism (Campbell et al., 2014). For recessive conditions like Alpers-Huttenlocher syndrome, siblings of an affected proband have a 25% chance to be affected and a 50% chance to carry a disease gene (Anagnostou, Ng, Taylor, & McFarland, 2016). Having this information available can guide family members as they navigate their reproductive landscape. Some families may decide not to have

additional biological children, some may choose preimplantation genetic screening or prenatal testing, and others may use it for post-pregnancy management considerations.

2.3.5 Testing considerations and limitations

The direct and indirect consequences of genetic testing should always be conveyed to patients prior to its pursuit. Beyond inheritance and reproductive planning lies a complicated constellation of outcomes. Familial stigma can occur and can be accompanied by shame and guilt (Kessler, Kessler, Ward, & Opitz, 1984). Interpretation of the result can often be difficult as well, as even a pathogenic/likely pathogenic variant found in an asymptomatic family member cannot predict the exact presentation of the disease in that individual (Kalviainen et al., 2008). Additionally, family members need to consider the limitations of their insurance protections under the Genetic Information Nondiscrimination Act (GINA). GINA only protects against health insurance and employment discrimination based on genetic health data and does not protect against life insurance or long-term disability discrimination (Tenenbaum & Goodman, 2017). Younger family members may want to take this into consideration before going through with testing. An adequately disclosed informed consent will cover many of these testing limitations during a genetic counseling session prior to testing.

2.3.6 Actionable genes associated with epilepsy

A 2017 study by Wang et al. identified 977 genes associated with epilepsy. The study team broke these genes into four groups: Epilepsy genes (84 genes), neurodevelopmental genes (73), epilepsy-associated genes (536), and potential epilepsy genes (284) (J. Wang et al., 2017). Since

2017, the number of newly discovered epilepsy-associated genes has continued to grow. Some commercial labs currently offer tests that sequence roughly 1,500 epilepsy-associated genes (GeneDx, 2019). However, not all of these genes are well-studied or actionable, meaning that finding a pathogenic or likely pathogenic variant in one of these genes may not lead to therapeutic change. This manuscript will focus on reviewing the following well-described, actionable epilepsy genes as well as their role in therapeutic decision-making: *ALDH7A1*, *CACNA1A*, *CDKL5*, *GABRG2*, *GRIN2A*, *KCNQ2*, *MECP2*, *PCDH19*, *POLG*, *PRRT2*, *SCN1A*, *SCN1B*, *SCN2A*, *SCN8A*, *SLC2A1*, *STXBP1*, *SYNGAP1*, *TCF4*, *TPP1*, *TSC1*, *TSC2*, and *ZEB2*.

2.3.6.1 *ALDH7A1*

Pathogenic/likely pathogenic variants in both copies of the autosomal recessive *ALDH7A1* gene lead to a deficiency in alpha-aminoadipic semi-aldehyde (alpha-AASA) dehydrogenase, which plays a role in brain lysine catabolism and exists in equilibrium with delta1-piperidine 6-carboxylate, the adduct partner of pyridoxal 5'-phosphate (PLP). PLP is an important enzyme cofactor in the body and the active vitamer of pyridoxine, or vitamin B6 (Gallagher et al., 2009). Decreased amounts of alpha-AASA in the body reduces active PLP and subsequent vitamin B6 levels (Gallagher et al., 2009). Classical presentation typically involves neonatal, drug-resistant generalized seizures, severely abnormal EEGs and a host of comorbidities including respiratory distress, hepatomegaly, and acidosis. Vitamin B6 supplementation is the current treatment and typically results in the resolution of these clinical features (Mills et al., 2010).

2.3.6.2 *CACNA1A*

CACNA1A encodes the transmembrane subunit of a P/Q-type or CaV2.1 voltage-gated calcium channel. Alteration of this subunit inhibits calcium ion-mediated neurotransmitter release,

which can cause autosomal dominant neurological disease (Kordasiewicz, Thompson, Clark, & Gomez, 2006). Pathogenic/likely pathogenic variants in *CACNA1A* are associated with a host of diseases, including early-onset epileptic encephalopathy, spinocerebellar ataxia type 6, episodic ataxia type 2, and familial hemiplegic migraine type 1 (Hayashida et al., 2018; Indelicato et al., 2019). Typically, drugs that target calcium channels, such as topiramate, are used to treat *CACNA1A*-related epileptic seizures (Damaj et al., 2015). However, one study suggests that lamotrigine, which targets sodium ion channels, can be effective in treating seizures associated with epileptic encephalopathy (Byers, Beatty, Hahn, & Gospe, 2016). Additionally, several studies have suggested that acetazolamide, or Diamox, is an effective therapy option for patients affected with *CACNA1A*-related episodic ataxia type 2 and/or familial hemiplegic migraine type 1 (Battistini et al., 1999; Scoggan, Friedman, & Bulman, 2006).

2.3.6.3 *GABRG2*

Gamma-aminobutyric acid (GABA) is a critically important inhibitory neurotransmitter in the human brain that acts on GABA receptors clustered in the endoplasmic reticulum of neuron synapses. These GABA receptors are part of ligand-gated chloride ion channels that regulate neuronal communications (Kang & Macdonald, 2016). *GABRG2* encodes a subunit of the GABA-A receptor isoform, which is associated with several autosomal dominant epilepsy phenotypes when mutated. These include afebrile seizures, febrile seizures, childhood absence epilepsy, generalized epilepsy with febrile seizures plus (GEFS+) and even Dravet syndrome (Macdonald, Kang, & Gallagher, 2010). Current therapies for *GABRG2* pathogenic/likely pathogenic variants focus on enhancing GABA-A receptor-mediated inhibition via allosteric modulation. This means that drugs such as benzodiazepines, phenobarbital, gabapentin, and topiramate change how GABA-A receptors respond to their ligand, GABA. This manner of receptor upregulation helps to

compensate for missing or defective receptors due to pathogenic/likely pathogenic variants in *GABRG2* (Balan et al., 2013).

2.3.6.4 *GRIN2A*

GRIN2A encodes the epsilon subunit of the N-methyl-D-aspartate receptor (NMDAR) channel, which allows permeation of sodium, potassium, and calcium ions and is activated by glutamate, the major excitatory neurotransmitter in humans (Matta, Ashby, Sanz-Clemente, Roche, & Isaac, 2011). When non-functional, the NMDAR channel cannot mediate synapse transmission, which interferes with neuron communication in the brain. *GRIN2A* pathogenic/likely pathogenic variants are associated with a range of autosomal dominant speech disorders in all cases and epilepsy in 90% of cases (Myers, 2016).

Different combinations of AEDs have been shown to have efficacy with *GRIN2A*-related seizures, with no one combination being specifically recommended. That being said, treatment with topiramate elicited a good response in one case of intractable epilepsy (Venkateswaran et al., 2014). In another study, memantine, an NMDAR inhibitor, was shown to significantly reduce seizures in a proband with a specific pathogenic/likely pathogenic variant (p.L812M) in *GRIN2A* (Pierson et al., 2014). The same study raised the possibility of using the NMDAR antagonist dextromethorphan as therapy for patients with a different *GRIN2A* pathogenic/likely pathogenic variant (p.N615K) (Pierson et al., 2014). Speech therapy, including use of augmented communication techniques or technology, may be beneficial in treating speech deficits, but should be personalized to the specific presentation of the patient (Murray, McCabe, & Ballard, 2014).

2.3.6.5 *KCNQ2*

Pathogenic/likely pathogenic variants in *KCNQ2* produce a spectrum of autosomal dominant neonatal phenotypes ranging from benign familial neonatal epilepsy to severe neonatal epileptic encephalopathy, which can have a significant impact on early neurodevelopment (Miceli F, 2010). The various disease phenotypes are caused by reduced or non-existent functionality in a voltage-gated potassium ion channel in the brain, a subunit of which is encoded by *KCNQ2* (Berkovic et al., 1994). Reduction or loss in functionality leads to a reduction in the control of neuronal excitability and subsequent seizure activity (Miceli F, 2010).

In mild cases, the most commonly used AEDs to treat seizures are phenobarbital and phenytoin. Rarely, these cases are refractory, at which point levetiracetam and topiramate have been shown to be effective (Painter et al., 1981; Tulloch, Carr, & Ensom, 2012). For cases of neonatal epileptic encephalopathy, various studies report isolated responses to different AEDs. However, most patients typically continue to have daily seizures while on medication. *KCNQ2*-related encephalopathy research has investigated the efficacy of drugs such as carbamazepine that target sodium channels rather than potassium channels, which are co-localized in the membranes of neurons. Findings showed that 40% of neonatal epileptic encephalopathy patients attained seizure freedom on carbamazepine, implicating it as an appropriate first-tier medication for infants with this *KCNQ2*-related phenotype (Pisano et al., 2015).

2.3.6.6 *MECP2*

MECP2 encodes methyl-CpG binding protein 2 (MeCP2), which regulates transcription by modifying chromatin in cells and is required for neuron maturation in the brain. *MECP2* pathogenic/likely pathogenic variants are 99% *de novo*, X-linked, and are male-lethal by age 2 (Kaur S, 2001). In females, a spectrum of phenotypes is observed ranging from mild Variant Rett

syndrome to Classic Rett syndrome. Features of Classic Rett syndrome include intellectual disability, skill regression (99%), decelerated head growth (80%), absent speech (99%), seizures (60-80%), hand stereotypies (100%), cold extremities (99%), gait issues (99%), and irregular breathing (99%). Variant Rett syndrome can manifest as mild learning disabilities only, or be characterized by regression with possible recovery or stabilization (99%), hand stereotypies (97.3%), gait issues (80-99%), seizures (6-80%), sleep disturbances (80-99%), agitation (80-99%), and irregular breathing (80-99%) (Kaur S, 2001).

Treatment of Rett syndrome in females should involve a multi-disciplinary care team. Brain MRI's and video EEG monitoring should be included in regular neurologic evaluations (Kaur S, 2001). Treatment of epileptic seizures can be achieved through AED monotherapy or polytherapy or VNS. The most effective AED for treating Rett-syndrome-associated epilepsy is carbamazepine, which has been shown to have a 71% seizure reduction rate (Huppke, Kohler, Brockmann, Stettner, & Gartner, 2007). VNS has been shown to have a 50% or greater seizure reduction rate and may be considered if AEDs are found to be ineffective (Engineer et al., 2017). Also, since individuals with Rett syndrome can present with prolonged QT intervals, it is recommended that electrocardiograms be a part of regular cardiology evaluations (Kaur S, 2001).

Duplications in *MECP2* are also associated with epileptic seizures in about 50% of patients and are fully penetrant in males. Whereas *MECP2* duplications are not as lethal as other pathogenic/likely pathogenic variants, approximately 50% of affected males die before age 25 (Van Esch, 2008). In addition to seizures, patients present with global delays, intellectual disability, absent speech, spasticity, hypotonia, and recurrent respiratory infections. Patients should be followed closely by a neurologic care team (Van Esch, 2008). Seizures may be treated with AED monotherapy or polytherapy, though one study suggests valproic acid monotherapy as

an effective treatment for patients resistant to other single medications or medication combinations (Rajaprakash, Richer, & Sell, 2018).

2.3.6.7 *PCDH19*

PCDH19 encodes protocadherin-19, a transmembrane cell adhesion molecule that is heavily dependent on calcium binding and highly expressed in the developing human brain (Depienne & LeGuern, 2012). Pathogenic/likely pathogenic variants in *PCDH19* impair the calcium binding function and subsequent cell adhesion, which negatively affects synaptic plasticity and neuronal migration (Depienne & LeGuern, 2012). *PCDH19* pathogenic/likely pathogenic variants have a unique, non-classic mode of X-linked inheritance. The *PCDH19*-related features of intractable infantile-onset epileptic encephalopathy and intellectual disability present only in heterozygous females, while hemizygous, non-mosaic males are completely asymptomatic (Depienne & LeGuern, 2012). It is theorized that this pattern of inheritance is a product of random X-inactivation and tissue mosaicism in females. Some cells in the body may inactivate the X chromosome with the pathogenic/likely pathogenic variant, while other cells may inactivate the other X chromosome. This would lead to impaired communication between different cell lines in the body tissues, including the brain, and subsequent seizure activity (Depienne & LeGuern, 2012).

Treating *PCDH19*-related seizures in infancy and childhood is a challenge, as most are intractable. However, it is noted that this intractability tends to decrease as children age, making treatment more manageable. By adolescence or adulthood, some patients on monotherapy alone are able to achieve complete therapy and seizure freedom (Depienne & LeGuern, 2012). One study assessed the efficacy of specific AEDs, with phenytoin, potassium bromide, and clobazam

showing the highest efficacy and carbamazepine showing the lowest efficacy for *PCDH19* patients (Higurashi, 2013).

2.3.6.8 *POLG*

Pathogenic/likely pathogenic variants in the *POLG* gene inhibit the function of polymerase gamma, a DNA polymerase that carries out both replication and repair of mitochondrial DNA. This leads to an accumulation of DNA damage in the mitochondria and a host of phenotypes that vary in severity, from the devastating Alpers-Huttenlocher syndrome to adult myoclonic epilepsy (Hikmat, Eichele, Tzoulis, & Bindoff, 2017). Epilepsy secondary to mitochondrial *POLG* disorders is fairly common and more than 180 *POLG* pathogenic/likely pathogenic variants have been linked to seizure phenotypes (Anagnostou et al., 2016). Both autosomal dominant and recessive inheritance patterns have been seen with *POLG* pathogenic/likely pathogenic variants, with the latter resulting in more severe conditions such as the aforementioned Alpers-Huttenlocher syndrome, which causes patients to suffer from drug-resistant seizures and liver failure (Anagnostou et al., 2016). *POLG*-related seizures are thought to be explained by critically low levels of available neuronal energy due to mitochondrial respiratory chain failure. This failure disrupts the brain's synaptic network and can even lead to focal necrosis and neuronal cell death (Anagnostou et al., 2016).

POLG pathogenic/likely pathogenic variants are a classic example of a contraindication outcome. Avoidance of valproic acid, or Depakote, is critical in *POLG* cases, as it can induce liver toxicity and failure (Stewart et al., 2010). One study provides experimental evidence suggesting that the mechanism of this toxicity involves overexpression of mitochondrial biosynthesis genes. It is proposed that valproic acid increases mitochondrial metabolic rates in liver cells. Cells that are *POLG*-deficient cannot account for this increased energy demand due to impaired replication

capabilities. This cellular exhaustion is then thought to cause the damage to the liver (Sitarz et al., 2014). As an alternative treatment to valproic acid, one study suggests a combination of the AEDs phenytoin, oxcarbazepine, and levetiracetam alongside a ketogenic diet or a low-glycemic index diet, which has been seen to be well-tolerated in some patients with *POLG* pathogenic/likely pathogenic variants (Martikainen, Päivärinta, Jääskeläinen, & Majamaa, 2012).

2.3.6.9 *PRRT2*

PRRT2 encodes a proline-rich transmembrane protein that plays a role in vesicle release at neuronal synapses. Loss of function *PRRT2* pathogenic/likely pathogenic variants cause impaired vesicle release and neuronal communication (Valente et al., 2016). This disease mechanism is associated with several paroxysmal movement disorders that are inherited in an autosomal dominant manner. These conditions include benign familial infantile epilepsy, hemiplegic migraine, and paroxysmal kinesigenic dyskinesia with or without infantile convulsions (Ebrahimi-Fakhari, 2018). Carbamazepine has been shown to be effective for treating *PRRT2*-related seizures and at lower doses than typical epileptic seizures (Ebrahimi-Fakhari, 2018).

2.3.6.10 *SCN1A*

SCN1A codes for the core alpha subunit of the Na_v 1.1 sodium ion channel. Na_v 1.1, along with other sodium ion channels, plays an important role in the development of the nervous system as well as in the excitation of neurons. At the initial axon segments of neurons, sodium ion channels initiate and propagate action potentials, which influences their excitability. Loss of function pathogenic/likely pathogenic variants can lead to an imbalance of this influence, overexcitability of neurons, and subsequent seizures (Oyrer et al., 2018; Zhang et al., 2017).

There are over 1,400 different mutations that have been found in *SCN1A*. Those that induce seizures have been found to be inherited in an autosomal dominant pattern and encompass a broad spectrum of phenotypes, ranging from mild generalized epilepsy with focal seizures plus (GEFS+), to the more severe Dravet syndrome (Fang, 2019; Zhang et al., 2017). Dravet syndrome is characterized by intellectual and developmental delay, intractable epilepsy, ambulatory issues, behavioral issues, and a high risk of status epilepticus (de Lange et al., 2018). It is also associated with a 15-20% mortality rate due to SUDEP and other comorbidities such as accidents and infections (Brunklaus et al., 2015; Meng et al., 2015). *SCN1A* pathogenic/likely pathogenic variants have been found to be highly associated with Dravet syndrome, with these variants identified in 70% of patients (Xu et al., 2012). About 50% of these pathogenic/likely pathogenic variants are nonsense variants and 90% are de novo (Jain et al., 2019; Oyrer et al., 2018).

The goal of Dravet syndrome treatment is to reduce seizure frequency and minimize comorbidities while limiting drug toxicity. Patients with confirmed *SCN1A*-related disorders have epilepsy mainly stemming from the inactivity of sodium ion channels in inhibitory neurons (Zhang et al., 2017). This information helps to guide AED prescription, which typically includes a combination of stiripentol, clobazam, and valproate. Of note, out of these three drugs, only stiripentol has been found to have formal efficacy in Dravet syndrome patients (Brigo & Igwe, 2015). This efficacy was determined in a randomized clinical trial in which the effects of stiripentol were supplementary to clobazam and valproate, which are thought to work by increasing signal inhibition by GABA-receptors (Johannessen, 2004). Stiripentol was found to inhibit cytochrome P₄₅₀ enzymes, which increased the levels of other AEDs in the blood. This was found to be especially true for clobazam (Chiron et al., 2000; Giraud et al., 2006).

Knowledge of the sodium ion channel mechanism in *SCN1A*-related disorders is also helpful to avoid certain medications for patients. For instance, it is common practice to avoid drugs that block sodium ion channel function, which would exacerbate the symptoms already caused by *SCN1A*-related disorders if administered. These contraindicated medications are phenytoin, carbamazepine, and lamotrigine (Snoeijen-Schouwenaars et al., 2015). There is little evidence supporting seizure increase in patients with *SCN1A*-related disorders who took phenytoin and carbamazepine, but there is one study that found significant seizure increase in the majority of patients who took lamotrigine (Guerrini et al., 1998).

2.3.6.11 *SCN1B*

SCN1B codes for the beta subunit of sodium ion channels, which are involved in cell adhesion, aggregation, and channel voltage dependence (Patino et al., 2009). Similar to *SCN1A*, heterozygous *SCN1B* pathogenic/likely pathogenic variants have been associated with conditions such as GEFS+ and Dravet syndrome (Patino et al., 2009). They can also cause heart arrhythmias, such as Brugada syndrome, which can cause sudden death and necessitate the installation of an internal defibrillator (Aeby et al., 2019). Homozygous *SCN1B* pathogenic/likely pathogenic variants can cause early onset infantile epileptic encephalopathy (EIEE). *SCN1B*-related seizures often remain refractory to treatment, though one study observed seizure improvement for some patients prescribed a combination of valproic acid, topiramate, clobazam, and fenfluramine and who followed the ketogenic diet (Aeby et al., 2019).

2.3.6.12 *SCN2A*

SCN2A encodes the alpha subunit of Na_v 1.2 sodium ion channels which localize in the synapses of excitatory neurons (Howell et al., 2015). Pathogenic/likely pathogenic variants in

SCN2A have been associated with benign familial neonatal infantile seizures (BFNIS), intellectual disability, epileptic encephalopathy, and epilepsy of infancy with migrating focal seizures (EIMFS) (Howell et al., 2015). Patients with *SCN2A* pathogenic/likely pathogenic variants have been shown to respond well to phenytoin, while vigabatrin and lamotrigine have been shown to exacerbate seizures in some patients (Berkovic, Howell, Hay, & Hopper, 1998).

2.3.6.13 *SCN8A*

SCN8A encodes the alpha subunit of the Na_v 1.6 sodium ion channel (O'Brien & Meisler, 2013). Na_v 1.6 is uniquely localized at the nodes of Ranvier and the axon initial segments of neurons and are involved in persistent, repetitive neuron firing. Pathogenic/likely pathogenic variants can cause either hypo or hyperexcitability in neurons, leading to intellectual disability and epileptic encephalopathy (O'Brien & Meisler, 2013). Treatment involving AED polytherapy is often necessary, as *SCN8A*-related encephalopathy is difficult to manage, with breakthrough seizures being a common occurrence. It is important to initialize treatment as soon as possible, as patients with this condition are at a 10% risk of SUDEP. High doses of sodium channel blockers such as phenytoin and carbamazepine have been shown to be effective in reducing seizures (Hammer, 2016).

2.3.6.14 *SLC2A1*

SLC2A1 encodes an important transporter protein in the brain that brings in glucose from the blood stream. The brain and CNS require large amounts of energy, and proper glucose levels maintain that energy. When problems occur in *SLC2A1*, loss of transport function can cause low brain glucose levels in the presence of normal blood glucose levels. This is called Glut1 deficiency syndrome (Glut1-DS) (Koch & Weber, 2019). Seizure onset for patients with *SLC2A1*

pathogenic/likely pathogenic variants typically occurs when they are infants and can be accompanied by significant developmental delay and spasticity. This is the case for the classic phenotype presentation of Glut1-DS, which accounts for about 80% of patients. The remaining cases fall along a spectrum of Glut1-DS phenotypes that vary in severity (Anand et al., 2011). Glut1-DS is inherited in an autosomal dominant pattern, but 90% of *SLC2A1* pathogenic/likely pathogenic variants are de novo. As mentioned previously, patients with Glut1-DS have been able to achieve complete seizure freedom through the use of the ketogenic diet (Klepper, 2008; D. Wang, Pascual, & De Vivo, 1993).

2.3.6.15 *STXBP1*

STXBP1 encodes a syntaxin-binding protein which plays a role in vesicle trafficking and cellular signaling. Non-functional protein product interferes with neurotransmitter release and causes excessive neuronal firing (Pevsner, Hsu, & Scheller, 1994). *STXBP1* pathogenic/likely pathogenic variants are associated with a wide range of epileptic encephalopathies and intellectual disability, including Dravet-like and Rett-like phenotypes (Khaikin, 2016). About 25% of affected patients are refractory to seizure medication and about 20% require multiple AED combinations to attain therapy (Khaikin, 2016). A variety of AEDs have been used effectively as treatment, with the most common being vigabatrin, valproic acid, and phenobarbital (Khaikin, 2016).

2.3.6.16 *SYNGAP1*

SYNGAP1 encodes a GTP-ase activating protein in the Ras pathway of brain dendrite synapses. This protein functions by suppressing NMDR signaling and subsequent neuronal signaling (Clement et al., 2012). When non-functional, excessive signaling leads to overexcitation in the neurons and subsequent seizures. *SYNGAP1* pathogenic/likely pathogenic variants are

associated with generalized epilepsy, developmental delays, intellectual disability, behavioral issues, and autism spectrum disorder (Holder, 2019). Management is multidisciplinary and based on the specific needs of each patient. AED selection is not guided, and any combination of standard AEDs may be considered for treatment of seizures. The ketogenic diet has also been used to effectively improve or control seizures in some patients (Holder, 2019).

2.3.6.17 *TCF4*

TCF4 encodes a transcription factor that regulates cellular proliferation and differentiation, especially in the central nervous system during early development (de Pontual et al., 2009). Pathogenic/likely pathogenic variants in *TCF4* are associated with Pitt-Hopkins syndrome (PHS), which is characterized by dysmorphic facial features, sleep disturbances, seizures, developmental delay, speech delay, intellectual disability, autism, and severe myopia (Sweetser, 2012). Management is multidisciplinary and should focus on improving neurodevelopment and speech through early intervention services. Epilepsy occurs in nearly 40% of patients with PHS, but guidelines for seizure management do not exist. Effective AEDs that have been documented so far include carbamazepine monotherapy and lamotrigine/clobazam polytherapy (Peippo & Ignatius, 2012).

2.3.6.18 *TPP1*

TPP1 encodes a lysosomal peptidase protein that removes N-terminal tripeptides from other small proteins (Mole, 2001). Pathogenic/likely pathogenic variants in *TPP1* and 13 other genes are associated with a group of lysosomal storage disorders called Neuronal Ceroid-Lipofuscinoses (NCL). They are characterized by seizures, neurodegeneration, and premature death by the second decade (Mole, 2001). Seizure management can improve quality of life, though

AED selection should be done carefully with consideration to the individual's age and stage of disease. Lamotrigine monotherapy has been shown to be the most effective AED for seizure control in NCL, with some patients achieving 100% seizure reduction. This is closely followed by valproic acid monotherapy which reduces seizures by 70% (Mole, 2001).

2.3.6.19 West Syndrome (*TSC1*, *TSC2*, and *CDKL5*)

West syndrome is an umbrella term for a spectrum of infantile spasms occurring typically before the age of two. These spasms are accompanied by developmental delay or regression and a pathognomonic electroencephalogram pattern known as hypsarrhythmia (Wheless et al., 2012). The classical presentation of West syndrome is associated with myriad etiologies, including brain trauma, infection, and pathogenic/likely pathogenic gene variants, such as those in autosomal dominant *TSC1* and *TSC2* and X-linked *CDKL5*.

Pathogenic/likely pathogenic variants in both *TSC1* and *TSC2* are causative of a condition called tuberous sclerosis complex (TSC), which results in benign tumor formation, developmental and intellectual disability, and epilepsy. As mentioned previously, *TSC1* and *TSC2* pathogenic/likely pathogenic variants are inherited in an autosomal dominant manner, but over two-thirds arise sporadically. Also, despite complete penetrance, phenotypic variability is high (Shelley & Goetzinger, 2018). When West syndrome appears clinically due to TSC, poor outcomes are predicted. Though one study did not observe mortality from TSC-related West syndrome, all patients with this condition combination were found to have severe intellectual disability. However, it was seen that with aggressive early intervention with AEDs, patients achieved a more than 50% reduction in their seizures, with some becoming seizure-free entirely (Husain et al., 2000).

Specifically, for patients found to have infantile seizures and tuberous sclerosis caused by either *TSC1* or *TSC2*, vigabatrin, a GABAergic drug, is an effective treatment. Seizure cessation has been seen in 95% of patients fitting this description despite resistance to other treatments (Curatolo, Verdecchia, & Bombardieri, 2001). Research suggests that drugs that inhibit the protein kinase mammalian target of rapamycin (mTOR) may also benefit patients with TSC and epilepsy. A clinical trial of the drug everolimus showed seizure reduction in 86% of patients with TSC-related subependymal giant cell astrocytomas (Rabito & Kaye, 2014).

Additionally, the identification of either *TSC1* or *TSC2* in a patient necessitates increased tumor surveillance. The 2012 International Tuberous Sclerosis Complex Consensus Conference recommends a brain MRI, kidney MRI, and echocardiogram every 1-3 years in newly diagnosed patients. Additionally, annual dermatology, dental, ophthalmology, and pulmonology evaluations are recommended (Krueger, Northrup, & International Tuberous Sclerosis Complex Consensus, 2013).

West syndrome can also appear due to pathogenic/likely pathogenic variants in the cyclin-dependent kinase-like 5 gene *CDKL5*, which cause seizures, global delays, and hand stereotypies (Muller et al., 2016). *CDKL5* is involved in normal brain development and the transmission of signals between neurons. Evidence suggests it modifies the activity of the Rett syndrome-associated *MECP2* gene, resulting in overlapping phenotypic features (Rosas-Vargas et al., 2008). Like *MECP2*, *CDKL5* is inherited in an X-linked dominant manner. As such, one copy is necessary to inflict disease status in females. For males, *CDKL5*-related West syndrome is lethal.

Treatment for patients with *CDKL5* pathogenic/likely pathogenic variants includes various AED combinations, the ketogenic diet, and VNS. Response rates for at least one AED or the ketogenic diet were found to be highest within 3 months of treatment initiation (69%) with steady

decline by 12 months post-treatment (24%), suggesting low levels of efficacy for these treatments over long periods of time (Muller et al., 2016). For patients that experience this situation, VNS treatment has been proven to be a safe and effective long-term treatment option (Lim et al., 2018).

2.3.6.20 *ZEB2*

ZEB2 encodes a 2-handed zinc finger/homeodomain protein, which represses transcription through DNA-binding and is critical during neural crest development (Verstappen et al., 2008). Pathogenic/likely pathogenic variants in *ZEB2* are associated with autosomal dominant Mowat-Wilson syndrome (MWS) (Adam, 2007). Individuals with MWS present with a constellation of features including facial dysmorphisms, agenesis or hypogenesis of the corpus callosum, seizures, microcephaly, absent speech, intellectual disability, heart defects, genitourinary abnormalities, and Hirschsprung disease (Adam, 2007). Patients with MWS should be closely managed by a multi-disciplinary care team to ensure optimal health outcomes. In regards to MWS-related seizures, standard AEDs are considered effective treatment (Adam, 2007).

2.4 Utilization Management

Broadly, utilization management (UM) is "a set of techniques used by or on behalf of purchasers of health care benefits to manage health care costs by influencing patient care decision-making through case-by-case assessments of the appropriateness of care prior to its provision" (Institute of Medicine, 1989). In a genetic testing context, UM is the process of reviewing, screening, and modifying genetic testing requests with the goal of ensuring optimal, cost-effective genetic test orders (C. E. Miller et al., 2014). This process involves careful examination of patient

medical records, investigation of prior genetic testing, and communication with providers (Kotzer et al., 2014). Genetic counselors perform many of these reviews in both the laboratory setting and hospital setting. The task of UM at CHP is undertaken by a team of highly trained genetic counselors that constitute the Genetic Testing Stewardship Program (GTSP).

2.4.1 Genetic testing stewardship at CHP

The roles of the GTSP are multi-faceted. Aside from reviewing outpatient UM cases, the program also helps to ensure appropriate inpatient genetic testing, educates providers about genetic testing best practices, offers direct counseling services through the same-day Genetic Testing Clinic (GTC), provides stewardship over laboratory samples, and vets reference laboratories for possible collaboration. The summation of these roles makes the GTSP both a clinical and lab-focused group.

Some of the ways in which the GTSP ensures optimal test ordering include cancellation, deferral, and modification of test orders (Anderson, 2012). Cancellations occur if the test order is a duplicate or outright inappropriate for the patient. Deferrals may occur if testing is deemed to be more appropriate at a later date. This may be the case when an individual is an inpatient, whose testing would be costly to the hospital if not deferred for when they become an outpatient. Some patients also may not have insurance or may be waiting for their insurance to change, in which case deferral is the best option to prevent unnecessary costs. Modification often occurs if the ordered test is appropriate, but a better option is available. An example of modification is choosing a different laboratory to run the ordered test. Considerations that factor into this decision include the types of tests made available by the lab (e.g., panels, whole exome), pricing, the gene content of the tests, and the technology used (e.g., Next-Gen Sequencing (NGS), Sanger sequencing).

The template from which the GTSP operates was initially formed in the clinical laboratory setting, where UM procedures have been well-established. This establishment arose from necessity, as it became apparent that 10-30% of laboratory tests in the United States were ordered inappropriately or deemed unnecessary for patient care (Zhi, Ding, Theisen-Toupal, Whelan, & Arnaout, 2013). Mathias et al. investigated a UM database of 1,400 genetic test orders and found that 3% of these orders were changed or cancelled completely due to error (2016). Additionally, these errors occurred disproportionately among various specialty settings. In decreasing order of total percent error, the results were Genetic Inpatient (8%), Non-Genetics Inpatient (6%), Non-Genetics Outpatient (5%), and Genetics Outpatient (1%) (Mathias et al., 2016).

The difference between nongenetic and genetic outpatient orders is not surprising, due to the complexity of genetic testing. Interestingly, the highest rate of error, comprising 8% of total error, was found in the inpatient genetic setting (Mathias et al., 2016). Mathias et al. attributes this error to resident inexperience and miscommunication between multiple consultant departments (Mathias et al., 2016). This level of error is concerning, given the context of inpatient billing. Typically, hospitals receive a lump sum of funds from an inpatient's insurance at the initiation of their hospitalization in order to cover the treatments necessary for their care. Genetic testing is expensive and when ordered incorrectly or too often in an inpatient setting, it can quickly become the fiscal responsibility of the hospital. Inappropriate ordering of genetic tests in the inpatient setting, then, can result in high costs for hospitals.

Given the extent of genetic testing errors, genetic counselors in laboratory settings have a tremendous responsibility to mitigate them as best they can. In the previously mentioned study by Mathias et al., UM intervention led to modification or cancellation of 18% of genetic test orders and sequential testing of 15% of those orders (Mathias et al., 2016). In another study taking place

over a 21-month period at Associated Regional University Pathologists (ARUP) Laboratories, 99 requested test changes or cancellations per month, on average, were made by genetic counselors (C. E. Miller et al., 2014). Cost-savings due to test cancellations averaged at about \$792 per test, \$48,000 per month, and nearly \$1.2 million over the course of the study (C. E. Miller et al., 2014). At Mayo Clinic and Cleveland Clinic, annual genetic testing cost avoidances of \$779,060 and \$300,000 respectively have been observed (Kotzer et al., 2014).

Due to the obvious impact UM has on cost-savings at institutions, there is occasionally a negative connotation associated with it. The term UM has, at times, been used in the context of reduced quality of care in favor of cost-savings (Dickerson et al., 2017). As such, the term stewardship has been suggested as an alternative, to revitalize the focus of the process on improving the value of healthcare for individual patients, which is defined as its quality relative to its cost (Dickerson et al., 2017). Stewardship, which is defined as “the careful and responsible management of something entrusted to one's care” by Merriam-Webster, better describes the role genetic counselors play in attenuating the negative outcomes of genetic testing for patients (Dickerson et al., 2017).

2.4.2 Health insurance policies

Health insurance companies face the challenge of determining when reimbursement is appropriate for genetic testing. This decision-making process is influenced by State and Federal laws, clinical evidence, and community demand (SACGHS, 2006). Once the coverage decision is made, the health insurance company then creates a policy dictating under what circumstances coverage occurs. Much like the initial coverage decision, policies are subject to change as new scientific discoveries are made or new laws are formed (SACGHS, 2006). Beyond the existence

or boundaries of a policy, individual cases can present for review of coverage in the context of pre-authorization. Typically in this case, providers argue on behalf of the patient that testing is medically necessary in order to obtain coverage (SACGHS, 2006).

Insurance companies and medical providers utilize a uniform coding language in order to keep track of and accurately communicate the tests and procedures associated with individual patients. This language utilizes Current Procedure Technology (CPT®) codes that serve as nomenclature for a wide variety of medical terms and processes. They are created and maintained by a branch of the American Medical Association (AMA) called the CPT® Editorial Board (CPT®, 2018). CPT® codes can be attached to health insurance policies to strengthen and clarify their terms of coverage, which streamlines the review process as well as the billing process (SACGHS, 2006).

However, there is not a specific CPT® code for all types of genetic testing. EPs, for instance, do not currently have a specific CPT® code and so are open to a degree of variance when reviewed for insurance coverage or are billed for by a laboratory. Also, not every major health insurance carrier has a policy specific to EPs. As such, clinics such as the GTSP have had to utilize their own sets of testing criteria to standardize EP testing and make convincing arguments for insurance coverage.

The EP criteria currently utilized by the GTSP have the prerequisite that patients must first present with at least a single seizure. Once this has been established, the patient's situation must include one or more of the following:

- *Breakthrough seizures (i.e., seizures occurring while on anti-epileptic medication)*
- *Management includes multiple medication (AED) combinations.*
- *Behavior changes, skill regression, or developmental delay.*

- *History of status epilepticus (SE) or epilepsy partialis continua (EPC).*

Aside from needing to meet one or more of the above criteria, an EP in consideration must include medically actionable epilepsy genes. Also, if the patient presents with epileptic seizures and multiple or systemic congenital anomalies, exome should be considered instead.

This manuscript proposes a fifth criterion, which was utilized alongside the established criteria during data collection and analysis:

- *History of 2 or more seizures in a first or second degree relative.*

The addition of this criteria takes into account family history of epilepsy, which in itself suggests a genetic etiology. Taken as a whole, the five criteria listed above comprise the foundation of this manuscript's retrospective study.

2.5 Testing Criteria

Testing criteria for genetic testing exist as an intricate part of the UM process and are used by genetic counselors as an evaluation tool. Each individual criterion is a situational or patient-related requirement that must be met to warrant testing. The establishment of a standard set of criteria from which one can evaluate the need for a genetic test helps to mitigate error potential in test ordering. Fewer errors in test ordering can lead to higher yield of positive results (i.e., higher diagnosis rates), which is a direct indication that the ordered test was appropriate (Geddes, Basel, Frommelt, Kinney, & Earing, 2017). The main goal for test criteria, then, is, “to [correctly] identify individuals for whom the likelihood of testing positive justifies the costs of undergoing genetic testing” (Cropper et al., 2017). In order to reach this goal, however, proper assessment of existing genetic testing criteria is imperative.

2.5.1 Developing testing criteria

The standardization of genetic testing criteria as a means to guide appropriate test selection for patients is a relatively new concept outside of the realm of cancer, which is beholden to the National Comprehensive Cancer Network (NCCN) Clinical Guidelines (Cropper et al., 2017). With the lack of published and standardized criteria for genetic testing, some institutions, such as health insurance companies or government-run subsidies have developed internal criteria. One such group in Ontario, Canada published a study detailing their epilepsy genetic testing criteria and how they were developed. The group, which was dubbed the Genetic Testing Advisory Committee (GTAC) and tasked with their project by the Ministry of Health and Long-Term Care (MOHLTC), was comprised of pediatric epileptologists, an adult epileptologist, and medical geneticists (Jain et al., 2019).

The GTAC began their criteria development by first defining epilepsy and several mandatory prerequisites. Epilepsy was split into “well-controlled” and “drug refractory” categories, with each requiring mandatory consultation by either a clinical geneticist or epileptologist respectively once diagnosed. Any suspicions of metabolic disorders were to be addressed by a geneticist with appropriate training (Jain et al., 2019). Following mandatory evaluation, the next prerequisite to testing involved acquiring brain imaging studies through the use of MRI, either with or without proton MR spectroscopy, and an EEG. When epilepsy presented with certain features, such as dysmorphia or intellectual disability, it was recommended, but not required, to pursue other testing routes first, such as CMA (Jain et al., 2019).

Once the mandatory prerequisites for this study were set and genetic testing deemed warranted post-evaluation, the type of genetic test was selected. The GTAC focused on EPs utilizing NGS technology for their criteria development, due to their superior cost-effectiveness

over CMA and WES. They further subdivided EPs into focused and comprehensive options (Jain et al., 2019). The GTAC EP criteria were as follows:

- *“If the clinical diagnosis is clear and genetic heterogeneity is low, focused gene panels are indicated.*
- *If a treatable epilepsy is under consideration, a STAT epilepsy panel focused on treatable conditions should be considered.*
- *Where the clinical diagnosis is clear and genetic heterogeneity is high, but the clinical diagnosis is not indicative of a treatable condition, either a focused gene panel or comprehensive epilepsy panel should be carried out.*
- *If the clinical diagnosis is not clear and genetic heterogeneity is unknown, either a focused gene panel or comprehensive epilepsy panel should be carried out”*(Jain et al., 2019).

These criteria, in some cases, are broad and allow providers the space to tailor to specific cases. To summarize, the GTAC in Ontario justified either focused or comprehensive EP testing in the settings listed above under the conditions that appropriate consultation, screening, and diagnosis had already been completed. This structured framework for testing is an excellent example of criteria development, but it is important to keep in mind the health insurance framework within which any developed criteria might exist.

Health insurance companies in the United States, as mentioned previously, rely on evidence-based codes and rigid policies to know when to cover genetic testing. Testing criteria are the backbone of these policies and need to be as sensitive and predictive as possible in order to provide effective results. Therefore, it is important to assess their performance. Established testing criteria that have been subjected to the scrutiny of research can be modified to increase their

sensitivity and potentially cut costs on unnecessary testing when incorporated into policy. Additionally, policies based on testing criteria that are predictive of disease can help to ensure that no patients are overlooked that should have testing.

2.5.2 Assessing testing criteria

The core question under investigation during the assessment process of genetic testing criteria is how well said criteria identify and predict disease status or pathogenic/likely pathogenic variant status. In order to answer this question, investigators must examine two major parameters: sensitivity and positive predictive value (PPV). Sensitivity in this context is the likelihood that a given criteria will be met when a pathogenic/likely pathogenic variant is present (Parikh, Mathai, Parikh, Chandra Sekhar, & Thomas, 2008). This represents the criteria's ability to correctly identify pathogenic/likely pathogenic variants in a given population. PPV is the probability that patients have a pathogenic/likely pathogenic variant when they meet testing criteria (Parikh et al., 2008). It is a measure of how well a set of criteria predict positive results in a theoretical population (i.e., untested individuals) and is the more useful of the two parameters from a health policy standpoint. This is because health policies that are based on criteria with high PPVs will spend less money on tests that do not yield actionable or medically relevant results (Altman & Bland, 1994). PPV can increase or decrease depending on the performance of each individual criterion but is ultimately reliant on the prevalence of pathogenic/likely pathogenic variants in a population. Both sensitivity and PPV have been used in other studies as assessment tools for testing criteria.

A study by LaDuca et al. utilized sensitivity as their measure of criteria performance. They reviewed the records of 165,000 patients who had undergone commercial multi-gene panel testing for hereditary cancer syndromes. These records included pedigrees, requisition forms, and

progress notes. Their goals were to identify pathogenic/likely pathogenic variant frequencies in cancer predisposition genes as well as to discover how often pathogenic/likely pathogenic variants were identified by *BRCA1/2* and Lynch syndrome testing criteria (LaDuca et al., 2020). LaDuca et al. discovered that 94.2% of *BRCA1/2* pathogenic/likely pathogenic variants met *BRCA1/2* criteria and 73.1% of mismatch repair gene pathogenic/likely pathogenic variants met Lynch syndrome criteria.

However, 67% of the pathogenic/likely pathogenic variants in the patients that met *BRCA1/2* criteria were in genes other than *BRCA1/2* and 53.8% of the pathogenic/likely pathogenic variants in the patients that met Lynch syndrome criteria were in genes other than those associated with mismatch repair. The conclusion that LaDuca et al. drew from these results was that even though the sensitivities of the two sets of criteria were high, their ability to predict the presence of pathogenic/likely pathogenic variants in their specific genes was low. This conclusion supported revising existing *BRCA1/2* and Lynch syndrome criteria to include a greater variety of cancer predisposition genes (LaDuca et al., 2020). Overall, this study is an example of the utility and limitations of the sensitivity parameter. Sensitivity is a useful assessment tool, but it is restricted to being a descriptive statistic for an existing population. For the purpose of outcome prediction in a theoretical population, PPV is the more appropriate parameter.

A team from The University of Texas MD, Anderson Cancer Center conducted a retrospective chart analysis in 2017 to assess the NCCN Clinical Guidelines for *BRCA1/2* testing. Their patient population included 1,123 individuals who were diagnosed with breast cancer and had genetic counseling between March 31, 2013 and June 30, 2014. Their methods involved calculating PPV for *BRCA1/2* testing criteria to determine how well they predicted true pathogenic/likely pathogenic variant status in breast cancer patients. They did this by identifying

those patients that were found to be positive for either BRCA1 or BRCA2 pathogenic/likely pathogenic variants and determining which of them met either one criterion or two or more criteria (Cropper et al., 2017).

The PPV was much higher in the two or more criteria category at 12.0% when compared to the one criterion category of 3.2%. Cropper et al. looked at each individual criterion separately to determine if any one criterion had a more substantial impact on PPV. It was found that none did, nor did any combination of two criteria outperform the rest. However, two criteria in particular severely underperformed on their own. Only 1.7% of patients who were diagnosed at age 45 or younger and met no other criteria were found to have a pathogenic/likely pathogenic variant. Also, none of the 37 patients who only met the criteria of having two relatives with breast cancer tested positive for *BRCA1/2* (Cropper et al., 2017). These results overall reinforce that some criteria may not be predictive of genetic risk, and that generally a combination of criteria predict pathogenic/likely pathogenic variant status better than individual criterion only.

In summary, an ideal set of testing criteria should have high sensitivity and a high PPV. Highly sensitive criteria identify a large number of positive test results when met. Criteria with a high PPV, when met, are more closely associated with positive results than negative results. This means that the proportion of untested individuals that meet criteria with pathogenic/likely pathogenic variants can be estimated. It should be noted, however, that PPV depends on the prevalence of the pathogenic/likely pathogenic variants in the population. Low pathogenic/likely pathogenic variant prevalence means that even for the most sensitive criteria, PPV will be low. Establishing effective testing criteria requires careful thought in the processes of development and assessment. The results of assessment should spur further development and vice versa until an optimal set of criteria is produced. The studies performed by the GTAC, Cropper et al., and LaDuca

et al. can serve as models when considering the processes of criteria development and assessment in settings other than hereditary cancer predisposition syndromes.

The focus of this study was the assessment and modification of criteria in the setting of epilepsy panel (EP) testing. As discussed in this literature review, epilepsy is a complex neurological condition and can be genetic in origin (Zhang et al., 2017). Determining an etiology for a patient's epilepsy in a timely manner is critical and genetic testing plays a role in this process for some patients (Kalilani et al., 2019). In these cases, proper stewardship requires that careful attention be paid to the patient's overall phenotype, the genetic testing modality utilized, and what criteria are met in order to ensure the most appropriate genetic test is ordered (Anderson, 2012; Cropper et al., 2017; Sanchez Fernandez et al., 2019). Most health insurers provide little in the way of standardized guidance in this setting and often operate under non-specific policies, especially in regards to EP coverage (Aetna, 2019; Cigna, 2019; UPMCHealthPlan, 2019). Vetted EP criteria stand to play a role in this arena by providing a foundation for a specific EP health insurance policy to be built, which would provide guidelines for providers as to when EP testing would be considered appropriate and covered. This study underwent the process of vetting the EP criteria utilized by the Genetic Testing Stewardship Program (GTSP) at UPMC Children's Hospital (CHP) for this purpose. The background and methods of this assessment process will be the point of discussion in the next section of this manuscript.

3.0 Manuscript

3.1 Background

Epilepsy panels (EPs), especially comprehensive NGS panels, are currently the most cost-effective genetic test for determining an etiology for idiopathic generalized epilepsy (IGE) when compared to whole-exome sequencing (WES) and chromosomal microarray analysis (CMA) (Sanchez Fernandez et al., 2019). Swift determination of said etiology spares patients and the healthcare system from long and expensive diagnostic odysseys comprised of neuroimaging, biopsies, and single gene tests (Joshi et al., 2016). It also has the potential to mitigate harm to patients caused by epilepsy comorbidities (Kalilani et al., 2019). Further benefits of determining a genetic epilepsy etiology include medication management, therapy options, and family planning information (Poduri, Sheidley, Shostak, & Ottman, 2014). It is in the best interest of patients and the healthcare system to have established policies directing providers when to order EPs and health insurance companies when to cover them.

The UPMC Health Plan has policies for WES, CMA, and a general genetic testing policy. However, they do not currently have a policy for EP genetic testing specifically (UPMCHealthPlan, 2019). This is also the case for other major health insurance companies, such as Cigna and Aetna. Cigna will cover genetic testing when “medically necessary” but offers no guidance for epilepsy panel coverage (Cigna, 2019). Aetna provides similar policy language with the addition of considering epilepsy-related genetic testing for anything other than Dravet syndrome to be “experimental” (Aetna, 2019). The incorporation of clinically validated testing

criteria into medical policy can rectify these information gaps and provide much needed direction for health insurance companies and ordering providers alike.

The Genetic Testing Stewardship Program (GTSP) is a clinical and lab-focused group that performs many roles at CHP, including reviewing outpatient utilization management (UM) cases, ensuring appropriate inpatient genetic testing, educating providers about genetic testing best practices, vetting reference laboratories for possible collaboration, and offering direct counseling services through the same-day Genetic Testing Clinic (GTC). Since early 2017, the genetic counselors in the GTSP have been using a set of criteria as a screening tool during the review process for EPs ordered for patients seen through Neurology, Medical Genetics, and the GTC at CHP. These screening criteria provide guidelines for when a comprehensive EP is considered appropriate for a patient and when it is not. In addition to the requirement for patients to present with at least one seizure, the four EP criteria in the set are: “Breakthrough seizures,” “Management includes multiple medication combinations,” “Behavior changes, skill regression, or developmental delay,” and “History of status epilepticus or epilepsy partialis continua.” This study proposes a fifth criterion, “History of 2 or more seizures in a first or second degree relative,” to be added to the EP criteria utilized by the GTSP.

3.2 Methods

This study was approved by the University of Pittsburgh Institutional Review Board (IRB) (PRO19050363) under Expedited Review. This approval included a waiver of informed consent and HIPAA authorization (Appendix A).

3.2.1 Study population

Patients who had an initial epilepsy evaluation and diagnosis through the Neurology department at UPMC Children's Hospital of Pittsburgh (CHP) between January 1st, 2016 and December 31st, 2018 were included in this study. These evaluations were strictly in the outpatient setting and did not include inpatient Neurology consultations. Epilepsy phenotypes, age of evaluation, and age of epilepsy diagnosis varied, making the population fairly heterogeneous. Genetic testing for patients was ordered through Neurology, Medical Genetics, or the GTC. Epilepsy panels (EPs) that were ordered for these patients between January 1st, 2016 and June 30th, 2019 were included in this study to account for delays in testing and allow for result availability. EP results that returned after June 30th, 2019 were included as long as they were ordered before that date. In total, 1,242 patient charts were reviewed.

3.2.2 Data identification

Patients for this study were ascertained by the Genetic Counseling Supervisor of Laboratory Services at CHP, prior to data collection, through ongoing quality improvement initiatives. Patients with service dates within the study timeframe were pulled from the CHP electronic medical record (EMR) by the following Visit Type: "new patient consult for epilepsy." The patient query was filtered by ICD-10 diagnosis codes beginning with G40, which are associated with various epilepsy diagnoses (**Table 1**). Inpatients and duplicates were identified and removed prior to data collection. Patient identifiers were stored via UPMC institutional encryption and were password protected. Each patient was given a working Case ID and identifiers were kept separate from all data collection files as per IRB protocol.

Table 1. Utilized epilepsy ICD-10 diagnosis codes

ICD-10 Diagnosis Code	Diagnosis Description
G40.001	Localization-related (focal) (partial) idiopathic epilepsy and epileptic syndromes with seizures of localized onset, not intractable, with status epilepticus
G40.009	Localization-related (focal) (partial) idiopathic epilepsy and epileptic syndromes with seizures of localized onset, not intractable, without status epilepticus
G40.011	Localization-related (focal) (partial) idiopathic epilepsy and epileptic syndromes with seizures of localized onset, intractable, with status epilepticus
G40.019	Localization-related (focal) (partial) idiopathic epilepsy and epileptic syndromes with seizures of localized onset, intractable, without status epilepticus
G40.109	Localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures, not intractable, without status epilepticus
G40.119	Localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with simple partial seizures, intractable, without status epilepticus
G40.209	Localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with complex partial seizures, not intractable, without status epilepticus
G40.219	Localization-related (focal) (partial) symptomatic epilepsy and epileptic syndromes with complex partial seizures, intractable, without status epilepticus
G40.301	Generalized idiopathic epilepsy and epileptic syndromes, not intractable, with status epilepticus
G40.309	Generalized idiopathic epilepsy and epileptic syndromes, not intractable, without status epilepticus
G40.319	Generalized idiopathic epilepsy and epileptic syndromes, intractable, without status epilepticus
G40.401	Other generalized epilepsy and epileptic syndromes, not intractable, with status epilepticus
G40.409	Other generalized epilepsy and epileptic syndromes, not intractable, without status epilepticus
G40.419	Other generalized epilepsy and epileptic syndromes, intractable, without status epilepticus
G40.802	Other epilepsy, not intractable, without status epilepticus
G40.804	Other epilepsy, intractable, without status epilepticus
G40.814	Lennox-Gastaut syndrome, intractable, without status epilepticus
G40.822	Epileptic spasms, not intractable, without status epilepticus
G40.824	Epileptic spasms, intractable, without status epilepticus
G40.89	Other seizures
G40.901	Epilepsy, unspecified, not intractable, with status epilepticus
G40.909	Epilepsy, unspecified, not intractable, without status epilepticus
G40.911	Epilepsy, unspecified, intractable, with status epilepticus
G40.919	Epilepsy, unspecified, intractable, without status epilepticus
G40.A09	Absence epileptic syndrome, not intractable, without status epilepticus
G40.A19	Absence epileptic syndrome, intractable, without status epilepticus
G40.B09	Juvenile myoclonic epilepsy, not intractable, without status epilepticus

3.2.3 Data collection

The generated patient list was used to complete the retrospective chart review. Patient names were entered into CHP's electronic medical record (Cerner®) and all retrieved data was kept on a separate Microsoft® Excel spreadsheet with no patient identifiers as per IRB protocol. Patient demographics were obtained, including age at date of service, sex, and race. Relevant provider notes were reviewed, including the Neurology note corresponding to the date of service as well as Medical Genetics and Genetic Counselor notes, in order to determine if the patient's phenotype met EP testing criteria and if an EP was attempted. Testing was confirmed by locating the scanned report in Cerner®. In the event that no test report was found, phone messages and authorization requests were investigated to determine the status of attempted EP testing. An EP testing attempt, in this study, is defined as the actions taken by a provider to pursue an EP following the initial conversation with the patient's family and confirmation of their interest in testing. These actions include ordering the EP and requesting authorization from insurance, provided said patient has insurance coverage.

Information pertaining to EP testing attempts was collected to identify whether or not providers mentioned and/or attempted to order EPs for their patients. From this data, educated assumptions were made regarding why EPs were not ordered for some patients. In order to succinctly document this information, each individual patient situation was categorized as shown in **Table 2**. For instance, the "No, previous testing positive" (67/1,242, 5.4%) category accounted for all of the positive genetic testing that occurred in the absence of an EP. In these cases, it was possible that an EP was not ordered because either WES, CMA, or another genetic test was diagnostic, or provided a genetic explanation, for the patient's epilepsy or seizure phenotype. Even if these tests were non-diagnostic, which was the case in the "No, WES" (19/1,242, 1.5%), "No,

CMA” (63/1,242, 5.1%), and “No, other genetic testing” (67/1,242, 5.4%) categories, it was possible that their completion alone was enough to prevent the ordering of an EP. For example, WES would have covered the necessary epilepsy genes, or a Comprehensive Brain Malformation panel may have been more appropriate than an EP given the patient phenotype.

Previous positive testing was not the only reason why EPs were not ordered. Some families were not interested in testing when it was presented during the initial evaluation (6/1,242, 0.5%). Others either did not follow up after insurance approval for EP testing (11/1,242, 0.9%) or were denied authorization by insurance (16/1,242, 1.3%). There were also some patients that had an EP outside of the study timeframe (29/1,242, 2.3%) or did not have any notes mentioning an EP documented in the EMR (855/1,242, 68.8%).

Table 2. EP attempt categories, definitions, and demographics

Was an EP attempted?	Definition	Number of patients (n=1,242)
Yes, lost to follow-up	- EP was approved by insurance but was not completed by the patient.	11 (0.9%)
Yes, insurance barrier	- EP was attempted but insurance denied it.	16 (1.3%)
Yes, EP	- EP was completed.	109 (8.8%)
No, WES	- WES was completed and was non-diagnostic. - EP was NOT attempted.	19 (1.5%)
No, other genetic testing	- A different panel or single gene test was completed and was non-diagnostic. - EP was NOT attempted.	67 (5.4%)
No, CMA	- CMA was completed and was non-diagnostic. - EP was NOT attempted.	63 (5.1%)
No, testing not mentioned	- The provider did not document mentioning an EP in the patient chart	855 (68.8%)
No, previous testing positive	- WES, CMA, or other genetic testing proved diagnostic - EP was NOT attempted.	67 (5.4%)
No, no family interest	- An EP was mentioned but the family did not wish to attempt it.	6 (0.5%)
No, other	- An EP was completed outside the study timeframe.	29 (2.3%)

During data collection of EP attempts, only one primary genetic test (e.g., “No, WES” or “No, CMA,” etc.) was selected for each patient. In some cases, patients had more than one genetic test completed, which necessitated the use of an organizational hierarchy for EP attempt selection. Because EPs were the target data and focus of this study, they were given the highest priority and selected as the testing attempt when observed. WES results followed EPs in priority and were selected as the testing attempt if an EP was not completed. Other genetic tests, such as single gene tests or other gene panels, followed WES in priority and were selected as the testing attempt only in the absence of an EP or WES. Only genetic testing relevant to an epilepsy phenotype was collected as data (i.e., tests for genes like *CFTR*, which is associated with cystic fibrosis, were not included). CMA had the lowest priority and was selected in the absence of all other testing. To summarize, the EP attempt selection method was used to succinctly organize and describe the EP ordering process. This helped rationalize why some EPs were not ordered for a large patient cohort (n=1,242). The demographics collected by this method are not representative of the total proportions of genetic testing for the study cohort, which are described later in this manuscript.

For patients that had EP testing (109/1,242, 8.8%), information about the specific test was gathered. This included the name of the test, the name of the performing laboratory, order date, result date, and the number of genes included in the panel, which ranged from 22 to 1,428 genes. Additionally, the results of each EP report were documented. Pathogenic or likely pathogenic variants in autosomal dominant genes and homozygous or compound heterozygous pathogenic or likely pathogenic variants in autosomal recessive genes were considered positive. Likewise, pathogenic or likely pathogenic variants in X-linked dominant genes as well as hemizygous X-linked recessive genes (in male patients) were considered positive.

For the purposes of this study, a pathogenic or likely pathogenic variant found in only one autosomal recessive gene was considered negative, as this type of result would not necessarily explain patient phenotype. This was also the case for heterozygous autosomal recessive Variants of Unknown Significance (VUS). Even if these results were to be reclassified to pathogenic or likely pathogenic, they likewise would not necessarily explain patient phenotype. Therefore, these VUS results were re-categorized as negative. All other VUS results were considered neither positive nor negative for the purposes of this study and were not included in any sensitivity or PPV calculations. Each affected gene was listed along with cDNA and protein nomenclature as well as inheritance pattern (Autosomal dominant [AD], Autosomal recessive [AR], X-linked dominant [XD], X-linked recessive [XR], or Mitochondrial [M]). The same was done for any VUS results that were reported. Reports that found no gene variants were classified and documented as negative EPs.

To determine if patients met testing criteria, each relevant progress note was scanned for suggestive language. Breakthrough seizures, for instance, were derived from terms such as “intractable” and “refractory,” yet could be seen due to AED non-compliance. Cases of non-compliance were not included as meeting the criterion. Developmental delays were derived from phrases such as “behind in early motor milestones” or “neurocognitive impairment.” Status epilepticus and epilepsy partialis continua did not have any euphemisms and so were readily identified when available. Medication combinations and failed medications were listed in Neurology progress notes. Family history, when not obtainable through a documented three-generation pedigree, was obtained from progress notes. For ease of collection and documentation, each criterion was abbreviated as: “Breakthrough seizures” (*BreakThr*), “Management includes multiple medication combinations” (*MedCombo*), “Behavior changes, skill regression, or

developmental delay” (*DevDelay*), “History of status epilepticus or epilepsia partialis continua” (*StatEpi*), and “History of 2 or more seizures in a first or second degree relative” (*FamHx*).

3.2.4 Data analysis

Descriptive statistics were created for all demographic data collected, including age, sex, race, and testing attempt, as a percentage of the total patient population (n=1,242). These demographics were stratified to indicate their association with positive, negative, or VUS epilepsy panel results. Screening criteria were evaluated for the entire cohort. However, sensitivity and PPV calculations were performed only with completed EP testing results (n=109). EP results were coded as positive (n=17) or negative (n=54) when possible. After re-categorization, the remaining VUS results (n=38) were not used to calculate sensitivity or PPV, as they represented neither a true positive nor true negative result. This ambiguity nullified the utility of these results in the screening criteria analysis.

Each positive and negative EP result was further analyzed to determine the proportions of screening criteria that were met. This was done in a hierarchical category system, based on the number of criteria considered. The goal of the screening criteria analysis was to track the performance of each individual criterion as more combinations were made available. The relationships between the criteria and the resulting trends in their values became apparent as the categories progressed, revealing the strongest performers in both sensitivity and PPV. The screening criteria data analysis in this study followed the “or” logic illustrated by the connecting lines in the example algorithm in **Figure 1**. This methodology will be the next topic of discussion.

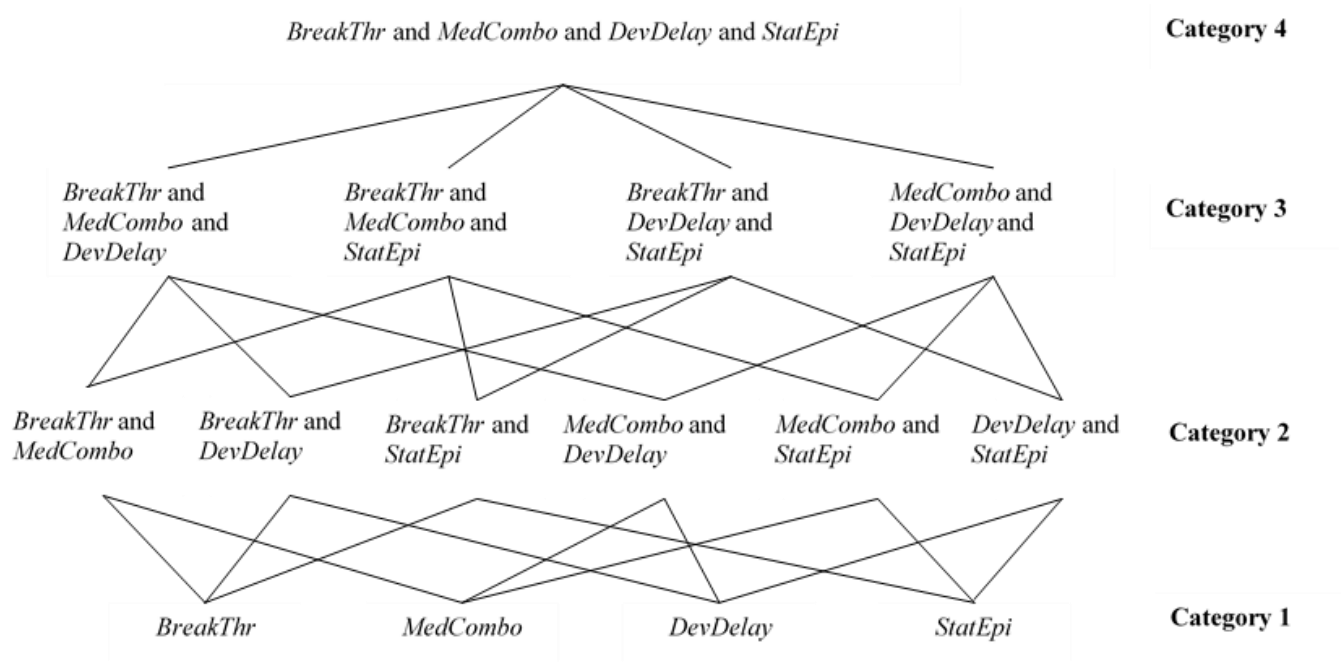


Figure 1. Example criteria hierarchy demonstrating progressive levels of data capture with each category

Beginning with screening Category 1, the proportion of the total number of positive and negative EP results meeting each individual criterion (*BreakThr*, *MedCombo*, *DevDelay*, *StatEpi* and *FamHx*) was calculated. The level of data capture in screening Category 1 was such that only individual criteria were accounted for, not criteria combinations. In other words, there were no instances in Category 1 where more than one criterion was considered for each patient. Each instance of a met criterion was treated as its own singular event. As such, the proportions from Category 1 were the smallest of any category and the derivative sensitivities and PPVs had the widest 95% confidence intervals (CIs).

In screening Category 2, not only were the singular criterion events from Category 1 captured, but two-criteria combinations were accounted for as well. In this case, the proportion of positive or negative EP results meeting one “or” both criteria in the two-criteria combinations were considered. In this way, the level of data capture increased, meaning it was then possible to analyze

the data of patients that met up to two criteria. The resulting two-criteria combinations carried with them the proportions of their individual components, the criteria from Category 1. As such, their sensitivities and PPVs were amalgams of the sensitivities and PPVs calculated from Category 1 data. Subsequent comparison revealed the beginnings of relationships between the criteria and trends in their values. For instance, two particular criteria, *DevDelay* and *StatEpi*, when considered together, meaning one or both could be met, had the highest PPV in Category 2. This meant that they, together or apart, were more closely associated with positive EP results than any other criteria or combination of criteria at this level of data capture.

By following the *DevDelay/StatEpi* example from Category 2 through **Figure 1**, it becomes evident that it was a part of two different Category 3 combinations, as illustrated by the connecting lines. The expanded data capture concept that was discussed for Category 2 persisted in Category 3 and 4 as well. This meant that patients meeting up to three criteria could be analyzed in Category 3 while accounting for all of the proportions and calculated values from the previous categories. For instance, the *DevDelay/StatEpi* combination, as well as its individual components, were accounted for during the assessment of the *BreakThr/DevDelay/StatEpi* criteria combination and the *MedCombo/DevDelay/StatEpi* criteria combination. Interestingly, neither of these combinations had the highest PPV in Category 3, demonstrating in this particular example how the values of strong Category 2 performers can be negatively affected when considered alongside certain other criteria. This situation exemplifies the concept of criteria relationships and their value trends throughout the different categories. The consideration of more criteria as the categories progressed provided additional insight into how the criteria interacted and affected each other's performance regarding the detection and prediction of positive EP results.

The epitome of criteria inclusion and evaluation was Category 4. No positive or negative EP results met all five testing criteria concurrently, hence the exclusion of a Category 5. Accounting for every possible criteria combination (i.e., one, two, three, or four criteria met) led to Category 4 having the largest proportions and narrowest CIs. This meant that Category 4 data was the most inclusive and reliable. This also meant that Category 4 was the final indicator for criteria performance, upon which all of the relationships and trends converged. In the end, the top-performing criteria consistently contributed to the highest sensitivities and PPVs throughout category progression and resulted in the highest values within Category 4.

In order to help facilitate the sensitivity and positive predictive value (PPV) calculations, the EP result proportions from each category were displayed in tables like the example shown in **Table 3**. Positive EPs that met a criterion or criteria combination were entered into box “A.” Negative EPs that met a criterion or criteria combination were entered into box “B.” Positive EPs that did not meet a criterion or criteria combination were entered into box “C.” Negative EPs that did not meet a criterion or criteria combination were entered into box “D”.

Table 3. Sample proportion table

Criterion/Criteria	Positive Epilepsy Panel (+)	Negative Epilepsy Panel (-)
Met (+)	A	B
Not Met (-)	C	D

Once all of the proportion tables were created, sensitivity and PPV for each criterion or criteria combination in Categories 1, 2, 3 and 4 were analyzed. Stata (version 15) software was

used to generate the 95% confidence intervals (CIs) for each calculated proportion. **Equation 1** was used to calculate sensitivity and **Equation 2** was used to calculate PPV.

$$\text{Equation 1. Sensitivity} = \frac{A}{A+C}$$

$$\text{Equation 2. PPV} = \frac{A}{A+B}$$

Then, the proportion of patients without genetic testing that met each criterion or criteria combination were calculated (n=507). These patients were pulled from the “No, no testing mentioned” and “No, no family interest” EP attempt categories and served as a hypothetical population on which the predictive capabilities of the EP criteria could be evaluated. The other EP attempt categories were excluded, as they accounted for either attempted or completed genetic testing (**Table 2**). The highest PPVs in each criteria category were multiplied by the total number of patients in the untested population that met the corresponding criterion or criteria combination. The goal of these calculations was to estimate the number of patients who did not have EP testing who have pathogenic/likely pathogenic epilepsy gene variants.

3.3 Results

3.3.1 Patient information

The demographics of the study population varied as seen in **Table 4**. The patients’ ages were evenly distributed from infancy to 17 years, with a marked drop-off at age 18 years. Most patients were age 12 years or younger (868/1,242, 69.9%), white (1,035/1,242, 83.3%), and male (696/1,242, 56%). Patients of Hispanic, American Indian, and Pacific Islander descent were

severely underrepresented, but it is possible that some may have been listed in the Other race category in the EMR. The majority of patients with positive EPs were white (16/17, 94.1%), males (11/17, 64.7%), and between infancy and 3 years of age (9/17, 53%). The majority of patients with VUS EPs were white (34/38, 89.5%), male (24/38 63.2%), and between 4 and 8 years of age (15/38, 39.5%). The majority of patients with negative EPs were white (43/54, 79.6%), male (33/54, 61.1%), and between infancy and 3 years of age (16/54, 29.6%). Patient ages were taken as of their initial neurology evaluation, or date of service, and do not align with test order dates, as some tests were ordered after the date of service.

Table 4. Demographics

Demographic	Number of Patients (n = 1,242)	Positive Epilepsy Panels (n=17)	VUS Epilepsy Panels (n = 38)	Negative Epilepsy Panels (n=54)
<u>Age (years)</u>				
0-3	214 (17.2%)	9 (53%)	10 (26.3%)	16 (29.6%)
4-8	335 (27%)	4 (23.5%)	15 (39.5%)	12 (22.2%)
9-12	319 (25.7%)	4 (23.5%)	6 (15.8%)	13 (24.1%)
13-17	329 (26.5%)	0 (0%)	4 (10.5%)	11 (20.4%)
18-21	41(3.3%)	0 (0%)	3 (7.9%)	2 (3.7%)
22+	4 (0.3%)	0 (0%)	0 (0%)	0 (0%)
<u>Sex</u>				
Male	696 (56%)	11 (64.7%)	24 (63.2%)	33 (61.1%)
Female	546 (44%)	6 (35.3%)	14 (36.8%)	21 (38.9%)
<u>Race</u>				
White	1,035 (83.3%)	16 (94.1%)	34 (89.5%)	43 (79.6%)
Black	141 (11.4%)	0 (0%)	3 (7.9%)	7 (12.9%)
Asian	21 (1.6%)	0 (0%)	1 (2.6%)	2 (3.7%)
Hispanic	0 (0%)	0 (0%)	0 (0%)	0 (0%)
American Indian	1 (0.1%)	0 (0%)	0 (0%)	0 (0%)
Pacific Islander	2 (0.2%)	0 (0%)	0 (0%)	1 (1.9%)
Other	42 (3.4%)	1 (5.9%)	0 (0%)	1 (1.9%)

Regarding genetic testing, a mixture of EPs (109/1,242, 8.8%), WES (52/1,242, 4.2%), other single gene tests or panels (134/1,242, 10.8%), CMAs (190/1,232, 15.3%,) and Fragile X tests (69/1,242, 5.6%) were completed for the study cohort as seen in **Table 5**. Some patients had more than one type of genetic testing (i.e., EP, WES, and CMA ordered for the same patient over the course of their treatment). However, only one of these cases led to multiple genetic tests identifying the same positive result.

For example, a male patient who presented with seizures, global developmental delay, ankyloglossia, fatty liver, dry skin, dry scalp, epicanthus, and hyperopia had CMA in 2015 followed by WES in 2019. Both tests identified a Xp22.31 deletion. This deletion encompassed the *STS* gene, which is associated with X-linked recessive ichthyosis, also called STS deficiency, and epilepsy in some patients (Malik et al., 2017). This patient is one of several in this study cohort with multisystemic features and epilepsy secondary to a genetic syndrome. In these cases, EPs may not be the most appropriate test.

As seen in **Table 5**, positive and negative EP results did not coincide with positive results from WES, CMA, or other gene tests. In one case, WES was diagnostic when a previously ordered EP yielded a VUS result in the *CACNA1A* gene. WES for a female patient that presented with sensorineural hearing impairment, mild intellectual disability, seizures, ataxia, Arnold-Chiari malformation, ectopic kidney, and movement abnormalities, revealed a heteroplasmic pathogenic variant in the mitochondrial *MT-TK* gene in addition to identifying the *CACNA1A* VUS. The *MT-TK* variant was consistent with a diagnosis of Myoclonic Epilepsy with Ragged Red Fibers (MERRF) syndrome (DiMauro & Hirano, 1993). This patient's EP was ordered in 2016 and her WES was ordered in 2018. Even though WES may be more appropriate than an EP for patients with multisystemic or syndromic features, some EPs on the current market include mitochondrial

DNA sequencing (BlueprintGenetics, 2020). The specific EP selected for this patient in 2016 did not, making this case an example of how test selection and available technology can have an impact on the timing of a genetic diagnosis.

Table 5. Proportions of positive genetic tests in relation to EPs

Genetic Test	Number of tests (n=554)	Percentage of Positive Results	Percent Positive when EP Positive	Percent Positive when EP VUS	Percent Positive when EP Negative
Epilepsy panel (EP)	109 (19.7%)	17 (15.6%)	-	-	-
Whole exome sequencing (WES)	52 (9.4%)	*17 (32.6%)	0 (0%)	•1 (3.7%)	0 (0%)
Chromosomal microarray analysis (CMA)	190 (34.3%)	25 (13.2%)	0 (0%)	0 (0%)	0 (0%)
Other genetic testing	134 (24.2%)	24 (17.9%)	0 (0%)	0 (0%)	0 (0%)
Fragile X testing	69 (12.4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

*In one case, a patient that had both WES and CMA had the same positive result identified on both tests.

•In one case, WES was diagnostic when a previously ordered EP yielded a VUS result.

Most of the positive WES, CMA, and other genetic testing results in the study cohort were related to epilepsy or had epilepsy as a secondary presenting feature (**Table 6**). For example, WES diagnoses included not only primary genetic epilepsy conditions such as MERRF syndrome (*MT-TK*) and infantile epileptic encephalopathy type 5 (*SPTAN1*), but also conditions such as Cornelia de Lange syndrome (*SMC1A*) and STS deficiency (Xp22.31del), which have been reported to be associated with epilepsy in some patients (Malik et al., 2017; Verrotti et al., 2013).

Additional genetic testing in the form of panels and single gene tests resulted in diagnoses such as Noonan syndrome (*BRAF*) and Sanfilippo syndrome (Homozygous *GNS* variants), which can present with epilepsy (Pierpont, 2016; Scarpa, Lourenço, & Amartino, 2017). Genetic causes

for holoprosencephaly (*ZIC2*) and lissencephaly (*TUBA1A*) were identified as well, though seizures in these cases were most likely associated with the major brain malformations themselves (Barratt & Arkell, 2018; Kumar et al., 2010). CMAs diagnosed some CNV-related conditions associated with epilepsy such as Wolf-Hirschhorn (4p deletion) syndrome and DiGeorge syndrome (22q11.21 deletion) (Kagitani-Shimono et al., 2005; Strehlow et al., 2016).

Some patients were given diagnoses that were not definitively associated with epilepsy. For example, some patients were found to have pathogenic/likely pathogenic variants in genes associated with cancer predisposition (*TP53*), cardiomyopathy (*TPM1*), or polycystic liver disease (*ALG8*) or lethal CNVs such as trisomy 13 (Besse et al., 2017; England et al., 2017; Schneider, Zelley, Nichols, & Garber, 1993). These patients had seizures in addition to their primary health concerns, but it is likely that their genetic testing was not primarily for epilepsy. One could make an argument for secondary epilepsy in some of these cases, such as with a brain tumor caused by a *TP53* variant, but for the sake of clarity, these and other similar results were excluded from the total Mendelian calculation in **Table 6**.

Table 6. Positive tests associated with epilepsy and at least one EP criteria

Genetic Test	Positive Results	Positive Results Related to Epilepsy	Positive Results Meeting at least one EP Criterion
EP (n=109)	17 (15.6%)	17 (100%)	16 (94.1%)
WES (n=52)	17 (32.6%)	13 (76.4%)	17 (100%)
CMA (n=190)	25 (13.2%)	13 (52%)	24 (96%)
Other genetic test (n=134)	24 (17.9%)	17 (70.8%)	22 (91.7%)
*Total: 60			

* 59/1,242 (4.8%) patients in this study cohort were confirmed to have a Mendelian explanation for their epilepsy. This number would likely increase with an uptake in genetic testing. The total was subtracted by one to account for the case of two positive test results for one patient.

3.3.2 Criteria performance

As mentioned previously, EP testing criteria were assessed in four different categories, which corresponded to the number of applicable criteria per assessment group. Individual criteria proportions along with associated abbreviations can be seen in **Table 7**. The most commonly met criterion for positive EPs was *MedCombo* (11/17, 65%). For negative EPs, the most commonly met criterion was *BreakThr* (37/54, 68.5%). Of note, 1 (5.9%) positive EP result did not meet any criteria and 5 (9.3%) negative EP results did not meet any criteria. None of the positive or negative EP results met all five criteria at the same time.

Table 7. Individual criteria proportions

Criteria Met	Positive Epilepsy Panel (n = 17)	Negative Epilepsy Panel (n = 54)
Breakthrough seizures (<i>BreakThr</i>)	10 (59%)	37 (68.5%)
Management includes multiple anti-epileptic medication combinations (<i>MedCombo</i>)	11 (65%)	30 (55.6%)
Behavior changes, skill regression, or developmental delay (<i>DevDelay</i>)	10 (59%)	22 (40.7%)
History of status epilepticus or epilepsy partialis continua (<i>StatEpi</i>)	3 (18%)	2 (3.7%)
History of 2 or more seizures in a first or second degree relative (<i>FamHx</i>)	7 (41%)	28 (51.9%)
None	1 (5.9%)	5 (9.3%)

The individual criteria proportions listed in **Table 7** were used to calculate their respective sensitivities and positive predict values (PPVs). These data comprised assessment Category 1, which is represented in **Table 8**. The criterion with the highest sensitivity, meaning the highest ability to detect a positive EP, was *MedCombo* (*M*) with a value of 64.7%. The criterion with the

highest PPV, or ability to predict a positive EP, was *StatEpi* (S) with a value of 60%. It should be noted that the CIs in Category 1 are substantially wider than those in the higher categories due to overall smaller sample sizes. When multiple criteria are considered, there is more opportunity to meet one. This leads to larger proportions, narrower CIs, and higher confidence in the result.

Table 8. Individual criteria sensitivity and positive predictive value (PPV); Category 1

Criteria	Sensitivity [95% CI]	PPV [95% CI]
<i>B</i>	58.8% [33%-82%]	21.3% [11%-36%]
<i>M</i>	64.7% [38%-56%]	26.8% [14%-43%]
<i>D</i>	58.8% [33%-82%]	31.3% [16%-50%]
<i>S</i>	17.6% [4%-43%]	60% [15%-95%]
<i>F</i>	41.2% [18%-67%]	20% [8%-37%]

B = Breakthrough seizures / *BreakThr*

M = Management includes multiple medication combinations / *MedCombo*

D = Behavior changes, skill regression, or developmental delay / *DevDelay*

S = History of status epilepticus or epilepsy partialis continua / *StatEpi*

F = History of 2 or more seizures in a first or second degree relative / *FamHx*

For the sake of clarity and brevity in the higher categories, the criteria were further abbreviated to single letters when assigned to tables, as in **Table 8**. Category 2 data was created from the results of two-criteria combinations (**Table 9**). As expected, the sensitivity of each two-criteria combination increased over those from single criteria alone. The positive predictive values were also more homogenous across Category 2. The criteria combination with the highest sensitivity in Category 2, *MedCombo/FamHx* (88%), included the criterion with the highest sensitivity in Category 1. The criteria combination with the highest PPV in Category 2, *DevDelay/StatEpi* (30.3%), included the criterion with the highest PPV in Category 1.

Table 9. Two-criteria combination sensitivity and PPV; Category 2

Criteria Combination	Sensitivity [95% CI]	PPV [95% CI]
<i>B/M</i>	70.5% [44%-90%]	22.6% [12%-36%]
<i>B/F</i>	82.4% [57%-96%]	23.3% [13%-36%]
<i>M/F</i>	88% [64%-99%]	24.6% [14%-37%]
<i>M/D</i>	76.5% [50%-93%]	27.1% [15%-42%]
<i>M/S</i>	64.7% [38%-86%]	26.2% [14%-42%]
<i>B/D</i>	76.5% [50%-93%]	24.1% [13%-38%]
<i>D/S</i>	58.8% [33%-82%]	30.3% [16%-49%]
<i>B/S</i>	64.7% [38%-86%]	23% [12%-37%]
<i>S/F</i>	64.7% [38%-86%]	26.8% [14%-43%]
<i>D/F</i>	82.4% [57%-96%]	25% [14%-38%]

B = Breakthrough seizures / *BreakThr*

M = Management includes multiple medication combinations / *MedCombo*

D = Behavior changes, skill regression, or developmental delay / *DevDelay*

S = History of status epilepticus or epilepsy partialis continua / *StatEpi*

F = History of 2 or more seizures in a first or second degree relative / *FamHx*

In Category 3, sensitivities continued to increase and became more homogenized than in Category 2 (**Table 10**). Overall PPVs decreased but remained similar to each other. The highest sensitivity criteria combinations were *BreakThr/DevDelay/FamHx* (94.1%) and *MedCombo/DevDelay/FamHx* (94.1%). The highest PPV criteria combination was *MedCombo/DevDelay/StatEpi* (27.1%). Again, these criteria combinations carried forward some of the highest-performing criteria from the previous categories. This trend continues into Category 4. Of note, the highest PPV in Category 3 was noted to have decreased slightly from Category 2.

Table 10. Three-criteria combination sensitivity and PPV; Category 3

Criteria Combination	Sensitivity [95% CI]	PPV [95% CI]
<i>B/M/D</i>	76.5% [50%-93%]	23.6% [13%-37%]
<i>B/D/F</i>	94.1% [71%-100%]	25% [15%-37%]
<i>B/M/F</i>	88% [64%-99%]	23.4% [14%-36%]
<i>B/D/S</i>	76.5% [50%-93%]	24.1% [13%-38%]
<i>B/S/F</i>	88% [64%-99%]	24.2% [14%-37%]
<i>B/M/S</i>	70.5% [44%-90%]	22.6% [12%-36%]
<i>M/D/F</i>	94.1% [71%-100%]	25.4% [15%-38%]
<i>M/D/S</i>	76.5% [50%-93%]	27.1% [15%-42%]
<i>M/S/F</i>	88% [64%-99%]	24.2% [14%-37%]
<i>D/S/F</i>	82.4% [57%-96%]	24.6% [14%-38%]

B = Breakthrough seizures / *BreakThr*

M = Management includes multiple medication combinations / *MedCombo*

D = Behavior changes, skill regression, or developmental delay / *DevDelay*

S = History of status epilepticus or epilepsy partialis continua / *StatEpi*

F = History of 2 or more seizures in a first or second degree relative / *FamHx*

The final level of criteria combinations, Category 4 (**Table 11**), resulted in the highest overall sensitivities, but capped out with the highest sensitivities matching those in Category 3 at 94.1%. The highest PPV belonged to *MedCombo/DevDelay/StatEpi/FamHx* (25.4%), which was lower than the highest PPV in Category 3. As the category levels increased, overall sensitivities increased and overall PPVs decreased and homogenized. CIs were narrower in the higher categories, which increased the reliability of the data.

Table 11. Four-criteria combination sensitivity and PPV; Category 4

Criteria Combination	Sensitivity [95% CI]	PPV [95% CI]
<i>B/M/D/S</i>	76.5% [50%-93%]	23.6% [13%-37%]
<i>B/M/D/F</i>	94.1% [71%-100%]	24.6% [15%-37%]
<i>S/F/B/M</i>	88% [64%-99%]	23.4% [14%-36%]
<i>M/D/S/F</i>	94.1% [71%-100%]	25.4% [15%-38%]
<i>D/S/F/B</i>	94.1% [71%-100%]	25% [15%-37%]

B = Breakthrough seizures / *BreakThr*

M = Management includes multiple medication combinations / *MedCombo*

D = Behavior changes, skill regression, or developmental delay / *DevDelay*

S = History of status epilepticus or epilepsy partialis continua / *StatEpi*

F = History of 2 or more seizures in a first or second degree relative / *FamHx*

Overall, sensitivities increased with each category and PPVs decreased and remained similar to each other in the higher categories. High sensitivities throughout indicated an acceptable ability to detect positive EPs in the study population. The majority of the PPVs were below average, with the highest PPV able to predict a positive result 60% of the time. However, the utility of these PPVs resides in their ability to predict the proportion of unidentified positive results in a hypothetical patient population that did not have genetic testing. For this study, the hypothetical population consisted of patients who did not have epilepsy-specific genetic testing attempted or completed (n=861). 507 (58.8%) patients from this untested population met at least one testing criterion and were included in the prediction calculations. EP testing may have identified an etiology for the epilepsy of these patients or may have provided a diagnostic result.

The criteria or criteria combinations with the highest PPVs are shown with their respective populations in **Figure 2**. The predicted number of unidentified positive results depended on both the PPV of the criteria or criteria combination and the initial number of patients meeting said criteria or criteria combination. In Category 1, the *StatEpi* criterion predicted 16 unidentified positive results. In Category 2, the *DevDelay/StatEpi* criteria combination predicted 60 unidentified positive results. In Category 3, the *MedCombo/DevDelay/StatEpi* criteria combination predicted 73 unidentified positive results. Finally, in Category 4, the *MedCombo/DevDelay/StatEpi/FamHx* criteria combination predicted 121 unidentified positive results. Based on these findings, it is estimated that 121 out of the 477 patients who did not receive EP testing but who met one or more of *MedCombo*, *DevDelay*, *StatEpi*, or *FamHx* criteria, would have had a positive EP result if they had been tested. The CIs for each criterion or criteria combination are displayed as error bars in **Figure 2**. With 95% confidence, the true proportions of the predicted unidentified results lie between the upper and lower boundaries of each CI. Even

though the CIs appear to be growing larger with each successive category in **Figure 2**, they are actually narrowing. The CI in Category 4, for instance, is the narrowest, but appears the widest because Category 4 accounts for the highest number of possible criteria combinations and, subsequently, the highest sample size (n=477).

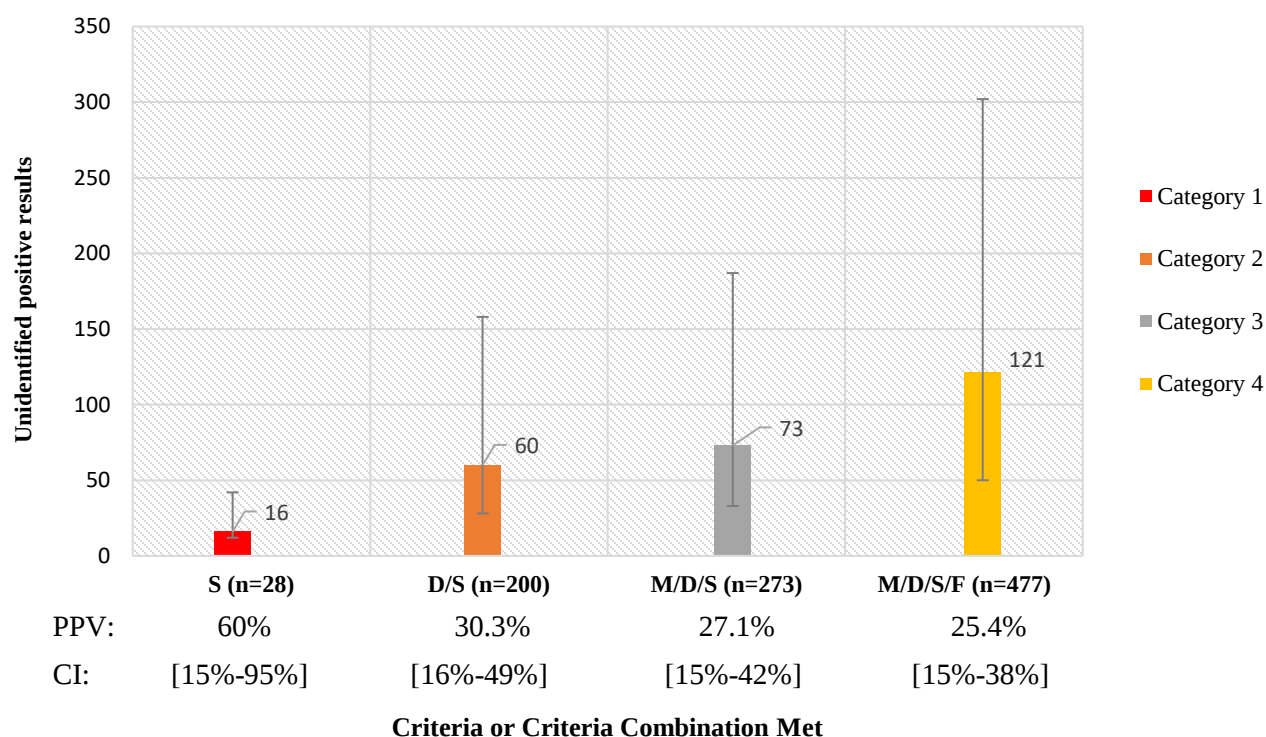


Figure 2. Bar graph representing the unidentified positive result predictions of the criteria or criteria combinations with the highest PPVs in each category

The criteria are abbreviated as follows: M = Management includes multiple medication combinations / *MedCombo*, D = Behavior changes, skill regression, or developmental delay / *DevDelay*, S = History of status epilepticus or epilepsy partialis continua / *StatEpi*, F = History of 2 or more seizures in a first or second degree relative / *FamHx*.

In order for the predictions in **Figure 2** to be accurate, the demographics and the distributions of met criteria for the positive/negative EP cohort and the untested patient cohort should be similar. This is because results cannot effectively be applied or generalized to a dissimilar patient population. The data in **Table 12** show that both patient cohorts did indeed have

similar demographics and proportions of met individual criteria. The most noticeable difference was in age, as the untested cohort tended to be older than the EP cohort. Also, a greater proportion of the untested cohort met the *FamHx* criterion (34.1%) when compared to the EP cohort (21.9%).

Table 12. Cohort comparison of demographics and individual criteria proportions

Positive/Negative EP Cohort	Untested Patient Cohort
Demographics	
n = 71	n = 861
0-3: 25 (35.2%)	0-3: 124 (14.4%)
4-8: 16 (22.5%)	4-8: 225 (26.1%)
9-12: 17 (24%)	9-12: 226 (26.2%)
13-17: 11 (15.5%)	13-17: 257 (29.9%)
18-21: 2 (2.8%)	18-21: 26 (3%)
22+: 0 (0%)	22+: 3 (0.4%)
Male: 44 (62%)	Male: 473 (55%)
Female: 27 (38%)	Female: 388 (45%)
White: 59 (83.1%)	White: 709 (82.3%)
Black: 7 (9.9%)	Black: 100 (11.6%)
Asian: 2 (2.8%)	Asian: 12 (1.4%)
American Indian: 0 (0%)	American Indian: 1 (0.12%)
Pacific Islander: 1 (1.4%)	Pacific Islander: 1 (0.12%)
Other: 2 (2.8%)	Other: 38 (4.4%)
Total times each criterion was met	
n = 160	n = 889
B: 47 (29%)	B: 208 (23.4%)
M: 41 (25.6%)	M: 163 (18.3%)
D: 32 (20%)	D: 187 (21%)
S: 5 (3.1%)	S: 28 (3.2%)
F: 35 (21.9%)	F: 303 (34.1%)

B = Breakthrough seizures / *BreakThr*

M = Management includes multiple medication combinations / *MedCombo*

D = Behavior changes, skill regression, or developmental delay / *DevDelay*

S = History of status epilepticus or epilepsy partialis continua / *StatEpi*

F = History of 2 or more seizures in a first or second degree relative / *FamHx*

Aside from determining the sensitivity and predictive capabilities of the EP testing criteria, this study further stratified positive EP results and investigated actionable gene findings (**Table 13**). In total, 15/17 (88.2%) positive EP results returned actionable findings. The phrase “actionable findings” refers to those genes discussed in detail earlier in this manuscript. These findings have the potential to heavily influence medication management for patients, especially in the cases of *POLG* and *SCN1A* pathogenic/likely pathogenic variants. Of note, 11 (73.3%) of these cases met Category 2, 3, or 4 criteria, suggesting that the higher criteria categories are more often associated with actionable findings than Category 1.

Table 13. Actionable gene findings for non-recessive conditions

Actionable Gene (Pathogenic/Likely Pathogenic)	Criteria Met (11/15, 73.3% Category 2, Category 3, or Category 4)
<i>CACNA1A</i>	M/D/S
<i>POLG</i>	
<i>GABRG2</i>	B/M/F
<i>KCNQ2</i>	None
<i>KCNQ2</i>	F
<i>PCDH19</i>	B/M
<i>POLG</i>	F
<i>POLG</i>	B/M/D/S
<i>POLG</i>	B/D/F
<i>PRRT2</i>	M/D/F
<i>PRRT2</i>	F
<i>SCN1A</i>	B/M/D/F
<i>SCN1A</i>	B/M/D
<i>SCN1A</i>	B/M/D/S
<i>SCN1A</i>	B/M/D/F
<i>SCN8A</i>	B/M/D

B = Breakthrough seizures / *BreakThr*

M = Management includes multiple medication combinations /
MedCombo

D = Behavior changes, skill regression, or developmental delay /
DevDelay

S = History of status epilepticus or epilepsy partialis continua /
StatEpi

F = History of 2 or more seizures in a first or second degree relative /
FamHx

3.4 Discussion

3.4.1 Criteria performance

The primary parameters that play a role in assessing the performance of testing criteria are the sensitivity and PPV statistics. Ideally, these should both be high with narrow CIs in informative criteria sets. Sensitivity is a retrospective measure of performance; it shows what proportion of positive results the criteria were able to detect. PPV is a prospective measure, in that it estimates the proportion of patients meeting criteria that will have a positive result in a hypothetical population. PPV is important for health insurance purposes, as a high PPV indicates that a test is likely to yield diagnostic results. The sensitivity and PPV results of this study will be discussed next.

For ease of discussion, the EP testing criteria will be referred to by their respective organizational categories (Categories 1, 2, 3, and 4). In Category 1, only one criterion was assessed at a time, which led to overall lower proportions and wider CIs than the higher categories. This is especially noticeable for the *StatEpi* criterion, which had the smallest proportions overall when assessed on its own. With only 3 positive EPs and 2 negative EPs meeting *StatEpi*, the observed PPV of 60% is unreliable, as indicated by its [15%-95%] CI. This is mirrored in the criterion's sensitivity, which indicates only 17.6% of positive EPs met this criterion.

Generally, status epilepticus and epilepsia partialis continua are rare. It was especially rare for a patient in the EP cohort to have either as a presenting feature without any other symptoms. Additionally, even though *StatEpi* was assessed individually due to the level of data capture in Category 1, it was never truly met on its own. This means that at least one other criterion was

always concurrently met with *StatEpi* in this patient cohort. This makes sense given what is known about status epilepticus.

Review of the literature suggested that status epilepticus is a more severe indicator of epilepsy phenotypes. For instance, status epilepticus is common in genetic syndromes such as Dravet syndrome and is strongly associated with refractory seizures (de Lange et al., 2018; Sillanpää, 1993). This study showed that while status epilepticus did occur in patients with breakthrough seizures, it performed better as a criterion and was more predictive when taken into account alongside developmental delay, rather than with breakthrough seizures (**Table 9**). This does not contradict the literature but suggests that patients with one or both of these two features are more likely to have positive EP results and a genetic explanation for their epilepsy. As the categories progressed beyond two-criteria combinations, the *StatEpi* criterion remained an important contributor to high PPVs overall (**Figure 2**).

The other criteria in Category 1 had larger proportions than *StatEpi*, but still lacked reliability and predictive power on their own. The highest sensitivity belonged to *MedCombo* (64.7%) with an 18% CI. This sensitivity is relatively low, especially when compared to the higher categories. Additionally, the PPVs for *BreakThr*, *MedCombo*, *DevDelay*, and *FamHx* were low to moderate with CIs ranging from 25-34% in width. The sensitivities in Category 1 tended to be the lowest of all the categories (17.6%-64.7%). The PPVs varied widely, ranging from the lowest PPV (20%) to the highest PPV (60%) of all the categories. However, the overall results of Category 1 suggested that the EP criteria performed better and more reliably when the opportunity to meet more than one was available.

In Category 2, overall sensitivities increased (58.8%-88%) while the PPVs decreased and homogenized (22.6%-30.3%). Additionally, CIs narrowed, providing more reliable results. This

was intrinsically due to proportions increasing when the individual criteria were considered together, which was a theme in Categories 3 and 4 as well. The highest sensitivity belonged to *MedCombo/FamHx* (88%) and the highest PPV belonged to *DevDelay/StatEpi* (30.3%). Of note, these combinations included the strong performers, *MedCombo* and *StatEpi*, from Category 1 respectively, which likely contributed to the trend of raising their values above those of the other combinations. The results of Category 2, aside from reinforcing the conclusion drawn from Category 1, indicated that this manuscript's proposed fifth criterion, *FamHx*, was associated with a high degree of sensitivity when considered alongside the *MedCombo* criterion.

The main take-a-ways from Category 3 were increased sensitivities (70.5%-94.1%), a lower maximum PPV belonging to *MedCombo/DevDelay/StatEpi* (27.1%), and a continued high-value trend between the four criteria *MedCombo*, *DevDelay*, *StatEpi*, and *FamHx*. At this point, the maximum sensitivity of 94.1%, attributed to both the *BreakThr/DevDelay/FamHx* and *MedCombo/DevDelay/FamHx* combinations, were the highest the criteria sensitivity would get. The maximum PPV dropped slightly in each category but also became more reliable, with maximum PPV CIs dropping from 80% in Category 1 to 33% in Category 2 and 27% in Category 3.

As mentioned previously, the maximum sensitivity for Category 3 and Category 4 was the same at 94.1%, which belonged to the *BreakThr/MedCombo/DevDelay/FamHx*, *MedCombo/DevDelay/StatEpi/FamHx*, and *DevDelay/StatEpi/FamHx/BreakThr* combinations. The minimum sensitivity in Category 4 did increase to 76.5%, however, indicating that the four criteria combinations had the highest overall sensitivities. This makes sense given the level of data capture intrinsic to Category 4. The PPVs in Category 4 continued to decrease and become more homogenized, with values ranging from 23.4% to 25.4%. The highest PPV (25.4%) in Category 4

belonged to the *MedCombo/DevDelay/StatEpi/FamHx* combination. The CI for the highest PPV in Category 4 was narrower than the highest PPVs in the prior categories (23%). The results of Category 4 indicated that the highest overall sensitivities and the most reliable PPVs were obtained when every possible EP criteria combination was considered. Looking at the categories as a whole, the most important contributors to sensitivity were *MedCombo* and *FamHx* and the most important contributors to PPV were *DevDelay* and *StatEpi*.

The results of the unidentified positive EP predictions in **Figure 2** were a measure of the potential utility of the EP testing criteria when implemented as a health insurance policy. If all the patients from the untested population of this study that met EP criteria had received testing, up to 121 additional positive results would have been obtained, depending on the criterion or criteria combination met. That is potentially 121 diagnostic odysseys ended or epilepsy medications altered due to hypothetical insurance coverage based on the EP criteria. Furthermore, this estimate is accurate, due to the similarities in demographics and distributions of met criteria between the EP cohort and the untested cohort (**Table 12**). It stands to reason, then, that a policy based on the EP criteria in this manuscript has the potential to benefit an increased number of patients that are affected with epilepsy and that mirror this study's population demographics.

All five criteria contributed to either the highest sensitivity or the highest PPV per category over the course of the assessment. There was no single poor performer. Additionally, sensitivities were highest and PPVs most reliable in Category 4, where at least one of any four of the five criteria were met. This data suggests that all five criteria should be used together, allowing for the maximum number of criteria combinations possible (Category 4). It is also important to note that the convergence of the criteria combinations in Category 4 to a relatively low PPV (25.4%) does not necessarily indicate that the EP criteria poorly predict positive results. PPV, in the setting of

this study, is directly related to the prevalence of epilepsy-associated pathogenic/likely pathogenic variants in the study population (Parikh et al., 2008). Therefore, the only way for the EP testing criteria PPVs to increase is for the prevalence of epilepsy-related pathogenic/likely pathogenic variants in the epilepsy testing population to increase. In other words, low PPVs are indicative of a low prevalence of Mendelian genetic causes for epilepsy in a population.

As discussed in the literature review, idiopathic generalized epilepsy (IGE) is characterized by complex inheritance in most cases. 40% of IGE can be attributed to genetic factors, with 1% of these factors being Mendelian (Zhang et al., 2017). This study determined that out of 1,242 patients with a mixture of IGE, juvenile myoclonic epilepsy, childhood absence epilepsy, and other unspecified seizures, 59 (4.8%) were found to have single gene or CNV-related causes for their symptoms (**Table 6**). This is higher than the rate described by Zhang et al. and is possibly due to a higher prevalence of pathogenic/likely pathogenic variants in the study population and/or improved testing methods. It should be noted that many patients in this cohort did not have genetic testing completed or had genetic testing done outside of the study timeframe. Had more patients been tested/included in this study, it is likely that more positive results would have been observed. If the pathogenic/likely pathogenic variant prevalence of 4.8% were to increase, PPV would increase as well. This would likely be the case in a larger patient cohort with a higher rate of genetic testing.

3.4.2 Actionable gene findings

Out of the 17 positive EP results, 15 (88.2%) reported pathogenic/likely pathogenic variants in actionable epilepsy genes (**Table 13**). Most of these cases met criteria in Categories 2, 3 and 4 (11/15, 73.3%). Variants in the sodium channel genes *SCN1A* and *SCN8A* met criteria in

Categories 3 and 4 only. One actionable finding in *KCNQ2* did not meet any criteria at all. It is possible that a more severe phenotype correlates with meeting more EP criteria but assessing specific patient phenotypes and outcomes is beyond the scope of this study. However, the identification of actionable gene variants likely provided new medication and treatment options for these patients as described in the literature. Specifically, with sodium channel blockers and Depakote being contraindicated for patients with *SCN1A* and *POLG* mutations respectively, it is likely that these medications were removed as a treatment possibility in these cases (Snoeijs-Schouwenaars et al., 2015; Stewart et al., 2010).

3.4.3 Overall positive genetic testing

Overall, the positive genetic testing results in this study demonstrated a broad range of patient phenotypes and reasons for a neurology evaluation. Not every patient in the cohort had epilepsy as a primary symptom, meaning they had other multisystemic features or unrelated health concerns with secondary epilepsy. Also, most of the positive genetic testing results met at least one EP criteria (**Table 6**). This demonstrates a tendency for the EP testing criteria to capture epilepsy secondary to other syndromes or disorders in addition to epilepsy as a primary genetic diagnosis. This emphasizes the importance of patient evaluation as a whole when deciding to order a genetic test and supports the use of larger, more comprehensive EP options when one is considered. EP testing criteria offer important guidance but are not perfect or exclusive when considering patients with multi-systemic features and medical needs. In these cases, genetic testing should be driven by overall patient phenotype and broad testing such as WES should be considered. This point is outlined in the existing EP criteria used at CHP.

3.4.4 Comparison to prior studies

This manuscript reviewed two prior studies in the literature relevant to criteria assessment. The first study, by LaDuca et al., used only sensitivity as its assessment parameter and the second study, by Cropper et al., used only PPV (2020, 2017). This study used both sensitivity and PPV to assess criteria performance and the results bear similarities and differences to those prior to it. Sensitivities in this study were high, as described previously, indicating that the EP criteria performed well in regards to detecting positive EP results.

Similarly, LaDuca et al. found that *BRCA1/2* criteria were 94.2% sensitive and Lynch syndrome criteria were 73.1% sensitive for their respective pathogenic/likely pathogenic variants (2020). However, neither the *BRCA1/2* criteria nor the Lynch syndrome criteria were exclusive to *BRCA1/2* or mismatch repair variants, meaning they often detected pathogenic/likely pathogenic variants in other cancer-related genes. This finding is somewhat similar to the overall genetic testing results for this study that were described in the previous section. The EP criteria were not exclusive to genes associated with primary epilepsy syndromes and detected positive results for conditions that included epilepsy as a secondary feature. LaDuca et al. recommended revising existing *BRCA1/2* and Lynch syndrome criteria to include a greater variety of cancer predisposition genes, and this study recommended not relying solely on EP criteria to make genetic testing decisions due to the importance of overall patient phenotype (2020). Both of these recommendations serve as a reminder that testing criteria do not act in isolation and are instead pieces of a larger diagnostic process that includes evaluation on the part of a provider prior to genetic testing.

The PPV results of this study, as previously described, converged to a final value of 25.4% when all possible combinations for the five EP criteria were considered. This study did not identify

an EP criterion that had a PPV of <10% when its individual proportions were assessed in Category 1. Cropper et al. conducted a somewhat similar assessment of 14 individual NCCN criteria for *BRCA1/2* genetic testing and found two individual criteria that predicted *BRCA1/2* pathogenic/likely pathogenic variants at a rate of <10%, which was selected as the poor performance cut-off (2017). Additionally, Cropper et al. determined that when patients met only one NCCN criterion, the PPV was 3.2%, which falls within the 1% to 6% range of *BRCA1/2* pathogenic/likely pathogenic variant rates in patients diagnosed with breast cancer in the “unselected,” or general, population (2017). The conclusion drawn here, was that two or more NCCN criteria needed to be met for them to be predictive, as the PPV in this setting increased to 12% (2017).

In comparison, this study did not assess criteria or criteria combinations in isolation, as seen in **Figure 1**. Each criterion was assessed according to how many times it was met and according to the contribution of its values when more criteria were added to the set for consideration. This was different from Cropper et al.’s methods, through which a criterion that was never met on its own always had a PPV of 0% (2017). This value is not an indication of poor predictive performance, but an indication that the criterion may perform better when partnered with other criteria in the set. It can also indicate that the criterion is rare and more closely associated with severe disease phenotype in the context of epilepsy. In summary, sequentially assessing the entire set of criteria as an amalgam of its components is an approach that better teases out the true predictive capabilities of each individual criterion, rather than assessing them on their own.

3.4.5 Study limitations

Even though this study effectively assessed the performance of established and proposed EP testing criteria, its retrospective methodology proved to be a limitation. The EMR documentation upon which this study relied was not standardized and may not have been an accurate representation of each patient's medical history. At times, genetic test reports were not scanned, or scanned with poor quality, and results had to be translated from progress notes. Often, progress note language was not standardized, and information such as family history or developmental delay was sometimes omitted for older patients. On other occasions, multiple progress notes from different departments had to be reviewed in order to collate the necessary information for one patient. All these variations in EMR documentation are examples of how the chart review process could have reduced study result accuracy.

VUS results, except for those that were re-categorized as negative, were not included in the EP criteria performance analysis due to their ambiguity. It is possible that some of these VUS results were reclassified over the course of this study, or will be reclassified in the future, due to new information or parental testing. Data collection for this study occurred at one point in time during each patient's medical history and likely did not capture any dynamic changes made to the interpretation of VUS results. Reclassification of every VUS result documented in this study would likely change the meaning of the study data significantly. Additionally, it should be noted that the large number of VUS results (n=38) is an indication that current genetic knowledge has not caught up with the ability to test for and find variants.

Other limitations of this study were the timing and multiplicity of genetic testing. Some of the patients in this study had testing outside of the assigned testing window and so were not represented in the results. This led to an underestimation of positive results and ultimately lower

sensitivities and PPVs. Also, EPs were ordered at different times from a variety of labs with variable gene content. Testing modalities change frequently throughout the years, and it is possible that technology and new gene discovery could have had an impact on the results. Additionally, multiple tests were often ordered for patients. The hierarchical documentation method of this study did not effectively account for this or determine the order in which multiple tests were performed in all cases. This blurred the lines of patient diagnosis and criteria utility. In some cases, it is likely that providers did not order an EP due to overall patient phenotype or previously positive genetic testing, rather than the patient's epilepsy history alone. Furthermore, beyond the few cases that are mentioned, this study offers little insight into patient phenotypes or outcomes which are important factors in determining test utility. A future longitudinal study could determine the specific outcomes of patients who met EP criteria and did or did not have genetic testing.

Furthermore, this study only collected data from outpatient neurology evaluations. Inpatient neurology evaluations during the study timeframe were excluded due to lack of data access and ambiguity regarding admission and discharge ICD-10 diagnosis codes. It is possible that this exclusion caused an underrepresentation of EPs and overall lower criteria proportions, sensitivities, and PPVs. For instance, one study found that 38.8% of acute-care ambulatory patients admitted for neurodevelopmental disorders with seizures received a diagnosis from WES (Soden et al., 2014). Though these results were in a different patient population and at a different institution, they demonstrated a diagnostic inpatient yield for WES that was higher than that found in this outpatient study (32.6%). Therefore, future studies may wish to include both outpatient and inpatient evaluations in order to represent the total patient population within the study timeframe.

As a final point, the results of this study are representative of a specific patient population at CHP. Other institutions may have significantly different patient demographics or prevalence

rates of epilepsy. Therefore, the data collected, and the inferences made by this study cannot be directly applied to patient populations at institutions outside of the UPMC hospital system. Collaboration between institutions in the future could help to rectify these demographic differences and lead to a more generalizable assessment of the EP criteria performance. Until this is done, the EP criteria should not be incorporated into a health policy, as doing so based on this study alone could possibly exacerbate health disparities in the community, given the lack of minority representation in this study's data.

3.4.6 Suggested criteria modification and future directions

The current EP criteria utilized at CHP are *BreakThr*, *MedCombo*, *DevDelay*, and *StatEpi*. Based on the results of this study, one modification is suggested for these criteria. The suggestion is the addition of the proposed criterion *FamHx*. In Category 2 and onward, *FamHx* was one of four well-performing criteria and was often associated with the highest sensitivities in each category. *FamHx* was also met by 8/15 (53.3%) of the actionable finding cases, which suggests the important role family history plays in patient management. Furthermore, as access to genetic counselors increases, so too will professionally documented three-generation pedigrees in the EMR, making a detailed family history a more standard part of all epilepsy evaluations.

Given the integral role *FamHx* played in increasing criteria sensitivity, omitting it would likely lower the ability of the EP criteria to detect positive results. *FamHx* was less involved than some of the other criteria when it came to the highest PPV in each category, but it contributed in Category 4, which was the most reliable. Finally, *FamHx* was the only criterion met by 3 (5%) of the 15 actionable findings cases, meaning the omission of this criterion in a future policy would have failed to capture these actionable results. For these reasons, “History of 2 or more seizures in

a first or second degree relative” is proposed as the fifth criterion to be added to the existing EP criteria at CHP.

Future studies have the potential of having larger sample sizes and higher rates of genetic testing, which, as discussed previously, could lead to a higher prevalence of pathogenic/likely pathogenic variants and higher criteria PPVs. This would also lead to narrower confidence intervals and increased data reliability. There is also the potential for collaboration with health insurance companies to develop a policy based on the modified criteria in this study. Another opportunity for future studies involves looking at populations within different institutions to account for ethnic and socioeconomic bias. The methodology behind this study is also applicable to testing criteria for many other genetic conditions, and future studies should continue to assess and develop new testing criteria to improve diagnostic yield in different settings. Given the widespread lack of guidance for genetic test ordering and insurance coverage, the test criteria produced by future studies stand to greatly benefit patients and the healthcare system at large.

3.4.7 Policy recommendations

The results of this study suggest that restrictive “and” logic should not be included in a health insurance policy for EP testing. In other words, health insurance policies should not require that a patient meets criteria X and Y before providing coverage for testing. Instead, using “or” logic, such that meeting criterion X, criterion Y, or both allows coverage, is a more appropriate and inclusive method. This conclusion is further reinforced by the actionable results of this study. Given that 3/15 (20%) of the actionable findings (**Table 13**) met only one criterion, it would not be recommended to require patients to meet two or more EP criteria in an insurance policy. Doing so would have led to unidentified actionable results in these cases.

During the criteria assessment process prior to policy development, the use of “and” logic prevents the visualization of trends and the assessment of individual criteria performance as more criteria are added to the policy. In **Figure 1**, “or” logic is used, as represented by the connecting lines between categories. In this way, the values of the individual criterion contribute to the combination values as more criteria are added, revealing the trends and relationships described in this manuscript. Use of “and” logic removes the contribution of the individual criteria and makes each new combination its own entity with its own values. In essence, the lines in **Figure 1** would disappear and each combination would stand alone in this case. The reason for this, is that not every patient that met criterion X, for instance, would also have met criterion Y. The result of “and” logic, then, is not only a reduction in overall proportions, sensitivities, and PPVs, but the loss of the relationship and trend information gleaned as the criteria levels progress, which was essential to the assessment of the criteria performance in this study.

Even though one patient out of 15 (6.7%) with an actionable finding did not meet any criteria, it should be noted that this patient had a *KCNQ2* pathogenic variant, which can present with a mild phenotype, and the patient was only 1 year of age. Patients evaluated for epilepsy in their infancy have the potential to meet more criteria as they age, meaning they could still benefit from an EP despite not meeting criteria at the time of their evaluation. Therefore, when developing an insurance coverage policy for EPs, age should be considered.

This conclusion is supported by the demographic data from **Table 4**. The majority of positive EP results were found in patients that were 3 years old or younger (9/17, 53%). Given this data, the point raised in the previous paragraph regarding age, and the often severe presentation of early-onset seizures, this study suggests that future EP policies include a clause stating that

coverage will be automatic for individuals diagnosed with epilepsy under the age of 3 years, regardless of meeting EP criteria.

3.5 Conclusion

This study presents data supporting the reliability and predictive capabilities of the EP testing criteria utilized by the GTSP at CHP with the suggested addition of a family history criterion. All five criteria should be utilized together to allow for the most criteria combination possibilities. There is currently little in the way of standardized guidance for providers deciding to order EPs for patients and for insurance companies determining coverage. This study offers a vetted solution for both parties to be adopted and utilized as each see fit. Furthermore, this study may serve as a model for other institutions seeking to assess their own sets of testing criteria across the various genetic specialties. This will open the door for future conversations about adopting evidence-based policies, which have the potential to improve patient access to genetic testing.

4.0 Research Significance to Genetic Counseling and Public Health

Since 2006, the National Society of Genetic Counselors (NSGC) has defined the service of genetic counseling as: "...the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease. This process integrates the following: Interpretation of family and medical histories to assess the chance of disease occurrence or recurrence. Education about inheritance, testing, management, prevention, resources and research. Counseling to promote informed choices and adaptation to the risk or condition." (Resta et al., 2006).

By this definition, genetic counselors assume a unique role in patient care that marries the communication of complex information and risk assessments with emotional and psychological support and guidance. This role translates across many genetic specialties, such as prenatal, hereditary cancer, pediatrics, adult genetics, and neurology. For each of these specialties, genetic counselors help patients to make informed decisions about genetic testing and facilitate the ordering process for them. By the very nature of this process, genetic counselors increasingly find themselves involved in insurance discussions with patients, which has since prompted the coinage of the term "genesurance." Genesurance is defined by one study as: "that portion of a genetic counseling session, whether intentional or non-intentional, that is devoted to the topic of costs and insurance/third party coverage (particularly for genetic testing)" (Brown et al., 2018)

Brown et al. conducted a survey on clinical genetic counselors to investigate the extent to which genesurance is involved in their practice. They discovered that 99% of the genetic counselors reported performing some form of genesurance counseling with their patients and that 10% of their time in session was spent doing it (Brown et al., 2018). Additionally, 95% reported

patient decisions changing based on the discussion and 74% reported clinical practice changing due to genesurance concerns. These results demonstrate how involved genetic counselors are in discussing insurance with patients as it relates to their genetic testing.

By knowing a patient's insurance company has adopted a set of genetic testing criteria into their coverage policies, a genetic counselor can transparently say to the patient whether their insurance will cover their testing based on whether or not they meet criteria. This is already standard of care in the hereditary breast and ovarian cancer sphere, with most insurance companies following the National Comprehensive Cancer Network (NCCN) clinical guidelines in order to make decisions about covering genetic testing of the *BRCA1/2* genes (Cropper et al., 2017).

When insurance companies align with clinical guidelines, it makes the decision to cover testing easier and eliminates the need for clinicians to appeal for coverage on the patient's behalf. Most insurance companies require a pre-authorization process for genetic testing orders, meaning they review whether they qualify for coverage based on the patient's health insurance plan. This process typically involves back-and-forth communication between the insurance company and the ordering clinicians. Often the insurance company requires information about how testing will affect medical management for the patient. The whole process can take several weeks to months and sometimes ends in the denial of coverage for the genetic test. With clinically validated, criteria-based policies in place, insurance companies need only follow guidelines to determine if testing is covered when providers request it, which would lessen the burden of the appeals process.

This streamlined process of coverage decision-making saves time, effort, and resources for both insurance companies and clinical providers. It also removes a source of stress for families wondering about their finances in relation to genetic testing and allows them to ultimately have testing done sooner, when clinically appropriate. Genetic counselors will be able to spend less time

providing genesurance counseling for families because of the guidance provided by criteria-based pay policies. Genetic counselors will also potentially be able to offer reassurance based on patients not meeting any criteria, on the grounds that phenotype is often less severe in these cases. This will allow more time to be spent in other areas of need, be they informational or psychosocial, and ultimately improve patient outcomes.

Before insurance companies can adopt testing criteria into their policies, however, they must first be proven to be effective. This study provides a model for conducting that process in the context of panel testing for epilepsy. Results will likely vary per study and patient population, but the concept of measuring sensitivity and PPV is the same. Determining these values to the most reliable degree is the basis of evaluating criteria performance. It is this evidence, then, that forms the foundation of evidence-based policy development.

Developing and assessing genetic testing criteria plays a critical role in the public health sphere. There are ten essential service components of public health that are recognized by the Center for Disease Control and Prevention (CDC) (CDC, 2018). These ten components fall under three activity categories: Assessment, Policy Development, and Assurance. This study aligns with the Assessment and Policy Development categories and specifically addresses the components of “investigation” and “education.” It was important for this study to investigate the performance of the EP testing criteria in order to determine if they were appropriately utilized in patient care and if they were providing a benefit in the form of increased diagnostic yield. Discussing the results of this investigation is the purpose of this manuscript and it serves to educate health insurance companies and providers alike about the role clinical testing criteria play in the processes of ordering and paying for genetic testing.

As genetic testing becomes more common, the need for standardized guidance in the areas of ordering and covering genetic testing increases. Lack of standardization can lead to access inequities, meaning some patients may be less likely than others to receive the testing they need. Some providers may not have access to a utilization management (UM) team or understand the process of ordering an EP for a patient, leading to errors. Also, some patients may be less likely to be covered under a certain health insurance plan. One study found that children with epilepsy and Medicaid insurance were faced with more barriers to genetic testing than those patients with commercial coverage (Kutscher, Joshi, Patel, Hafeez, & Grinspan, 2017). However, if newly developed guidelines, in the form of vetted testing criteria, become widely disseminated and incorporated into policy by most major health insurers, both private and public, coverage decisions could simplify and broaden, potentially leading to greater patient access to genetic testing (Kutscher et al., 2017). Additionally, streamlined, criteria-based policies have the potential of informing providers and simplifying the ordering process, which could reduce the likelihood of ordering an inappropriate test for a patient. In summary, evidence-based policies based on vetted genetic testing criteria have the potential to improve patient access to genetic testing.

Appendix: Institutional Review Board Approval Letter

University of Pittsburgh
Institutional Review Board

Human Research Protection Office
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APPROVAL OF SUBMISSION (Exempt)

Date:	July 24, 2019
IRB:	STUDY19050363
PI:	Christine Munro
Title:	Epilepsy Panel Testing Criteria: A Clinical Assessment
Funding:	None

The Institutional Review Board reviewed and approved the above referenced study. The study may begin as outlined in the University of Pittsburgh approved application and documents.

Approval Documentation

Review type:	Initial Study
Approval Date:	7/24/2019
Exempt Category:	(4) Secondary research on data or specimens (no consent required)

Determinations:	• Waiver of HIPAA authorization • Children
Approved Documents:	• EpiPanel - Power and Sample Size Considerations, Category: Other; • HRP-723 - WORKSHEET - Exemption_Secondary Data.Specimens_Version_0.01.docx, Category: IRB Protocol

As the Principal Investigator, you are responsible for the conduct of the research and to ensure accurate documentation, protocol compliance, reporting of possibly study-related adverse events and unanticipated problems involving risk to participants or others. The HRPO Reportable Events policy, Chapter 17, is available at <http://www.hrpo.pitt.edu/>.

Clinical research being conducted in an UPMC facility cannot begin until fiscal approval is received from the UPMC Office of Sponsored Programs and Research Support (OSPARS).

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, [Dana DiVirgilio](#).

Please take a moment to complete our [Satisfaction Survey](#) as we appreciate your feedback.

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