The impact of next-generation sequencing on diagnosis and management of children with epilepsy: from molecular testing to cost-effectiveness analysis

By

Tran Thi Thu Ha

A thesis submitted to the School of Graduate Studies

in partial fulfillment of the requirements for

the degree of Master of Science in Pharmacy

Faculty of Pharmacy

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

July 2020

Abstract

Genetic testing is essential for diagnosis and treatment selection for children with epilepsy. Conventional approaches to genetic testing such as chromosomal microarray (CMA), and single-gene sequencing are time-consuming and expensive. Meanwhile, next-generation sequencing (NGS) allows simultaneous examination of all or most genes, which permits comprehensive and timely diagnosis of genetic etiology. Although NGS methods such as epilepsy panel (EP), whole-exome sequencing (WES), and whole-genome sequencing (WGS) are increasingly used, there is limited evidence about their diagnostic yield, clinical utility, and the cost-effectiveness of NGS-incorporated diagnostic strategies. The goal of this thesis, therefore, is two-fold: first, to conduct a systematic review and a meta-analysis of diagnostic yield and clinical utility of EP, WES, and WGS in comparison with CMA in pediatric epilepsy and second, to evaluate the cost-effectiveness of different NGS-incorporated diagnostic strategies from the health care system's perspective.

A systematic review of PUBMED and EMBASE database identified 56 studies investigating diagnostic yields of EP, WES, WGS, and CMA (Chapter 2). Our random-effects meta-analysis of these 56 studies revealed that diagnostic yield was highest for WGS (0.66; 95% CI 0.00-1.00, two studies, 211 children, I^2 =99%), followed by WES (0.37; 95% CI 0.30-0.44, eighteen studies, 1322 children, I^2 =86%), and EP (0.25; 95% CI 0.22-0.28, thirty five studies, 14,265 children, I^2 =86%). CMA provided the lowest diagnostic yield (0.10; 95% CI 0.07-0.13, seventeen studies, 2,306 children, I^2 =85%). Clinical utility regarding to clinical management of WES (0.15, 95% CI 0.09-0.22, p=0.15, two studies of 289 children) was higher than that of EP (0.10, 95% CI 0.07-0.13, p<0.01, eleven studies of 11,044 children). Given their high diagnostic yield and clinical utility, NGS should be adopted in routine genetic investigation of pediatric epilepsy.

A decision-analytic model was developed to evaluate the cost-effectiveness of EP and WES in diagnosis and clinical management of epilepsy (Chapter 3). All EP and WES-related strategies were more effective than conventional diagnostic strategy. Among all diagnostic strategies, "WES as second-tier test" was the most cost-effective (ICER of 26,070 CAD per QALY). I also found that although the "WES and CMA as first-tier tests" strategy generated the highest QALYs, it was not cost-effective relative to "WES as second-tier test" (ICER > 100,000 CAD per QALY). Given the high cost of WES, "WES and CMA as first-tier tests" could become a cost-effective

strategy when cost of WES decreases or the proportion of patients with etiology identified by WES and CMA increases.

Acknowledgments

I would like to acknowledge and give special thanks to my supervisor, Dr. Hai Nguyen, for his guidance, academic advice, and daily support throughout my Master's program. I would like to thank the Translational Personalized Medicine Initiative of Memorial University and School of Graduate Studies for their financial support and training opportunities. I would also like to thank my family and friends for their encouragement during my graduate studies.

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List of Abbreviations

AEDs - anti-epilepsy drugs

ALDH7A1 - aldehyde dehydrogenase 7 family, member A1

BRAT1 - BRCA1 associated ATM activator 1

CAU - care as usual

CBZ – carbamazepine

CDKL5 - cyclin-dependent kinase-like 5

CI - confidence interval

CLB – clobazam

CMA - chromosomal microarray

CNVs - copy-number variants

DD - developmental delay

DEE - developmental and epileptic encephalopathy

DEPDC5 - DEP domain containing 5

Dx - diagnostic utility

EE - epileptic encephalopathy

EEG - electroencephalogram

EOEE - early-onset epileptic encephalopathy

ESM – ethosuximide

FOLR1 - folate receptor alpha

FOXG1 -forkhead box G1

GBP – gabapentin

GGC - Greenwood Genetic Center

GRIN2A/2B/2D - glutamate ionotropic receptor NMDA type subunit 2A/2B/2D

GTAC - Genetic Testing Advisory Committee

ICER – Incremental Cost-Effectiveness Ratio

ID - intellectual disability

ILAE - International League Against Epilepsy

KCNQ2 - potassium channel, voltage-gated, KQT-like subfamily member 2

KCNT1 - potassium channel subfamily T member 1

KKI - Kennedy Krieger Institute

LEV – levetiracetam

LTG – lamotrigine

MEF2C - myocyte enhancer factor 2C

MeSH - Medical Subject Heading

MNG - Medical Neurogenetics Laboratories

MOH - Ministry of Health

MRI - magnetic resonance imaging

mTOR - mammalian target of rapamycin

N.A. - not applicable

NAC - not adequately controlled

NGS - Next-generation sequencing

NSF - not seizure-free

OHIP - Ontario Ministry of Health and Long-term Care Schedule of Benefits for Physician Services

OXC – oxcarbazepine

PB – phenobarbital

PCDH19 - Protocadherin 19

PHT – phenytoin

PNPO - pyridoxamine 5’-phosphate oxidase

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analyses

QALYs - Quality-Adjusted Life Years

SCN1A - sodium channel, voltage-gated type I, alpha subunit

SCN2A - sodium channel, voltage-gated type II, alpha subunit

SCN8A - sodium channel, voltage-gated, type VIII alpha subunit

SF - seizure-free

SLC2A1 - solute carrier family 2 member 1

STRADA SYNGAP1 - synaptic RAS-GTPase-activating protein 1

STXBP1 -syntaxin-binding protein 1

TPM – topiramate

TSC1 - tuberous sclerosis 1

TSC2 - tuberous sclerosis 2

VPA – valproate

WES - Whole-exome sequencing

WGS - Whole-genome sequencing

WTP – Willingness To Pay

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Chapter 1: Introduction

1.1 Background and Rationale

1.1.1 Definition and Epidemiology of Epilepsy

Epilepsy is a chronic neurologic disorder characterized by "an increased predisposition to develop seizures and by the neurologic, cognitive, psychological and social manifestations". Epileptic seizures are characterized by "a transient occurrence of symptoms due to abnormal excessive or synchronous epileptic neuronal activity in the brain" [1]. To facilitate the application of clinical definition of epilepsy as a diagnostic term, the International League Against Epilepsy (ILAE) Task Force broadened the definition in 2014 [2] (Table 1.1).

Table 1. 1 International League Against Epilepsy (ILAE) operational definition of epilepsy (2014)

Operational definition of epilepsy [2]
1. ≥2 unprovoked or reflex seizures occurring > 24 hours apart. 2. An unprovoked or reflex seizure with a probability of other seizures close to the recurrence probability after 2 reflex seizures (≥60%) in the next 10 years. 3. Epileptic syndrome diagnosed. Resolved epilepsy is a definition for patients with an age-dependent epileptic syndrome who passed the applicable age or those who were seizure-free for ≥10 years, with no anti-epileptic drugs for ≥5 years [2].

Epilepsy is one of the most common neurologic disorders that affect infants and children in the world. According to the World Health Organization report in 2019, around 50 million people worldwide suffered from epilepsy [3]. A recent systematic review and meta-analysis reported the annual incidence and prevalence of epilepsy worldwide at 61.4 per 100,000 and 760 per 100,000, respectively [4]. It was estimated that 10.5 million children worldwide had epilepsy [5]. The active period prevalence of epilepsy for children less than 18 years of age was estimated at 4.8/1,000 worldwide. The lifetime prevalence of children with epilepsy was 7.2/1,000 [4]. The

incidence of epilepsy in children was estimated at 0.82/1000, which was more than two times higher than in adults [6].

1.1.2. Epilepsy classification

The framework for epilepsy classification is presented in Figure 1.1. The classification of epilepsy includes (1) seizure classification, (2) epilepsy classification, (3) syndrome identification, and (4) etiology investigations [7] (Figure 1.1)

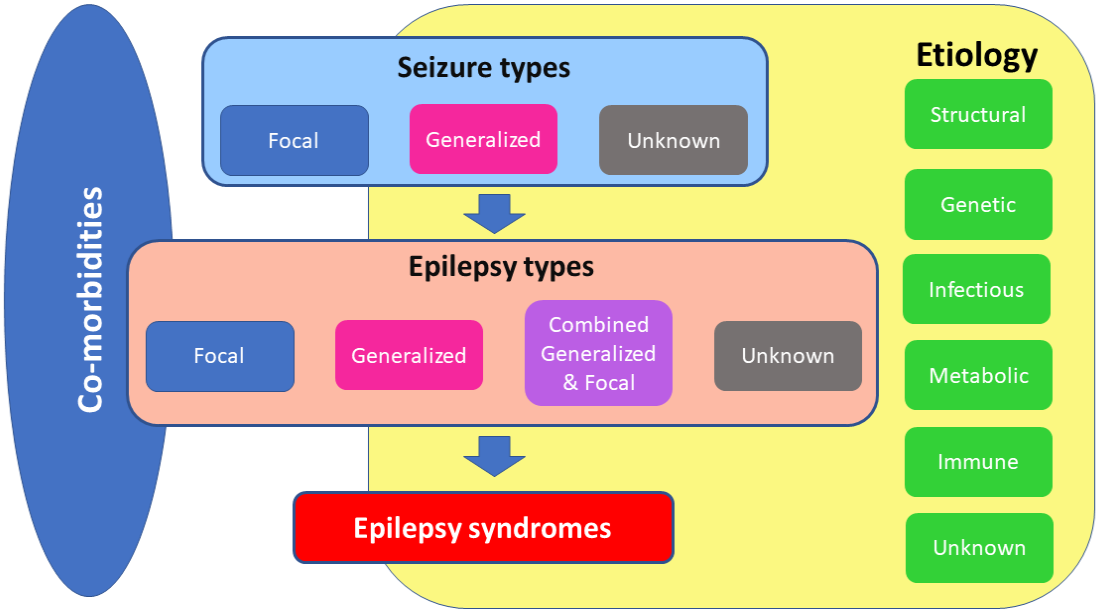


Figure 1. 1 Framework for epilepsy classification adapted from Scheffer et al, 2017 (p.515) [7]

Classification of seizures

Seizures are classified by the type of seizure onset which includes focal, generalized, and unknown (Figure 1.1). While focal-onset seizures originate from single brain hemisphere, generalized-onset seizures arise from some points of brain and rapidly distribute within networks [8]. If the origin of seizure is not determined, the seizure is categorized as unknown onset. Unknown seizures can be reclassified whenever updated information is available.

Classification of epilepsies

Epilepsy types include focal epilepsies, generalized epilepsy, combined generalized and focal epilepsy and unknown category. Focal epilepsies include seizures involving one brain hemisphere while generalized epilepsy patients may present with generalized seizures (Fig 1.1). When patients have both generalized and focal seizures, the epilepsy is categorized as a combined generalized and focal epilepsy. Unknown epilepsy is classified for patients with epilepsy who are not determined with the exact epilepsy type [7]. Epilepsy types can be determined from seizure types, electroencephalogram (EEG), and imaging studies.

Epilepsy syndromes

An epilepsy syndrome is characterized by a cluster of features including seizure types, EEG features, imaging findings, comorbidities, age-dependent onset, and sometimes prognosis. Although the ILAE has not defined a list of epilepsy syndromes, there are several well-known syndromes (Table 1.2). A full list of epilepsy syndromes is available at the ILAE website (epilepsydiagnosis.org)."

Table 1. 2 Examples of recognized epilepsy syndromes

In neonatal period
Benign neonatal seizure
Benign familial neonatal epilepsy
Early infantile epileptic encephalopathy with suppression burst (Ohtahara syndrome)
In infancy
Benign infantile epilepsy
West syndrome
Dravet syndrome
In childhood
Panyiotopoulos syndrome
Lennox-Gastaut syndrome
Landau-Kleffner syndrome
Benign epilepsy of childhood with centro-temporal spikes

1.1.3 Etiology

Six epilepsy etiologies as categorized by the ILAE are: structural, genetic, infectious, metabolic, immune, and unknown causes [2]. Some patients can be identified into more than one etiologic category (Figure 1.2). The proportion of each etiology in epilepsy children was obtained from Howell et al. (2018), Wirrell et al. (2011), and Ackermann et al. (2019) [9-11].

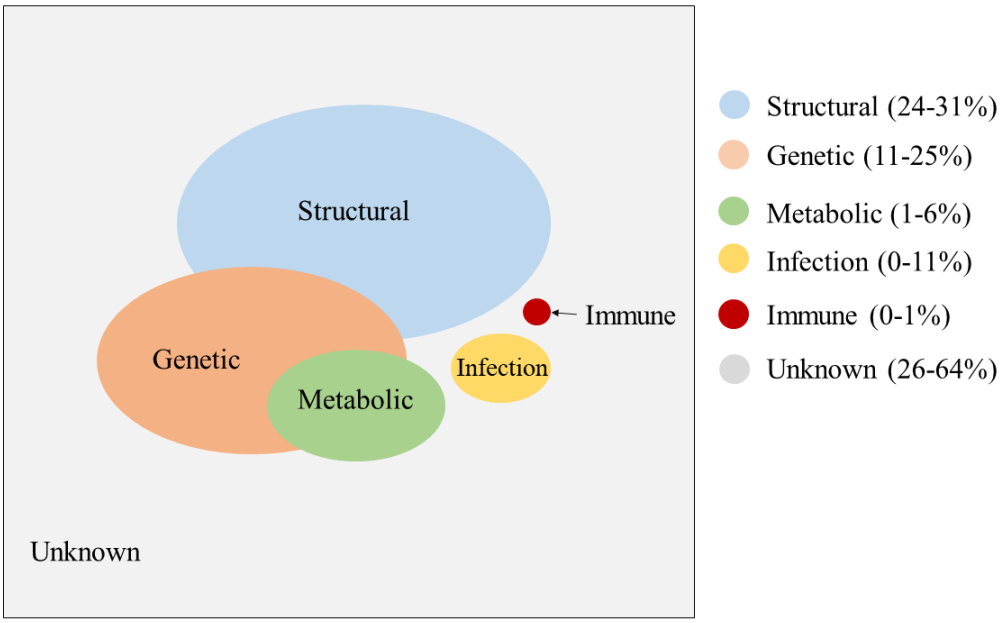


Figure 1. 2 Etiologies of epilepsies defined by the ILAE

Structural etiology

A structural etiology is associated with abnormalities in structural imaging. Specific magnetic resonance imaging (MRI) epilepsy protocols have been used to identify a subtle structural lesion [12]. Based on abnormalities in imaging findings together with an electroclinical assessment, possible causes of epilepsies can be reasonably inferred. The structural etiologies may be genetic or acquired, or both. Acquired structural etiologies can be identified in patients as a result of stroke, trauma, infection, and hypoxic-ischemic encephalopathy. A structural etiology may have a genetic basis originated from mutations in TSC1 or TSC2 genes. In this case, structural or genetic etiology terms can be used.

Genetic etiology

Genetic epilepsy directly results from a known or presumed genetic mutation. Genetic etiology can be identified by investigating family history or clinical symptoms in patients with the same syndrome. For example, most families of patients with benign familial neonatal epilepsy have genetic mutations in KCNQ2, or KCNQ3 genes which are known to be related to the potassium channel. Genetic etiology can explain the causes of severe and mild epilepsy. Causative mutations in epilepsy genes have been identified in 30-50% of infants with epileptic encephalopathies and severe developmental delay [13]. Genetic etiology may also imply treatment. Interpretation of specific gene mutations and understandings of phenotypes is critical to enable prediction of the outcome.

Genetic etiology of epilepsies can be identified as monogenic causes in which a particular gene is mutated (e.g., SCN1A mutations in Dravet syndrome). Monogenic epilepsies include familial epilepsies and severe epilepsies referred to as epileptic encephalopathies [13]. Monogenic causes remain the main focus for diagnosis and gene discovery that will contribute to the improvement of diagnosis and management of epilepsies [14, 15]. In addition, monogenic cause recognition can aid in interpretation of genetic testing results and identification of family members who might be at risk for epilepsies [16].

Despite an increasingly recognized mechanism of monogenic inheritance in severe epilepsies, common types of idiopathic generalized epilepsies (e.g., childhood absence epilepsy, juvenile absence epilepsy, and juvenile myoclonic epilepsy) are thought to have complex genetic inheritance [17-19]. The genetic risk factor for these epilepsies remains unclear that results in difficulties in genetic counseling [14, 16]. Under these circumstances, careful investigation of family history may provide better estimates for recurrence risk for families [16].

Infectious etiology

Infectious etiology refers to epilepsies resulted from an acute infection, namely meningitis and encephalitis. Examples of infectious etiology include neurocysticercosis, tuberculosis, HIV, cerebral malaria, subacute sclerosing panencephalitis, cerebral toxoplasmosis, and congenital infection [7]. An infectious etiology may have implications for specific treatment.

Metabolic etiology

Metabolic epilepsies may have a known or presumed metabolic cause. Metabolic causes include porphyria, uremia, aminoacidopathies, or pyridoxine-dependent seizures. A large proportion of metabolic epilepsies has a genetic etiology. However, some cases such as cerebral folate deficiency are due to acquired metabolic etiology. A metabolic etiology of epilepsies may inform specific treatment and possible prevention of cognitive impairment [20].

Immune etiology

Epilepsies can occur in patients with immunological diseases such as autoimmune-mediated inflammation of the central nervous system. Immune epilepsy may require targeted treatment with immunotherapies [21, 22].

Unknown etiology

Unknown etiology is defined as the cause of epilepsies, which cannot be found. Many patients with epilepsies remain in this category. Determination of the cause can be influenced by the availability of diagnostic tests that is varied depending on health care settings and countries.

1.1.4 Diagnosis of epilepsy

Epilepsy requires a complex diagnosis in which phenotypic evaluation is the key. Phenotypic evaluations include investigations of epilepsy features, personal history, family history, physical and neurological examination, and instrumental findings [23].

Epilepsy features

A detailed epilepsy history should be achieved. Epilepsy and seizure types can be determined by using the ILAE classification based on age of onset, frequency and response to treatment [8]. An ictal video-EEG or home-video recordings can be useful for a diagnosis of epilepsy syndrome and assessment of recurrence risk [24, 25]. Other relevant features such as the duration of the episodes, triggering factors, the setting of the seizures (e.g., presence of flickering lights and sound, cognitive performances) should be indicated. Drug response and cognitive impairment should be documented to provide the overall prognosis.

Personal history

It is essential to collect information on pregnancy (e.g., week of gestation, abortion threats, fetal movements, and infections) and delivery (e.g., eutocic or dystocic, respiratory distress, peri- and

neonatal course). Psychomotor development with regards to motor and language skills and neurological conditions should be investigated (e.g., attention deficit hyperactivity disorder, autism disorder, and movement disorders). Non-neurological comorbidities such as metabolic conditions and structural abnormalities should be evaluated [23].

Family history

Family history information can be useful for suggesting the possible inheritance pattern and the genetic risk in the relatives of the patient. For example, siblings of epilepsy patient suspected with genetic etiology have higher chance of developing epilepsy compared to the general population (3-5% higher risk of developing epilepsy versus 1-2% in general population). When the patient is the mother, siblings have a 4-6% higher risk [26, 27].

A three-generation pedigree should be investigated and that can be expanded for further generations whenever possible [28]. Parental consanguinity, twin pregnancies, abortions or miscarriages, and infantile deaths should be noticed.

Physical and neurological examination

Physical examination is conducted to investigate growth parameters, facial dysmorphic features, and limb abnormalities. Neurological examination and movement disorders should be evaluated.

Instrumental investigations

EEG is most helpful to determine epilepsy types. EEG can be also performed to identify possible abnormalities in unaffected members of certain families. However, EEG has technical and temporal limits, such as the presence of age-dependent abnormalities and variability between individuals.

Standard laboratory tests (e.g., blood, glucose, electrolytes, and ammonia investigations) should always be conducted in epilepsy patients with developmental delay, treatment resistance, or progressive neurological deterioration [29]. A comprehensive metabolic examination can provide an underlying mechanism of metabolic conditions with possible etiology-specific treatment. Early diagnosis and timely initiation of appropriate treatment for treatable metabolic epilepsy can stabilize or reverse neurological symptoms [29, 30]. In addition to standard laboratory workup, first-line metabolic screening should be also performed (e.g., plasma and urine amino acid levels, urine organic acids, blood spot acylcarnitine profile, and urine creatine/creatinine ratio) [30]. In patients suspected with specific disorders, second-line tests can be further undergone. For

example, the analysis of plasma and urine biotin dose and serum biotinidase enzyme activity can reveal metabolic disorders in epilepsy neonatal with neuro-opthalmological, and cutaneous manifestations [30]. An early diagnosis of biotinidase deficiency in these patients can initiate biotin supplementation, allowing seizure control, stabilization or reversal of neurological complications [30, 31].

Neuroimaging is essential for the diagnosis of brain abnormalities in epilepsy. Brain MRI should be performed to rule out cortical migration defects, cortical development abnormalities, vascular anomalies, and defects of the corpus callosum and cerebellum. In some conditions, brain MRI can support the selection of appropriate genetic tests [32, 33].

1.1.5 Genetic tests

Chromosomal microarray

A chromosomal microarray (CMA) is the first-tier clinical diagnostic test for patients with epilepsy and developmental disability, intellectual impairment, and/or dysmorphism. CMA is able to detect copy-number variants (CNVs). CNVs were found in 28% of patients with genetic generalized epilepsy and intellectual disability. Patients with early-onset genetic generalized epilepsy and developmental disability before 4 years old have the highest diagnostic yield [34]. When all epilepsies such as generalized or focal epilepsy, and epileptic encephalopathy are considered, the diagnostic yield of CMA is less than 5% of patients [35]. In a study of 805 epilepsy patients, an estimated 5% of patients were explained by CNVs [36].

Karyotype

Traditional karyotyping can identify structural and numerical chromosome abnormalities and mosaicism. Its diagnostic yield is low because it cannot detect abnormalities smaller than 5-10 megabases [37]. Due to increasing availability and affordability of CMA, karyotyping is used in certain circumstances, such as ring chromosome 20 syndrome and Killian syndrome.

Single-gene testing

Single-gene testing identifies changes in a single gene (point mutations, exomic deletions, small CNVs). It is considered in patients with a particular epilepsy syndrome. Single-gene testing is highly accurate but time-consuming due to targeting only one gene at a time. Patients with Dravet syndrome are often considered with single-gene sequencing since SCN1A mutation can be

identified and explained in 80% of patients [38]. Rett syndrome is caused by MECP2 mutation in addition to genes with similar symptoms (FOXG1, CDKL5, and GRIN2B).

Epilepsy panel (EP)

Epilepsy panel (EP) available for genetic testing can be used to analyze a selected group of genes considerably varying from a small number of genes (e.g., TSC1 and TSC2) to large panels (more than 400 genes). Notably, costs and turn-around time of small EPs and single-gene testing sometimes may be close to large EPs. The diagnostic yield of EP is from 15% to 25% depending on clinical presentations, time of onset, and family history [39, 40].

Whole-exome sequencing (WES)

WES sequences most of the proteins encoding exons and splice junction. The overall diagnostic yield of WES in patients with epilepsies was in the range of 20% and 40% [41, 42]. A meta-analysis on the diagnostic yield of CMA, WES and panel testing in epilepsy showed that WES has the highest diagnostic rate [43].

Whole-genome sequencing (WGS)

WGS analyzes most of the DNA in the whole genome. Compared to WES, WGS has advantages such as the capabilities of identifying CNVs, a high coverage of the genome, and more in-depth coverage to detect mosaicism. Currently, WGS is not routinely used in clinical practice due to its high cost. In the near future, since the sequencing costs reduce, WGS would be more accessible in clinics [44].

1.1.6 Clinical utility of genetic tests

Before genetic testing is widely adopted into clinical practice, “clinical utility” is defined as “the ability to prevent or ameliorate adverse health outcomes such as mortality, morbidity, or disability through the adoption of efficacious treatments conditioned on test results” [45]. However, “clinical utility” in this sense may be too restrictive. Thanks to the increasing use of genetic test, the conceptualization of clinical utility of genetic testing expands. We now provide an overview of clinical utility of genetic testing in epilepsy.

Genetic testing provides an etiologic diagnosis in a proportion of patients with epilepsy. Diagnostic yield of genetic testing in epilepsy ranged from 13 to 73% depended on type of genetic test, patient characteristics and age of onset [43]. A genetic diagnosis may end the

"diagnostic odyssey" and reduce the diagnostic delay from 3.4 years to 21 days [46, 47]. Surveillance for longer-term health issues such as gait issues in Dravet syndrome and multi-system problems in tuberous sclerosis are also benefits of diagnosis [48]. An etiologic diagnosis may lead to a more accurate prognosis for the patients and their families [49-51], informed recurrence risk and avoidance of further investigations [49, 52].

Genetic testing can also impact clinical management in epilepsy. There are a number of therapies specific to genetic epilepsies in which mutations are identified by genetic tests (e.g. pyridoxine for ALDH7A1) (Table 1.3). A recent retrospective study reported a large cohort of 9769 children referred for EP testing. It was found that genetic etiology might allow therapy initiation in 33% of 1502 patients with a positive genetic diagnosis [53].

Clinical utility of genetic testing is also referred to as "the likelihood that the test will lead to an improved health outcome" [54]. In this sense, genetic testing is associated with clinical effectiveness resulted from a selection of effective therapies. Recently, Na et al. (2020) reported that in a cohort of 150 patients with early-onset developmental and epileptic encephalopathy, 35% of patients received positive genetic diagnoses [55]. Among patients with genetic mutations, KCNQ2 was the most frequently identified. Patients with KCNQ2 mutations initiated ketogenic diet which led to more than 50% reduction of seizure after treatment in 6 of 10 patients [55].

1.1.7 Conventional diagnostic strategy in epilepsy children

Howell et al. (2018) described the conventional diagnostic strategy consisted of a combination of standard three-tier tests for children with epilepsy (Figure 1.3) [9]. In this strategy, an epilepsy patient underwent standard first-tier tests including clinical investigations, metabolic screening, neuroimaging, CMA and targeted single-gene sequencing for suspected causative mutations. When the first-tier tests were negative, the patient further underwent complex metabolic tests and targeted single-gene sequencing known as standard second-tier tests. After nondiagnostic second-tier tests, the patient was offered invasive tests, i.e., skin or liver biopsies to get samples for cytogenetic analysis.

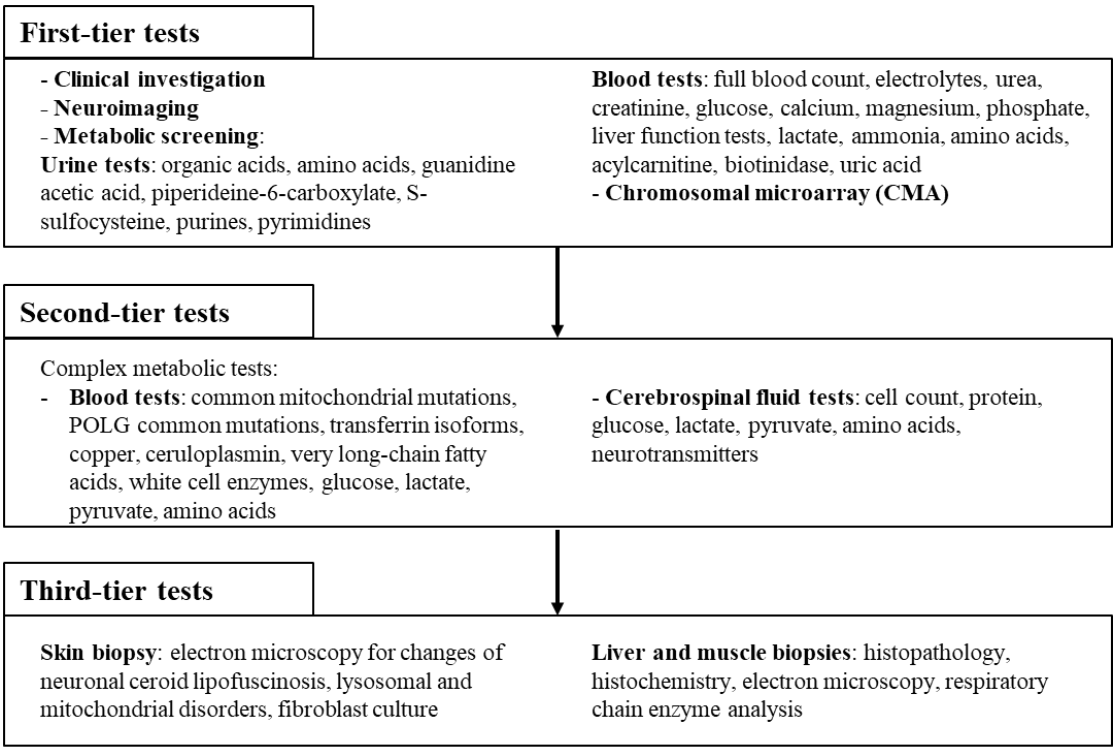


Figure 1. 3 Conventional diagnostic strategy in children with epilepsy adapted from Howell et al., 2018 (p. 1178) [9]

1.1.8 Treatment options for pediatric epilepsies

Many therapeutic options are available for pediatric epilepsy including anti-epileptic drugs (AEDs), ketogenic diet, vagus nerve stimulation, and surgery. AEDs are used to control the seizure frequency and its severity [56, 57]. Over 20 AEDs have been available until now. Selection of AEDs depends on types of seizure or epilepsy syndromes [58, 59]. Table 1.3 shows examples of treatment options for a variety of epilepsy syndromes which are adapted from Guerrini et al. (2006) [5].

Anti-epileptic drugs (AEDs)

AEDs are the effective treatment which can reduce seizure frequency in most epilepsy children. Moreover, several AEDs were shown to affect patient's developmental and cognitive functioning [60, 61]. Approximately two-thirds of children experienced seizure reduction after consumption of AEDs. However, AEDs failed to control seizure in about 20-30% of children [62, 63]. Seizure may not last beyond childhood, but epileptic children have potential long-term psychological consequences despite no longer suffering from seizure [64].

Ketogenic diet

A ketogenic diet is a high-fat and low-carbohydrate diet. A ketogenic diet is an alternative for children with epilepsy when their parents decide to not treat them with AEDs [65]. A ketogenic diet is also given for refractory epilepsy after 2 or 3 attempts of AEDs. In patients with refractory epilepsy, a ketogenic diet was associated with a 50% seizure reduction [66]. Moreover, developmental improvement was found in patients treated with a ketogenic diet [67, 68].

Surgery

Surgery can be an option for epilepsy patients who fail to achieve seizure reduction from a ketogenic diet [69]. Studies showed that 70% of patients undergoing surgery became seizure free eventually [69-72]. Surgery can be associated with motor improvement [73-75]. It is also related to the decrease of verbal memory up to 2 years after a left temporal lobectomy [76].

Table 1. 3 Therapeutic options for epilepsy syndromes with an indication of age of onset adapted from Guerrini et al (2006) [5]

	Specific syndromes	**Age at onset**	**Monotherapy or add-on**	**Possible add-on**	**Surgery**
Idiopathic focal epilepsies of infancy and childhood	Benign infantile seizures (non-familial)	Infant	PB	…	No
	Benign childhood epilepsy with centrotemporal spikes	3-13 years	VPA, CBZ	…	No
	Early and late onset idiopathic occipital epilepsy	2-8 years, 6-17 years	VPA, CBZ	…	No
Familial (autosomal dominant) epilepsies	Benign familial neonatal convulsions	Newborn young infant	PB	…	No
	Benign familial infantile convulsions	Infant	CBZ, PB	…	No
	Autosomal dominant nocturnal frontal lobe epilepsy	Childhood	CBZ, OXC, TPM, PHT, GBP	LEV, PHT, PB, CLB	No
	Familial lateral temporal lobe epilepsy	Childhood-adolescence	CBZ, OXC, VPA, TPM, PHT, GBP	LEV, PHT, PB, CLB	No
	Generalized epilepsies with febrile seizures plus	Childhood - adolescence	VPA, ESM, TPM, LTG	CLB, LEV	No
Symptomatic (or probably symptomatic) focal epilepsies	*Limbic epilepsy*				
	Mesial temporal lobe epilepsy with hippocampal sclerosis	School age or earlier	CBZ, VPA, OXC, TPM, PHT, GBP	LEV, PHT, PB, CLB	Temporal resection
	Mesial temporal lobe	Variable	CBZ, VPA,	LEV, PHT,	Temporal

	epilepsy defined by specific causes		OXC, TPM, PHT, GBP	PB, CLB	resection
	Other types defined by location and causes	Variable	CBZ, VPA, OXC, TPM, PHT, PHT, GBP	LEV, PHT, PB, CLB	Lesionectomy +/- cortical resection
	Neocortical epilepsies				
	Rasmussen syndrome	6-12 years	Plasma-pheresis, immune-globulins	PHT, CBZ, PB, TPM, CLB	Functional hemispherectomy
	Hemiconvulsion – hemiplegia syndrome	1-5 years	CBZ, VPA, OXC, TPM, PHT, GBP	LEV, PT, PB, CLB	Functional hemispherectomy
	Other types defined by location and cause	Variable	CBZ, VPA, OXC, TPM, PHT, GBP	LEV, PHT, PB, CLB	Lesionectomy +/- cortical resection
	Migrating partial seizures of early infancy	Infant	PB, PHT, CBZ, TPM, VPA	BDZ	No
Idiopathic generalized epilepsies	Benign myoclonic epilepsy in infancy	3 months – 3 years	VPA	BDZ	No
	Epilepsy with myoclonic astatic seizures	3-5 years	VPA, ESM, TPM	BDZ, LTG, LEV	No
	Childhood absence epilepsy	5-6 years	VPA, ESM, LTG	…	No
	Epilepsy with myoclonic absences	1-12 years	VPA, ESM	BDZ	No
	Idiopathic generalized epilepsies with variable phenotypes				
	Juvenile absence epilepsy	10-12 years	VPA, ESM, LTG	BDZ	No
	Juvenile myoclonic epilepsy	12-18 years	VPA, TPM	BDZ, PRM, PB	No
	Epilepsy with generalized tonic-clonic seizures only	12-18 years	VPA, LTG, TPM, CBZ	BDZ, LVT	No
Reflex epilepsies	Idiopathic photosensitive occipital lobe epilepsy	10-12 years	VPA	LEV, BDZ	No
	Other visual sensitive epilepsies	2-5 years	VPA	LEV, BDZ	No
	Startle epilepsy	Variable	CBZ, VPA, OXC, TPM, PHT, GBP	LEV, PHT, PB, CLB	Lesionectomy +- cortical resection
Epileptic encephalo-pathies	Early myoclonic encephalopathy and Ohtahara syndrome	Newborn-infant	Steroids, PB	BDZ	No
	West syndrome	Infant	Steroids, PB	BDZ, TPM BDZ	Lesionectomy +- cortical resection
	Dravet's syndrome	Infant	Stiripentol,	BDZ	No

	(severe myoclonic epilepsy in infancy)		GVG		
	Lennox-Gastaut syndrome	3-10 years	…	BDZ, LTG	Collosotomy
	Landau-Kleffner syndrome	3-6 years	VPA, ESM, steroids	BDZ, LTG	Multiple subpial transections
	Epilepsy with continuous spike waves during slow-wave sleep	4-7 years	VPA, ESM, steroids		No
Progressive myoclonic epilepsies	Unverricht-Lundborg, Lafora, ceroido-lipofuscinoses, etc	Late infant-adolescent	VPA, TPM	BDZ, PB	No
Seizures not necessarily needing a diagnosis of epilepsy	Benign neonatal seizures	Newborn	PB	….	No
	Febrile seizures	3-5 years	VPA if repeated and prolonged	…	No
	Reflex seizures	Variable	…	…	No
	Drug or other chemically- induced seizures	Variable	…	…	No
	Immediate and early post-traumatic seizures	Variable	…	…	No

Note. PB, phenobarbital; VPA, valproate; CBZ, carbamazepine; OXC, oxcarbazepine; TPM, topiramate; PHT, phenytoin; GBP, gabapentin; ESM, ethosuximide; LTG, lamotrigine; LEV, levetiracetam; CLB, clobazam

1.1.9 Precision medicine in pediatric epilepsy

Providing a genetic diagnosis can be useful for patients to initiate a personalized treatment approaches for children with epilepsy. The term “precision medicine” was chosen in this thesis. Precision medicine was defined as “treatments targeted to the needs of individual patients on the basis of genetic, biomarker, phenotypic, or psychological characteristics” [77]. In epilepsy, precision medicine implies a treatment approach that targets the underlying causes [78]. The concept of precision medicine was suitable to use in this thesis where genetic tests were integrated into the diagnostic strategy to better understand the underlying mechanisms of pediatric epilepsy that in turn potentially deliver effective treatment.

A good example of precision medicine in epilepsy is sodium channel blocking such as carbamazepine and phenytoin which is the gold standard therapy for epilepsy caused by mutations in SCN8A and KCNQ2 [5, 79]. Another example of precision medicine is rapamycin for patients with TSC mutations which is approved for this indication. Table 1.4 further provides examples of personalized therapies for gene mutations in epilepsy.

Table 1. 4 Gene mutations and personalized therapies in genetic epilepsy

Gene mutations	Therapy	References
ALDH7A1	Pyridoxine, lysine-restricted diet and arginine supplement	[80-85]
BRAT1	Zonisamide	[86]
CDKL5	Vigabatrin and zoninsamide	[87]
DEPDC5	mTOR inhibitors (rapamycin)	[88, 89]
FOLR1	Folinic acid supplementation	[90]
FOXG1	Adrenocorticotropin	[91-95]
KCNQ2	Carbamazepine	[96-98]
KCNT1/ KCNT2	Quinidine	[99-101]
GRIN2A	N-methyl D-aspartate receptor inhibitors such as memantine	[102, 103]
GRIN2B	N-methyl D-aspartate receptor inhibitors such as memantine	[104, 105]
GRIN2D	N-methyl D-aspartate receptor inhibitors such as memantine	[106]
MEF2C	Valproate	[107, 108]
mTOR	mTOR inhibitors (rapamycin)	[109-111]
PCDH19	Clobazam, bromide	[112]
PNPO	Pyridoxine	[82, 84, 113-116]
SLC2A1	Ketogenic diet	[117-122]
SCN1A	Valproate and benzodiazepine	[123]
SCN2A	Sodium channel blockers including carbamazepine, lacosamide, lamotrigine, phenytoin, oxcarbazepine, zonisamide	[124]
SCN8A	Sodium channel blockers including carbamazepine, lacosamide, lamotrigine, phenytoin, oxcarbazepine, zonisamide	[125-127]
STXBP1	Adrenocorticotropin	[128]
STRADA	mTOR inhibitors (rapamycin)	[129, 130]

SYNGAP1	Valproate, lamotrigine	[131]
TSC1/ TSC2	Rapamycin	[123]

Note. ALDH7A1, aldehyde dehydrogenase 7 family, member A1; BRAT1, BRCA1 associated ATM activator 1; CDKL5, cyclin-dependent kinase-like 5; DEPDC5, DEP domain containing 5; FOLR1, folate receptor alpha; FOXG1, forkhead box G1, KCNQ2, potassium channel, voltage-gated, KQT-like subfamily member 2; KCNT1, potassium channel subfamily T member 1; GRIN2A/2B/2D, glutamate ionotropic receptor NMDA type subunit 2A/2B/2D; MEF2C , myocyte enhancer factor 2C, mTOR, mammalian target of rapamycin, PCDH19. Protocadherin 19; PNPO, pyridoxamine 5'-phosphate oxidase; SLC2A1, solute carrier family 2 member 1; SCN1A, sodium channel, voltage-gated type I, alpha subunit SCN2A, sodium channel, voltage-gated type II, alpha subunit; SCN8A, sodium channel, voltage-gated, type VIII alpha subunit; STXBP1, syntaxin-binding protein 1; STRADA SYNGAP1, synaptic RAS-GTPase-activating protein 1; TSC1, tuberous sclerosis 1; TSC2, tuberous sclerosis 2

1.1.10 Gaps in the literature

Genetic epilepsy manifests early in life with a broad clinical spectrum including benign, self-limited epilepsy, epilepsy resulted from inborn errors of metabolism, epilepsy with intellectual disabilities and early-onset, severe epileptic encephalopathies [13, 132]. However, it is challenging to recognize genetic epilepsy based on clinical investigations. Genetic testing has played a crucial role in the diagnosis and treatment of epilepsy. Early correct determination of genetic etiology can help to avoid unnecessary investigations and allow the selection of more effective treatment in certain cases [133]. A growing number of studies supported NGS-based genetic tests as diagnostic tools for epilepsy. Various genetic tests available for diagnosis of epilepsy make it challenging for genetic specialists to choose a test. Several considerations should be addressed before adopting the widespread use of these genetic tests, such as diagnostic yield, clinical utility (i.e., improving medical management, access to genetic counseling and family planning), and cost-effectiveness.

Despite its importance, thus far, only one recent meta-analysis and three cost-effectiveness analyses tried to answer these questions [9, 43, 134]. The meta-analysis investigated the diagnostic yield of WES in comparison with EP and CMA [43] in the general population with epilepsy. However, given the differences in diagnostic yield between children and adults [135], its findings may not be applicable for children. Moreover, this meta-analysis did not include data from recent studies, which may affect the conclusion. The value of elucidating a molecular diagnosis includes improvement of clinical management, access to genetic counseling and reproductive plan. While the clinical utility of genetic tests is more relevant for considerations to

improve health outcomes of affected patients and their families, no study has compared the clinical utility of different genetic tests in children with epilepsy.

Regarding cost-effectiveness of WES in epilepsy patients, Palmer et al. (2018) investigated a small population of 32 individuals with epileptic encephalopathy who were undiagnosed after standard first-tier diagnostic tests, including metabolic investigations, magnetic resonance imaging, electroencephalogram, and CMA [134]. In this cohort, a diagnostic approach with WES for patients undiagnosed after standard second-tier tests (i.e., metabolic investigations, further neuro-imaging, and single-gene sequencing) led to 14 additional diagnosed cases compared to second-tier testing without WES (16/32, 50% vs. 2/32, 6.2%, respectively) with lower total cost (AU$ 9,536 vs. AU$ 11,827, respectively). Incorporating WES into a standard diagnostic approach was cost-effective with approximately 10 times less costly than a standard diagnostic approach (AU$ 19,074 vs. AU$ 189,243 per diagnosis) [134]. The second study conducted a cost-effectiveness analysis of WES in patients with severe epilepsies of infancy. It found that incorporating WES into standard diagnostic pathway increased costs and diagnostic yield but cost per diagnosis was lower (15,378 USD vs. 16,951 USD, respectively) [9]. It also found that early WES cost less than late WES. Early WES with limited metabolic testing achieved higher diagnostic yield (46/86 vs. 39/86) with lower cost per diagnosis (9,904 USD vs. 16,951 USD) [9]. The third study compared the cost-effectiveness of genetic testing strategies (i.e., CMA, EP, WES and combination strategies) in patients with epilepsy [43]. Among individual tests, WES was the most cost-effective at an incremental cost-effectiveness ratio (ICER) of 14,114 USD per diagnosis compared to EP. They also concluded that WES followed by EP and CMA (ICER of 15,336 USD) was the most cost-effective among combination strategies [43].

Existing evidence on the cost-effectiveness of EP and WES for patients with epilepsy is both limited and based on small study populations. All these studies used diagnostic yield as the outcome measure and thus did not capture improvements in health outcomes of the patient population as a result of a positive diagnosis and subsequent changes in patient management. There is a need to perform a more comprehensive economic evaluation that includes quality-adjusted life years (QALYs) gained by changes in treatment to better inform decision-makers on the value of integrating new genetic tests for epilepsy in routine clinical practice.

1.2 Purpose of study

This thesis aimed to assess the molecular diagnostic yield and clinical utility of different genetic tests as well as the cost-effectiveness of NGS-incorporated testing strategies in unselected epileptic children younger than 18 years old.

1.3 Specific research objectives

The specific research objectives of this thesis were:

1. To compare the diagnostic yield and clinical utility of EP, WES, WGS, and CMA in children with epilepsy by conducting a systematic review of the literature (Chapter 2).

2. To evaluate the cost-effectiveness of EP and WES diagnostic strategies for children with epilepsy from the healthcare system's perspective (Chapter 3). We estimated and compared the cost and QALYs of these different diagnostic strategies that include not only diagnostic results but also treatment therapies that follow the testing results.

1.4 Study framework

The thesis involved a systematic review of the literature (Chapter 2) and a cost-effectiveness analysis (Chapter 3). Evidence from Chapter 2 was used to provide inputs for a model developed in Chapter 3. Figure 1.4 illustrated the study framework which included three domains: (1) process, (2) study methods and (3) outcomes.

The diagnostic process was prompted by the integration of new interventions which were genetic tests, namely EP and WES. The primary outcome of diagnostic process was the proportion of patients diagnosed with a specific etiology which was measured by the diagnostic yield. While the diagnostic yield of conventional diagnostic tests was retrieved from the literature, the diagnostic yield of genetic tests was measured from our systematic review (Chapter 2). The secondary outcome of diagnostic process was healthcare costs. Measuring both costs and diagnostic yield, we evaluated the cost-effectiveness of EP and WES-related strategies in diagnostic stage (Chapter 3).

After a diagnostic process, clinical management was initiated and followed up to 2 years. In this treatment phase, patients underwent either usual care or personalized therapy. The proportion of patients initiating precision-therapy approach was measured by the clinical utility of genetic tests through our systematic review in chapter 2. Clinical management determined patient's health

outcomes and treatment costs. We estimated both measures to evaluate the cost-effectiveness of EP and WES in this treatment stage (Chapter 3).

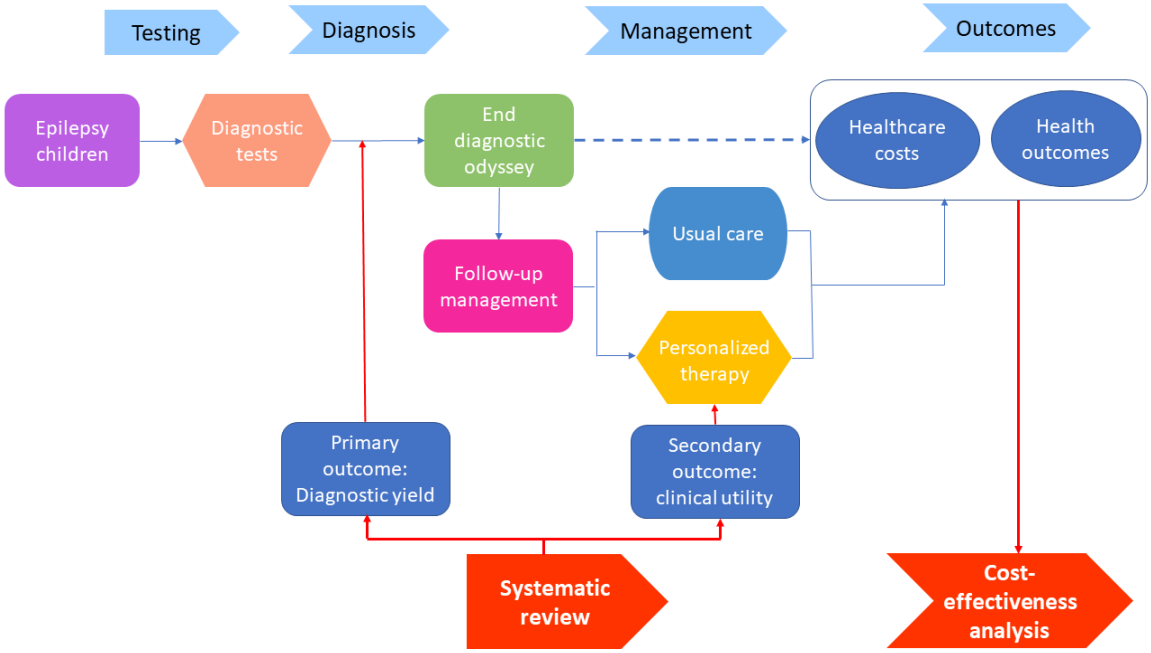


Figure 1. 4 Study framework

1.5 Significance of the study

Accurate diagnosis based on genetic testing has the potential to impact and optimize children's clinical management and their health for their entire life. Our study will provide updated evidence on the molecular diagnostic yield and clinical utility of WGS, WES, EP, and CMA in epileptic children. These results provide timely evidence to guide clinicians and policymakers in choosing optimal diagnostic strategies. The findings from our systematic literature review and meta-analysis will also guide the choice of NGS-integrated diagnostic strategies for cost-effectiveness analysis. Meanwhile, understanding the economic value of implementing WES and EP in the diagnosis and treatment will inform the selection and implementation of cost-effective diagnostic strategies in children with epilepsy.

Chapter 2: Diagnostic yield and clinical utility of epilepsy panel, whole-exome sequencing, whole-genome sequencing, and chromosomal microarray in epilepsy children: A systematic review and meta-analysis

2.1 Introduction

Epilepsy is one of the most common neurologic disorders, affecting more than 50 million people in the world [136]. Epilepsy incidence is age-dependent with the highest incidence rate (54-144 per 100,000) in children under the age of 10 [137]. While children with epilepsy experience a high burden of cognitive and behavioral comorbidity [138], the diagnostic process of pediatric epilepsy is challenging with a considerable risk of misdiagnosis [137].

20-30% of epilepsy causes are due to acquired insults such as birth trauma, brain injury, and tumors, while the remaining 70-80% are due to genetic factors [139]. Genetic epilepsy tends to manifest earlier in life with a large clinical spectrum ranging from benign, self-limited epilepsy, epilepsy resulted from inborn errors of metabolism, epilepsy with intellectual disabilities and early-onset, severe epileptic encephalopathies [13, 132]. However, it is challenging to recognize genetic epilepsy based on clinical investigations. Thus, genetic testing is increasingly used as a diagnostic tool in children with epilepsy. Genetic testing allows not only early determination of genetic etiology but also an approach to select optimal treatment to improve outcomes and in some cases, leads to life-saving therapy [140, 141].

A chromosomal microarray (CMA) is known as the first-tier genetic testing for individuals with epilepsy and developmental delay (DD), intellectual disability (ID), and/or dysmorphic features. However, in all epilepsy (with and without ID and DD), the diagnostic yield of CMA is low (8%) that requires many other tests to find an etiologic diagnosis, representing a diagnostic odyssey [43]. Next-generation sequencing (NGS) is a new technology that can sequence many genes simultaneously and allow comprehensive ascertainment of causal mutations. NGS includes epilepsy panel (EP), whole-exome sequencing (WES), and whole-genome sequencing (WGS). EP screens multiple potentially relevant genes while WES targets the protein-coding regions in the genome and WGS sequences the entire genome.

With an increase in options for genetic tests, it is essential to understand their diagnostic yield and clinical utility before the widespread application of these tests in clinical practice. However, only one meta-analysis compared the diagnostic yield of WES and EP with CMA in the general population with epilepsy [43]. Given that diagnostic yield differed between adults and children [135], findings from a general population may not be applicable for children. Furthermore, no study compares the clinical utility of different genetic tests in children with epilepsy [43, 142]. To fill the gap in the literature, in this chapter, we compared the diagnostic yield and clinical utility of EP, WES, WGS and CMA in pediatric epilepsy by conducting a systematic review and meta-analysis.

2.2 Materials and methods

2.2.1 Data sources and record identification

The literature search for the diagnostic yield and clinical utility of genetic tests aimed to systematically identify relevant studies of whole-genome sequencing, whole-exome sequencing, epilepsy panel, and chromosomal microarray for children with epilepsy. The 4 sets of terms were combined using the operator AND. Search terms regarding to diagnostic yield and clinical utility were combined using the operator OR. The literature search was performed in July 2019.

Searching articles from PubMed was performed by using the combination of following Medical Subject Heading (MeSH) terms:

((((((((((gene* panel) OR epilepsy panel) OR whole-exome sequencing) OR whole* sequencing) OR whole-genome sequencing) OR chromosomal microarray) OR comparative genomic hybridization)) AND (((diagnos*) OR utility) OR yield)) AND ((epilep*) OR seiz*)) AND ((((child*) OR infant*) OR adolescen*) OR pediatri*)

We searched EMBASE using the following terms:

('diagnosis'/exp OR diagnosis OR utility OR 'yield'/exp OR yield) AND ('epilepsy'/exp OR epilepsy OR 'seizure'/exp OR seizure) AND ('gene panel' OR (('gene'/exp OR gene) AND panel) OR 'epilepsy panel' OR (('epilepsy'/exp OR epilepsy) AND panel) OR 'whole exome sequencing'/exp OR 'whole exome sequencing' OR 'whole genome sequencing'/exp OR 'whole genome sequencing' OR 'chromosomal microarray'/exp OR 'chromosomal microarray' OR 'comparative genomic hybridization'/exp OR 'comparative genomic hybridization' OR 'next

generation sequencing'/exp OR 'next generation sequencing') AND ('child'/exp OR child OR 'infant'/exp OR infant OR 'adolescent'/exp OR adolescent OR 'pediatrics'/exp OR pediatrics)

The search of PubMed was updated in March 2020, and an additional 5 articles were included to further discuss the clinical utility of genetic tests. The literature search was performed by a single reviewer using the following terms:

(((((gene* panel OR epilepsy panel OR whole-exome sequencing OR whole* sequencing OR whole-genome sequencing OR chromosomal microarray OR comparative genomic hybridization))) AND ((epilep* OR seiz*))) AND ((child* OR infan* OR adolescen* OR pediatri*))) AND (clinical management OR reproductive OR counsel OR outcome OR response OR drug OR treatment)

2.2.2 Study screening and eligibility

Study screening was performed in two steps. Firstly, the reviewer screened and reviewed all titles and abstracts and identified all potentially eligible articles. Studies investigating the molecular diagnostic yield and/or clinical utility of EP, WES, WGS, and CMA were included. Studies in which patients younger than 18 years old with a broad spectrum of epilepsy were eligible. Then, all eligible full-text articles were obtained and imported into a spreadsheet.

2.2.3 Inclusion criteria and data extraction

All full-text articles accepted through abstract screening were reviewed by the reviewer against the inclusion and exclusion criteria.

Articles that met the following criteria were included: (1) proband less than 18 years of age at the time of sequencing, and (2) evaluated the molecular diagnostic yield and/or clinical utility of genetic testing.

Exclusion criteria were: (1) did not investigate the population of interest; (2) did not discuss WGS, WES, EP and CMA; (3) basic science; (4) did not include molecular diagnostic yield; (5) methodology or review paper; (6) include <10 participants; (7) conference abstract or not in English; (8) animal studies.

Patients: affected children (younger than 18 years) with one of two categories: epilepsy or epilepsy plus neurodevelopmental disorders (DD, ID, or autism spectrum disorder)

Diagnostic tests: WGS, WES, EP, and CMA for identifying the etiology of epilepsy

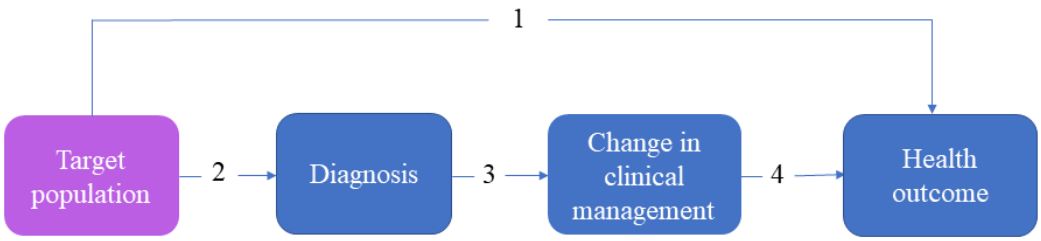
Comparator: the treatment groups were participants undergoing WGS, WES, and EP. Patients performing CMA was defined as a reference group.

Outcomes: molecular diagnostic yield and clinical utility.

Diagnostic yield was the proportion of patients receiving a diagnosis of pathogenic or likely pathogenic variants affecting genes associated with phenotypes.

The clinical utility of a genetic test included the impact on clinical management, genetic counseling, and treatment effectiveness. To avoid the heterogeneity in definitions of clinical utility among studies, only impact on clinical management was considered in our meta-analysis [143]. The remained effects were further discussed without any analysis.

Figure 2.1 illustrates the analytic framework for relationship between a variety of evidence in Section 2.2.3



Research questions

1. Direct evidence that genetic test impact health outcome
2. Diagnostic yield
3. Impact of genetic test on clinical management
4. Impact of clinical management on health outcome

Figure 2. 1 Analytical framework for relationship between a variety of evidence

Settings: genetic tests can be performed in hospital laboratories or reference laboratories as in clinical setting or in research laboratories as in experimental setting.

Statistical analysis:

We performed random-effect models to determine the diagnostic yield and clinical utility of genetic tests. We chose the random effect models because of heterogeneity in study designs, patient populations, and interventions. The statistical heterogeneity between studies was assessed

by the I^2 statistic with suggested thresholds for low (25-50%), moderate (50-75%) and high (>75%) heterogeneity, respectively [144]. For each analysis, the I^2 and p values of statistical heterogeneity were presented.

We investigated heterogeneity between studies by performing univariate analysis. The heterogeneity resulted from the year of study publication and the number of probands was examined in our analysis. The associations of variables (year of publication and number of probands) with heterogeneity were examined by meta-regression.

Forest plots were used to show findings from a single study and pooled groups. We defined $P<0.05$ as statistically significant. The "meta" and "metaphor" packages in R were used in our analyses.

2.2.4 Assessment of methodological quality

A revised tool of Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) was used for the assessment of the quality of included studies [145]. The reviewer evaluated four domains including patient selection, index test, reference standard and flow and timing. The reviewer answered signaling questions and judged risk of bias and applicability concern to each included study (Table 2.1). Signaling questions were answered with a 'yes', 'no' or 'unclear'. Studies responded with 'unclear' or 'no' answer for one or more signaling questions were judged to have 'unclear or high risk of bias'. Applicability concern were judged as having 'high', 'unclear' or 'no' concern (Table 2.1).

Table 2. 1. QUADAS-2 tool for assessing quality of included studies

Domain 1. Patient selection	Signaling question	Was a consecutive random sample of patients enrolled?
		Was a case-control design avoided?
		Did the study avoid inappropriate exclusions?
	Risk of bias	Could the selection of patients have introduced bias?
	Concerns about applicability	Are there concerns that the included patients and setting do not match the review question?
Domain 2. Index test	Signaling question	Were the index test results interpreted without knowledge of the results of the reference standard?

		If a threshold was used, was it prespecified?
	Risk of bias	Could the conduct or interpretation of the index test have introduced bias?
	Concerns about applicability	Are there concern that the index test, its conduct, or interpretation differ from the review question?
Domain 3. Reference standard	Signaling question	Is the reference standard likely to correctly classify the target condition?
		Were the reference standard results interpreted without knowledge of the results of the index test?
	Risk of bias	Could the reference standard, its conduct, or its interpretation have introduced bias?
	Concerns about applicability	Are there concern that the index test, its conduct, or interpretation differ from the review question?
Domain 4. Flow and timing	Signaling question	Was there an appropriate interval between index test and reference standard?
		Did all analyzed patients receive the reference standard?
	Risk of bias	Were all patients included in the analysis?
	Concerns about applicability	Could the patient flow have introduced bias?

2.3 Results

2.3.1 Study selection

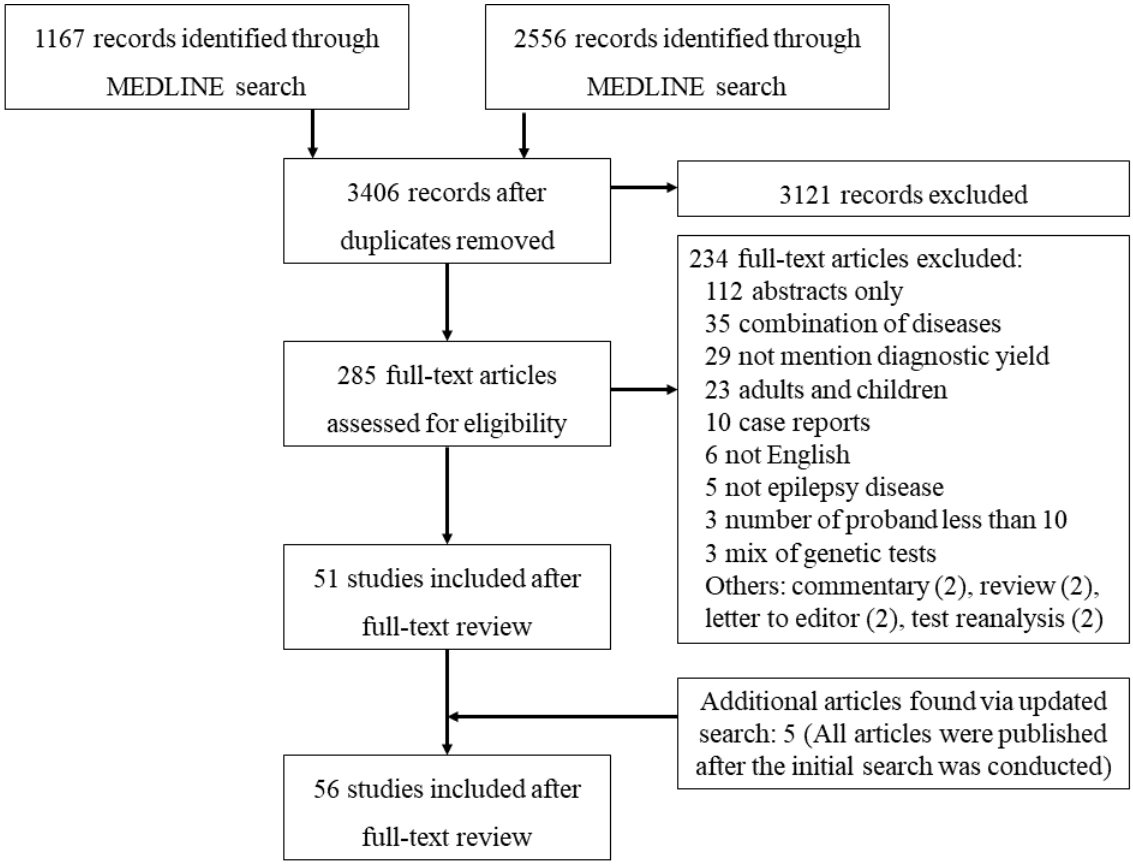


Figure 2. 2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart

The study selection procedure was shown as a PRISMA flow diagram in Figure 2.2. EMBASE and MEDLINE search yielded 3,406 records after removing duplicated articles. After a review of abstracts, 285 studies were included for full-text review. After reviewing the full-text studies, 51 articles were selected, and 234 studies were excluded. Our second search conducted in March 2020 resulted in additional 5 studies. As a result, 56 studies were included in our analysis (Figure 2.2).

2.3.2 Study characteristics

Table 2.2 presents the characteristics of 56 included studies, including the inclusion criteria, molecular diagnostic yield, and clinical utility of EP, WES, WGS, and CMA in pediatric

epilepsy. Patients were diverse in terms of clinically defined syndromes (e.g., West syndrome, infantile spasms), age of onset (e.g., onset before 18 years, early-onset epilepsy) and neurological features (e.g., developmental delay, co-occurring with autism spectrum disorder). In 14 studies, EP was performed as a second-tier test after a normal CMA results and/or normal metabolic and MRI results. In 18 studies, EP was used as a first-tier test. WES was performed as a second-tier test in 4 studies and as a first-tier technique in the remaining 7 studies. CMA was used as a first-tier test and only in probands. The majority of studies reported sequence analysis of EP in probands (singleton-EP) whereas only 2 studies investigated EP in probands and their parents (trio-EP). The number of studies reporting WES performed in probands (singleton-WES) and proband-parents (trio-WES) was 7 and 6 articles, respectively. Regarding laboratory settings, there was no study of genetic tests experimentally performed in laboratories. There were 15 studies reporting WES performed in the hospital laboratories while no study reported WES in the reference laboratories. The hospital laboratories in which EP was performed were shown in 18 articles, while the reference laboratories performing EP were stated in 11 articles. CMA was more likely performed in the hospital laboratories rather than in the reference laboratories (11 versus 6 articles). Neurological features including intellectual delay and developmental delay were reported in most studies.

Table 2. 2 Characteristics of 56 studies included in our study

First author, year	Study population	Type of genetic test	Singleton or Trio	Molecular diagnostic yield (%)	Clinical utility (%)
WGS					
Ostrander et al., 2018 [146]	EOEE	WGS	Trio	100% (14/14)	N.A.
Hamdan et al., 2017 [147]	DDE and pharmaco-resistant seizure	WGS	Trio	32% (63/197)	N.A.
WES					
Costain et al., 2019 [148]	Childhood epilepsy	WES	Both	37% (40/109)	19% (21/109)
Yang et al., 2019 [123]	Epilepsy children with onset within first year of life	WES	Both	42% (108/257)	N.A.
Demos et al., 2019 [149]	Early onset epilepsy	WES	Both	33% (59/180)	39% (23/59)
Long et al., 2019 [150]	Epilepsy co-occurring with autism spectrum disorder	WES	Singleton	56% (34/61)	N.A.
Jiao et al., 2019 [151]	Epilepsy	WES	Both	37% (63/172)	N.A.
Tsang et al., 2019 [152]	Drug-resistant epilepsy	WES	Singleton	12% (6/50)	N.A.
Papuc et al., 2019 [142]	EOEE	WES	Both	33% (20/60)	N.A.
Yuskaitis et al., 2018 [153]	Infantile spasm	WES	Trio	15% (15/100)	N.A.
Peng et al., 2019 [154]	Drug-resistant epilepsy	WES	Singleton	18% (13/74)	55% (34/62)
Palmer et al., 2018 [134]	EE	WES	Trio	50% (15/30)	N.A.

Bruun et al., 2018 [155]	Neonatal encephalopathy	WES	Trio	36% (5/14)	N.A.
Howell et al., 2018 [9]	Severe epilepsies of infancy	WES	Singleton	27% (18/66)	N.A.
Berg et al., 2017 [156]	Early-life epilepsy	WES	Singleton	31% (16/51)	N.A.
Kobayashi et al., 2016 [157]	EOEE with infantile movement disorder	WES	Singleton	80% (8/10)	N.A.
Dimassi et al., 2016 [158]	Sporadic infantile spasms	WES	Trio	40% (4/10)	N.A.
Allen et al., 2016 [159]	EOEE	WES (analyze with 137 genes)	Singleton	22% (11/50)	N.A.
Michaud et al., 2014 [160]	Infantile spasms	WES	Trio	73% (13/18)	N.A.
Veeramah et al., 2013 [161]	EE	WES	Trio	70% (7/10)	N.A.
EP					
Na et al., 2020 [55]	EOEE	EP (172 genes)	Both	35% (52/150)	18% (27/150)
Truty et al., 2019 [53]	Childhood epilepsy	EP (up to 183 genes)	Both	15% (1502/9769)	5% (491/9769)
Costain et al., 2019 [148]	Childhood epilepsy	EP (up to 666 genes)	Both	19% (31/163)	2% (4/163)
Hoezl et al., 2020 [162]	Pediatric epilepsy	EP (up to 434 genes)	Both	18% (16/91)	8% (7/91)
Yang et al., 2019 [123]	Epilepsy children with onset within first year of life	EP	Singleton	27% (127/476)	N.A.
Symonds et al., 2019 [163]	Children with epilepsy younger than 36 months	EP (104 genes)	Singleton	25% (52/307)	N.A.

Yamamoto et al., 2019 [164]	Children with epilepsy	EP	Both	29% (13/45)	N.A.
Long et al., 2019 [150]	Co-occurring epilepsy with autism disorder spectrum	EP	Singleton	29% (6/14)	N.A.
Balciuniene et al., 2019 [165]	Idiopathic epilepsy	EP	Both	11% (16/151) 18% (27/151)	N.A. N.A.
Wang et al., 2019a [166]	Children with unexplained epilepsy	EP (437 genes)	Singleton	28% (22/120)	N.A.
Angione et al., 2019 [167]	Epilepsy with myoclonic-atonic seizures	EP (38 to 89 genes)	Singleton	4% (2/51)	N.A.
Liu et al., 2018a [48]	Pediatric refractory epilepsy	EP (153 genes)	Singleton	23% (40/172)	48% (19/40)
Miao et al., 2018 [168]	Pediatric epilepsy	EP (480 genes)	Singleton	28% (39/141)	N.A.
Gieldon et al., 2018 [169]	Seizure with ID	EP	Both	23% (7/31)	N.A.
Peng et al., 2019 [154]	Drug-resistant epilepsy	EP (epilepsy and Mendelian-related genes)	Singleton	36% (72/199)	N.A.
Kothur et al., 2018 [170]	EE	EP (71 genes)	Singleton	29% (30/105)	N.A.
Oates et al., 2018 [47]	Onset <2 years, resistant or familial epilepsy	EP (45 to 102 genes)	Singleton	20% (19/94)	63% (12/19)
Stanek et al., 2018 [171]	Severe childhood epilepsy	EP (97 to 112 genes)	Both	28% (42/151)	N.A.
Fung et al., 2017 [172]	Cryptogenic neonatal/infantile EE	EP (430 genes)	Singleton	29% (9/31)	N.A.
Ko et al., 2018 [173]	DEE	EP (172 genes)	Singleton	37% (103/278)	27% (28/103)

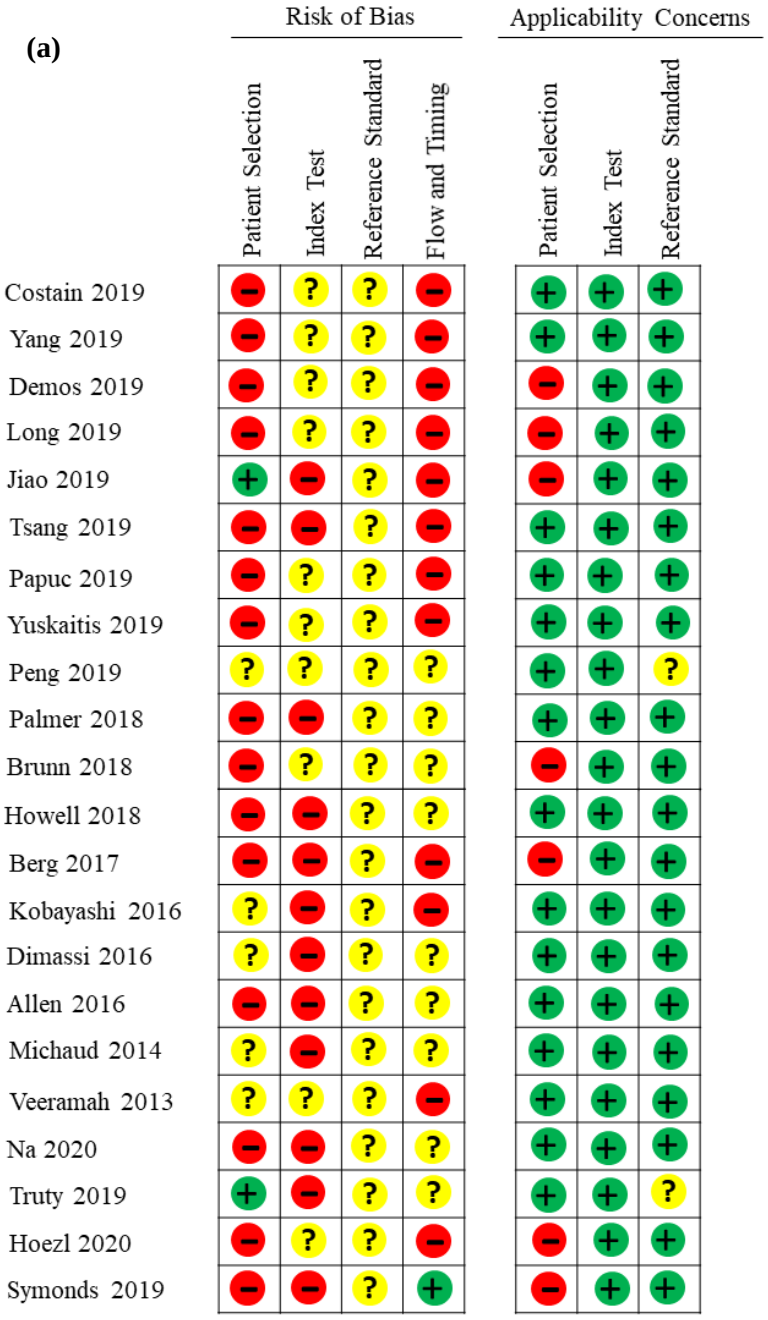
Rim et al., 2018 [174]	Intractable early onset epilepsy	EP (172 genes)	Singleton	38% (28/74)	N.A.
Zhou et al., 2018 [175]	EE	EP (480 genes)	Trio	34% (24/70)	N.A.
Ortega-Moreno et al., 2017 [176]	Pediatric epilepsy and DD	EP (83 to 106 genes)	Singleton	20% (17/87)	N.A.
Berg et al., 2017 [156]	Early-life epilepsy	EP	Singleton	28% (59/210)	N.A.
Arafat et al., 2017 [177]	Early infantile EE	EP (308 genes)	Singleton	19% (13/68)	N.A.
Zhang et al., 2017 [178]	EOEE	EP (17 genes)	Singleton	32% (56/175)	N.A.
Gokben et al., 2017 [179]	EOEE	EP (16 genes)	Singleton	40% (12/30)	N.A.
Segal et al., 2016 [180]	Medication resistant epilepsy	EP	Singleton	57% (28/49)	N.A.
Trump et al., 2016 [181]	Early-onset epilepsy and disorders of severe DD	EP (46 genes)	Singleton	18% (71/400)	N.A.
Zhang et al., 2015 [182]	Epilepsy and ID/DD	EP (300 genes)	Both	18% (46/253)	N.A.
Mercimek-Mahmutoglu et al., 2015 [40]	Unexplained pediatric epilepsy	EP (38-70 genes)	Singleton	15% (14/93)	N.A.
Ream and Mikati, 2014 [183]	Pediatric drug resistant epilepsy	EP (38-53 genes)	Singleton	46% (6/13)	N.A.
(Della Mina et al., 2015 [184]	Epilepsy < 4 years old	EP (67 genes)	Singleton	47% (9/19)	N.A.
Wirrell et al., 2015 [185]	Infants with newly diagnosed West syndrome	EP	Singleton	32% (11/34)	N.A.
CMA					

Jiao et al., 2019 [151]	Children with rare neurological disorders	CMA	Singleton	17% (22/132)	N.A.
Tsang et al., 2019 [152]	Drug-resistant epilepsy	CMA	Singleton	0% (0/50)	N.A.
Angione et al., 2019 [167]	Epilepsy with myoclonic-atonic seizures	CMA	Singleton	3% (1/37)	N.A.
Papuc et al., 2019 [142]	EOEE and combined DEE	CMA	Singleton	12% (6/50)	N.A.
Oates et al., 2018 [47]	Onset <2 years, resistant or familial epilepsy	CMA	Singleton	22% (16/74)	N.A.
Berg et al., 2017 [156]	Early-life epilepsy	CMA	Singleton	14% (40/289)	N.A.
Mercimek-Mahmutoglu et al., 2015 [40]	Unexplained pediatric epilepsy	CMA	Singleton	2% (2/110)	N.A.
Ream and Mikati, 2014 [183]	Drug-resistant epilepsy	CMA	Singleton	17% (2/12)	N.A.
Wang et al., 2019b [186]	Epilepsy and DD/ID	CMA	Singleton	27% (13/49)	N.A.
Vlaskamp et al., 2017 [187]	Pediatric epilepsy	CMA	Singleton	11% (24/226)	N.A.
Allen et al., 2015 [188]	Unexplained severe early-onset epilepsy	CMA	Singleton	6% (3/51)	N.A.
Wirrell et al., 2015 [185]	Infants with newly diagnosed West syndrome	CMA	Singleton	14% (11/34)	N.A.
Boutry-Kryza et al., 2015 [189]	Infantile spasms	CMA	Singleton	15% (11/73)	N.A.
Michaud et al., 2014 [160]	Infantile spasms	CMA	Singleton	14% (6/44)	N.A.
Olson et al., 2014 [190]	Pediatric epilepsy	CMA	Singleton	5% (40/805)	N.A.

Helbig et al., 2014 [191]	Childhood epilepsy and complex phenotypes including structural brain lesions	CMA	Singleton	7% (16/223)	N.A.
Du et al., 2014 [192]	Infantile spasms	CMA	Singleton	9% (4/47)	N.A.

Note. N.A. not applicable, EE, epileptic encephalopathy; EOEE, early-onset epileptic encephalopathy; DEE, developmental and epileptic encephalopathy; DD, developmental delay; ID, intellectual delay

2.3.3. Quality of studies



(a)

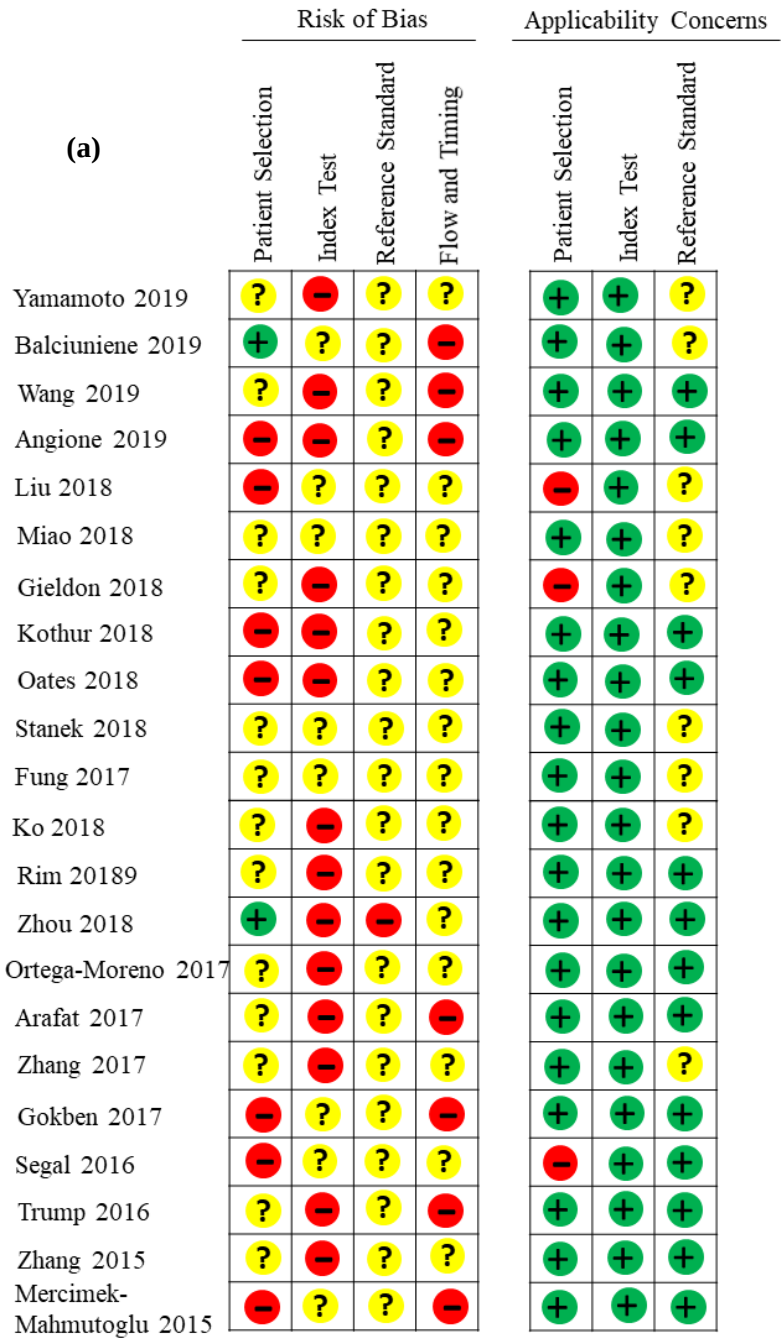

Risk of Bias
Applicability Concerns
Patient Selection
Index Test
Reference Standard
Flow and Timing
Patient Selection
Index Test
Reference Standard
Yamamoto 2019
Balciuniene 2019
Wang 2019
Angione 2019
Liu 2018
Miao 2018
Gieldon 2018
Kothur 2018
Oates 2018
Stanek 2018
Fung 2017
Ko 2018
Rim 20189
Zhou 2018
Ortega-Moreno 2017
Arafat 2017
Zhang 2017
Gokben 2017
Segal 2016
Trump 2016
Zhang 2015
Mercimek-Mahmutoglu 2015

Figure 2. 3. Risk of bias and applicability concerns summary (a); risk of bias and applicability concerns graph (b)

The quality of included studies was assessed using the QUADAS-2 tool with detailed information shown in Appendix 1. The risk of bias and applicability concerns are shown in Figure 2.3 (a) for each study and graphically summarized across all included studies in Figure 2.3 (b).

Most studies showed low risk for patient selection, reference standard, and flow and timing bias. As for index test bias, xx studies were at unclear risk because information was insufficient to

ensure that index test results were interpreted without knowledge of the results of the reference standard. The majority of studies included in this meta-analysis inspired low concern about applicability (Figure 2.3b)

Risk of bias

In the 'patient selection' domain, the judgement for risk of bias was influenced largely by non-random or non-consecutive selection of patients. 28 studies (52%) were considered at high risk of bias because all of these studies had non-random and non-consecutive patient selection. 22 studies (41%) were categorized unclear risk of bias because they did not show whether patients were consecutive or randomly recruited.

Regarding the 'index test' domain, no study was considered at low risk of bias. The risk of bias was judged to be high in 31 studies (57%) and unclear in the remaining 23 studies (43%). Of the 31 studies assessed at high risk of bias, 26 studies (84%) had the index test interpreted with knowledge of the reference standard results.

For the reference standard domain, all studies were judged to be at unclear risk of bias because it was unclear that the reference standard correctly classify the target condition.

In the 'flow and timing' domain, 24 studies were considered to have high risk of bias while 22 studies were judged to be at unclear risk of bias. Only one study provided information about the appropriate interval between the index test and reference standard.

Applicability concerns

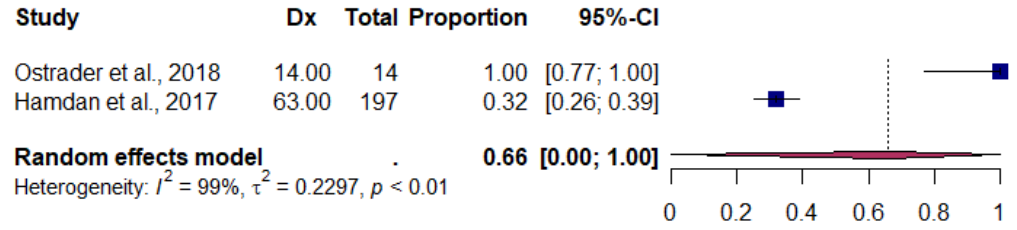
For the 'patient selection' domain, 12 studies (22%) were considered to be of high applicability concerns. These studies focused on patients with different risk of genetic etiology. This population did not represent a real-life situation of pediatric patients clinically tested with genetic tests. The majority of studies were considered to have low applicability concerns for the 'index test' and 'reference standard' domain because the studies matched the review question (Figure 2.3b).

2.3.3 Diagnostic yield in studies of EP, WES, WGS, and CMA

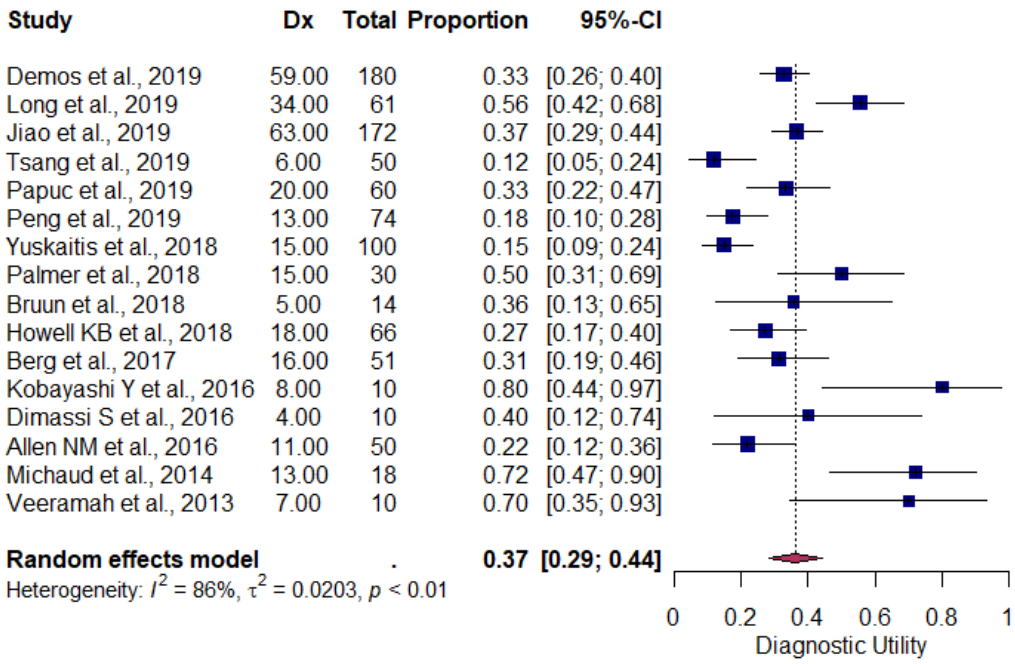
A random-effects meta-analysis of the 51 articles showed that molecular diagnostic yield was highest for WGS (0.66; 95% CI 0.00-1.00, two studies, 211 children, I^2 =99%), followed by WES (0.37; 95% CI 0.29-0.44, sixteen studies, 956 children, I^2 =86%), and EP (0.26; 95% CI 0.22-

0.29, thirty studies, 3,616 children, I^2 =86%). The molecular diagnostic yield of CMA was lowest (0.10; 95% CI 0.07-0.13, seventeen studies, 2,306 children, I^2 =85%). High heterogeneity (I^2 >75%) occurred in all test groups (WGS, WES, EP, and CMA). The funnel plots revealed that the high molecular diagnostic yield of WGS was likely due to a small number of studies (Figure 2.4).

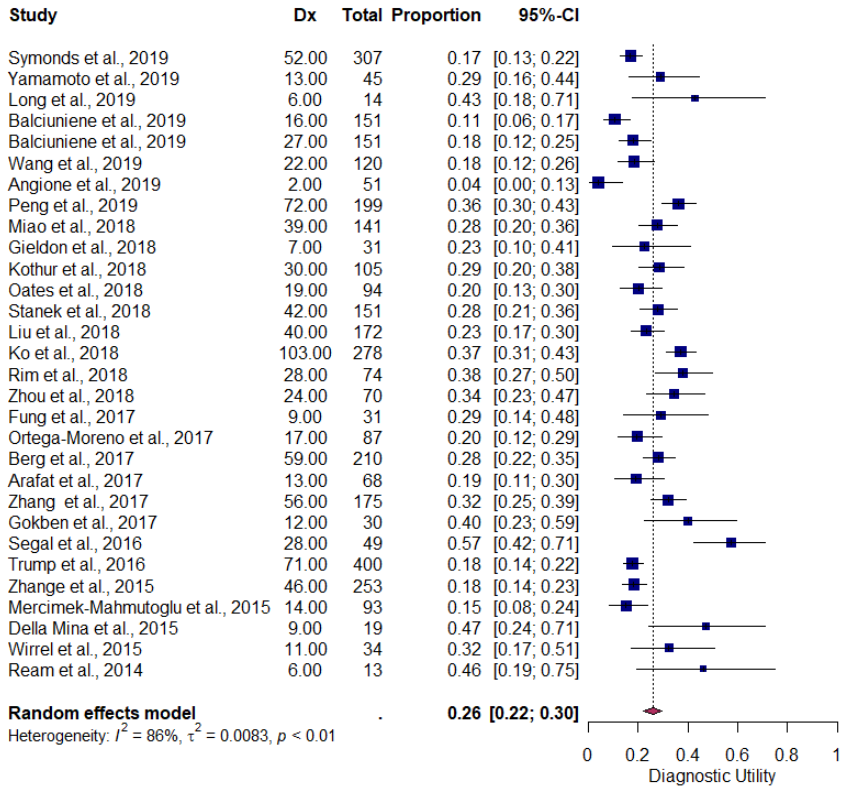
WGS



WES



EP



CMA

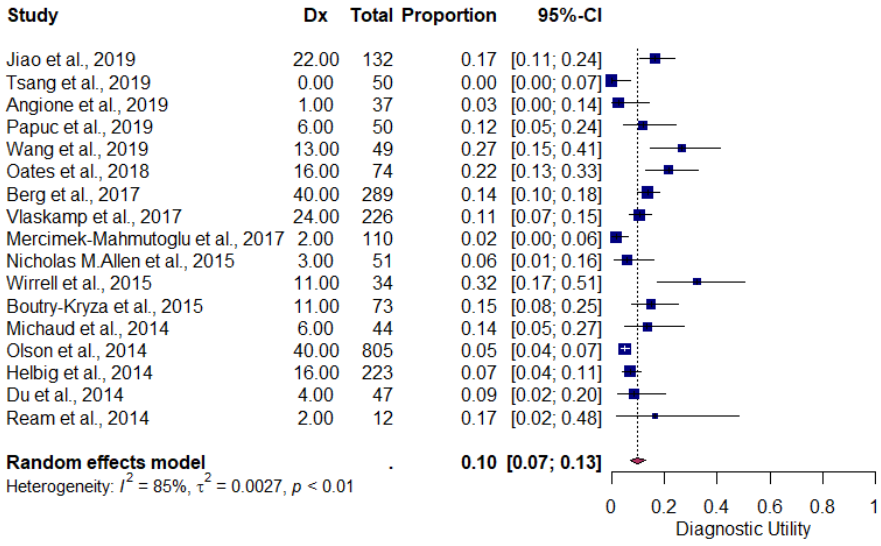


Figure 2. 4 Meta-analysis of the molecular diagnostic yield of the different genetic tests

Note. CI, confidence interval; WGS, whole-genome sequencing; WES, whole-exome sequencing; EP, epilepsy panels; CMA, chromosomal microarray

2.3.4 Heterogeneity analyses of molecular diagnostic yield in studies of EP, WES, WGS, and CMA

Heterogeneity between studies reporting the molecular diagnostic yield of EP, WES, WGS, and CMA was analyzed by meta-regression. Studies investigating the molecular diagnostic yield of WES and EP were published during the period 2013-2019. We found that the odds of diagnosis identified by WES or EP decreased by 18% every year (Figure 2.5a, P =0.0182). Our results also demonstrated that the odds of diagnosis achieved by CMA grew by 6% every year (Figure 2.5c, P =0.2167).

Sample size in studies of WES and EP ranged from 10 to 400 probands. We found a modest association of study sample size with molecular diagnostic yield of WES/EP. The odds of diagnosis by WES/EP reduced by 7% when the number of probands increased by 100 (Figure 2.5b, P =0.0295). The number of probands in studies of CMA varied between 12 and 805. Our meta-regression demonstrated that an increase of 100 probands reduced the odds of diagnosis by CMA by 23% (Figure 2.5d, P =0.0058).

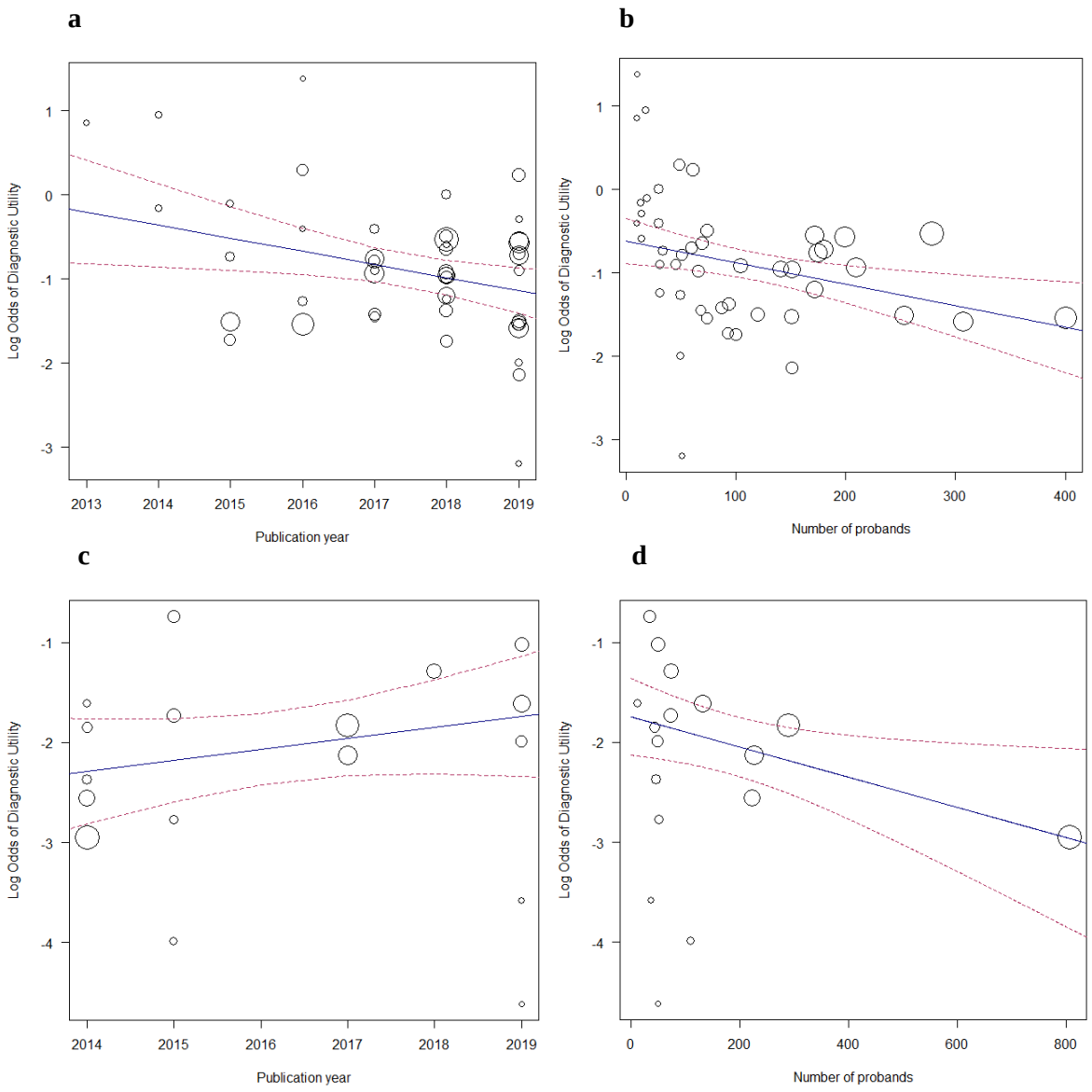


Figure 2. 5 Heterogeneity of molecular diagnostic yield in WES and EP and CMA studies a. Scatterplot of diagnostic yield by WES/EP versus year of publication b. Scatterplot of diagnostic yield by WES/EP versus the number of probands c. Scatterplot of diagnostic yield by CMA versus year of publication d. Scatterplot of diagnostic yield by CMA versus the number of probands

2.3.5 Diagnostic yield by age of onset

We divided patients into six subgroups by age of seizure onset: (1) neonatal-onset epilepsy, (2) infant-onset epilepsy, (3) toddler-onset epilepsy, (4) early childhood-onset epilepsy, and (5) middle-onset epilepsy, and (6) adolescent-onset epilepsy. However, there was no studies on diagnostic yield of WES and EP in adolescent-onset epilepsy.

The overall diagnostic yield of WES and EP was the highest among neonatal-onset epilepsies (61%) followed by infant-onset epilepsies (36%). The diagnostic yields of WES/EP in older onset age of seizures including toddler, early childhood and middle childhood were comparable (26%, 30% and 24%). We compared two groups categorized by age of onset using the Fisher's exact test. Significant relationships between the age of onset and diagnostic yield were observed for neonatal compared with the remained group ($p<0.05$) and infants compared with toddler ($p<0.05$). However, no significant difference was observed when comparing infants with early childhood ($p=1$), and toddler with middle childhood ($p=0.7544$).

In neonatal-onset epilepsy, the diagnostic yield of WES and EP considerably ranged from 36% to 80%. This variability was probably due to both the small number of studies and the small number of patients in all included studies. In neonatal-onset patients, there was 4 studies reporting diagnostic yield of EP while only one study investigated the diagnostic yield of WES [47, 55, 171, 173]. The diagnostic yield of EP in neonatal-onset epilepsies was higher than that of WES (61% versus 36%) (Figure 2.6). Significant difference was observed between EP and WES in this patient group ($p=0.0084$; the result is significant at $p<0.05$). The unusual high diagnostic yield of EP can be explained by a small number of patients. Moreover, WES was reported from a single study. Therefore, future studies with larger cohort of patients might provide a reasonably unbiased estimate of diagnostic yield of WES/EP in neonatal-onset epilepsy.

In infant-onset epilepsy, the diagnostic yield of WES and EP ranged from 15% to 80%. In particular, a diagnostic yield of WES was reported at 44% which was higher than that of EP (31%) (Figure 2.7). However, no significant difference was observed in the diagnostic yields between WES and EP ($p=0.27$). The higher diagnostic yield by WES was due to an extremely high estimate from small cohorts by Michaud et el. (2014) (72%, 13/18) and Kobayashi et al. (2016) (80%, 8/10) [157, 160]. We then excluded these two studies from the analysis. We found that the diagnostic yield of WES became comparable to EP (30.5% vs 30.8%). This may be due to selecting the most appropriate patients for testing and having a well-designed EP.

In toddler-onset epilepsy, the diagnostic yield of EP was reported at 26%. While there was a large number of studies reporting the diagnostic yield of EP, only two studies investigated that of WES. The diagnostic yield of WES was comparable to EP (24% vs 26%) (Figure 2.8).

In epilepsy onset at early and middle childhood, the diagnostic yield of WES/EP was 30% and 24%, respectively. In these subgroups, the diagnostic yield of WES was higher than that of EP

(34% versus 24% and 39% versus 19%, respectively) (Figure 2.9 and 2.10). However, there was no significant difference observed between WES and EP in these patient population (p=0.006 and p=0.86). It should be noted that in the older age group (seizure onset >5 years old), there was a small number of studies and a small number of patients. Moreover, the diagnostic yield of WES and EP in this older age of seizure onset was relatively lower than that of young age of onset. This can be explained by fewer genes discovered and more complex etiology in older onset epilepsies. Therefore, further efforts in gene discovery will be needed for future diagnosis and management of epilepsies.

The diagnostic yield of CMA between different groups of seizure onset was relatively low and remained unchanged, ranging from 10% to 15% (Figure 2.7c, 2.8c, 2.9c, and 2.10c).

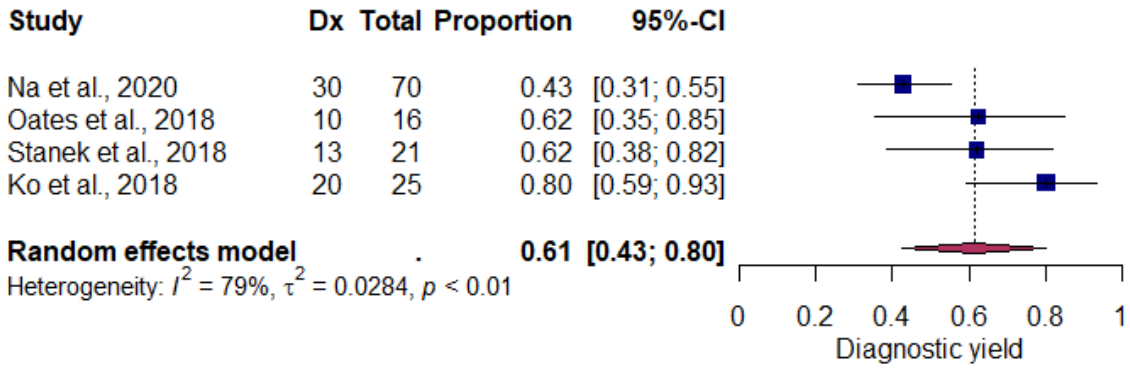
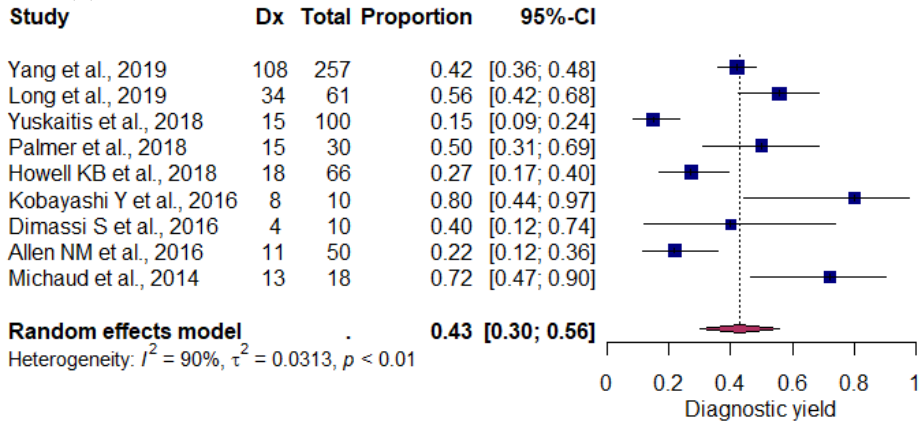
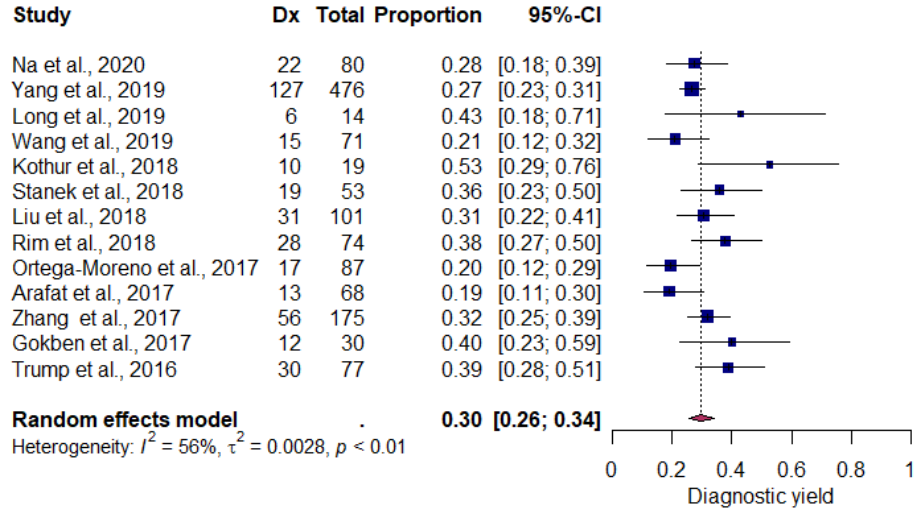


Figure 2. 6 Diagnostic yield of EP in neonatal-onset epilepsy

WES (a)



EP (b)



CMA (c)

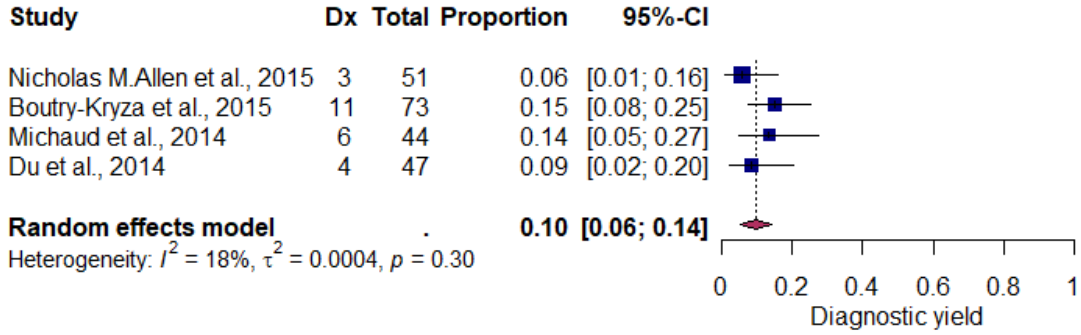
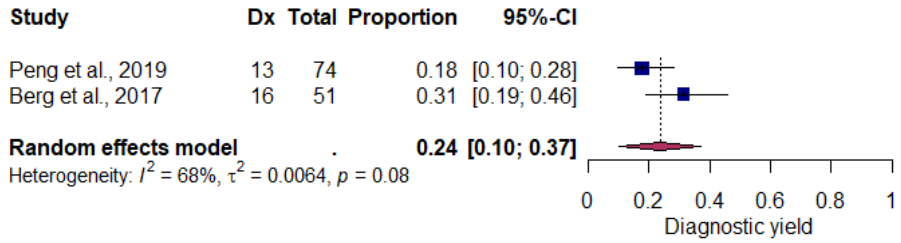
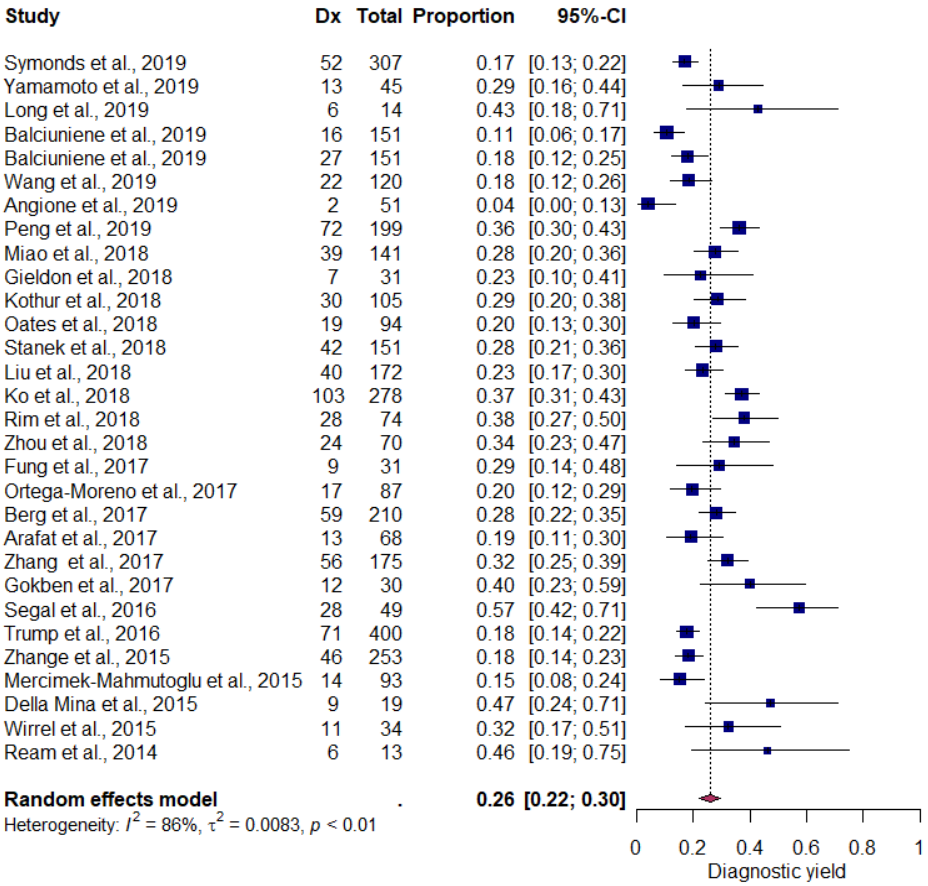


Figure 2. 7 Diagnostic yield of WES (a), EP (b), and CMA (c) in infant-onset epilepsy

WES (a)



EP (b)



CMA (c)

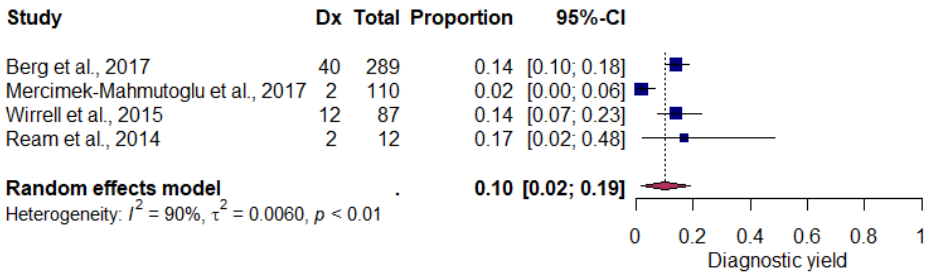
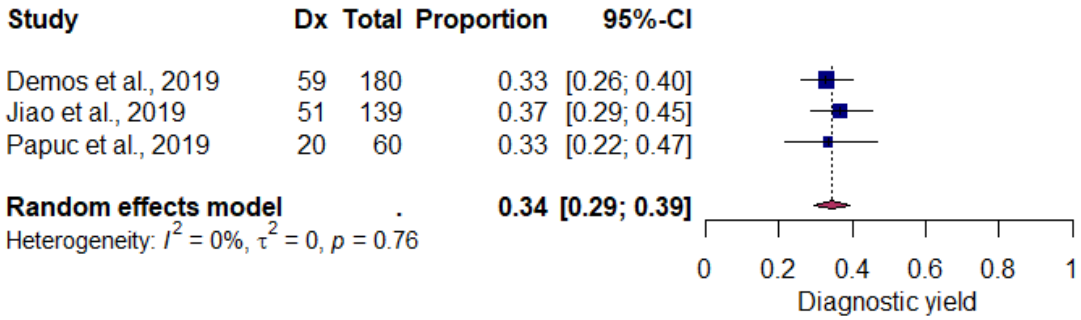
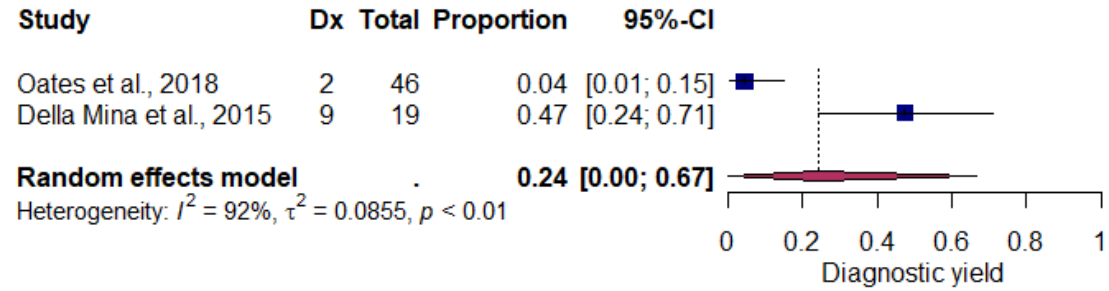


Figure 2. 8 Diagnostic yield of WES (a), EP (b), and CMA (c) in toddler-onset epilepsy

WES (a)



EP (b)



CMA (c)

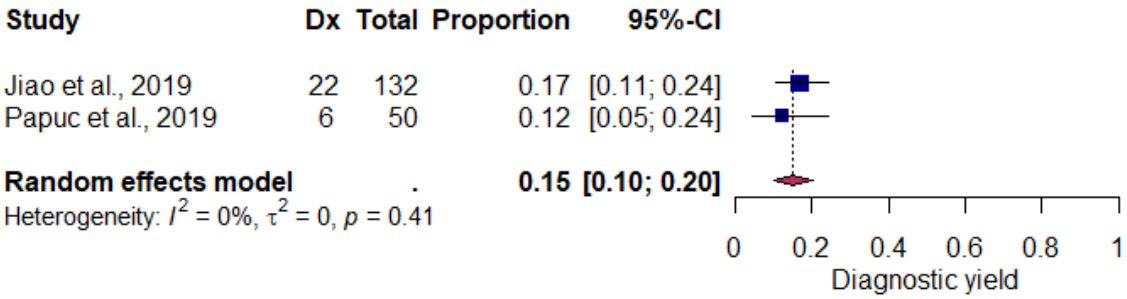
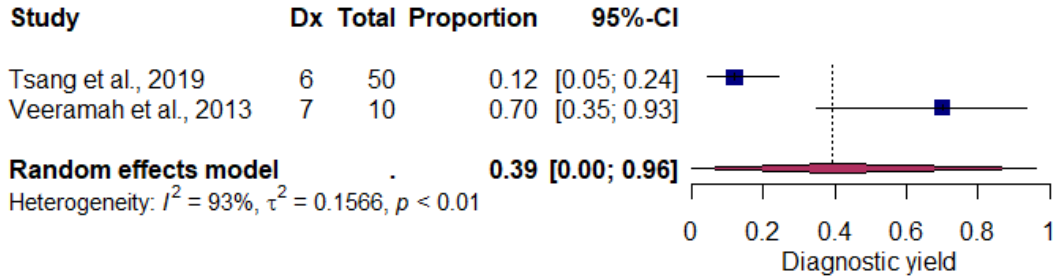
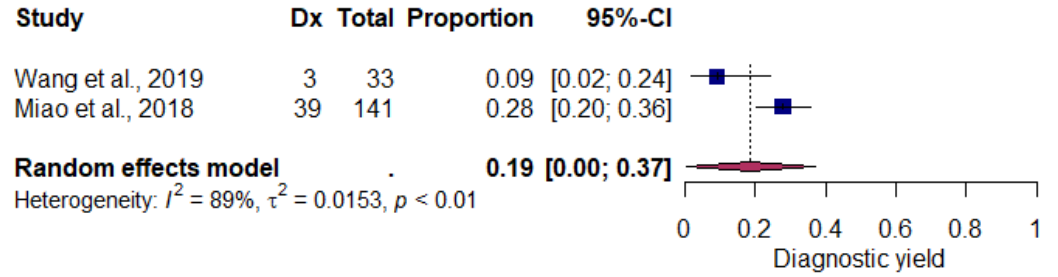


Figure 2. 9 Diagnostic yield of WES (a), EP (b) and CMA (c) in early childhood-onset epilepsy

WES (a)



EP (b)



CMA (c)

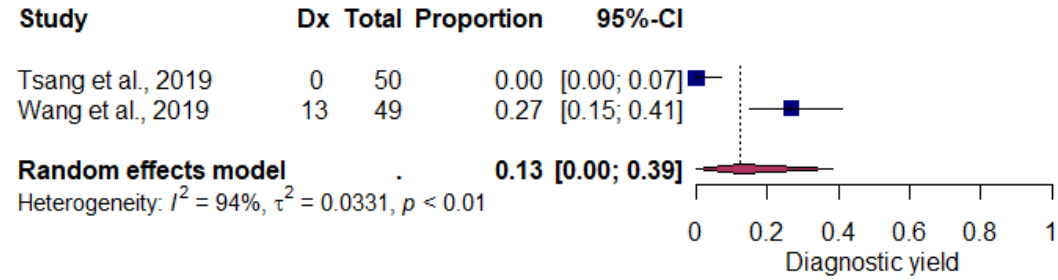


Figure 2. 10 Diagnostic yield of WES (a), EP (b), and CMA (c) in middle childhood-onset epilepsy

2.3.6 Diagnostic yield by neurological features

We aimed to stratify patients into three subgroups: (1) epilepsy plus (epilepsy and neurodevelopmental disorders such as developmental, intellectual delay, and/or behavior issues), (2) mixed epilepsy and (3) epilepsy only. However, there was no study investigating children with epilepsy only. Therefore, epilepsy plus was defined as a population with more than 80% of patients having neurodevelopmental features. The mixed epilepsy group was defined as the population with less than 80% of patients concomitant with neurodevelopmental disorders or epilepsy and neurodevelopmental features but not reporting the specific proportion of patients with epilepsy and neurodevelopmental issues.

The diagnostic yield of genetic tests in epilepsy plus neurodevelopmental disorders was higher than that in mixed epilepsy (26% versus 24%, p=0.1497) but no statistical significance was found. The diagnostic yield of WES in epilepsy plus was significantly higher than that in mixed epilepsy (48% versus 33%, p<0.05) (Figure 2.11). As the majority of causative variants in these studies was *de novo* [142, 150, 157], the higher diagnostic yield of WES in epilepsy plus can be explained by the ability of identifying *de novo* mutations [139]. However, there was no difference in diagnostic yield between epilepsy plus and mixed epilepsy in patients diagnosed by EP and CMA (26% versus 26% and 10% versus 10%, respectively) (Figure 2.12 and 2.13).

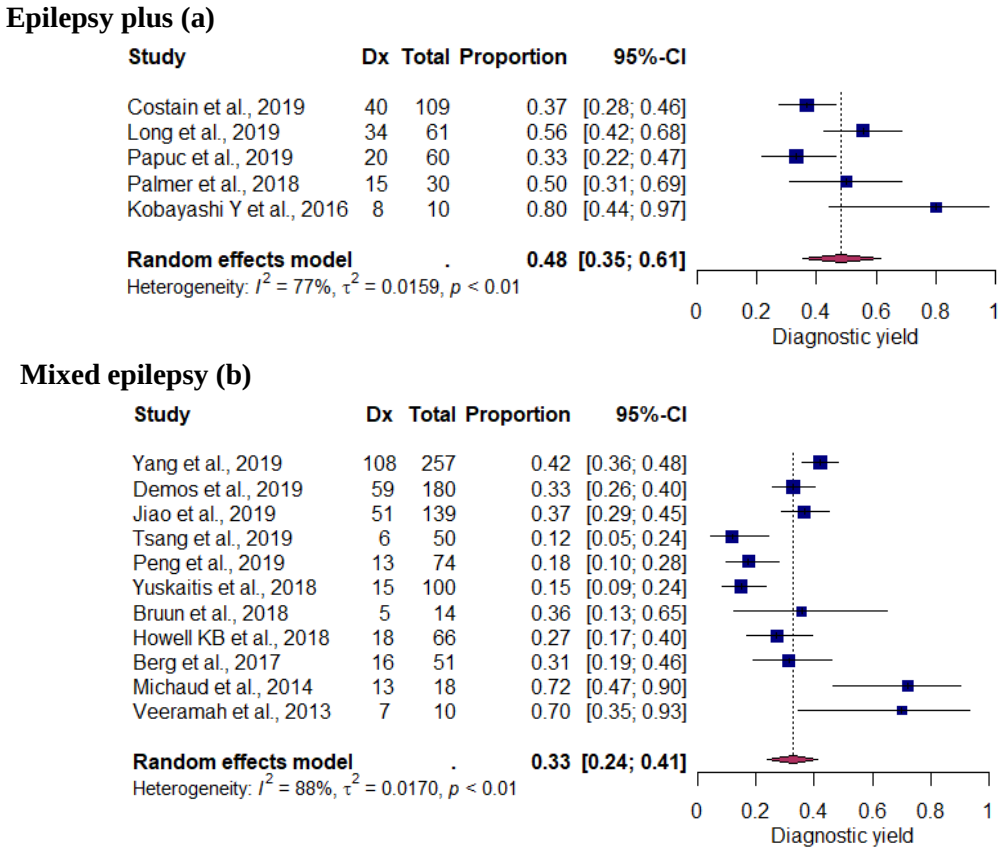
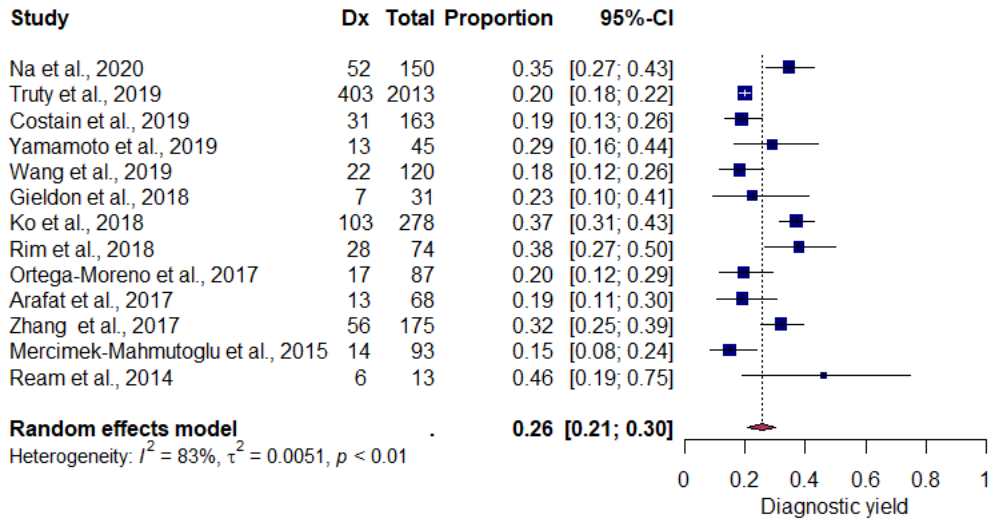


Figure 2. 11 Diagnostic yield of WES in epilepsy plus (a) and mixed epilepsy (b)

Epilepsy plus (a)



Mixed epilepsy (b)

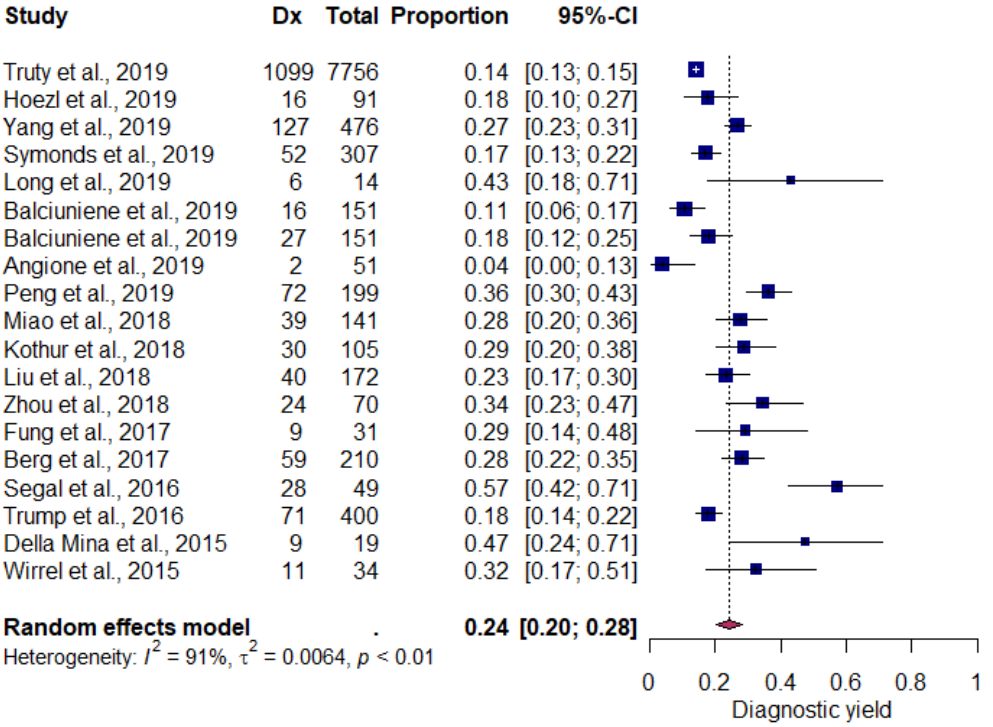
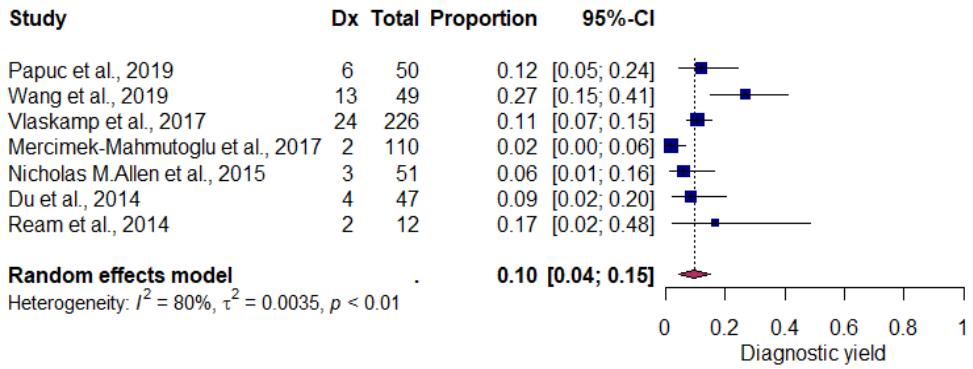


Figure 2. 12 Diagnostic yield of EP in epilepsy plus (a) and mixed epilepsy (b)

Epilepsy plus (a)



Mixed epilepsy (b)

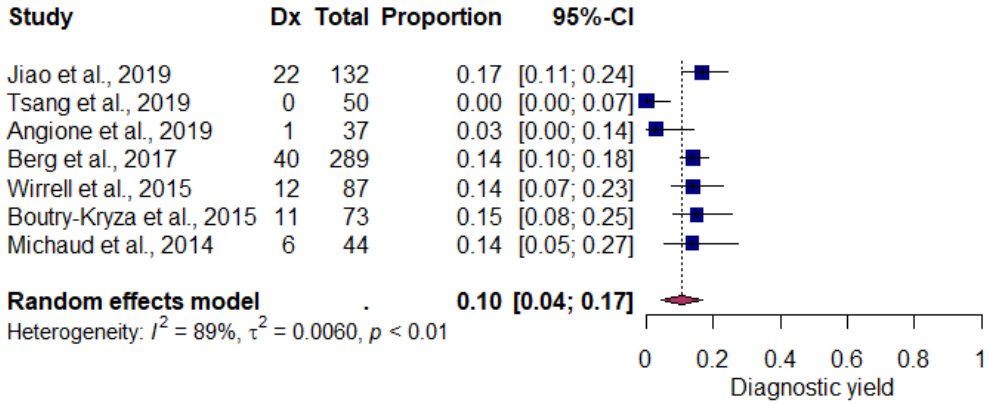


Figure 2. 13 Diagnostic yield of CMA in epilepsy plus (a) and mixed epilepsy (b)

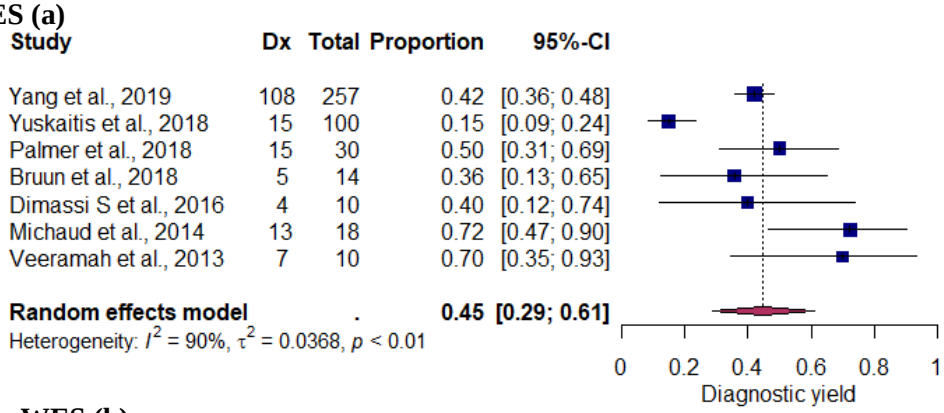
2.3.7 Diagnostic yield of genetic testing in trios versus singleton

Genetic tests can be performed either in probands (singleton) or trios (probands and their parents). All studies reported CMA performed in singleton setting while EP and WES were performed in both settings, namely singleton and trios.

There was no direct comparison in the diagnostic yield of trio-WES and singleton-WES within a study. Meta-analysis was performed in 13 studies. Trio-WES had a higher diagnostic yield than singleton-WES (45% versus 33%) (Figure 2.14). In these studies, the odds of diagnosis using trios was less than double of using singletons (95% Cl 0.77 – 1.73, p=0.4893). However, no significant difference was observed. Therefore, a direct comparison of the diagnostic yield of trio-WES with singleton-WES within the same cohort is needed.

Balciuniene et al. (2019) is the only study performing both trio-EP and single-EP within the cohort of childhood-onset epilepsy [165]. The diagnostic yield of trio-EP was higher than that of singleton-EP (15% versus 11%). No significant difference was observed (OR=1.5, p=0.3032). Estimating the diagnostic yield of trio-EP and singleton-EP across 26 studies, we found that trio-EP had a comparable diagnostic yield with singleton-EP (25% versus 26%) (Figure 2.15). It should be noted only 2 studies reported the diagnostic yield of trio-EP whereas 24 studies performed singleton-EP. Our failure to show a superiority in the diagnostic yield of trio-EP to singleton-EP was due to a significant heterogeneity between studies. Therefore, additional studies comparing trio-EP and singleton-EP with a larger sample size are needed.

Trio-WES (a)



Singleton-WES (b)

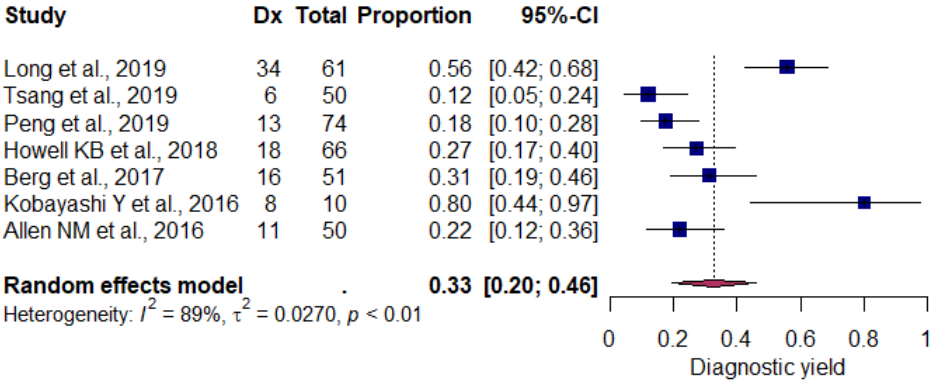
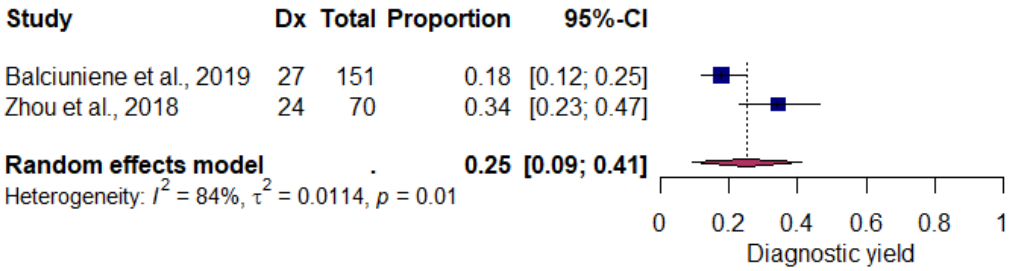


Figure 2. 14 Diagnostic yield of trio-WES (a), and singleton-WES (b)

Trio-EP (a)



Singleton-EP (b)

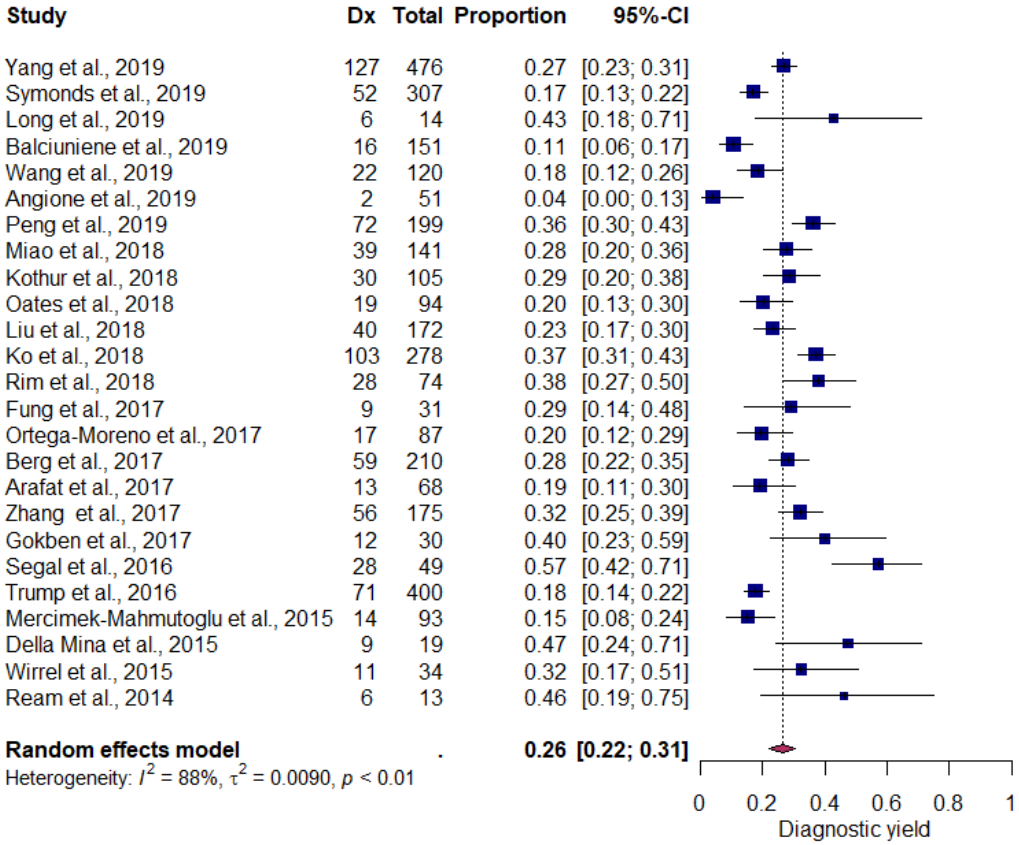


Figure 2. 15 Diagnostic yield of trio-EP (a) and singleton-EP (b)

2.3.8 Diagnostic yield by different laboratory settings

Genetic tests were performed in three settings, namely hospital laboratories, reference laboratories and experimental laboratories. Clinically testing in hospital laboratories was facilitated by communication between clinicians and geneticists. In reference laboratories communication of clinicians and geneticists was limited. Genetic tests experimentally performed in laboratories were used for research purpose of novel methods or gene discovery. In our

systematic review, no study investigating the diagnostic yield of genetic tests in experimental laboratories was found.

In 16 studies, the diagnostic yield of WES by hospital laboratories was 38% (Figure 2.16) and by reference laboratories was 12%. However, only one study reported the diagnostic performance of WES in reference laboratories [152]. The higher diagnostic yield of WES by hospital laboratories can be explained by the availability of phenotype information. In hospital setting, phenotypic information can be retrieved from medical records, concomitant investigations and probably from discussions with clinicians when needed. In reference laboratory, phenotypic information was provided in genetic orders that had fewer content. In addition, the difference between hospital and reference laboratories highlighted the complexity in interpretation of WES findings. Our findings suggest that the availability of phenotypic information and discussions between clinicians and geneticists are highly encouraged to better provide accurate diagnosis.

In contrast to WES, the diagnostic yield of EP and CMA was not different between hospital and reference laboratories (27% versus 25% and 10% versus 10%, respectively) (Figure 2.17 and 2.18). This was probably due to the mature interpretation of EP and CMA.

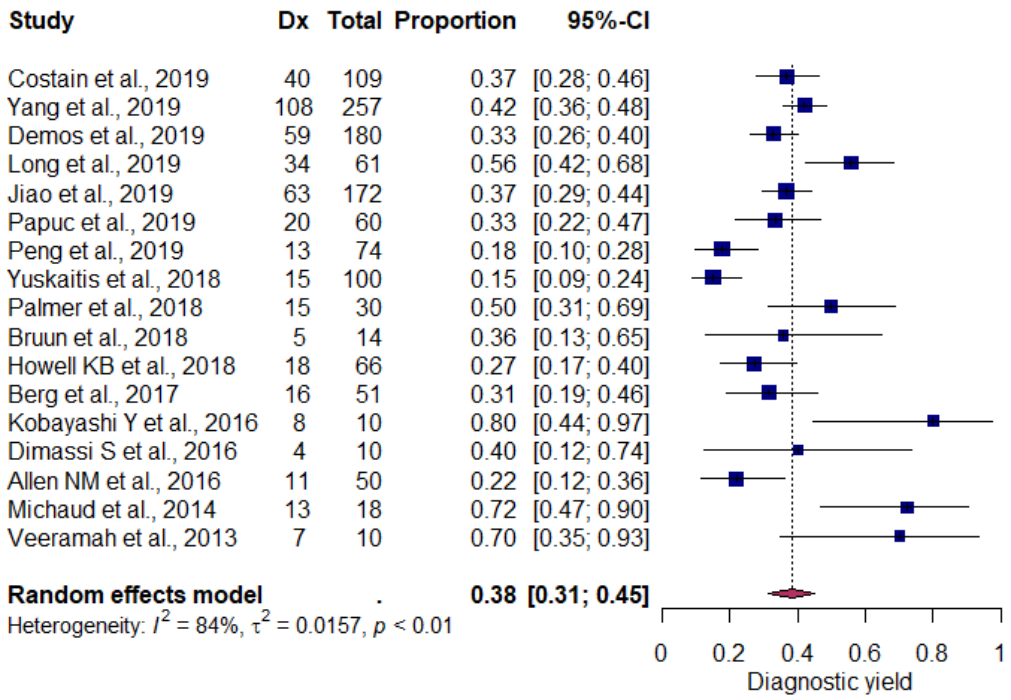
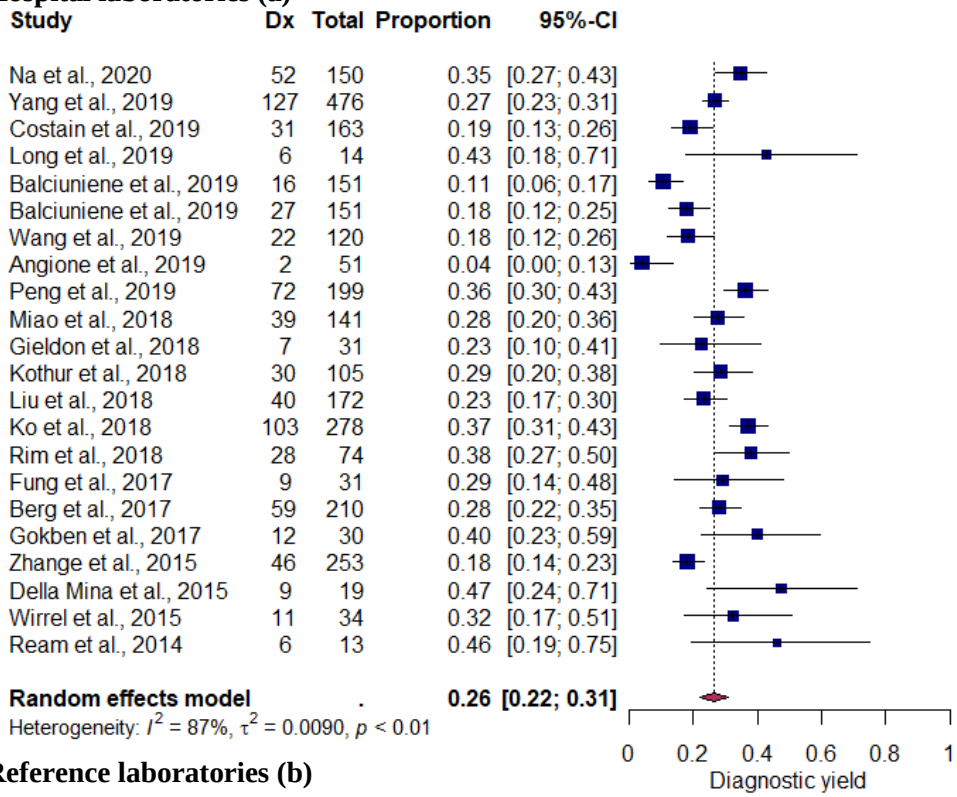


Figure 2. 16 Diagnostic yield of WES in hospital laboratories

Hospital laboratories (a)



Reference laboratories (b)

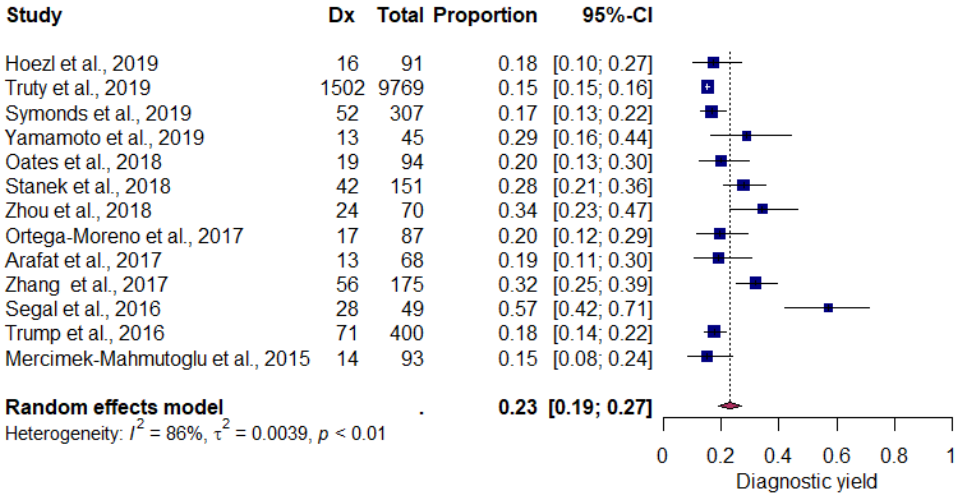
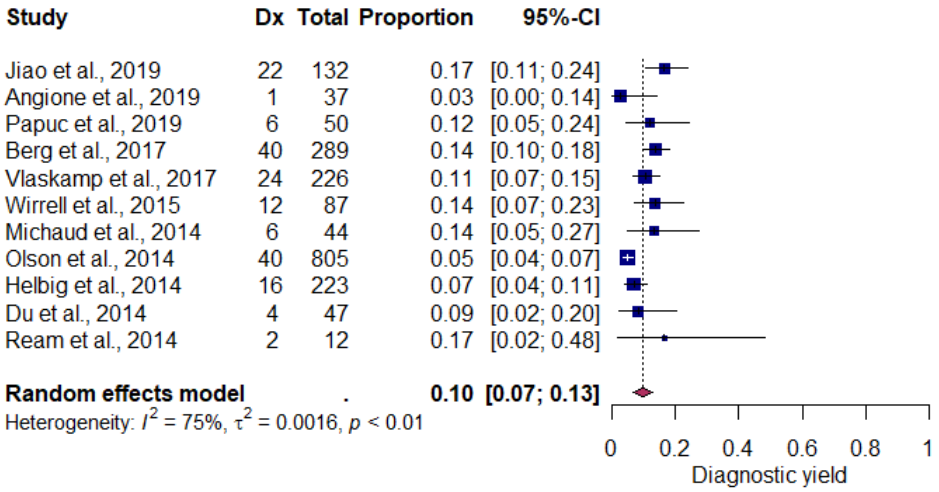


Figure 2. 17 Diagnostic yield of EP in hospital laboratories (a), reference laboratories (b)

Hospital laboratories (a)



Reference laboratories (b)

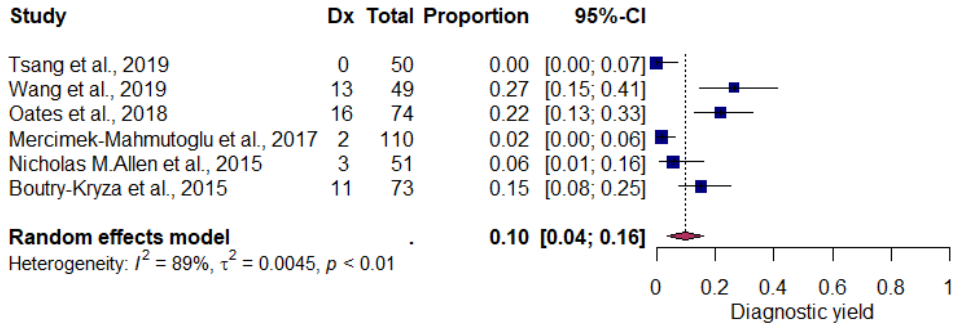


Figure 2. 18 Diagnostic yield of CMA in hospital laboratories (a), reference laboratories (b)

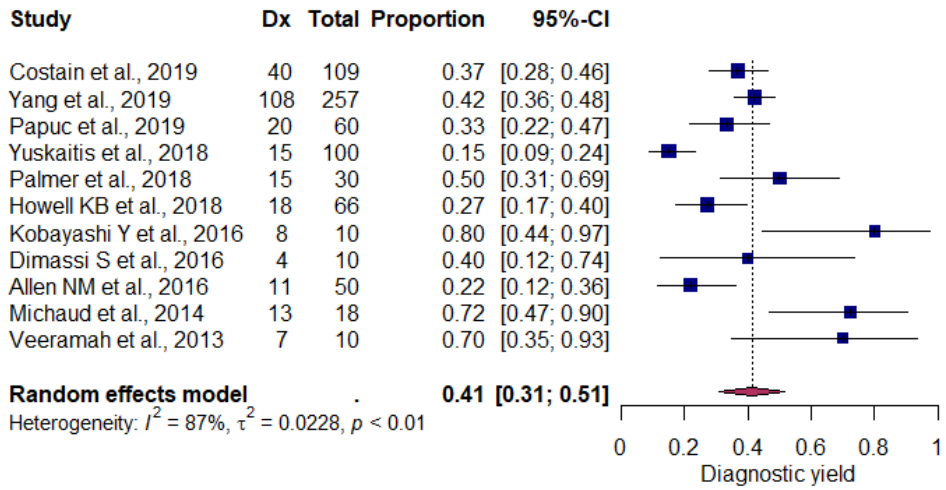
2.3.9 Diagnostic yield by second-tier versus first-tier

A factor that influences diagnostic yield is whether or not the patient has had extensive prior diagnostic testing [193]. If all structural or metabolic etiologies were ruled out by conventional first-tier tests, the diagnostic yield of genetic test would be likely high.

Diagnostic yield of WES as a second-tier test was higher than that as a first-tier test (41% versus 31%) (Figure 2.19). We did not find any difference in diagnostic yield of EP between using as a second-tier and first-tier test (26%) (Figure 2.20). However, after excluding studies published before 2017, the diagnostic yield of EP as second-tier test (30%) was significantly higher than first-tier test (24%) (OR=1.5, p<0.0001). The difference in diagnostic yield of WES and EP as second-tier versus first-tier illustrated the value of prior investigations to exclude cases of non-genetic epilepsy.

In patients undiagnosed after first-tier tests, WES gained higher diagnostic yield than EP (41% versus 26%). Similarly, WES was also better than EP in term of achieving a genetic diagnosis when using as a first-tier test (31% versus 26%).

WES as second-tier (a)



WES as first-tier (b)

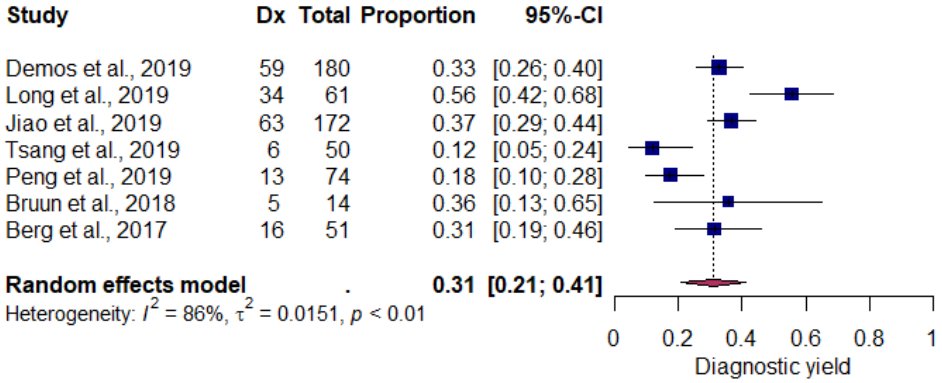
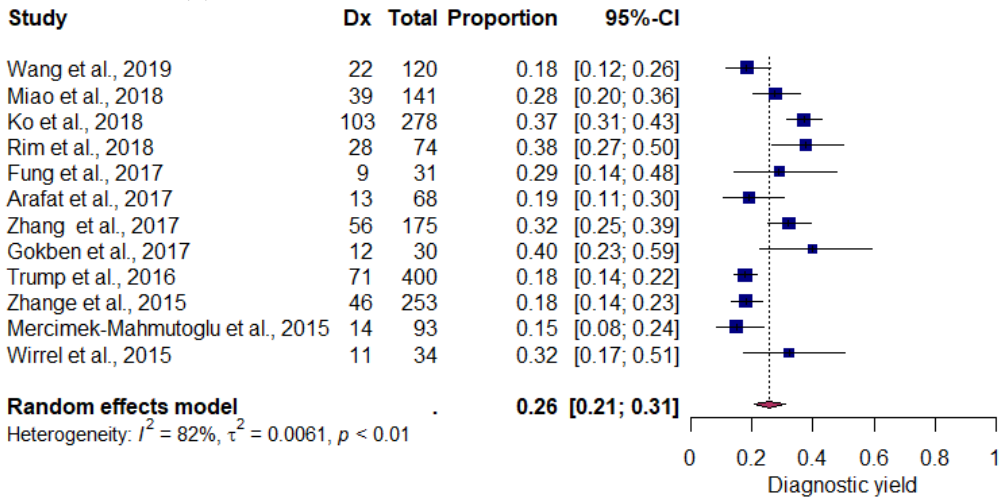


Figure 2. 19 Diagnostic yield of WES as second-tier (a) and first-tier (b)

EP as second-tier (a)



EP as first-tier (b)

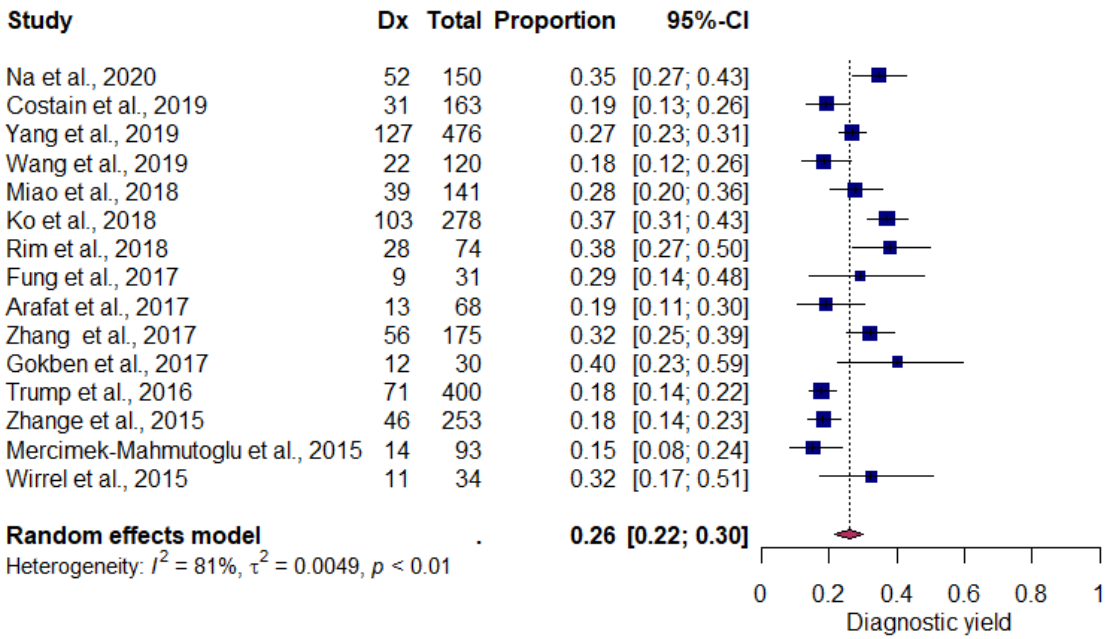


Figure 2. 20 Diagnostic yield of EP as second-tier (a), and first-tier (b)

2.3.10 Diagnostic yield of EP, WES, and CMA in early-life epilepsy

Heterogeneity between studies was mild when focusing on studies of early-life epilepsy published since 2017 with a sample size larger than 30 individuals (Figure 2.21). In 11 studies of 1,235 children with early-life epilepsy published since 2017, the diagnostic yield of WES (0.33, 95% CI 0.29-0.37, I^2=0%, P =0.71) was greater than EP (0.28, 95% CI 0.23-0.32, I^2 =47%, P =0.08). The

diagnostic yield CMA remained the lowest (0.15, 95% CI 0.12-0.18, I^2 =0%, P =0.40). No heterogeneity between studies investigating the diagnostic yield of WES and CMA (I^2=0%) for this subgroup. Moderate heterogeneity between studies providing information about the diagnostic yield of EP (I^2 =47%) (Figure 2.21).

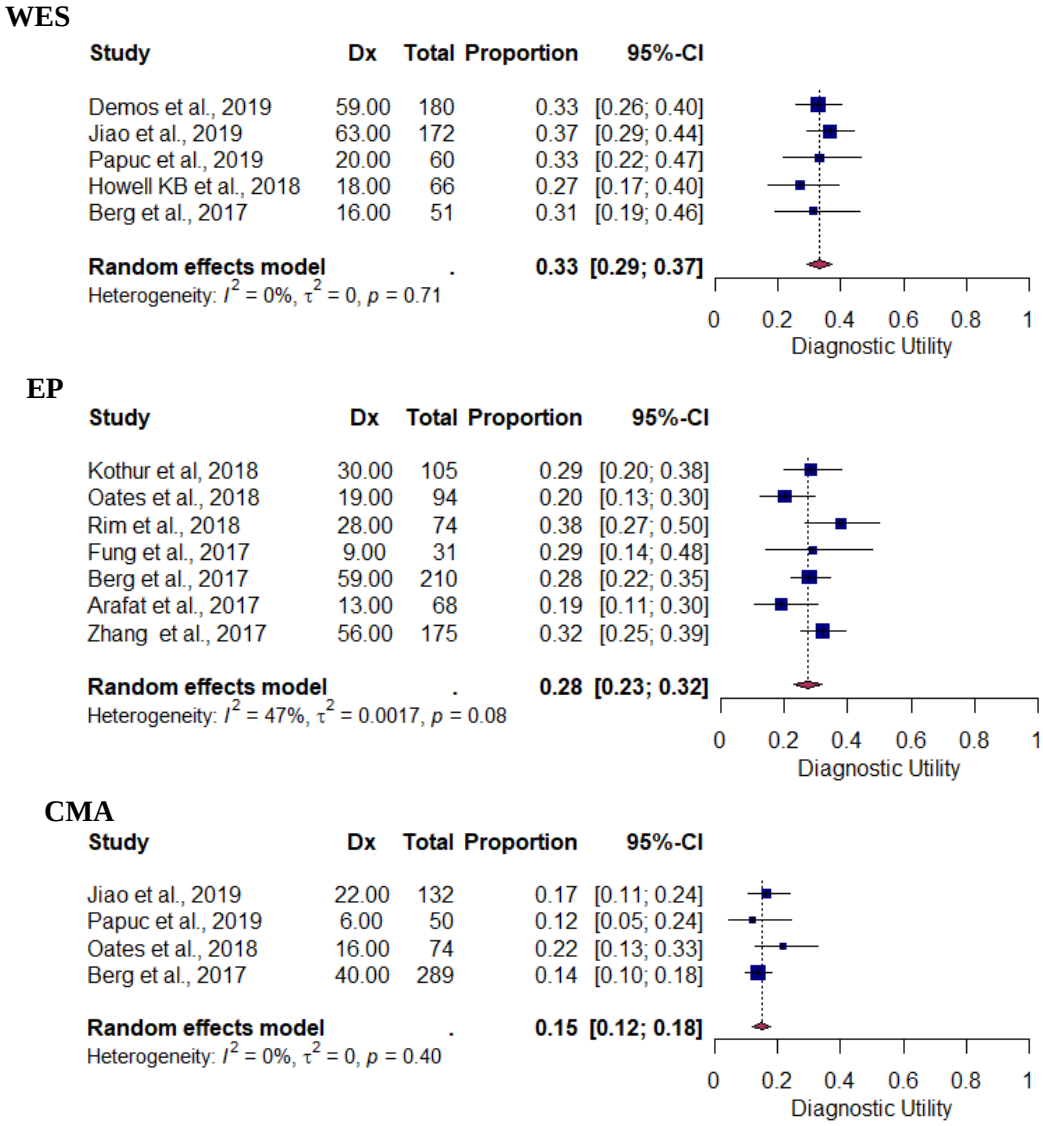


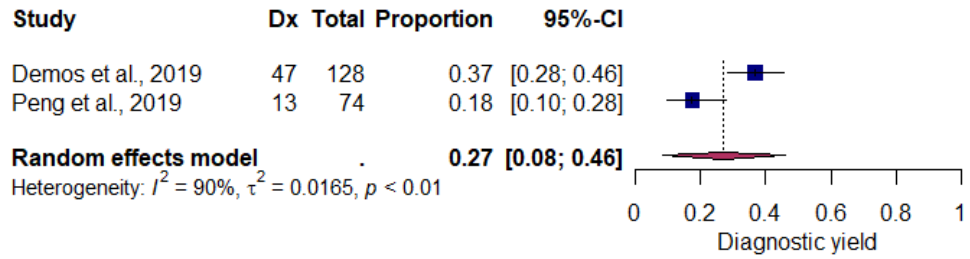
Figure 2. 21 Diagnostic yield of WES, EP, and CMA in early-life epilepsy by studies published since 2017 with sample size ≥ 30 patients

2.3.11 Diagnostic yield of EP, WES and CMA in drug-resistant epilepsy

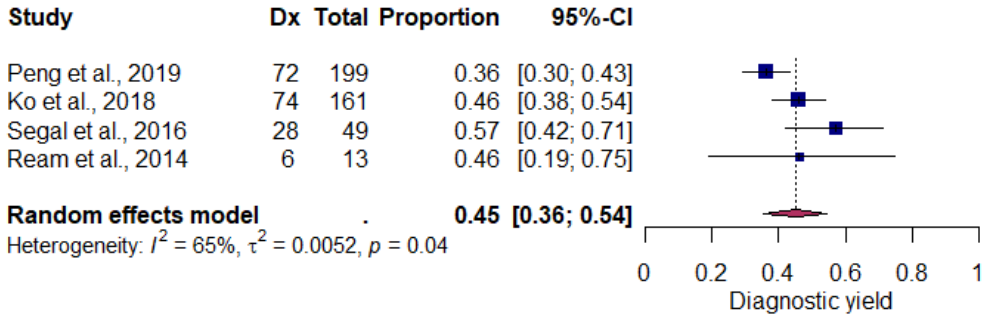
The diagnostic yield of CMA in drug-resistant epilepsy was the lowest (12%) (Figure 2.22). We might have expected a higher diagnostic yield of WES than EP in drug-resistant epilepsy. However, we did not find an increased diagnostic yield by WES compared to EP across the patients with drug-resistant epilepsy (27% versus 45%) (Figure 2.22). While two studies reporting the diagnostic yield of WES which ranged from 18% to 37%, the diagnostic yield of EP ranged from 36% to 57%. While most studies reported diagnostic yield nearly 40%, Peng et al. (2018) reported the extreme lowest yield (18%) and Segal et al (2016) reported the extreme highest yield (57%) [154, 180]. This low diagnostic yield of 18% was due to the unfinished work of identifying novel candidate of gene mutations [154]. When this is done, the higher diagnostic yield will be expected. The 57% rate was due to selection bias of a retrospective cohort of patients with high presumed genetic etiology (29%) [180].

Whether drug-resistant epilepsy has a more likelihood of identifying a genetic cause remains controversial. In the Ko et al. (2018), treatment resistance was associated with the increased probability of receiving a positive diagnostic result [173]. The odds ratios for a positive diagnostic result in children resistant to the treatment was 2.57 (p=0.0004) [173]. Conversely, in the Demos et al. (2019), no significant difference between drug-resistant epilepsy and non drug-resistant epilepsy was reported (37% versus 39%, p=0.84) [149].

WES



EP



CMA

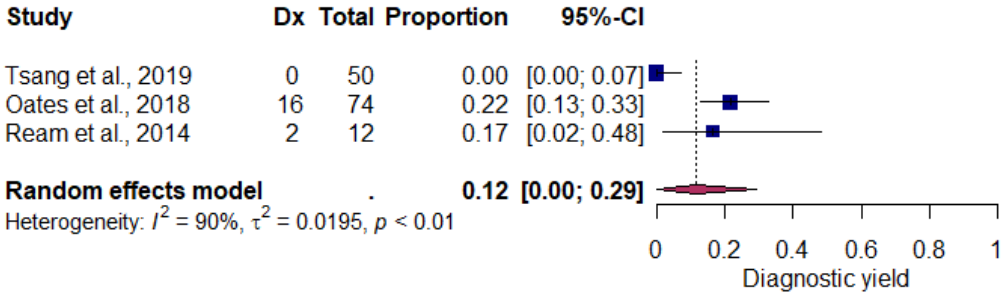


Figure 2. 22 Diagnostic yield of WES, EP, and CMA in drug-resistant epilepsy

2.3.12 Clinical utility of WES, EP

While it is well-known that genetic testing can provide an etiology diagnosis, the clinical utility of genetic testing is still poorly documented.

Impact of genetic testing on clinical management

No study reported the clinical utility of CMA and WGS in pediatric epilepsy. Seven studies discussed implications of etiology results on the medical management of epilepsy patients. Among these, two studies provided specific examples of effect of WES on medical management while five studies reported this utility of EP. Impact on clinical management was more frequently discussed in larger studies than in small cohorts.

Our meta-analysis showed that WES results prompted a change of clinical management in 15% of patients (95% CI 0.09-0.22, p=0.15, two studies of 289 children). The proportion of children whose clinical management changed after EP results was 10% (95% CI 0.07-0.13, p<0.01, eleven studies of 11,044 children) (Figure 2.23). The clinical utility of WES regarding to clinical management was significantly higher than that of EP (OR=2.9, p<0.0001).

The majority of illustrating the genetic diagnosis to guide clinical management is to provide indications and contraindication for certain AEDs. Truty et al. (2019) reported the impact of genetic diagnosis by EP in a largest cohort of 9,769 epilepsy chidren [53]. This study found that EP led to 15% of total cohort with genetic diagnosis. 33% of genetic diagnosis was defined as "actionable" results. The authors identified that more than 50% of actionable results was associated with avoidance of contraindications. While 40% of actionable results was related to the indication of effective medications, about 10% of actionable diagnoses was about metabolic disorders [53].

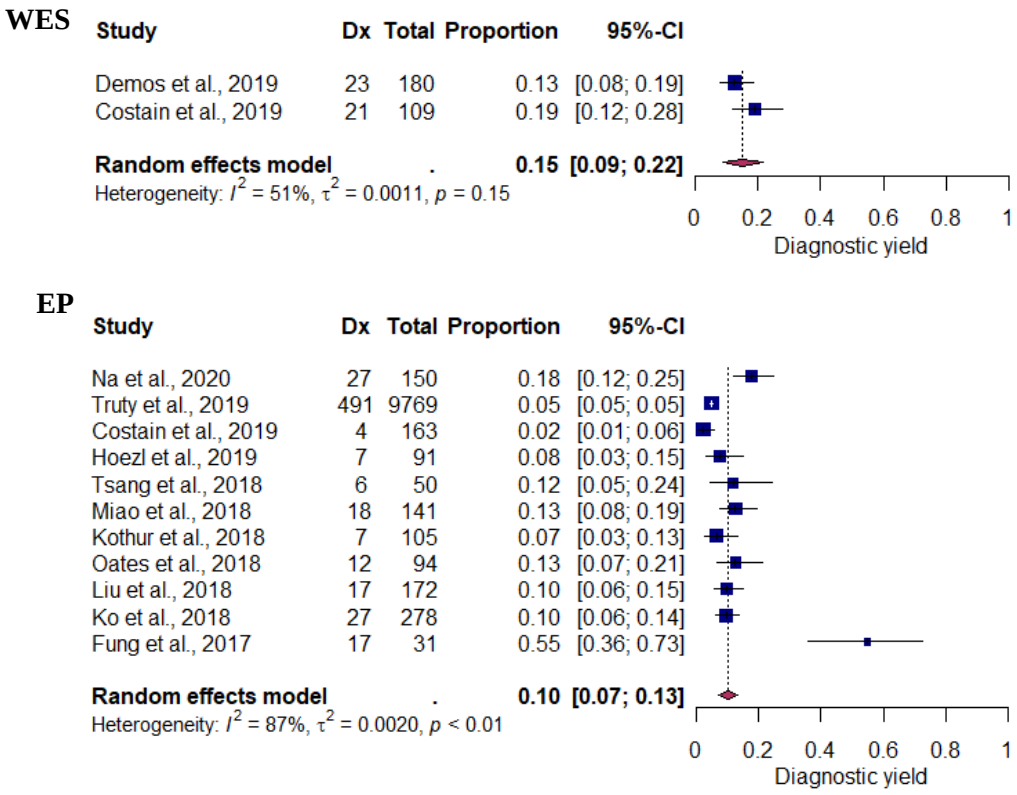


Figure 2. 23 Clinical utility of WES and EP in terms of clinical management

Impact of genetic testing on treatment effectiveness

Beyond clinical management, several studies reported other clinical utility measures of the downstream effect of genetic testing. Study authors usually chose clinically interesting example to highlight. By nature of this report, the data of outcomes were not fully reported.

Miao et al. (2018) examined the impact of EP in 141 children with epilepsy [168]. A genetic diagnosis of 39 patients led to a change in clinical management in 18 patients (46%) or 13% of all patients. This study also reported response to anti-epilepsy drugs in these 18 patients. Two patients with KCNQ2 variants and one patient with KCNT1 variant became seizure-free when treated with lacosamide and phenobarbital, respectively. Two Ohtahara patients with SCN2A variants achieved good response to phenytoin while one patient with KCNQ2 variants exhibited a 50% seizure reduction. Five Dravet patients with SCN1A variants, a patient with KCNT1 variant and a patient with STXBP1 variant were refractory to multiple anti-epilepsy drugs. Four patients with CDKL5, KCNB1, KCNA2 and KCNJ6 variants did not receive any special or precision medications [168].

Yang et al. (2019) investigated the diagnostic yield of EP and WES in children with epilepsy onset within the first year of life [123]. The former revealed 27% of the 476 patients with positive genetic results, whereas the latter one provided a genetic diagnosis in 42% of the 257 patients. The authors reported the treatment response of 84 patients with the 3 most commonly genes, namely SCN1A, KCNQ2 and TSC2. 42 patients (50%) were found to be seizure free or seizure reduction while 34 patients (40%) did not respond to treatment. 8 patients (10%) were lost to follow-up [123].

In the most recent study, Na et al. (2020) found 35% of the 150 early-onset developmental and epileptic encephalopathy patients with positive genetic diagnoses [55]. The authors reported the treatment effectiveness in 27 patients with the 3 most frequently genes, namely KCNQ2, STXBP1 and CDKL5. 70% of patients (19 patients) showed a good response to treatment. 7 of 9 patients with STXBP1 variants and 6 of 10 patients with KCNQ2 variants experienced more than 50% reduction of seizure after a treatment with ketogenic diet. Among 8 patients with CDKL5 variants, 5 patients treated with high dose of prednisolone while one patient underwent corpus callosotomy and vagus nerve stimulation experience a reduction of more than 50% in seizure [55].

Impact of genetic testing on genetic counseling

Another benefit of genetic testing is to provide genetic counseling of the patient and their family to inform reproductive decisions and identify other family members at risk. Howell et al. (2018) identified genetic diagnoses in 21% of 86 infants with severe epilepsies [9]. Genetic diagnoses informed reproductive counseling in all infants and prognostic counseling in most patients. Genetic counseling after genetic testing also established a significant recurrence risk in five families [9]. Oates et al. (2018) also reported benefits of EP on providing counseling in epilepsy children with onset < 2 years with a genetic diagnosis (20%, 19/96 patients) [47]. 31% of 19 patients with genetic etiology were identified with additional affected relatives. Genetic counseling also supported further observations in some families to identify members at risk [47]. Tsang et al. (2018) found that 12% of 50 children with drug-resistant epilepsy had genetic etiology [152]. Genetic counseling in children with genetic diagnosis advised two fathers of patients to have MRI and EEG and extended family members to undergo genetic tests whether they have genetic or non-genetic malformations [152].

2.4 Discussion

Our systematic review identified 56 publications investigating diagnostic yield of EP, WES, WGS, and CMA in pediatric epilepsy. The diagnostic yield of WGS was highest (0.66, 95% CI 0.00-1.00) followed by WES (0.37, 95% CI 0.30-0.44) and EP (0.25, 95% CI 0.22-0.28). CMA gained the lowest molecular diagnostic yield (0.1, 95% CI 0.07-0.13). However, it should be noted that the highest molecular diagnostic yield of WGS was due to a small number of studies, and one of them found striking results [146]. Further research is needed to more accurately measure the molecular diagnostic yield of WGS. Impacts of confounding factors including the year of study publication and sample size on the molecular diagnostic yield of WES/EP and CMA were investigated. The odds of diagnosis identified by CMA was found to increase by 6% every year. This improvement in the molecular diagnostic yield of CMA can be explained by the mature use over time. The odds of diagnosis identified by WES/EP reduced by 18% every year that can be explained by broader use for children with less severe and late-onset epilepsy.

To our knowledge, this is the first systematic literature review and meta-analysis comparing the diagnostic yield as well as the clinical utility of EP, WES, WGS, and CMA in pediatric epilepsy. A previous meta-analysis only investigated the diagnostic yield of WES, EP, and CMA in general population with epilepsy [43]. That review included 20 studies and demonstrated a higher

diagnostic yield by WES compared to our estimate (45% versus 37%, respectively). This can be explained by more studies of small cohorts with striking results in the previous meta-analysis. However, since adjusting for publication bias, their estimate on the diagnostic yield of WES was consistent with our results (32% versus 33%, respectively).

It was also found that the diagnostic yield of genetic tests varied depending on different factors such as age of seizure onset, concomitantly neurological features, test settings, and laboratory settings. This suggested that genetic testing might be more useful for certain groups.

Regarding the age of seizure onset, the diagnostic yield of WES/EP in neonatal-onset epilepsy was higher than that in older-onset epilepsy. This was consistent with the higher yield of EP observed in neonatal-onset group than infant-onset epilepsy in Stanek et al. (2018) (62% versus 28%) [171]. Oates et al. (2018) also found the varying diagnostic yield of EP, ranging from 80% for epilepsy at first month of life to 21% for epilepsy onset at infancy and toddler [47]. This could be explained by the genetic testing at early age which might deplete patients with causative gene mutations from the cohort of older age of seizure onset. Moreover, fewer genes discovered and more complex etiology in older onset epilepsies [47]. Therefore, further efforts in gene discovery will be needed for better epilepsy diagnosis.

In addition to differences in the diagnostic yield across age of seizure onset, there was variation across neurological features. Overall, the diagnostic yield was reported at 37% for WES and 26% for EP. Compared with a diagnostic yield of 33% by WES for epilepsy with less than 80% of cases with intellectual disability/developmental delay, an increased to 43% was achieved when more than 80% of patients were associated with developmental disorders. Although the increase in diagnostic yield of EP was modest, our review found a higher yield for epilepsy with intellectual disability/developmental delay (26% versus 24%). This suggested that WES provided a valuable diagnostic tool of increase diagnostic yield to diagnose genetic etiology, especially in epilepsy children with intellectual disability/developmental delay.

Apart from the characteristics of patients, the diagnostic yield of NGS depends on sequencing method. A higher diagnostic yield was achieved with trio-WES compared to singleton-WES (46% versus 33%). Data from sequencing the exomes of proband and both parents could be important because short reads did not allow for identifying whether the variant occurred de novo[194]. Although trio-WES increased the diagnostic yield, trio sequencing also increased

costs of test. Therefore, given no funding limitations, trio-WES should be performed to achieve the highest diagnostic yield.

The higher diagnostic yield of WES performed in hospital laboratories than reference laboratories (38% versus 12%) could give the potential benefits of the collaboration and discussion between clinicians and laboratory professionals. Laboratories might need extensive data of phenotypic features such as access to medical records, imaging results to prioritize variant analysis. Therefore, active discussion with clinicians could provide comprehensive patient information and interpretation without assumptions. Of course, it would be argued that only one study reported WES in reference laboratory, active relationship between clinicians and laboratory professionals could facilitate variant interpretation and diagnosis. Thanks to benefits of clinicians and laboratory professionals, the Association for Molecular Pathology recommended this collaboration [195].

Our study found that half of WES/EP studies included in our meta-analysis investigated the diagnostic yield of WES/EP as a first-tier test. In the remained studies, WES/EP was used as a second-tier test when patients received a normal biochemical, neuroimaging and CMA results. Our findings suggest that WES or EP could be performed as a second-tier test or as a first-tier test concurrent with CMA. Although WES might find copy-number variants with the diagnostic yield similar to CMA, significant bioinformatics analyses of exome database are needed [196]. Therefore, with the current technique, WES does not appear to be an only first-tier test. Despite the lowest diagnostic yield, the utility of CMA as the first-tier test still warrants in the diagnostic approach of pediatric epilepsy.

Our review found 14 studies described the clinical utility of NGS, ranging from changes in clinical management to family counseling. With respect to impact of genetic tests in clinical management, WES was associated with better utility than EP (15% versus 10%). A previous meta-analysis reported the clinical utility of different genetic tests in children with suspected monogenic disorders [197]. Our estimate in the clinical utility of WES in pediatric epilepsy was similar to its utility in children suspected with monogenic disorders (15% versus 17%, respectively) [197]. Apart from the impact on clinical management, genetic diagnosis was also associated with downstream consequences of treatment and initiation of genetic counseling. The discussion of these impacts of genetic tests was provided in our review. Determining the effectiveness of NGS to achieve patients' health outcomes could be useful to understand clinical

utility of NGS. However, there is a limited number of studies measure the clinical effectiveness of NGS which requires future studies for more evidence. Moreover, effectiveness analysis is also essential for economic evaluation. Together, effectiveness and economic evidence of NGS would be necessary to inform efficient integration of NGS into clinical practice.

Our study found that thanks to the molecular diagnostic yield and clinical utility, WES and EP should be incorporated into the clinical practice. Half of WES/EP studies included in our meta-analysis investigated the molecular diagnostic yield of WES/EP as a first-tier test. In the remained studies, WES/EP was used as a second-tier test when patients received a normal biochemical, neuroimaging and CMA results. Our findings suggest that WES or EP could be performed as a second-tier test or as a first-tier test concurrent with CMA. Although WES might find copy-number variants with the molecular diagnostic yield similar to CMA, significant bioinformatics analyses of exome database are needed [196]. Therefore, with the current technique, WES does not appear to be an only first-tier test. Despite the lowest molecular diagnostic yield, the utility of CMA as the first-tier test still warrants in the diagnostic approach of pediatric epilepsy.

This meta-analysis has several strengths. First, our meta-analysis included more than twice the number of studies in the previous study (56 studies versus 20 studies), although our patient population was restricted to children with epilepsy. Added recently published studies and the increased number of studies and patients enhanced our statistical accuracy. Second, no time restriction was applied for the literature search to collect all existing literature. Third, subgroup analysis was conducted to compare the diagnostic yield of WES and EP in a similar population and to control the heterogeneity associated with different clinical presentations in the molecular diagnostic yield.

We acknowledged several limitations. First, the generation of evidence on diagnostic yield and clinical utility of genetic tests from the randomized controlled trials would be ideal. However, no such randomized controlled trials were available. Therefore, our review summarized all the best available evidence until now. Second, acquiring results from different studies introduced a heterogeneity between included studies to estimate the average diagnostic yield. Given this heterogeneity, we took into account a variety of factors which might influence the diagnostic yield such as age of seizure onset, neurological features, sequencing method and laboratory setting.

While numerous studies investigated the molecular diagnostic yield and clinical utility of EP and WES, the evidence on WGS is limited. Given the promising benefits of WGS, further studies investigating molecular diagnostic yield and impact on clinical management are needed before its adoption as a routine diagnostic test. While NGS-based tests have the potential to improve diagnostic yield and impact clinical management, their cost-effectiveness is not rigorously investigated. Future cost-effectiveness analyses are needed to inform neurologists, geneticists and other clinicians in selecting optimal diagnostic strategies in the management of children with epilepsy. Moreover, cost-effectiveness of diagnostic strategies using NGS will provide evidence for decision-makers in reimbursement policy for this new technology to ensure its access. Therefore, in the next chapter (Chapter 3), a cost-effectiveness analysis of NGS-incorporated diagnostic strategies will be conducted using findings from this chapter (Chapter 2) to better inform decision-making.

2.5 Conclusion

NGS is a relatively new technology with expanded applications to support diagnostics and clinical management of pediatric epilepsy. Given its high molecular diagnostic yield and clinical utility, NGS should be adopted in routine genetic investigation of pediatric epilepsy. In the next chapter, we will assess the cost-effectiveness of NGS-incorporated diagnostic strategies for children with epilepsy to support its implementation in health care systems.

Chapter 3: Cost-effectiveness of genetic testing in children with epilepsy in precision medicine era

3.1. Introduction

Epilepsy is one of the most common neurologic disorders, affecting 10.5 million children worldwide [5, 198]. Epilepsy presents a heavy burden by its high prevalence, high morbidity, and high costs [199-201]. The conventional diagnostic strategy involves multiple laboratory tests, imaging studies, biopsies, chromosomal microarray (CMA) and sequencing of one or more genes. This approach is lengthy, costly and complex with burdensome procedures [40, 202]. Despite the so-called diagnosis odyssey, about 55% of infants with epilepsy remained with unknown etiology [9]. A high rate of unexplained epilepsy translating into huge cost [203] highlights the need for a cost-effective diagnostic strategy to optimize the healthcare utilization.

Epilepsy panel (EP) and whole-exome sequencing (WES) have been increasingly supported by clinical evidence to be useful genetic tests in epilepsy. It is thanks to their diagnostic yield of 13%-73% depending on sample size, clinical indications, the time-point in the diagnostic odyssey, and how the sequencing was performed [43]. While EP sequences genes relevant to a particular phenotype, WES offers an extensive evaluation in which most of the protein-coding regions are sequenced. Since WES is not limited to sequencing specific genes, WES has the potential to provide more diagnoses than EP [204]. A recent meta-analysis showed that the diagnostic yield of WES was higher than EP in patients with epilepsy (45% vs. 23%) [43]. While WES is better than EP in identifying causative genes [204], WES is more expensive and time-consuming [43, 205]. Therefore, careful evaluation of EP and WES utilization in epilepsy can inform the optimal selection among diagnostic strategies to maximize effectiveness and optimize costs.

To date, there are only three cost-effectiveness analyses of genetic testing in patients with epilepsy. Palmer et al. (2017) investigated the cost-effectiveness of WES in 32 children with epileptic encephalopathy who were undiagnosed after standard first-tier tests [134]. This study compared the standard second-tier tests followed by WES with standard second-tier tests alone. The WES-incorporated strategy increased diagnostic yield (50% vs. 6.2%) and saved 3,710 USD per additional diagnosis [134]. Howell et al. (2018) published a cost-effectiveness study of WES

with bioinformatic analysis limited to 341 infantile-onset epilepsy genes (namely targeted WES) in 86 infants with severe epilepsies [9]. Seven diagnostic strategies included a conventional strategy and six strategies in which targeted WES was gradually incorporated into the diagnostic procedures. Among the first five WES-integrated strategies which resulted in 48 diagnoses, strategy 5 was the least costly (533,431 USD). Strategy 6 was the next best effective strategy with 46 diagnoses and the total cost of 455,597 USD. The incremental cost-effectiveness ratio (ICER) of strategy 6 compared to strategy 5 was 38,917 USD per addition diagnosis [9]. A more recent study identified the cost-effectiveness of different genetic testing (i.e., CMA, EP, WES and combination strategies) in a hypothetical patient population with epilepsy [43]. Compared to no genetic testing, WES had the highest ICER (34,500 USD) followed by CMA (17,887 USD) and EP (15,848 USD). The authors concluded that among individual tests, WES and EP were the most cost-effective. They also declared that WES followed by EP and CMA (ICER of 15,336 USD) was the most cost-effective strategy among combination strategies [43]. However, it should be noted that no threshold of willingness-to-pay per diagnosis was established. Therefore, caution is needed for the interpretation of these results.

The main limitation of these cost-effectiveness analyses is to use diagnostic yield as an outcome measure rather than quality-adjusted life years (QALYs) which are recommended for economic evaluations [206]. Given this, the interpretation of previous findings is problematic to inform decision-making. This is due to a lack of commonly accepted willingness-to-pay threshold per diagnosis. Furthermore, all previous studies only considered short-term costs and consequences, valuing cost of diagnosis and diagnostic yield as outcomes. Since the elucidation of genetic etiology can guide clinical management [207-211] that results in costs of treatment and changes in patient's quality of life, significant health economic impact can be expected. For this reason, a comprehensively methodological approach measuring costs of both diagnosis and treatment stages and QALYs gained by subsequent changes in clinical management following an etiologic diagnosis is needed. The comprehensive cost-effectiveness analysis will better inform decision-makers and guide reimbursement policy.

To fill these gaps, we aimed to assess the cost-effectiveness of EP and WES diagnostic strategies in children with epilepsy. Developing a decision-analytic model, we assessed costs and QALYs of different diagnostic strategies incorporating EP and WES in comparison with conventional strategy. Given the limitations of previous studies, costs of diagnostic and treatment stages, as

well as QALYs gained by medical management aided by etiologic diagnosis were included in our model.

3.2. Methods

3.2.1. Overview

A decision-analytic model was developed to compare costs and effectiveness of different diagnostic strategies by taking into account both diagnosis and management of children with epilepsy. The primary effectiveness outcome was QALYs gained by clinical management suggested by an etiologic diagnosis. The secondary effectiveness outcome was the diagnostic yield. Adopting the health care system perspective, we estimated direct medical costs of diagnostic work-up as well as follow-up costs incurred by clinical management for two years since diagnosis made. Due to a short-term evaluation, a discount rate was not applied for costs as well as QALYs gained.

3.2.2 Diagnostic strategies

We evaluated four diagnostic strategies, including three strategies of incorporating EP and WES into clinical practice as well as a conventional diagnostic strategy. A schematic representation of these 4 diagnostic strategies was displayed in Figure 3.1.

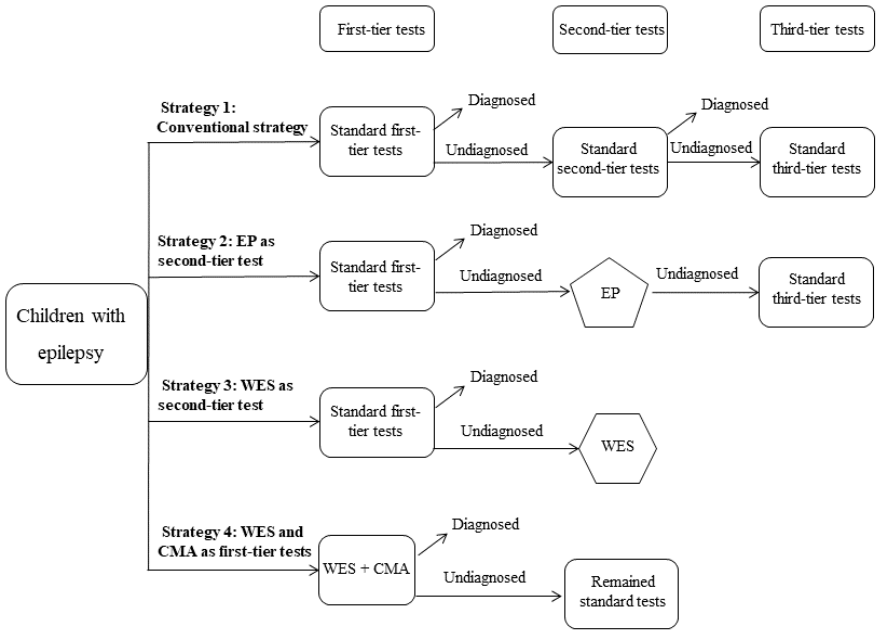


Figure 3. 1. Schematic representation of different diagnostic strategies

Strategy 1: Conventional diagnostic strategy

Strategy 1 (Conventional diagnostic strategy) represented the current practice in epilepsy diagnosis. The conventional strategy consisted of a combination of standard three-tier tests (Figure 1.2, Chapter 1) [9]. In this strategy, an epilepsy patient underwent standard first-tier tests including clinical investigations, metabolic screening, neuroimaging, CMA and targeted single-gene sequencing for suspected causative mutations. When the first-tier tests were negative, the patient further underwent complex metabolic tests and targeted single-gene sequencing known as standard second-tier tests. After nondiagnostic second-tier tests, the patient was offered invasive tests, i.e., skin or liver biopsies to get samples for cytogenetic analysis.

Strategies 2: EP as second-tier test

Strategy 2 (EP as second-tier test) was constructed based on an algorithm developed by Mercimek-Mahmutoglu et al. (2015) [40]. In this strategy, for patients undiagnosed after standard first-tier tests, EP was offered as second-tier test. If patients remained undiagnosed after EP, they would undergo biopsies to collect tissue samples for cytogenetic analysis.

Strategy 3: WES as second-tier test

Strategy 3 (WES as second-tier test) aligned with the algorithm developed by Costain et al. (2019) [148]. WES would replace standard second-tier and third-tier tests. These invasive tests namely cerebrospinal fluid examination and biopsies can be useful for diagnosis of metabolic etiology in a small percent of patients [212]. However, previously suspected metabolic etiology was frequently diagnosed in epilepsy patients through NGS [184, 213, 214]. Moreover, a genetic diagnosis by WES can help avoid further biopsies [46]. Therefore, this strategy was used to avoid giving complex metabolic tests, and invasive biopsies which were burdensome for patients [148].

Strategy 4: WES and CMA as first-tier tests

In strategy 4 (WES and CMA as first-tier tests), the combination of CMA and WES was used as first-line diagnostic tests. Strategy 4 was modelled to illustrate the situation when access and affordability of WES increased. This strategy was examined in Berg et al. (2017) and Jiao et al. (2019) [151, 156]. The rationale for the concurrent use of WES and CMA was to find single nucleotide variants and copy number variants, respectively. CMA can be seen as a complement to WES because until now, WES are limited in their capacity to detect copy number variants [215].

Patients undiagnosed after CMA and WES would undergo conventional diagnostic tests excluding genetic tests.

3.2.3 Model structure

We developed a decision-analytic model for cost-effectiveness analysis of different diagnostic strategies that included two stages, namely diagnostic and treatment stage (Figure 3.2 and Figure 3.3). The model started with a decision tree comparison of EP and WES-related diagnostic strategies with conventional strategy. Results from these diagnostic strategies led to treatment nodes that terminated in health outcomes experienced by patients after treatment (Figure 3.4).

Diagnostic stage:

We populated our model with children with epilepsy. Children with epilepsy were diagnosed either by conventional or EP or WES-incorporated diagnostic strategies, that was represented by each branch in the model. Each branch was divided into 2 further branches, representing a positive and negative result of diagnostic strategy. While "negative result" branch remained un-divided, "positive result" branch was further divided into "genetic etiology" and "non-genetic etiology". Genetic etiology potentially aided the selection of treatment options based on the identified mutations. However, it should be noted that identifying genetic mutations did not always translate into the use of precision medicine. Regarding the availability of mutation-specific therapies, each branch representing genetic etiology was divided into "actionable" and "non-actionable" alteration (Figure 3.2).

Treatment stage:

Each branch in the treatment stage represented a treatment option for patients with epilepsy corresponding to their etiologic diagnosis (Figure 3.3). Treatment options were divided into three groups. In group 1, patients identified with clinically actionable mutations received comprehensive genetic counseling and initiated precision medicine specific to those mutations. Based on current literature, we modeled targeted therapies for 15 frequent actionable mutations (i.e., ALDH7A1, KCNQ2, PNPO. SCN1A, SLC2A1, TSC1/2, GRIN2A, KCNT2, FOXG1, GRIN2B, KCNT1, SCN2A, SCN8A, STXBP1, and CDKL5). These were gene mutations known to have treatment with available evidence on effectiveness. Patients positive for a mutation among those 15 genes would receive targeted treatment according to their mutations identified (Table 1.4, Chapter 1). In group 2, patients who tested positive for non-actionable mutations

would undergo comprehensive genetic counseling and care as usual (CAU) with conventional anti-epilepsy drugs (AEDs). In group 3, patients with negative test results would continue CAU commonly given to epilepsy patients.

Each treatment branch was followed by a subtree that represented health outcome of patients at 2-year follow-up after treatment. Patient outcomes included seizure-free (SF), not seizure-free (NSF, 50%-99% reduction in seizures), and not adequately controlled (NAC, <49% reduction in seizures). We assumed that death rate between strategies was the same; thus, it was not included in the analysis (Figure 3.3). This assumption was based on the findings of two studies, namely Palmer et al. (2018) and Mercimek-Mahmutoglu et al. (2015) [40, 134]. In both studies, no death was recorded from either conventional strategy or WES or EP-related strategies [40, 134].

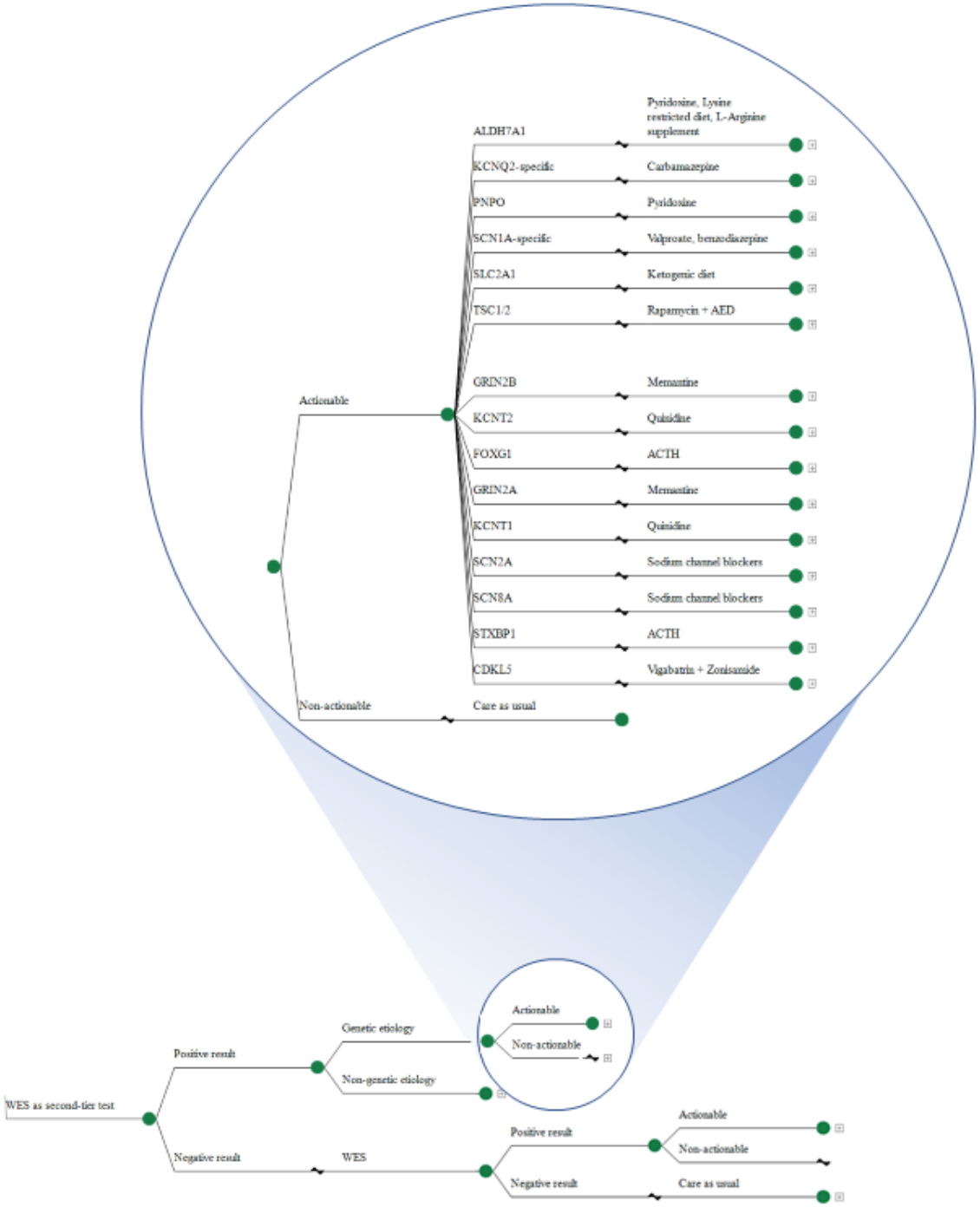


Figure 3. 2 Decision-analytic model represented strategy 3 ("WES as second-tier test"), diagnostic stage (a) and treatment stage (b)

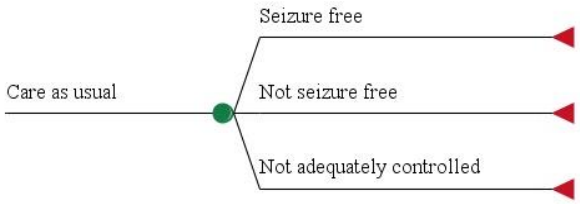


Figure 3. 3 Treatment effectiveness

3.2.4 Time horizon

Although a lifetime horizon should be adopted in modelling, the long-term prognosis of patients with epilepsy has been poorly documented [216]. To date, all clinical data on efficacy categorized by etiology diagnosis were reported at a range of 3 months and 5 years. Therefore, our model considered a 24-month time horizon. We assumed that the efficacy of treatment remained unchanged during the 24-month period.

3.2.5 Input parameters

Probabilities

The proportion of patients with an etiology identified by the conventional diagnostic strategy was obtained from Howell et al. (2018), Wirrell et al. (2011), and Ackermann et al. (2019) [9-11]. Patients in these studies were diagnosed by conventional tests without the availability of EP and WES. The average estimate from these three studies was 47.1% which we used as a base-case probability (Table 3.1).

Diagnoses made by strategies 2 and 3 included diagnoses obtained by standard first-tier tests and EP as second-tier test followed by biopsies (strategy 2) or WES as second-tier test (strategy 3). For the former, the proportion of patients with an etiology identified by first-tier tests was estimated based on the diagnostic yield of CMA, neuroimaging, metabolic screening and clinical investigations. The diagnostic yield of CMA was taken from our systematic review described in chapter 2. Resulted from seventeen studies of 2,306 children with epilepsy, etiology by CMA was established in 10% of patients. Diagnostic yield of neuroimaging in children with epilepsy ranged from 12.7% to 23.4 % [217-219]. Diagnostic yield of clinical investigations and metabolic screening was taken from Howell et al. (2018) and Mercimek-Mahmutoglu et al. (2015) [9, 40]. Therefore, we estimated that etiology was identified in 28.3%-43.3% of patients by first-tier tests.

The mean value was 35.83% used as the base-case estimate for the proportion of patients identified by first-tier tests (Table 3.1).

To determine the proportion of patients identified by EP or WES among patients with normal first-tier tests, we performed a systematic literature review described in chapter 2. This review of 36 studies provided data on the diagnostic yield of EP and WES in a large population of 4,572 children with epilepsy. For the base-case, the proportion of patients with monogenic cause identified by EP or WES as a second-tier test was estimated at 0.2699 and 0.3069, respectively (Figure 2.17 and 2.18, Chapter 2). The proportion of patients identified by biopsies as third-tier test was taken from Mercimek-Mahmutoglu et al. (2015) [40] (Table 3.1).

The proportion of patients with etiology identified by concurrent use of WES and CMA as first-tier tests was based on Jiao et al. (2019) and Berg et al. (2017) [151, 156]. These two studies reported the etiology established in 40.9%-47.8% of children with epilepsy. The average of these proportions was estimated at 43.4% which was used in the base-case analysis (Table 3.1).

Assuming that patients undiagnosed after WES and CMA (strategy 4) underwent conventional tests except for genetic tests, non-genetic etiologies would be identified. Given 47.1% of patients with etiology identified by conventional strategy, it was estimated that 16% of patients with genetic etiology and 31.1% of patients with non-genetic etiology. This estimate was based on Howell et al. (2018), Wirrell et al. (2011), and Ackermann et al. (2019) [9-11]. Among non-genetic etiologies, the structural abnormality was the most frequent cause (69%-100%) and metabolic etiology accounted for the minor proportion (0%-3%). While almost all gene mutations causing metabolic epilepsy were found [220], genetic etiology was identified in 44% of patients with structural abnormalities [219]. We assumed that WES and CMA identified all genes causing genetic-metabolic/structural etiology. Thus, conventional tests further identified acquired structural etiology in patients undiagnosed after WES and CMA. Given non-genetic etiology of 31.1% among our model cohort, we therefore estimated that 17.5% of patients with non-genetic etiology would be established by conventional tests after WES and CMA (i.e., 31.1% x 56%) (Table 3.1).

Table 3. 1. Probabilities used in the model

Probabilities	Value	Reference
Proportion of patients with etiology identified by conventional diagnostic strategy	0.4711	[9-11]
Proportion of patients with etiology identified by standard first-tier tests	0.1218	[9, 40]
Proportion of patients with etiology identified by EP in normal first-tier tests	0.2669	[9, 39, 40, 159, 168, 172-174, 177-179, 181, 182, 185]
Proportion of patients with etiology identified by biopsy in normal EP	0.125	[40]
Proportion of patients with etiology identified by WES in normal first-tier tests	0.3069	[134, 142, 153, 160]
Proportion of patients with genetic etiology identified by WES and CMA as first-tier tests	0.4344	[151, 156]
Proportion of patients with non-genetic etiology identified by conventional tests	0.175	Assumption

Mutation proportion

The proportion of each mutation among all mutations identified by EP or WES-incorporated strategies (strategies 2, 3, and 4) was estimated from 36 studies reporting diagnostic yield of EP and WES in pediatric epilepsy previously described in our systematic review (chapter 2). Studies included in this review provided data on mutation frequencies in a large population of 4,572 children with epilepsy. We quantified the proportion of actionable and non-actionable gene mutations from aggregated data on mutation frequencies. Further, we estimated the proportion of each actionable mutation among 15 actionable gene mutations by pooling 36 studies reporting mutation frequencies (Appendix 5). The proportion of actional gene mutations and the proportion of each mutation among all actionable gene mutations identified by conventional strategy (strategy 1) was estimated from Mercimek-Mahmutoglu et al. (2015) and Howell et al. (2018) [9, 40]. The resulting proportion of gene mutation was provided in Appendix 5.

Costs

Adopting the health care system perspective, we estimated direct medical costs incurred during diagnosis and treatment. Specific categories included costs of different diagnostic tests, costs of comprehensive genetic counseling, costs of special diet and medication. We did not include costs of surgery and vagal nerve stimulator which were two other treatment options for epilepsy. This assumption was made since surgery only occurred in 1.8% of patients and vagal nerve stimulator was implanted in only 2.6% of patients [216]. All costs were presented in 2018 Canadian dollars using average exchange rates by the Bank of Canada and inflate rates if needed (See Appendix 6 for detailed costs).

Diagnostic costs

Costs of conventional and diagnostic strategies in which EP or WES was incorporated into clinical practice were determined. Costs of conventional diagnostic strategy comprised costs of investigations that occurred for diagnostic purposes in a three-tier fashion (strategy 1). Costs of biopsies would be estimated for patients undiagnosed after EP (strategy 2) while no further costs of diagnosis would be incurred for patients undiagnosed after WES (strategy 3). If WES and CMA (strategy 4) did not provide a diagnosis, it was assumed that costs of all conventional diagnostic tests excluding genetic tests would be incurred. Costs of genetic counseling were determined for all patients undergoing genetic tests.

Treatment costs

Treatment was identified as targeted therapy and care as usual. Costs of targeted therapy included special diet and specific medication which was corresponded to patients identified with actionable gene mutations. Costs of usual care were defined as costs of conventional AEDs relative to treatment effectiveness. We based our estimates of treatment costs on studies available in the literature (Table 3.2).

Resource use

Costs of diagnostic tests included costs of imaging, biochemical screening, metabolic investigations, biopsies, and genetic tests. Costs of biochemical tests, imaging, metabolic investigation, and biopsies were based on the listed fee by the British Colombia Ministry of Health (MOH). When tests' costs were not available in the B.C Schedule of Fees for the Laboratory Services Outpatient, the costs were collected from different laboratories including The

Kennedy Krieger Institute (KKI), the Greenwood Genetic Center (GGC), and Medical Neurogenetics Laboratories (MNG) in the United States (See Appendix 6 for detailed costs).

Costs of genetic tests included costs of genetic counseling, costs of CMA, single-gene sequencing, EP, and WES. Costs of comprehensive genetic counseling were from the Ontario Ministry of Health and Long-term Care Schedule of Benefits for Physician Services (OHIP). Costs of CMA were based on the estimates by Dragojlovic et al. (2019) [221]. Costs of single-gene sequencing were obtained from the LifeLabs in Canada (www.lifelabs.genetics.com, accessed September 4, 2019). Due to a large number of different single-gene sequencing, modeling separate tests was not viable. Thus, the average costs of various single-gene sequencing were measured.

Costs of EP and WES included costs of Sanger sequencing and the average of commercially available prices of EP or WES. Prices of EP and WES were obtained from prices listed by different laboratories (i.e., LifeLabs, GGC, MNG, Centogene, University of Chicago's Genetic Services Laboratory). Due to prices collected from different labs, prices of EP and WES were estimated by averaging the available prices. Of note, we used costs of "trio" EP and WES in which both patients and their parents were tested.

Table 3. 2. Base case costs

Costs	Value (CAD)	Source
Cost of diagnostic tests		
Cost of standard first-tier tests:		See Appendix 6 for detailed unit costs and source
- Cost of biochemistry	1,182	
- Cost of neuroimaging	1,233	
- Cost of chromosomal microarray	785	
Cost of standard second-tier tests:	2,926	
Cost of standard third-tier tests:	3,400	
Cost of EP	4,382	
Cost of WES	7,230	
Cost of Sanger sequencing in trios	160	
Cost of treatment		
Cost of ALDH7A1:	104.89	[222]

- Cost of pyridoxine (300 mg/day)	52,523.68	[222]
- Cost of L-arginine (660 mg/kg/day)		
Cost of CDKL5:	3950.9	https://www.canadadrugmart.com
- Cost of vigabatrin (70.625 mg/kg/day)	686.44	https://www.canadapharmacy.com
- Cost of zonisamide (5.85 mg/kg/day)		
Cost of FOXG1:	201,945	[223]
- Cost of adrenocorticotropic hormone	2,706.75	[224]
- Cost of topiramate		
Cost of GRIN2A/ 2B:	3328	[225]
- Cost of memantine (0.5 mg/kg/day)	215.74	[224]
- Cost of valproate (25 mg/kg/day)		
Cost of KCNQ2:	615.17	[224]
- Cost of carbamazepine (25 mg/kg/d)		
Cost of PNPO:	104.89	[222]
- Cost of pyridoxine (300mg/day)	975	[195]
Cost of mono AED:	2,631.52	[195]
Cost of 2 AEDs:	5,031.52	[195]
Cost of 3 AEDs:		
Cost of SCN1A:	565.96	[224]
- Cost of valproate (36 mg/kg/day)		
Cost of SCN2A/ SCN8A:	5,536.53	[226]
- Cost of phenytoin (7.50 mg/kg)	7,566.34	[226]
Cost of SLC2A1 – ketogenic diet		
Cost of STXBP1:	40,243.3	[223]
- Cost of adrenocorticotropic hormone		
Cost of KCNT1/ KCNT2:	607.53	[227]
- Cost of quinidine (33mg/kg/d)		
Cost of TSC1/ TSC2:	9,089.46	[226]
- Cost of rapamycin		

Effectiveness

Effectiveness was measured as QALYs, a measure of length of life adjusted by the quality of life. QALYs was calculated by multiplying the utility value for each health outcome by time horizon. We also measured diagnostic yield as secondary effectiveness.

Three health outcomes regarding seizure reduction included seizure-free (SF), not seizure-free (NSF), and not adequately controlled (NAC). Probabilities regarding the effectiveness of treatment specific for each "actionable" gene mutation were estimated from case reports, cohort studies of patients with the mutations of interest. Probabilities of SF, NSF, and NAC by usual care for patients with non-actionable gene mutations, non-genetic etiology and unknown etiology were from a retrospective study of patients with early-onset epilepsy [228] (Appendix 7).

A review from the literature found no published utility values specific to all epilepsy syndromes except for Lennox-Gastaut syndrome – an epileptic encephalopathy at childhood onset. We based utility estimate for each health outcome on estimates by Elliott et al. (2018) [229]. Table 3.3 contained the specific health utilities which were used in our study.

Table 3. 3. Base case utilities

Health outcome	Value	Source
Seizure-free (SF)	0.699	[229]
Not seizure-free (NSF)	0.605	[229]
Not adequately controlled (NAC)	0.427	[229]

3.2.6 Cost-effectiveness analysis

We used the decision-analytic model to determine costs, effectiveness, and incremental cost-effectiveness of 4 diagnostic strategies. To estimate incremental cost-effectiveness, we first ranked the strategies in order of rising effectiveness. Next, strategies that were less or equally effective and more costly were considered dominated and removed. Among the undominated strategies, the incremental cost-effectiveness ratio (ICER) was calculated by dividing the difference between costs by the difference in effectiveness compared to the next effective strategy. Strategies that had lower effectiveness and higher ICERs were extendedly dominated and excluded. Strategies were cost-effective if their ICERs were lower than the commonly accepted willingness-to-pay (WTP) threshold of 100,000 CAD per QALY [230].

3.2.7 Sensitivity analyses

Sensitivity analyses were conducted to explore the robustness of our findings. In one-way sensitivity analyses, values of key inputs (namely cost of EP, cost of WES, the proportion of patients identified with etiology, and utility) were varied within a range of ± 25%. (Appendix 8).

We also examined a scenario in which a time horizon of 20 years was adopted to investigate whether our conclusion changed when looking beyond the short-term.

In the probabilistic sensitivity analysis, distributions were assigned to all input parameters and 10,000 Monte Carlo simulations were performed. Normal distributions were assigned to costs and utilities while beta distributions were adopted to probabilities.

3.3 Results

3.3.1 Base-case cost-effectiveness analysis of genetic testing through diagnostic trajectory

Cost-effectiveness results of different diagnostic strategies through diagnostic trajectory were shown in Table 3.4. All strategies incorporating either EP or WES were more effective than the conventional diagnostic strategy. In particular, strategy 4 (WES and CMA as first-tier tests) was the most effective strategy, achieving the highest diagnostic yield of 60.9%. Strategy 2 (EP as second-tier test) and strategy 3 (WES as second-tier test) were more effective than conventional strategy (54% and 56% vs. 47%) (Panel A of Table 3.4). The higher effectiveness of EP and WES-related strategies was due to more patients achieving genetic etiology by EP and WES. While the conventional diagnostic strategy identified only the suspected gene mutations suggested by phenotypic features, EP and WES detected almost all known disease-causing mutations. Moreover, WES could identify frequent mutations included in EP as well as less common mutations which were often excluded from EP, resulting in higher diagnostic yield. Therefore, EP and WES could help to facilitate the transition from targeted sequencing of specific genes to simultaneously testing mutations in many genes.

Cost of strategy 2 (EP as second-tier test) and strategy 3 (WES as second-tier test) was lower than strategy 1 (conventional strategy) (8,115 and 8,165 vs. 9,318 CAD) (Panel A of Table 3.4). This was due to the cost savings from avoiding complex metabolic tests, biopsies, and targeted single-gene sequencing that were offset by the costs of EP or WES. Implementing the most effective strategy, WES and CMA as first-tier tests (strategy 4), would increase diagnosis costs by 39% compared to strategy 3 (WES as second-tier test) (11,363 vs. 8,164 CAD). Although patients diagnosed by strategy 4 avoided greater costs resulted from additional clinical investigations (i.e., neuroimaging, metabolic screening) than strategy 3, these savings could not offset high costs of WES used as first-tier test. This could be explained by more patients offered with WES in strategy 4 than strategy 3.

Strategy 4 (WES and CMA as first-tier tests) was the most effective and most costly that resulted in an ICER of 59,477 CAD per diagnosis compared to the next best effective strategy (WES as second-tier test) (Panel A of Table 3.4). Compared to conventional strategy, strategy 4 produced an ICER of 14,712 CAD per diagnosis (Panel B of Table 3.4).

Table 3. 4. Cost-effectiveness results of diagnostic strategies through diagnostic trajectory

Strategy	Cost (CAD)	Incremental cost (CAD)	Diagnostic yield	Incremental diagnostic yield	Incremental cost-effectiveness ratio (ICER)
Panel A: All strategies					
Strategy 1 - Conventional strategy	9,318	—	0.47	—	Dominated
Strategy 2 – EP as second-tier test	8,115	-1,203	0.5355	—	—
Strategy 3 – WES as second-tier test	8,164	49	0.5552	0.0197	2,515
Strategy 4 – WES and CMA as first-tier tests	11,363	3,199	0.609	0.0736	59,477
Panel B: "WES and CMA as first-tier tests" vs. conventional strategy					
Strategy 1 – Conventional strategy	9,318	—	0.47	—	—
Strategy 4 – WES and CMA as first-tier tests	11,363	2,045	0.609	0.139	14,712

3.3.2 Base-case cost-effectiveness analysis of genetic testing taking into account changes in clinical management

Cost-effectiveness results of different diagnostic strategies accounting for clinical management aided by diagnosis were shown in Table 3.5. Among all strategies, strategy 4 (WES and CMA as first-tier tests) gained the highest QALYs (1.1056 QALYs). Compared to conventional diagnostic strategy, the strategies 2 and 3 (EP or WES as second-tier test) resulted in higher QALYs (1.0950 and 1.0975 vs. 1.0843 QALYs) (Panel A of Table 3.5). Higher QALYs gained by strategies 2, 3 and 4 were due to more patients achieved a genetic diagnosis and received effective targeted therapies.

Total costs of strategies 2 and 3 (EP or WES as second-tier test) were comparable to conventional diagnostic strategy (19,295 CAD and 19,362 CAD vs. 19,511 CAD) (Panel A of Table 3.5). This was due to the savings in diagnosis (as shown in Table 3.4) offset by the increase in treatment cost that came from changing clinical management (+987 CAD and +996 CAD). Although many targeted therapies were less costly than conventional AEDs, these savings could not offset the increase in switching from conventional AEDs to costly targeted treatment (i.e., L-arginine, ketogenic diet, adrenocorticotropic hormone). Strategy 4 (WES and CMA as first-tier tests) incurred the highest costs (22,810 CAD). In addition to increased costs in diagnosis (as shown in Table 6), patients in strategy 4 experienced additional costs resulted from switching to targeted therapies (+1,254 CAD). More patients switched to targeted therapies by strategy 4 than either strategy 2 or strategy 3. Therefore, additional costs of treatment in strategy 4 were the highest among all EP or WES-related strategies (+1,254 CAD vs. +987 CAD and +996 CAD).

Conventional strategy was dominated by strategy 2 (EP as second-tier test) (more costly and less effective). After excluding the dominated strategies, 3 remained strategies were considered to measure the ICER. The ICER of strategy 3 (WES as second-tier test) versus strategy 2 (EP as second-tier test) was 26,070 CAD per QALY. Compared with the conventional WTP threshold of 100,000 CAD per QALY, strategy 3 was cost-effective relative to strategy 2. Strategy 4 (WES and CMA as first-tier tests) gained the highest QALYs (1.1059 QALYs), but it was not cost-effective relative to strategy 3 (ICER of 426,517 CAD per QALY) (Panel A of Table 3.5). Compared to conventional strategy, strategy 4 remained not cost-effective with an ICER of 154,538 CAD per QALY (Panel B of Table 3.5).

Table 3. 5. Cost-effectiveness results of diagnostic strategies accounting for changes in clinical management

Strategy	Cost (CAD)	Incremental cost (CAD)	QALYs	Incremental QALYs	Incremental cost-effectiveness ratio (ICER)
Panel A: All strategies					
Strategy 1 – Conventional strategy	19,511	—	1.0843	—	Dominated
Strategy 2 – EP as second-tier test	19,295	—	1.0950	—	—
Strategy 3 – WES as second-tier test	19,362	67	1.0975	0.0025	26,070
Strategy 4 – WES and CMA as first-tier tests	22,810	3,448	1.1056	0.0081	426,517
Panel B: "WES and CMA as first-tier tests" vs. conventional strategy					
Strategy 1 – Conventional strategy	19,511	—	1.0843	—	—
Strategy 4 – WES and CMA as first-tier tests	22,810	3,299	1.1056	0.0213	154,538

3.3.3 One-way sensitivity analyses

A series of one-way sensitivity analysis demonstrated that our findings were robust to the impact of uncertainty within input parameters. Conventional strategy was dominated in all scenarios when input parameters including the proportion of patients with identified etiology, the proportion of genetic etiology, the proportion of actionable gene mutations, costs of genetic tests, utility values were varied in examined ranges of base-case values (Appendix 7). Sensitivity analyses also found that strategy 3 (WES as second-tier test) would be no longer cost-effective relative to strategy 2 (EP as second-tier test) when cost of EP reduced by 25% (Table 3.6). In this case, strategy 2 would become the most cost-effective strategy. When cost of WES reduced by 25% or the proportion of patients with etiology identified by WES and CMA as first-tier tests increased by 25%, strategy 4 remained not cost-effective relative to strategy 3 despite its improved ICER (ICER of 344,932 and 171,325 CAD per QALY). We also found that when we projected our model to a time horizon of 20 years, strategy 4 would become cost-effective (ICER

of 66,920 CAD per QALY). In this analysis, strategies 2 and 3 were dominated by strategy 4, making strategy 4 the most cost-effective among all diagnostic strategies (Table 3.6).

The Tornado diagram as displayed in Figure 3.6 showed the impact of a change in inputs on the ICER of WES and CMA as first-tier tests (strategy 4) versus conventional strategy. The ICER was the most sensitive to the proportion of patients with etiology identified by WES and CMA, currently estimated as 43.44%. When the proportion of patients with etiology identified by WES and CMA increased by 25% (54%), strategy 4 would become cost-effective (ICER of 96,665 CAD per QALY). We also considered the lower cost of WES compared to the base-case cost (5,521 vs. 7,362 CAD). As expected, strategy 4 would become a cost-effective strategy, with ICER of 68,314 CAD per QALY (Figure 3.5).

Table 3. 6. Results of one-way sensitivity analyses

Variable	Values	ICER (cost per QALY)			
		Strategy 1- Conventional strategy	**Strategy 2- EP as second-tier test**	**Strategy 3- WES as second-tier test**	**Strategy 4- WES and CMA as first-tier tests**
Cost of EP	3,597	Dominated	—	325,758	426,517
Cost of WES	5,521	Dominated	Dominated	—	344,932
Proportion of patients with etiology identified by WES and CMA as first-tier tests	0.5429	Dominated	—	26,070	171,325
Time horizon	20	—	Dominated	Dominated	66,920

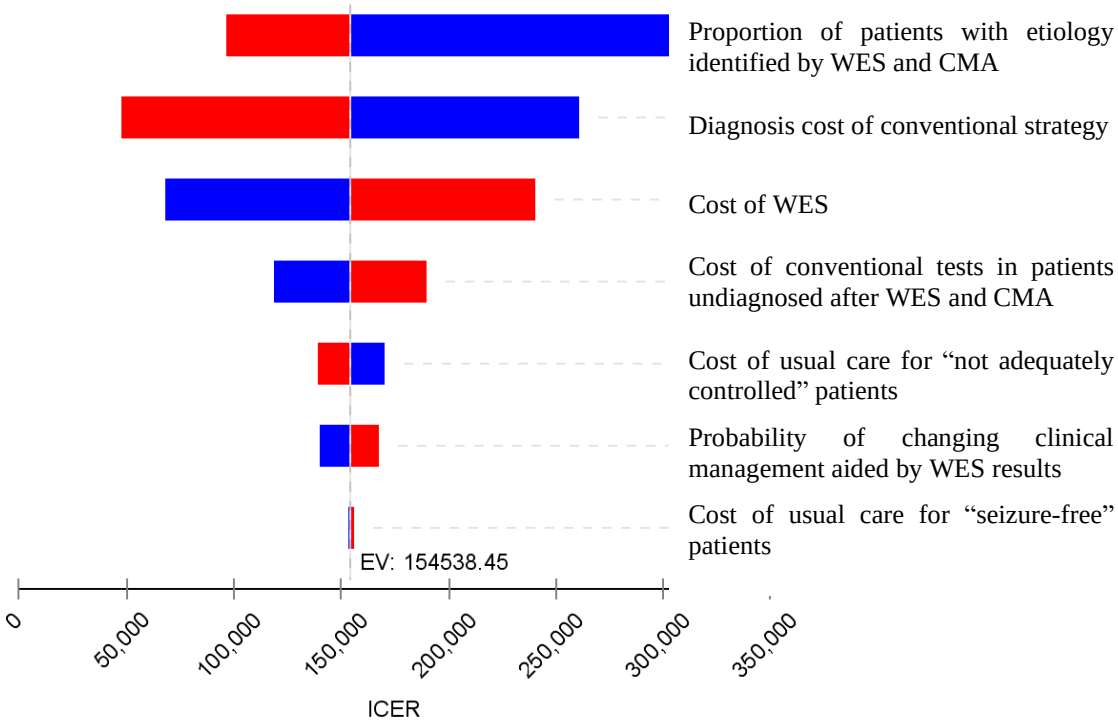


Figure 3. 4 Tornado diagram, ICER of WES and CMA as first-tier tests (strategy 4) vs. conventional strategy

3.3.4 Probabilistic sensitivity analysis

We performed 10,000 microsimulations where all input variables were varied simultaneously along with distributions. The cost-effectiveness acceptability curve (Figure 3.6) showed that at any WTP threshold, strategy 3 (WES as second-tier test) would gain the highest chance to be cost-effective among all strategies. We also found that at the commonly applied WTP threshold of 100,000 CAD per QALY, strategy 4 (WES and CMA as first-tier tests) was cost-effective in only 5% of 10,000 iterations.

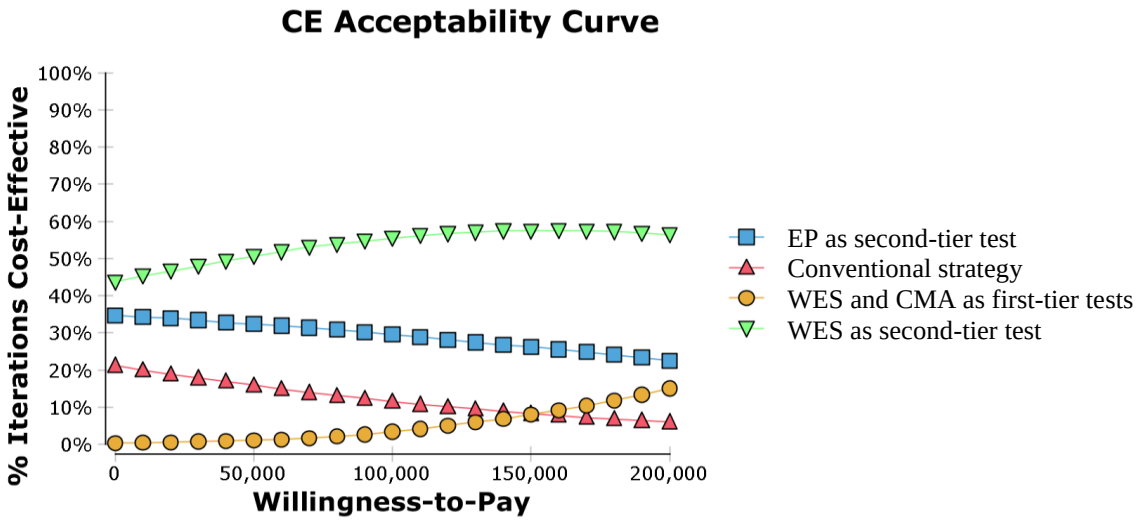


Figure 3. 5 Cost-effectiveness (CE) acceptability curve

3.4 Discussion

Our study is the first to assesses the cost-effectiveness of EP and WES diagnostic strategies in children with epilepsy using QALYs as a health outcome. All EP and WES-related strategies were more effective than conventional diagnostic strategy. Improvement in QALYs was due to more patients diagnosed and going on effective therapies. Among all diagnostic strategies, "WES as second-tier test" was the most cost-effective (ICER of 20,881 CAD per QALY). We also found that although the "WES and CMA as first-tier tests" strategy generated the highest QALYs, it was not cost-effective relative to conventional strategy (ICER of 151,794 CAD per QALY). These findings were robust to several sensitivity analyses relating to inputs used in the modelling.

Our findings on costs of conventional strategy per patient were comparable to Howell et al. (2018) (9,318 CAD versus 9,954 CAD (7,687 USD), respectively) [9]. However, while we found that "WES and CMA as first-tier tests" resulted in an ICER of 14,712 CAD per diagnosis relative to conventional strategy, Howell et al. (2018) found a similar strategy was cost-saving. This can be explained by lower estimated cost of WES in the Howell et al. (2018) (2,130 CAD (1,639 USD) versus 7,230 CAD, respectively). It should be noted that our study estimated cost of trio-WES which was performed in both children and their parents. In contrast, Howell et al. (2018) used singleton targeted WES in which WES was performed only in patients with analysis limited to 341 infantile-onset epilepsy genes. Therefore, our estimate on cost of WES was higher than Howell et al. (2018) that explained the difference between conclusions. Notably, when our model

used cost of WES from Howell et al. (2018), we also found that "WES and CMA as first-tier tests" more effective and less costly than conventional strategy.

Receiving a genetic diagnosis in epilepsy can lead to changes in clinical management [207-211], which are associated with downstream costs. Therefore, estimating treatment costs may change the conclusion on cost-effectiveness of EP and WES. Our study showed that using EP or WES as second-tier test reduced diagnosis cost compared to conventional strategy. However, when accounting for treatment followed by diagnostic results, costs of EP and WES-related strategies were comparable to conventional strategy. This was due to these cost savings from diagnosis offset by an additional treatment costs associated with switching from conventional AEDs to targeted therapy.

As the first study of cost-effectiveness of EP and WES that include costs and benefits of clinical management following the genetic testing results for children with epilepsy, it was not straightforward to compare our findings directly with other studies because of differences in patient characteristics, study method, time horizon, etc. In a broader literature, Schofield et al. (2019) found that WES was cost-effective over a time horizon of 20 years in suspected monogenic disorders (ICER of 28,362 CAD (AU$ 31,144) per QALY) [231]. This finding was in line with our sensitivity analysis on the cost-effectiveness of WES for long-term (20 years). However, in that study, there was no reference strategy. Therefore, future comprehensive research on long-term cost-effectiveness of WES are needed.

Our study has a number of strengths. It is the first cost-effectiveness analysis to consider the benefits of genetic diagnosis in informing optimal treatments for epilepsy patients and use QALYs as an effectiveness outcome. It is also the only study that evaluates both EP and WES-related strategies and conventional diagnostic strategy in epilepsy. As such, our study provides a more comprehensive comparison among these diagnostics strategies to inform policymakers. Lastly, this study uses input parameters that were obtained from a large number of studies and up-to-date data in the literature. As more precision therapies become available for epilepsy gene mutations and improved diagnostic performance, our framework is useful for further economic evaluations of genetic tests.

We acknowledged some limitations. First, given the translation of genetic etiology into precision medicine, the patient's health outcome was likely to be underestimated in our study. We did not capture the benefits of avoiding burdensome procedures such as biopsies on health utility.

Further, potential benefits of reducing diagnostic odyssey and informing reproductive choices were not included in our study. Although quantifying these downstream implications of genetic diagnosis can improve cost-effectiveness of EP and WES, data associated with these benefits have not been available in the literature. Second, data on treatment outcomes were sourced from the studies with small-to-moderate sample size and short-to-medium follow-up duration. However, these data are the best available to date. Given that EP and WES are increasingly used in pediatric neurology, our study highlighted the need for future clinical data in larger cohorts with longer follow-up time required for comprehensive cost-effectiveness analyses. Third, WES could be better than EP in terms of diagnostic yield since the novel genes can be identified by re-analyzing the available data [232]. However, our study focused more on clinical care and we did not consider the possibility of WES re-analysis. Fourth, although the 2-year time horizon used in our model is mostly longer than that in existing studies, it might still be short. Future studies should use a longer time horizon when longer-term data on outcomes become available. Lastly, we did not include death outcome in our model. However, this is unlikely to have a major impact on our results as death rates were likely to be similar across all strategies within the two year time horizon.

3.5 Conclusion

Among all strategies, “WES as second-tier test” was found to be the most cost-effective. “WES and CMA as first-tier tests” generated the highest QALYs, but was not cost-effective relative to conventional strategy. Given its high costs of WES, “WES and CMA as a first-tier tests” would become a cost-effective strategy when cost of WES decreased or the proportion of patients with etiology identified by WES and CMA increased. Although integration of genetic testing into routine practice is currently a strong focus for precision therapies, there are still challenges with implementation including the lack of evidence on economic value of genetic testing. Our cost-effectiveness analysis of EP and WES in children with epilepsy provides timely evidence on cost effectiveness of genetic testing for children with epilepsy to help guide its implementation.

Chapter 4: Summary

4.1 Overview

The main objective of this thesis was to assess diagnostic yield and clinical utility of NGS-based genetic tests and to evaluate cost-effectiveness of NGS-incorporated diagnostic strategies for children with epilepsy. This aim was achieved by conducting a systematic literature review and meta-analysis and carrying out a modelling-based cost-effectiveness analysis of incorporating NGS into diagnostics and clinical care for pediatric epilepsy.

Our meta-analysis that compared diagnostic yield of NGS-based tests (i.e., WGS, WES, and EP) with CMA showed that the former had a higher diagnostic yield than the latter. Our study also found that the clinical utility of WES was higher than EP in children with epilepsy, while there is no reported clinical utility of WGS and CMA (Chapter 2).

Findings from a systematic literature review in Chapter 2 were used as input parameters of a decision-analytic model to evaluate the cost-effectiveness of different diagnostic strategies. Our study showed that incorporating EP and WES into diagnostic trajectory was more effective than conventional diagnostic strategy over 2-year time horizon. We also found that "WES as second-tier test" was the most cost-effective. "WES and CMA as first-tier tests" generated the highest QALYs, but was not cost-effective relative to "WES as second-tier test" (Chapter 3).

This chapter will discuss (1) the main findings; (2) strengths and limitations of the meta-analysis and cost-effectiveness analysis; (3) the implications of the study findings; and, (4) future research.

4.2 Main findings

Chapter 2 of this thesis focused on the systematic literature review and meta-analysis of the diagnostic yield and clinical utility of current genetic tests in pediatric epilepsy. This study showed that the molecular diagnostic yield of WGS and WES and EP were higher than that of CMA (66%, 37%, 25%, respectively versus 10%). By classifying the population into different subgroups based on several factors such as age of seizure onset, neurological features, sequencing method and laboratory settings, we found that genetic testing might be useful for certain subgroups. The clinical utility of WES regarding to clinical management was higher than that of

EP (15% versus 10%). Clinical utility of WGS and CMA has not been reported yet, suggesting that future studies examining their clinical utility are needed.

Chapter 3 of this thesis evaluated the cost-effectiveness of incorporating either WES or EP into clinical care for pediatric epilepsy from the health care system's perspective and covered the 2-year period of clinical management since diagnosis was made. Cost-effectiveness results of different diagnostic strategies through diagnostic trajectory are shown in Table 3.4. All strategies incorporating either EP or WES were more effective than conventional diagnostic strategy. The most effective strategy, WES and CMA as first-tier tests, would result in an ICER of 59,477 CAD per diagnosis compared to the next best strategy (i.e., WES as second-tier test). Concerning clinical management aided by a diagnosis, WES as second-tier test was the most cost-effective (ICER of 26,070 CAD per QALY). It was also found that although the "WES and CMA as first-tier tests" strategy generated the highest QALYs, it was not cost-effective relative to "WES as second-tier test" (ICER > 100,000 CAD per QALY). Given the high costs of WES, "WES and CMA as first-tier tests" could become a cost-effective strategy only when cost of WES decreases or the proportion of patients with etiology identified by WES and CMA increase.

4.3 Strengths and limitations

The systematic review in this thesis was comprehensive as it included many different databases. However, the review included only observational studies due to the lack of randomized controlled trial comparing diagnostic yield and clinical utility of these genetic tests. It is therefore difficult to determine whether improvements or decline in diagnostic and clinical utility were due to the intervention of NGS (WGS, WES, EP) or other confounders. Moreover, the number of studies reporting the clinical utility of WES and EP is small. This presents a challenge for performing clinical utility reviews. Further research is needed to examine the clinical utility of genetic testing in children with epilepsy.

Our cost-effectiveness analysis also has several advantages. First, a variety of genetic etiology of epilepsy presents a challenge to model building by introducing multiple gene mutations and respective targeted therapies. However, by modeling 16 commonly actionable gene mutations, which were previously identified with potentially effective therapy, our approach made existing data manageable. Second, our study is the first to account for treatments that follow genetic diagnostics and use QALYs as an outcome. This approach facilitates comparisons of the

strategies considered in our study with other diagnostic/interventions in terms of costs and effectiveness to inform decision making regarding healthcare resource allocation.

The cost-effectiveness analysis had some limitations. We did not include WGS as an option of NGS-based genetic test. This is due to its low uptake in routine practice [44]. Although WGS sequences the entire genome which would lead to higher diagnostic yield and higher likelihood at identifying actionable gene mutation, the cost of WGS is still 2-3 times higher than that of WES [233]. A lower cost of WGS in the future could lead to increased use of WGS in clinical practice and warrant future research on the cost-effectiveness of WGS.

4.4 Implications of the current study findings

Our findings can help inform patients, health professionals, and decision-makers on the value of NGS in improving diagnostic yield compared to CMA. Incorporating the NGS into the routine practice was more effective and less costly than the conventional diagnostic strategy. Positive results by NGS could impact patient management that in turn improved patient's QALYs while not increasing utilization of health care services.

These results have important implications for patients, health professionals, and decision-makers. For patients, they provide comfort that NGS has a positive impact on identifying causative genes and actionable gene mutations. For health professionals, the study provides evidence that NGS-based tests are beneficial for diagnosis and clinical management, which in turn result in higher QALYs. For decision-makers, given the high prevalence of pediatric epilepsy and the provision of EP and WES as a diagnostic tool, these findings help to inform evidence-based care in epilepsy care.

As EP and WES make their way into routine practice for epilepsy care, our findings in terms of costs, diagnostic yield, and health outcomes of different strategies for incorporating EP and WES into epilepsy diagnosis and care provide timely evidence to inform decision-makers during the implementation process.

4.5 Future research

Given lower costs and faster time-to-diagnosis, NGS has been increasingly adopted in clinical practice. As the number of NGS applications continues to increase, the importance and relevance of future research on the effectiveness and cost-effectiveness of NGS will increase. Several directions for future research are identified.

First, while WGS potentially has the highest diagnostic yield among NGS-based genetic tests, studies on diagnostic yield and clinical utility of WGS are limited. Future studies investigating the effectiveness of WGS in children with epilepsy will better inform clinicians in clinical practice.

Furthermore, given the rapid technological development and decreasing sequencing costs of NGS, the cost-effectiveness of NGS might improve. With more accurate data on costs and effectiveness, future cost-effectiveness analysis could provide better estimates of economic value of NGS for diagnosis and treatment for children with epilepsy.

Lastly, health economic evaluations typically measure the effect of technology only for the affected individual. However, genetic diagnostic results could provide valuable information on genetic etiology, and health risks of family members of patients. Therefore, health service use and health-related utility of family members should also be considered in future economic evaluations.

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APPENDICES

Appendix 1. The detailed quality information of the included studies

	Patient Selection				Index test			Reference Standard			Flow and timing		
	SQ1	SQ2	SQ3	App	SQ1	SQ2	App	SQ1	SQ2	App	SQ1	SQ2	SQ3
Costain et al., 2019 [148]	N	Y	Y	High risk	U	Y	High risk	U	U	Low risk	U	N	Y
	High RoB				Unclear RoB			Unclear RoB			High RoB		
Yang et al., 2019 [123]	N	Y	Y		U	Y	High risk	U	U	Low risk	U	N	Y
	High RoB				Unclear RoB			Unclear RoB			High RoB		
Demos et al., 2019 [149]	N	Y	Y	High risk	U	Y	High risk	U	Y	High risk	N	N	Y
	High RoB				Unclear RoB			Unclear RoB			High RoB		
Long et al., 2019 [150]	N	Y	Y	High risk	U	Y	High risk	U	U	High risk	U	N	Y
	High RoB				Unclear RoB			Unclear RoB			High RoB		
Jiao et al., 2019 [151]	Y	Y	Y	High risk	N	Y	High risk	U	U	High risk	U	N	N
	Low RoB				High RoB			Unclear RoB			High RoB		
Tsang et al., 2019 [152]	N	Y	Y	High risk	N	Y	High risk	U	U	High risk	U	N	Y
	High RoB				High RoB			Unclear RoB			High RoB		
Papuc et al., 2019 [142]	N	Y	Y	Low risk	U	Y	Low risk	U	U	High risk	U	N	N
	High RoB				Unclear RoB			Unclear RoB			High RoB		
Yuskaitis et al., 2018 [153]	N	Y	Y	High risk	U	Y	Low risk	U	U	High risk	U	N	Y
	High RoB				Unclear RoB			Unclear RoB			High RoB		

Peng et al., 2019 [154]	U Y Y Unclear RoB	High risk	U Y Unclear RoB	High risk	U U Unclear RoB	Unclear	U U Y Unclear RoB	
Palmer et al., 2018 [134]	N Y Y High RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB	
Bruun et al., 2018 [155]	N Y N High RoB	Low risk	U Y Unclear RoB	Low risk	U U Unclear RoB	High risk	U U Y Unclear RoB	
Howell et al., 2018 [9]	N Y Y High RoB	Low risk	U N High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB	
Berg et al., 2017 [156]	N Y Y High RoB	Low risk	U N High RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB	
Kobayashi et al., 2016 [157]	U Y Y Unclear RoB	Low risk	U N High RoB	High risk	U U Unclear RoB	High risk	U N N High RoB	
Dimassi et al., 2016 [158]	U Y Y Unclear RoB	High risk	U N High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB	
Allen et al., 2016 [159]	N Y Y High RoB	Low risk	N Y High RoB	Low risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB	
Michaud et al., 2014 [160]	U Y Y Unclear RoB	High risk	N Y High RoB	Low risk	U U Unclear RoB	Low risk	U U Y Unclear RoB	
Veeramah et al., 2013 [161]	U Y Y Unclear RoB	Low risk	U Y Unclear RoB	Low risk	U U Unclear RoB	High risk	U N Y High RoB	

Na et al., 2020 [55]	N Y Y High RoB	Low risk	N Y High RoB	Low risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB
Truty et al., 2019 [53]	Y Y Y Low RoB	High risk	N Y High RoB	Low risk	U Y Unclear RoB	Unclear	U U Y Unclear RoB
Hoezl et al., 2020 [162]	N Y Y High RoB	High risk	U Y Unclear RoB	High risk	U U Unclear RoB	Low risk	U N Y High RoB
Symonds et al., 2019 [163]	N Y Y High RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	High risk	Y Y Y Low RoB
Yamamoto et al., 2019 [164]	U Y Y Unclear RoB	High risk	N Y High RoB	Low risk	U U Unclear RoB	Unclear	U U Y Unclear RoB
Balciuniene et al., 2019 [165]	Y Y Y Low RoB	Low risk	U Y Unclear RoB	High risk	U U Unclear RoB	Unclear	U U N High RoB
Wang et al., 2019a [166]	U Y Y Unclear RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB
Angione et al., 2019 [167]	N Y Y High RoB	High risk	N N High RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB
Liu et al., 2018a [48]	N Y Y High RoB	Low risk	U Y Unclear RoB	Low risk	U U Unclear RoB	Unclear	U U Y Unclear RoB
Miao et al., 2018 [168]	U Y Y Unclear RoB	High risk	U Y Unclear RoB	High risk	U U Unclear RoB	Unclear	U U Y Unclear RoB

Gieldon et al., 2018 [169]	U Y Y Unclear RoB	High risk	N Y High RoB	Low risk	U U Unclear RoB	Unclear	U N Y Unclear RoB
Kothur et al., 2018 [170]	N Y Y High RoB	High risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB
Oates et al., 2018 [47]	N Y Y High RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB
Stanek et al., 2018 [171]	U Y Y Unclear RoB	Low risk	U Y Unclear RoB	High risk	U U Unclear RoB	Unclear	U U Y Unclear RoB
Fung et al., 2017 [172]	U Y Y Unclear RoB	Low risk	U Y Unclear RoB	High risk	U U Unclear RoB	Unclear	U U Y Unclear RoB
Ko et al., 2018 [173]	U Y Y Unclear RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	Unclear	U U Y Unclear RoB
Rim et al., 2018 [174]	U Y Y Unclear RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U U Y Unclear RoB
Zhou et al., 2018 [175]	Y Y Y Low RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	High risk	U U Y Unclear RoB
Ortega-Moreno et al., 2017 [176]	U Y Y Unclear RoB	High risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U U Y Unclear RoB
Arafat et al., 2017 [177]	U Y Y Unclear RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U N Y High RoB

Zhang et al., 2017 [178]	U Y Y Unclear RoB	Low risk	N U High RoB	High risk	U U Unclear RoB	Unclear	U U Y Unclear RoB	
Gokben et al., 2017 [179]	U Y N High RoB	Low risk	U U Unclear RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB	
Segal et al., 2016 [180]	N Y Y High RoB	High risk	U U Unclear RoB	High risk	U U Unclear RoB	Low risk	U U Y Unclear RoB	
Trump et al., 2016 [181]	U Y Y Unclear RoB	Low risk	N U High RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB	
Zhang et al., 2015 [182]	U Y Y Unclear RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB	
Mercimek-Mahmutoglu et al., 2015 [40]	N Y Y High RoB	Low risk	U Y Unclear RoB	High risk	U U Unclear RoB	Low risk	U N Y High RoB	
Ream and Mikati, 2014 [183]	N Y Y High RoB	High risk	N Y High RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB	
Della Mina et al., 2015 [184]	Y N Y Low RoB	Low risk	N Y High RoB	High risk	U U Unclear RoB	High risk	U N Y High RoB	
Wirrell et al., 2015 [185]	U Y Y Unclear RoB	High risk	U N High RoB	High risk	U U Unclear RoB	Low risk	Y N Y High RoB	
Wang et al., 2019b [186]	N Y Y High RoB	High risk	U Y Unclear RoB	Low risk	U U Unclear RoB	High risk	U N Y High RoB	

Vlaskamp et al., 2017 [187]	N Y Y High RoB	Low risk	U Y Unclear RoB	Low risk	U U Unclear RoB	Unclear	U N Y High RoB
Allen et al., 2015 [188]	N Y Y High RoB	Low risk	U U Unclear RoB	Unclear	U U Unclear RoB	Unclear	U U Y High RoB
Boutry-Kryza et al., 2015 [189]	U Y Y Unclear RoB	High risk	N Y High RoB	High risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB
Olson et al., 2014 [190]	N Y Y High RoB	High risk	N U High RoB	High risk	U U Unclear RoB	Low risk	U U Y Unclear RoB
Helbig et al., 2014 [191]	U Y Y Unclear RoB	High risk	U Y Unclear RoB	Low risk	U U Unclear RoB	High risk	U N Y High RoB
Du et al., 2014 [192]	U Y Y Unclear RoB	High risk	U Y Unclear RoB	Low risk	U U Unclear RoB	Low risk	U Y Y Unclear RoB

Note. App, Applicability concern; SQ1, signaling question 1; SQ2, signaling question 2; SQ3, signaling question 3; Y, yes; N, No; U, unclear; RoB: risk of bias

Appendix 2. R codes of comparison of diagnostic yield

```
> install.packages(c("metafor", "meta"))

> library(metafor)

> library(meta)

> data1 <- read.csv("regression.CMA.csv", as.is=TRUE)

> data1

>pes.summary = metaprop(Dx, Total, Authoryear, data=data1, sm="PRAW")

> forest(pes.summary, xlim=c(0,1),
```

```
+ rightcols=FALSE,
+ leftcols=c("studlab", "Dx", "n", "effect", "ci"),
+ leftlabs=c("CMA", "Dx", "Diagnostic yield", "95% C.I."),
+ xlab="Diagnostic yield", smlab="",
+ weight.study="random", squaresize=0.5, col.square="navy",
+ col.square.lines="navy",
+ col.diamond="maroon", col.diamong.lines="maroon",
+ pooled.totals=FALSE,
+ comb.fixed=FALSE,
+ fs.hetstate=10,
+ print.tau2=TRUE,
+ print.pval.Q=TRUE,
+ print.I2=TRUE,
+ digits=2)
```

Appendix 3. R codes of regression by publication year

```
>dat=read.csv("regression.CMA.csv",header=T,sep=",")
>ies.logit=escalc(xi=Dx, ni=Total, measure="PLO", data=dat)
>metareg.year=rma(yi,vi,data=ies.logit,mods=~Year,method="REML")
> print(metareg.year)
>wi=1/sqrt(ies.logit$vi)
> size=1+3*(wi-min(wi))/(max(wi)-min(wi))
>preds.year=predict(metareg.year,newmods=c(1985:2020))
> plot(ies.logit$Year,ies.logit$yi,cex=size,pch=1,xlab="Publication year",
+ ylab="Log Odds of Diagnostic yield", las=1)
```

> lines(1985:2020,preds.year$pred,col="navy")

> lines(1985:2020,preds.year$ci.lb,lty="dashed", col="maroon")

> lines(1985:2020,preds.year$ci.ub,lty="dashed", col="maroon")

Appendix 4. R codes of regression by number of probands

> dat2=read.csv("regression.CMA.csv",header=T,sep=",")

> ies.logit2=escalc(xi=Dx, ni=Total, measure="PLO", data=dat2)

> subganal.size2=rma(yi,vi,data=ies.logit2,mods=~Total,method="DL")

> print(subganal.size2)

> preds.size2=predict(subganal.size2,newmods=c(0:2),transf=transf.ilogit)

>wi=1/sqrt(ies.logit2$vi)

>size=1+3*(wi-min(wi))/(max(wi)-min(wi))

> plot(ies.logit2$Total,ies.logit2$yi,cex=size,pch=1, xlab="Number of probands", ylab="Log Odds of Diagnostic yield", las=1)

> preds.size2=predict(subganal.size2,newmods=c(0:1000))

> lines(0:1000,preds.size2$pred,col="navy")

> lines(0:1000,preds.size2$ci.lb,lty="dashed",col="maroon")

> lines(0:1000,preds.size2$ci.ub,lty="dashed",col="maroon")

Appendix 5. Proportion of each actionable gene mutation

Probabilities	Value
Proportion of actionable mutation among all mutations detected by strategy 1 (conventional diagnostic strategy):	0.2833
Proportion of each mutation among analyzed "actionable" mutations by strategy 1 (conventional diagnostic strategy):	
ALDH7A1	0.1
KCNQ2	0.1
PNPO	0.2
SCN1A	0.2

SCN8A	0.1
SLC2A1	0.1
TSC1/2	0.2
Proportion of actionable mutation among all mutations detected strategy 2 – EP as second-tier test:	0.5609
Proportion of each mutation among analyzed "actionable" mutations by strategy 2 – EP as second-tier test:	
ALDH7A1	0.01333
KCNQ2	0.2
PNPO	0.01333
SCN1A	0.22667
SLC2A1	0.013
TSC1/2	0.00767
FOXG1	0.02
GRIN2A	0.02
KCNT1	0.06
SCN2A	0.1
SCN8A	0.06
STXBP1	0.133
CDKL5	0.133
Proportion of actionable mutation among all mutations detected by strategy 3 – WES as second-tier test:	0.5049
Proportion of each mutation among analyzed "actionable" mutations by strategy 3 – WES as second-tier test:	
ALDH7A1	0.0130
KCNQ2	0.1948
PNPO	0.0130
SCN1A	0.2208
SLC2A1	0.0130
TSC1/2	0.0195
GRIN2B	0.0065
KCNT2	0.0065
FOXG1	0.0195
GRIN2A	0.0195
KCNT1	0.0584

SCN2A	0.0974
SCN8A	0.0584
STXBP1	0.1299
CDKL5	0.12993
Proportion of actionable mutation among all mutations detected by strategy 4 – WES plus CMA as first-tier tests:	0.4969
Proportion of each mutation among analyzed "actionable" mutations by strategy 4 – WES plus CMA as first-tier tests:	
ALDH7A1	0.0186
KCNQ2	0.1925
PNPO	0.0186
SCN1A	0.2112
SLC2A1	0.0124
TSC1/2	0.0248
GRIN2B	0.0062
KCNT2	0.0062
FOXG1	0.0186
GRIN2A	0.0186
KCNT1	0.0559
SCN2A	0.1056
SCN8A	0.0621
STXBP1	0.1242
CDKL5	0.1242

Appendix 6. Cost of laboratory tests

Test	**Specimen**	**Unit cost**	**Source**	**First-tier (FT) or Second-tier (ST)**
Basic biochemistry				
Urea – blood	Blood	1.57	MOH	**FT**
Electrolytes:			MOH	**FT**
- Sodium – urine	Urine	2.72		
- Potassium – urine	Urine	1.39		
- Sodium – blood	Blood	1.38		

- Potassium – blood	Blood	5.57		
Lactate	Blood	7.64	MOH	**FT**
Ammonia	Blood	7.41	MOH	**FT**
Liver biochemistry:	Blood		MOH	**FT**
AST		1.73		
ALT		1.47		
Alkaline phosphatase	Blood	7.3	MOH	**FT**
Uric acid:	Blood	1.06	MOH	**FT**
Creatine and creatinine	Urine	18.53	MOH	**FT**
Venous blood gas:	Blood		MOH	**FT**
pH pCO_2 pO_2		36.18		
Carbon monoxide		17.58		
Full blood count	Blood	10.96	MOH	**FT**
TORCH screen:	Blood		MOH	**FT**
Toxoplasma		104		
Rubella		119		
Cytomegalovirus		36.92		
HSV congenital infection screen		27.9		
Urine metabolic screen:	Urine		MOH	**FT**
Urea		1.76		
Uric acid		1.06		
Glucose		1.06		
Protein		34.58		
Keto acids		16.43		
Organic acids		105.41		
Amino acids		54.27		
Vitamin B12	Blood	14.38	MOH	**FT**
Copper	Urine	49.78	MOH	**FT**
Ceruloplasmin	Blood	10.15	MOH	**FT**

Selenium	Blood	49.77	MOH	**FT**
Zinc	Blood	102.44	MOH	
Plasma amino acids	Blood	78.42	MOH	**FT**
Urine AASA	Urine	258.34	MNG	**FT**
Iron studies:	Blood	7.56	MOH	
Vitamin D: 1,2,5 dihydroxy	Blood	94.49		**ST**
Thyroid function including T3/T4:	Blood		MOH	**ST**
Total T3		12.12		
T3-free		9.35		
T4 or total thyroxine		12.12		
T4-free		12.12		
Thyrotropin-releasing hormone TRH stimulation test		55.91		
Thyroid-stimulating hormone TSH		9.9		
7 dehydrocholesterol	Blood	202	KKI	**ST**
Total homocysteine	Blood	22.97	MOH	**ST**
While cell enzymology (lysosomal enzymes)	Blood	42.36	MOH	**ST**
Transferrin isoforms	Blood	98.05	MOH	**ST**
Acyl carnitine profile	Blood	41.28	MOH	**ST**
Buffy coat electron microscopy	Blood	412.19	MOH	**ST**
Very long-chain fatty acids	Blood	91.69	MOH	**ST**
Biotinidase	Blood	270	GGC	**ST**
Purine and pyrimidines	Urine	63.34	MOH	**ST**
Urinary oligosaccharides	Urine	337	GGC	**ST**

EEG:		125.9	MSP	**FT**
-Procedure		85.92		
-Consultation				
MRI head scan:				**FT**
-MRI brain without contrast, General Anesthesia		899.36	[221]	
-Anesthesia fee		72.2	MSP	
-Interpretation fee (neurologist)		52.48	MSP	
PET head scan:			Saskatchewan Medical Association	**FT**
-PET partial body		772		
-Interpretation		197		
CT head scan:				**FT**
-CT brain without contrast monitored care		855.6	(Cost of Canada)	
-Procedure fee		89.01	MSP	
-Anesthesiologist fee		72.2	MSP	
-Interpretation fee (neurologist)		52.48	MSP	
Cerebrospinal fluid:				**ST**
-Spinal tap, local anesthetic		376.47	[221]	
-Lumbar punctures		67.34	MSP	
-Consultation, neurosurgeon		77.8	MSP	
CSF: blood lactate	CSF	1.06	MOH	**ST**
CSF: blood glucose	CSF	7.91	MOH	**ST**
CSF: neurotransmitters	CSF	270	MNG	**ST**
CSF: amino acids	CSF	331	MNG	**ST**
Respiratory chain enzymology: muscle				
-Muscle biopsy, general anesthesia		2,163.4	[221]	
-Consultation		77.8	MSP	

-Anesthesiologist, 30 minutes	65.26	MSP
- Anatomical pathologist, 30 minutes	88.5	MSP
Respiratory chain enzymology: fibroblasts		
-Skin biopsy, local anesthetic	304.05	[221]
-Procedure fee	50.29	MSP
-Consultation	47.55	MSP
-Skin culture	603.88	MOH
Genetic testing		
CMA – proband	785	[221]
Single gene sequencing	1100	Lifelabs
Screening for KCNQ2	1100	Lifelabs
Screening for KCNT1	1100	Lifelabs
Screening for SCN2A	1100	Lifelabs
Screening for ALDH7A1	1100	Lifelabs
Screening for CDKL5	1100	Lifelabs
Sequencing of MECP2	900	Lifelabs
Sequencing of SCN1A	1100	Lifelabs
Sequencing of STXBP1	1100	Lifelabs
Sequencing of SCN8A	1100	Lifelabs
Sequencing of PNPO	1300	Lifelabs
WES		
Cost of Sanger sequencing per variant in trio	160	Sickkid
Cost of trio exome sequencing:		
-WES (gold)	3,500	Lifelabs

-WES (platinum)	5,300	Lifelabs
- (10-14 days turnaround)	6,342	MNG
- (2-3 weeks turnaround)	5,695	MNG
-Trio WES	7,515	University of Chicago
-Trio WES (STAT)	15,030	
Cost of EP:		
-Epilepsy	4,283	MNG
-Early infantile epileptic encephalopathy	4,822	Centogene
	4,535	Greenwood Genetic Center
	3,887	University of Chicago

Appendix 7. Treatment effectiveness

		Value	Source
ALDH7A1, seizure-free (SF)		0.7272	[234]
Not seizure-free (NSF)		0.1364	[235]
Not adequately controlled (NAC)		0.1364	
CDKL5,	SF	0.25	[87]
	NSF	0.375	
	NAC	0.375	
STXBP1,	SF	0.6	[128]
	NSF	0.2	
	NAC	0.2	
SCN8A,	SF	0.3148	[79, 236]
	NSF	0.4815	
	NAC	0.2037	
SCN2A,	SF	0.3654	[237]
	NSF	0.3269	
	NAC	0.3077	
KCNT2,	SF	0	[238]
	NSF	1	

	NAC	0	
KCNT1,	SF	0.0513	[235, 239]
	NSF	0.3590	
	NAC	0.5897	
GRIN2A/B,	SF	0	[103]
	NSF	1	
	NAC	0	
FOXG1,	SF	1	[94]
	NSF	0	
	NAC	0	
TSC1/2,	SF	0.4444	[123]
	NSF	0.3333	
	NAC	0.2222	
SLC2A1,	SF	1	[240-242]
	NSF	0	
	NAC	0	
SCN1A,	SF	0.2375	[123]
	NSF	0.2375	
	NAC	0.525	
PNPO,	SF	0.6666	[243]
	NSF	0.1667	
	NAC	0.1667	
KCNQ2,	SF	0.655	[244]
	NSF	0.202	
	NAC	0.143	
Care as usual for genetic cause			[228]
SF		0.33	
NSF		0.335	
NAC		0.335	
Care as usual for non-genetic cause			[228]
SF		0.26	

NSF	0.37	
NAC	0.37	
Care as usual for unknown cause		[228]
SF	0.51	
NSF	0.245	
NAC	0.245	

Appendix 8. Results of one-way sensitivity analyses

Variable	Values	ICER (cost per QALY)			
		Strategy 1- Conventional strategy	**Strategy 2- EP as second-tier test**	**Strategy 3- WES as second-tier test**	**Strategy 4- WES and CMA as first-tier tests**
Proportion of patients with etiology identified by EP as second-tier test	0.2002	Dominated	—	19,734	426,517
	0.3336	Dominated	9,362	—	691,009
Proportion of patients with etiology identified by WES as second-tier test	0.2302	Dominated	44,442	—	329,986
	0.3836	Dominated	—	34,138	2,141432879
Proportion of patients with etiology by WES and CMA as first-tier tests	0.3258	Dominated	—	26,070	Dominated
	0.5429	Dominated	—	26,070	171,325
Proportion of patients with etiology identified by standard first-tier test	0.2687	—	118,012	26,070	191,850
	0.4479	Dominated	—	26,070	2,123,815
Cost of EP	3,597	Dominated	—	325,758	426,517
	5,995	Dominated	Dominated	—	426,517
Cost of WES	5,521	Dominated	Dominated	—	344,932
	9,202	Dominated	—	486,100	508,103

Proportion of actionable genes identified by EP as second-tier test	0.4207	Dominated	—	105,858	426,517
	0.7011	Dominated	Dominated	—	426,517
Proportion of actionable genes identified by WES as second-tier test	0.3787	Dominated	Dominated	—	426,821
	0.6311	Dominated	—	106,895	426,162
Proportion of actionable genes identified by WES and CMA as first-tier tests	0.3727	Dominated	—	26,070	430,306
	0.6211	Dominated	—	26,070	423,671
Utility of "seizure-free"	0.52	Dominated	—	189,013	10,256,528
	0.87	Dominated	—	14,001	217,787
Utility of "not seizure-free"	0.45	Dominated	—	20,202	300,814
	0.76	Dominated	—	36,742	732,692
Utility of "not adequately controlled"	0.32	Dominated	—	19,726	330,496
	0.53	Dominated	—	38,430	601,185
Time horizon	20	—	—	Dominated	Dominated