TECHNOLOGY REVIEW (MINI-HTA)

WHOLE EXOME SEQUENCING FOR CHILDREN WITH SUSPECTED GENETIC DISEASE

Malaysian Health Technology Assessment Section (MaHTAS)
Medical Development Division
Ministry of Health Malaysia
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EXECUTIVE SUMMARY

Background

Rare diseases (RD) posed an important public health issue greatly impacting the lives of patients, their family and caregivers, healthcare systems and society, and constituted a diagnostic challenge as they include a very heterogeneous group of disorders that can affect any system of the body. Rare diseases (RD) are a group of an estimated 6,000 to 9,000 known severe, chronic, degenerative, and often life-threatening conditions defined as diseases affecting less than 1 in 2,000 (5 in 10,000) people in Europe, while World Health Organization has suggested a frequency of less than 6.5 to 10 per 10,000 people to define RD. Majority of the countries defined RD as to be between 0.01% to 0.05% of their population. In Malaysia, RD as stipulated is a life-threatening and/or chronically debilitating rare condition affecting fewer than 1 in 4,000 people, as listed in the Malaysian RD list.

There are more than 6,000 genetic diseases, including single gene disorders, genomic structural defects and copy number variants, which are leading cases of childhood mortality and posed substantial financial implication. Estimated range of total inpatient charges for US pediatric patients related to suspected genetic diseases was US$ 14 to US$ 57 billion (2012), which is 11% to 46% of all pediatric inpatient charges.

Approximately 80% of RD have a genetic origin. However, more than half of these patients remain without a definite diagnosis. Twenty-five percent of patients with a RD are waiting from five to 30 years for confirmatory diagnosis and during this time 40% receive a misdiagnosis. The challenge is contributed by factors namely, genetic heterogeneity, clinical heterogeneity, frequent comorbidity and disease progression which is faster in children, switching the diagnostic odyssey to a race against time. Hence, establishing an early genomic diagnosis is important for timely management and optimal outcomes, particularly in guiding decision such as therapeutic selection and surgery.

Traditionally, establishment of molecular diagnosis was made by serial testing guided by differential diagnosis. Serial testing employs tests including newborn screening panels, metabolic testing, cytogenetics, single gene sequencing and panel sequencing. Conventional molecular testing of patients with genetic disorders has earlier relied primarily on single gene or panel testing or microarrays. However, it was estimated that up to 50% of patients fail to receive a molecular diagnosis after such testing and embark on a diagnostic odyssey which is both slow and costly for health-care providers. Traditional sequential gene sequencing is not expedient in neonates owing to high cost, turnaround time and heterogeneity of phenotype at this young age.

WGS and WES have gained interest as they permit comprehensive and timely diagnosis of genetic diseases by allowing concomitant examination of all of most genes in the differential diagnosis. Exome sequencing has increasingly available following improvements in massively parallel sequencing and bioinformatics tools for data analysis, which have lowered the cost and decreased the turnaround time. Approximately 95% of the exome can be sequenced with currently available techniques. WES which is the targeted sequencing of the subset of the human genome that codes for proteins, helps to resolve undiagnosed genetic conditions, improves the diagnostic yield, guide treatment decision and the management of patients.

Genome sequencing identified and analysed the sequence of all coding and non-coding nuclear DNA, thus is costlier than exome sequencing due to high cost of data analysis. However, the diagnostic utility (20% to 30%) is almost similar with WES. Besides, although genome sequencing can identify variants outside of the coding regions, determination of pathogenicity of these variants is often not possible.

According to the requestor, to date there are more than 9,000 RD; which often comprise of serious multisystem disease that assume a disproportionate amount of healthcare resources. Genetic conditions incur higher direct heal-care costs (3.5 to 8.3 times higher per patient) and resource use. WES could transform the field of genetic disease diagnostics with rapid, high-throughput, which is needed to end the diagnostic odyssey and improve disease management in these patients. However, currently WES is not available in the MOH facilities. Hence, this necessitates the review which is conducted following the request by Clinical Geneticist from the Genetic Unit, Hospital Pulau Pinang to assess the evidence on WES to be used in diagnosing children with suspected genetic disease.

Objective/ aim

The objective of this technology review is to assess the effectiveness, safety and cost-effectiveness of WES to be used in diagnosing children with suspected genetic disease.

Results and conclusion

A total of 21 studies were included in this review. There were fourteen studies retrieved on the effectiveness of WES as diagnostic option for children with suspected genetic disease, of which five studies were on infant. The fourteen studies were comprised of two SR, one scoping review, one RCT, nine cohort studies and one case series. Two studies retrieved were on safety (qualitative studies) and another five studies were on cost-effectiveness, as well as one HTA report on WES as diagnostic option for children with suspected genetic disease. The studies were originated from US, Australia, Taiwan, Hong Kong, France, Brazil, Germany and UK. The included SR reviewed evidences from multiple countries, mainly from US and Europe. Total participants enrolled in this review were 21,937. Study sample size varied from 29 to 500 patients.

The 21 included studies investigated the use of WES or rapid WES in children with a variety of suspected genetic conditions, the most common being developmental abnormality or delay, or neurodevelopmental disorders, with six studies investigating WES or rapid WES exclusively in newborns, and two studies investigating impact of WES among caregivers. The WES conducted varies from proband to trio in the included studies. The longest follow up reported was up to 33 months.

Effectiveness

Based on the above review, there was fair level of evidences on WES to be used in diagnosing children including infant with suspected genetic disease.

WES including rapid WES showed beneficial effect in diagnostic yield and clinical utility (change in clinical management) in diagnosing children including infant with suspected genetic disorder. WES appeared better in terms of providing diagnosis rate to patients and relatives; and the benefit of diagnosis, namely impact on clinical management to patients and relatives, than standard care.

The WES was carried out as standard WES or rapid WES, either singleton (proband) or trio; in a variety of genetic conditions in clinical practice; from children with neurodevelopmental disorders, congenital anomalies, developmental delay, intellectual disability, undiagnosed developmental abnormality, medical condition requiring rapid diagnosis, cases with suspected monogenic disease, symptomatic patients with rare disease or ill infants; commonly being neurological or neurodevelopmental disorder.

Diagnostic yield ranged from 31.6% to 52.0%, and from 36.7% to 57.5% following WES in children and infants with suspected genetic disease, respectively. Mean turn around time was 40 days (range 25 to 100 days).

Following the use of rapid WES, diagnostic yield ranged from 20% to 52.5% in critically ill infants. Time to report ranged from 5.3 to 16 days.

Impact on clinical management ranged from 26% to 52% for children or infant with suspected genetic disease following use of WES, and ranged from 57% to 88% following rapid WES in critically ill infant or patients beyond infancy.

Safety

In terms of safety, WES Constituent Device was registered as Class II medical device by USFDA. The WES constituent device consists of reagents, instrumentation, software and instructions. There is no psychosocial impact upon receiving VUS on caregivers following WES, with most had a good understanding and the result had no impact on their perception of their child's condition. WES test results, evoked relief as well as worries, identified advantages and disadvantages, irrespective of the type of result among parents with children whom underwent WES.

Cost-effectiveness

In terms of cost-effectiveness, evidence demonstrated that pathways with earlier WES testing were more likely to be cost savings compared to pathways that used WES later in the testing pathway or used WES as a last-resort strategy.Cost estimates for a single test ranged from $555 to $5,169 for WES and from $1,906 to $24,810 for WGS. Cost estimates for a trio ranged from £2,658 ($3,825) to £6,466 ($9,304).

CEA conducted in Australia from healthcare system perspective found using WES to replace most investigations (as a first line) results in a savings per additional diagnosis of AU$2,182 (US$1,702). WES as a first-line test replacing most investigations is dominant. Another CEA in Australia found if WES performed at initial tertiary presentation, the resulted incremental cost savings was A$9020 (US$6838) per additional diagnosis compared with standard diagnostic pathway. However, adding WES to the standard diagnostic pathway does not offer a cost savings, but incurs an additional cost of A$5760 (US$4371) per diagnosis.

Another CEA done in UK from NHS perspective found if WES was introduced later in the testing pathway, the ICER per additional positive genetic diagnosis was £3,171; while if the test used as a near first-line test, the ICER per additional genetic diagnosis was £2,201, compared to the usual testing approach. Sensitivity analyses showed that the largest driver of cost was the cost of the genetic testing, including cost of the exome sequencing and the associated bioinformatics analysis.

Cost analysis in a German cohort of children with NDD/epilepsy found genetic examinations had the highest cost savings potential amounting to 302,947.07€ (90.2%) out of 335,837.49€ [a total of 687,168.02€ was spent on genetic diagnostics]. This corresponds to total savable cost of 3,025.56€ per individual, compared to saving of 197.33€ for cMRI examinations and 98.98€ for metabolic testing in this cohort.

Economic implication

A cost calculation was conducted to estimate the potential cost implication should WES be integrated earlier in the diagnostic pathway for patients with suspected genetic diseases. It involves four scenario analyses which offer WES or CMA either as the first-tier or second-tier test. All patients were assumed not to undergo any prior genetic testing upon presentation at the genetic clinic, and beyond these two tests, the costs for any further testing were not considered. In a population without a clear differential diagnosis, as a first-test, the number of patients with positive results from WES was almost quadruple the number achieved with CMA, at a cost per diagnosis less than a quarter of the cost per diagnosis estimated for CMA. In all scenarios, integrating WES as the first-tier test have resulted in a lower cost per diagnosis as well as cost per patient. Even when a higher test cost for WES was applied, a similar trend was observed. This may indicate that the diagnostic yield of a genetic test plays a significant role in affecting the cost per diagnosis or the cost per patient.

Organizational

In terms of organizational, WES is conducted by laboratories that are accredited by the Clinical and Laboratory Improvement Act (CLIA) to conduct high complexity testing. This test is commonly only conducted in laboratories associated with large, tertiary medical centers or commercial genetics laboratories due to the equipment and software involved (particularly the bioinformatics platform).

Creating reasonable expectations, establishing an understanding of the value and limitations of testing, creating awareness of the potential harms, and allowing the family to make informed choices is a mainstay of informed consent. Elements of counseling should include a three-generation family pedigree; discussion of pathogenic/likely pathogenic results, benign results, and variants of uncertain significance; detection of misattributed paternity or consanguinity, and secondary findings unrelated to the reason for testing.

The ACMG 2021 guideline recommended ES and GS as a first-tier or second-tier test for patients with one or more congenital anomalies prior to one year of age, or for patients with Developmental Disorder/Intellectual Disability with onset prior to 18 years of age. The EuroGentest and the European Society of Human Genetics (2016) guidelines on the evaluation and validation of next-generation sequencing (NGS) applications for the diagnosis of genetic disorders; highlighted the importance of diagnostic utility, informed consent, information to the patient and clinician, validation and reporting.

In terms of reimbursement, several commercial payers covered WES with specific criteria have to be met by the beneficiaries. Several eligibility criteria have to be met for funding by Medicare (Australia) on WES, which are:-

- If the child is strongly suspected of having a single gene disorder and is aged 10 years or younger.

- The child has a non-informative chromosome microarray (CMA) test. Negative Fragile X testing and urine metabolic screening is also desirable.
- A clinical geneticist has been consulted about the test indications.
- The family has given informed consent using the appropriate consent forms.

The Washington State Health Authority stated that WES is a covered benefit with conditions. The test is considered medically necessary for the evaluation of unexplained congenital or neurodevelopmental disorders in a phenotypically affected individual when all of the criteria are met (in the document). Similarly, whole exome or WGS is considered medically necessary by several commercial payers such as Kaiser Permanente, Cigna, Aetna when criteria listed (in the document) are met. Pre- and post-test genetic counseling is required for any individual undergoing whole exome or WGS.

Ethico-legal

In terms of ethico-legal, the Genetic Information Nondiscrimination Act (GINA, 2008) protects the US citizen from discrimination based on their genetic information in both health insurance and employment.

Methods

Studies were identified by searching electronic databases. The following databases were searched through the Ovid interface: MEDLINE(R) In-process and other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to present. EBM Reviews-Cochrane Database of Systematic Reviews (2005 to April 2024), EBM Reviews-Cochrane Central Register of Controlled Trials (April 2024), EBM Reviews – Database of Abstracts of Review of Effects (1st Quarter 2024), EBM Reviews-Health Technology Assessment 1st Quarter 2024), EBM Reviews-NHS Economic Evaluation Database (1st Quarter 2024). Parallel searches were run in PubMed. Appendix 3 showed the detailed search strategies. No limits were applied to the search. The last search was run on 31 March 2024. Additional articles were identified from reviewing the references of retrieved articles. Among the tools used to assess the risk of bias and methodological quality of the articles retrieved is the Cochrane ROBIS risk of bias tool, ROB-2 tool and CASP checklist. All full text articles were then graded based on guidelines from the US/Canadian Preventive Services Task Force.

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ABBREVIATION

AE	Adverse event
ACMG	American College of Medical Genetic & Genomic
CA	Congenital anomalies
CASP	Critical Appraisal Skilled Programme
CEA	Cost-effectiveness analysis
CMA	Chromosomal microarray
CI	Confidence Interval
CVS	Chorionic villus sampling
DD	Developmental delay
ES	Exome sequencing
GINA	Genetic Information Nondiscrimination Act
gDNA	genomicDNA
HR	Hazard Ratio
HRQOL	Health related quality of life
ID	Intellectual disability
IPD	Individual patient data
IQR	Interquartile Range
MOH	Ministry of Health
NDD	Neurodevelopmental disorder
NGS	Next generation sequencing
NICU	Neonatal Intensive Care Unit
PICU	Paediatric Intensive Care Unit
QOL	Quality of Life
RCT	Randomised controlled trial
RD	Rare disease
RR	Relative risk
SE	Standard error
SOI	Severity of Illness
TAT	Turn around time
vs	Versus
VUS	Variant uncertain significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
WMD	Weighted mean difference
WTP	Willingness to pay

1.0 BACKGROUND

Rare diseases (RD) imposed an important public health issue, greatly impacting the lives of people living with such conditions, their family and caregivers, healthcare systems and society.[1] They remain an important health issue and a diagnostic challenge as they include a very heterogeneous group of disorders that can affect any system of the body.[2] Rare diseases (RD) are a group of an estimated 6,000 to 9,000 known severe, chronic, degenerative, and often life-threatening conditions defined as diseases affecting no more than 1 in 2,000 (5 in 10,000) people in Europe.[3] While the World Health Organization (WHO) has suggested a frequency of less than 6.5 to 10 per 10,000 people to define RD. In the United States of America, a number fewer than 200,000 people has been defined for RD, with less than 50,000 people in Japan and less than 2,000 people in Australia.[4,5] Majority of the countries defined RD as to be between 0.01% to 0.05% of their population.[6] In Malaysia, RD is defined as a life-threatening and/or chronically debilitating rare condition affecting fewer than 1 in 4,000 people, as listed in the Malaysian RD List.[7] The top three RD in Malaysia are Marfan syndrome, Prader-Willi syndrome, and Osteogenesis Imperfecta. [6]

There are more than 6,000 human genetic diseases.[8] Although genetic diseases are individually rare, they collectively affect approximately 1 in 17 individuals.[9] It was estimated that the range of total inpatient charges for US pediatric patients related to suspected genetic diseases in 2012 was US$ 14 to US$ 57 billion, which is 11% to 46% of all pediatric inpatient charges.[10]

Genetic diseases including single gene disorders, genomic structural defects and copy number variants are leading cases of childhood mortality.[11] Over 20% of infant death in the US are caused by chromosomal abnormalities, congenital malformation, and deformation.[12] Approximately 80% of RD have a genetic origin and the new genomic technologies revolutionized diagnostic approach.[13] More than half of patients with the suspected rare genetic disease remain without a definite diagnosis. Eurordis study found that 25% of patients with a RD are waiting from five to 30 years for confirmatory diagnosis and during this time 40% receive a misdiagnosis. [14] The diagnostic odyssey typically involves assessment and many investigations, many of which are invasive and costly. The diagnostic trajectory can be prolonged, and many children continue to be undiagnosed.[15]

Establishing an etiologic diagnosis in children with suspected genetic disease is important for timely management and optimal outcomes, particularly in guiding decision such as therapeutic selection and surgery.[16] Etiologic diagnosis for genetic disease require identification of causative molecular basis and is challenging following genetic heterogeneity (there are over 5200 genetic disorders for which the molecular basis has been established), clinical heterogeneity, frequent comorbidity obscuring clinical manifestation, and faster disease progression in children, switching diagnostic odyssey to a race against time.[16,17]

Traditionally, establishment of molecular diagnosis was made by serial testing guided by differential diagnosis. Whole genome sequencing (WGS) and whole-

exome sequencing (WES) are relatively new method for diagnosing genetic disease, while chromosomal microarray (CMA) is well established. Chromosomal microarray is the recommended first line genomic test for children with several type of genetic disease. Serial testing employs test such as newborn screening panels, metabolic testing, cytogenetic, chromosomal fluorescence in situ hybridization, single gene sequencing and sequencing a panel of genes.[18] Conventional molecular testing of patients with genetic disorders has earlier relied primarily on single gene or panel testing or microarrays. However, estimates suggest that up to 50% of patients fail to receive a molecular diagnosis after such testing and embark on a diagnostic odyssey which is both slow and costly for health-care providers.[19] Traditional sequential gene sequencing is not expedient in neonates owing to high cost, turnaround time and heterogeneity of phenotype at this young age. [20]

WGS and WES have the potential to permit comprehensive and timely diagnosis of genetic diseases by allowing concomitant examination of all of most genes in the differential diagnosis.[14] Exome sequencing is designed to identify and analyse the sequence of all protein-coding nuclear genes in the genome. Approximately 95% of the exome can be sequenced with currently available techniques. The diagnostic utility of exome sequencing was estimated around 20% to 30%, and among more than 2000 cases with suspected Mendelian disorders the rate was 25%. Exome sequencing has increasingly available following continuous improvements in massively parallel sequencing and bioinformatics tools for data analysis, which have lowered the cost and decreased the turnaround time.[21] WES which is the targeted sequencing of the subset of the human genome that codes for proteins, helps to resolve undiagnosed genetic conditions, improves the diagnostic yield, guide treatment decision and the management of patients.[22] Genome sequencing identified and analysed the sequence of all coding and non-coding nuclear DNA. Genome sequencing is more costly than exome sequencing due to high cost of data analysis. However, the diagnostic utility (20% to 30%) is almost similar with WES. Besides, although genome sequencing can identify variants outside of the coding regions, determination of pathogenicity of these variants is often not possible.[23]

Rapid WES examines 2% of the genome, representing almost all exons and immediate flanking intronic region typically within 10-20 base pairs of the exons. Rapid WGS in contrast, examines all exons and introns and 90% of the genome. Evidence examining utility of rWGS and rWES as diagnostic test in infant in genetic disease revealed rates of diagnosis of simple genetic disease ranged from 42% to 57%, changes in medical management from 30% to 72%, and altered outcome from 24% to 34%. The NHS in UK will offer rWGS as part of the care for all seriously ill children. [24]

According to the requestor, to date, there are more than 9000 RD; which often comprise of serious multisystem disease that assume a disproportionate amount of health care resources. Genetic conditions incur higher direct heal-care costs (3.5 to 8.3 times higher per patient) and resource use. WES could transform the field of genetic disease diagnostics with rapid, high-throughput, which is needed to end the diagnostic odyssey and improve disease management in these patients. However, currently WES is not available in the MOH facilities. Hence, this necessitates the review which is conducted following the request by Clinical Geneticist from the

Genetic Unit, Hospital Pulau Pinang to assess the evidence on WES to be used in diagnosing children with suspected genetic disease.

2.0 OBJECTIVE / AIM

The objective of this technology review is to assess the effectiveness, safety and cost-effectiveness of WES in diagnosing children with suspected genetic disease.

3.0 TECHNICAL FEATURES

The haploid human genome (23 chromosomes) is made up of approximately 3.1 billion base pairs (the letters A,T,G and C) and contains more than 20,000 distinct genes.[25] Only 1 to 2% of the entire DNA (genome) can form protein. This portion of the DNA (the coding region), is known as the exome. The human exome includes all coding nuclear DNA sequences, approximately 180,000 exons that are transcribed into mature RNA. (Mitochondrial DNA is not included in the exome). Comprising only 1% to 2% of the human genome, the exome however contains majority of currently recognized disease-causing variants.[26] Although exomes account for 1% of the physical size of the genome, 85% of the reported disease-causing mutations are located on it. [25] (Figure 1)

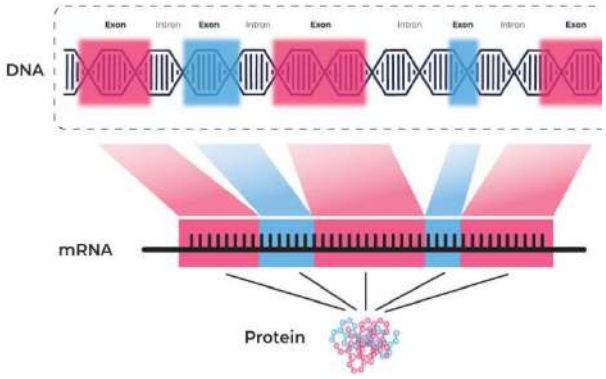

Figure 1:
Exon:1-2% of genome that encode protein
Exome: total exon of the genome

A mutation is defined as a permanent change in the nucleotide sequence, whereas a polymorphism is defined as a variant with a frequency above 1%. The terms “mutation” and “polymorphism,” which have been used widely, however often lead to confusion thus, it is recommended that both terms be replaced by the term “variant” with the following modifiers: (i) pathogenic, (ii) likely pathogenic, (iii) uncertain significance, (iv) likely benign, or (v) benign. Although these modifiers may not address all human phenotypes, they comprise a five-tier system of classification for variants relevant to Mendelian disease. The ACMG guideline 2015 recommended that all assertions of pathogenicity (including “likely pathogenic”) be reported with respect to a condition and inheritance pattern (e.g.,

c.1521_1523delCTT (p.Phe508del), pathogenic, cystic fibrosis, autosomal recessive).In the past decade, sequencing technology has evolved rapidly with the advent of high-throughput next-generation sequencing (NGS). By adopting and leveraging next-generation sequencing, clinical laboratories are now performing increasing catalogue of genetic testing spanning genotyping, single genes, gene panels, exomes, genomes, transcriptomes, and epigenetic assays for genetic disorders. [26]

Next-generation sequencing provides a new strategy for diagnosing genetic disorders. In recent years, WES has been shown to have a unique value in the diagnosis of monogenic diseases. WES could be used as a first-tier test for children with suspected monogenic disorders. [28] WES is used in clinical settings for identifying rare variants and discovering Mendelian disorders. Mendelian disorders are a type of genetic disorder that occurs when an alteration in single genes is inherited from the parents, resulting in disease. WES can identify mutations in genes associated with inherited conditions, diagnose rare diseases, and guide treatment decisions. WES which is the targeted sequencing of the subset of the human genome that codes for proteins, helps to resolve undiagnosed genetic conditions. This approach improves the diagnostic yield and the management of patients. The diagnostic rate of proband WES in a series of more than 2000 cases with suspected Mendelian disorders was 25%.[22]

Analysis of genomic data can include: [29]

i. The whole genome (introns and exons)
ii. The whole exome (exons only)
iii. A gene panel (genes of interest only)

Whole exome sequencing (WES) does not detect all genetic changes, it cannot detect DNA changes in the mitochondria or introns, variations in copy number, aneuploidy, polyploidy or chromosome translocations. It is a diagnostic test used to identify the genetic cause of an individual's health condition. The WES can be performed for an individual (singleton testing) or in combination with both biological parents (trio testing).

Diagnostic WES testing is ordered by a physician or other health care professional and is conducted in a clinical diagnostic laboratory to aid in the diagnosis of a patient. Parents' or siblings' genes may be sequenced to help interpret identified variants (Trio WES). WES uses NGS technologies; **NGS makes many copies of the target genome, then cuts them into random sequences, and simultaneously sequences the resulting fragments**. After this sequencing step, WES requires a series of bioinformatics analyses to interpret the sequencing.[29]

WES Methods [30]

- DNA sources and extraction

The first step of WES involves the acquisition of high-quality genomic DNA (gDNA) from biological samples, most commonly extracted from peripheral blood leukocytes. Common extraction methods include the traditional 'salting out' technique and spin column-based methods. Noteworthy, gDNA can also be

extracted from saliva, which provides a non-invasive alternative to venesection, but at the expense of quantity and quality, particularly pertaining to risk of DNA contamination from oral microflora and food remnants. Formalin-fixed paraffin embedded (FFPE) samples are another viable source, i.e. in archival histopathology specimens and also in cancers.

- Exome library preparation

The preparation of an exome enrichment library follows DNA extraction, summary as in Table 1. Agilent, Illumina and NimbleGen are three commonly used exome capture kits. Product selection is influenced by platform-specific strengths and weaknesses. Despite differences, all capture technologies obey the same three basic principles: (1) DNA fragmentation, (2) adaptor ligation and (3) target enrichment.

Table 1: Comparison of exome capture kits

Characteristic	Agilent HaloPlex	Agilent HaloPlex	Agilent HaloPlex	Ilumina Nextera Rapid Capture Expanded Exome	Illumina TruSeq Exome Enrichment
Target size	37 MB	50 MB	64 MB	62 MB	64 MB
Probe size (bases)	161-75	120	55–105	95	95
Number of targeted exons	557 999	335 765	300 000	201 121	201 121
Reads on target (%)	80	80	>70	60	>65
Fragmentation method	Transposomes	Ultrasonication	Ultrasonication	Transposomes	Ultrasonication

- Exome sequencing

Following exon enrichment, the resultant captured library is subject to high-throughput, massively parallel sequencing to produce millions of short reads. The exome-sequencing methodological workflow is visualized in Figure 1. Current sequencing platforms include Life Technologies SOLiD, Roche's 454 Genome Sequencer, Pacific Bioscience's RS, Life Technologies Ion Proton and the current market leader, Illumina's HiSeq range of sequencers, which use a sequencing by synthesis approach. Figure 2 and 3 illustrates the workflow of WES.

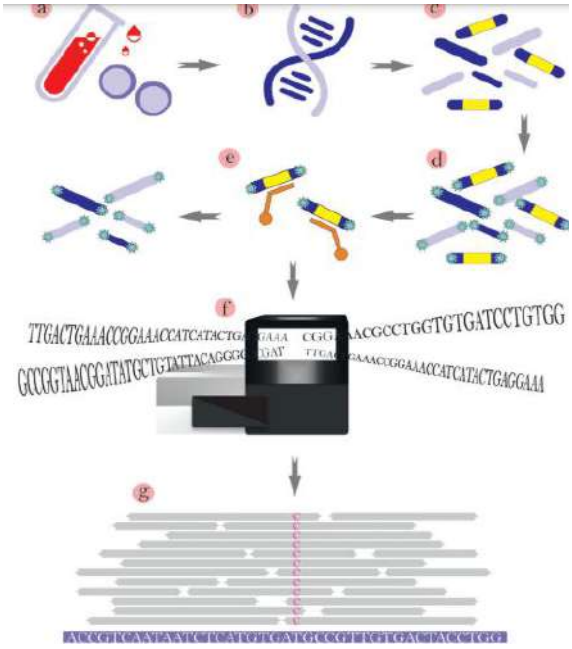

Figure 2: Workflow example of WES (Source: Seaby EG 2016)

(A) A blood sample is collected containing peripheral lymphocytes. (B) gDNA is extracted from white blood cells using extraction kits or the salting-out method, and the quality and quantity is assessed. (C) DNA is fragmented either by sonication or enzymatic methods, which vary between library preparations. (D) Fragment ends are repaired by removing overhanging nucleotides, and the ends are ligated to adaptors (stars). Rectangular regions within fragments represent exons present in DNA fragments. (E) Aqueous-phase hybridization capture enriches exonic sequences (rectangular regions) by ligation of fragments to biotinylated baits (probes) as used by most enrichment platforms. Hybridized fragments are recovered by biotin-streptavidin-based magnetic bead pulldown. Uncaptured regions are washed away. The enriched library of exonic fragments are eluted and amplified. (F) The resultant exome library is sequenced using massively parallel sequencing technologies, producing millions of sequenced reads. (G) Raw data are aligned to the human genome reference sequence and downstream in silico tools analyse output data

Training medical professionals in genomics will improve communication between clinical and research disciplines; interpretation of exome data requires analysis by genomic informaticians with limited clinical knowledge. There is a demand to train clinicians in genomic informatics to be able to close the gap between the clinical and research disciplines and truly demonstrate personalized, translational medicine.

Exome sequencing is done at certified laboratories. WES is done using saliva or a blood sample, and the DNA is sequenced with NGS technology. DNA sequencing determines the order of the four letters (A, T, G, and C) that make up the DNA molecule. The human genome data will then be analysed by the geneticists and bioinformaticians to look for mutations and variation.[31]

Currently, diagnostic WES is still considered a highly complex and expensive test that requires a chain of significant informatics infrastructure for data storage and management, specialized expertise in bioinformatics and medical genomics, all of which could lead to difficulty in achieving a short turn-around time in routine practice at smaller genetics centres. The standard turnaround times in several laboratories vary from 11 to 21 weeks, with an average of 18 weeks. In the leader, the Baylor College of Medicine, the standard turn-around time for results is 15 weeks. [32]

Rapid WES (rWES) and rapid WGS (rWGS) provide a much faster turnaround time (TAT) in days, as contrasted to a succession of different conventional diagnostic tests that may take several months to return, enabling diagnosis-predicated precision medicine and a shorter diagnostic odyssey. [3]

Advantages of exome sequencing and genome sequencing over multigene panels include the former do not require the clinician to determine which disorders (and, hence, which genes) are likely to be involved; thus, testing can be ordered earlier in a patient's diagnostic evaluation. [31] Exome sequencing and genome sequencing can detect the presence of two or more genetically distinct disorders (the phenotypic presentation of which may have complicated diagnosis) in the same individual. Using a multigene panel forces the clinician to select the best panel for the patient, which is often difficult because:

- The less well defined the patient's phenotype, the more difficult it is to identify the most appropriate multigene panel;
- Genes for RD or newly discovered genes may not be included in a multigene panel;
- The clinical sensitivity (which can vary widely among multigene panels) is not provided for some panels

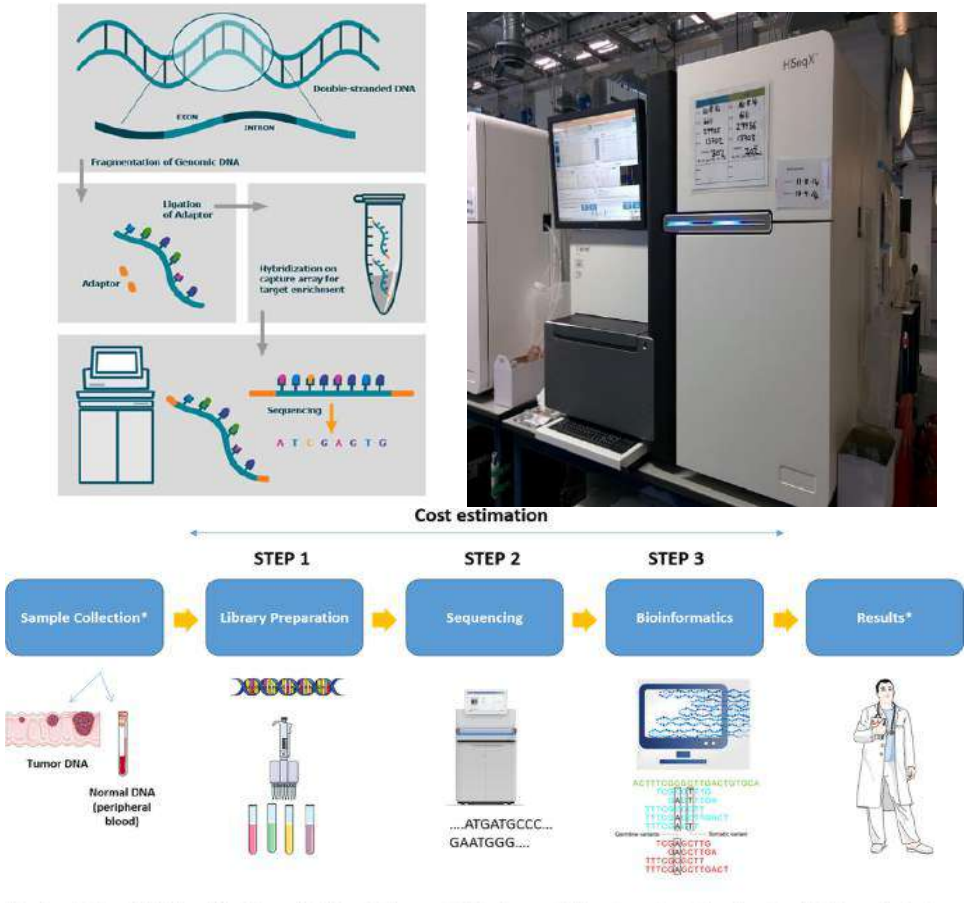

Figure 3: Sequencing platform (above) and steps in whole exome sequencing (Source: Bayle A et al 2021)[33]

4.0 METHODS

4.1 SEARCHING

Electronic databases searched through the Ovid interface:

- MEDLINE(R) In-Process and Other Non-Indexed Citations and Ovid MEDLINE (R) 1946 to present
- EBM Reviews – Cochrane Central Registered of Controlled Trials – February 2024
- EBM Reviews – Database of Abstracts of Review of Effects – 1st Quarter 2024
- EBM Reviews – Cochrane Database of Systematic Reviews – 2005 to February 2024
- EBM Reviews – Health Technology Assessment – 1st Quarter 2024
- EBM Reviews - NHS Economic Evaluation Database – 1st Quarter 2024

Other databases:

- PubMed
- Horizon Scanning database (National Institute of Health research (NIHR) Innovation Observatory, i-HTS International Network)
- Other websites: US FDA, INAHTA, MHRA

General databases such as Google and Yahoo were used to search for additional web-based materials and information. Additional articles retrieved from reviewing the bibliographies of retrieved articles or contacting the authors. The search was limited to articles on human. No limitation in the study design. There was no language limitation in the search. Appendix 1 showed the detailed search strategies. The last search was conducted on the 31st March 2024.

4.2 SELECTION

Two reviewers screened the titles and abstracts against the inclusion and exclusion criteria and then evaluated the selected full-text articles for final article selection. The inclusion and exclusion criteria were:

Inclusion criteria

Population	Children with suspected genetic disorder
Interventions	Whole exome sequencing
Comparators	Whole genome sequencing, Sanger sequencing (single gene testing), other sequencing approach, panel testing, chromosomal microanalysis (CMA) as singleton (proband) or trio (parents and child), no comparator

Outcomes	Diagnostic yield/utility (proportion of patients tested who received genetic diagnoses), rate of clinical utility (proportion of patients tested in whom the diagnosis changed clinical management), sensitivity and specificity, acute clinical usefulness, reporting of incidental findings, quality-adjusted life years (QALYs), time to diagnosis, mortality, parent satisfaction, psychosocial impact
Study design	Systematic reviews (SR), randomised control trials (RCTs), cohort study, case series
Type of publication	English, full text articles

Exclusion criteria

Study design	Survey, anecdotal, animal studies
Type of publication	Non-English
Setting	Studies evaluating WES in clinical setting, testing was performed in hospital or reference laboratories, or research laboratories

4.3 RISK OF BIAS ASSESSMENT

Relevant articles were critically appraised according to the study design. Systematic review was appraised using ROBIS, RCT using Cochrane ROB-2 appraisal tool; cohort studies and cost-effectiveness analysis were appraised using CASP checklist. Evidences were graded according to the US/Canadian Preventive Services Task Force (See Appendix 2). Data were extracted from included studies using a pre-designed data extraction form (evidence table as shown in Appendix 6) and presented qualitatively in narrative summaries. No meta-analysis was conducted for this review.

5.0 RESULTS

A total of 455 titles were identified through the Ovid interface: MEDLINE(R) In-process and other Non-Indexed Citations and Ovid MEDLINE(R) 1946 to present, EBM Reviews-Cochrane Database of Systematic Reviews (2005 to April 2024), EBM Reviews-Cochrane Central Register of Controlled Trials (April 2024), EBM Reviews-Database of Abstracts of Review of Effects (1st Quarter 2024), EBM Reviews-Health Technology Assessment (1st Quarter 2024), EBM Reviews-NHS Economic Evaluation Database (1st Quarter 2024) and PubMed. Three articles were identified from references of retrieved articles. After removal of duplicates, 60 titles were screened. A total of 60 titles were found to be potentially relevant and abstracts were screened using the inclusion and exclusion criteria. Forty potentially relevant abstracts were retrieved in full text. Thirty-six articles were assessed for eligibility, and finally

21 full text articles were included and the remaining articles were excluded. (Figure 4).

The review included a total of 21 studies in this review, which comprised of two SR, one scoping review, one RCT, nine cohort studies and one case series (on effectiveness), followed by two studies retrieved on safety (qualitative studies) and another five studies included were on cost-effectiveness.

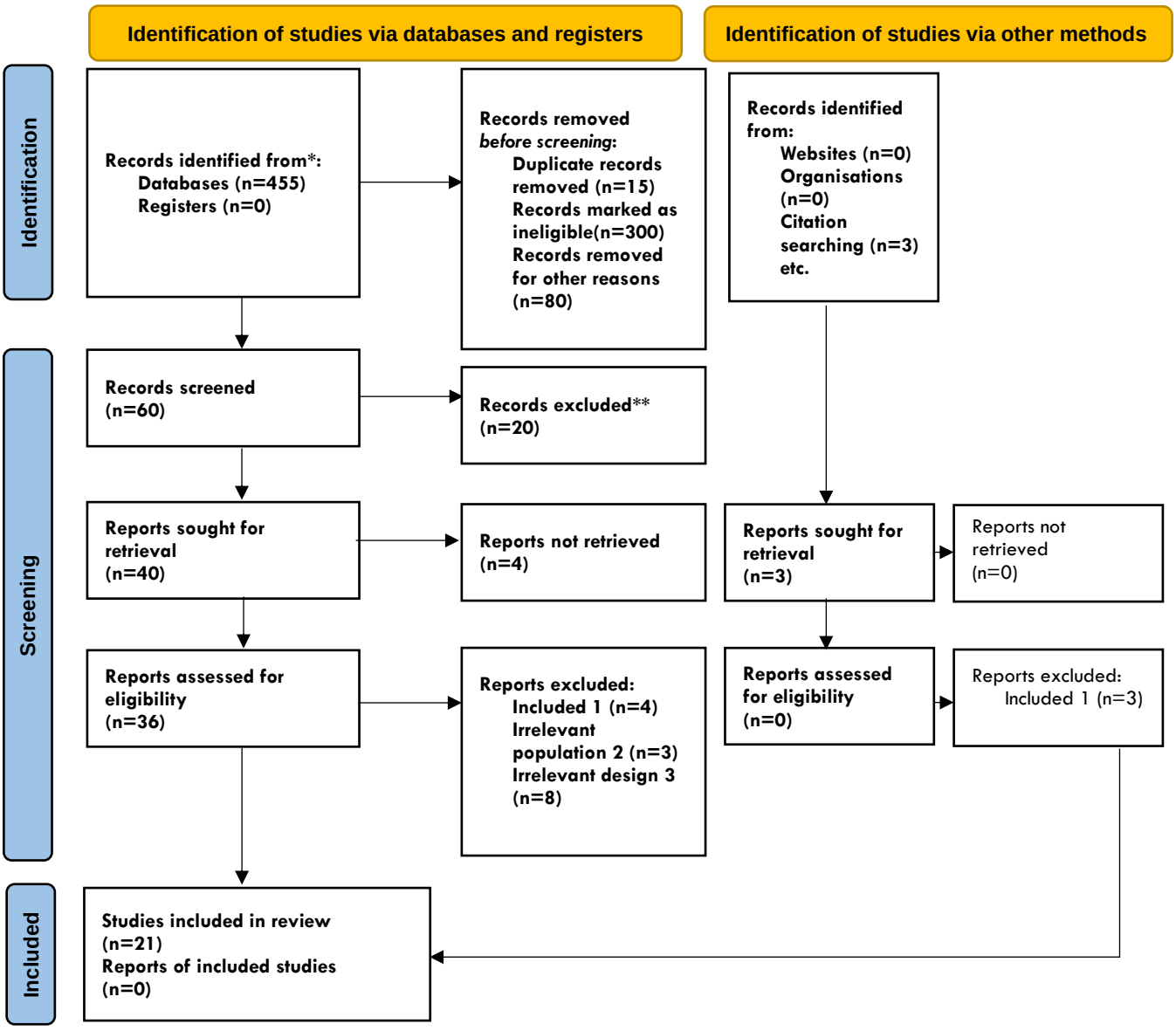

Figure 4: PRISMA 2020 flow diagram of retrieval of articles used in the results

A total of 21 studies were included in this review. There were fourteen studies retrieved on the effectiveness of WES as diagnostic option for children with suspected genetic disease, of which five studies were on infant. The fourteen studies were comprised of two SR, one scoping review, one RCT, nine cohort studies and one case series. Two studies retrieved were on safety

(qualitative studies) and another five studies were on cost-effectiveness, as well as one HTA report on WES as diagnostic option for children with suspected genetic disease. The studies were originated from US, Australia, Taiwan, Hong Kong, France, Brazil, Germany and UK. The included SR reviewed evidences from multiple countries, mainly from US and Europe. Total participants enrolled in this review were 21,937. Study sample size varied from 29 to 500 patients.

The 21 included studies investigated the use of WES or standard WES, or rapid WES or critical WES, in children with a variety of suspected genetic conditions, the most common being developmental abnormality or delay, or neurodevelopmental disorders, with six studies investigating WES or rapid WES exclusively in newborns, and two studies investigating impact of WES among caregivers. The WES conducted varies from singleton proband to trio in the included studies. The longest follow up reported was up to 33 months.

The SR was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist guideline.

5.1 RISK OF BIAS ASSESSMENT OF INCLUDED STUDIES

The risk of bias (methodology quality) of all included studies were assessed according to the type of the study design; using the relevant checklist of National Collaborating Centre for Methods and Tools (ROBIS) for Systematic Review and meta-analysis, ROB-2 for RCT and Critical Appraisal Skill Programme (CASP) for cohort and economic studies.

These assessments involved answering a pre-specified question of those criteria assessed and assigning a judgement relating to the risk of bias. The risk of bias of the included studies was assessed independently by two reviewers, RS and FNM. Any disagreements were resolved through discussion until consensus was reached.

Risk of bias assessment for included systematic review and meta-analysis

For SR, assessment was done following the domain-based evaluation (ROBIS), addressing these domains: bias arising from the study eligibility criteria, identification and selection of studies, data collection and study appraisal, synthesis and finding; as well as the overall risk of bias. The plot of the domain-level judgements for each individual result was generated using robvis, a web app designed for visualizing risk-of-bias assessments. The results were illustrated in the figure as below.

Two SR were included in this assessment. The risk of bias of each of the included study is displayed in the figure below. All of the included SR were considered as having low risk of bias. (Figure 5).

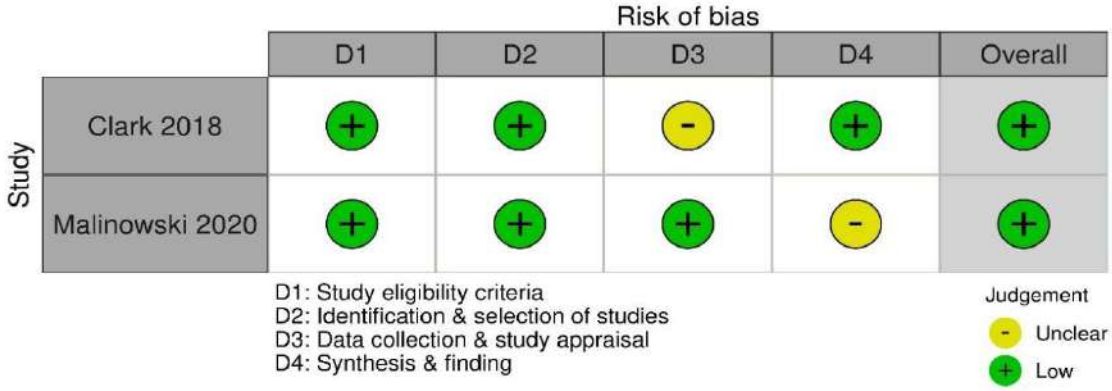

Figure 5: Summary of risk of bias assessment for systematic review using ROBIS

Risk of bias assessment for included RCT

For RCT, assessment was done following the domain-based evaluation (CASP), addressing these domains: bias due to randomization process, bias due to deviation from intended intervention, bias due to missing outcome data, bias in measurement of outcome, and bias in selection of reported result. The results were illustrated in the Figure 6 as below.

One RCT was included in this review, and the risk of bias of the study is low.

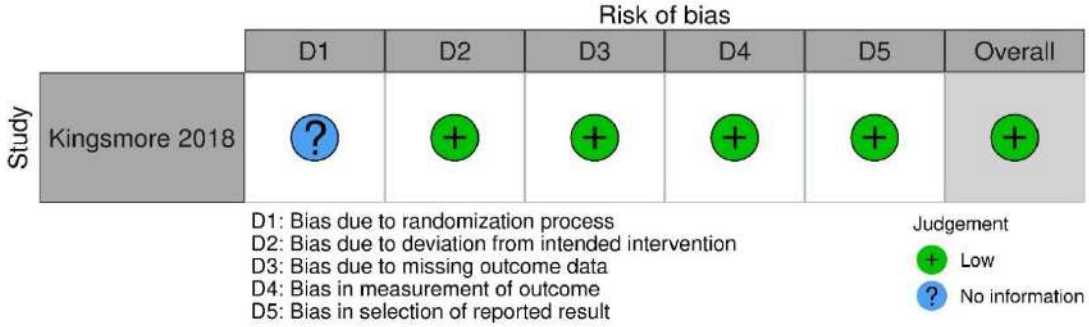

Figure 6: Summary of risk of bias assessment for systematic review using ROB-2

Risk of bias assessment for included cohort study

For cohort study, assessment was done following the domain-based evaluation (CASP), addressing these domains: selection of cohort, exposure accurately measured, outcome accurately measured, confounding factors and follow-up. The results were illustrated in the Figure 7 as below.

Nine cohort studies were included in this assessment. The risk of bias of each of the included study is displayed in the figure below. Studies having low risk of bias for at least three of the domains were considered as having overall low risk of bias. All of the included cohort studies were having low risk of bias, though there were unclear information on confounding, and follow up, in several of the studies.

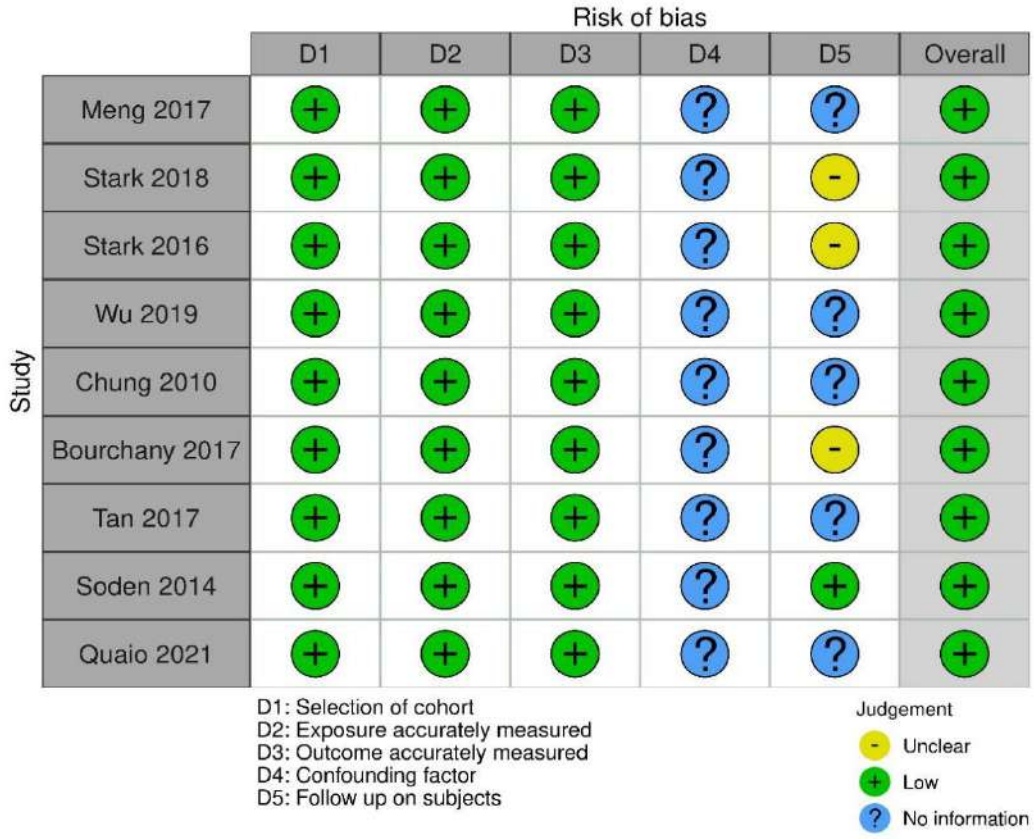

Figure 7: Quality assessment of cohort studies (CASP)

Risk of bias assessment for included economic evaluation

Criteria assessed	Klau et al 2021	Stark et al 2017	Sagoo et al 2017
A well-define question posed?	+	+	+
Comprehensive description of competing alternative given?	+	?	+
Effectiveness established?	+	+	+
Effects of intervention identified, measured and valued appropriately?	?	+	+
All important and relevant resources required and health outcome costs for each alternative identified, measured in appropriate units and valued credibly?	+	+	+
Costs and consequences adjusted for different times at which they occurred (discounting)?	?	?	?
Results of the evaluation?	+	+	+
Incremental analysis of the consequences and costs of alternatives performed?	?	+	+
Sensitivity analysis performed?	?	+	+

Figure 8: Summary of risk of bias assessment for economic evaluation using CASP checklist

Three cost-effectiveness analyses were included in this assessment and risk of bias of individual CEA were summarised in Figure 8. Overall, the included studies were considered as having low risk of bias, however information for several domains were not clearly stipulated in the included studies as illustrated above.

5.2 EFFECTIVENESS

There were fourteen studies retrieved on the effectiveness of WES as diagnostic option for children with suspected genetic disease, of which five studies were on infant. The fourteen studies were comprised of two SR, one scoping review, one RCT, nine cohort studies and one case series.

PAEDIATRIC PATIENTS WITH SUSPECTED GENETIC DISORDER

Clark et al. (2018) in a meta-analysis conducted, compared the diagnostic utility (rate of causative, pathogenic, or likely pathogenic genotypes in known disease genes) and clinical utility (proportion in whom medical or surgical management was changed by diagnosis) of WGS, WES, and chromosomal microarray (CMA) in children with suspected genetic diseases by systematic review of the literature (January 2011 to August 2017) and meta-analysis, following MOOSE/ PRISMA guidelines. They found from the 37 studies, comprising of 20,068 children; **diagnostic utility** of WGS (0.41, 95% CI 0.34 to 0.48, I^2=44%) and **WES (0.36, 95%CI 0.33 to 0.40, I^2= 83%)** were qualitatively greater than CMA (0.10, 95%CI 0.08 to 0.12, I^2= 81%). Among studies published in 2017, the diagnostic utility of WGS was significantly greater than CMA (p<0.0001, I^2=13% and I^2=40%, respectively). Among studies featuring within-cohort comparisons, the diagnostic utility of WES was significantly greater than CMA (p<0.001, I^2=36%). The **diagnostic utility of WGS and WES were not significantly different**.

In studies featuring within-cohort comparisons of WGS/WES, the likelihood of diagnosis was significantly **greater for trios** than singletons (odds ratio 2.04, 95%CI 1.62 to 2.56, I^2=12%). Diagnostic utility of WGS/WES with **hospital-based interpretation** (0.42, 95%CI 0.38 to 0.45, I^2=48%) was qualitatively higher than that of reference laboratories (0.29, 95%CI 0.27 to 0.31, I^2 = 49%); this difference was significant among studies published in 2017 (p<0.0001, I^2 = 22% and I^2 = 26%, respectively).

The clinical utility of WGS (0.27, 95%CI 0.17 to 0.40, I^2 = 54%) and WES (0.17, 95%CI 0.12 to 0.24, I^2 = 76%) were higher than CMA (0.06, 95% CI 0.05 to 0.07, I^2 = 42%); this difference was significant for WGS vs CMA (p< 0.0001). They concluded that in children with suspected genetic diseases, the diagnostic and clinical utility of WGS/WES were greater than CMA. Subgroups with higher WGS/WES diagnostic utility were trios and those receiving hospital-based interpretation. WGS/WES should be considered a first-line genomic test for children with suspected genetic diseases. [14 level I]

Srivastava S 2019 in a scoping review aimed to compare the yield of exome sequencing (ES) with that of chromosomal microarray (CMA), the current first-tier test for NDDs. They selected articles from PubMed focusing on ES and NDD from 1 January 2014 to 29 June 2018.This effort began as an initiative of the Translational Neuroscience Center (TNC) at Boston Children's Hospital (BCH), following multiple discussions with experts in the field. They defined NDD as global developmental delay, intellectual disability, and/or autism spectrum disorder. They classified the included articles as belonging to one of two categories: (1) isolated NDD: the study population pertained to NDD; or (2) NDD plus associated conditions: the study population pertained to NDD plus a specific clinical finding (defined as any additional neurological, systemic, syndromic, or other clinical characteristic, e.g., microcephaly, neutropenia, or Coffin–Siris syndrome). The consensus development conference included input from genetics professionals,

paediatric neurologists, and developmental behavioural paediatricians. They identified **30 articles** with data on molecular diagnostic yield in individuals with isolated NDD, or NDD plus associated conditions. The 30 articles were diverse in their patient representations, articles originated from United States, Europe, Middle East, and Asia. Among the articles focused on NDD plus associated conditions, the features included features of a clinically defined syndrome (e.g., Coffin–Siris syndrome, DOOR syndrome, Nicolaides–Baraitser syndromes, Rett syndrome, Smith–Magenis syndrome); systemic findings (unexplained metabolic phenotype); associated medical problems (neutropenia); and neurological features (microcephaly, macrocephaly).

In terms of **diagnostic yield**; the yield of ES was 36% overall, 31% for isolated NDD, and 53% for the NDD plus associated conditions. ES yield for NDDs is markedly greater than previous studies of CMA (15 to 20%). **Meanwhile, in terms of change in management**; among those with a diagnosis by ES, changes in medical/medication management occurred in 30% (range: 2 to 46%; n = 6 studies). Four studies discussed impact on reproductive planning and found that 80% (range: 42 to 100%) of diagnoses were informative for reproductive planning. For those for whom the diagnosis was not informative for reproductive planning, the reasons were not entirely clear. They concluded that ES consistently outperforms CMA for evaluation of unexplained NDDs. They suggested placing ES at the beginning of the evaluation of unexplained NDDs.[34]

Malinowski J et al 2020 in another systematic review assessed the outcomes from exome and genome sequencing for paediatric patients with congenital anomalies (CA) or intellectual disability (ID). The systematic search identified primary literature from January 2007 to March 2019 describing health, clinical, reproductive, and psychosocial outcomes resulting from ES/GS in patients with CA/Developmental Delay (DD)/ID. A narrative synthesis of results was performed. The review outcomes were the extent to which studies reported a measurable impact on health, reproductive, psychological, and behavioural outcomes for the patient or the patient's family. The inclusion criteria were:

- Patients with CA with age of onset ≤1 year, patients with DD/ID diagnosed by 18 years
- English literature including case studies/series, observational studies, RCT
- Intervention either ES or GS
- Health and clinical management outcomes reported

The studies identified were 167 studies (case series/case report), with finally 36 studies with a patient population ≥20 were included. For the 36 studies included, the sample size ranged from 22 to 278 patients. Most studies reported ES results (n=27), while seven studies reported health or clinical management outcomes following GS; two studies used both methods. Studies were predominantly in the United States (n=15).

They found for change in clinical management, the most frequently reported outcomes from ES/GS were: **changes to clinical management or reproductive decision-making**. Two studies reported on the **reduction of mortality or morbidity or impact on quality of life** following ES/GS. Changes in clinical management include changes in medication/diet, change in procedure or surveillance, referral to specialist, redirection of care.

- Change in medication/diet

Alterations to a patient's existing diet were mentioned in nine studies. Coenzyme Q10 supplementation and a ketogenic diet were introduced following ES results, in a patient diagnosed with coenzyme Q10 deficiency.

- Change of procedures or surveillance

Changes to planned procedures (surgery, imaging, and/or diagnostic studies) or surveillance strategies were specified in 19 studies.For example, following ES, 54% (84/155) of patients with CA/DD/ID underwent additional (unspecified) molecular testing, imaging, or biochemical testing. In other studies, the ES/GS results led to discontinuation of unnecessary procedures. The need for diagnostic tissue biopsy was eliminated for two patients diagnosed with mitochondrial disorders through ES. Additional invasive testing/ procedures were halted in 2% (2/115) of patients after ES, in a case series of patients with CA/DD/ID.

- Referral to specialists

As a consequence of ES/GS results, patients were referred to specialists in a variety of disciplines for follow-up care (six studies). Subspecialty referrals were initiated or changed for ~25% of patients who underwent ES/GS and received a diagnosis

- Redirection of care

In 66 neonatal patients with CA/DD/ID, dysmorphic features, and other clinical symptoms from a clinical laboratory, rapid ES identified a pathogenic variant in SOX10, which is associated with peripheral demyelinating neuropathy, central demyelination, where the family requested ventilator support be withdrawn due to the prognosis.

Other outcomes reported were:-

- Eligibility for clinical trials

Outcomes pertaining to enrolment in or eligibility for clinical trials was specifically reported by six studies. In a large case series of patients with CA 31% (36/115) were enrolled in research studies following ES.

- Family-focused outcomes

Twelve studies reported outcomes following ES/GS that had an impact on family members of the patient, such as cascade genetic testing, referral to specialists. Cascade testing enabled a diagnosis in 12 relatives of the infants in an Australian cohort with multiple CA who had ES results.

- Reproductive-focused outcomes

Impact of ES/GS on outcomes related to reproductive planning for families of patients in 20 studies, including the decisions to become pregnant, terminate a pregnancy, use assisted reproductive technologies, use preimplantation genetic diagnosis, use donor sperm/egg, and undergo previously unplanned additional prenatal testing such as chorionic villus sampling (CVS) or amniocentesis. For 95% (20/21) of families who received a result from ES in a German study of 72 patients with CA/DD/ID, the results were important for family planning for either the parents or for the unaffected siblings of patients, and 19% (4/21) of families decided to undergo additional prenatal testing for a subsequent pregnancy. In another case series of CA/DD/ID patients from the Middle East, North Africa, and Central Asia, 9% (10/118) of families had genetic counseling, carrier testing, or prenatal diagnostic options following ES results in the proband. Malinowski J et al. concluded that there is evidence that ES/GS for patients with CA/ DD/ID informs clinical and reproductive decision-making, which could lead to improved outcomes for patients and their family members. Further research is needed to generate evidence regarding health outcomes to inform robust guidelines regarding ES/GS in the care of patients with CA/DD/ID. [35] level I

Wu et al. (2019) assessed the feasibility of whole-exome sequencing (WES) as a tool to improve the efficacy of rare diseases diagnosis for paediatric patients with severe illness. The study setting involved a tertiary referral Children's Hospital in Taiwan. Critically ill PICU patients suspected of having a genetic disease and newborns who were suspected of having a serious genetic disease after newborn screening were enrolled. They employed a fast but standard whole-exome sequencing platform together with text mining-assisted variant prioritization in PICU setting over a one-year period. Exome capture was performed using either the TruSeq exome or TruSeq Rapid exome capture kit (Illumina, San Diego, CA), and trio (patient and both parents) sequencing was performed using the Next-Seq500 (Illumina). The NextSeq500 mid out system run three WES at a time. The patients' ages at the time of molecular analysis ranged from 0.2 months to 13.1 years (mean 2.2 year; median 7.9 months). In this study, around 50,000 to 100,000 variants were obtained for each of the 40 patients in 5 days after blood sampling. Eleven patients were immediately found be affected by previously reported mutations after searching mutation databases. Another seven patients had a diagnosis among the top five in a list ranked by text mining. **Overall 40 patients were examined over a one-year period, and 21 patients (52.5%) received a genetic diagnosis, in 6.2 ± 1.1 working days** (range, 4.3 to 9 days). Most of the diagnoses were first recognized in Taiwan. Specific medications were recommended for 10 patients (10/21, 47.6%), transplantation was advised for five, and hospice care was suggested for two patients. **Overall, clinical management was altered in time for 81.0%** of patients who had a molecular diagnosis. They concluded the current whole-exome sequencing algorithm, balanced in cost and speed, uncovered genetic conditions in infants and children in PICU, helped their managements in time and promoted better utilization of PICU resources. [36] level II-3

PAEDIATRIC PATIENTS WITH SUSPECTED MONOGENIC DISORDER OR RARE DISEASE

Chung et al. (2020) examined the diagnostic and clinical utility, and the economic impact on clinical management of rapid wide exome sequencing (rWES) in patients beyond infancy and ICU setting. In this study, rWES was performed on a prospective cohort of patients recruited from June 2016 to February 2020 with suspected monogenic disorder referred from territory-wide paediatric ICUs and non-ICUs in Hong Kong urging for rapid genetic diagnosis. All eligible families were invited. A rapid turnaround time (TAT) of 14 days was aimed. Library preparation began when they received samples from two or more families indicated for rWES in the same batch of sequencing. The DNA libraries were sequenced using Illumina NextSeq500, with the aim to obtain an average depth of 100X for each sample. Variant analysis and interpretation were reviewed by multidisciplinary team members, including genome analyst, bioinformatician, clinical geneticist, neonatologist, and paediatric subspecialist as required. The pathogenicity of the variants was assessed using the American College of Medical Genetics and Genomics (ACMG) guideline. **Clinical utility and costs associated with clinical management were assessed** in diagnosed cases. Economic impact of clinical management brought about by the rWES genetic diagnosis was estimated from the healthcare system perspective. The cost of rWES includes the cost of human exome library preparation, sequencing, reagents, data storage, server fees, sanger validation, labware, and labour. All costs in this study were reported in Hong Kong dollars (HKD) with an exchange rate of about 10.1 per British pound sterling (GBP) at the time of study. Actual quantitative changes in healthcare utilisation were compared with a counterfactual diagnostic trajectory and/or with matched historical control whenever possible.

The study found rWES were offered to 102 families and 32/102 patients received a molecular diagnosis, corresponding to a **diagnostic yield of 31.4%, with a median TAT of 11 days. Clinical management changed in 28 of 32 diagnosed patients (88%),** including but not limited to modifications in treatment, avoidance of surgeries, and informing decisions on redirection of care.

Cost analysis was performed in eight patients. rWES was estimated to reduce hospital length of stay by 566 days and decrease healthcare costs by HKD$8,044,250 (GBP£796,460) for these eight patients. The net cost-savings after inclusion of rWES costs were estimated to be HKD$5,325,187 (GBP£527,246). This study illustrates that rWES in the paediatric and adolescent clinical setting in Hong Kong is feasible, has high diagnostic and clinical utility, reduces healthcare utilisation costs, and is comparable to international standards. **rWES merits consideration as a first-tier diagnostic tool for patients with urgent needs in the clinical setting**. Further research is needed to identify the long-term clinical and economic impact from the individual, family, and healthcare system perspective. [37] level II-2

Bourchany et al. (2017) in a pilot study evaluated the feasibility of reducing the turnaround times for diagnostic WES in a primary genetic center in France for patients requiring rapid diagnostic orientation for their care. The WES was proposed to 29 patients with severe undiagnosed disorders with developmental abnormalities and faced with medical situations requiring rapid diagnosis. Each family gave consent. Genomic DNA was extracted from peripheral-blood samples from the proband and both parents via standard procedures using the GentraPuregene tissue kit (Qiagen). Whole-exome capture and sequencing were performed in the proband only at the Integragen platform on 1.5 mg of genomic DNA per individual using the SureSelect Human All Exon V5 kit (Agilent) for 22 samples and the SureSelect XT Clinical Research Exome kit (Agilent) for 7 samples. The resulting libraries of extracted DNA were sequenced on a NextSeq 500 (Illumina). Data were analyzed following the standard procedures. Variants were interpreted using in-house software. Each rare variant affecting protein sequences with clinical relevance was tested for familial segregation. The cohort comprised of 15 males and 14 females with a mean age of 5.8 years. The study found **the diagnostic rate was 44.8% (13/29**), with a mean turnaround time of 40 days (range 25 days to 100 days) from reception of the specimen to delivery of results to the referring physician. Besides permitting genetic counseling, the rapid diagnosis for positive families led to two pre-natal diagnoses and two inclusions in clinical trials. This pilot study demonstrated the feasibility of reducing the turnaround times for diagnostic WES in cases requiring a rapid answer in a primary genetics center, with a diagnostic yield at least similar to that in the literature. In such cases, the implementation of WES as a first-line strategy appears to be the best option for the benefit of patients, especially in highly heterogeneous disorders. [38]
level II-2

Tan TY et al (2017) conducted another study on diagnostic impact and cost-effectiveness of WES for ambulant children with suspected monogenic condition. The aim was to investigate the impact of WES in sequencing-naive children suspected of having a monogenic disorder and evaluate its cost-effectiveness if WES had been available at different time points in their diagnostic trajectory. This study involved ambulant children aged 2 to 18 years suspected of having a monogenic condition from the outpatient clinics of the Victorian Clinical Genetics Services at the Royal Children's Hospital, Melbourne, Australia, were prospectively recruited from May 1 through November 30, 2015, by clinical geneticists after referral from general and subspecialist pediatricians. All children had nondiagnostic single-nucleotide polymorphism microarray and no prior single-gene or panel sequencing. All children underwent singleton WES with targeted phenotype-driven analysis. This prospective study was part of the Melbourne Genomics Health Alliance demonstration project. They only assessed the pathogenicity of variants relevant to the participant's phenotype, and classification was based on the American College of Medical Genetics and Genomics standards for interpretation of sequence variants. Sanger sequencing was used to confirm pathogenic and likely pathogenic variants of clinical significance. The study

examined the clinical utility of a molecular diagnosis and the cost-effectiveness of alternative diagnostic trajectories, depending on timing of WES.For costing approach, cost data in Australian dollars for all children that included initial presentation to tertiary services for diagnostic purposes, first clinical genetics assessment, enrollment, and WES rep were collected from medical records. Three diagnostic counterfactual scenarios were considered, and the actual trajectory and compared their costs and diagnostic yields to investigate which option was most cost-effective. First is the standard diagnostic pathway without WES, which included all investigations and clinical assessments that occurred primarily for diagnostic purpose. Second, we considered the standard diagnostic pathway with WES. Third scenario was WES at the first genetics appointment, which included all costs up to and including the first genetics appointment with cost of WES (test, Sanger validation, and genetic service delivery) and fourth was WES at initial tertiary presentation. Sensitivity analysis using a higher cost of singleton WES in another Australian laboratory (A$2300) was conducted. A total of 44 children involved, with age at enrolment ranged from 2-10 years (n=30 (68%)), and from 10-18 years (n= 14 (32%)).

They found, in terms of clinical utility/diagnostic rate; of 61 children originally assessed, 44 (21 [48%] male and 23 [52%] female) aged 2 to 18 years (mean age at initial presentation, 28 months; range, 0-121 months) were recruited, and **diagnosis was achieved in 23 (52%) by singleton WES.** The diagnoses were unexpected in 8 of 23 (35%), and **clinical management was altered in 6 of 23 (26%).** The mean **duration of the diagnostic odyssey was 6 years,** with each child having a mean of 19 tests and 4 clinical genetics and 4 non-genetics specialist consultations, and 26 (59%) underwent a procedure while under general anaesthetic for diagnostic purposes. They concluded that singleton WES in children with suspected monogenic conditions has high diagnostic yield, and cost-effectiveness is maximized by early application in the diagnostic pathway. It was suggested that paediatricians should consider early referral of children with undiagnosed syndromes to clinical geneticists. [39 level II-2]

Soden S et al. (2014) in another study assessed effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders (NDD). The aim was to evaluate effectiveness of a WGS and WES sequencing program for children with NDD. This is a retrospective analysis of patients enrolled in a biorepository at a children's hospital in the central United States. The repository comprised all families enrolled in a research WGS and WES program established to diagnose paediatric monogenic disorders, but without a definitive diagnosis. Over a 33-month period, 155 families with heterogeneous clinical conditions were enrolled into the repository and analyzed by WGS or WES for diagnostic evaluation. Of these, 100 families with 119 children affected by NDD received diagnostic WGS and/or WES of parent-child trios, wherein the sequencing approach was guided by acuity of illness. Standard WES or rapid WGS was performed on the basis of acuity of illness: 85 families with affected children followed in ambulatory clinics received non-expedited

WES, followed by non-expedited WGS if WES was unrevealing; 15 families with infants who were symptomatic at or shortly after birth and in NICU or PICU received immediate, rapid WGS.

They outlined diagnostic yield and an initial analysis of the impact on time to diagnosis, cost of diagnostic testing (total cost of prior negative diagnostic testing for children who received a diagnosis), and subsequent clinical care. For costing estimation, laboratory tests, radiologic procedures, electromyograms, and nerve conduction velocity studies performed for diagnostic purposes were included.The study population consisted of **100 families had 119 children with NDDs**. Mean age of the affected children in the ambulatory clinic group was about seven years at enrolment. Among ambulatory care clinic patients, the mean age at symptom onset was 6.6 months (range, 0 to 90 months), enrollment was at 83.7 months (range, 1 to 252 months), and confirmed and reported diagnosis was at 95.3 months (range, 16 to 262 months). Among infants who received a diagnosis via rapid WGS sequencing, the median age of symptom onset was 0 day (mean, 8.2 days; range, 0 to 90 days), median age at enrollment was 38 days (range, 2 to 154 days), and median age at confirmed and reported diagnosis was 50 days (range, 8 to 521 days).Characteristic pf patients and families were as in (Table 2 &Table 3).

They found in terms of diagnostic rate, 45% received molecular diagnoses of genetic disorder, (in 45 of the 100 NDD families (53 of 119 affected children) following WES or WGS and confirmed by Sanger sequencing.The **diagnostic rate differed** between the acutely ill infants and non-acutely ill older patients. An accelerated sequencing modality, **rapid WGS, yielded diagnoses in 73%** of families with acutely ill children (11 of 15). Forty percent of families with children with nonacute NDD, followed in ambulatory care clinics (34 of 85), **received diagnoses: 33 by WES** and 1 by staged WES then WGS.

In terms of change in care, a change in clinical care or impression of the pathophysiology was reported in **49%** of the 45 newly diagnosed families (n=22). A change in drug or dietary treatment either occurred or was planned was demonstrated in 10 families (23%). Change in clinical care includes new treatment, treatment discontinued, comorbidity evaluated, change in impression and others.

In terms of time to diagnosis, if WES or WGS had been performed at symptom onset, genomic diagnoses may have been made 77 months earlier than occurred in this study. The time to diagnosis with 50-hour WGS was much shorter than routine WES or WGS (Table 3). Among the 11 families receiving 50-hour WGS, the fastest times to final report of a confirmed diagnosis were 6 days (n = 1), 8 days (n = 1), and 10 days (n = 2) (Table 3).They concluded WGS and WES provided prompt diagnoses in a substantial minority of children with NDD who were undiagnosed despite extensive diagnostic evaluations. Preliminary analyses suggested that WES was less costly than continued conventional diagnostic testing of children

with NDD in whom initial testing failed to yield a diagnosis. They suggested that **initial diagnostic evaluation of children with NDD should include trio WGS or WES**, with extension of accelerated sequencing modalities to high-acuity patients. [40 level II-2]

Table 2: Characteristics of families with NDD enrolled for acuity-guided genome- or exome-based diagnostic testing

	Number		
	Total	**Exome**	**Rapid genome**
Families	100	85	15
Affected children	119	103	16
Consanguineous families	4	4	0
NICU enrollments	11	0	11

Table 3: Time to diagnosis

	Exome sequencing (months)		Rapid genome sequencing (days)		
	Mean	**Range**	**Mean**	**Median**	**Range**
Symptom onset	6.6	0-90	8.2	0	0-90
Enrollment	83.8	1-252	43.2	38	2-154
Molecular diagnosis	95.3	16-262	107.5	50	8-521

Quaio et al. (2021) in another study assessed the diagnostic power and clinical impact of exome sequencing in a cohort of 500 patients with rare diseases. The objective was to investigate the clinical usefulness of this approach in diagnosis and estimate its impact on the outcome of patients with rare diseases. The study population involved a cohort comprised of 500 symptomatic patients who had undergone exome sequencing for diagnostic purposes. The exome sequencing (ES) findings of the first 500 samples of adult, paediatric patients obtained from the service were reviewed. All the samples were collected from 2016 to 2020 in facilities of the Fleury Group, a tertiary diagnostic laboratory with subsidiaries in nine states of Brazil. The inclusion criteria were symptomatic patients who had undergone molecular investigation for suspected diseases of genetic etiology. Patients who refused to share genomic data or receive information on medically actionable findings were excluded from this cohort. Additionally, exome analysis performed for other reasons than clinical diagnostic (such as research protocols or prenatal genetic counselling) were excluded. The clinical features were as follows:(a) neurodevelopmental disorders (including autism, intellectual disability, and behaviour anomaly), (b) seizure (c) other neurological anomaly (including hypotonia, hypertonia, neuromuscular disease, hearing loss, visual impairment, and ataxia), (d) syndromic/malformative conditions (including microcephaly, macrocephaly, skeletal anomalies, and malformations), (e) growth anomaly (including intrauterine growth restriction, short stature, tall stature, failure to thrive, and obesity), (f) Immune or haematological anomalies (g) cancer, (h) cardiovascular disease (i) gastrointestinal/liver disease, (j) consanguinity, (k) metabolic/hormonal anomaly, and (l) other. DNA from the proband and both

parents were extracted in a clinical setting from peripheral blood leukocytes, saliva or prenatal samples of villus biopsy or amniotic fluid after appropriate cell culture. Exome capture was also conducted in a clinical setting using Agilent Clinical Research Exome v1. Sequencing was performed using an Illumina NextSeq platform.

In terms of diagnostic yield, in total, 164 primary findings were reported in 158 patients, representing an **overall diagnostic yield of 31.6%.** Most of the findings (61.6%) corresponded to autosomal dominant conditions, followed by autosomal recessive (25.6%) and X-linked (12.8%) conditions. These patients harboured 195 variants, among which 43.6% are novel in the literature. The **rate of molecular diagnosis was considerably higher** for prenatal samples (67%; 4/6), younger children (44%; 24/55), consanguinity (50%; 3/6), gastrointestinal/liver disease (44%; 16/36) and syndromic/malformative conditions (41%; 72/175).

For 15.6% of the cohort (78 patients), they observed a direct **potential for the redirection of care** with targeted therapy, tumour screening, medication adjustment and monitoring for disease-specific complications. This include eligibility for targeted therapies or diets in 3.2% (n = 16) of cases; potential benefit from the established guidelines of tumour screening for 1.6% (n = 8); benefit from medication optimization and adjustments in 4.4% (n = 22) of cases. **They concluded that** ES is a powerful method to identify the molecular bases of monogenic disorders and redirect clinical care. [41 level II-2]

INFANTS OR NEONATES

Meng et al. (2017) evaluated the use of exome sequencing for infants in ICU ascertainment of severe single-gene disorders and effect on medical management. The study aimed to determine the diagnostic yield and use of clinical exome sequencing in critically ill infants. Clinical exome sequencing was performed for 278 unrelated infants within the first 100 days of life who were admitted to Texas Children's Hospital in Houston, Texas, during a 5-year period between December 2011 and January 2017. Exome sequencing types included proband exome, trio exome, and critical trio exome, a rapid genomic assay for seriously ill infants. Main outcomes were indications for testing, diagnostic yield of clinical exome sequencing, turnaround time, molecular findings, patient age at diagnosis, and effect on medical management among a group of critically ill infants who were suspected to have genetic disorders. They found the mean (SEM) age for infants participating in the study was 28.5 (1.7) days; of these, the mean (SEM) age was 29.0 (2.2) days for infants undergoing **proband exome sequencing, 31.5 (3.9) days for trio exome, and 22.7 (3.9) days for critical trio exome**. Clinical indications for exome sequencing included a range of medical concerns. Overall, a **molecular diagnosis was achieved in 102 infants (36.7%) by clinical exome sequencing**, with relatively low yield for cardiovascular abnormalities. The diagnosis **affected medical management for 53 infants (53/102) (52.0%)** and had a substantial effect on informed redirection of care, initiation of new subspecialist care, medication/dietary modifications, and furthering life-saving procedures in

select patients. **Critical trio exome (rapid WES) sequencing revealed a molecular diagnosis in 32 of 63 infants (50.8%**) at a mean (SEM) of 33.1 (5.6) days of life with a mean (SEM) turnaround time of 13.0 (0.4) days. Clinical care was altered by the diagnosis in 23 of 32 patients (71.9%). **The diagnostic yield, patient age at diagnosis, and medical effect in the group that underwent critical trio exome sequencing were significantly different compared with the group who underwent regular exome testing**. For deceased infants (n=81), genetic disorders were molecularly diagnosed in 39 (48.1%) by exome sequencing, with implications for recurrence risk counselling. This study provides evidence that clinical exome sequencing uncovers monogenic disorders in a significant number of infants in NICUs and pediatric ICUs who were suspected to have genetic disorders, **significantly affecting the medical care of more than half of infants who receive diagnoses**. Exome sequencing is a powerful tool for the diagnostic evaluation of critically ill infants with suspected monogenic disorders in the neonatal and pediatric intensive care units and its use has a notable effect on clinical decision making. [42 level II-2]

Kingsmore et al. 2019 conducted RCT to compare effectiveness and outcomes of the analytic and diagnostic performance of singleton proband and trio, rapid genome and exome sequencing in ill infants. The second Newborn Sequencing in Genomic Medicine and Public Health (NSIGHT2) study was a randomized, controlled trial of the effectiveness of rapid whole-genome or -exome sequencing (rWGS or rWES, respectively) in acutely ill infants with diseases of unknown etiology, primarily from the NICU, PICU, and CVICU at Rady Children's Hospital, San Diego. The inclusion criteria were infants' age <4months and time from admission or time from development of a feature suggestive of a genetic condition of <96hours. Genome interpretation was performed as singleton probands. Infants undiagnosed as singletons were re-analyzed as familial trios. Clinical urWGS, rWGS, and rWES were performed in laboratories accredited by the College of American Pathologists and certified through the Clinical Laboratory Improvement Amendments. Genomic DNA was isolated with an EZ1 Advanced XL robot and the EZ1 DSP DNA Blood kit (QIAGEN). Sequencing libraries were analyzed with a Library Quantification Kit (KAPA Biosystems) and High Sensitivity NGS Fragment Analysis Kit (Advanced Analytic). Sample preparation and sequencing for rWES was performed by an external clinical laboratory (GeneDx). In this RCT, comparisons of analytic and diagnostic performance of rWGS and rWES were evaluated. There were 578 infants (46%) had disease of unknown etiology eligible for enrolment out of 1,248 ill inpatient infants age of <4 months screened shortly after admission. Those screened represented 98% of Rady Children Hospital regional NICU, PICU, and CVICU admissions. Of the 578 eligible infants, 213 (37%) were enrolled within 96 hours of admission. A total of 24 infants (11%) were very ill and received ultrarapid whole-genome sequencing (urWGS). The remaining 189 infants were randomized, with 95 receiving rWES and 94 rWGS. The age at enrollment was median (range); 5 (1 to 121) days for rWES, and 4 (1 to 105) days for rWGS.

They found overall, the analytic performance of rWGS was superior to rWES, including variants likely to affect protein function, and ClinVar pathogenic/likely pathogenic variants (p<0.0001). The WGS compared to WES identified; **(1) 12% more coding domain variants (median 26,080 [range 24,305 to 31,345] versus 23,421 [range 22,193 to 28,390**], p<0.0001), (2) 37% more variants of types likely to affect protein function (missense, nonsense, altered splice sites, frameshift indels, disrupted start codons, in-frame indels, copy number variants, and variants predicted to create cryptic splice sites; median 1,028 [range 762 to 3,585] versus 766 [range 555 to 2,930],p<0.0001), and (3) t**wice as many variants annotated as pathogenic or likely pathogenic by ClinVa**r (median 6.0 [range 2 to 16] versus 3.0 [range 0 to 8], p<0.0001. Comparison of analytical performance of rWES and rWGS is as illustrated in Table 4. Whole-genome sequencing provided more even coverage than WES, (Table 4) with median proportion of nucleotides with >than 10-fold coverage was 98.0% (range 97.7% to 98.5%) with WGS and 94.5% (range 93.8% to 95.1%) with WES.

Table 4: Comparison of analytical performance of rWES and rWGS

Diagnostic	Value	Total variants	Copy number variants	Heterozygous variants	Homozygous variants	Coding variants	%coding Nt with≥10x coverage
rWES	Median	38,901	7.0	**22,134**	13,157	23,421	94.5
rWGS	Median	4669,310	9.0	**2132,709**	1420,249	26,080	98.0
	Fold difference	121	1.3	108	112	1.12	1.04

The **diagnostic performance of rWGS and rWES were similar (18 diagnoses in 94 infants [19%] versus 19 diagnoses in 95 infants [20%], respectively), as was time to report of result** (median 11.0 versus 11.2 days, was not significantly different respectively). However, **the median time from sample accessioning to interpretation of rWGS (3.2 days) was shorter than rWES** (5.3 days, p < 0.0001). The proportion diagnosed by urWGS (11 of 24 [46%]) was higher than rWES/rWGS (p<0.004) and time to result was less (median 4.6 days, p<0.0001). (Table 5)

The diagnostic rate of trio sequencing was not significantly higher than singleton sequencing. The incremental diagnostic yield of reflexing to trio after negative proband analysis was 0.7% (1 of 147). The authors concluded that rapid genomic sequencing can be performed as a first-tier diagnostic test in inpatient infants. The urWGS had the shortest time to result, which was important in unstable infants, and those in whom a genetic diagnosis was likely to impact immediate management. Further comparison of urWGS and rWES is warranted because genomic technologies and knowledge of variant pathogenicity are evolving rapidly.[43 level II-I]

Table 5: rate of molecular diagnosis and time to diagnosis with rWGS, rWES and urWGS in NICU, PICU and CVICU infants

	rWES (n=95)	rWGS (n=94)	urWGS (n=24)	rWES vs rWGS, p value
Infants diagnosed, n	19(20%)	18(19%)	11(46%)	0.88

Time sample receipt to first positive/negative report (days),median (range)	11.2(4.3-38.6)	11.0(3.3-49.1)	4.6(1.1-1.4)	0.65
Time sample receipt to first positive report (days),median (range)	11.4(8.1-38.6)	12.4(3.3-41.2)	2.3(1.1-1.4)	1.00
Time sample receipt to interpretation (days),median (range)	**5.3(2.6-11.9)**	**3.2(1.6-16.4)**	**2.2(0.9-3.3)**	<0.0001

Powis Z et al. (2017) evaluated diagnostic rates, characteristics and time to diagnosis following exome sequencing in neonates. This study involved 66 patients 1 month of age and under. A total of 56 neonatal patients from birth to 1 month old at the time of testing) were identified sequentially through clinical samples sent to Ambry Genetics Laboratory (Aliso Viejo, CA) for DES. An additional 10 neonatal patients were referred for research testing through a university medical centre. The clinical histories and results of 66 neonatal patients undergoing DES were retrospectively reviewed. All patients' clinical and testing histories, along with pedigrees provided by referring physicians, were reviewed and summarized by a team of board-certified genetic counsellors with previous clinical experience. DNA isolation, exome library preparation, sequencing, bioinformatics, and data analysis were performed as previously described. Genes were classified as characterized (known to cause Mendelian disease) or uncharacterized (not previously associated with disease) based on Ambry's clinical validity assessment criteria. They found **clinical DES identified potentially relevant findings in 25 patients** (37.9%). (Table 6) Of the 25 patients, 14 had positive findings in characterized genes (ACTG2, ASXL1, CLPB, FBXL4, GNB5, HNF4A, LRP5, MAGEL2, NOTCH1, NSD1, RAB23, RECQL4, SF3B4, and TUBB3). Eleven patients had likely positive findings in characterized genes (ACTA1, COL2A1, EP300, MYBPC3, PNKP, RBM10, RYR1, SMARCA4, SON, SOX10, and XYLT1). The majority of patients had structural anomalies such as birth defects, dysmorphic features, cardiac, craniofacial, and skeletal defects. **In term of time to diagnosis**; the average time from initiation of testing to report was 72 days, for cases within the past 2 years 61 days. **The average time, for clinical rapid testing, until the healthcare provider was notified of the result was 8 days**, and for Sanger confirmation and a written report it was 15 days. They concluded the utility of family-based exome sequencing in neonatal patients was demonstrated. When assessing neonatal patients with a suspected genetic condition, clinical DES may be superior to traditional, comprehensive genetic-testing approaches for cases in which the clinical phenotype suggests one or more characterized syndromes. The option for rapid testing with results in 8 days gives the medical provider information that can potentially improve outcomes for this vulnerable population. This work suggests that appropriate use of clinical DES may increase the rate of genetic diagnosis for neonatal patients.[44 level III]

Table 6: Diagnostic result of neonates following WES

Category	Diagnostic rates
All cases	25/66 (37.9%)

Trios	22/58 (37.9%)
Clinical rapid cases	3/6 (50.0%)
All clinical cases	24/56 (42.9%)

Stark et al. (2018) in another study, prospectively evaluate the outcomes of a rapid genomic diagnosis program at two pediatric tertiary centers in Australia. The study involved 40 infants, recruited by the clinical genetics services at the two tertiary paediatric hospitals in Melbourne, Australia, the Royal Children's Hospital (RCH) and Monash Children's Hospital (MCH), between April 2016 and September 2017. The median age at enrolment was 28 days (range 3 days to 4 years). Inclusion criteria were paediatric patient (0 to 18 years), likely monogenic disorder, and complexity, as defined by:-

- Multiple organ systems involved and/or
- Severe condition with high morbidity and mortality and/or
- Severe limitations on function and activities of daily living High acuity:
- Inpatient in an ICU or
- Other medical indication (e.g., awaiting transplantation or acute neurological deterioration)

They found, in terms **of diagnostic utility; o**f 40 enrolled patients, **21 (52.5%) received a diagnosis, with median time to report of 16 days** (range 9 to 109 days). A result was provided during the first hospital admission in 28 of 36 inpatients (78%).

- **Change/impact in clinical management:**
  Clinical **management changed in 12 of the 21 diagnosed patients (57%),** including the provision of lifesaving treatment, avoidance of invasive biopsies, and palliative care guidance.

- **Cost analysis**
  The cost of providing rWES was AU$3,959 per patient. The total cost of diagnostic assessments and investigations, including rWES, in this cohort, was AU$281,143 and the cost per diagnosed patient was AU$13,388 (US$10,453using rWES.**(Table 7)**
  Additional cost savings from avoidance of planned tests and procedures and reduced length of stay are estimated to be around AU$543,178 (US$424,101).

- **Impact on clinician time**
  Referrals for inpatient genetics consultation at the recruitment centre have nearly doubled during the course of the study, from 150 per year in 2014 to 273 in 2016. The average number of genetic counsellors–inpatient contacts per rWES family was 1.8 (range 0 to 4 contacts), and overall duration 68 min (range 15 to 150 min). The average number of clinical geneticist–inpatient contacts per rWES family was 4 (range 1 to 6 contacts), with an estimated 30 min spent with families who did not receive a diagnosis and 90 min with those who received a diagnosis. They concluded rapid genomic testing in acute paediatrics is not only feasible but also cost-effective, and has high diagnostic and clinical utility. It requires a whole-of-system approach for

successful implementation. Developing the capability to reliably produce rapid genomic results for selected patients has applications outside of acute paediatric care, notably in the oncology and prenatal settings, and requires investment to further optimize test performance and equity of access. [45] level II-2

In the earlier study, Stark et al. (2016) prospectively evaluated the diagnostic and clinical utility of singleton WES as a first-tier test in infants with suspected monogenic disease. This study involved a total of 80 infants, with average age at enrolment was 8 months (range: 1 week to 34 months). Inclusion criteria were infants (0 to 2 years of age) who presented with 1) Multiple congenital abnormalities and dysmorphic features or 2) Other features strongly suggestive of monogenic disorders, for example, neurometabolic conditions and skeletal dysplasias, were recruited by the genetics service at the Royal Children's Hospital (RCH), Melbourne, Australia, between February 2014 and May 2015. Singleton WES was performed as a first-tier sequencing test in infants recruited from a single paediatric tertiary centre. This occurred in parallel with standard investigations, including single- or multigene panel sequencing when clinically indicated. The diagnosis rate, clinical utility, and impact on management of singleton WES were evaluated. The study was part of the Melbourne Genomics Health Alliance demonstration project. Parents provided written informed consent after genetic counselling regarding the testing.

They found, in terms of diagnosis rate, of the 80 enrolled infants, 46 received a molecular genetic diagnosis through singleton WES (57.5%) compared with 11 (13.75%) who underwent standard investigations in the same patient group. (Table 8).Twenty-one of 80 participants (26%) had one or more genetic tests (Sanger sequencing of single genes, NGS gene panels, methylation studies, and mitochondrial mutation panels) as part of standard clinical care. The median time to report WES was 134 days (range: 83 to 278 days)**.**

They also observed clinical management changed following exome diagnosis in 15 of 46 diagnosed participants (32.6%). Three participants had additional treatment started, one had treatment stopped, and four had modifications to existing treatment regimens. Nine participants had additional surveillance for known complications of their condition, and one was discharged from surveillance based on an erroneous clinical diagnosis. **Eleven of the 15 participants who had a change in clinical management** as a result of WES were not diagnosable by standard care in infancy. **Twelve relatives received a genetic diagnosis** following cascade testing (only five would have been diagnosed by standard care) and 28 **couples were identified as being at high risk** (25 or 50%) **of recurrence in future pregnancies**. Standard care would have identified 13 of these couples. They concluded this prospective study provides evidence for increased diagnostic and clinical utility of singleton WES as a first-tier sequencing test for infants with a suspected monogenic disorder. Singleton WES outperformed standard care in terms of diagnosis rate and the benefits of a

diagnosis, namely, impact on management of the child and clarification of reproductive risks for the extended family in a timely manner. [46 level II-2]

Table 8: Patient characteristics, phenotypic features and diagnostic rate of single WES in selected group

Characteristic	Number (%)	Diagnostic rate
Age at enrolment		
0-6 month	37 (46%)	
6-12 month	25 (31%)	-
12-36 month	18 (23%)	
Parental consanguinity	17 (21%)	8/17 (47%)
Affected first degree relative	16 (20%)	-
Prenatal presentation	9 (11%)	-
Principal phenotypic feature		
• Congenital abnormalities and dysmorphic feature	43 (54%)	21/43 (49%)
• Neurometabolic disorder	19 (24%)	14/19 (74%)
• Skeletal dysplasia	6 (7%)	4/6 (67%)
• Eye	3 (4%)	2/3 (67%)
• Other (GI, renal, immunological)	9 (11%)	5/9 (55%)
Primary indication for WES		
No clinical diagnosis	40 (50%)	16/40 (40%)
Clinical diagnosis of a genetically heterogenous condition	33 (41%)	26/33 (78%)
Suspected dual diagnosis	3 (4%)	1/3 (33%)
Commercial test not available	4 (5%)	3/4(75%)
Referral source		
Inpatient consultation	44 (55%)	24/44 (54%)
Neonatal ICU	33 (41%)	16/33 (48%)
Paediatric ICU	4 (5%)	3/4 (75%)
Other inpatient consultation	7 (8%)	5/7 (71%)
Outpatient consultation	36 (45%)	22/36 (61%)

5.3 SAFETY

There were two evidences retrieved on the safety of WES as diagnostic option for children suspected of having genetic disease.

Whole Exome Sequencing Constituent Device **was registered as Class II medical device by USFDA.** The WES constituent device consists of reagents, instrumentation, software and instructions. A whole exome sequencing constituent device is for germline whole exome sequencing of genomic deoxyribonucleic acid (DNA) isolated from human specimens. The DNA sequence generated by this device is intended as input for clinical germline DNA assays that have FDA marketing authorization and are intended for use with this device.[47] The US Food and Drug Administration (FDA) approves sequencing platforms when they are sold to conduct clinical NGS, including WES. FDA approval is based on the demonstration of analytic validity, in other words that the sequencing machines correctly sequence DNA specimens. The FDA does not regulate WES as a diagnostic

test, which involves both the sequencing component and the bioinformatics and variant interpretation component. [29]

Helix, the leading population genomics company, has received *de novo* authorization from the US Food and Drug Administration (FDA) for the Helix® Laboratory Platform, (class II, regulation number 21 CFR 866.6000), a whole exome sequencing platform with coverage of approximately 20,000 genes. The analytical validation of the sequencing platform was conducted using a novel representative sampling-based approach in conjunction with rigorous quality metrics to establish accuracy and reproducibility of the device. The Helix Laboratory Platform is a qualitative in vitro diagnostic device intended for exome sequencing and detection of single nucleotide variants (SNVs) and small insertions and deletions (indels) in human genomic DNA extracted from saliva samples collected with Oragene ®•Dx OGD-610. The Helix Laboratory Platform is only intended for use with other devices that are germline assays authorized by FDA for use with this device. The device is performed at the Helix laboratory in San Diego, CA.[48]

Li X et al (2019) evaluated caregivers' perception of and experience with variants of uncertain significance (VUS) from whole exome sequencing for children with undiagnosed conditions. This study aimed to explore the psychosocial impact of receiving a VUS from pediatric WES on caregivers and to identify implications for clinical practice. Fourteen telephone **interviews** were conducted with parents or legal guardians who received VUS results from their child's WES to assess their understanding of the result, affective responses, perceived impact, and adaptation. Our content analysis showed that most participants had a good understanding of the purpose of the test and the majority of them recalled the result category. Most participants deemed the result had no impact thus far on their perception of their child's condition. However, one participant reported **feelings of fear** related to the VUS. **Most participants experienced a range of emotions** from receiving the result. The majority of participants reported that this result **did not significantly alter their child's care or their ability to take care** of their child, and three participants reported empowerment. Additionally, several participants expressed an interest in research studies and peer support groups dedicated to families with a VUS identified on WES. Our study elicited new information about the psychosocial impact of receiving a VUS from WES. This insight may help to guide pre- and post-WES counseling in the future.[49 level III]

Krabbenborg L et al (2016) evaluated the understanding of the Psychosocial Effects of WES Test Results on Parents of Children with Rare Diseases. While a lot of attention is now given to pre-test counselling procedures for WES, little is known about how parents experience the (positive, negative, or inconclusive) WES results in daily life. To fill this knowledge gap, data were gathered through in-depth interviews with parents of 15 children who underwent WES analysis. WES test results, like results from other genetic tests, evoked relief as well as worries, irrespective of the type of result. Advantages of obtaining a conclusive diagnosis included becoming more accepting towards the situation, being enabled to attune care to the needs

of the child, and better coping with feelings of guilt. Disadvantages experienced included a loss of hope for recovery, and a loss by parents of their social network of peers and the effort necessary to re-establish that social network. While parents with conclusive diagnoses were able to re-establish a peer community with the help of social media, parents receiving a possible diagnosis experienced hurdles in seeking peer support, as peers still needed to be identified. These types of psychosocial effects of WES test results for parents are important to take into account for the development of successful genetic counselling strategies.[50 level III]

5.4 ECONOMIC EVALUATION / FINANCIAL IMPLICATION

There were five studies retrieved on the cost-effectiveness or cost implication of WES for children with suspected genetic disease.

Testing for RD requires high infrastructure cost, including special equipment or instruments, reagents, and human resource skills. In previous years, Malaysia has not had the capacity for in-house testing. The majority of lysosomal disorder testing had to be sent to Australia and Taiwan. Since 2014, tests for majority lysosomal disorder can be carried out at the Institute for Medical Research, which reduces the cost from AU$ 2,5000 to AU$ 240–750 (MYR 800 to 2,000).[6]

Scwarze K et al. (2018) conducted a systematic review to summarize the current health economic evidence for whole exome sequencing (WES) and whole-genome sequencing (WGS). Relevant studies were identified in the EMBASE, MEDLINE, Cochrane Library, EconLit and University of York Centre for Reviews and Dissemination databases from January 2005 to July 2016. Publications were included in the review if they were economic evaluations, cost studies, or outcome studies. The review focused specifically on WES and WGS, as these are the newest NGS technologies, with potentially the highest costs. For the purposes of this review no restrictions were placed on the comparisons made within these economic evaluations: studies comparing WGS with WES were eligible for inclusion, as were studies comparing these testing approaches against other forms of testing, such as single-gene tests. Publications were further categorized by type of economic evaluation, with five approaches considered; cost consequence analysis, cost minimization analysis, CEA, CUA and CBA. All cost estimates for WES and WGS testing were extracted from the included studies, including both costs and commercial prices. Cost estimates were converted to British pounds and US dollars based on purchasing-power parities (PPP). The PPP reflect the cost of purchasing a standardized set of goods and services in different countries and are subject to fewer fluctuations than exchange rates. Costs were then inflated to 2016 values using a UK health-care inflation index. Finally, minimum and maximum costs were calculated for each sequencing approach.The review included 36 studies, which comprised of full EE (8), partial EE (13), cost study (7), outcome study

(8). According to sequencing approach: WES (24), WGS (5), both WES & WGS (5), bacterial WGS (2); and according to study population: children or newborn (13), adults (9), both adults and children (6), population unclear/no study population (8). Study sample of the included studies were: not reported (8), <20 (9), 20-99 (10), >100 (9). Meanwhile, according to study setting: USA (13), Canada (6), Netherland, Australia (5), UK (4), Spain, Germany, France (1). The 36 included studies investigated the use of WES and WGS in a variety of genetic conditions in clinical practice, the most common being neurological or neurodevelopmental disorders (seven studies), with 13 (36%) publications investigating WES and WGS exclusively in children or newborns. Study sample size varied from a single child to 2,000 patients.

Diagnostic yield

The lowest diagnostic yield for WES (3%) was estimated in a patient group with colorectal cancer. The highest rate for WES (79%) was reported for individuals with childhood-onset muscle disorders. Around a third of the included publications investigated WES or WGS in a population group that was difficult to diagnose.

Table 9 : Summary of diagnostic yield estimates for different sequencing approach (Schwarze 2018)

Sequencing approach	Number of estimates	Minimum yield (%)	Maximum yield (%)
WES	27	3	79
WGS	3	17	73

Cost estimates

Twenty-nine studies reported cost estimates, of which 18 reported costs for WES.

Cost estimates for a single test ranged from $555 to $5,169 for WES and from $1,906 to $24,810 for WGS. The highest estimate for a single test (£3,592) was based on commercial prices. The highest estimate of the actual costs of a single WES test (i.e. not commercial prices) was £1,808 ($2,602). **Cost estimates for a trio ranged from £2,658 ($3,825) to £6,466 ($9,304).** The costs for reagents ranged from £291 ($420) to £1,171 ($1,685). Cost estimates varied little over time. The lowest estimate for a single WES test was £736 ($1,060) in 2013 and £736 ($1,070) in 2017.Few cost analyses presented data transparently and many publications did not state which components were included in cost estimates.[51]

Table 10: Summary of cost estimates for different sequencing approaches

Sequencing approach	Number of estimates	Minimum cost (GBP)[a]	Maximum cost (GBP)
WES	18	382	3,592
WGS	8	1,312	17,243
Bacterial WGS	2	40	487

GBP-British pound. [a]All costs are for a single sample unless stated otherwise.

Stark et al. (2017) examined the cost-effectiveness of three scenarios for implementing WES as a routine clinical test for infants with suspected monogenic disorders. This was a prospective study of patients attending a tertiary level publicly funded children's hospital in Australia. The study was part of the Melbourne Genomics Health Alliance demonstration project. The study was conducted to determine whether testing of individual patients (singletons) using WES as a first-line test is more cost-effective than standard investigations and to identify the optimal timing of WES in the diagnostic pathway. Cohort of infants (N = 40) attending tertiary children hospital who underwent singleton WES in parallel to usual diagnostic care (including commercial single-gene or multigene panel sequencing when clinically indicated). Eligible infants were if they presented with multiple congenital abnormalities and dysmorphic features or other features strongly suggestive of monogenic disorders such as neurometabolic conditions and skeletal dysplasias. Health economic analysis was undertaken from the **funded hospital system perspective** encompassing costs per patient, costs per diagnosis, and incremental costs per additional diagnosis for three alternative strategies for integrating WES into the diagnostic trajectory. Cost data for diagnosis-related investigations and assessments were collected for a prospective, sequential clinical cohort of infants (N = 40) who underwent singleton WES in parallel to usual diagnostic care. Cost data was collected using a bottom-up approach, by identifying all of the resources that were used to provide a service and by assigning a value to each of those resources. The costs of investigations and patient encounters were obtained from the hospital, state government, and testing laboratories. The costs in overseas currencies was converted into Australian dollars (AU$) based on the exchange rate of AU$1=US$0.78 (2015). They determined costs per patient, costs per diagnosis, and incremental costs per additional diagnosis for three alternative strategies for integrating WES into the diagnostic trajectory. The three diagnostic pathway models of care incorporating the use of WES into standard diagnostic care as the "last resort" diagnostic approach (model 1) and at earlier points in the diagnostic trajectory (models 2 and 3).

They found standard care achieved an average cost per diagnosis of AU$27,050 (US$21,099) compared with AU$5,047 (US$3,937) for singleton WES. If WES had been performed after exhaustive standard investigation, then there would have been an incremental cost per additional diagnosis of US$ 6,327 (AU$8,112) (95% CI: $5,850.95 to 11,966.87) (Model 1). Using WES to replace some investigations decreases this incremental cost to US$2,045 (AU$2,622) (95% CI: $847.09 to 4,459.16) (Model 2), whereas using it to replace most investigations (as a first line) results in a savings per additional diagnosis of AU$2,182 (US$1,702) (Model 3).The cost-effectiveness plane demonstrates that WES as a first-line test replacing most investigations (model 3) is dominant (i.e., less cost with a higher number of diagnoses) compared with standard diagnostic care. (Table 11). They concluded the use of WES early in the diagnostic pathway more than triples the diagnostic rate for one-third the cost per diagnosis, providing strong support for reimbursement as a clinical test.[52]

Table 11: Cost and cost-effectiveness of the use of WES at three points in the diagnostic trajectory compared with standard diagnostic care

Item	**Standard diagnostic care**	**Model 1: WES as a last resort**	**Model 2: WES replacing some investigations**	**Model 3: WES as first-line test**
Total cost	189,352.53	335,365.31	236,551.49	150,071.60
Total number of patients	40	40	40	40
Total patients undergoing WES	0	27	40	40
Total number of diagnosis	7	25	25	25
Average cost per patient (95%CI)	4,773.81 (3693.33 to 5894.96)	8,384.13 (7,078.51 to 9,619.00)	5,913.79 (5242.69 to 6640.83)	**3751.79 (3751.79 to 3751.79)**
Average cost per diagnosis (95%CI)	27,050.36 (15,365.51 to 68,529.77)	13,414.61 (10,164.61 to 18,351.32)	9462.06 (7497.48 to 12618.76)	**6002.86 (4841.02 to 7898.51)**
Incremental cost per additional diagnosis (95%CI)	-	8,111.82 (5,850.95 to 11,966.87)	2622.16 (847.09 to 4459.16)	**-2182.27 (-5855.02 to 129.92)**

Klau J, et al. 2021 in a single-center study aims to determine the time, diagnostic procedure, and cost saving potential of early exome sequencing in a cohort of 111 individuals with genetically confirmed neurodevelopmental disorders. They retrospectively collected data regarding diagnostic time points and procedures from the individuals' medical histories and developed criteria for classifying diagnostic procedures in terms of requirement, followed by a cost allocation. All 111 individuals had received a molecular diagnosis using NGS methods at the Institute of Human Genetics at Leipzig University Medical Center (UKL), a tertiary care centre in Germany, between 2017 and 2020.The UKLs patient information systems were used to curate genetic/clinical information from the individuals' medical history. This involved all procedures performed from birth to the final molecular report. They classified each procedure by its requirement. Only the final NGS-based investigations leading to diagnosis and their validation was classified as required for genetic analyses. They recorded diagnostic time points (t1–t6) on the structured diagnostic pathway. Associated diagnostic costs were determined using a retrospective bottom-up approach by inferring the total health care costs based on individual procedures.The study involved 111 individuals with pediatric-onset NDD and/ or epilepsy from a diagnostic cohort of 2128 cases based on a series of filtering steps.Of the 111 individuals included, 49 (44.1%) were female and 62 (55.8%) were male. Due to the inclusion criteria described earlier, all individuals enrolled were under 20 years of age at the time of diagnosis

They found in term of diagnostic procedure and cost, the diagnostic categories with the highest amounts of potentially non-required diagnostics are genetic, metabolic, and cranial MRI. Out of 407 performed genetic examinations, 296 (72.7%) were classified as potentially dispensable. They

calculated the costs for the three diagnostic categories with the largest number of non-required diagnostic procedures: genetic diagnostics, metabolic diagnostics, and cMRI.

In the cohort, a total of 687,168.02€ was spent on genetic diagnostics. Thereof, 302,947.07€ (44.1%) are associated with dispensable examinations. Of the 82,589.20€ spent on cMRI, 21,903.37€ (26.5%) were considered not required if the final genetic diagnosis would have been known and considered. From 35,980.43€ issued for metabolic examinations, a portion of 10,987.05€ (30.5%) was classified as not required. Thus, genetic examinations show the highest cost savings potential with 302,947.07€ (90.2%) out of 335,837.49€. On average, the total potentially savable costs in the study amounted to €3,025.56 per individual. This corresponds to an average of 2729.25€ for genetic diagnostics, 197.33€ for cMRI examinations and 98.98€ for metabolic testing regarding potential cost savings.Cost savings by first tier exome sequencing lie primarily in genetic, metabolic, and cMRI testing in this German cohort, underscoring the utility of performing exome sequencing at the beginning of the diagnostic pathway and the potential for saving diagnostic costs and time.[53]

Sagoo GS et al. (2017) estimated the budget impact and cost-effectiveness of introducing whole-exome sequencing-based virtual gene panel tests into routine clinical genetics. Two scenarios were presented: Scenario 1)The exome sequencing-based virtual gene panel test is offered in addition to the genetic tests already conducted, and 2) The exome sequencing-based virtual gene panel test is presented as the 'near' first-line test. This analysis will use the limited perspective of the diagnostic clinical genetics service which is provided and funded by the NHS. It was decided not to discount either outcomes or costs because it is assumed that all patients could receive their test results within a single year time-frame. The expected general turn-around time (from sample collection to diagnostic test result) for the exome sequencing test is 168 days. Costs have been reported in UK £s for the year (2015). The comparator intervention is 'usual testing' and for this study will be taken as all known genetic diagnostic testing undertaken on these patients to date. Testing can include existing disease gene panel tests containing any number of genes that do not use clinical exome, whole exome or whole genome-based testing.Ninety-six patients were included in this cost-effectiveness study. The basic demographic characteristics of these 96 patients were 51 male (53.1%), 45 female (46.9%), with an average age at the end of 2016 of 24.5 years old, a median age of 16 years old, and ranging from 3 years old to 82 years old. **The use of exome sequencing produced a diagnostic yield of 42.5% across this mixed-patient group.**

When WES was introduced **later in the testing pathway, the ICER per additional positive genetic diagnosis was £3,171** when compared to the usual testing approach in these 96 patients. When WES test used as a **near first-line test, the ICER per additional genetic diagnosis of £2,201** when compared to the usual testing approach in these 96 patients (Table 12).

Table 12: Summary cost effectiveness of WES versus usual testing in different scenario

Test strategy	Total budget	Clinical cost (%)	Testing cost (%)	Mean cost per patient (range)	Mean cost per positive diagnosis	ICER (versus usual testing)
Usual testing	£171,899	53.6%	46.4%	£1,791 (£0 to £8,466)	-	-
Scenario 1 (WES in addition to genetic test already conducted)	£301,926	31.5%	69.5%	£3,145 (£1,317 to £9,783)	£7,364	£3,171
Scenario 2 (near first line)	£262,122	35.2%	64.8%	£2,730 (£1,317 to £7,117)	£6,393	£2,201

Scenario 1 will always be the most expensive option as the costs of usual testing are incurred before going on to use exome sequencing in those patients in which the genetic tests conducted are negative. The usual testing strategy, where exome sequencing is not used, will always be the cheapest option, except if in scenario 2 the cost of the genetic tests conducted and the clinical workload could be reduced in these patients. If the cost of the genetic testing for these 96 patients could be brought down by £943 in scenario 2, then the overall budget required to test the 96 patients would be slightly cheaper than the actual incurred by the usual testing strategy (£171,593 vs £171,899). An additional benefit is a potential increase in the diagnostic yield of 42.7%. Sensitivity analyses showed that the largest driver of cost was the cost of the genetic testing, including the cost of the exome sequencing and the associated bioinformatics analysis. In terms of budget impact, for the usual testing pathway, the overall budget and the average per-patient testing pathway cost were £171,899 and £1,791, respectively based on these 96 patients. Of this £171,899 budget, £92,160 is accounted for by the clinical appointments and clinical work-up (53.6%) and £79,739 (46.4%) is accounted for by the genetic testing costs. [54]

Van Nimwegen KV (2017) in a HTA of next-generation sequencing compared the costs and outcomes associated with WES (using the HiSeq 4000) with those of conventional diagnosis for pediatric neurological disorders (which includes magnetic resonance imaging scans, electroencephalography, and muscle biopsies). In the author's first analysis, WES was treated as a last-resort test, with an incremental cost per additional diagnosis of £8,319 ($12,092). The second analysis treated WES as a first-line test, and in many plausible scenarios this resulted in cost savings. The cost-effectiveness of diagnostic WES in complex pediatric neurology is empirically examined. Health care resource use associated with the conventional diagnostic trajectory of 100 patients was measured prospectively, from inclusion in this study until a diagnosis was reached, or when no diagnosis was reached, one year after inclusion. As all patients underwent both the conventional diagnostic pathway and WES, the real costs of the WES trajectory could not be determined and were assumed to equal the costs of two physician visits and a WES trio. The primary

effectiveness outcome was diagnostic yield of both trajectories. In addition, to explore whether it would indeed be cost-saving to perform WES as a first-tier test, a model-based CEA was performed with the retrospectively collected cost data (from the first hospital visit in our university hospital until a diagnosis was reached, or in absence of a diagnosis, one year after inclusion) of these patients.

They found WES increases diagnostic yield substantially, from 8% to 30% in this patient population. The average costs of the conventional diagnostic trajectory of these patients were €1,883; when retrospectively collected costs were included, these amounted to €9,337. With an average cost of €3,809 in the WES trajectory, an incremental cost-effectiveness ratio (ICER) of €9,016 per additional diagnosis was found, for WES as an add-on test. Whether this is deemed cost-effective depends on the willingness-to-pay for an additional diagnosis. Implementing WES as a first-tier test on the other hand, might substitute a substantial amount of conventional diagnostics, and thereby result in cost savings. The model-based CEA showed that the application of WES as a first-tier test seems only justifiable if at least 28% of all paediatric neurology patients are complex ones.[55]

Tan TY et al (2017) in another study on diagnostic impact and cost-effectiveness of WES for ambulant children with suspected monogenic condition investigated the impact of WES in sequencing-naive children suspected of having a monogenic disorder and evaluate its cost-effectiveness if WES had been available at different time points in their diagnostic trajectory. This study involved ambulant children aged 2 to 18 years suspected of having a monogenic condition from the outpatient clinics of the Victorian Clinical Genetics Services at the Royal Children's Hospital, Melbourne, Australia, were prospectively recruited from May 1 through November 30, 2015. The study examined the clinical utility of a molecular diagnosis and the cost-effectiveness of alternative diagnostic trajectories, depending on timing of WES. For costing approach, cost data in Australian dollars for all children that included initial presentation to tertiary services for diagnostic purposes, first clinical genetics assessment, enrollment, and WES rep were collected from medical records. Three diagnostic counterfactual scenarios were considered, and the actual trajectory and compared their costs and diagnostic yields to investigate which option was most cost-effective. First is the standard diagnostic pathway without WES, which included all investigations and clinical assessments that occurred primarily for diagnostic purpose. Second, they considered the standard diagnostic pathway with WES. Third scenario was WES at the first genetics appointment, which included all costs up to and including the first genetics appointment with cost of WES (test, Sanger validation, and genetic service delivery) and fourth was WES at initial tertiary presentation. Sensitivity analysis using a higher cost of singleton WES in another Australian laboratory (A$2300) was conducted. A total of 44 children involved, with age at enrolment ranged from 2 to10 years (n=30 (68%)), and from 10 to 18 years (n= 14 (32%)).

They found WES performed at initial tertiary presentation had the lowest cost per patient (A$5186 [US$3933]; 95% CI, A$4637-A$5811 [US$3517-US$4407]).Economic analyses of the diagnostic trajectory identified that WES performed at initial tertiary presentation resulted in an incremental cost savings of A$9020 (US$6838) per additional diagnosis (95% CI, A$4304 to A$15 404 [US$3263 to US$11 678]) compared with the standard diagnostic pathway. Even if WES were performed at the first genetics appointment, there would be an incremental cost savings of A$5461 (US$4140) (95% CI, A$1433 to A$10 557 [US$1086 to US$8004]) per additional diagnosis compared with the standard diagnostic pathway. Adding WES to the standard diagnostic pathway does not offer a cost savings, but it incurs an additional cost of A$5760 (US$4371) (95% CI, A$4692 to A$7799 [US$3561 to US$5918]) per diagnosis.They concluded that singleton WES in children with suspected monogenic conditions has high diagnostic yield, and cost-effectiveness is maximized by early application in the diagnostic pathway.[39]

Soden D et al. (2014) estimated that WES would be cost-effective in pediatric neurodevelopmental disorders, compared with existing nongenomic investigative approaches (including laboratory tests, radiologic procedures, and electromyograms) on a cost-per-diagnosis basis if the cost of WES was no more than £2,123 ($3,063) per individual.The cost of prior negative tests in the nonacute patients was $19,100 per family, suggesting sequencing to be cost-effective at up to $7640 per family. Excluding all costs other than that of prior tests, genomic sequencing of ambulatory care patients was cost-effective at a cost of no more than $7640 per family.Assuming **WES of an average of 2.55 individuals per family**, as occurred when they sought to enrol trios, it would be **cost-effective as long as the cost was no more than $2996** per individual. The nonacute, ambulatory clinic patients were older children (average age, 83.6 months) and had received a much longer period of subspecialty care and considerable prior diagnostic testing. These patients had received an average of **13.3 prior tests/panels (range, 4 to 36) with a mean cost of $19,100 (**range, $3248 to $55,321), whereas the acute care group had received, on average, **7 prior diagnostic tests (range, 1 to 15) with a mean cost of $9550 (range** $3873 to $14,605).[40]

Nolan D and Carlson M (2016) estimated cost following WES in Pediatric Neurology Patients. They performed a retrospective chart review of 135 patients who were evaluated in the University of Michigan Paediatric Neurology clinic between 6/2011 and 6/2015. Patients were referred to the Paediatric Neurology clinic for a variety of symptoms including developmental delay and epilepsy. They recorded previous diagnostic testing, indications for whole exome sequencing, and whole exome sequencing results. Whole exome sequencing was sent to either GeneDx Laboratory (Gaithersburg, MD) or Baylor Molecular Genetics Laboratory (Houston, TX) and performed on genomic DNA obtained from each patient as well as parents and any affected siblings, if available. The authors collected information regarding the genetic and metabolic laboratory

diagnostic work-up done prior to obtaining WES. Estimated charges were based on available pricing from private laboratories.

They found results were available for 50 patients. Insurance barriers often precluded whole exome sequencing. The mean patient age at which patients initially presented for neurologic evaluation was approximately two years and nine months (range: two days to 12 years and 11 months). The mean age at which WES was resulted was seven years of age and five months (range:10 months to 16 years). The overall presumptive diagnostic rate for the cohort was 48% (n= 24 of 50 with WES results available).

The initial genetic testing consisted of a karyotype, chromosomal microarray, Fragile X testing, and methylation PCR. First tier metabolic laboratory investigation depended highly on the age of the patient but most often included at least urine organic acids, plasma amino acids, acylcarnitine profile, and lactate level. The secondary genetic tests most often included single gene tests or gene panels. They found the average charges for secondary genetic and metabolic testing were higher in patients who had a negative WES result ($3270.59 and $624.62, respectively) compared to those patients with a positive WES result ($1660.67 and $380.42, respectively).The estimated charge for whole exome sequencing changed significantly depending on the year it was performed and ranged from $15 000 several years ago to more recently an estimated charge of around $2000-$9000.Compared to current second tier testing, WES can result in lower long-term charges. They concluded overcoming barriers related to WES insurance authorization could allow for more efficient and fruitful diagnostic neurological evaluations.[56]

5.4.1 FINANCIAL IMPLICATION (LOCAL)

Approximately 80 per cent of rare diseases have a genetic basis, and among, 70 per cent manifest during childhood.[57] Presently, most of the patients referred to the genetic clinics are without definitive diagnosis. Several diagnostic procedures are accessible for patients referred to the genetic clinics within tertiary centres under the Ministry of Health (MOH) which include karyotyping, metabolic analysis, and single gene testing. Nevertheless, apart from the limited availability of chromosomal microarray (CMA), the financial burden for other genetic examinations, including multigene panel testing, is typically borne by the patients themselves.[58]

Objective

Genetic diseases are often complex to diagnose and exhibit heterogenous phenotypes.[59] Next-generation sequencing (NGS), such as whole exome sequencing (WES) is typically carried out after several genetic tests have been done or at the last step in a patient's diagnosis trajectory. Hence, this analysis aims to explore the potential cost implication of WES should it be integrated at earlier time points in the diagnostic pathway of patients with suspected genetic disorder.

Scenario Analyses

Initial investigations of genetic diseases typically involve clinical and genetic evaluations guided by patients' observed characteristics. However, there are no published local data on the standard diagnostic pathway or clinical investigations for this population. Moreover, the type as well as the number of tests undertaken between one patient and another may vary greatly depending on the complexity of the disorder. Hence, no estimation on the potential cost saving resulting from the integration of WES in the current diagnostic pathway will be attempted. Instead, scenario analyses considering WES as the first-tier or as the second-tier upon initial visit to the genetic clinic in a tertiary hospital will be explored.

A hypothetical population of 30 patients with unclear differential diagnosis was used in this analysis. This figure corresponded to the average number of patients per month seen in one of the MOH genetic clinic over a period of 17 months from August 2020 to December 2021.[58] Based on the findings from the systematic review, four alternative diagnostic strategies were proposed, in which the population would be subjected to:

1) CMA as the first-tier test upon initial visit to the genetic clinic in a tertiary centre, or
2) CMA as the first-tier test followed by WES as the second-tier test for those with negative results from the first-tier test, or
3) WES as the first-tier test upon initial visit to the genetic clinic in a tertiary centre, or
4) WES as the first-tier test followed by CMA as the second-tier test for those with negative results from the first-tier test.

In this analysis, only costs for each genetic test and specialist outpatient clinic visit would be considered. These costs were used to estimate cost per diagnosis as well as cost per patient for each of the proposed scenarios mentioned above. The cost for the genetic tests was provided by L. H. Moey, MRCPCH (written communication, April 2024). It was mentioned that samples for either CMA or WES test will be sent to a genetic laboratory abroad for analysis as it is more affordable. Hence, the test's cost was converted from USD to MYR using recent currency exchange rate (May 2024). The cost estimated for the specialist outpatient clinic visit was according to the amount stated in the Fees (Medical) (Cost of Services) Order 2014. Additionally, the turnaround time for both tests may take around two to three months. Therefore, the costs were estimated for the plausible positive results achieved through the utilisation of these tests within the first six months of the patient's follow-up at the genetic clinic. The detection rate used in this analysis was based on the results of the meta-analysis conducted by Clark et al. (2018). A conservative estimate of two specialist outpatient clinic visits per patient for each test performed (one prior to the genetic testing or sample collection, and another for consultation of the test

results) was applied in the calculation. The input parameters are as displayed in **Table 13** below.

Table 13: Input parameter for scenario analysis

Parameters		Base estimate	95% CI lower limit	95% CI upper limit	Source
Diagnostic yield following testing strategy					
	CMA	0.1	0.08	0.12	Clark, 2018
	WES	0.36	0.33	0.4	Clark, 2018
Costs (MYR)			**Lower bound**	**Upper bound**	
	CMA	2,000.00	1,600.00	2,400.00	varied by +/- 20%
	WES	1,659.00	1,327.20	1,990.80	
	Specialist Clinic Visit for each test	240.00	NA	NA	Fees (Medical) (Cost of Services) Order 2014

Alternative scenario

An alternative scenario in which the price or cost of WES was varied according to the market survey in Malaysia was explored.

Results and discussion

In an ideal situation, by offering WES early in the diagnostic trajectory, those with positive results were almost four times than that of CMA with a potential cost saving in the total cost of approximately MYR 10,000, as shown in **Table 14 below**. The number of patients with positive results from the use of WES as the second-tier diagnostic tool was five times more than those detected with CMA, even though there was an increase in the total cost of nearly MYR 8,000 for the WES test.

Irrespective of the test sequence between CMA and WES, the total number of patients with positive as well as negative results were similar after undergoing these two tests over a period of at least six months. However, the total costs implicated were lesser for strategy 4, amounted to almost MYR 100,000 for 13 patients with positive results. Whereas, for the same number of patients managed to achieve definitive diagnoses for their genetic conditions, employing strategy 2 has raised the total costs to almost MYR 19,000 more than that of strategy 4

Table 14: Total costs for patients with positive results from WES or CMA, either as the first-tier or the second-tier in the diagnostic pathway

Strategy	# Patients with positive results	# Patients with negative results	Test costs	Specialist clinic visit costs	Total costs
First-tier					
CMA	3	27	60,000.00	7,200.00	67,200.00

Table 15 : Cost per diagnosis and cost per patient for each scenario

Scenario	Cost per diagnosis	Cost per patient
Strategy 1	22,400.00	2,240.00
Strategy 2	9,313.92	2,078.47
Strategy 3	5,275.00	1,899.00
Strategy 4	7,859.91	2,032.07

As illustrated in Table above**,** the lowest cost per diagnosis of about MYR 5,000 was obtained in the scenario with strategy 3, that is by employing WES as the first-tier test in the diagnostic pathway. On the other hand, the integration of WES as the second-tier test as in strategy 2, has led to 18 per cent increase in the cost per diagnosis compared to that of strategy 4. Similar trend was observed in the total cost per patient. As cost per diagnosis is largely dependent on the diagnostic yield associated with a test, the difference between the costs per diagnosis and the costs per patient for all scenarios was substantial with the latter being only around 10 to 36 per cent of that of the former. Hence, the better the diagnostic yield or detection rate a genetic test, the lower the cost per diagnosis would be.

In the alternative scenario, the cost for WES was 75 per cent more than the cost for CMA used in the base case analysis. Its impact on the potential cost implication is as shown in Table 16. Despite such increase in the WES cost, the costs per diagnosis with CMA as the first-tier test in strategy 1 and 2 were still higher than that of strategy 3 and strategy 4.

In this hypothetical cohort, due to the low diagnostic yield associated with CMA and WES, 42 per cent of the population had to undergo a minimum of two genetic tests to reach a clear diagnosis within a period of at least six months.

Given the lack of locally published data to support a more detailed analysis, this analysis did not aim to be exhaustive in estimating all possible costs that may have occurred with the integration of WES earlier in the diagnostic pathway.

Table 16: Cost per diagnosis and cost per patient for alternative scenario

Strategy	Cost per diagnosis	Cost per patient
Strategy 1		
Strategy 2	13,221.70	2,950.53
Strategy 3	10,388.89	3,740.00
Strategy 4	12,201.89	3,154.63

Instead, a conservative potential cost implication was carried out, hence there are several assumptions underlying this analysis. Firstly, it was assumed that the population was suspected to suffer from diverse rare genetic diseases who had not undergone any prior genetic investigation. Secondly, any other genetic test performed after the specified strategy(s) for those who were still without a clear diagnosis were not considered in this estimation.

Limitations of this cost analysis include but not limited to the lack of comparison to the current diagnostic odyssey experienced by Malaysians in achieving a definitive diagnosis about their conditions. A retrospective study using local data that investigate the costs incurred along patients' diagnostic trajectory would be meaningful in providing basis for estimating potential cost saving with NGS, especially WES in terms of reducing the number of unnecessary tests, particularly the invasive ones as well as the change in clinical management as a shorter period to diagnosis would have led to reduced complications arising from undiagnosed or misdiagnosed conditions. Availability of such data would enable the estimation of cost associated with the clinical utility reported for a genetic test to be conducted. In addition, as WES is not yet a standard procedure in Malaysia, the reanalysis cost of WES was not considered. It is possible with the improvement in the NGS technology, reanalysis of samples from those with negative or inconclusive results would increase further detection rate, thus reduced cost of reanalysis and its likelihood of making further diagnoses

might decrease the cost per diagnosis further. This analysis also did not take into the consideration the rate of inconclusive results that have may resulted from using either genetic test.

Conclusion of cost estimation

In a population without a clear differential diagnosis, more patients (79%) would have diagnoses with WES within the first 3 months compared to CMA at a cost per diagnosis less than a quarter of the cost per diagnosis estimated for CMA. Furthermore, integrating WES as the first-tier test upon patients' initial symptoms onset or visit to the tertiary centres may have resulted in a lower cost per diagnosis as well as cost per patients. Even when higher test cost for WES was applied, similar trend was observed. This may indicate that the diagnostic yield of a genetic test plays a significant role in affecting cost per diagnosis or cost per patient.

5.5 ORGANISATIONAL

The WES is conducted by laboratories that are accredited by the Clinical and Laboratory Improvement Act (CLIA) to conduct high complexity testing. Because of the equipment and software involved (particularly for the bioinformatics platform), this test is generally only conducted in laboratories associated with large, tertiary medical centers or commercial genetics laboratories. [29]

Guideline

The American College of Medical Genetics and Genomics **(**ACMG) Guideline (2021) group stated that evidence supports the clinical utility and desirable effects of ES/GS on active and long-term clinical management of patients with Congenital Anomalies (CA)/Developmental Delay (DD)/Intellectual Disability (ID), and on family-focused and reproductive outcomes with relatively few harms. Compared with standard genetic testing, ES/GS has a higher diagnostic yield and may be more cost-effective when ordered early in the diagnostic evaluation. They recommended that ES/GS be considered as a first- or second-tier test for patients with CA/DD/ID.

This guideline recommends ES and GS as a first-tier or second-tier test (guided by clinical judgment and often clinician–patient/ family shared decision making after CMA or focused testing) for patients with one or more CAs prior to one year of age or for patients with DD/ID with onset prior to 18 years of age.

According to the ACMG guidelines, patients with clinical presentations highly suggestive of a specific genetic diagnosis should undergo targeted testing first. This may include patients with suspicion of a chromosomal disorder, known family history of a disorder, or strong clinical suspicion of a diagnosis in which sequencing may not be diagnostic, such as Prader–Willi/Angelman related methylation abnormality or fragile X syndrome**.**They emphasized the

importance of sharing variant interpretation in open databases to improve diagnostic yield and reduce VUS. Continued provider education will be an important component to adoption of ES/GS as a first- or second-line test for patients with CA/DD/ID. [60]

Genetic counselling

The value of genetic counseling in ES/GS is well-established. Creating reasonable expectations, establishing an understanding of the value and limitations of testing, creating awareness of the potential harms, and allowing the family to make informed choices is a mainstay of informed consent for ES/GS. **Elements of counseling should include a three-generation family pedigree; discussion of** pathogenic/likely pathogenic results, benign results, and variants of uncertain significance; detection of misattributed paternity or consanguinity, and secondary findings unrelated to the reason for testing. The ACMG Secondary Findings v3.0 is the recently released minimum set of genes recommended for evaluation in a diagnostic exome or genome. Limits of testing should be discussed, including limited disease-gene known associations. Post-test counseling should include the opportunity for reanalysis and reclassification of variants that may lead to amended interpretation and issuing a new report.[60]

The American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology, in 2015 published standards and guidelines **for the interpretation of sequence variants**. By adopting and leveraging next-generation sequencing, clinical laboratories are performing an ever-increasing catalogue of genetic testing spanning genotyping, single genes, gene panels, exomes, genomes, transcriptomes, and epigenetic assays for genetic disorders. By virtue of increased complexity, this shift in genetic testing has been accompanied by new challenges in sequence interpretation. Hence, the ACMG, the Association for Molecular Pathology (AMP), and the College of American Pathologist revisit and revise the standards and guidelines for the interpretation of sequence variants. [26]

These recommendations primarily apply to the breadth of genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. This report recommends the use of specific standard terminology; “pathogenic,” “likely pathogenic,”“uncertain significance,” “likely benign,” and “benign”, to describe variants identified in genes that cause Mendelian disorders. Moreover, this recommendation describes a process for classifying variants into these five categories based on criteria using typical types of variant evidence (e.g., population data, computational data, functional data, segregation data). Because of the increased complexity of analysis and interpretation of clinical genetic testing described in this report, the ACMG strongly recommends that clinical molecular genetic testing should be performed in a Clinical Laboratory Improvement Amendments–approved laboratory, with results interpreted by a board-certified clinical molecular geneticist or molecular genetic pathologist or the equivalent.[26]

The EuroGentest and the European Society of Human Genetics (2016) published guidelines for the evaluation and validation of next-generation sequencing (NGS) applications for the diagnosis of genetic disorders. The work was performed by a group of laboratory geneticists and bioinformaticians, and discussed with clinical geneticists, industry and patients' representatives, and other stakeholders in the field of human genetics. These guidelines mostly deal with NGS testing in the context of rare and mostly monogenic diseases. They mainly focus on the targeted analysis of gene panels, either through specific capture assays, or by extracting data from whole-exome sequencing. The benefit of implementing NGS in diagnostics is the introduction of testing many genes at once in a relatively short time and at relatively low costs, and thereby yielding more molecular diagnoses. These are the guideline recommendations:-[61]

Diagnostic utility

i. For diagnostic purpose, only genes with a known (i.e, published and confirmed) relationship between the aberrant genotype and the pathology should be included in the analysis.
ii. For the sake of comparison, to avoid irresponsible testing, for the benefit of the patients, 'core disease gene lists' should be established by the clinical and laboratory experts.
iii. A simple rating system on the basis of coverage and diagnostic yield, should allow comparison of the diagnostic testing offer between laboratories.

Informed Consent and Information to The Patient and Clinician

i. The laboratory has to provide for each NGS test the following: the diseases it targets, the name of the genes tested, their reportable range, the analytical sensitivity and specificity, and, if possible, the diseases not relevant to the clinical phenotype that could be caused by mutations in the tested genes.
ii. Laboratories should provide information on the chance of unsolicited findings.
iii. If a clinical centre or a laboratory decides to offer patients an opt-in, opt-out protocol to get carrier status for unrelated diseases and secondary findings all the logistics need to be covered.
iv. The local policy about dissemination of unsolicited and secondary findings should be clear for the patient.
v. It is recommended to provide a written information leaflet or online available information for patients.

Validation

i. All NGS quality metrics used in diagnostics procedures should be accurately described.
ii. The diagnostic laboratory has to implement a structured database for relevant quality measures for (i) the platform, (ii) all assays, and (iii) all samples processed
iii. Accuracy and precision should be part of the general platform validation, and the work does not have to be repeated for individual methods or tests

iv. The diagnostic laboratory has to validate all parts of the bioinformatic pipeline (public domain tools or commercial software packages) with standard data sets whenever relevant changes (new releases) are implemented
v. The diagnostic laboratory has to take steps for long-term storage of all relevant data sets.

Reporting

i. The report of a NGS assay should summarize the patient's identification and diagnosis, a brief description of the test, a summary of results, and the major findings on one page.
ii. Laboratories should have a clearly defined protocol for addressing unsolicited and secondary findings prior to launching the test.

Distinction between research and diagnostics

i. Diagnostic tests that have as their primary aim to search for a diagnosis in a single patient should be performed in an accredited laboratory.
ii. Research results have to be confirmed in an accredited laboratory before being transferred to the patient.
iii. The frequency of all variants detected in healthy individuals sequenced in a diagnostics and/or research setting should be shared.
iv. All reported variants should be shared by submission to federated, regional, national, and/or international databases [58]

Washington State Health Care Authority in the HTA conducted by University of North Carolina (2019) included a total of 57 studies from 60 publications published between 2014 and 2019. In this evaluation, some studies enrolled patients with diverse phenotypes, while others enrolled patients with a single phenotype (e.g., epilepsy). The degree of diagnostic testing prior to WES testing that was received by participants enrolled in these studies varied; most had received some initial diagnostic evaluation (specialty consultation,13-25 laboratory, imaging). Many had also received some genetic testing (e.g., single or multigene panels, CMA). They calculated the pooled estimate for diagnostic yield from the individual studies as 38% (95% CI, 35.7% to 40.6%). The pooled diagnostic yield of traditional testing pathways (4 studies) as 21% (95% CI, 5.6% to 36.4%) and the diagnostic yield of gene panels (6 studies) as 27% (95% CI, 13.7% to 40.5%). The likelihood of a genetic cause and therefore the diagnostic yield of WES varied by patient age and phenotype. The diagnostic yield decreased as the age of participants increased: 42% among infants, 38% among children, and 20% among adults. There were seven disorders or groups of related disorders for which there were more than two studies of diagnostic yield. The seven disorders with their respective diagnostic yield are i) Epilepsy (40%) ii) Intellectual or Developmental Disability (29%) iii) Neurologic Disorders (33%) iv) Neurodevelopmental Disorders (28%) v) Limb-girdle Muscular Dystrophy (48%) vi) Peripheral Neuropathy (32%) and vii) Undiagnosed After Standard Workup (31%). They also found a diagnosis from WES resulted in a change in clinical management of 12% to 100% across 18 studies that enrolled diverse phenotypes and 0% to 31% across 5 studies enrolling participants with epilepsy. Seven studies reported on diverse health outcomes. Four studies among hospitalized pediatric patients reported mortality, which ranged from 17% to 57%. Management changes based on WES resulted in improved seizure control or

behavior management in 0% to 3% of patients with epilepsy. The **pooled proportion of patients with a medically actionable secondary finding was 3.9%** across 13 studies; most patients and families did not experience psychosocial harms from receiving negative or uncertain WES results. The cost of a WES test ranged from $1,000 to $15,000 across 15 studies. In both single-phenotype and diverse phenotype populations, **testing pathways that included WES identified more diagnoses and either cost less or cost somewhat more** (highest reported estimate was $8,599 more) per additional diagnosis. **Pathways with earlier WES testing were more likely to be cost savings** compared to pathways that used WES later in the testing pathway or used WES as a last-resort strategy.It was concluded that WES increases the yield of molecular diagnosis over standard diagnostic testing. A diagnosis from WES changes clinical management for some patients, but the certainty in the estimate of this frequency is very low. The evidence regarding the impact of WES testing on health and most safety outcomes is limited, though they have low certainty that the proportion of patients tested who receive a medically actionable secondary finding is about 3.9%. WES may be cost-effective in terms of diagnostic success; however, the certainty is very low.[29]

USFDA in 2016 produced a guidance for Stakeholders and Food and Drug Administration Staff, on Considerations for Design, Development, and Analytical Validation of Next Generation Sequencing (NGS) – Based In Vitro Diagnostics (IVDs) Intended to Aid in the Diagnosis of Suspected Germline Diseases. The term “germline diseases or other conditions” encompasses those genetic diseases or other conditions arising from inherited or de novo germline variants. Examples of signs and symptoms of suspected germline diseases could include developmental delay, congenital dysmorphologies, or other clinical features. This guidance does not address tests intended for use in the sequencing of healthy individuals.[62]

In the presentation for test performance (WES based tests), these parameters need to be described; describe how known, clinically relevant regions of the exome are defined, and the relevant metrics (e.g., coverage) for those regions.
In the summary performance information, this information is to be included:

- Results for test accuracy and precision/reproducibility presented in a tabular format, across the regions queried by the test, by variant type and size (e.g., sizes that include distribution of results by size, separately for deletions and insertions, by polymorphic and non-polymorphic regions), summarized as a mean percentage agreement and disagreement with the reference sequences and 95% CI, separately for positive and negative results, and broken down by whether results were generated from clinical specimens, contrived samples, cell lines, or reference sample sets.
- For results of reproducibility studies, list the number of replicates for each variant/variant type, and conditions tested (e.g., number of runs, days, instruments, reagent lots, sites, operators, specimens/type, etc.).
- Indicate the average depth of coverage and the percentage of target regions covered at the minimum depth of coverage.

Availability and reimbursement

In Australia, eligibility criteria for Medicare funded WES are:-[63]

- If the child is strongly suspected of having a single gene disorder and is aged 10 years or younger.
- The child has a non-informative chromosome microarray (CMA) test. Negative Fragile X testing and urine metabolic screening is also desirable.
- A clinical geneticist has been consulted about the test indications.
- The family has given informed consent using the appropriate consent forms.
- Whenever possible trio testing including child and parents is recommended. Samples (2 to 5 ml EDTA blood*) are ideally required from both parents and child. Stored DNA from previous diagnostic tests can also be used. Saliva samples are accepted by some laboratories.

Consent

Consent should include the possible outcomes of testing:-

- Genetic cause identified; Pathogenic/likely pathogenic variant in a known gene.
- Uninformative result; a genetic cause is not identified. This does not exclude a genetic diagnosis. This may be because:-
  - ❖ the test did not examine the gene causing the condition OR
  - ❖ the gene causing the condition is not yet known OR
  - ❖ the gene variant causing the condition cannot be found by the test.

  Future re-analysis is possible.
- Gene variant of uncertain significance (VUS); unclear result, may require testing in other family members, and/or may require review in the future
- Incidental finding; in rare cases (<1% of reports) a gene change is found that is not related to the patient's clinical features but may have implications for the child or relative's current or future health

Impact/implication to patients

Onward referrals may be made for screening or management based on results. An incidental finding in an adult relative based on this testing may affect applications for life/income protection insurance, but not medical/private health insurance. Sometimes a genomic test result in one person has implications for another persons' or relatives health care. Trio testing could reveal non-paternity, non-maternity or unexpected family relationships.

Data will be stored securely in databases according to the Australian standards. Limitations of testing is that this is not a general health test and will not identify all gene changes that could contribute to health problems.

The **Washington State Health Authority** stated that WES is a covered benefit with conditions. The reimbursement determination includes the following:[64]

- Whole exome sequencing (WES) is considered medically necessary for the evaluation of unexplained congenital or neurodevelopmental disorders in a phenotypically affected individual when ALL of the following criteria are met:

i. A board-certified or board-eligible Medical Geneticist, or an Advanced Practice Nurse in Genetics (APGN) credentialed by either the Genetic Nursing Credentialing Commission (GNCC) or the American Nurses Credentialing Center (ANCC), who is not employed by a commercial genetic testing laboratory, has evaluated the patient and family history, and recommends and/or orders the test; and

ii. A genetic etiology is considered the most likely explanation for the phenotype, based on EITHER of the following; and
    - Multiple abnormalities affecting unrelated organ systems, (e.g. multiple congenital anomalies); or
    - TWO of the following criteria are met:
    - Significant abnormality affecting at minimum, a single organ system,
    - Profound global developmental delay, or intellectual disability as defined below,
    - Family history strongly suggestive of a genetic etiology, including consanguinity,
    - Period of unexplained developmental regression (unrelated to autism or epilepsy),
    - Biochemical findings suggestive of an inborn error of metabolism where targeted testing is not available;

iii. Other circumstances (e.g. environmental exposures, injury, infection) do not reasonably explain the constellation of symptoms; and

iv. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative genomic hybridization/chromosomal microarray analysis [CMA]) is available; and

v. The differential diagnosis list and/or phenotype warrant testing of multiple genes and ONE of the following:
    - WES is more efficient and economical than the separate single-gene tests or panels that would be recommended based on the differential diagnosis (e.g., genetic conditions that demonstrate a high degree of genetic heterogeneity); or
    - WES results may preclude the need for multiple invasive procedures or screening that would be recommended in the absence of testing (e.g. muscle biopsy); and

vi. A standard clinical work-up has been conducted and did not lead to a diagnosis; and

vii. Results will impact clinical decision-making for the individual being tested; and

viii. Pre- and post-test counselling is performed by an American Board of Medical Genetics or American Board of Genetic Counselling certified genetic counsellor.

The WES is not covered for:

- Uncomplicated autism spectrum disorder, developmental delay, mild to moderate global developmental delay.
- Other circumstances (e.g. environmental exposures, injury, infection) that reasonably explain the constellation of symptoms.
- Carrier testing for “at risk” relatives.
- Prenatal or pre-implantation testing.

Cigna Healthcare in their Medical Coverage Policy no 0519 (2024) on Whole Exome and Whole Genome Sequencing for Non-Cancer Indications highlighted that pre- and post-test **genetic counseling is required** for any individual undergoing whole exome or whole genome sequencing.[65] Whole exome or whole genome sequencing is **considered medically necessary** when criteria listed below are met and when a **recommendation for testing is confirmed** by ONE of the following:

• an independent Board-Certified or Board-Eligible Medical Geneticist
• an American Board of Medical Genetics and Genomics or American Board of Genetic Counseling-certified Genetic Counselor not employed by a commercial genetic testing laboratory
• a genetic nurse credentialed as either a Clinical Genomics Nurse (CGN) or an Advanced Clinical Genomics Nurse (ACGN) by the Nurse Portfolio Credentialing Commission, Inc. OR a genetic nurse with an Advanced Genetics Nursing Certification (AGN-BC) renewed by the American Nurses Credentialing Center (ANCC) who is not employed by a commercial genetic testing laboratory, who:
  - has evaluated the individual
  - completed a three-generation pedigree
  - intends to engage in post-test follow-up counseling

Whole exome or whole genome sequencing is considered medically necessary when ALL of the following General Criteria are met:-

General Criteria

- individual has been evaluated by a board-certified medical geneticist or other board-certified specialist physician specialist with specific expertise in the conditions and relevant genes for which testing is being considered
- testing results will directly impact clinical decision-making and/or clinical outcome for the individual being tested
- no other causative circumstances (e.g., environmental exposures, injury, prematurity, infection) can explain symptoms
- clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative

genomic hybridization [CGH]/chromosomal microarray analysis [CMA]), is available

- the differential diagnosis list and/or phenotype warrant testing of multiple genes and ONE of the following:
  - ❖ Whole exome or whole genome sequencing is more practical than the separate single gene tests or panels that would be recommended based on the differential diagnosis.
  - ❖ Whole exome or whole genome sequencing results may preclude the need for multiple and/or invasive procedures, follow-up, or screening that would be recommended in the absence of testing.

Disease Specific Criteria

Whole exome or whole genome sequencing is considered medically necessary for **ANY** of the following clinical scenarios when ALL of the general criteria listed above are also met:

• Phenotype suspicious for a genetic diagnosis: ANY of the following:

- ❖ individual with multiple major structural or functional congenital anomalies affecting unrelated organ systems, including metabolic disorders
- ❖ individual with one major structural congenital anomaly and two or more minor structural anomalies
- ❖ individual with at least two of the following:
  - o major structural congenital anomaly affecting a single organ system
  - o neurological features including at least two of the following:
    - ➢ autism
    - ➢ severe psychological/psychiatric disturbance (e.g., self-injurious behavior, reversed sleep-wake cycles) or severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome)
    - ➢ symptoms of a complex neurodevelopmental disorder (e.g., dystonia, ataxia, alternating hemiplegia, neuromuscular disorder)
  - o family history strongly implicating a genetic etiology
  - o period of unexplained developmental regression (unrelated to autism or epilepsy)

• **Epilepsy:**

- ❖ Individual with known or suspected developmental and epileptic encephalopathy (onset before three years of age) for which likely non-genetic causes of epilepsy (e.g. environmental exposures; brain injury secondary to complications of extreme prematurity, infection, trauma) have been excluded

• **Hearing loss:**

- ❖ Individual with confirmed bilateral sensorineural hearing loss of unknown etiology

- **Global developmental delay:**
  - ❖ Individual diagnosed with global developmental delay* following formal assessment by a developmental pediatrician or neurologist

- **Intellectual disability:**
  - ❖ Individual diagnosed with moderate/severe/profound intellectual disability** following formal assessment by a developmental pediatrician or neurologist

- **Fetal testing, when ALL of the following criteria are met:**
  - ❖ standard diagnostic genetic testing (chromosomal microarray analysis (CMA) and/or karyotype) of the fetus has been performed and is uninformative
  - ❖ testing is performed on direct amniotic fluid/chorionic villi, cultured cells from amniotic fluid/chorionic villi or DNA extracted from fetal blood or tissue
  - ❖ at least one of the following is present:
    - multiple fetal structural anomalies affecting unrelated organ systems
    - fetal hydrops of unknown etiology
    - a fetal structural anomaly affecting a single organ system and family history strongly suggests a genetic etiology

**Global developmental delay is defined as significant delay in younger children, under age five years, in at least two of the major developmental domains: gross or fine motor; speech and language; cognition; social and personal development; and activities of daily living.*
***Moderate/severe/profound intellectual disability as defined by Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria, diagnosed by 18 years of age.*

The Maryland Department of Health in its coverage policy (2022) highlighted that WES will be considered for coverage when all of the criteria below are met, and confirmed with supporting medical documentation. [65] Whole exome sequencing may be considered medically necessary for the evaluation of unexplained congenital anomalies or neurodevelopmental disorders in children when all the following criteria are met:-

● Test is ordered by one of the following provider types, who has evaluated the patient and family history, and recommends and/or orders the test:
- ❖ Neurologist in collaboration with a medical geneticist or certified genetic counselor.
- ❖ Developmental paediatrician in collaboration with a medical geneticist or certified genetic counselor
- ❖ Psychiatrist in collaboration with a medical geneticist or certified genetic counselor.

● The patient has been evaluated by a board-certified clinician with expertise in clinical genetics and counseled about the potential risks of genetic testing.
- Pre- and post-test counselling is performed by an American Board of Medical Genetics or American Board of Genetic Counselling certified genetic counsellor.

● The patient and/or parents/legal guardians (if applicable) have been appropriately counseled about the testing by a qualified professional (same or similar to ordering providers) who is involved in the member's care.

- The patient has one of the following:
  - ❖ Profound global developmental delay or intellectual disability.
  - ❖ Family history strongly suggests a genetic etiology, including consanguinity.
  - ❖ Period of unexplained developmental regression (unrelated to autism or epilepsy).

● Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative genomic hybridization / chromosomal microarray analysis) is available.

● A genetic etiology is the most likely explanation for the phenotype or clinical scenario despite previous genetic testing (e.g., chromosomal microarray analysis and/or targeted single gene testing), OR when previous genetic testing has failed to yield a diagnosis and the affected individual is faced with invasive procedures or testing as the next diagnostic step (e.g., muscle biopsy).

- ❖ WES is more practical than the separate single gene tests or panels that would be recommended based on the differential diagnosis.
- ❖ WES results may preclude the need for multiple and/or invasive procedures.

● No other causative circumstances (e.g. environmental exposures, injury, or infection) can explain the symptoms.
● WES results have a reasonable potential to directly impact patient management and clinical outcome for the individual being tested.

Whole exome **reanalysis** of previously obtained uninformative whole exome sequence is medically necessary when one of the following criteria is met:-

- There has been an onset of additional symptoms that broadens the phenotype assessed during the original exome evaluation.
- There has been the birth or diagnosis of a similarly affected first-degree relative that has expanded the clinical picture.

WES is not covered in the following scenarios*.

- WES is not covered for uncomplicated Autism Spectrum Disorder, developmental delay, and mild to moderate global developmental delay.
- WES is not covered when environmental exposures, injury, or infection may reasonably explain the patient's constellation of symptoms.
- WES is considered investigational for:
  - ❖ Prenatal screening for fetal diagnosis.
  - ❖ Preimplantation testing of an embryo.
  - ❖ Purpose of genetic carrier screening.

- ❖ Genetic disorders in all other situations

Aetna (2019) in its policy, highlighted that WES is considered medically necessary for the evaluation of unexplained congenital or neurodevelopmental disorder in children </= 21 years of age when all of the following criteria are met: [66]

A. A genetic etiology is considered the most likely explanation for the phenotype, based on either of the following:
   1. Multiple congenital abnormalities affecting unrelated organ systems; or
   2. Two of the following criteria are met:
      - Abnormality affecting at minimum a single organ system (e.g., brain),
      - Significant developmental delay, intellectual disability (e.g., characterized by significant limitations in both intellectual functioning and in adaptive behaviour), symptoms of a complex neurodevelopmental disorder (e.g., self-injurious behaviour, reverse sleep-wake cycles, dystonia, hemiplegia, spasticity, epilepsy, muscular dystrophy), and/or severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome),
      - Family history strongly suggestive of a genetic etiology, including consanguinity,
      - Period of unexplained developmental regression,
      - Biochemical findings suggestive of an inborn error of metabolism, and

B. The member and family history have been evaluated by a Board-Certified or Board-Eligible Medical Geneticist, and

C. Member receives pre- and post-test counseling by an appropriate independent provider (not an employee of the genetic testing laboratory), such as an American Board of Medical Genetics or American Board of Genetic Counseling-certified Genetic Counselor, or an Advanced Practice Nurse in Genetics (APGN) credentialed by either the Genetic Nursing Credentialing Commission (GNCC) or the American Nurses Credentialing Center (ANCC), and

D. Alternate etiologies have been considered and ruled out when possible (e.g., environmental exposure, injury, infection), and

E. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available, and

F. WES is more efficient than the separate single-gene tests or panels that would be recommended based on the differential diagnosis (e.g., genetic conditions that demonstrate a high degree of genetic heterogeneity), and

G. A diagnosis cannot be made by standard clinical workup, excluding invasive procedures such as muscle biopsy, and

H. WES is predicted to have an impact on health outcomes, including:
   1. Guiding prognosis and improving clinical decision-making, which can improve clinical outcome by:
      - application of specific treatments as well as withholding of contraindicated treatments for certain rare genetic conditions,
      - surveillance for later-onset comorbidities,
      - initiation of palliative care,

- withdrawal of care; or

2. Reducing diagnostic uncertainty (e.g., eliminating lower-yield testing and additional screening testing that may later be proven unnecessary once a diagnosis is achieved); or
3. For persons planning a pregnancy, informing genetic counseling related to recurrence risk and prenatal diagnosis options; and

I. Family trio testing (WES of the biologic parents or sibling of the affected child) is considered medically necessary when criteria for WES of the child are met.

Kaiser Permanente (KP) in its coverage policy stated that WES is considered medically necessary for a phenotypically affected individual when ALL of the following criteria are met:-

1. Individual has been evaluated by a board-certified medical geneticist (MD) or other board-certified physician specialist with specific expertise in the conditions and relevant genes for which testing is being considered
2. Results have the potential to directly impact clinical decision-making and clinical outcomes for the patient
3. A genetic etiology is the most likely explanation for the phenotype as demonstrated by EITHER of the following:
   A. multiple abnormalities affecting unrelated organ systems OR
   B. TWO of the following criteria are met:
   - abnormality affecting a single organ system
   - significant intellectual disability, symptoms of a complex neurodevelopmental disorder (e.g. self-injurious behaviour, reverse sleep-wake cycles) or severe neuropsychiatric condition (e.g., schizophrenia, bipolar disorder, Tourette syndrome)
   - family history strongly implicating a genetic aetiology
   - period of unexplained developmental regression (unrelated to autism or epilepsy)
   - dysmorphic facial features
   - abnormal growth not otherwise explained
4. No other causative circumstances (e.g. environmental exposures, injury, infections) can explain symptoms
5. Clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available
6. The differential diagnosis list and/or phenotype warrant testing of multiple genes and ONE of the following:
   A. WES is more practical than the separate single-gene tests or panels that would be recommended based on the differential diagnosis
   B. WES results may precede the need for multiple and/or invasive procedures, follow-up, or screening that would be recommended in the absence of testing.

All requests must be approved by a KP geneticist, regardless of whether they have seen the patient.

5.6 SOCIAL

There is no evidence retrieved on social implication on WES for children with suspected genetic disorder.

5.7 ETHICAL / LEGAL

There is no evidence retrieved on legal and ethical implication on WES for children with suspected genetic disorder.

In the US, several federal agencies regulate genetic tests including the Food and Drug Administration (FDA), the Centres for Medicare and Medicaid Services (CMS), and the Federal Trade Commission (FTC). Genetic and Genomic tests, like other types of diagnostic tests, were evaluated and regulated on the following three criteria, analytical validity, clinical validity and clinical utility. Analytical Validity refers to how well the test predicts the presence or absence of a particular gene or genetic change. Clinical Validity, refers to how well the genetic variant(s) being analyzed is related to the presence, absence, or risk of a specific disease. Meanwhile Clinical Utility, refers to whether the test can provide information about diagnosis, treatment, management, or prevention of a disease that will be helpful to patients and their providers. [68]

The CMS implements regulations to control the analytical validity of clinical genetic tests, but there is no federal oversight of the clinical validity of most genetic tests. In light of this, FDA has proposed new policies to enhance analytical validity regulation and expand oversight of the clinical validity of genetic tests. Neither agency has issued formal plans to regulate the clinical utility of genetic tests, but typically, health care insurers like CMS draw on data from the research and medical communities to determine the clinical utility of medical treatments and procedures. Since clinical genomics is a relatively new field, frameworks to evaluate the clinical utility of genetic tests are still being developed.

In many circumstances there is uncertainty whether if participating in genetics research or undergoing genetic testing will lead to being discriminated against based on their genetics. In the US, the Genetic Information Nondiscrimination Act (GINA) describes what protections GINA **does** and **does not** offer.The GINA (2008) protects the US citizen from discrimination based on their genetic information in both health insurance (Title I) and employment (Title II). Title I amends the Employee Retirement Income Security Act of 1974 (ERISA), the Public Health Service Act (PHSA), and the Internal Revenue Code (IRC), through the Health Insurance Portability and Accountability Act of 1996 (HIPAA), as well as the Social Security Act, to prohibit health insurers from engaging in genetic discrimination. Title II of GINA is implemented by the Equal Employment Opportunity Commission (EEOC) and prevents employers from using

genetic information in employment decisions and prevents employers from requesting and requiring genetic information from employees or those applying for jobs.[69]

In Title I (Health Insurance), GINA prohibits health insurers from discrimination based on the genetic information of enrolees. Specifically, health insurers may not use genetic information to determine if someone is eligible for insurance or to make coverage, underwriting or premium-setting decisions. Furthermore, health insurers may not request or require individuals or their family members to undergo genetic testing or to provide genetic information. As defined in the law, genetic information includes family medical history, manifest disease in family members, and information regarding individuals' and family members' genetic tests decisions such as hiring, firing, promotions, pay, and job assignment.

Title II of GINA is implemented by the Equal Employment Opportunity Commission (EEOC) and prevents employers from using genetic information in employment. Furthermore, GINA prohibits employers or other covered entities (employment agencies, labour organizations, joint labour-management training programs, and apprenticeship programs) from requiring or requesting genetic information and/or genetic tests as a condition of employment. The regulations governing implementation of GINA in employment took effect on January 10, 2011.[69]

The items highlighted in the Ministry of Health's Guidelines On Ethical Issues In The Provision Of Medical Genetics Services In Malaysia provides further relevant information pertaining to the specific service provision in the health facility. [70]

5.8 LIMITATION

Our review has several limitations. Although there was no restriction in language during the search, only English full text articles were included in the report. Among the SR included was a review which included observational studies (case series) with variable study population, vary in outcome measurement, lack in long term outcome, and without head-to-head comparator in objectively assessing the effectiveness or performance of WES in diagnosing children with suspected genetic disorder. Notably, the highest level of evidence, RCT appears unlikely to be performed in these population group. Hence, observational studies including case series are important, and often the only evidence available for this patient group. Varying or heterogeneity in the study population or phenotype is another limitation of the included studies, limiting generalizability of the results. This is translated to small number of study population eligible for similar intervention, hence challenging measurement of robust outcome in several of the studies. Some study outcomes, such as mortality requires long time horizon which impose further limitation. Heterogeneity of outcomes being measured limiting quantitative summary of results. Included studies with high

risk of bias may affect methodological quality of this review. Lack of local data on cost and utility of the interventions and comparator in the population of interest prohibit the generation of local cost-utility analysis.

6.0 CONCLUSION

Based on the above review, there was fair level of evidences on WES to be used in diagnosing children including infant with suspected genetic disease.

WES including rapid WES showed beneficial effect in diagnostic yield and clinical utility (change in clinical management) in diagnosing children including infant with suspected genetic disorder. WES appeared better in terms of providing diagnosis rate to patients and relatives; and the benefit of diagnosis, namely impact on clinical management to patients and relatives, than standard care.

The WES was carried out as standard WES or rapid WES, either singleton (proband) or trio; in a variety of genetic conditions in clinical practice; from children with neurodevelopmental disorders, congenital anomalies, developmental delay, intellectual disability, undiagnosed developmental abnormality, medical condition requiring rapid diagnosis, cases with suspected monogenic disease, symptomatic patients with rare disease or ill infants; commonly being neurological or neurodevelopmental disorder.

Diagnostic yield ranged from 31.6% to 52.0%, and from 36.7% to 57.5% following WES in children and infants with suspected genetic disease, respectively. Mean turn around time was 40 days (range 25 to 100 days).

Following the use of rapid WES, diagnostic yield ranged from 20% to 52.5% in critically ill infants. Time to report ranged from 5.3 to 16 days.

Impact on clinical management ranged from 26% to 52% for children or infant with suspected genetic disease following use of WES, and ranged from 57% to 88% following rapid WES in critically ill infant or patients beyond infancy.

In terms of safety, WES Constituent Device was registered as Class II medical device by USFDA. The WES constituent device consists of reagents, instrumentation, software and instructions. There is no psychosocial impact upon receiving VUS on caregivers following WES, with most had a good understanding and the result had no impact on their perception of their child's condition. WES test results, evoked relief as well as worries, identified advantages and disadvantages, irrespective of the type of result among parents with children whom underwent WES.

In terms of cost-effectiveness, evidence demonstrated that pathways with earlier WES testing were more likely to be cost savings compared to

pathways that used WES later in the testing pathway or used WES as a last-resort strategy.

Cost estimates for a single test ranged from $555 to $5,169 for WES and from $1,906 to $24,810 for WGS. Cost estimates for a trio ranged from £2,658 ($3,825) to £6,466 ($9,304).

CEA conducted in Australia from healthcare system perspective found using WES to replace most investigations (as a first line) results in a savings per additional diagnosis of AU$2,182 (US$1,702). WES as a first-line test replacing most investigations is dominant. Another CEA in Australia found if WES performed at initial tertiary presentation, the resulted incremental cost savings was A$9020 (US$6838) per additional diagnosis compared with standard diagnostic pathway. However, adding WES to the standard diagnostic pathway does not offer a cost savings, but incurs an additional cost of A$5760 (US$4371) per diagnosis.

CEA in UK from NHS perspective found if WES was introduced later in the testing pathway, the ICER per additional positive genetic diagnosis was £3,171; while if the test used as a near first-line test, the ICER per additional genetic diagnosis was £2,201, compared to the usual testing approach. Sensitivity analyses showed that the largest driver of cost was the cost of the genetic testing, including cost of the exome sequencing and the associated bioinformatics analysis.

Cost analysis in a German cohort of children with NDD/epilepsy found genetic examinations had the highest cost savings potential amounting to 302,947.07€ (90.2%) out of 335,837.49€ [a total of 687,168.02€ was spent on genetic diagnostics]. This corresponds to total savable cost of 3,025.56€ per individual, compared to saving of 197.33€ for cMRI examinations and 98.98€ for metabolic testing in this cohort.

A cost calculation was conducted to estimate the potential cost implication should WES be integrated earlier in the diagnostic pathway for patients with suspected genetic diseases. It involves four scenario analyses which offer WES or CMA either as the first-tier or second-tier test. All patients were assumed not to undergo any prior genetic testing upon presentation at the genetic clinic, and beyond these two tests, the costs for any further testing were not considered. In a population without a clear differential diagnosis, as a first-test, the number of patients with positive results from WES was almost quadruple the number achieved with CMA, at a cost per diagnosis less than a quarter of the cost per diagnosis estimated for CMA. In all scenarios, integrating WES as the first-tier test have resulted in a lower cost per diagnosis as well as cost per patient. Even when a higher test cost for WES was applied, a similar trend was observed. This may indicate that the diagnostic yield of a genetic test plays a significant role in affecting the cost per diagnosis or the cost per patient.

In terms of organizational, WES is conducted by laboratories that are accredited by the Clinical and Laboratory Improvement Act (CLIA) to conduct high complexity testing. This test is commonly only conducted in laboratories associated with large, tertiary medical centers or commercial genetics laboratories due to the equipment and software involved (particularly the bioinformatics platform).

Creating reasonable expectations, establishing an understanding of the value and limitations of testing, creating awareness of the potential harms, and allowing the family to make informed choices is a mainstay of informed consent. Elements of counseling should include a three-generation family pedigree; discussion of pathogenic/likely pathogenic results, benign results, and variants of uncertain significance; detection of misattributed paternity or consanguinity, and secondary findings unrelated to the reason for testing.

The ACMG 2021 guideline recommended ES and GS as a first-tier or second-tier test for patients with one or more congenital anomalies prior to one year of age, or for patients with Developmental Disorder/Intellectual Disability with onset prior to 18 years of age. The EuroGentest and the European Society of Human Genetics (2016) guidelines on the evaluation and validation of next-generation sequencing (NGS) applications for the diagnosis of genetic disorders; highlighted the importance of diagnostic utility, informed consent, information to the patient and clinician, validation and reporting.

In terms of reimbursement, several commercial payers covered WES with specific criteria have to be met by the beneficiaries. Several eligibility criteria have to be met for funding by Medicare (Australia) on WES, which are: -

- If the child is strongly suspected of having a single gene disorder and is aged 10 years or younger.
- The child has a non-informative chromosome microarray (CMA) test. Negative Fragile X testing and urine metabolic screening is also desirable.
- A clinical geneticist has been consulted about the test indications.
- The family has given informed consent using the appropriate consent forms.

The Washington State Health Authority stated that WES is a covered benefit with conditions. The test is considered medically necessary for the evaluation of unexplained congenital or neurodevelopmental disorders in a phenotypically affected individual when all of the criteria are met (in the document). Similarly, whole exome or WGS is considered medically necessary by several commercial payers such as Kaiser Permanente, Cigna, Aetna when criteria listed (in the document) are met. Pre- and post-test genetic counseling is required for any individual undergoing whole exome or WGS.

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APPENDIX 1: HIERARCHY OF EVIDENCE FOR EFFECTIVENESS

DESIGNATION OF LEVELS OF EVIDENCE

I Evidence obtained from at least one properly designed randomized controlled trial.

II-I Evidence obtained from well-designed controlled trials without randomization.

II-2 Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group.

II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence.

III Opinions or respected authorities, based on clinical experience; descriptive studies and case reports; or reports of expert committees.

SOURCE: US/CANADIAN PREVENTIVE SERVICES TASK FORCE (Harris 2001)

APPENDIX 2: SEARCH STRATEGY

Ovid MEDLINE® In-Process & Other Non-indexed Citations and Ovid MEDLINE® 1946 to present

Database: Ovid MEDLINE(R) ALL <1946 to May 01, 2024>
Search Strategy:
1 RARE DISEASES/ (14485)
2 (disease* adj1 orphan).tw. (1371)
3 (disease* adj1 rare).tw. (33532)
4 CONGENITAL ABNORMALITIES/ (35471)
5 (abnormalit* adj1 congenital).tw. (8222)
6 (birth adj1 defect*).tw. (12314)
7 (congenital adj1 defect*).tw. (5564)
8 deformit*.tw. (81007)
9 (fetal adj1 anomal*).tw. (2821)
10 (fetal adj1 malformation*).tw. (2358)
11 DEVELOPMENTAL DISABILITIES/ (22660)
12 (child adj2 development deviation*).tw. (0)
13 (child adj2 development disorder*).tw. (10)
14 (developmental adj2 delay disorder*).tw. (20)
15 (developmental adj1 disabilit*).tw. (7377)
16 (disabilit* adj1 developmental).tw. (7377)
17 (child adj3 development disorders specific).tw. (0)
18 INTELLECTUAL DISABILITY/ (60922)
19 (deficienc* adj1 mental).tw. (1662)
20 (retardation adj1 mental).tw. (27232)
21 (intellectual adj2 development disorder*).tw. (31)
22 (intellectual adj1 disability*).tw. (21288)
23 (psychosocial adj2 mental retardation*).tw. (3)
24 idiocy.tw. (542)
25 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 (290577)
26 WHOLE EXOME SEQUENCING/ (8384)
27 (complete adj2 exome sequencing*).tw. (23)
28 (whole adj2 exome sequencing*).tw. (19563)
29 (complete adj2 transcriptome sequencing*).tw. (9)
30 (whole adj2 transcriptome sequencing*).tw. (1218)
31 26 or 27 or 28 or 29 or 30 (24371)
32 GENETIC TESTING/ (46003)
33 (genetic adj2 predictive testing).tw. (28)
34 (genetic adj2 predisposition testing).tw. (30)
35 (genetic adj1 screening*).tw. (7486)
36 (genetic adj1 testing).tw. (32667)
37 SEQUENCE ANALYSIS, DNA/ (171894)
38 (analys#s adj2 dna sequence).tw. (7105)
39 (dna adj2 sequence determination*).tw. (177)
40 (dna adj1 sequencing).tw. (32915)

41 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 (270516)
42 25 and 31 and 41 (559)
43 limit 42 to (english language and humans) (455)

OTHER DATABASES	
EBM Reviews – Cochrane Central Registered of Controlled Trials	Similar MeSH, keywords, limits used as per MEDLINE search
EBM Reviews – Database of Abstracts of Review of Effects	
EBM Reviews – Cochrane database of systematic reviews	
EBM Reviews – Health Technology Assessment	
NHS economic evaluation database	
PubMed	Similar MeSH, keywords, limits used as per MEDLINE search
INAHTA	
US FDA	

APPENDIX 3: CHECKLIST FOR A HIGH QUALITY FERTILITY PRESERVATION PROGRAM

CHECKLIST FOR A HIGH-QUALITY FP PROGRAM: THE ESHRE GUIDELINE 2020

An FP program should fulfil the following requirements:

- The legal framework of the country should be considered with regards to:
  i) administrative/legal facilities agreement,
  ii) authorization and accreditation when imposed by local/national regulatory authorities;
  iii) ethical approval for aspects that are considered research
- Referral pathways need to be established and require continuous maintenance.
- The following material and methods should be available:
  - Appropriate equipment
  - Qualified/authorized personnel (training programs)
  - Standard operating procedures (SOP):
    • Manipulation procedures, cryopreservation procedures, transport conditions, media conditions
  - Certified and/or registered media/supplements and equipment used as per local legislation
- Administrative forms related to patients' assessment should be available, including:
  - Oncologists/other medical specialists written approval for FP, where appropriate
  - Report containing diagnosis and status of the disease and medical treatment proposed

  - Assessment and recording of patient's medical history, including assessment of specific factors relevant to FP e.g. risk of thrombosis/infection, previous treatment that may impact ovarian reserve/response to ovarian stimulation
  - Assessment of patient's serology (obligatory as part of regulatory rules in some countries)
- Multidisciplinary staff should officially participate in decision-making
- Written informed patients consent forms should be available outlining the following:
  - the risks/benefits of the procedure/intervention to be applied to recipient and to their gametes/tissue; it is suggested to use the EuroGTPII tool (http://www.goodtissuepractices.eu/)
  - the known or unknown outcomes
  - any applicable age limits or other criteria for using cryopreserved oocytes/embryos or ovarian tissue a psychosocial screening regarding the welfare of the child might be part of the procedure before using their stored material
  - choices regarding the destiny of the material in case of non-use within centre's determined period of time, for instance disposal, or donation for research
  - acknowledging centres policy for long-term storage, including time limitations and costs

APPENDIX 3: EVIDENCE TABLE

Only available upon request.

WHOLE EXOME SEQUENCING FOR CHILDREN WITH SUSPECTED GENETIC DISEASE

e ISBN 978-967-2887-85-0

MAHTAS
(online)