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TruSight™ Software Suite

Bringing efficiency and high confidence to case management, variant analysis, and interpretation in rare disease.

Highlights

- **Comprehensive genomic evaluation**
  Analyze, visualize, and interpret small variants, structural variants, mitochondrial variants, repeat expansions, runs of homozygosity, and *SMN1/SMN2* variants
- **Ready-made, integrated workflow**
  Keep pace with evolving technology using a ready-made infrastructure and simplified integration of diverse analytical tools to optimize the benefits of next-generation sequencing

Introduction

Whole-genome sequencing (WGS) and whole-exome sequencing (WES) using next-generation sequencing (NGS) technologies are powerful methods for investigating variants linked to genetic disease. In a meta-analysis of literature from January 2011 to August 2017, 37 studies comprising 20,068 children were reviewed for the diagnostic utility of three testing approaches: chromosomal microarray (CMA), WES, and WGS. Results showed 8.3× greater odds of diagnosis with NGS methods, compared to CMA (Figure 1).[1]

WGS and WES provide a high-resolution, unbiased view across the entire genome to discover causative variants associated with rare diseases. However, the vast amounts of data produced by these methods represent a significant bottleneck and require comprehensive data analysis tools that can efficiently translate the raw sequencing data into meaningful, interpretable results. To address this challenge, Illumina offers TruSight Software Suite. This software as a service (SaaS) integrates with BaseSpace™ Sequence Hub and Illumina sequencing systems to access run monitoring, run metrics, and automated sequencing data upload. It includes cloud-based access to the DRAGEN™ Bio-IT Platform, enabling comprehensive, streamlined secondary and tertiary analysis workflows for NGS (Figure 2).

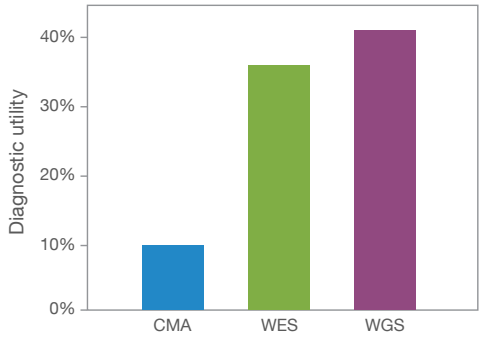

Figure 1: WGS and WES have higher diagnostic utility than CMA—Quantitative analyses of 37 studies comprising 20,068 children for diagnostic utility of first-line genomic tests showed 36% and 41% utility for WES and WGS, respectively, compared to 10% for CMA. 95% CI: 4.7-14.9, P < 0.0001.

Variant analysis in TruSight Software Suite

Secondary analysis includes:

- Alignment and variant calling using the DRAGEN platform

Tertiary analysis includes:

- Variant annotation
- Variant filtering and triage
- Variant visualization
- Variant curation
- Variant interpretation and customized reporting

Powered by the DRAGEN platform

TruSight Software Suite is powered by the DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform, providing secondary analysis of genomic data. Fundamental features of the DRAGEN platform address common challenges in genomic analysis, such as lengthy

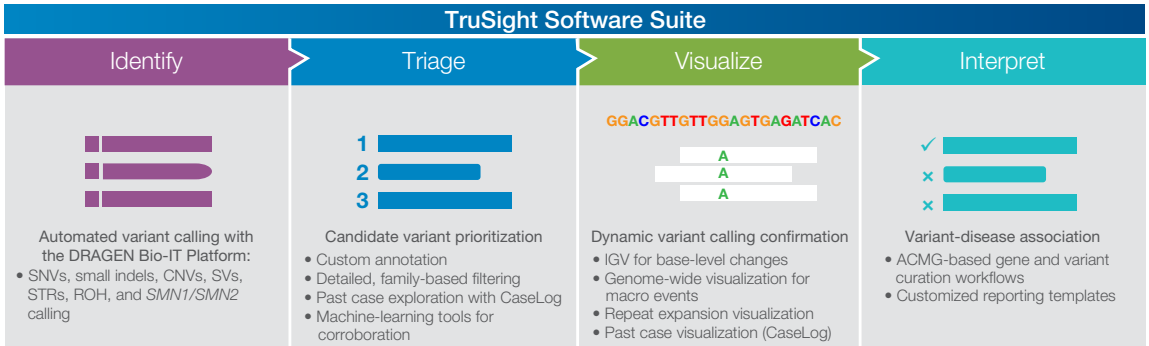

Figure 2: Variant analysis in TruSight Software Suite—Variant analysis in TruSight Software Suite begins with automatic alignment and variant calling using the DRAGEN platform, requiring approximately three hours for a 30× trio, followed by triaging, visualizing, and interpreting variants.

compute times and massive volumes of data. Without compromising accuracy, the DRAGEN platform delivers quickness, flexibility, and cost efficiency, enabling labs of all sizes and disciplines to do more with their genomic data. Comprehensive variant calling includes single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), structural variants (SVs), short tandem repeats (STRs), repeat expansions, runs of homozygosity (ROH), *SMN1/SMN2* calling, and more (Figure 3). While TruSight Software Suite is compatible with both WGS and WES outputs, the DRAGEN platform only supports repeat expansion calling and *SMN1/SMN2* calling with whole-genome samples. See the TruSight Software Suite user guide for additional details.

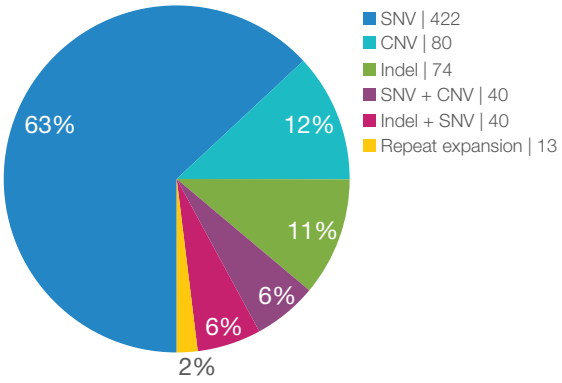

Figure 3: Variant types identified with TruSight Software Suite—Analysis of WGS data resulted in detection of different variant types. Percentages indicate number of cases out of 669 total cases.

Integration with other platforms and systems

Many labs struggle to keep pace with integrating new genomics technology, instruments, and methodologies. TruSight Software Suite simplifies the process, integrating seamlessly with the NovaSeq™ 6000, NextSeq™ 2000, or other systems via BaseSpace Sequence Hub for automating WGS and WES analysis. Furthermore, TruSight Software Suite represents the final piece in the rare disease workflow of an integrated, DNA-to-report WGS solution, including Illumina DNA PCR-Free Prep, Tagmentation, and the NovaSeq 6000 System. Compatibility with Application Programming Interfaces (APIs) enables integration with other institutional laboratory information management systems (LIMS). The software provides a complete data storage architecture to manage short- and long-term storage of FASTQ, VCF, BAM files, etc, in a cost-effective and secure manner.

Simplified, customizable case management

TruSight Software Suite features a Case Management Portal that allows users to create new cases, import data files, and associate sequencing data files with each case. Alternatively, this information can be easily imported via an API. Users enter familial relationship information about each case (up to five individuals), including family structure, proband gender, proband phenotypic features (optional), and affected status of family members to improve variant filtering and prioritization.

Cases can be assigned to specific roles or functions within a laboratory to improve efficiency. Real-time updates of case status are displayed in the TruSight Software Suite dashboard, a single view to monitor a laboratory's entire caseload. This allows managers and other personnel to monitor progress through the analysis workflow.

Intuitive, high-powered interpretation

TruSight Software Suite uses critical data aggregation, variant visualization, variant curation, and machine-learning tools to promote efficient and informed interpretation.

Variant triage

Using the Interpretation tab in TruSight Software Suite, variants can be filtered following a custom plan or a prebuilt filter plan. Family-based filtering in TruSight Software Suite enables comparison of the proband with other family members. Additional options include filtering on population frequencies from sources such as the Genome Aggregation Database (gnomAD), variant consequences, modes of inheritance, ClinVar pathogenicity, and more.

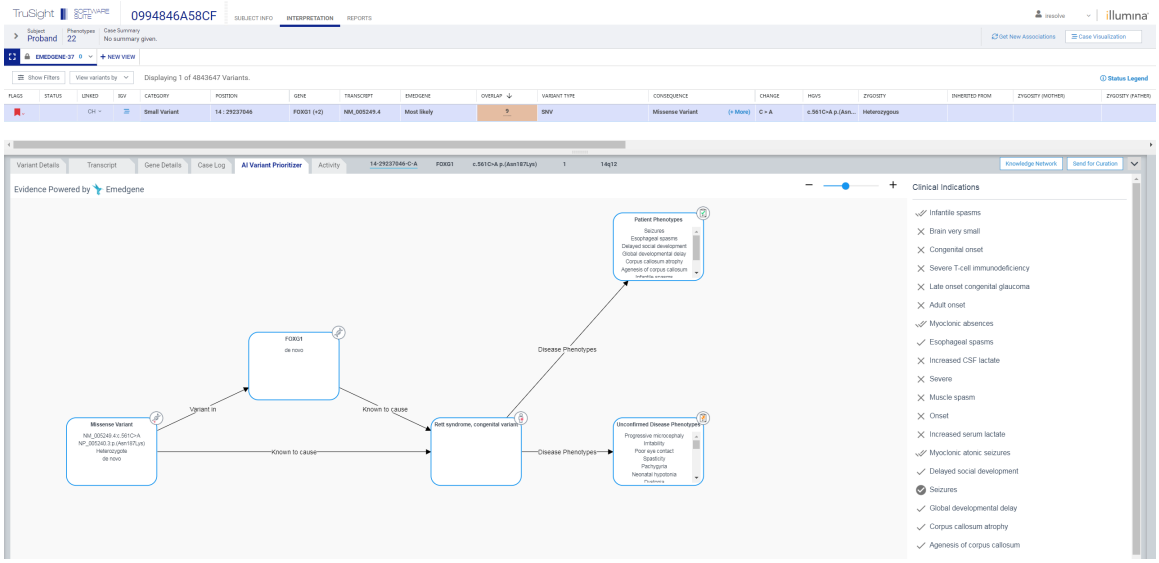

Figure 4: Variant filtering and prioritization—Family-based variant filtering enables identification of inherited and *de novo* variants. The variant grid, which is customizable by each user, shows information on category of variant, chromosomal position, gene affected, overlap (number of overlapped phenotypes for the variant), consequence of the variant, population frequency of the variant (if known), and more.

Variant analysis with machine learning

TruSight Software Suite incorporates a genomics artificial intelligence (AI) engine, powered by Emedgene, to rank variants and highlight the most-likely candidates. The engine generates a knowledge-graph showing supporting evidence for the variant prioritization, like disease–gene relationships, generated by the application of natural language processing (NLP) to various data sources (Figure 4).

Illumina worked with collaborators at Stanford University, University of California, San Francisco, University of Florida, University of Chicago, and the Broad Institute to develop the SpliceAI and PrimateAI analysis tools. These state-of-the-art deep neural networks are powered by machine learning to find disease-causing mutations. SpliceAI and PrimateAI provide unbiased, highly accurate classification of mRNA splice sites and missense variants, respectively.[1-3] Using these automated prioritization tools, users can add depth to their analysis and quickly filter out millions of variants to focus on the top, candidate variants of interest for visualization and interpretation.

Variant visualizations

TruSight Software Suite features embedded visualization tools, such as the Integrative Genomics Viewer (IGV), for further inspection of genomic data, including read alignments, variants, B-allele frequency, and coverage tracks for all subjects within a case. In addition to variant-level visualizations, the IGV offers views of an entire chromosome or whole genome to look for large anomalies.

Variant interpretation and curation

TruSight Software Suite offers various features to help determine which prioritized variants are relevant to the current case, enabling interrogation of gene– and variant–disease associations with overlapping phenotypic features similar to those in the proband.

TruSight Software Suite aggregates and integrates data from preferred external databases, such as the Online Mendelian Inheritance in Man (OMIM) catalog, ClinVar, and others, into the Variant Details tab. The aggregated data can be viewed to gain valuable insights into a variant, eliminating the need for repeated online searches in separate databases. Additionally, TruSight Software Suite integrates standard terminology for variant classification developed by the American College of Medical Genetics (ACMG), which helps with recording variant details and associations. This enables access and storage of gene-level information (eg, tolerance to loss-of-function variation, etc.) and characteristics of gene–disease relationships (via preferred external databases). Transcript-level information is also displayed for each variant, and both canonical and noncanonical transcripts can be selected for interpretation. Features such as the Note field can be used to add case-specific notes pertaining to a specific variant. The Comments field can be used to record case-independent information about the variant or gene, which may be valuable if observed in future cases.

CaseLog: a customer-specific database

CaseLog is used to view and aggregate gene, variant, and phenotypic information for each case across both private and public data sets (Figure 5). This interactive database stores public, rare disease data sets and cases previously seen by a lab to inform curation, interpretation, and reporting of genes or variants of interest based on new discoveries in the scientific community.

Results and custom report generation

Interpretation is complete when variants have been identified and curated with known disease associations. Customers can use templates in TruSight Software Suite to customize reports of gene and variant associations relevant to cases (Figure 6). The report can be

Figure 5: CaseLog—The CaseLog feature in TruSight Software Suite enables visualization of aggregate data for both variants and genes of interest.

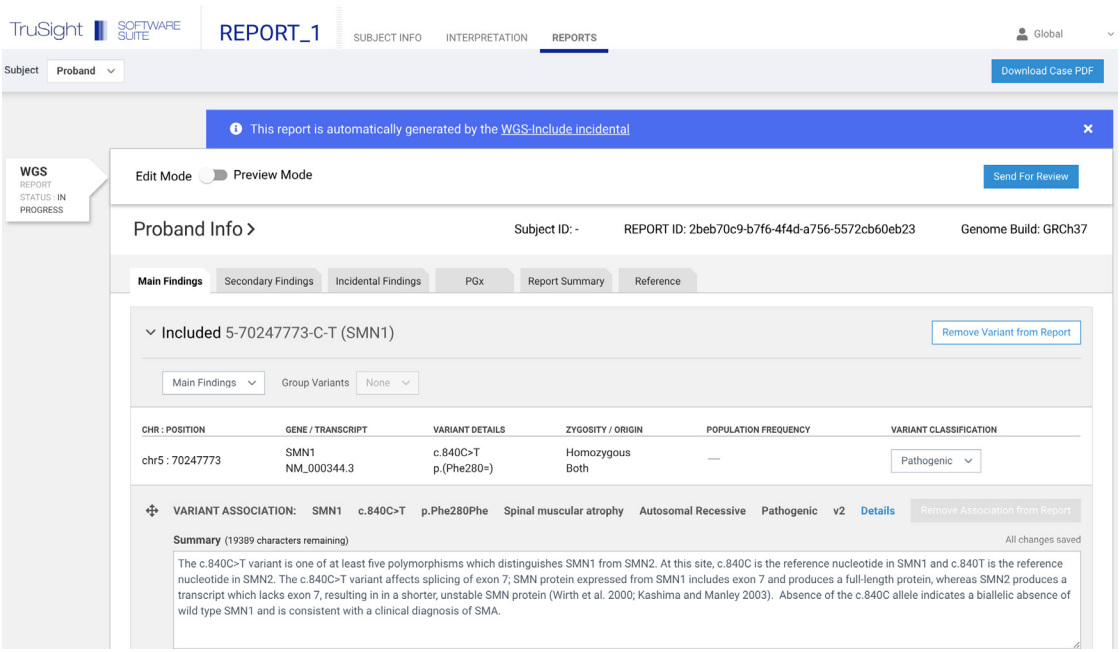

Figure 6: Customizable report generation—TruSight Software Suite offers a template for customization of reports of gene and variant associations relevant to the case.

sent for additional review and approval within the software. For ease of data sharing, reports can be downloaded in a PDF or JSON format.

Secure, compliant environment

TruSight Software Suite is ISO-27001 and ISO-13485 certified and complies with Health Insurance Portability and Accountability Act (HIPAA) (third-party audited) and the principles of the General Data Protection Regulation (GDPR). TruSight Software Suite also offers options to integrate with a lab's single sign-on policy and other security settings.

Summary

TruSight Software Suite offers an intuitive and comprehensive rare disease analysis and interpretation solution. It integrates with Illumina sequencing systems and includes the DRAGEN Bio-IT Platform for ultra-rapid variant calling and features tools to visualize, triage, and interpret variants associated with genetic disease. Results can be output using customizable templates for customer-specific reports.

Learn more

Learn more about TruSight Software Suite at www.illumina.com/trusight-software-suite

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Ordering information

Illumina offers a 30-day free trial, allowing customers to work with example cases available in TruSight Software Suite or upload and evaluate their own cases within the software.

Product	No. of samples	Catalog no.
TruSight Software Suite	48 WGS/96 WES	20041943
TruSight Software Suite	96 WGS/192 WES	20041944
TruSight Software Suite	288 WGS/576 WES	20041945
TruSight Software Suite	480 WGS/960 WES	20041946
TruSight Software Suite	960 WGS/1920 WES	20041947
TruSight Software Suite	2400 WGS/4800 WES	20041948
TruSight Software Suite	4800 WGS/9600 WES	20041949
TruSight Software Suite	9600 WGS/19,200 WES	20042010
TruSight Software Suite 30 day, 15 sample free trial		20042019
TruSight Software Suite Training at customer site (1 day)		20042020
TruSight Software Suite Training at Illumina Solutions Center (1 day)		20042021

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Using Next-Generation Sequencing to Study Rare Undiagnosed Genetic Disease

Whole-exome and transcriptome sequencing prove beneficial in uncovering mutations and pathways associated with rare disease.

Introduction

A rare disease affects only a small group of individuals. Yet, the term small is relative. In the United States, a rare disease is defined as affecting 200,000 people or fewer, according to the National Center for Advancing Translational Sciences.[1] In Europe, a disease is considered rare when it affects fewer than 1 in 2000 individuals.[2] In Korea, the Korean Undiagnosed Disease Program (KUDP) has determined that a condition is rare when it affects fewer than 20,000 people or when the prevalence is unknown due to its rarity.[3] All these small groups add up. With as many as 7000 known rare diseases, and many yet to be discovered, tens of millions of people all over the world are affected. Unfortunately, many of them are likely to go undiagnosed and untreated due to a lack of reliable and accurate clinical tools. Murim Choi, PhD is working to change the future for some of these patients.

Dr. Choi, Associate Professor at the Seoul National University (SNU) College of Medicine, works closely with local clinicians to study pediatric neurodevelopmental disorders. As a Principal Investigator in the SNU College of Medicine Functional Genomics Lab and a contributor to the KUDP's numerous research efforts, Dr. Choi's goal is to understand the molecular mechanisms of various human diseases using next-generation sequencing (NGS) and other forms of genetic analysis. He is a pioneer in using whole-exome sequencing (WES) to identify genetic variants that might play a causal role in different rare disease conditions. Early in his career, he taught himself the Perl programming language to enhance his ability to understand the data that his studies produce. Ultimately, Dr. Choi's goal is to contribute to the diagnosis of rare disease patients who often present with puzzling symptoms.

iCommunity spoke with Dr. Choi about his love of Perl programming, his use of WES over the last decade, and how NGS will be used in the future for rare disease research.

Q: What first sparked your interest in a career in genetics research?

Murim Choi (MC): I was originally trained as a developmental biologist, receiving a Bachelor's degree in Molecular Biology. I always wanted to learn about heart development and ended up at Duke University studying how the heart developed in the mouse system. It wasn't genomics, more embryology and mouse genetics. In early 2000, I was studying phenotypic variation due to a genetic variant that was causing heart problems in a specific mouse strain. It was a project that led me into the world of genetics and the genotype-phenotype relationship. I decided that I wanted to study human genetics.

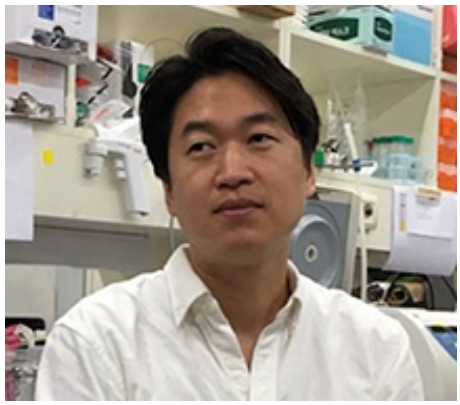
Q: When did you begin studying the genetics of human disease?

MC: In 2007, I started working in Dr. Richard Lifton's lab at Yale University. We were using Illumina SNP arrays to look at genotype, heterozygosity, and copy number variation (CNV) on disease genomes. We were also performing genome-wide association studies (GWAS). It was a whole new world to me.

Q: What prompted you to teach yourself the Perl coding language?

MC: I decided I needed to study coding so that I could manage the data that we were producing in Dr. Lifton's lab. I went to the bookstore with my one-year-old son and picked up a book on Perl, which was the main tool for bioinformatics then. During the day, I was in the lab preparing DNA and performing GWAS. After I put my son to bed at night, I studied Perl. I really enjoyed it.

There were no public tools for GWAS data analysis, so I used my knowledge of Perl to design the genomic alteration and downstream analysis. I showed Dr. Lifton the GWAS data of the cohort that I had been working on and he liked the analysis. He said that I shouldn't go back to the lab bench and recommended that I had the skill to analyze and manage the genetic data, which I agreed with. It felt quite powerful to be able to digest and make sense of the data.



Murim Choi, PhD is an Associate Professor at SNU, a Principal Investigator in the SNU College of Medicine Functional Genomics Lab, and a KUDP research contributor.

Q: Dr. Lifton's lab was known for its work on cardiovascular diseases. How did you become involved in rare and undiagnosed genetic disease research?
MC: My work in Dr. Lifton's lab allowed me to look at an individual's genome in a very broad way. He was kind enough to provide me with all the data sets he had for analysis. When the first NGS systems were introduced in 2009, Dr. Lifton asked me to set up the WES pipeline in the lab. One of the first projects was using NGS to exome sequence a patient who had been diagnosed with a rare disease.

> "Today, the cost of NGS has dropped dramatically and we perform WES routinely as one the first steps in assessing for rare genetic disease."

Q: Why are some of these rare diseases so difficult to diagnose?
MC: Rare diseases are mostly Mendelian and monogenic. Their symptoms can be diverse and complex, with onset early in development. These features make it hard for clinicians to diagnose. Therefore, where the patient lives and the experience of the clinician play a significant role in diagnosing rare disease. If it is an easy and straightforward rare disease, the patient is diagnosed instantly in a clinic close to their home. However, not all physicians have enough experience diagnosing rare diseases, raising the possibility that they might misdiagnose the patient. In South Korea, local pediatricians might refer those patients to clinicians at the SNU Children's Hospital. I'm affiliated with the hospital, which is right across the street from my lab. My lab is situated in a great location to study these diseases.

Q: What can genetic assessments offer pediatric patients with rare disease?
MC: Even with a genetic assessment, we don't always have a treatment available for patients with rare disease. If it's a *de novo* mutation, we can let the parents know that subsequent children won't likely to be afflicted by the disease. If the disease is the result of recessive variants, we can advise the parents to be prescreened for the mutation before having a second child.

Sometimes, we can provide direct help to the child and the family. One scenario is when the identified mutations are in the enzyme of a metabolic pathway, resulting in the lack of an important metabolite. In those cases, we can supplement those metabolites to ameliorate the symptoms.

Another scenario is when we identify variants where there are current drugs available that target those mutations for non-rare diseaes. For example, several years ago there were two cases where we identified patients who harbored *de novo* mutations in genes critical for autoimmune and hyperinflammatory responses, respectively. A functionally mimicking antibody to enhance defective gene function alleviated the severe autoimmune systems in one patient.[4] A signaling inhibitor to reduce increased gene function relieved vasculopathy in the other patient.[5]

However, most of the patients that we analyze are pediatric neurology patients and their cases are complex. Neurological disease is where breakthroughs in understanding the underlying genetics are most needed.

Q: What are the advantages of NGS techniques over older genetic technologies in identifying rare disease-associated variants?
MC: When I first joined the Lifton lab, I was a young geneticist and ignorant about what NGS could offer. I heard people talking about how sequencing was getting more robust and I wasn't sure what that meant. The only sequencing that I knew of was Sanger sequencing and I couldn't understand the advantage of making it faster.

I soon realized how much faster NGS was and that it provided the best coverage of the genome. Yet, at SNU, when I first collaborated with the clinicians, we were careful not to use NGS immediately because of its cost. We would start with Sanger or focused screening approaches based on clinical interpretations. If they didn't provide the answer, we'd move to WES because it provided more coverage for unknown genes and could identify mutations in unexpected genes, providing explanations for novel syndromes and unrecognized diseases.

Today, the cost of NGS has dropped dramatically and we perform WES routinely as one the first steps in assessing for rare genetic disease. It is no longer cost prohibitive to use NGS to resequence every rare disease patient to obtain a better understanding of the genetics of their conditions. It enables us to stratify patients by genotypes that were unrecognized previously. I think we'll see the use of NGS increase for rare disease studies.

> "...our group is performing more functional genomic techniques, including CRISPR and single cell-based NGS analyses."

Q: How does NGS enable your own research?
MC: We perform two kinds of NGS studies in our laboratory; patient genome sequencing, such as whole-genome sequencing (WGS) and WES, and functional genomics. Both approaches make good use of NGS techniques. Functional genomics studies assess the impact of variants of unknown significance on cellular function. We can use that information as we follow rare disease patients over time. It provides us with a very comprehensive reading.

Q: What inspired you to begin using WES in your studies?
MC: We used WES in Dr. Lifton's lab to analyze six subject samples, all without any known mutations and five from patients conceived from consanguineous marriages. At the time, it was

much easier to detect homozygous variants in those individuals. One of the five patients had been diagnosed with Bartter syndrome. We didn't find any of the mutations associated with Bartter syndrome, but did identify a mutation in the chloride channel gene. We discussed the finding with a postdoc who had insight into the biology of Bartter Syndrome in renal disease. She suggested that the cause of the patient's symptoms was due to disruption of the chloride channel associated with congenital chloride diarrhea rather than Bartter syndrome. Fortunately, there's a drug to treat congenital chloride diarrhea and it alleviated some of the subject's symptoms. It was the first study to show how NGS techniques could inform diagnosis in rare disease patients.[6]

> "Illumina sequencing data has gotten better and better over the last 10 years. We have a quality control pipeline, but it rarely raises a red flag because the data are very stable."

Q: How has your use of WES evolved in your research?
MC: It's been 10 years since we reported that WES could be used to help with the diagnosis of rare disease. We've used WES to identify *TONSL* mutations in SPONASTRIME dysplasia,[7] *GLBI* mutations in GM1 gangliosidosis,[8] and *GABBR2* mutations in Rett syndrome.[9] The value of WES isn't a secret anymore and it's now moving into routine use in applied industry and clinics. The pediatric clinical laboratory that we collaborate with now performs WES routinely. When they find something interesting, they bring it to us to study in greater depth.

WES is a very stable technique. However, as basic scientists, we feel that WES should be covered by the clinical labs. That's why our group is performing more functional genomic techniques, including CRISPR (clustered regularly interspaced short palindromic repeats)–based screening methods and and single cell-based NGS analyses. While our methods may have changed, our objectives have not. We are still interested in understanding phenotype-genotype relationships. We're using our functional genomics data to make sense of the patient phenotypic data to identify the presence of rare disease.

Q: What is the focus of the SNU School of Medicine Functional Genomics Lab?
MC: Our lab is focused on the genetics of ultrarare disease. We have a pool of cohorts and have made some interesting discoveries of novel genotype-phenotype correlations. We are using the information to establish collaborations using GeneMatcher, a platform to share gene and phenotype information with clinicians and researchers worldwide. It's given us a window into the biology of what's really going on in the cell, enabling us to see if the novel gene we've identified could be a potential therapeutic target.

We are also performing data analysis studies. We have accumulated approximately 700 probands and their parents' data. While it is a small study compared to the large data projects currently underway in the United Kingdom and by major consortium, we can use the data for controls and comparisons as we build the database.

As I stated earlier, we don't have consanguineous marriages or many homozygous variants in Korea. However, we do have a high occurrence of compound heterozygous variant patients. We believe that if we can increase the number of proband families in our database, we'll have the information necessary to create a gene panel for use in prescreening potential parents for rare disease and associated mutations. As NGS becomes less expensive, the cost of the test will become more affordable and available to couples thinking of having children.

> "I think we'll see more clinical labs performing WES on rare disease patient samples. There is also a place for WGS in the clinic."

Q: What NGS sequencing systems do you use?
MC: We used the HiSeq™ 2000 System when we set up our WES pipeline in Dr. Lifton's lab. In our Functional Genomics Lab, we outsource the sequencing to a service lab that uses HiSeq and NovaSeq™ 6000 Systems. Illumina sequencing data has gotten better and better over the last 10 years. We have a quality control pipeline, but it rarely raises a red flag because the data are very stable.

Q: How does your research team contribute to the KUDP?
MC: Dr. Jong-Hee Chae is a pediatric clinician, a principal investigator at the KUDP, and one of my closest collaborators. She runs the KUDP pediatric neurology program and has created a database of 5000 neurodevelopment cohorts. Recently, we successfully transplanted our WES pipeline into her lab. I'm involved in the genetic interpretation of the different variants found and participate in clinician meetings to review the analysis.

Q: What do you see as the future of NGS in studying rare and undiagnosed genetic diseases?
MC: I think the use of NGS methods will continue to grow in the future. There will be more of a distinction between the methods clinical laboratories perform and the methods used in basic science labs.

I think we'll see more clinical labs performing WES on rare disease patient samples. There is also a place for WGS in the clinic. Linkage analysis studies can be performed with WGS to narrow down the region being investigated. RNA-Seq of tissue samples can also be used to pin down the gene of interest. Ultimately, I believe that WGS will be used routinely in Korean clinical labs.

In basic science labs studying rare disease, we'll continue to perform RNA-Seq if we have a solid hypothesis of what we are looking for in the genome. We'll also run more functional genomics screening experiments using CRISPR and other synthetic primers so that we can eventually compare our discoveries with the phenotype information from patients.

Learn more about the products and systems mentioned in this article:

NovaSeq 6000 System, www.illumina.com/systems/sequencing-platforms/novaseq.html

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ARTICLE OPEN

Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases

Michelle M. Clark[1], Zornitza Stark[2], Lauge Farnaes[1,3], Tiong Y. Tan[2,4], Susan M. White[2,4], David Dimmock[1] and Stephen F. Kingsmore[1]

Genetic diseases are leading causes of childhood mortality. Whole-genome sequencing (WGS) and whole-exome sequencing (WES) are relatively new methods for diagnosing genetic diseases, whereas chromosomal microarray (CMA) is well established. Here we 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 CMA in children with suspected genetic diseases by systematic review of the literature (January 2011–August 2017) and meta-analysis, following MOOSE/PRISMA guidelines. In 37 studies, comprising 20,068 children, diagnostic utility of WGS (0.41, 95% CI 0.34–0.48, $I^2 = 44\%$) and WES (0.36, 95% CI 0.33–0.40, $I^2 = 83\%$) were qualitatively greater than CMA (0.10, 95% CI 0.08–0.12, $I^2 = 81\%$). 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 1.62–2.56, $I^2 = 12\%$; $P < 0.0001$). Diagnostic utility of WGS/WES with hospital-based interpretation (0.42, 95% CI 0.38–0.45, $I^2 = 48\%$) was qualitatively higher than that of reference laboratories (0.29, 95% CI 0.27–0.31, $I^2 = 49\%$); this difference was significant among studies published in 2017 ($P < .0001$, $I^2 = 22\%$ and $I^2 = 26\%$, respectively). The clinical utility of WGS (0.27, 95% CI 0.17–0.40, $I^2 = 54\%$) and WES (0.17, 95% CI 0.12–0.24, $I^2 = 76\%$) were higher than CMA (0.06, 95% CI 0.05–0.07, $I^2 = 42\%$); this difference was significant for WGS vs CMA ($P < 0.0001$). In conclusion, 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.

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INTRODUCTION

Genetic diseases (single-gene disorders, genomic structural defects, and copy number variants) are a leading cause of death in children less than ten years of age.[1–8] Establishing an etiologic diagnosis in children with suspected genetic diseases is important for timely implementation of precision medicine and optimal outcomes, particularly to guide weighty clinical decisions such as surgeries, extracorporeal membrane oxygenation, therapeutic selection, and palliative care.[9] With the exception of a few genetic diseases with pathognomonic findings at birth, such as chromosomal aneuploidies, etiologic diagnosis requires identification of the causative molecular basis. In practice, this is remarkably difficult for several reasons: firstly, genetic heterogeneity—there are over 5200 genetic disorders for which the molecular basis has been established.[10] Secondly, clinical heterogeneity—genetic disease presentations in infants are frequently *formes frustes* of classic descriptions in older children (see, for example Inoue et al.[11]). Thirdly, comorbidity is frequent in infants with genetic diseases—including prematurity, birth trauma, and sepsis—obfuscating clinical presentations.[2] Fourthly, approximately four percent of children have more than one genetic diagnosis.[12] Finally, disease progression is faster in children, switching the diagnostic odyssey to a race against time.[9,13,14]

Traditionally, establishment of molecular diagnoses was by serial testing guided by the differential diagnosis. CMA is the recommended first-line genomic test for children with several types of genetic diseases.[15,16] Serial testing employs many other tests—including newborn screening panels, metabolic testing, cytogenetics, chromosomal fluorescence in situ hybridization, single-gene sequencing, and sequencing of panels of genes associated with specific disease types (such as sensorineural deafness, cardiac dysrhythmias, or epilepsy).[15] Iterative inquiry of differential diagnoses, however, frequently incurs a diagnostic odyssey and rarely allows etiologic diagnosis in time to influence acute management. Thus, inpatient management of children with suspected genetic diseases largely remains empiric, based on clinical diagnoses.[9]

Over the past five years, WGS and WES have started to gain broad use for etiologic diagnosis of infants and children with suspected genetic diseases.[17–48] By allowing concomitant examination of all or most genes in the differential diagnosis, WGS and WES have the potential to permit comprehensive and timely

[1]Rady Children's Institute for Genomic Medicine, San Diego, CA, USA; [2]Murdoch Children's Research Institute, Melbourne, Australia; [3]Department of Pediatrics, University of California San Diego, San Diego, CA, USA and [4]Department of Paediatrics, University of Melbourne, Melbourne, Australia
Correspondence: Stephen F. Kingsmore (skingsmore@rchsd.org)

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ascertainment of genetic diseases. Timely molecular diagnosis, in turn, has the potential to institute a new era of precision medicine for genetic diseases in children. During this period, WGS and WES methods have improved substantially. While numerous studies have been published,[17–48] there are not yet guidelines for their use by clinicians. Here we report a literature review and meta-analysis of the diagnostic and clinical utility of WGS and WES, compared with CMA, in children (age 0–18 years) with any suspected genetic disease.

RESULTS

WGS and WES are relatively new methods for diagnosis of childhood genetic diseases. We compared the diagnostic utility of WGS and WES with that of CMA, the recommended first-line genomic test for genetic diseases in children with intellectual disability, developmental delay, autism spectrum disorder, and multiple congenital anomalies.[15,16] A total of 2093 records were identified by searches for studies of the diagnostic utility of WGS, WES, and CMA in affected children with a broad range of suspected genetic diseases (Figure S1). Thirty seven of these, featuring 20,068 children, met eligibility criteria and were included in qualitative analyses (Tables 1 and 2).[17–54] Thirty-six were case studies; one was a randomized controlled trial.[26] In these, the pooled diagnostic utility of WGS was 0.41 (95% CI 0.34–0.48, seven studies, 374 children, $I^2 = 44\%$), which was qualitatively greater than WES (0.36, 95% CI 0.33–0.40, 26 studies, $n = 9014$, $I^2 = 83\%$) or CMA (0.10, 95% CI 0.08–0.12, 13 studies, $n = 11{,}429$, $I^2 = 81\%$, Fig. 1a). Severe heterogeneity ($I^2 > 75\%$) within the WES and CMA groups precluded statistical comparisons.

Analysis of heterogeneity of diagnostic utility in studies of WGS, WES, and CMA

We used meta-regression to model heterogeneity in the diagnostic utility of WGS, WES, and CMA. Studies of WES and WGS varied in size from 22 to 1745 probands; Meta-regression showed a modest relationship between study size and diagnostic utility: on average, an increase of 1000 subjects decreased the odds of diagnosis by 28% (Fig. 2a, $P = 0.01$). Studies were published between 2013 and 2017; meta-regression showed that the odds of diagnosis by WES or WGS increased by 16% each year (Fig. 2c, $P = 0.01$) while the odds of diagnosis by CMA decreased by 14% (Fig. 2c, $P < 0.001$). The rate of consanguinity varied between 0% and 100%. It was not significantly associated with the odds of diagnosis ($P > 0.05$). The proportion of diagnoses in which causal variants occurred de novo (rather than inherited) ranged from 0.18–0.70; meta-regression showed that a 10% increase in the rate of consanguinity in studies of WES and WGS decreased the odds of de novo variant diagnoses by 21% ($P < 0.001$; Fig. 2d). Heterogeneity of diagnostic utility in disease type and proband age subgroups precluded quantitative analysis (Figure S2).

Subgroup comparisons of diagnostic utility of WGS, WES, and CMA

Heterogeneity within WGS and CMA groups was mild following removal of variance associated with year of publication. In eleven studies of 1962 children published in 2017, the pooled diagnostic utility of WGS (0.42, 95% CI 0.34–0.51, $I^2 = 13\%$) was significantly greater than CMA (0.05, 95% CI 0.03–0.09, $I^2 = 40\%$; $P < 0.0001$, Fig. 1b).[23,25,26,33,35,36,38,40–42,44]

Only two studies, featuring 138 children, compared WES and WGS within cohorts. The diagnostic utility of WES (0.29 and 0.37) did not differ significantly from that of WGS (0.34 and 0.50, respectively; $P > 0.05$).[24,36] Since the diagnostic utility of WES and WGS was not significantly different, we pooled WGS and WES studies in remaining subgroup analyses. Seven studies directly compared the proportion diagnosed by WGS or WES and CMA in 697 children; in each study, the diagnostic utility of WGS/WES was at least three-fold higher than CMA.[23,26,33,34,36,38,46] Four of these manuscripts contained enough information to estimate the marginal odds ratios of receiving a diagnosis among subjects that received both WGS/WES and CMA.[26,33,34,46] In them, the odds of a diagnosis by WGS/WES was 8.3 times greater than CMA (95% CI, 4.7–14.9, $I^2 = 36\%$; $P < 0.0001$, Fig. 1c).

Comparison of singleton and trio genomic sequencing and effect of site of testing

WGS/WES tests were either of affected probands or trios (proband, mother, father). In eighteen studies, comprising 3935 probands, the heterogeneity of diagnostic utility of singleton and trio WGS/WES was too great to permit quantitative analysis (Figure S3). Meta-analysis was performed in five studies (3613 children) that compared the diagnostic utility of WGS/WES by singleton and trio testing within cohorts.[18,21,22,28,33] In these studies, the odds of diagnosis using trios was double that using singletons (95% CI 1.62–2.56; $I^2 = 12\%$, $P < 0.0001$, Fig. 3).

Studies were performed in three settings: (i) Research studies of novel methods or disease gene discovery; (ii) Clinical testing with hospital-based interpretation, where a deep phenotype was ascertained from the medical record at interpretation, and clinicopathologic correlation was facilitated by communication between clinicians and interpreters; and (iii) Clinical testing and interpretation in reference laboratories, where phenotype information was limited to that provided in test orders, and communication between clinicians and interpreters was not possible. In nineteen studies, comprising 1597 probands, the diagnostic utility of hospital-based genomic sequencing was 0.42 (95% CI 0.38–0.45, $I^2 = 48\%$), and by reference laboratory-based genomic sequencing was 0.29 (95% CI 0.27–0.31, $I^2 = 49\%$, eleven studies, 6140 probands, Fig. 4a). Both hospital and reference laboratory subgroups demonstrated significant heterogeneity. However, heterogeneity was reduced in ten studies published in 2017 ($I^2 = 22\%$, $P = 0.25$, and $I^2 = 26\%$, $P = 0.26$, respectively).[23,25,26,33,35,36,38,40,41,44] In these, the diagnostic utility of hospital genomic sequencing was 0.42 (95% CI 0.38–0.46, $I^2 = 22\%$), which was significantly higher than reference laboratories (0.31, 95% CI 0.27–0.34, $I^2 = 26\%$; $P < 0.0001$, Fig. 4b). Of note, hospital studies had an average of 84 subjects, while reference laboratory studies had an average of 558 subjects, providing a possible explanation for the inverse relationship between-study size and rate of diagnosis (Fig. 1a).

Clinical Utility of WGS, WES, and CMA

To decrease the heterogeneity in definitions of clinical utility between studies, we excluded cases in which the only change in clinical management was genetic counseling or reproductive planning.[55] The proportion of children receiving a change in clinical management by WGS results was 0.27 (95% CI 0.17–0.40, $I^2 = 54\%$, four studies of 136 children), compared with 0.17 (95% CI 0.12–0.24, $I^2 = 76\%$, twelve studies of 992 children) by WES, and 0.06 (95% CI 0.05–0.07, $I^2 = 42\%$, eight studies of 4271 children) by CMA (Fig. 5). Meta-analysis of WGS and CMA groups, for which heterogeneity was not significant ($P = 0.09$ and $P = 0.10$, respectively), demonstrated that the rate of clinical utility of WGS was higher than CMA ($P < 0.0001$).[26,33,35,36,38,46,51–53]

DISCUSSION

Current guidelines state that CMA is the first-line genomic test for children with intellectual disability, developmental delay, autism spectrum disorder, and congenital anomalies.[15,49–54,56–58] Since 2011, WGS and WES have gained relatively broad use for etiologic diagnosis of genetic diseases, but guidelines do not yet exist for their use. A systematic review identified 37 publications in the period January 2011–August 2017, comprising 20,068 affected

Table 1. Characteristics of studies reporting diagnostic or clinical utility of WES or WGS

Citation	Site	Number of proband children	Genetic diseases tested	Proband age (mean or median)	Reference Lab Or Hospital Or Research Test	WES or WGS	Singleton or Trio	Consanguinity[a]	Molecular diagnosis rate	De novo variant diagnosis rate	Rate of clinical utility[b]
Zhu et al.[17]	US	119	Any	9.5 yr	Research	WES	T	8%	24%	45%	n.d.
Lee et al.[18]	US	520	Any	<18 yr	RefLab	WES	Both[c]	6%	26%	50%	n.d.
Yang et al.[19]	US	1745	Any	6 yr	RefLab	WES	S	n.d.	26%	47%	n.d.
Yang et al.[20]	US	218	Any	<18 yr	Reflab	WES	S	n.d.	27%	47%	n.d.
Sawyer et al.[21]	CA	362	Any	all	Research	WES	Both[c]	21%	29%	n.d.	6%
Retterer et al.[22]	US	3040	Any	7 yr	RefLab	WES	Both[c]	n.d.	29%	43%	n.d.
Vissers et al.[23]	NL	150	Neuro	5 yr 7 mo	RefLab	WES	T	5%	29%	70%	n.d.
Taylor et al.[d24]	UK	68	Any	n.d.	Research	WES	Both	17%	29%	n.d.	n.d.
Trujillano et al.[25]	Mixed	820	Any	<15 yr	RefLab	WES	T	45%	30%	24%	n.d.
Petrikin et al.[26]	US	37	Any	<4 mo	H	WGS	T	3%	30%	61%	41%
Valencia et al.[27]	US	40	Any	7 yr	H	WES	T	n.d.	30%	33%	18%
Farwell et al.[28]	US	417	Any	<18 yr	RefLab	WES	Both[c]	n.d.	31%	49%	n.d.
DDD[29,30]	UK	1133	NDD	5.5 yr	Research	WES	T	3%	31%	64%	n.d.
Iglesias et al.[31]	US	91	Any	<18 yr	H	WES	S	11%	32%	41%	18%
Thevenon et al.[32]	FR	43	Neuro	<18 yr	H	WES	S	n.d.	33%	n.d.	9%
Meng et al.[33]	US	178	Any	28 days	H	WES	S	n.d.	33%	n.d.	n.d.
Taylor et al.[d24]	UK	68	Any	n.d.	Research	WGS	Both	17%	34%	18%	n.d.
Stavropoulos et al.[34]	CA	100	Any	5.5 yr	H	WGS	S	8%	34%	57%	n.d.
Bick et al.[35]	US	22	Any	<18 yr	H	WGS	S	n.d.	36%	n.d.	27%
Lionel et al.[36]	CA	70	Any	<18 yr	H	WES	S	9%	37%	n.d.	n.d.
Soden et al.[e37]	US	85	NDD	7 yr	H	WES	T	5%	40%	50%	21%
Farnaes et al.[38]	US	42	Any	<1 yr	H	WGS	T	2%	40%	38%	31%
Srivastava et al.[39]	US	78	NDD	9 yr	H	WES	S	4%	41%	56%	27%
Baldridge et al.[40]	US	155	Any	6 yr	RefLab + H	WES	S	4%	43%	38%	5%
Meng et al.[33]	US	100	Any	28 days	H	WES	T	n.d.	44%	n.d.	19%
Monies et al.[41]	SA	270	Any	<18 yr	H	WES	S	49%	45%	22%	n.d.
Eldomery et al.[f42]	US	63	Any	<18 yr	Research	WES	T	n.d.	48%	n.d.	n.d.
Kuperberg et al.[43]	IS	57	Neuro	<18 yr	H	WES	S	n.d.	49%	61%	9%
Lionel et al.[36]	CA	70	Any	<18 yr	H	WGS	T	9%	50%	n.d.	n.d.
Tan et al.[44]	AU	44	Any	2–18 yr	H	WES	S	n.d.	52%	61%	14%
Charng et al.[45]	SA	31	NDD	all	Research	WES	Both	90%	55%	24%	n.d.
Willig et al.[46]	US	35	Any	26 days	H	WGS	T	3%	57%	65%	37%
Stark et al.[47]	AU	80	Any	8 mo	H	WES	S	21%	58%	35%	23%
Tarailo-Graovac et al.[48]	CA	41	Any	6 yr	Research	WES	T	15%	68%	39%	44%
Sum/Average	**28**	**10392**						**17%**	**31%**	**44%**	**18%**
Range		**22-3040**						**2–90%**	**24–68%**	**18–70%**	**5–44%**

AU Australia, *CA* Canada, *IS* Israel, *NDD* neurodevelopmental disabilities, *Neuro* neurologic, *NL* Holland, *SA* Saudi Arabia, *UK* United Kingdom
The statistics in bold are calculated across all rows.
[a]By history or based on long runs of homozygosity
[b]Other than reproductive plans
[c]Statistical difference between S and T within study
[d]Bespoke methods for de novo variants
[e]Corrected to omit infants reported in ref. [25]
[f]Unsolved by singleton WES

Table 2. Characteristics of studies reporting diagnostic or clinical utility of chromosomal microarray

Citation	Site	Number of proband children	Genetic diseases tested	Proband age (mean or median)	Consanguinity	Diagnostic utility	De novo variant diagnosis rate	Rate of clinical utility
Lionel et al.[36]	CA	44	Any	<18 yr	9%	0%	n.d.	0%
Vissers et al.[23]	NL	150	Neuro	5 yr 7 mo	3%	3%	100%	n.d.
Meng et al.[33]	US	237	Any	28 days	n.d.	5%	n.d.	3%
Willig et al.[46]	US	25	Any	26 days	3%	4%	n.d.	0%
Petrikin et al.[26]	US	48	Any	<4 mo	5%	6%	n.d.	2%
Farnaes et al.[38]	US	18	Any	<1 yr	6%	17%	n.d.	6%
Stavropoulos et al.[34]	CA	100	Neuro	5.5 yr	n.d.	8%	n.d.	n.d.
Ho et al.[49]	US	5487	[a]	7.2 yr	n.d.	9%	n.d.	n.d.
Zilina et al.[50]	ES	1072	Any	Postnatal	8%	11%	22%	n.d.
Tao et al.[51]	HK	327	Any	<18 yr	n.d.	11%	n.d.	9%
Henderson et al.[52]	US	1780	[a]	<18 yr	n.d.	13%	n.d.	6%
Coulter et al.[53]	US	1792	[a]	<18 yr	n.d.	13%	n.d.	6%
Battaglia et al.[54]	IT	349	[a]	<18 yr	n.d.	16%	45%	n.d.
Sum/Average	**13**	**11,429**			**7%**	**11%**	**31%**	**6%**

ES Estonia, IT Italy, HK Hong Kong, CA Canada, NL Holland
The statistics in bold are calculated across all rows
[a]Intellectual disability, developmental disorders, autism spectrum disorder, multiple congenital anomalies

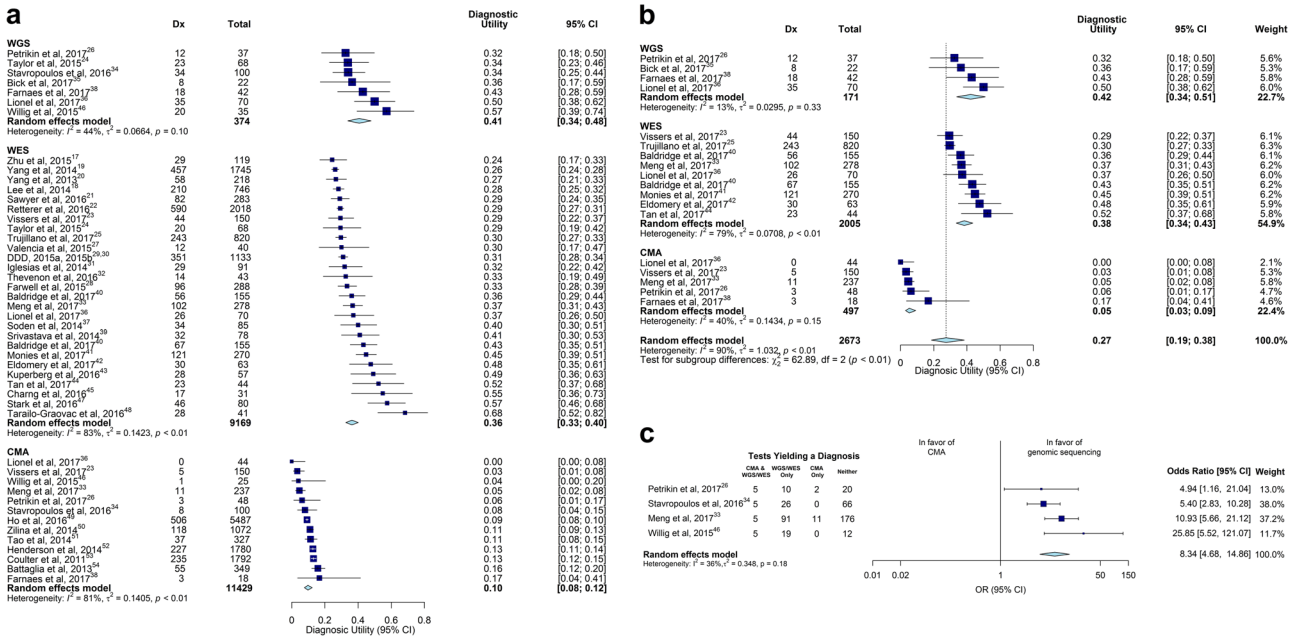

Fig. 1 Comparison of diagnostic (Dx) utility of WGS, WES and CMA. **a** The pooled diagnostic utility of WGS and WES were both greater than of CMA. However, severe heterogeneity precluded quantitative analysis. **b** The subset of studies published in 2017 showed reduced heterogeneity for all subgroups. The pooled diagnostic utility with WGS was significantly higher than with CMA ($P < 0.0001$). **c** Among manuscripts that provided complete data for the frequency of diagnoses made by WES and CMA, the pooled odds of diagnosis was 8.3 times greater for WGS ($P < 0.0001$)

children, which reported the diagnostic utility of WGS, WES, and/or CMA.[17–54] Since only thirteen (35%) of these reported results of a comparator test, pooling made comparisons susceptible to confounding from factors including clinical setting, patient factors, eligibility criteria, study quality, clinical expertise, and testing procedures. Meta-regression showed that the odds of diagnosis by WES or WGS increased by 16% each year, while the odds of diagnosis by CMA decreased by 14% each year between 2013 and 2017. For WES and WGS, which are evolving technologies, this likely was due to methodologic improvements; for CMA, which is a mature technology, this was likely due to broader use with time following allowance of reimbursement. Meta-analysis of studies published in 2017, which removed variance associated with year of publication, showed that the diagnostic utility of WGS (0.42, 95% CI 0.34–0.51, $I^2 = 13\%$) was significantly greater than CMA (0.05, 95% CI 0.03–0.09, $I^2 = 40\%$; $P < 0.0001$).[23,25,26,33,35,36,38,40–42,44] Similarly,

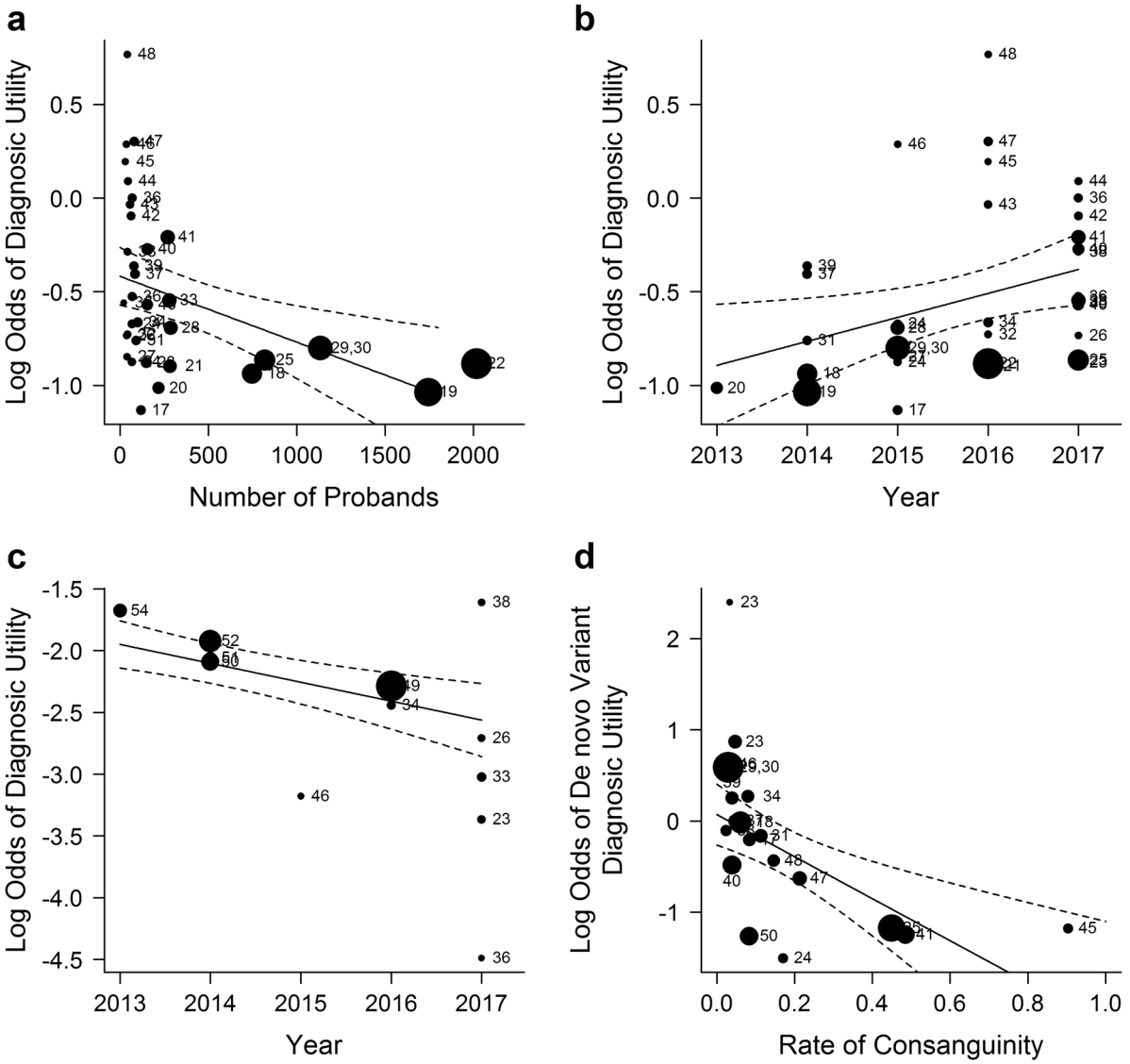

Fig. 2 Exploration of heterogeneity of diagnostic utility in WGS and WES studies. **a** Meta-regression scatterplot for study size. On average, an increase of 1000 subjects decreased the odds of diagnosis by 28% ($P = 0.01$). Size of data point corresponds to the study's inverse-variance weight. **b** Meta-regression scatterplot for diagnostic utility of WGS/WES vs year of study publication. On average, the odds of diagnosis increased by 16% per annum since 2013 ($P = 0.01$). **c** Meta-regression scatterplot for the diagnostic utility of CMA vs year of study publication. The odds of diagnosis decreased by an average of 14% per year between 2013 and 2017 ($P < 0.001$). **d** The rate of diagnosis associated with de novo variation varied inversely with consanguinity. On average, increasing the rate of consanguinity by 10% decreased the odds of de novo variant diagnoses by 21% ($P < 0.001$)

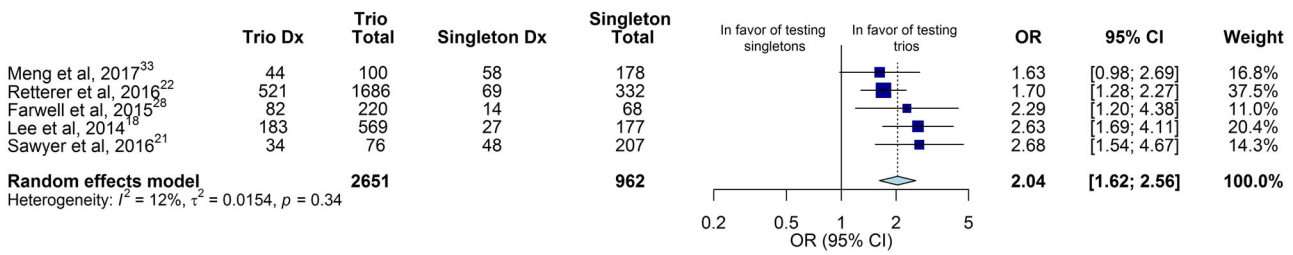

Fig. 3 Comparison of diagnostic (Dx) utility of singleton and trio WGS/WES in studies where both analyses were performed. In five studies that conducted within-cohort comparisons of singleton and trio genomic sequencing, the pooled odds of diagnosis for trios was twice that of singletons ($P < 0.0001$)

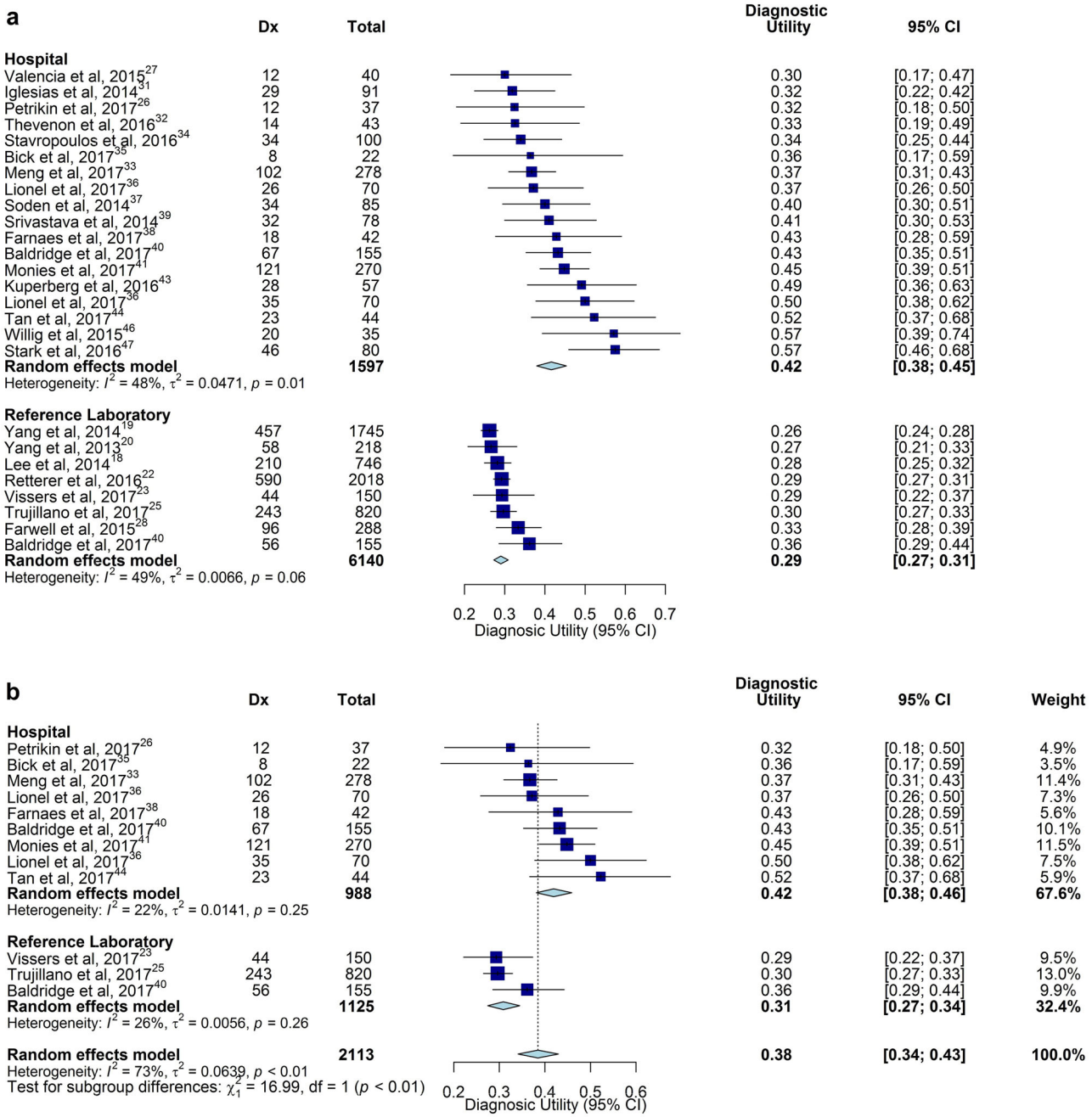

Fig. 4 Comparison of diagnostic (Dx) utility of WGS/WES in hospital laboratories and reference laboratories. **a** The pooled diagnostic utility of hospital-based testing was greater than reference laboratory testing. However, substantial heterogeneity was observed. **b** The subset of studies published in 2017 showed reduced heterogeneity for both subgroups. The pooled diagnostic utility was significantly greater in hospitals than in reference laboratories ($P = 0.004$)

meta-analysis of studies featuring within-cohort comparisons showed that the odds of a diagnosis by WGS or WES was 8.3 times greater than CMA (95% CI, 4.7–14.9, $I^2 = 36\%$; $P < 0.0001$).[26,33,34,46] These results suggest that CMA should no longer be considered the test with highest diagnostic utility for childhood genetic diseases. Rather, WGS or WES should be considered a first-line genomic test for etiologic diagnosis of children with suspected genetic diseases.

While diagnostic utility is an important measure of the value of a clinical test, the relative clinical utility of WGS, WES, and CMA are more relevant for clinicians seeking to improve outcomes of rare childhood genetic diseases through implementation of targeted treatments (precision medicine).[9] Given the genetic and clinical heterogeneity of genetic disease[10] and consequent myriad potential therapeutic interventions, it has been difficult to nominate meaningful, generally applicable measures of clinical utility. A previous approach was to collapse all interventions that were temporally and causally related to a molecular diagnosis into an overall "actionability" rate.[26,36,38,46,51–53,55] Such interventions were either based on practice guidelines endorsed by a professional society or peer-reviewed publications making medical management recommendations. While this has been applied in seven WGS and WES studies to date, definitions of actionability have varied. Furthermore, the evidence base for efficacy of ultra-rare genetic disease treatments is often qualitative rather than quantitative. Nevertheless, after excluding cases in which the only

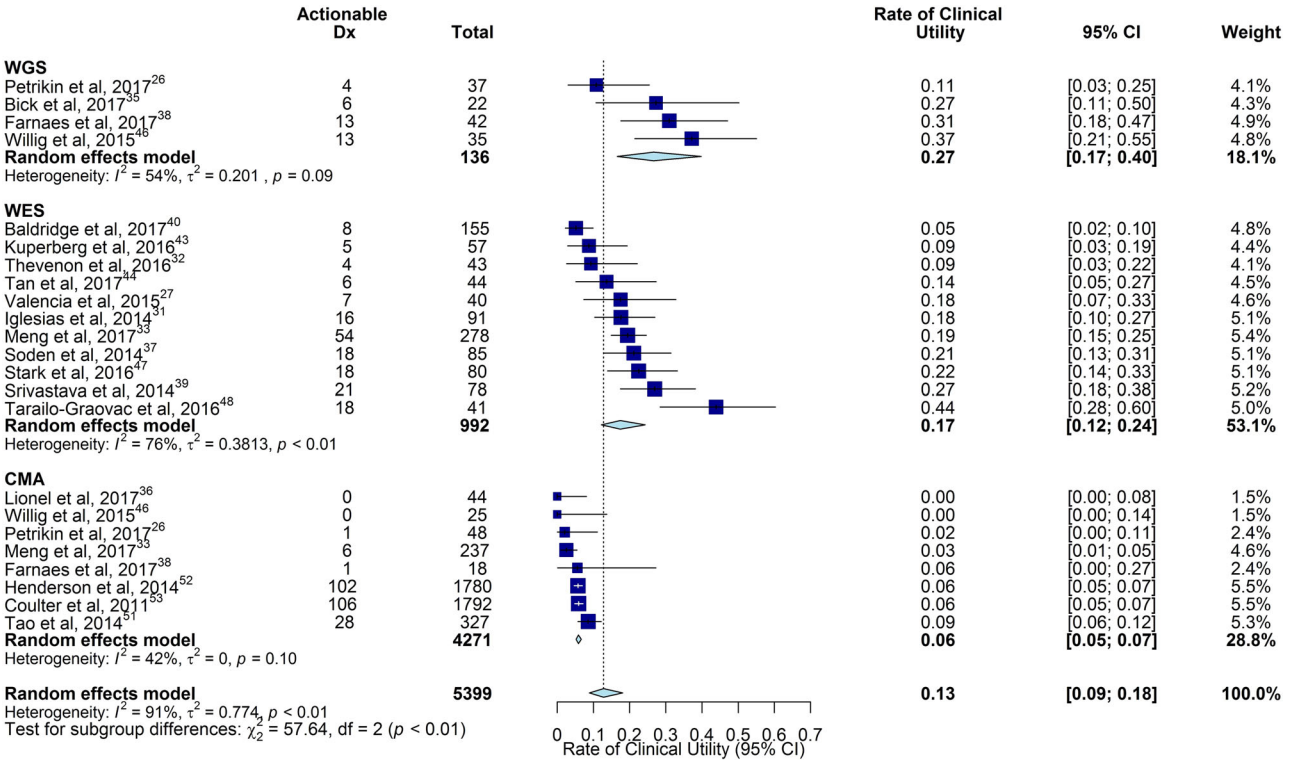

Fig. 5 Comparison of the rate of clinical utility of WGS, WES, and CMA. The rate of clinical utility was the proportion of children tested who received a change in medical or surgical management as a result of genetic disease diagnosis. The pooled rate of clinical utility of WGS and WES were both greater than of CMA. However, there was severe heterogeneity in the WES subgroup. Testing for subgroup differences amongst groups with low to moderate heterogeneity, we found that WGS diagnoses lead to an improved rate of clinical utility over CMA diagnoses

changes were ending the diagnostic odyssey or reproductive planning, WGS and WES had a higher actionability rates than CMA (0.27 [95% CI 0.17–0.40], 0.17 [95% CI 0.12–0.24], and 0.06 [95% CI 0.05–0.07], respectively). This difference was significant for WGS and CMA ($P < 0.0001$), in which within-group heterogeneity was not significant. One caveat was that children tested by CMA in these studies more frequently had multiple congenital anomalies, developmental delay, intellectual disability, or autism spectrum disorders, which were a subset of the presentations of children tested by WGS. Unfortunately, no study has yet reported the relationship between clinical utility of WGS, WES, or CMA and outcomes in children with genetic diseases.

Since WGS is about twice as expensive as WES, which is about twice as expensive as CMA, it is important to identify factors associated with high diagnostic utility. One such factor was the test setting: Hospital laboratory testing had a higher diagnostic utility (0.42, 95% CI 0.38–0.45) than reference laboratory testing (0.29, 95% CI 0.27–0.31). This difference was statistically significant ($P < 0.0001$) among studies published in 2017, in which within-subgroup heterogeneity was not significant. This difference was supported by a study of double interpretation of WES of 115 children, first at a reference laboratory and second at the hospital caring for the children; the diagnostic utility of reference laboratory interpretation was 0.33, and rate of false positive diagnoses was 0.03. The diagnostic utility of hospital interpretation was 0.43, and there were no false positives.[40] The major difference between hospital and reference laboratory interpretation is the quality and quantity of phenotype information available at time of interpretation. In hospital testing, phenotypic features are ascertained from the medical record, include findings by subspecialist consultants, results of other concomitantly ordered tests, negative findings, and, in difficult cases, are supplemented by discussion with clinicians to ascertain material negative findings or clarify conflicting findings. In reference laboratories, the available phenotypic features are those provided in test orders. They tend to be fewer in number and have less information content. One reference laboratory study found an association between the number of phenotypes available at interpretation and diagnostic yield: the diagnostic utility was 0.26 with one to five phenotype terms, 0.33 with six to fifteen terms, and 0.39 with more than fifteen terms.[25] This was observed for all phenotypes, family structures, and inheritance patterns. Additional studies are needed to evaluate the reason for the apparent difference in diagnostic utility of hospital and reference laboratory WES/WGS. In the interim, it is suggested that "send out" WES and WGS tests should be accompanied by as much phenotypic information as possible, and open discussion should be encouraged between the laboratory and referring clinician after the results are available to provide a better diagnosis.

De novo variants accounted for the majority of genetic disease diagnoses, except in studies with high rates of consanguinity. Consanguinity is known to increase the population incidence of homozygous recessive genetic diseases. Herein, consanguinity was associated with decreased likelihood of attribution of diagnosis to de novo variants: Meta-regression of 29 studies found the rate of consanguinity to be inversely related to the odds of diagnoses attributed to de novo variants ($P < 0.001$). Consanguinity is thought to increase the diagnostic utility of WGS and WES: In one study, the diagnostic utility of WES was 0.35 in 453 consanguineous families, and 0.27 in 443 non-consanguineous families.[25] However, meta-analysis failed to show a significant association between the rate of consanguinity and diagnostic utility. Unfortunately, most studies did not report the proportion of probands with a family history of a similar illness, which was also anticipated to increase diagnostic utility.

Testing of parent–child trios is considered superior to singleton (proband) testing for genetic disease diagnosis, since trios facilitate detection of de novo variants and allow phasing of compound heterozygous variants during interpretation (rather than during confirmatory testing). However, trio testing is about twice as costly as singleton testing. Meta-analysis of five studies that compared the diagnostic utility of singleton and trio testing within cohorts showed trio testing to have twice the odds of diagnosis than singleton testing (95% CI 1.62–2.56, $P < 0.0001$).[18,21,22,28,33] This result was supported by a study in which 36% of unsolved singleton WES cases were diagnosed when re-analyzed as trios.[19,20,42] Additional studies are needed to guide clinicians with regard to the choice of initial trio or singleton testing. Factors to be considered include cost, time-to-result, and presence of consanguinity or family history of a similar condition.

Clinical WES has been much more broadly used than clinical WGS, since WGS was very expensive until recently, and remains ~$6000 per proband. WES examines almost all known exons and several hundred intronic nucleotides at ends of exons, or approximately two percent of the genome. WGS examines all exons and 90% of the genome. Only seven studies have reported the diagnostic utility of clinical WGS in 374 children.[24,26,34–36,38,46] Meta-analysis did not show the difference in the diagnostic utility of WGS and WES to be significant. Subsequent to the meta-analysis, one very recent study directly compared the diagnostic utility of clinical WGS and WES in 108 subjects. Three patients (3%) received diagnoses by WGS that were completely unidentified by WES.[59] Additional studies are needed since the diagnostic utility of WGS and WES are increasing disparately as a result of improved identification of disease-causing copy number and structural variations, repeat expansions, and non-exonic regulatory and splicing variations.[34,36,42,57,58,60–64] In one recent study, these increased diagnostic utility by 36%.[42] Recent research has shown WGS to have higher analytic sensitivity for copy number and structural variations than CMA, particularly small structural variations (less than 10,000 nucleotides[34,36,64]), suggesting that WGS may become the single first-line genomic test for etiologic diagnosis of most children suspected to have a genetic disease. However, the published data do not yet support superiority of WGS over WES.

This meta-analysis had several limitations. We used published diagnostic rates at face value; we did not reclassify diagnoses according to the strength of evidence of gene-disease relationships.[65] Comparisons should be interpreted with caution due to heterogeneity of pooled averages of the published data. We were unable to control for heterogeneity in diagnostic utility associated with different types of clinical presentations or "cherry picking" (enrichment for children considered a priori to have high likelihood of a genetic etiology of disease. The highest level of evidence for clinical interventions is meta-analyses of randomized controlled trials (Level I).[66] For WGS and WES, only one such study has yet been published.[26] Published studies constitute Level II evidence (controlled studies or quasi-experimental studies) and Level III evidence (non-experimental descriptive studies, such as comparative studies, correlation studies, and case-control studies). The meta-analysis did not include diagnostic specificity (which has only been directly examined in one manuscript),[40] nor the relative cost-effectiveness of WGS, WES, and CMA, either in terms of the cost of the diagnostic odyssey or long-term impact on healthcare utilization. It excluded next-generation sequencing-based panel tests, which are frequently used for specific presentations, such as epilepsy. It did not include subgroup analysis of the diagnostic utility or clinical utility by affected organ system, which might have identified subgroups of children who are most likely to benefit from testing. While, on average, the CMA studies were one or two years older than the WGS/WES studies, the diagnostic utility of CMA did not increase with time. In several of the WGS/WES studies, patients had previously received negative CMA tests, diminishing the relative diagnostic utility of WGS/WES.

CONCLUSIONS

In meta-analyses of 37 studies of children with suspected genetic diseases, the diagnostic utility of WGS (0.41, 95% CI 0.34–0.48) and WES (0.36, 95% CI 0.33–0.40) were higher greater than CMA (0.10, 95% CI 0.08–0.12), the current first-line genomic test for certain childhood genetic disorders. The same was true for the rate of clinical utility (WGS 0.27, 95% CI 0.17–0.40; WES 0.17, 95% CI 0.12–0.24; CMA 0.06, 95% CI 0.05–0.07). Additional randomized controlled studies are needed, particularly studies that examine the diagnostic determinants of optimal outcomes for children with rare genetic diseases.[67]

METHODS

Data sources and record identification

We searched PubMed from 1 January 2011, to 4 August 2017 with the terms ("exome sequencing" or "whole-genome sequencing" or "chromosomal microarray"), and ("diagnosis" or "clinical"), and "genetic disease" (Figure S1). We manually searched journals not indexed by PubMed that published articles related to clinical genomic testing. There were no language restrictions.

Study screening and eligibility

Studies that evaluated the diagnostic utility (proportion of patients tested who received genetic diagnoses) or clinical utility (proportion of patients tested in whom the diagnosis changed medical or surgical management) of WGS, WES, and/or CMA were eligible. We limited eligibility to studies of cohorts with a broad range of genetic diseases, rather than one or a few disease types or clinical presentations, and in which the majority of probands were less than 18 years old. The systematic review and meta-analysis were performed according to the MOOSE and PRISMA guidelines (Table S1 and Figure S1).

Inclusion criteria and data extraction

Data extraction was manual. Data were reviewed for completeness and accuracy by at least two expert investigators and disparities were reconciled by consensus. The QUADAS-2 tool was used to assess the quality of the included studies (Table S2). The PICOTS typology of the criteria for inclusion of studies in quantitative analyses was:

Patients: Data extraction was limited to affected children (age less than 18 years) with suspected genetic disease.

Intervention: WGS, WES, and/or CMA for etiologic diagnosis of a suspected genetic disease.

Comparator: The groups compared were subjects tested by WGS, WES, and CMA. CMA was treated as the Reference Standard. Subgroups were patients tested with WGS, WES, or CMA as singletons (proband) and trios (parents and child). Trios did not include the use of parental DNA for confirmatory phasing by Sanger sequencing.

Outcomes: Diagnostic utility, rate of clinical utility. Molecular diagnoses were defined as pathogenic or likely pathogenic diplotypes (pairs of haplotypes) affecting genes or genomic variations with definitive, strong, or moderate associations with phenotypes that overlapped at least part of the clinical features of the affected patient, and that were reported to the patient's clinician.[65] Variants of uncertain significance and secondary findings were not extracted. The definition of clinical utility conformed to a position statement of the American College of Medical Genetics and Genomics, but was limited to changes in management for individual patients.[55]

Timing: Where more than one publication reported results from a cohort, we included the most recent value for diagnostic utility. Clinical utility was assessed acutely (typically within six months of enrollment of the last patient).

Settings: Testing was performed clinically in hospital laboratories and reference laboratories, and experimentally in research laboratories. Hospital and reference laboratory clinical tests were defined primarily by the site of testing and, as disclosed in the methods, and, secondarily, by the affiliations of the authors. Clinical testing was defined as testing under fixed protocols that were attested to comply with state or national regulatory guidelines for in vitro diagnostic testing. Experimental research tests were those that explored the utility of novel or bespoke methods of testing or analysis.

Study Design: There were no study design restrictions.

Statistical Analysis

Between-study heterogeneity was explored by univariate analysis. Potential sources of heterogeneity included year of publication, number of probands, genetic disease tested, and consanguinity. The variable for genetic disease tested was treated as having four categories in the publications examined: any genetic disease, genetic diseases that included neurodevelopmental and metabolic disorders, neurodevelopmental disabilities alone, and infants (average proband age less than one year at testing). The effect of disease tested on heterogeneity was explored with a random-effects model as described below. We used meta-regression to study associations of continuous variables (year, study size, and the rate of consanguinity) and heterogeneity.

When comparing rates between studies, raw proportions (i.e., molecular diagnostic and clinical utility rates) for individual studies were logit transformed due to small sample sizes and low event rates.[68] For each comparison, only the relevant subsets of patients reported in each relevant study were retained. Pooled subgroup proportions and their variances were obtained by fitting an inverse-variance weighted logistic-normal random-effects model to the data. 95% confidence intervals (CIs) for individual studies were derived using the Clopper–Pearson exact method.[69] Pooled proportions and CIs were back-transformed for interpretation. For studies which conducted within-cohort comparisons, an inverse-weighted random-effects model was used to estimate pooled odds ratios (ORs). Due to the paired nature of the data, the marginal cross-over OR estimator of Becker and Balagtas[70,71] was used for the meta-analysis of studies that conducted within-cohort comparisons of WES and CMA diagnostic rates. For all analyses, between-study heterogeneity was assessed using between-study variance (τ^2), the I^2 statistic[72] and Cochran's Q test.[73] I^2 values of 25, 50, and 75% indicate mild, moderate, and severe heterogeneity, respectively.[72] Subgroup analyses were conducted to minimize severe heterogeneity between studies. Subgroup differences in rates and ORs were tested when there was not significant evidence of within-group heterogeneity. Forest plots were used to summarize individual study and pooled group meta-analysis statistics. Two-tailed $P \le 0.05$ were considered statistically significant. All statistical analyses were conducted using the 'meta' (version 4.8.1) and 'metafor' (version 2.0.0) packages in R (version 3.3.3).[74–76]

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AUTHOR CONTRIBUTIONS

S.F.K. conceived the study, performed the literature searches, analyzed the data, and wrote the first draft of the manuscript. M.M.C. checked accuracy of the data, performed the statistical analyses, prepared the figures, and helped write and revise the manuscript. L.F. and D.D. helped write and revise the manuscript and assisted in data collection. Z.S. helped conceive the study and helped write and revise the manuscript. T.T. and S.W. helped write and revise the manuscript.

ADDITIONAL INFORMATION

Supplementary information accompanies the paper on the *npj Genomic Medicine* website (https://doi.org/10.1038/s41525-018-0053-8).

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