Knowledge translation in the era of precision diagnostics: Examining the use of clinical exome and genome sequencing for rare genetic disease diagnosis

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Dissertation Preface

Taila Hartley, the named doctoral candidate of this dissertation, was the lead for all aspects of the dissertation including its conceptualization, design, data collection, analysis, and drafting each of the manuscripts presented. Detailed co-author contributions are provided within each of the relevant Chapters.

Positionality Statement

Taila Hartley is a cis gendered, English speaking, able bodied self-identified person of Mixed descent born in Canada in the 1980s. She has been educated in Canada and completed a Bachelor of Science degree in Biochemistry (Queen's University) and two Master of Science degrees in Biochemistry (University of Toronto) and Genetic Counselling (University of British Columbia). She worked as a Certified Genetic Counsellor with Care4Rare Canada at the CHEO Research Institute for five years prior to pursuing her doctoral degree in Population Health. Her knowledge and experience is therefore primarily from Canadian academic institutions and healthcare settings. Her research is based on a

constructivist epistemology, with recognition that her experiences in the world affect her observations and interpretations.

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Dissertation Abstract

In just over a decade, exome sequencing and genome sequencing (ES/GS) has transitioned from a research method to an implemented test for patients with suspected rare genetic diseases (RGDs) worldwide, and healthcare systems are challenged to optimize its use within their jurisdictions. This thesis aimed to examine the rapidly evolving scientific evidence base related to ES/GS and how it has been translated into diagnostic care for families with RGDs to inform practice and policy in the future. Guided by the Knowledge-to-Action (KTA) conceptual framework, I designed and conducted three original studies: two aimed to generate evidence related to the KTA concept of Knowledge Creation, and the third studied the Action Cycle. In Article 1, we examined the knowledge base and determined that evidence related to the etiologies of RGDs and analytical processes related to ES/GS testing are progressing at a pace that has diagnostic implications. Next, in Article 2, we examined knowledge refinement and found that one knowledge user, organizations representing genetics professionals, have produced clinical recommendations related to a broad range of topics connected to ES/GS for RGD diagnosis, but the quality of clinical guidance documents is low, overall, and with specific reference to the rigour of the methods developers used. Finally, in Article 3, we studied the Action Cycle and found that implementing publicly-funded ES/GS using a set of clinical eligibility criteria in the Ontario healthcare system resulted in clinically-valid diagnoses for patients that met provincial benchmarks for diagnostic yield. Together, the results of these studies informed eight considerations for optimizing ES/GS testing with implications for healthcare practitioners, patients, guidance developers, payers, and researchers. Importantly, this thesis provides evidence of the necessity for continued evaluation and improved guidance development related to ES/GS to optimize this testing. It offers a foundation for future studies that may investigate knowledge translation into policy and practice in this and other rapidly evolving fields. Ultimately, these findings will enable better diagnostic care for families with RGDs.

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List of Acronyms and Abbreviations

AGREE	Appraisal of Guidelines for Research and Evaluation
AD	autosomal dominant
ACMG	American College of Medical Genetics and Genomics
AGNC	Association of Genetic Nurses and Counsellors
AR	autosomal recessive
BAM	binary alignment map
CADTH	Canadian Agency for Drugs and Technologies
CCMG	Canadian College of Medical Geneticists
CHEO	Children's Hospital of Eastern Ontario
CIHR	Canadian Institutes of Health Research
CORD	Canadian Organization for Rare Disorders
COVID-19	Coronavirus disease of 2019
CPG	clinical practice guideline
CRAM	compressed reference-oriented alignment map
DD	developmental disability
DNA	deoxyribonucleic acid
ES	exome sequencing
ES/GS	exome sequencing and genome sequencing
ESHG	European Society of Human Genetics
GA4GH	Global Alliance for Genomics and Health
GFH	German Society of Human Genetics
gnomAD	Genome Aggregation Database
GS	genome sequencing
GTAC	Genetic Testing Advisory Committee
GUS	gene of uncertain significance
HPO	human phenotype ontology
iKT	integrated knowledge translation
IQR	interquartile range
KT	knowledge translation
KTA	Knowledge-to-Action
ID	intellectual disability

LP	likely pathogenic
LP/P	likely pathogenic/pathogenic
LSR	living systematic reviews
MCA	Multiple congenital anomalies
MOH	Ministry of Health
MME	Matchmaker Exchange
OECD	Organization for Economic Cooperation and Development
OHIP	Ontario Health Insurance Plan
OMIM	Online Mendelian Inheritance in Man
P	pathogenic
PRESS	Peer Review Electronic Search Strategies
PRISMA-ScR	Preferred Reporting Items for Systematic reviews and Meta-Analyses - Scoping Review
REB	research ethics board
RGD	rare genetic disease
RNA	ribonucleic acid
SAGER	Sex And Gender Equity in Research
SFTP	secure file transfer portal
SIGU	Italian Society of Human Genetics
STROBE	Strengthening The Reporting of OBservational studies in Epidemiology
VCF	variant calling file
VKGL	Dutch Society for Clinical Genetic Laboratory Diagnostics
VKGN	Association of Clinical Genetics Netherlands
VUS	variant of uncertain significance

Please note that gene names are presented throughout this dissertation using Human Genome Organisation (HUGO) Gene Nomenclature (HGNC) approved gene symbols; abbreviations and full gene names are not included here.

Chapter 1: Introduction

1.1. Rare genetic diseases as a population health concern

The field of population health is concerned with improving the health of entire populations by reducing health inequities among specific population groups (Government of Canada, 2013). A range of health indicators can be used to measure the health of populations and may be used to advocate for equity-deserving groups, hold organizations accountable, improve the quality of initiatives for population health, or stimulate research (Etches et al., 2006). One such indicator includes the study of health system interventions for particular populations, ranging from health promotion and disease prevention to treatments and diagnostics. Recently, there has been interest in applying public and population health approaches to individuals with rare genetic diseases (RGDs) (Valdez et al., 2016), a group that consists of thousands of distinct diseases, each affecting fewer than 1 in 2,500 people in the population by definition and caused by innate mutations in a person's DNA (i.e., genetic mutations) (Ferreira, 2019).

While their low prevalence and innate primary cause make RGDs an unlikely candidate for a population health approach, there are compelling reasons to apply a population health lens to RGDs. Despite being individually rare, collectively, RGDs affect at least 1 in 16 individuals, more than 300 million people worldwide (Nguengang Wakap et al., 2020). They have significant familial impacts, with over half of RGDs affecting children (Batshaw et al., 2014). Further, very few RGDs are preventable or curable, frequently resulting in severe health consequences, often including premature death (Ferreira, 2019). There are also several significant and shared challenges for people with RGDs related to their medical care. Healthcare systems are not designed well to identify and coordinate the complex and expensive care of patients with RGDs, resulting in, for example, delayed diagnoses, poor surveillance, poorly defined molecular and physiologic mechanisms, undefined standards of care, scarce longitudinal data collection and research opportunities, slow development of medical treatments, and a scarcity of

screening strategies (Marshall et al., 2019; Valdez et al., 2016). Given these challenges, it is hard to ignore the potential benefit of new interventions to improve the provision of clinical care for RGDs.

1.2. Diagnostic care for RGDs and the introduction of exome sequencing and genome sequencing (ES/GS)

One of the first significant hurdles experienced by families with RGDs is the establishment of the correct etiologic diagnosis. A survey of Canadian families with suspected RGDs in 2015 documented that, on average, families waited 3-5 years, visited more than five different clinical specialties, and often received three or more misdiagnoses on what is commonly referred to as a 'diagnostic odyssey' (Canadian Organization for Rare Disorders, 2015a). While the vast majority of RGDs do not have a therapy approved by the US Food and Drug Administration or European Medicines Agency (Boycott & Ardigo, 2018), an accurate genetic diagnosis can play a vital role in creating an optimal care plan, informed by prognostic and clinical management information, potential lifestyle modifications, and the provision of genetic risk information for family members (Sawyer et al., 2016). A confirmed diagnosis can also have psychological benefit for patients and their families (Kole & Faurisson, 2010).

There are several reasons why RGDs are notoriously difficult to diagnose. First, they are genetically heterogeneous; there are over 6,000 RGDs for which the molecular basis is known (Nguengang Wakap et al., 2020). Second, they are clinically heterogeneous; there can be clinical overlap between different diseases and significant variability within one disease. Third, the clinical presentations in 5-7 percent of individuals with RGDs are the result of multiple RGDs conflating to appear as one (Balci et al., 2017; Posey et al., 2017). Fourth, despite considerable progress in the past decade, we still have not identified all the genetic causes of RGDs; it is estimated that 1000s are yet to be delineated (Bamshad et al., 2019). And finally, until recently, available diagnostic genetic tests have been highly reliant on a physician's understanding of their patient's clinical phenotypes and awareness of the thousands of currently known

RGDs. This is because the establishment of a molecular diagnosis has previously been pursued through serial diagnostic testing of one to a few genes at a time, guided by the physician's differential diagnosis. The outcome of these diagnostic challenges is that more than half of the families with a suspected RGD are ultimately left without a genetic diagnosis following the traditional diagnostic evaluation in medical genetics (Shashi et al., 2014).

This testing paradigm is shifting, however, with the introduction of new DNA sequencing approaches called exome sequencing and genome sequencing (ES/GS). Exome sequencing (ES) is a capture-based method that restricts DNA sequencing to the 2% of the genome that encodes proteins (i.e., the exons hence 'exome') whereas genome sequencing (GS) does not involve a capture step and results in sequencing across the entire genome. While they are different techniques, during both ES and GS, all ~20,000 of a patient's genes are sequenced at once, enabling concurrent assessment for most known RGDs. The first application of ES for RGDs was as a research method to enable the discovery of the genes responsible for a given disease, sometimes referred to as 'gene discovery' (Ng et al., 2010). Prior to the introduction of ES in 2010, the conventional gene discovery methods were laborious and required significant pre-test knowledge, including the genomic location of interest and function of the candidate genes. Many diseases were intractable to such methods and the field plateaued at approximately 150 published genetic discoveries per year (Bamshad et al., 2019). The new computational approaches that came with ES/GS were a disruptive innovation in the field, and quickly replaced previous research methods as the dominant tool for RGD gene discovery. In the last decade, the discoveries made using ES/GS have accounted for 36% of all genetic discoveries ever made for RGDs (Bamshad et al., 2019; Boycott et al., 2017). This pace of knowledge inquiry has not subsided; between 200 and 300 new disease-gene associations continue to be published in the scientific literature each year (Bamshad et al., 2019).

Beyond gene discovery, it did not take long for clinical researchers to recognize that ES/GS technology could also have a significant impact on diagnostic care for RGD patients. As a diagnostic test, ES and GS are quite similar, with the ability to identify many overlapping mechanisms of RGDs. While GS has the potential to identify additional mechanisms of RGDs [i.e., non-coding variants, small insertions-deletions, copy-number variations (CNVs), and chromosomal rearrangements] and hence provide the opportunity for higher diagnostic yields than ES, thus far studies that have compared ES and GS have not identified significant differences in yield (Clark et al., 2018). Given this, we consider the two technologies together throughout this dissertation as an approach to screen all known RGDs, with the expectation that if studies show added utility for GS, and costs of DNA sequencing decrease such that cost-effectiveness studies encourage the use of GS, the field may move towards performing clinical GS rather than clinical ES.

Clinical ES/GS has two unique features that differentiate it from other diagnostic investigations for RGDs. First, when applied to large groups of patients with suspected RGDs, it **results in a significant increase in the proportion of cases that receive a molecular diagnosis** (i.e., increased diagnostic utility) compared to targeted traditional clinical investigations (Stark et al., 2016; Tan et al., 2017). Even when applied to the most difficult-to-diagnose Canadian patients, ES/GS can result in diagnoses for an additional 29% of patients tested (Sawyer et al., 2016). More than 9,000 children with suspected RGDs have been reported in the scientific literature to have had this testing, and the diagnostic yield of ES/GS has been found to be approximately 36% (95% Confidence Interval 33-40%) (Clark et al., 2018). This rate varies depending on several factors, including the clinical features of the patients (e.g., neurodevelopmental delay vs isolated autism), family history (e.g., whether there are others affected with the same disease, or if the patient's parents are related by blood/consanguinity thereby increasing

the chances of an autosomal recessive condition), the sequencing strategy used (e.g., patient sequenced on their own or with their unaffected parents as a trio), and the timing of the testing (e.g., as a first tier test vs at the end of their diagnostic odyssey).

The second unique feature of ES/GS is that its **clinical effectiveness increases the more the test is performed**. As more RGD patients are sequenced, detected DNA variants (whether pathogenic or benign) in genes that are known to cause human disease are deposited into freely accessible, public databases. For example, ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), a public database financially supported by the National Institutes of Health in the United States of America, contains data for over 120,000 unique genetic variants, with clinical significance interpretations provided by over 390 clinical laboratories and research groups (Harrison et al., 2016). In addition, the 200-300 new gene discoveries made by the scientific community each year (Bamshad et al., 2019) are curated by RGD databases like Online Mendelian Inheritance in Man (www.omim.org) and Orphanet (www.orpha.net) and made available for clinical interpretation. These and other contributions to biomedical knowledge, in combination with improvements in bioinformatics approaches, make interpretation of genomic variants easier and, in turn, impact the future diagnostic performance of the test. A patient who receives a non-diagnostic result at first interpretation may therefore benefit from reanalysis of that original test data in the future since the genomic knowledge base on which interpretation rests increases over time. Indeed, studies are now being published that show up to 83% of cases may be diagnosed with reanalyzed ES/GS data that was previously reported as 'non-diagnostic' (Need et al., 2017; Tan et al., 2020). Optimal patient care may therefore be reliant on ensuring relevant biomedical knowledge related to RGDs and new computational approaches are translated efficiently, dynamically, and appropriately into clinical practice.

1.3. Implementation of ES/GS into healthcare systems

Despite the clinical research that has shown a potential improvement in RGD diagnostic outcomes with the use of ES/GS technology, for those who are tasked with implementing this technology in the healthcare system, further research is needed to make decisions regarding clinical use and reimbursement (Clyne et al., 2014; Phillips et al., 2020). For healthcare professionals, some have said there is limited evidence for the generation of Clinical Practice Guidelines (CPGs) regarding how ES/GS technology should be applied as a diagnostic test for RGD patients (Burke et al., 2019). For healthcare policymakers and funders, including in Canada, there is little jurisdiction-specific health system and services research examining the diagnostic utility, clinical utility (e.g., the extent to which an ES/GS-based etiologic diagnosis changes clinical care or patient outcomes), and cost-effectiveness (e.g., value for money) of clinically-performed ES/GS (Weymann et al., 2022). Given this, one might have expected slow clinical implementation of ES/GS, but this does not appear to be the case. Healthcare systems around the world are actively implementing this testing for RGD diagnostics (Phillips et al., 2021; Stark et al., 2019). In a report from the Global Alliance for Genomics and Health (GA4GH) in 2017, tens of thousands of patients around the world with RGDs were noted as receiving ES/GS through their healthcare system (Birney et al., 2017). However, the number of tests was highly variable between jurisdictions. For example, at the time of publication, whereas in the United Kingdom (population 66 million) 20,000 patients with suspected RGD were anticipated to receive clinical ES/GS testing in 2018, only 2,000 patients were expected to be tested in Canada (population 35 million) (Birney et al., 2017). This five-fold difference in access between the United Kingdom and Canada implies that different testing approaches are being developed and implemented.

The variability in the numbers of patients receiving clinical ES/GS worldwide implies that multiple healthcare systems are actively engaged in implementing genomic-based diagnostics for RGD diagnostic

care, but the same body of knowledge is being translated into different clinical approaches. There are a multitude of reasons for which this might be the case, given that these are complex systems in which multiple knowledge users (i.e., individuals who use research results to make informed decisions) and other factors are at play in making decisions. It is likely that one of the elements contributing to these differences in clinical practice is the rapidly changing knowledge base (i.e., the set of information or data that is available to draw on) related to genomic medicine. Further complicating matters, knowledge users have described feeling that they are working in isolation, without the benefit of learning from others (Manolio et al., 2015). Indeed, only a minority of published studies (<1%) in genomics are related to the development of evidence-based recommendations, integration of those recommendations into clinical practice, and investigating the ensuing health outcomes and population impact (Clyne et al., 2014).

1.4. Knowledge translation

All healthcare systems are challenged to improve the quality of care they provide using the best evidence available (Madon et al., 2007). For decades it has been known that healthcare systems fail to use evidence optimally (Balas & Boren, 2000; Davis et al., 2003; Morris et al., 2011), and evidence related to genomics is no exception (Burke & Korngiebel, 2015). In response, the field of Knowledge Translation (KT) has emerged to bridge the gaps between knowledge and practice and is a vital component of the evidence-based practice movement (Straus et al., 2011). The Canadian Institutes of Health Research defines KT (now referred to as knowledge mobilization) as "a dynamic and iterative process that includes the synthesis, dissemination, exchange, and ethically-sound application of knowledge to improve the health of Canadians, provide more effective health services and products, and strengthen the healthcare system" (Canadian Institutes of Health Research, 2016). It is a broad concept that encompasses everything from the creation of new knowledge through to its application to

yield beneficial outcomes to society. KT research aims to understand and close the knowledge-to-practice gaps in healthcare by increasing the usability of high-quality knowledge to ensure evidence can be accessed and understood by relevant knowledge users (Straus et al., 2011). Knowledge users are a particularly important group of individuals who have the capacity to act on research findings and are ultimately the ones who make the decision to adopt best practices (Jull et al., 2019). Within the healthcare system this might include policymakers, healthcare professionals, and healthcare consumers. Knowledge translation can be particularly challenging in areas of healthcare like genomics in which both biomedical research (including gene discovery) and clinical research (such as investigations with new patient groups) outpaces an already speedy translation of the technology into the clinic (Burke & Korngiebel, 2015; Khoury et al., 2012). In this scenario, it is a complex balance to ensure genomic technologies are not underused, overused, or misused based on premature evidence, any of which can lead to unnecessary costs and potential harm.

There are a few methods by which KT researchers might increase the engagement of knowledge users with research and facilitate appropriate use of research findings. Two broad categories by which this is done include end-of-grant KT and integrated KT (iKT) (Canadian Institutes of Health Research, 2016). End-of-grant KT is more commonly employed and consists of researchers developing and implementing a plan for making potential knowledge users aware of the knowledge gained by their project and its potential implications for decision-making. This could be, for example, through dissemination of study findings into particular journals, at targeted conferences, or through policy briefs. It might also include more intensive dissemination activities such as interactive educational sessions with stakeholders and media engagement. By contrast, in iKT, potential knowledge users are engaged throughout the research process so that research teams produce findings that are more likely to be directly relevant to, and used by, knowledge users. This includes shaping the research questions together, deciding on appropriate

methodology together, helping one another with data collection and tool development, interpreting the study findings together, and then moving the research results into practice. In doing so, the intent is to place the knowledge producers and knowledge users on equal footing with the intent to address the specific research needs of the knowledge users (Harrison & Graham, 2021).

1.5. Goal of this thesis

Healthcare systems are challenged to deliver optimal, evidence-based, ES/GS-based diagnostics for patients in the context of a rapidly changing knowledge base related to RGDs and a paucity of jurisdiction-specific evidence. **Therefore, this thesis aims to examine this rapidly evolving knowledge base and how it has been translated into diagnostic care for families with RGDs to inform practice and policy, and ultimately enable improved diagnostic care in the future.**

1.6. Knowledge-to-Action (KTA) Conceptual Framework

There are a number of theories, models, and frameworks related to knowledge translation that can assist in planning, implementation, evaluation and sustainment of change (Nilsen et al., 2015). Here, we were interested in conceptualizing knowledge and the process by which knowledge is created, refined and applied. The studies included in this dissertation were guided by the Knowledge-to-Action (KTA) conceptual framework (**Figure 1.1**), which helps to define the steps from the generation of scientific evidence to clinical application (Graham et al., 2006). This framework was developed through synthesis of over 30 planned action theories. It consists of two distinct but related concepts that relay the key elements of KT: Knowledge Creation and the Action Cycle. Within the framework, Knowledge Creation is depicted as a funnel in which primary studies (i.e., first generation knowledge) are gradually refined for professionals and policymakers via works of knowledge synthesis (i.e., second generation knowledge) and then knowledge tools like Clinical Practice Guidelines (i.e., third generation knowledge). The Action

Cycle encircles the funnel and is a series of phases that depict how knowledge might be applied in practice. This process includes (1) identifying a problem that needs to be addressed, (2) identifying and reviewing relevant knowledge, (3) adapting the knowledge to a specific context, (3) assessing barriers to using the knowledge, (4) choosing and tailoring an intervention to overcome barriers to use the knowledge, (5) monitoring the knowledge use, (6) evaluating the outcomes, and (7) sustaining ongoing knowledge use (Graham et al., 2006). Overall, the phenomenon is complex, dynamic, and iterative; the boundaries between Knowledge Creation, the Action Cycle, and their internal steps, are fluid and permeable.

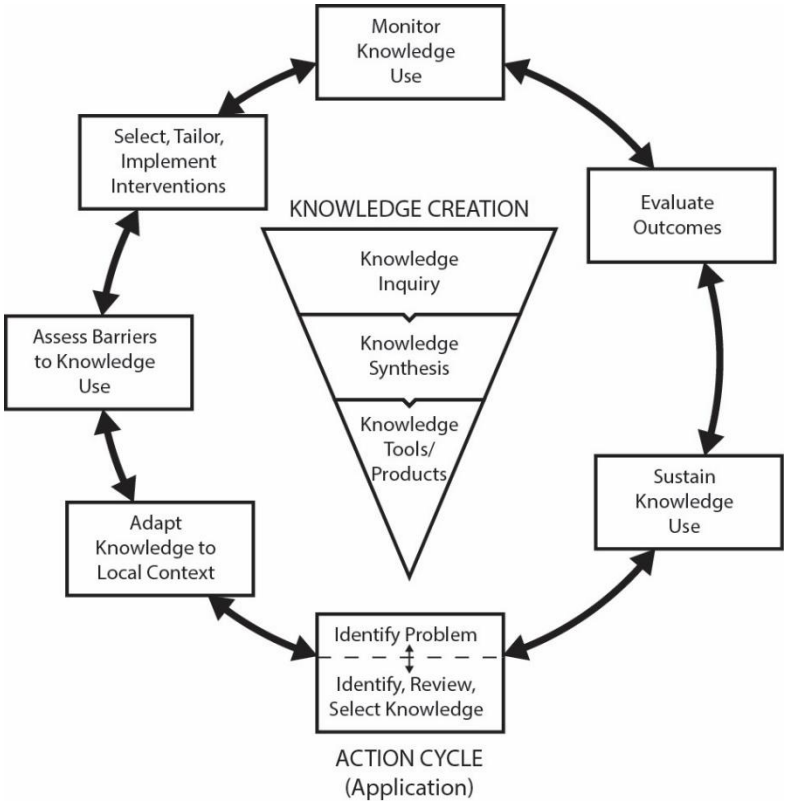

Figure 1.1. The Knowledge-to-Action (KTA) Framework (reproduced with permission from Graham et al., 2006).

1.7. Thesis objectives and study design

Based on the KTA framework, three objectives were delineated for this thesis:

1. To examine how the knowledge base related to ES/GS is changing over time and its diagnostic implications;
2. To examine how knowledge users have refined this ever-evolving knowledge base into clinical guidance; and,
3. To examine whether implementation of ES/GS based on this knowledge base results in diagnoses for families with RGDs.

Each of these objectives aligns to a different aspect of the KTA framework and the theoretical translation of knowledge related to ES/GS into clinical practice (**Figure 1.2**). The first two objectives relate to the Knowledge Creation process and seek to understand the challenges associated with the knowledge base on which implementation of ES/GS is occurring. The third objective relates to the Action Cycle and the real-world diagnostic outcomes within a healthcare system that are occurring based on this knowledge base. Each of these objectives was used to design a unique study that corresponds to an article in this dissertation.

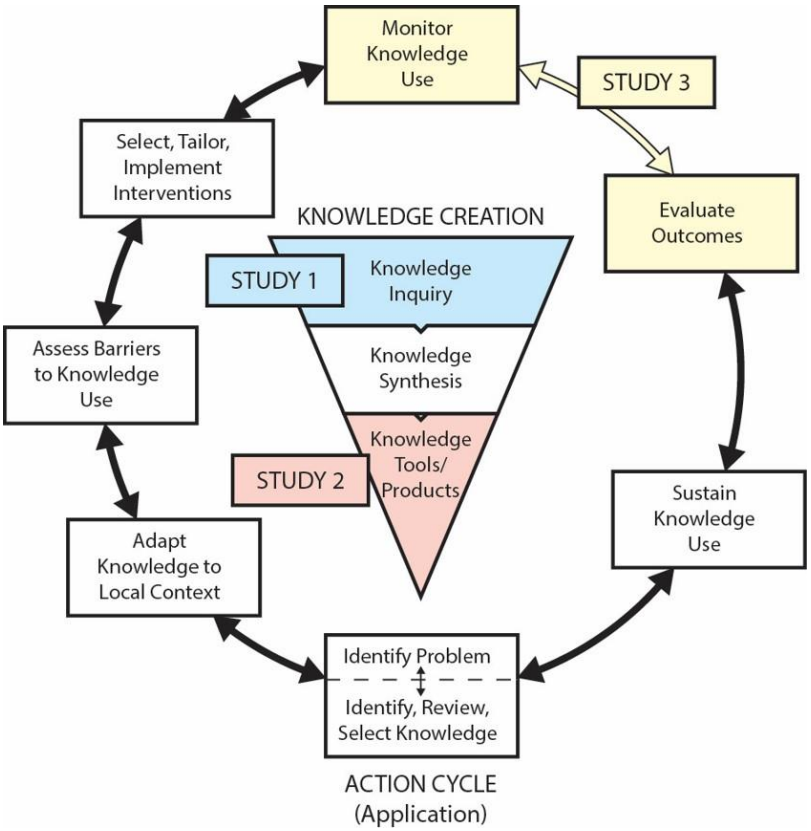

Figure 1.2. Components of the KTA framework that are targeted by the three articles within this dissertation (adapted with permission from Graham et al., 2006).

1.8. Study setting

The province of Ontario, Canada has a publicly funded health insurance plan, called the Ontario Health Insurance Plan (OHIP), for its approximately 15 million residents (defined as those who live in Ontario for a minimum of 153 days each year and are Canadian citizens, residents, or Indigenous persons) (Government of Ontario, 2017, 2020). This program is government-run, by the Ministry of Health (MOH), and funds medically necessary hospital and physician services, including specialist medical consultations (such as from a Clinical Geneticist) and approved laboratory tests, all of which are billed directly to them and free to patients at the point of use (Government of Ontario, 2017).

When clinical laboratories began offering clinical ES in 2011 (Baylor Genetics, 2023) it became accessible to Ontario patients only through "special access" requests for testing outside of the country, which anecdotally took significant physician time to prepare and were laborious to review and approve at the MOH (Ontario Ministry of Health, 2018). In 2014, the MOH created the Genetic Testing Advisory Committee (GTAC), to provide advice on the provision of new and existing genetic tests for the province (Ontario Ministry of Health and Long-Term Care, 2018). In 2016, the GTAC released a report with recommendations for routine publicly-funded ES for Ontarians (Genetic Testing Advisory Committee, 2016). To make these recommendations, GTAC convened a working group with expertise in clinical genetics, laboratory genetics, genetic counselling, and health services research. This working group was tasked with making general recommendations for the implementation of ES/GS in Ontario, creating eligibility criteria for publicly funded testing, and providing implementation guidance for the MOH. Within their recommendations, GTAC strongly encouraged the MOH to implement routine testing in parallel to a robust evaluative process given the paucity of high-quality evidence related to the use of ES/GS. They also stressed the importance of the MOH establishing criteria for both the frequency and funding for reanalysis of non-diagnostic ES/GS data [recommendation 5.6]. At the time that the eligibility criteria were approved by the MOH in 2017, no laboratories within Ontario were performing clinical grade ES. Testing was therefore performed at accredited laboratories outside of Ontario (typically in the United States of America), severely limiting the jurisdiction's ability to evaluate this testing within their patient population. The MOH therefore started engaging with the research group Care4Rare Canada (www.care4rare.ca) in 2017, with the intent of evaluating publicly-funded ES aligned with the roll-out of routine testing in April 2018. Ontario healthcare decision-makers are therefore actively engaged in making decisions related to whether and how ES/GS technologies should be made available for the diagnostic care of RGD patients in their jurisdiction.

Care4Rare Canada is a pan-Canadian, multi-site research consortium devoted to conducting studies related to the use of new technologies that might improve diagnostic care for patients affected with RGDs (Boycott et al., 2022). The consortium was first launched in 2010, shortly after the development of Next-Generation Sequencing technologies and ES/GS approaches (Beaulieu et al., 2014), and continues to engage hundreds of interdisciplinary researchers, members of partnering patient organizations, and front-line clinicians, including most of the certified clinical and laboratory geneticists spread across 21 clinical genetics centers in Canada (Boycott et al., 2022). The impact of the consortium has been primarily in three areas: advancing clinical genomic knowledge related to RGDs; facilitating access to testing by engaging and educating knowledge users (including front-line clinicians and policymakers); and supporting the development of knowledge tools (like clinical guidance documents and reports) with organizations representing genetics healthcare professionals [like the Canadian College of Medical Geneticists (Boycott et al., 2015; Lazier et al., 2022; Zawati et al., 2014)] and patients [like the Canadian Organization for Rare Disorders (Canadian Organization for Rare Disorders, 2015b)] (Boycott et al., 2022).

In 2018, Care4Rare launched the Care4Rare-SOLVE project which had two goals. The first was to facilitate access to testing via integrated KT projects with provincial healthcare systems, including the Ontario MOH. Here, they would leverage their network across Ontario to prospectively enroll and evaluate publicly-funded ES testing performed in clinical laboratories outside of Canada for families with suspected RGD. The second goal was to provide diagnoses to families with RGD across Canada following uninformative ES testing. This was done using a step-wise approach that first reanalyzed the existing ES data from 1,000 families across Canada (many of whom reside in Ontario and received publicly-funded ES performed at clinical laboratories outside of Canada), followed by a discovery protocol that utilizes other technologies not yet available as clinical tests (Boycott et al., 2022). Care4Rare Canada has

therefore been actively engaging with new genomic knowledge related to ES/GS in the reanalysis of ES data, has been engaging with knowledge users in the development of documents related to ES testing, and has a network of front-line clinicians in Ontario that can support the data collection required to evaluate ES/GS testing conducted outside of Canada.

Together, the Ontario policy context and Care4Rare Canada infrastructure provided an opportunity to investigate my dissertation objectives.

1.9. Study design to address dissertation objectives

In my first article (Chapter 2), I targeted Knowledge Inquiry and the knowledge base that is rapidly evolving. Here, I conducted a proof-of-principle study that examined the extent to which evolving knowledge regarding the causes of RGDs [e.g., new knowledge inquiry] impacted RGD diagnosis. I leveraged the opportunity of the existing reanalysis process, within Care4Rare-SOLVE, to collate and analyze the results from the reanalysis of 297 families from Ontario who received publicly-funded clinical ES performed in clinical laboratories outside of Canada. This experience provided valuable evidence regarding the diagnostic implications of both new genomic knowledge and evolving analytical processes. It provided insight for policymakers regarding the utility of ongoing reanalysis of ES/GS data. This study laid the foundation for Article 2 and Article 3 by investigating the implications of a rapidly evolving knowledge base and its potential impact on ES/GS, which in turn generates a need for responsive programs and policies.

In my second article (Chapter 3), I examined how knowledge users make use of this evolving knowledge base in the generation of Knowledge Tools/Products for clinical use. Here, I conducted a scoping review of clinical guidance documents generated by organizations representing genetics healthcare

professionals and examined the range of topics considered by these documents and strengths and weaknesses of the methodological processes used in their development. This study has important implications for future guidance developers and generates knowledge that helps us understand how professional groups are synthesizing and responding to the evidence to support its translation into health policy and patient care.

Finally, in my third article (Chapter 4), I prospectively studied two phases of the Action Cycle in the Ontario healthcare system, by using the Care4Rare research infrastructure to monitor and evaluate the implementation of clinical eligibility criteria and corresponding diagnostic outcomes from publicly-funded ES performed outside of Canada. This study is affiliated with the integrated KT study with the Ontario MOH and sought to monitor how ordering physicians (primarily clinical geneticists) are applying the set of provincial eligibility criteria, report the range of laboratory results for Ontario families with suspected RGD, determine whether those results are providing clinically-valid diagnoses, and speculate on the diagnostic impact of ES/GS based on hypothetical care pathways provided by the ordering physicians. This study specifically addresses questions posed by healthcare decision-makers at the Ontario MOH, needed to better understand the implication of implementing ES/GS policies on patient care in this jurisdiction.

From this collection of articles, we learned about the potential impact of a changing knowledge base on the diagnostic benefit of ES/GS; how professional organizations have mobilized over time to refine knowledge and translate it into recommendations and tools to inform policy and practice; and how such policies and programs are implemented by stakeholders and translated into diagnoses, based on a single jurisdiction as an example. Each of these articles targets one or more phases of the KTA framework, and

so together they paint a picture of knowledge translation practices, challenges, and future opportunities related to ES/GS for RGD diagnostics.

1.10. References

Balas, E. A., & Boren, S. A. (2000). Managing clinical knowledge for health care improvement. *Yearb Med Inform*, (1), 65-70.

Balci, T. B., Hartley, T., Xi, Y., Dyment, D. A., Beaulieu, C. L., Bernier, F. P., Dupuis, L., Horvath, G. A., Mendoza-Londono, R., Prasad, C., Richer, J., Yang, X. R., Armour, C. M., Bareke, E., Fernandez, B. A., McMillan, H. J., Lamont, R. E., Majewski, J., Parboosingh, J. S., . . . Boycott, K. M. (2017). Debunking Occam's razor: Diagnosing multiple genetic diseases in families by whole-exome sequencing. *Clin Genet*, *92*(3), 281-289. https://doi.org/10.1111/cge.12987

Bamshad, M. J., Nickerson, D. A., & Chong, J. X. (2019). Mendelian gene discovery: Fast and furious with no end in sight. *Am J Hum Genet*, *105*(3), 448-455. https://doi.org/10.1016/j.ajhg.2019.07.011

Batshaw, M. L., Groft, S. C., & Krischer, J. P. (2014). Research into rare diseases of childhood. *JAMA*, *311*(17), 1729-1730. https://doi.org/10.1001/jama.2013.285873

Baylor Genetics. (2023). *Our History*. Retrieved January 26, 2023 from https://www.baylorgenetics.com/history/

Beaulieu, C. L., Majewski, J., Schwartzentruber, J., Samuels, M. E., Fernandez, B. A., Bernier, F. P., Brudno, M., Knoppers, B., Marcadier, J., Dyment, D., Adam, S., Bulman, D. E., Jones, S. J., Avard, D., Nguyen, M. T., Rousseau, F., Marshall, C., Wintle, R. F., Shen, Y., . . . Boycott, K. M. (2014). FORGE Canada Consortium: Outcomes of a 2-year national rare-disease gene-discovery project. *Am J Hum Genet*, *94*(6), 809-817. https://doi.org/10.1016/j.ajhg.2014.05.003

Birney, E., Vamathevan, J., & Goodhand, P. (2017). Genomics in healthcare: GA4GH looks to 2022. *bioRxiv*. https://doi.org/10.1101/203554

Boycott, K., Hartley, T., Adam, S., Bernier, F., Chong, K., Fernandez, B. A., Friedman, J. M., Geraghty, M. T., Hume, S., Knoppers, B. M., Laberge, A. M., Majewski, J., Mendoza-Londono, R., Meyn, M. S., Michaud, J. L., Nelson, T. N., Richer, J., Sadikovic, B., Skidmore, D. L., . . . Canadian College of

Medical Geneticists. (2015). The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet*, *52*(7), 431-437. https://doi.org/10.1136/jmedgenet-2015-103144

Boycott, K. M., & Ardigo, D. (2018). Addressing challenges in the diagnosis and treatment of rare genetic diseases. *Nat Rev Drug Discov*, *17*(3), 151-152. https://doi.org/10.1038/nrd.2017.246

Boycott, K. M., Hartley, T., Kernohan, K. D., Dyment, D. A., Howley, H., Innes, A. M., Bernier, F. P., Brudno, M., & Care4Rare Canada, C. (2022). Care4Rare Canada: Outcomes from a decade of network science for rare disease gene discovery. *Am J Hum Genet*, *109*(11), 1947-1959. https://doi.org/10.1016/j.ajhg.2022.10.002

Boycott, K. M., Rath, A., Chong, J. X., Hartley, T., Alkuraya, F. S., Baynam, G., Brookes, A. J., Brudno, M., Carracedo, A., den Dunnen, J. T., Dyke, S. O. M., Estivill, X., Goldblatt, J., Gonthier, C., Groft, S. C., Gut, I., Hamosh, A., Hieter, P., Hohn, S., . . . Lochmuller, H. (2017). International cooperation to enable the diagnosis of all rare genetic diseases. *Am J Hum Genet*, *100*(5), 695-705. https://doi.org/10.1016/j.ajhg.2017.04.003

Burke, W., Clayton, E. W., Wolf, S. M., Berry, S. A., Evans, B. J., Evans, J. P., Hall, R., Korngiebel, D., Laberge, A. M., LeRoy, B. S., & McGuire, A. L. (2019). Improving recommendations for genomic medicine: Building an evolutionary process from clinical practice advisory documents to guidelines. *Genet Med*, *21*(11), 2431-2438. https://doi.org/10.1038/s41436-019-0549-3

Burke, W., & Korngiebel, D. M. (2015). Closing the gap between knowledge and clinical application: challenges for genomic translation. *PLoS Genet*, *11*(2), e1004978. https://doi.org/10.1371/journal.pgen.1004978

Canadian Institutes of Health Research. (2016). *Knowledge Mobilization - About us*. Retrieved January 26 from https://cihr-irsc.gc.ca/e/29418.html

Canadian Organization for Rare Disorders. (2015a). *CORD Rare Disease Patient Survey*. Retrieved January 24, 2023, from http://www.raredisorders.ca/content/uploads/CORD-Patient-Survey-Summary.pdf

Canadian Organization for Rare Disorders. (2015b). *Now is the Time: A Strategy for Rare Diseases is a Strategy for all Canadians - Canada's Rare Disease Strategy*. Retrieved January 24, 2023, from https://www.raredisorders.ca/canadas-rare-disease-strategy/

Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M., Dimmock, D., & Kingsmore, S. F. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*, *3*, 16. https://doi.org/10.1038/s41525-018-0053-8

Clyne, M., Schully, S. D., Dotson, W. D., Douglas, M. P., Gwinn, M., Kolor, K., Wulf, A., Bowen, M. S., & Khoury, M. J. (2014). Horizon scanning for translational genomic research beyond bench to bedside. *Genet Med*, *16*(7), 535-538. https://doi.org/10.1038/gim.2013.184

Davis, D., Evans, M., Jadad, A., Perrier, L., Rath, D., Ryan, D., Sibbald, G., Straus, S., Rappolt, S., Wowk, M., & Zwarenstein, M. (2003). The case for knowledge translation: Shortening the journey from evidence to effect. *BMJ*, *327*(7405), 33-35. https://doi.org/10.1136/bmj.327.7405.33

Etches, V., Frank, J., Di Ruggiero, E., Manuel, D. (2006) Measuring population health: A review of indicators. *Annu Rev Public Health*. *27*, 29-55. https://doi.org/ 10.1146/ annurev.publhealth.27.021405.102141

Ferreira, C. R. (2019). The burden of rare diseases. *Am J Med Genet A*, *179*(6), 885-892. https://doi.org/10.1002/ajmg.a.61124

Genetic Testing Advisory Committee. (2016). *Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario*. Retrieved January 24, 2023, from http://www.health.gov.on.ca/en/pro/programs/gtac/reports.aspx

Government of Canada. (2013). *What is the Population Health Approach?* Retrieved July 23, 2022, from https://www.canada.ca/en/public-health/services/health-promotion/population-health/population-health-approach.html

Government of Ontario. (2017, September 22, 2022). *Health cards, eligibility, and coverage*. Retrieved January 27 from https://www.ontario.ca/page/apply-ohip-and-get-health-card

Government of Ontario. (2020, December 15, 2022). *Ontario Demographic Quarterly: Highlights of first quarter*. Retrieved January 27 from https://www.ontario.ca/page/ontario-demographic-quarterly-highlights-first-quarter

Graham, I. D., Logan, J., Harrison, M. B., Straus, S. E., Tetroe, J., Caswell, W., & Robinson, N. (2006). Lost in knowledge translation: time for a map? *J Contin Educ Health Prof*, *26*(1), 13-24. https://doi.org/10.1002/chp.47

Harrison, M. B., & Graham, I. D. (2021). *Knowledge Translation in Nursing and Healthcare: A Roadmap to Evidence-informed Practice* (Vol. 1). John Wiley & Sons, Inc. https://doi.org/10.1002/9781119123316

Harrison, S. M., Riggs, E. R., Maglott, D. R., Lee, J. M., Azzariti, D. R., Niehaus, A., Ramos, E. M., Martin, C. L., Landrum, M. J., & Rehm, H. L. (2016). Using ClinVar as a Resource to Support Variant Interpretation. *Curr Protoc Hum Genet*, *89*, 8 16 11-18 16 23. https://doi.org/10.1002/0471142905.hg0816s89

Jull, J. E., Davidson, L., Dungan, R., Nguyen, T., Woodward, K. P., & Graham, I. D. (2019). A review and synthesis of frameworks for engagement in health research to identify concepts of knowledge user engagement. *BMC Med Res Methodol*, *19*(1), 211. https://doi.org/10.1186/s12874-019-0838-1

Khoury, M. J., Gwinn, M., Dotson, W. D., & Schully, S. D. (2012). Knowledge integration at the center of genomic medicine. *Genet Med*, *14*(7), 643-647. https://doi.org/10.1038/gim.2012.43

Kole, A., & Faurisson, F. (2010). Rare diseases social epidemiology: Analysis of inequalities. *Adv Exp Med Biol*, *686*, 223-250. https://doi.org/10.1007/978-90-481-9485-8_14

Lazier, J., Hartley, T., Brock, J. A., Caluseriu, O., Chitayat, D., Laberge, A. M., Langlois, S., Lauzon, J., Nelson, T. N., Parboosingh, J., Stavropoulos, D. J., Boycott, K., Armour, C. M., & Canadian College of Medical Geneticists. (2022). Clinical application of fetal genome-wide sequencing during pregnancy: position statement of the Canadian College of Medical Geneticists. *J Med Genet*, *59*(10), 931-937. https://doi.org/10.1136/jmedgenet-2021-107897

Madon, T., Hofman, K. J., Kupfer, L., & Glass, R. I. (2007). Public health. Implementation science. *Science*, *318*(5857), 1728-1729. https://doi.org/10.1126/science.1150009

Manolio, T. A., Abramowicz, M., Al-Mulla, F., Anderson, W., Balling, R., Berger, A. C., Bleyl, S., Chakravarti, A., Chantratita, W., Chisholm, R. L., Dissanayake, V. H., Dunn, M., Dzau, V. J., Han, B. G., Hubbard, T., Kolbe, A., Korf, B., Kubo, M., Lasko, P., . . . Ginsburg, G. S. (2015). Global implementation of genomic medicine: We are not alone. *Sci Transl Med*, *7*(290), 290ps213. https://doi.org/10.1126/scitranslmed.aab0194

Marshall, D. A., Benchimol, E. I., MacKenzie, A., Duque, D. R., MacDonald, K. V., Hartley, T., Howley, H., Hamilton, A., Gillespie, M., Malam, F., & Boycott, K. M. (2019). Direct health-care costs for children diagnosed with genetic diseases are significantly higher than for children with other chronic diseases. *Genet Med*, *21*(5), 1049-1057. https://doi.org/10.1038/s41436-018-0289-9

Morris, Z. S., Wooding, S., & Grant, J. (2011). The answer is 17 years, what is the question: Understanding time lags in translational research. *J R Soc Med*, *104*(12), 510-520. https://doi.org/10.1258/jrsm.2011.110180

Need, A. C., Shashi, V., Schoch, K., Petrovski, S., & Goldstein, D. B. (2017). The importance of dynamic re-analysis in diagnostic whole exome sequencing. *J Med Genet*, *54*(3), 155-156. https://doi.org/10.1136/jmedgenet-2016-104306

Ng, S. B., Buckingham, K. J., Lee, C., Bigham, A. W., Tabor, H. K., Dent, K. M., Huff, C. D., Shannon, P. T., Jabs, E. W., Nickerson, D. A., Shendure, J., & Bamshad, M. J. (2010). Exome sequencing identifies the cause of a Mendelian disorder. *Nat Genet*, *42*(1), 30-35. https://doi.org/10.1038/ng.499

Nguengang Wakap, S., Lambert, D. M., Olry, A., Rodwell, C., Gueydan, C., Lanneau, V., Murphy, D., Le Cam, Y., & Rath, A. (2020). Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. *Eur J Hum Genet*, *28*(2), 165-173.

Nilsen, P. (2015) Making sense of implementation theories, models, and frameworks. *Implementation Science*, *10*:53. https://doi.org/10.1186/s13012-015-0242-0

Ontario Ministry of Health. (2018). *OHIP Out of Country Prior Approval Program*. Retrieved November 6, 2022, from https://www.health.gov.on.ca/en/public/programs/ohip/outofcountry/prior_approval.aspx#1

Ontario Ministry of Health and Long-Term Care. (2018). *Genetic Testing Advisory Committee*. Retrieved January 27 from https://www.health.gov.on.ca/en/pro/programs/gtac/

Phillips, K. A., Douglas, M. P., & Marshall, D. A. (2020). Expanding use of clinical genome sequencing and the need for more data on implementation. *JAMA*, *324*(20), 2029-2030. https://doi.org/10.1001/jama.2020.19933

Phillips, K. A., Douglas, M. P., Wordsworth, S., Buchanan, J., & Marshall, D. A. (2021). Availability and funding of clinical genomic sequencing globally. *BMJ Glob Health*, *6*(2). https://doi.org/10.1136/bmjgh-2020-004415

Posey, J. E., Harel, T., Liu, P., Rosenfeld, J. A., James, R. A., Coban Akdemir, Z. H., Walkiewicz, M., Bi, W., Xiao, R., Ding, Y., Xia, F., Beaudet, A. L., Muzny, D. M., Gibbs, R. A., Boerwinkle, E., Eng, C. M., Sutton, V. R., Shaw, C. A., Plon, S. E., . . . Lupski, J. R. (2017). Resolution of disease phenotypes resulting from multilocus genomic variation. *N Engl J Med*, *376*(1), 21-31. https://doi.org/10.1056/NEJMoa1516767

Sawyer, S. L., Hartley, T., Dyment, D. A., Beaulieu, C. L., Schwartzentruber, J., Smith, A., Bedford, H. M., Bernard, G., Bernier, F. P., Brais, B., Bulman, D. E., Warman Chardon, J., Chitayat, D., Deladoey, J., Fernandez, B. A., Frosk, P., Geraghty, M. T., Gerull, B., Gibson, W., . . . Boycott, K. M. (2016). Utility of whole-exome sequencing for those near the end of the diagnostic odyssey: Time to address gaps in care. *Clin Genet*, *89*(3), 275-284. https://doi.org/10.1111/cge.12654

Shashi, V., McConkie-Rosell, A., Rosell, B., Schoch, K., Vellore, K., McDonald, M., Jiang, Y. H., Xie, P., Need, A., & Goldstein, D. B. (2014). The utility of the traditional medical genetics diagnostic evaluation in the context of next-generation sequencing for undiagnosed genetic disorders. *Genet Med*, *16*(2), 176-182. https://doi.org/10.1038/gim.2013.99

Stark, Z., Dolman, L., Manolio, T. A., Ozenberger, B., Hill, S. L., Caulfied, M. J., Levy, Y., Glazer, D., Wilson, J., Lawler, M., Boughtwood, T., Braithwaite, J., Goodhand, P., Birney, E., & North, K. N. (2019). Integrating genomics into healthcare: A global responsibility. *Am J Hum Genet*, *104*(1), 13-20. https://doi.org/10.1016/j.ajhg.2018.11.014

Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., McGillivray, G., Downie, L., Ekert, P. G., Theda, C., James, P. A., Yaplito-Lee, J., Ryan, M. M., . . . White, S. M. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet Med*, *18*(11), 1090-1096. https://doi.org/10.1038/gim.2016.1

Straus, S. E., Tetroe, J. M., & Graham, I. D. (2011). Knowledge translation is the use of knowledge in health care decision making. *J Clin Epidemiol*, *64*(1), 6-10. https://doi.org/10.1016/j.jclinepi.2009.08.016

Tan, N. B., Stapleton, R., Stark, Z., Delatycki, M. B., Yeung, A., Hunter, M. F., Amor, D. J., Brown, N. J., Stutterd, C. A., McGillivray, G., Yap, P., Regan, M., Chong, B., Fanjul-Fernandez, M., Marum, J., Phelan, D., Pais, L. S., White, S. M., Lunke, S., & Tan, T. Y. (2020). Evaluating systematic reanalysis

of clinical genomic data in rare disease from single center experience and literature review. *Molec Genet Genomic Med*, *8*, e1508 https://doi.org/10.1002/mgg3.1508

Tan, T. Y., Dillon, O. J., Stark, Z., Schofield, D., Alam, K., Shrestha, R., Chong, B., Phelan, D., Brett, G. R., Creed, E., Jarmolowicz, A., Yap, P., Walsh, M., Downie, L., Amor, D. J., Savarirayan, R., McGillivray, G., Yeung, A., Peters, H., . . . White, S. M. (2017). Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. *JAMA Pediatr*, *171*(9), 855-862. https://doi.org/10.1001/jamapediatrics.2017.1755

Valdez, R., Ouyang, L., & Bolen, J. (2016). Public health and rare diseases: Oxymoron no more. *Prev Chronic Dis*, *13*, E05. https://doi.org/10.5888/pcd13.150491

Weymann, D., Dragojlovic, N., Pollard, S., & Regier, D. A. (2022). Allocating healthcare resources to genomic testing in Canada: Latest evidence and current challenges. *J Community Genet*, *13*(5), 467-476. https://doi.org/10.1007/s12687-019-00428-5

Zawati, M. H., Parry, D., Thorogood, A., Nguyen, M. T., Boycott, K. M., Rosenblatt, D., & Knoppers, B. M. (2014). Reporting results from whole-genome and whole-exome sequencing in clinical practice: a proposal for Canada? *J Med Genet*, *51*(1), 68-70. https://doi.org/10.1136/jmedgenet-2013-101934

Chapter 2: Examining the impact of a rapidly evolving knowledge base

2.1. Preface

Since the introduction of exome sequencing and genome sequencing (ES/GS) as a research method for the investigation of unsolved rare genetic diseases (RGDs) in 2010 (Ng et al., 2010), there have been thousands of gene discoveries in the scientific literature, and it is expected that this pace of discovery will continue for years to come (Bamshad et al., 2019). This progress in scientific evidence related to the genetic etiologies of RGDs creates both opportunities and challenges.

The opportunity from a rapidly evolving knowledge base includes the potential to provide more genetic diagnoses for families with RGDs. Clinical research has shown that ES/GS provides an informative test result (e.g., one or more molecular diagnoses) to approximately 36% of patients with a suspected RGD at the initial analysis (Clark et al., 2018). This leaves over 60% of patients, and their care teams, without a diagnosis. While some of these families may have etiology representing genetic mechanisms that are outside of the technological specifications of ES/GS, evidence is now accumulating that for others, the mutation is within the original test data but was not recognized at the time of analysis. Broadly, there are two reasons why these diagnoses are missed: either the bioinformatics pipeline failed to present the pathogenic variant(s) to the analyzer (Eldomery et al., 2017; Shamseldin et al., 2017); or the clinical information or scientific knowledge available to assess the DNA variant was insufficient to interpret it as pathogenic at the time of analysis (Basel-Salmon et al., 2018; Shashi et al., 2019). Recently, studies have shown that the systematic reanalysis of previously non-diagnostic ES/GS test data can result in diagnoses for up to 83% of patients (Need et al., 2017; Tan et al., 2020). Therefore, the evolving knowledge base can potentially inform new diagnoses in families with non-diagnostic test data.

Thus far, studies that have examined reanalysis have typically included small cohorts of less than 50 patients and have reported little information regarding timing since the initial analysis. Therefore, a challenge for decision-makers is determining whether genomic knowledge and computational approaches are progressing at a pace that warrants implementing reanalysis into clinical practice. If it is, this challenge includes determining what aspects of the initial ES/GS analysis should be re-done or done differently and the frequency at which genomic data should be re-examined. It is a policy challenge highlighted to the Ontario Ministry of Health by the Genetic Testing Advisory Committee (GTAC) Report (Genetic Testing Advisory Committee, 2016).

Here, I investigated Objective 1 of my dissertation "To examine how the knowledge base related to ES/GS is changing over time." I retrospectively examined the utility of reanalysis in identifying diagnoses for Ontario families that received publicly-funded ES/GS sequencing performed at clinical laboratories outside of Canada and received a research reanalysis with the Care4Rare-SOLVE project. Specifically, my sub-objectives for this Article were:

I. To determine if the reanalysis of non-diagnostic clinical exome sequencing data, using Care4Rare's infrastructure, resulted in additional diagnoses;
II. To examine how improvement in genomic knowledge, in comparison to other aspects of the Care4Rare infrastructure (a different bioinformatics pipeline, the incorporation of the clinical care team in analysis) has contributed to the diagnostic yield that results from reanalysis; and,
III. To examine whether the amount of time since initial analysis influences the proportion of diagnoses that can be made from reanalysis, and hence identify any implications for knowledge user decisions about the frequency of incorporation of new genomic knowledge into clinical practice.

2.2. Statement of permission for use of copyrighted material

The following chapter consists of the manuscript entitled “Bridging clinical care and research to maximize diagnoses and discoveries from reanalysis of clinical exome sequencing data: Results from a multi-site study in Ontario, Canada”. This manuscript has been published in *Clinical Genetics*.

2.3. Contribution

Taila Hartley led the study design, developed the protocol, led the submission to the research ethics board, contributed to the analyses of the exome sequencing data from the 287 families, attended the majority of meetings between study teams and clinical teams, designed the data collection form, performed the majority of data extractions from medical charts and research charts, conducted all data analysis and interpretation of findings, and drafted the manuscript. The specific contributions of all co-authors are available in the section Author Contributions of the Main Manuscript (Section 2.4).

2.4. Main manuscript

Bridging clinical care and research in Ontario, Canada: Maximizing diagnoses from reanalysis of clinical exome sequencing data

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ABSTRACT

We examined the utility of clinical and research processes in the reanalysis of publicly-funded clinical exome sequencing data in Ontario, Canada. In partnership with 8 sites, we recruited 287 families with suspected rare genetic diseases tested between 2014 and 2020. Data from 7 laboratories were reanalyzed with the referring clinicians. Reanalysis of clinically relevant genes identified diagnoses in 4% (13/287); 4 were missed by clinical testing. Translational research methods, including analysis of novel candidate genes, identified candidates in 21% (61/287). Of these, 24 families have additional evidence through data sharing to support likely diagnoses (8% of cohort). This study indicates few diagnoses are missed by clinical laboratories, the incremental gain from reanalysis of clinically-relevant genes is modest, and the highest yield comes from validation of novel disease-gene associations. Future implementation of translational research methods, including continued reporting of compelling genes of uncertain significance by clinical laboratories, should be considered to maximize diagnoses.

KEYWORDS: Reanalysis, exome sequencing, matchmaking, rare disease, healthcare system

INTRODUCTION

The introduction of exome sequencing (ES) to investigate the molecular etiologies of rare genetic diseases (RGD) has been transformative for the field of Medical Genetics. In parallel, ES is being implemented into healthcare systems as an efficient and broad diagnostic test for patients with RGDs (Birney et al., 2017; Clark et al., 2018) while also rapidly advancing genomic knowledge as a significant contributor to the more than 250 new disease-gene associations (Bamshad et al., 2019) and over 9,200 variant-disease associations (Wenger et al., 2017) reported in the scientific literature each year. This creates a unique challenge for those implementing this testing, who are trying to maximize the diagnostic potential of a technology that is reliant on a rapidly advancing genomic knowledge base. This challenge is particularly relevant for the approximately two thirds of patients with suspected RGDs who receive non-diagnostic results from ES (Clark et al., 2018). While some families may have a disease etiology representing a genetic mechanism that is outside of the technological specifications of ES, for others, their etiology is within the original sequencing data but is not recognized as such. This could be due to technical limitations or insufficient available evidence to interpret the variant(s) as diagnostic. For these families there is an opportunity in the reanalysis of existing clinical ES data.

Reflecting this opportunity, recent studies have shown that reanalysis of previously non-diagnostic ES data can yield additional diagnoses (Robertson et al., 2022; Tan et al., 2020). A recent literature review examined 27 studies that reanalyzed both ES and genome sequencing (GS) data and found the range in proportion of families that receive a diagnosis was extremely variable (from 0-83%) (Need et al., 2017; Tan et al., 2020). Factors that appear to influence the proportion of patients that receive a diagnosis include the patient population examined, time elapsed since the initial analysis, the bioinformatic tools employed, the inclusion of the referring clinician in the reanalysis, and whether additional research methods that could have diagnostic implications (translational research methods) are employed, like the

analysis of novel candidate genes, data sharing, and laboratory studies (Robertson et al., 2022). What aspects of the original analysis process are re-done or done differently may also have an effect. We and others have therefore proposed that patients with non-diagnostic clinical ES warrant an 'enhanced' reanalysis that includes re-phenotyping and deep-phenotyping, re-processing of the ES data, re-prioritization of DNA variants in collaboration with referring clinicians, re-classification of DNA variants, and additional data sharing, of genotypic and phenotypic information, to help resolve DNA variants within both known and novel disease genes (Hartley et al., 2020). For healthcare system policymakers and payers interested in implementing funded reanalysis, a better understanding of the diagnostic utility of these processes in their own patient population is necessary to maximize clinical diagnoses from reanalysis without overburdening healthcare systems.

In the Canadian province of Ontario, clinical ES has been available to patients with suspected RGDs through the publicly funded healthcare system since 2014 but, until April 2021, all sequencing had been done outside of Canada, providing limited opportunities to understand the utility of reanalysis. Since 2017, the Ontario Ministry of Health has been engaged with Care4Rare Canada, a pan-Canadian RGD research consortium, to generate evidence of the diagnostic and clinical utility of ES, including the potential utility of generating data within Ontario to facilitate subsequent reanalysis. The objective of this study was to generate evidence to inform the clinical and research processes for reanalysis of ES data in Ontario. We aimed to recruit families with non-diagnostic ES results, collect the ES data from laboratories outside of Canada, re-process the data using Care4Rare bioinformatics pipelines, and re-prioritize variants in collaboration with the referring clinicians. We first evaluated the diagnostic yield from a 'clinical reanalysis,' which focused on genes known to be associated with the primary indication for testing; if found to be of benefit, this approach could later be implemented in several settings (e.g.,

clinical laboratories or by clinicians themselves). Next, we applied a 'translational research reanalysis' protocol that might be considered for future implementation as part of standard clinical care.

METHODS

Study design

This study was conducted at eight Medical Genetics programs in collaboration with 46 physicians. According to provincial policy, patient samples were sent for clinical ES following suspicion of an undiagnosed monogenic disorder that typically met any two of the clinical presentation eligibility criteria listed in **Table 2.1** (Genetic Testing Advisory Committee, 2016).To be eligible for this study, the referring physician had to attest that the clinical ES results were non-diagnostic, with any of: 1) no DNA variants of interest; 2) DNA variants of uncertain significance (VUS) in genes with a known disease-gene association or genes of uncertain significance (GUS) ; or, 3) pathogenic variants in genes associated with only part of the participant's clinical presentation and thus high suspicion of a second undiagnosed genetic disease.

Table 2.1. Clinical presentation eligibility criteria for publicly funded ES in Ontario (must meet 2 or more)

Criteria	Description
1	Moderate to severe developmental or functional impairment
2	Multisystem involvement
3	Progressive clinical course
4	A differential diagnosis that includes ≥ 2 well defined conditions requiring evaluation by multiple targeted gene panels
5	A suspected severe genetic syndrome for which multiple family members are also affected, or where parents are consanguineous

The study was conducted with approval from the Clinical Trials Ontario Streamlined Research Review System (CTO-1577, approvals available in Appendix 1) in 2018 and individual institutional Research Ethics Board approvals prior to 2018. All families provided informed consent for this study and for data release from the clinical laboratory.

Data collection

Data relating to phenotype, demographics, and the clinical ES were extracted manually by local study team members from medical records and stored in the Care4Rare database Genomics4RD (www.genomics4rd.org) and/or PhenomeCentral (www.phenomecentral.org), both of which enable phenotyping using human phenotype ontology (HPO) terms. The original clinical laboratory reports were used to extract the report date for the initial analysis, the test name, the sequencing strategy, and the molecular results.

Data transfer and re-processing

Bespoke data transfer methods were established in collaboration with each of the clinical laboratories. Patients gave consent for their data to be accessed by the Care4Rare research team using the clinical laboratory's data release forms. We set up a secure file transfer portal (SFTP) or Citrix Sharefile account, to facilitate data transfers and collected all available sequencing files (FASTQ, BAM, CRAM, and/or VCF) for all available family members and consented into the study. There was no restriction in the length of time since initial analysis, beyond the laboratory restrictions for data availability. Whenever possible, we used the rawest form of data and processed FASTQ files through the most recent iteration of our previously described bioinformatics pipeline (Kernohan et al., 2018).

Clinical reanalysis

Family-based analysis was completed at the Children's Hospital of Eastern Ontario and the Hospital for Sick Children. Each reanalysis team included a minimum of five team members, including the local referring clinician, a clinical geneticist, a clinical laboratory geneticist, a genetic counselor, and a post-doctoral fellow. The 'clinical reanalysis' was limited to rare variants in genes associated with human

disease in the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org), Orphanet database (https://www.orpha.net), and custom gene-panels produced using the patient's HPO terms and HPO-gene annotations (https://hpo.jax.org/). Variants in known disease genes were only considered compelling candidates when the referring clinician provided feedback that the patient's presentation could be explained by the particular disease-gene association. Sanger sequencing was conducted if variants were of insufficient quality or would benefit from segregation. Splicing studies were conducted, either using RNA sequencing or splicing assays on human cell lines, as functional evidence in the assessment of the impact of DNA variant(s) when appropriate and if samples were available. All variants were interpreted by a genetic counselor and clinical laboratory geneticist. A definitive diagnosis in a known disease-gene was made once the DNA variant(s) segregated appropriately for the given condition and met American College of Medical Genetics and Genomics (ACMG) criteria for likely pathogenic (LP) or pathogenic (P) (Richards et al., 2015).

Translational research reanalysis

Next, the analysis was extended to include additional research methods that could have diagnostic implications (translational research methods). This began with expanding the analysis to include genes of uncertain significance (GUS). Variants within GUS were prioritized based on the following: 1) the segregation of the variants fit with the associated pedigree; 2) the variant was sufficiently rare within internal and control databases (e.g., gnomAD) to be compatible with the associated RGD; 3) it was plausible that the aberration could lead to the patient's disease (e.g., pathway, type of protein); and, 4) the gene was significantly intolerant to the types of variants observed. Additional translational research studies were conducted as appropriate and when samples were available. This included, primarily, the identification of additional families through our internal Care4Rare database, data sharing with the Matchmaker Exchange (MME) (https://www.matchmakerexchange.org/), and identification of newly

published case reports. Our methods for patient matchmaking through the MME have been reported elsewhere (Osmond et al., 2022). We also conducted Sanger sequencing and functional assays to assess the segregation and functional impact of the DNA variant(s). A family was only considered to have a likely diagnosis in a GUS once two families (e.g., a cohort of 3 or more families) with the same RGD was established.

Data Analysis

The proportions of reanalysis results were stratified based on proband sex assigned at birth, age of onset, phenotype, sequencing strategy, and year of the initial analysis. We examined the impact of time on reanalysis in two ways. We stratified the cohort into an earlier cohort that had testing up until December 2018 and a later cohort that had testing from January 2019 onwards. Next, we stratified the cohort based on the length of time between the clinical test report and our reanalysis. These time periods were examined in 12-month increments to examine the proportions of results in each period. Chi-square tests of independence were used to examine the relationship between the reanalysis results and groupings.

RESULTS

Demographics

In total, 287 probands and their relevant family members were recruited. The characteristics of the 287 probands are summarized in **Table 2.2**. Most of the cohort were affected with a sporadic disease that had onset in childhood, which included syndromic intellectual disability (ID) or developmental delay (DD). The provision of self-reported ethnicity information was left to the discretion of families. Amongst the 151 (52%) who provided these details, ethnicities from more than 100 different countries were

described, and almost half of the families (74/151, 49%) self-identified as having ancestry from more than one country. Consanguinity was confirmed or suspected in 9% of families.

Table 2.2. Cohort demographics and details on initial testing

Characteristics	No. Probands (%)	Characteristics	No. Probands (%)
Sex Assigned at Birth		**Year of Clinical Report**	
Male	150 (52)	2014	8 (3)
Female	137 (48)	2015	10 (3)
		2016	38 (13)
Age of Symptom Onset		2017	53 (18)
Congenital/Infantile	230 (80)	2018	44 (15)
Childhood	49 (17)	2019	101 (35)
Adult	8 (3)	2020	33 (11)
Family History		**Initial Results**	
Sporadic	241 (84)	Negative	116 (40)
Dominant	6 (2)	Uncertain (VUS or GUS)	164 (57)
Recessive	27 (7)	Partial diagnosis	7 (2)
X-linked	3 (1)		
Adopted	7 (2)	**Sequencing Strategy**	
Unclear	3 (1)	Singleton (proband alone)	51 (18)
		Duo (proband + parent)	20 (7)
Parental Consanguinity	26 (9)	Trio (proband + parents)	201 (70)
		Quad (affected sibs + parents)	11 (4)
Phenotype		Other	4 (1)
Syndromic ID/DD	192 (67)		
MCA without ID/DD	28 (10)	**Age at Initial Analysis**	
Multisystem	44 (15)	Age not provided	2 (1)
Single System	21 (7)	Infancy (0-2)	86 (30)
Isolated severe ID	2 (1)	Childhood (3-17)	161 (56)
		Adulthood (>18)	38 (13)

Abbreviations: DD = developmental disability, ID = intellectual disability, MCA = multiple congenital anomalies

Original laboratory results and timing of reanalysis

Clinical sequencing was performed at seven laboratories, but the majority was performed at GeneDx (240/287; 84%). An additional 17 families had testing through the University of Chicago, 10 from Baylor,

nine from BluePrint, six from Emory, four from Prevention Genetics, and one from Fulgent. The most common sequencing strategy was trios (181, 69%) followed by singletons (51/287; 18%). The initial laboratory results included 116 reports (40%) that were completely negative, 164 (57%) that had VUS in known disease genes and/or GUS, and seven (2%) had likely pathogenic or pathogenic variants in known disease genes that the ordering clinician felt represented only a partial diagnosis. The time between the initial clinical laboratory reports and reanalysis was variable. Data were analyzed as they became available between January 2017 and November 2021. The mean time since initial analysis was 22 months (SD 14 months) with a range from 1 to 73 months.

Clinical reanalysis results

Clinical reanalysis resulted in new diagnoses for nine of 287 (3%) families without the need for additional evidence through segregation or functional studies (**Table 2.3**). Of the nine diagnoses, five (56%) were attributed to new genomic knowledge; including three new gene-disease associations (*WDR37, CDK13, ARF1*), one phenotype expansion (*ATP1A3*), and one variant-disease association (*DNMT3A*). The other four diagnoses were in genes with well-established disease-gene relationships at the time of clinical report, indicating that they may have been missed by some portion of the laboratory's analysis workflow. These included two variants that had been previously classified in ClinVar as LP or P; an intronic variant in *DNM1* that was predicted to result in a new splice acceptor site, and a missense variant in *G6PD*. The other two diagnoses that met LP/P criteria were truncating variants in genes for which haploinsufficiency is a known mechanism of disease; a hemizygous stopgain in the X-linked gene *AMER1*, and a low-quality frameshift variant in *SYNGAP1* that required Sanger sequencing for validation.

Table 2.3. Diagnoses made from the reanalysis of genes with known disease-gene associations										
Method of Diagnosis	**Family**	**Disorder**	**Gene(s)**	**OMIM Disease**	**OMIM ID**	**Inh**	**Variant(s)**	**ACMG**	**On the initial clinical report?**	**Potential reason(s) for diagnosis**
ES data alone	934	GDD, bilateral coloboma, brain malformations	WDR37	Neurooculocardiogenitourinary syndrome	618652	AD	NM_014023.3:c.356C>T:p.Ser119Phe	LP	Yes, as GUS	New genomic knowledge (disease-gene association)
	936	Epileptic encephalopathy	DNM1	Developmental and epileptic encephalopathy 31	616346	AD	NM_001005336.1:c.1335+1638G>A	LP	No	Difference in analysis process, new genomic knowledge (phenotype-disease association)
	1106	GDD, FTT, dysmorphic features, complex congenital heart malformations	CDK13	Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder	617360	AD	NM_003718.4:c.2570G>T:p.Gly857Val	P	No	New genomic knowledge (disease-gene association)
	1126	GDD, choanal atresia, dysmorphic features, macrocephaly	AMER1	Osteopathia striata with cranial sclerosis	300373	XLD	NM_152424.3:c.261delG	P	No	Difference in analysis process
	1170	GDD, partial agenesis of corpus callosum	ARF1	Periventricular nodular heterotopia	618185	AD	NM_001024226.1:c.55C>T:p.Arg19Cys	LP	Yes, as GUS	New genomic knowledge (disease-gene association)
	1213	DD and Lennox Gastaut	SYNGAP1	Mental retardation, autosomal dominant 5	612621	AD	NM_001130066.1:c.380_383dup:p.Ser129AlafsTer24	P	No	Difference in analysis process
	1476	Infantile epileptic encephalopathy	ATP1A3	CAPOS syndrome	601338	AD	NM_001256213.1:c.2781C>G:p.Ile927Met	LP	Yes, as VUS	New genomic knowledge (phenotype-disease association)
	1835	Seizures, ataxia, GDD, dysarthria, tremulousness	G6PD	Hemolytic anemia, G6PD deficient	300908	XLD	NM_000402.3:c.934G>C:p.Asp312His	LP	No	Difference in analysis process, Clinical correlation
	1953	GDD, ID, dysmorphic features, long slender fingers, widely spaced nipples	DNMT3A	Tatton-Brown-Rahman syndrome	615879	AD	NM_022552.4:c.2207G>A:NP_072046.2:p.Arg736His	LP	Yes, as VUS	New genomic knowledge (variant-disease association)
Additional supporting evidence (segregation or splicing assays)	1129	Severe ID, microcephaly, dysmorphic features	HUWE1	Mental retardation, X-linked syndromic, Turner type	309590	XL	NM_031407.5:c.12067C>T:p.Arg4023Cys	LP	Yes	Segregation
	1257	GDD, midbrain atrophy, dysmorphic features, seizures	TRAPPC12	Encephalopathy, progressive, early-onset, with brain atrophy and seizures	617669	AR	NM_001321102.1:c.1531-3C>A NM_001321102.1:c.1776+3A>G	LP/LP	No	New genomic knowledge (disease-gene association), splicing studies
	1477	Hypotonia, nystagmus, cerebral atrophy, sensorimotor polyneuropathy	ATAD3A	Harel-Yoon syndrome	617183	AD	NM_001170535.2:c.384+3A>G	LP	No	New genomic knowledge (disease-gene association), splicing studies
	1897	Recurrent infections, myopia, hearing loss, polydactyly	ARR3	Myopia 26, X-linked, female-limited	301010	XL	NM_004312.3:c.298C>T:p.Arg100Ter	P	Yes	Segregation

Note: ACMG = American College of Medical Geneticists classification, AD = autosomal dominant, AR = autosomal recessive, Inh = inheritance, LP = likely pathogenic, P = pathogenic XL = X-linked, XLD = X-linked dominant

Clinical reanalysis also identified candidates in known genes in 30 additional families (30/287, 10%) that could explain all or part of the family's presentation. This included 15 candidates in autosomal recessive (AR) disease genes (six biallelic VUS, and nine families in which single LP/P variants were identified), nine VUSs in autosomal dominant (AD) disease genes, and seven VUSs in X-linked disease genes. Thus far, additional evidence has been gathered to support pathogenicity for four families (4/287; 1%) and we have since re-classified these variants to be likely pathogenic (**Table 2.3**). In two X-linked families, additional samples were collected and the VUSs segregated appropriately in distantly related affected family members (Families 1129 and 1897). In Families 1257 and 1477, splicing assays resolved the impact of splicing extended variants. This included compound heterozygous VUSs in *TRAPPC12* and a single heterozygous VUS in *ATAD3A* (Hanes et al., 2020). Of the 30 candidates in known genes, 14 (46%) were not included in the initial laboratory report. Details for the remaining 26 families are presented in **Supplemental Table 2.1**.

In summary, we have identified compelling candidates in known disease genes for 39 of 287 (14%) families, 13 (5%) of which have sufficient evidence to support pathogenicity based on ACMG variant classification criteria (**Figure 2.1**).

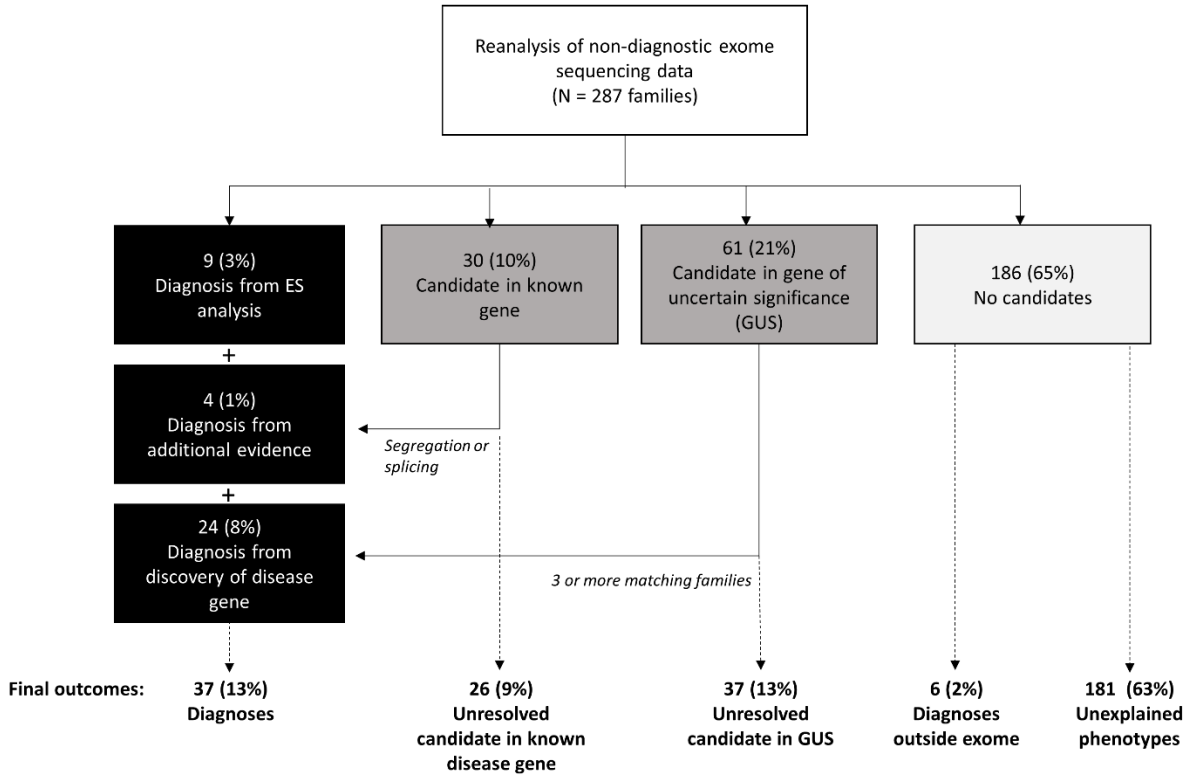

Figure 2.1. Results from the reanalysis of non-diagnostic clinical exome sequencing (ES) data for 287 families from Ontario, Canada. Reanalysis resulted in 37 diagnoses (13%). Nine were made in known genes from the review of ES data alone and four via upgrading VUSs in known genes using laboratory studies. The majority of diagnoses (24, 8% of cohort) were made through the analysis of genes of uncertain significance (GUSs) and additional data-sharing to identify cohorts with the same novel rare genetic disease. An additional 63 families (22%) have candidates in known genes and GUS. No candidates were identified in 186 families (65%), although six families (2% of cohort) have been found to have diseases caused by mechanisms intractable to ES.

Translational research reanalysis results

An additional 61 of the 287 families (21%) had candidate variants identified in a GUS (**Figure 2.1**). Since we identified the same compelling candidate gene (*AGO2*) in two families, there were 60 unique GUSs (**Supplemental Table 2.2**) for which additional evidence was sought. To identify additional families with rare variants in the same candidate genes and overlapping phenotypes, we queried our own internal database, submitted each gene to the Matchmaker Exchange, and periodically queried the scientific

literature for new publications. Variants were upgraded to likely diagnoses when the referring clinician believed we had identified two additional independent families. Thus far, we have upgraded variants to likely diagnoses for 23 of the 60 genes (38%). Ten of these genes have been published, in collaboration with our group, as novel disease-gene associations (Cheng et al., 2018; Huang et al., 2022; Johnstone et al., 2020; Lee et al., 2022; Lee et al., 2020; Lemire, Ito, et al., 2021; Lemire, Zheng, et al., 2021; Lessel et al., 2020; Nabais Sá et al., 2020; Radio et al., 2021; Schnur et al., 2021). Another four families have benefited from recent cohort publications by external research programs (Bott et al., 2021; Cogné et al., 2019; Granadillo et al., 2020; Mirzaa et al., 2020). The remaining nine genes are part of ongoing collaborations.

We have identified a single additional family for five of the 60 genes (8%). Three matches were made through the MME (*KIF26A, SEPT11, SCAI*). In the fourth case, we identified a *de novo* missense variant in *LONP1* [NM_004793.3:c.902G>A, p.(Arg301Gln)] in a proband exhibiting dystonia, hearing loss, and seizures. A recent publication reported a patient with an overlapping phenotype and a *de novo* missense at the same amino acid [c.901C>T, p.(Arg301Trp)] (Besse et al., 2020), providing additional evidence that dominant variants in *LONP1* may be a cause of mitochondrial encephalopathy. Finally, in the fifth case, after making no matches through data sharing, we published the phenotype and functional studies for a proband with Yunis-Varon syndrome in whom we identified biallelic missense variants in *VAC14* (Lines et al., 2017). Since that publication, we have had one clinician reach out to us with a phenotypically similar family.

We have therefore identified likely diagnoses in 24 families (8% of cohort) by reviewing GUSs and identifying additional matching families. An additional 37 families (13% of cohort) have candidates. Of

these, five have some additional evidence that indicates they may be disease-causing, whether it be a single additional family or evidence of functional perturbation.

Additional diagnoses made in the cohort

Six families were diagnosed within the clinic while awaiting research reanalysis. Three families had genetic diagnoses identified outside of the exome (two CNVs found by microarray and one pathogenic mitochondrial variant) and another three families received clinical diagnoses with non-monogenic etiology (two autoimmune diseases and one teratogenic exposure).

Impact of patient variables, initial sequencing strategy, and time on reanalysis results

We observed no difference in results based on biological sex assigned at birth and were unable to make meaningful comparisons based on age of onset, family history, phenotype, and sequencing strategy because most of the cohort were children, with sporadic syndromic ID/DD, and were run as a trio (**Table 2.4**).

Table 2.4. Reanalysis results stratified based on demographics, sequencing strategy, year of initial clinical report								
		Clinical Reanalysis			Research Reanalysis		Unsolved	
	N	Diagnosis in known gene (from ES data)	Diagnosis in known gene (from segregation or splicing)	Candidate in known gene	Diagnosis in novel gene (3 or more families with same RGD)	Candidate in GUS	Clinical diagnosis outside exome	No candidates
Total	287	9 (3%)	4 (1%)	27 (9%)	24 (8%)	37 (13%)	6 (2%)	180 (63%)
Biological Sex								
Male	150	5 (3%)	1 (1%)	11 (7%)	13 (9%)	20 (13%)	3 (2%)	97 (64%)
Female	137	4 (3%)	3 (2%)	16 (11%)	11 (8%)	17 (12%)	3 (2%)	83 (61%)
Age of Symptom Onset								
Congenital/infantile	230	8 (3%)	4 (2%)	21 (9%)	21 (9%)	25 (11%)	4 (2%)	147 (64%)
Childhood	49	1 (2%)	0 (0%)	5 (10%)	3 (6%)	11 (22%)	1 (2%)	28 (57%
Adult	8	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (13%)	1 (13%)	5 (63%)
Family History								
Sporadic	241	8 (3%)	2 (1%)	22 (9%)	21 (9%)	34 (14%)	6 (2%)	148 (61%)
Recurrence	36	1 (3%)	2 (6%)	3 (8%)	3 (8%)	3 (8%)	0 (0%)	24 (61%)
Unknown	10	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	8 (80%)
Consanguinity	26	1 (4%)	0 (0%)	6 (23%)	1 (4%)	3 (12%)	0 (0%)	15 (58%)
Phenotype								
Syndromic ID/DD	192	8 (4%)	3 (2%)	18 (9%)	21 (11%)	27 (14%)	2 (1%)	113 (59%)
MCA without ID/DD	28	0 (0%)	0 (0%)	3 (11%)	2 (7%)	2 (7%)	1 (4%)	20 (71%)
Multisystem	44	1 (2%)	1 (2%)	4 (9%)	0 (0%)	3 (7%)	1 (2%)	34 (77%)
Single system	21	0 (0%)	0 (0%)	2 (10%)	1 (5%)	5 (24%)	2 (10%)	11 (52%)
Isolated Severe ID	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)
Sequencing Strategy								
Trio	201	8 (4%)	3 (1%)	24 (12%)	19 (9%)	19 (9%)	4 (2%)	124 (62%)
Singleton	51	1 (2%)	1 (2%)	7 (14%)	2 (4%)	7 (14%)	2 (4%)	31 (61%)
Other	35	0 (0%)	0 (0%)	2 (6%)	3 (9%)	5 (14%)	0 (0%)	25 (71%)
Year of Initial Report								
2018 or earlier	153	8 (5%)	3 (2%)	11 (7%)	15 (10%)	23 (15%)	6 (4%)	87 (57%)
2019 or later	134	1 (<1%)	1 (<1%)	15 (11%)	9 (7%)	14 (10%)	0 (0%)	94 (70%)

Abbreviations: ES = exome sequencing, ID/DD = intellectual disability/developmental delay, GUS = gene of uncertain significance, RGD = rare genetic disease

The impact of time was studied in two ways. We stratified the cohort into an earlier cohort that had clinical testing up until December 2018 (n=153) and a later cohort that had testing from January 2019 onwards (n=134) (**Table 2.4**). The eligibility criteria for clinical ES testing were the same at both time points and therefore we assumed the likelihood of a genetic diagnosis would be similar amongst the two groups. A chi-square test of independence showed there was a significantly higher number of diagnoses in those that had testing in 2018 or earlier, $\chi^2(1,N=287)=4.72$, $p=0.03$. Similarly, the earlier period was associated with an increased likelihood of receiving a diagnosis outside of the exome by alternative clinical methods, $\chi^2(1,N=287)=5.37$, $p=0.02$. There was no association between the time periods and the likelihood of a diagnosis in a novel gene, $\chi^2(1,N=287)=0.78$, $p=0.38$. Next, we stratified the cohort based on the length of time between the initial analysis and reanalysis in 12-month increments (**Figure 2.2**). We could not perform statistical analyses due to the small numbers in each of the subgroups but identified a trend towards a higher proportion of families receiving diagnoses in clinically relevant genes when reanalysis was performed after two years (6%) compared to when performed at less than two years (2%). In contrast, the time since initial analysis did not appear to affect the proportion of families for whom we identified VUSs by clinical reanalysis (range 8% to 14%). The largest proportion of result-type amongst all the time groupings were candidates in GUS (range 14% to 30%). Of note, these latter results represent findings from reviewing the ES data, and do not include additional studies to better understand the potential utility of the data in isolation.

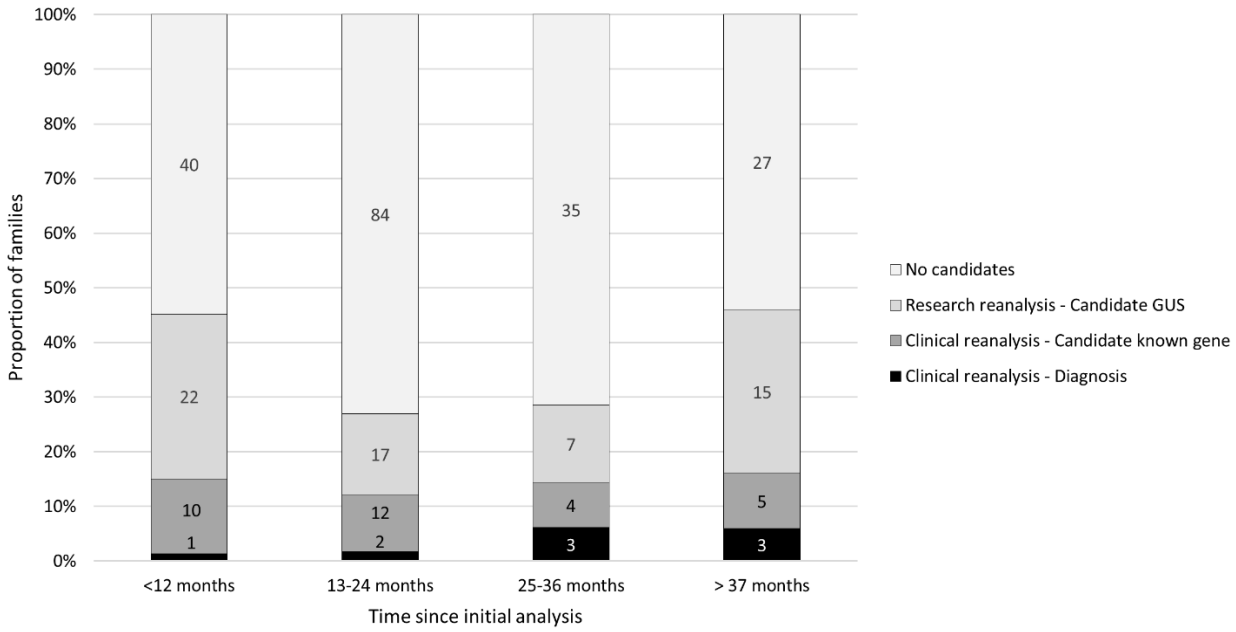

Figure 2.2. The proportion of families with distinct types of results following clinical and research reanalysis of exome sequencing data in 12 month increments since initial non-diagnostic clinical analysis. These data represent results from reanalysis of data alone and does not include additional laboratory investigations (segregation and splicing assays) or matchmaking. The proportion of families with diagnoses in known genes appears to be higher after 24 months compared to prior to 24 months since the initial analysis. In contrast, there is no trend in proportion of families with a GUS identified in different time periods but it is consistently higher than variants identified in known genes.

DISCUSSION

This cohort study investigated 287 families from Ontario who met provincial criteria for publicly funded clinical ES. We aimed to better understand the potential of implementing clinical reanalysis in a few settings (e.g., clinical laboratories, research laboratories, or by clinicians themselves), and defined the added benefit of ongoing translational research. Overall, this protocol yielded new diagnoses for 37 families (13%) (**Figure 2.1**).

Clinical reanalysis

Our 'clinical reanalysis' protocol resulted in candidate DNA variants for 39 (14%) families (**Figure 2.1**), of which 13 families (5%) received new diagnoses. We attributed the diagnostic yield from this approach

primarily to three contributing factors: new genomic knowledge, re-interrogation of data by a different group that used different analysis protocols and input from the clinical team, and the addition of segregation and splicing studies to resolve VUSs (**Table 2.3**).

New genomic knowledge contributed to nine of 13 diagnoses (69%) made through clinical reanalysis and included new disease-gene associations, new variant-disease association, and phenotype expansions for known diseases. Further, new genomic knowledge contributed to the identification of new VUSs, in new disease genes, that were not previously reported. The diagnostic yield from the application of new genomic knowledge has been well recognized in the literature, attributed to be the predominant factor in 93% of studies reported to date (Tan et al., 2020).

Four diagnoses were within genes with well-established disease-gene associations at the time of initial analysis, indicating they were missed by part of the clinical laboratories' analysis process. Our study highlights the potential utility of using a separate group and different analytic protocols to interrogate the same ES data. Using a similar approach, Shashi *et al.* was able to identify two diagnoses in 35 exomes reanalyzed, that were within the capture design of the ES but not reported by the original laboratories (Shashi et al., 2019). This included a variant that was missed by the filtering process and a CNV that is typically more difficult to call in ES data due to software limitations. While some of our findings may have been missed by the bioinformatics pipelines and analysis protocols at the clinical laboratories, we attributed some results to the inclusion of the clinical care team in the reanalysis protocol. In doing so, clinicians provided additional phenotypic information. This is most apparent in the six families recruited under the suspicion of having multiple genetic diseases. In these cases, a single molecular diagnosis was made on initial testing but the clinicians did not feel it accounted for the full phenotype of their patient, and provided details of what phenotypes remained unexplained. A second

diagnosis was identified in just one of these families. In Family 1835, a participant with seizures, ataxia, DD, sparse hair, severe eczema, and tremulousness received a diagnosis of *KCNH1* on initial analysis by the clinical laboratory. This diagnosis did not resolve the cyclical nature of their symptoms. In reanalysis, a pathogenic mutation in *G6PD*, associated with hemolytic anemia, was identified and accounted for the remaining phenotype. It was the contribution of the referring clinician that made this complete diagnosis possible. In Basel-Salmon et al. (2019), most of the diagnoses identified (10 of 13) from the reanalysis of 84 probands were attributed to incorrect interpretation of the clinical context and the absence of an OMIM entry. Similarly, in our study 18 of 39 findings (9 diagnoses and 30 candidates) in known disease genes had not been reported. Of these, seven of the disease genes had publications at the time of initial analysis but were not yet curated by OMIM, which may have contributed to them being missed. Our results suggest that additional phenotypic information from the clinical team and regular updates of new genomic knowledge into the analysis pipeline is imperative for optimizing test sensitivity.

Finally, in four families, we generated evidence, via Sanger sequencing and splicing studies, to re-classify variants as pathogenic based on ACMG guidelines. These studies demonstrate the utility of further investigating VUSs, an important approach given 48% of the over 500,000 variants listed in ClinVar in 2019 were interpreted to be of uncertain significance (Wai et al., 2020).

We achieved a diagnostic yield from clinical reanalysis of 5% (13/287), which is within the range of results from reanalysis of known disease genes. Tan et al. (2020) identified no new diagnoses in 57 cases in re-reviewing the data they had generated in their own clinical laboratory 12 months after initial analysis. By contrast, Shashi et al. (2019) identified diagnoses in known disease genes in 9 of 35 (26%) datasets that were generated by external groups. Small cohort sizes and differing methods and

timeframes between analyses make it difficult to compare findings. In one of the larger cohorts reported, Bowling *et al.* described how reanalysis, considering new genomic knowledge and updated bioinformatics pipelines, led to the identification of new diagnoses for 4% of their cohort of 365 individuals (Bowling et al., 2017).

Translational research reanalysis

The addition of translational research approaches for variants identified by reanalysis in novel candidate genes, including data-sharing strategies, identified the most diagnoses, improving our yield by an additional 8% (24/387), double that of clinical reanalysis alone. Our findings are in keeping with Eldomery *et al.* who performed a research reanalysis following clinical testing at a single clinical laboratory (Eldomery et al., 2017). They identified diagnoses in novel genes in eight of 74 (11%) cases and candidate genes in 13 of 74 (18%) cases for a total of 28% potential novel contributory variants. Their findings support our observation that there remains significant potential for novel disease-gene discovery in data from families with suspected RGD.

Impact of time to reanalysis

Families who had testing prior to 2018 were more likely to receive a diagnosis via our reanalysis; eight of the nine diagnoses in known disease genes were within families tested more than three years ago. This suggests that the analysis protocols of clinical laboratories are improving such that fewer diagnoses are missed. In addition, it highlights the utility of re-examining data after sufficient time has passed as the likelihood of new genomic knowledge will be greater. This is supported by our observation that as time between initial analysis and reanalysis increased, the proportion of participants that receive diagnoses in known disease genes increased. Notably, the proportion of families with candidates in novel disease genes did not differ between those that were tested earlier compared to later and was high at all time

periods following initial analysis (**Figure 2.2**), reflecting the growing body of genomic knowledge that remains far from complete. Until we have the complete catalog of molecular etiologies of all RGDs, we will continue to see this trend of increased likelihood of diagnosis over time and steady discovery rate.

Incorporating translational research activities into clinical care

Our findings suggest that an enhanced care pathway that includes some translational research may have utility for years to come and further assessment of the effectiveness of implementing these types of clinic models will be helpful. The addition of translational research approaches is not necessarily beyond the scope of clinical care. Many of these genes are already reported by clinical laboratories (22 of 61 candidates in our cohort), and data-sharing through the Matchmaker Exchange does not explicitly require a research consent if the phenotypic and genotypic information is non-identifiable (Dyke et al., 2017). We advocated for these methods to be considered, depending on resources and expertise available, as part of clinical care for patients with non-diagnostic ES data (Hartley et al., 2020).

Patients who remain undiagnosed

Despite our efforts, 63% of our cohort remains without a definitive diagnosis. For some of these patients, their etiology may be outside of the exome. We learned of six such families, which is likely an underestimate given the range of sub-specialists typically involved in complex pediatric disease in Canada (Canadian Organization for Rare Disorders, 2015). Regardless, for those who remain without an understood etiology, additional reanalysis in the future, or further investigation with other technologies can be considered. Interestingly, we heard from clinicians that negative reanalysis of ES data gave them further confidence that their patient's disease was not genetic in etiology. This is an important impression as further studies in these families may not optimize use of limited resources and shows how even a negative result can refine a physician's diagnostic thinking.

Study limitations

Our study has several limitations. First, our results may not accurately reflect the diagnostic utility of reanalysis in this population. Recruitment relied on Ontario clinicians identifying families, which introduced selection bias. For example, clinicians may be less likely to recruit participants with compelling VUSs if they believe them to be diagnostic or may only refer patients for whom they believe there is a high suspicion of monogenic disease, skewing our results. Second, the time intervals were not standardized. Although we observed an increase in diagnoses based on both year of initial testing and time since initial analysis, our data are not able to specify an appropriate timeframe in which periodic reanalysis should be completed. Our findings do seem to indicate, however, that the diagnostic utility of reanalysis is greater at 3 years or later. Further research is needed to characterize the timeframe that would maximize the clinical impact of reanalysis while minimizing its burden on the laboratory and healthcare system.

Conclusion

This multi-site study in the province of Ontario found that there remains significant potential utility in the non-diagnostic clinical ES data of patients with suspected RGD. On its own, the reanalysis of ES data identified several new diagnoses, including some that could have been made at initial analysis, and others that required the generation of new genomic knowledge. We find that most of the potential of this data lies, however, in the addition of translational research processes. While the analysis of novel candidate genes, and ensuing data sharing to resolve them, is not currently part of standard clinical practice, these practices should be considered, with appropriate consent, to maximize diagnostic potential. Importantly, clinical laboratories should continue to highlight GUS as part of their analysis.

This study highlights the benefits of reanalysis as a useful approach to increase genomic diagnosis that bridges current clinical knowledge with translational research into novel disease-gene associations.

DATA AVAILABILITY

The exome datasets supporting this study have been deposited in Genomics4RD, the official database for Care4Rare Canada. All candidate genes and corresponding phenotypes are available through the PhenomeCentral node of the Matchmaker Exchange.

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AUTHOR CONTRIBUTIONS:

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CONFLICT OF INTERESTS

The authors declare no competing interests.

2.5. References

Bamshad, M. J., Nickerson, D. A., & Chong, J. X. (2019). Mendelian gene discovery: Fast and furious with no end in sight. *Am J Hum Genet*, *105*(3), 448-455. https://doi.org/10.1016/j.ajhg.2019.07.011

Basel-Salmon, L., Orenstein, N., Markus-Bustani, K., Ruhrman-Shahar, N., Kilim, Y., Magal, N., Hubshman, M. W., & Bazak, L. (2019). Improved diagnostics by exome sequencing following raw data reevaluation by clinical geneticists involved in the medical care of the individuals tested. *Genet Med*, *21*(6), 1443-1451. https://doi.org/10.1038/s41436-018-0343-7

Besse, A., Brezavar, D., Hanson, J., Larson, A., & Bonnen, P. E. (2020). LONP1 de novo dominant mutation causes mitochondrial encephalopathy with loss of LONP1 chaperone activity and excessive LONP1 proteolytic activity. *Mitochondrion*, *51*, 68-78. https://doi.org/10.1016/j.mito.2020.01.004

Birney, E., Vamathevan, J., & Goodhand, P. (2017). Genomics in healthcare: GA4GH looks to 2022. *bioRxiv*. https://doi.org/10.1101/203554

Bott, L. C., Forouhan, M., Lieto, M., Sala, A. J., Ellerington, R., Johnson, J. O., Speciale, A. A., Criscuolo, C., Filla, A., Chitayat, D., Alkhunaizi, E., Shannon, P., Nemeth, A. H., Angelucci, F., Lim, W. F., Striano, P., Zara, F., Helbig, I., Muona, M., . . . Rinaldi, C. (2021). Variants in ATP6V0A1 cause progressive myoclonus epilepsy and developmental and epileptic encephalopathy. *Brain Commun*, *3*(4), fcab245. https://doi.org/10.1093/braincomms/fcab245

Bowling, K. M., Thompson, M. L., Amaral, M. D., Finnila, C. R., Hiatt, S. M., Engel, K. L., Cochran, J. N., Brothers, K. B., East, K. M., Gray, D. E., Kelley, W. V., Lamb, N. E., Lose, E. J., Rich, C. A., Simmons, S., Whittle, J. S., Weaver, B. T., Nesmith, A. S., Myers, R. M., . . . Cooper, G. M. (2017). Genomic diagnosis for children with intellectual disability and/or developmental delay. *Genome Med*, *9*(1), 43. https://doi.org/10.1186/s13073-017-0433-1

Canadian Organization for Rare Disorders. (2015). CORD Rare Disease Patient Survey. Retrieved January 24, 2023, from http://www.raredisorders.ca/content/uploads/CORD-Patient-Survey-Summary.pdf

Cheng, H., Dharmadhikari, A. V., Varland, S., Ma, N., Domingo, D., Kleyner, R., Rope, A. F., Yoon, M., Stray-Pedersen, A., Posey, J. E., Crews, S. R., Eldomery, M. K., Akdemir, Z. C., Lewis, A. M., Sutton, V. R., Rosenfeld, J. A., Conboy, E., Agre, K., Xia, F., . . . Lyon, G. J. (2018). Truncating variants in NAA15 are associated with variable levels of intellectual disability, autism spectrum disorder, and congenital anomalies. *Am J Hum Genet*, *102*(5), 985-994. https://doi.org/10.1016/j.ajhg.2018.03.004

Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M., Dimmock, D., & Kingsmore, S. F. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*, *3*, 16. https://doi.org/10.1038/s41525-018-0053-8

Cogné, B., Ehresmann, S., Beauregard-Lacroix, E., Rousseau, J., Besnard, T., Garcia, T., Petrovski, S., Avni, S., McWalter, K., Blackburn, P. R., Sanders, S. J., Uguen, K., Harris, J., Cohen, J. S., Blyth, M., Lehman, A., Berg, J., Li, M. H., Kini, U., . . . Campeau, P. M. (2019). Missense variants in the histone acetyltransferase complex component gene TRRAP cause autism and syndromic intellectual disability. *Am J Hum Genet*, *104*(3), 530-541. https://doi.org/10.1016/j.ajhg.2019.01.010

Dyke, S. O. M., Knoppers, B. M., Hamosh, A., Firth, H. V., Hurles, M., Brudno, M., Boycott, K. M., Philippakis, A. A., & Rehm, H. L. (2017). "Matching" consent to purpose: The example of the Matchmaker Exchange. *Hum Mutat*, *38*(10), 1281-1285. https://doi.org/10.1002/humu.23278

Eldomery, M. K., Coban-Akdemir, Z., Harel, T., Rosenfeld, J. A., Gambin, T., Stray-Pedersen, A., Kury, S., Mercier, S., Lessel, D., Denecke, J., Wiszniewski, W., Penney, S., Liu, P., Bi, W., Lalani, S. R.,

Schaaf, C. P., Wangler, M. F., Bacino, C. A., Lewis, R. A., . . . Lupski, J. R. (2017). Lessons learned from additional research analyses of unsolved clinical exome cases. *Genome Med*, *9*(1), 26. https://doi.org/10.1186/s13073-017-0412-6

Genetic Testing Advisory Committee. (2016). *Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario*. Retrieved March 30, 2023, from http://www.health.gov.on.ca/en/pro/programs/gtac/reports.aspx

Granadillo, J. L., A, P. A. S., Guo, H., Xia, K., Angle, B., Bontempo, K., Ranells, J. D., Newkirk, P., Costin, C., Vironт, J., Stumpel, C. T., Sinnema, M., Panis, B., Pfundt, R., Krapels, I. P. C., Klaassens, M., Nicolai, J., Li, J., Jiang, Y., . . . Shinawi, M. (2020). Pathogenic variants in TNRC6B cause a genetic disorder characterised by developmental delay/intellectual disability and a spectrum of neurobehavioural phenotypes including autism and ADHD. *J Med Genet*, *57*(10), 717-724. https://doi.org/10.1136/jmedgenet-2019-106470

Hanes, I., McMillan, H. J., Ito, Y., Kernohan, K. D., Lazier, J., Lines, M. A., & Dyment, D. A. (2020). A splice variant in ATAD3A expands the clinical and genetic spectrum of Harel-Yoon syndrome. *Neurol Genet*, *6*(4), e452. https://doi.org/10.1212/nxg.0000000000000452

Hartley, T., Lemire, G., Kernohan, K. D., Howley, H. E., Adams, D. R., & Boycott, K. M. (2020). New diagnostic approaches for undiagnosed rare genetic diseases. *Annu Rev Genomics Hum Genet*, *21*, 351-372. https://doi.org/10.1146/annurev-genom-083118-015345

Huang, Y., Lemire, G., Briere, L. C., Liu, F., Wessels, M. W., Wang, X., Osmond, M., Kanca, O., Lu, S., High, F. A., Walker, M. A., Rodan, L. H., Wangler, M. F., Yamamoto, S., Kernohan, K. D., Sweetser, D. A., Boycott, K. M., & Bellen, H. J. (2022). The recurrent de novo c.2011C>T missense variant in MTSS2 causes syndromic intellectual disability. *Am J Hum Genet*, *109*(11), 2092. https://doi.org/10.1016/j.ajhg.2022.10.001

Johnstone, D. L., Nguyen, T. T. M., Zambonin, J., Kernohan, K. D., St-Denis, A., Baratang, N. V., Hartley, T., Geraghty, M. T., Richer, J., Majewski, J., Bareke, E., Guerin, A., Pendziwiat, M., Pena, L. D. M., Braakman, H. M. H., Gripp, K. W., Edmondson, A. C., He, M., Spillmann, R. C., . . . Campeau, P. M. (2020). Early infantile epileptic encephalopathy due to biallelic pathogenic variants in PIGQ: Report of seven new subjects and review of the literature. *J Inherit Metab Dis*, *43*(6), 1321-1332. https://doi.org/10.1002/jimd.12278

Kernohan, K. D., Hartley, T., Naumenko, S., Armour, C. M., Graham, G. E., Nikkel, S. M., Lines, M., Geraghty, M. T., Richer, J., Mears, W., Boycott, K. M., & Dyment, D. A. (2018). Diagnostic clarity of exome sequencing following negative comprehensive panel testing in the neonatal intensive care unit. *Am J Med Genet A*, *176*(7), 1688-1691. https://doi.org/10.1002/ajmg.a.38838

Lee, R. G., Balasubramaniam, S., Stentenbach, M., Kralj, T., McCubbin, T., Padman, B., Smith, J., Riley, L. G., Priyadarshi, A., Peng, L., Nuske, M. R., Webster, R., Peacock, K., Roberts, P., Stark, Z., Lemire, G., Ito, Y. A., Boycott, K. M., Geraghty, M. T., . . . Filipovska, A. (2022). Deleterious variants in CRLS1 lead to cardiolipin deficiency and cause an autosomal recessive multi-system mitochondrial disease. *Hum Mol Genet*, *31*(21), 3597-3612. https://doi.org/10.1093/hmg/ddac040

Lee, Y. R., Khan, K., Armfield-Uhas, K., Srikanth, S., Thompson, N. A., Pardo, M., Yu, L., Norris, J. W., Peng, Y., Gripp, K. W., Aleck, K. A., Li, C., Spence, E., Choi, T. I., Kwon, S. J., Park, H. M., Yu, D., Heo, W. D., Mooney, M. R., . . . Schwartz, C. E. (2020). Mutations in FAM50A suggest that Armfield XLID syndrome is a spliceosomopathy. *Nat Commun*, *11*(1), 3698. https://doi.org/10.1038/s41467-020-17452-6

Lemire, G., Ito, Y. A., Marshall, A. E., Chrestian, N., Stanley, V., Brady, L., Tarnopolsky, M., Curry, C. J., Hartley, T., Mears, W., Derksen, A., Rioux, N., Laflamme, N., Hutchison, H. T., Pais, L. S., Zaki, M. S., Sultan, T., Dane, A. D., Gleeson, J. G., . . . Boycott, K. M. (2021). ABHD16A deficiency causes a

complicated form of hereditary spastic paraplegia associated with intellectual disability and cerebral anomalies. *Am J Hum Genet*, *108*(10), 2017-2023. https://doi.org/10.1016/j.ajhg.2021.09.005

Lemire, G., Zheng, B., Ediae, G. U., Zou, R., Bhola, P. T., Chisholm, C., de Nanassy, J., Lo, B., Wang, C., Shril, S., El Desoky, S., Shalaby, M., Kari, J. A., Wang, X., Kernohan, K. D., Boycott, K. M., Hildebrandt, F., & Sawyer, S. L. (2021). Homozygous WNT9B variants in two families with bilateral renal agenesis/hypoplasia/dysplasia. *Am J Med Genet A*, *185*(10), 3005-3011. https://doi.org/10.1002/ajmg.a.62398

Lessel, D., Zeitler, D. M., Reijnders, M. R. F., Kazantsev, A., Hassani Nia, F., Bartholomäus, A., Martens, V., Bruckmann, A., Graus, V., McConkie-Rosell, A., McDonald, M., Lozic, B., Tan, E. S., Gerkes, E., Johannsen, J., Denecke, J., Telegrafi, A., Zonneveld-Huijssoon, E., Lemmink, H. H., . . . Kreienkamp, H. J. (2020). Germline AGO2 mutations impair RNA interference and human neurological development. *Nat Commun*, *11*(1), 5797. https://doi.org/10.1038/s41467-020-19572-5

Lines, M. A., Ito, Y., Kernohan, K. D., Mears, W., Hurteau-Miller, J., Venkateswaran, S., Ward, L., Khatchadourian, K., McClintock, J., Bhola, P., Campeau, P. M., Boycott, K. M., Michaud, J., van Kuilenburg, A. B., Ferdinandusse, S., & Dyment, D. A. (2017). Yunis-Varón syndrome caused by biallelic VAC14 mutations. *Eur J Hum Genet*, *25*(9), 1049-1054. https://doi.org/10.1038/ejhg.2017.99

Mirzaa, G. M., Chong, J. X., Piton, A., Popp, B., Foss, K., Guo, H., Harripaul, R., Xia, K., Scheck, J., Aldinger, K. A., Sajan, S. A., Tang, S., Bonneau, D., Beck, A., White, J., Mahida, S., Harris, J., Smith-Hicks, C., Hoyer, J., . . . Bamshad, M. J. (2020). De novo and inherited variants in ZNF292 underlie a neurodevelopmental disorder with features of autism spectrum disorder. *Genet Med*, *22*(3), 538-546. https://doi.org/10.1038/s41436-019-0693-9

Nabais Sá, M. J., El Tekle, G., de Brouwer, A. P. M., Sawyer, S. L., Del Gaudio, D., Parker, M. J., Kanani, F., van den Boogaard, M. H., van Gassen, K., Van Allen, M. I., Wierenga, K., Purcarin, G., Elias, E. R., Begtrup, A., Keller-Ramey, J., Bernasocchi, T., van de Wiel, L., Gilissen, C., Venselaar, H., . . . de Vries, B. B. A. (2020). De novo variants in SPOP cause two clinically distinct neurodevelopmental disorders. *Am J Hum Genet*, *106*(3), 405-411. https://doi.org/10.1016/j.ajhg.2020.02.001

Need, A. C., Shashi, V., Schoch, K., Petrovski, S., & Goldstein, D. B. (2017). The importance of dynamic re-analysis in diagnostic whole exome sequencing. *J Med Genet*, *54*(3), 155-156. https://doi.org/10.1136/jmedgenet-2016-104306

Ng, S. B., Buckingham, K. J., Lee, C., Bigham, A. W., Tabor, H. K., Dent, K. M., Huff, C. D., Shannon, P. T., Jabs, E. W., Nickerson, D. A., Shendure, J., & Bamshad, M. J. (2010). Exome sequencing identifies the cause of a Mendelian disorder. *Nat Genet*, *42*(1), 30-35. https://doi.org/10.1038/ng.499

Osmond, M., Hartley, T., Dyment, D. A., Kernohan, K. D., Brudno, M., Buske, O. J., Innes, A. M., Boycott, K. M., & Care4Rare Canada, C. (2022). Outcome of over 1500 matches through the Matchmaker Exchange for rare disease gene discovery: The 2-year experience of Care4Rare Canada. *Genet Med*, *24*(1), 100-108. https://doi.org/10.1016/j.gim.2021.08.014

Radio, F. C., Pang, K., Ciolfi, A., Levy, M. A., Hernández-García, A., Pedace, L., Pantaleoni, F., Liu, Z., de Boer, E., Jackson, A., Bruselles, A., McConkey, H., Stellacci, E., Lo Cicero, S., Motta, M., Carrozzo, R., Dentici, M. L., McWalter, K., Desai, M., . . . Tartaglia, M. (2021). SPEN haploinsufficiency causes a neurodevelopmental disorder overlapping proximal 1p36 deletion syndrome with an episignature of X chromosomes in females. *Am J Hum Genet*, *108*(3), 502-516. https://doi.org/10.1016/j.ajhg.2021.01.015

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus

recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, *17*(5), 405-424. https://doi.org/10.1038/gim.2015.30

Robertson, A. J., Tan, N. B., Spurdle, A. B., Metke-Jimenez, A., Sullivan, C., & Waddell, N. (2022). Reanalysis of genomic data: An overview of the mechanisms and complexities of clinical adoption. *Genet Med*, *24*, 798-810. https://doi.org/10.1016/j.gim.2021.12.011

Schnur, R. E., Yousaf, S., Liu, J., Chung, W. K., Rhodes, L., Marble, M., Zambrano, R. M., Sobreira, N., Jayakar, P., Pierpont, M. E., Schultz, M. J., Pichurin, P. N., Olson, R. J., Graham, G. E., Osmond, M., Contreras-García, G. A., Campo-Neira, K. A., Peñaloza-Mantilla, C. A., Flage, M., . . . Hufnagel, R. B. (2021). UBA2 variants underlie a recognizable syndrome with variable aplasia cutis congenita and ectrodactyly. *Genet Med*, *23*(9), 1624-1635. https://doi.org/10.1038/s41436-021-01182-1

Shamseldin, H. E., Maddirevula, S., Faqeih, E., Ibrahim, N., Hashem, M., Shaheen, R., & Alkuraya, F. S. (2017). Increasing the sensitivity of clinical exome sequencing through improved filtration strategy. *Genet Med*, *19*(5), 593-598. https://doi.org/10.1038/gim.2016.155

Shashi, V., Schoch, K., Spillmann, R., Cope, H., Tan, Q. K., Walley, N., Pena, L., McConkie-Rosell, A., Jiang, Y. H., Stong, N., Need, A. C., & Goldstein, D. B. (2019). A comprehensive iterative approach is highly effective in diagnosing individuals who are exome negative. *Genet Med*, *21*(1), 161-172. https://doi.org/10.1038/s41436-018-0044-2

Tan, N. B., Stapleton, R., Stark, Z., Delatycki, M. B., Yeung, A., Hunter, M. F., Amor, D. J., Brown, N. J., Stutterd, C. A., McGillivray, G., Yap, P., Regan, M., Chong, B., Fanjul-Fernandez, M., Marum, J., Phelan, D., Pais, L. S., White, S. M., Lunke, S., & Tan, T. Y. (2020). Evaluating systematic reanalysis of clinical genomic data in rare disease from single center experience and literature review. *Molec Genet Genomic Med*, *8*, e1508 https://doi.org/10.1002/mgg3.1508

Wai, H. A., Lord, J., Lyon, M., Gunning, A., Kelly, H., Cibin, P., Seaby, E. G., Spiers-Fitzgerald, K., Lye, J., Ellard, S., Thomas, N. S., Bunyan, D. J., Douglas, A. G. L., & Baralle, D. (2020). Blood RNA analysis can increase clinical diagnostic rate and resolve variants of uncertain significance. *Genet Med*, *22*(6), 1005-1014. https://doi.org/10.1038/s41436-020-0766-9

Wenger, A. M., Guturu, H., Bernstein, J. A., & Bejerano, G. (2017). Systematic reanalysis of clinical exome data yields additional diagnoses: implications for providers. *Genet Med*, *19*(2), 209-214. https://doi.org/10.1038/gim.2016.88

2.6. Supplemental tables

Supplementary Table 2.1. Candidates in genes with established disease-gene associations

Gene(s)	OMIM disease	OMIM number	Inh.	Candidate details	Variant(s)	Captured by lab
DENND5A	Epileptic encephalopathy, early infantile, 49	617281	AR	Biallelic VUS in AR disease	NM_001243254.1:c.3699G>A;p.Leu1233Leu / NM_001243254.1:c.1363T>C:p.Ser455Pro (comp het)	no
POLG	Mitochondrial DNA Depletion Syndrome	613662	AR	Biallelic VUS in AR disease	NM_001126131.1:c.2354G>A:p.Gly785Asp / NM_001126131.1:c.830A>T:p.His277Leu (comp het)	yes
GLI3	Greig cephalopolysyndactyly syndrome	175700	AD	VUS in AD disease	NM_000168.5:c.575C>T:p.Pro192Leu (het)	no
LAMA1	Poretti-Boltshauser syndrome	615960	AR	Biallelic VUS in AR disease	NM_005559.3:c.767G>A:p.Arg256His (hom)	yes
ADAR	Aicardi-Goutieres syndrome 6	615010	AR	Biallelic VUS in AR disease	NM_001025107.2:c.2748G>T:p.Trp916Cys/ NM_001025107.2:c.-309C>G:NA (comp het)	yes
KMT2B	Dystonia 28, childhood-onset	617284	AD	VUS in AD disease	NM_014727.1:c.7945C>T:p.Arg2649Cys (het)	yes
TTN	Myopathy, myofibrillar, 9, with early respiratory failure	603689	AD	VUS in AD disease	NM_001256850.1:c.22148-6T>C:NA (het)	no
TUBGCP2	Pachygyria, microcephaly, developmental delay, and dysmorphic facies, with or without seizures	618737	AR	Biallelic VUS in AR disease	NM_001256617.1:c.2195C>T:p.Pro732Leu (hom)	no
SLC39A8	Congenital disorder of glycosylation, type 2n	616721	AR	Single LP/P in AR disease	NM_001135146.1:c.1233+2T>A (het)	yes
CACNA1S	Hypokalemic periodic paralysis, type 1	170400	AD	VUS in AD disease	NM_000069.2:c.3026C>A:p.Thr1009Lys (het)	no
ATRX	Mental retardation-hypotonic facies syndrome, X-linked	309580	XLR	VUS in XLR disease	NM_000489.3:c.2557G>A:p.Glu853Lys (hemi)	yes

GH1	Growth hormone deficiency, isolated, type 1A	262400	AR	Biallelic VUS in AR disease	NM_000515.3:c.-185T>C (hom)	yes
MBOAT7	Mental retardation, autosomal recessive 57	617188	AR	Biallelic VUS in AR disease	NM_001146056.1:c.604G>A:p.Gly202Ser (hom)	yes
CNTNAP1	Hypomyelinating neuropathy, congenital, 3	618186	AR	Biallelic VUS in AR disease	NM_003632.2:c.2198A>G:p.Asp733Gly (hom)	no
TAF1	Mental retardation, X-linked, syndromic 33	300966	XLR	VUS in XLR	NM_001286074.1:c.2748G>C:p.Glu916Asp (hemi)	yes
LMX1B	Nail-patella syndrome	161200	AD	VUS in AD disease	LMX1B NM_001174146.1:c.883C>A:p.Gln295Lys (het)	yes
PLA2G6	Neurodegeneration with brain iron accumulation 2B	610217	AR	Single LP/P in AR disease	NM_001004426.1:c.1186+1131del:NA (het)	yes
CRB2	Ventriculomegaly with cystic kidney disease	219730	AR	Single LP/P in AR disease	NM_173689.7:c.3089_3104dup:p.Gly1036AlafsTer43	no
AUTS2	Mental retardation, autosomal dominant 26	615834	AD	VUS in AD disease	NM_001127231.3:c.2841C>G:p.Tyr947Ter	no
ATP8A2	?Cerebellar ataxia, mental retardation, and dysequilibrium syndrome	615268	AR	Single LP/P in AR disease	NM_001313741.1:c.647_648insCCAGTTCA:p.Leu216PhefsTer19 (het)	yes
KMT2C	Kleefstra syndrome 2	617768	AD	VUS in AD disease	NM_170606.2:c.12053A>G:p.Tyr4018Cys (het)	no
ZDHHC9	Intellectual developmental disorder, X-linked, syndromic, Raymond Type	300799	XL	VUS in XL disease	NM_001008222.2;c.981C>T,p.Ala327Ala (hemi)	no
PQBP1	Renpenning syndrome	309500	XLR	VUS in XLR disease	NM_001032381.1:c.397C>G:p.Arg133Gly (hemi)	no
AIMP1	Hypomyelinating leukodystrophy 3	260600	AR	Single LP/P in AR disease	NM_001142415.1:c.773-2A>C:NA (het)	yes
ATRX	Mental retardation-hypotonic facies syndrome, X-linked, 1	309580	XLR	VUS in XLR disease	ATRX: NM_000489.5:c.6653C>T:p.Pro2218Leu (hemi)	yes
SLC25A46	Hereditary motor and sensory neuropathy typ VIB	616505	AR	Single LP/P in AR disease	NM_138773.4:c.800del:p.Pro267LeufsTer2 (het)	no

Supplementary Table 2.2. Candidates in genes of uncertain significance

Family ID	Disorder	Gene(s)	Number of additional families with same RGD	Mechanism of evidence	Relevant PMIDs	Reported on initial analysis
937	Short stature, DD, dysmorphic features	*FAM50A*	>2	matchmaking (cohort)	PMID: 32703943	yes
1008	DD, ADHD, Aortic Sclerosis	*SNRNP70*	0			yes
1062	Bone fragility, Hypotonia and Gross Motor Delay	*NRP2*	0			no
1071	Muscle disease	*KIF26A*	1	matchmaking (single family)		no
1074	Cerebellar ataxia	*SSFA2*	0			no
1081	Ectrodactyly, Cardiac VSD, Microcephaly, DD	*UBA2*	>2	matchmaking (cohort)	PMID: 34040189	no
1088	HSP	*GRIK2*	0			no
1092	Skeletal dysplasia/ Yunis-Varon-like	*VAC14*	1	functional, matchmaking (single family)	PMID: 28635952	yes
1107	GFF, FTT and hypotonia	*TNRC6B*	>2	since published	PMID: 32152250	no
1116	DD, Hydrocephalus, Laryngeal Cleft +	*FGF2*	0			no
1122	Thumb duplication, polymicrogyria, GDD	*SPEN*	>2	matchmaking (cohort)	PMID: 33596411	no
1123	GDD, hepatomegaly, Hx of polyhydramnios	*PIGQ*	>2	matchmaking (cohort) and functional	PMID: 32588908	no
1127	ID, reflux, laryngeal cleft, abdominal pain	*COPB2*	0			no
1128	DD, SNHL, short stature, dysmorphism	*TRRAP*	>2	since published	PMID: 30827496	no
1139	Mitochondrial	*UBR5*	>2	matchmaking (cohort)		yes
1152	Short stature, microcephaly and SNHL	*SPOP*	>2	matchmaking (cohort)	PMID: 32109420	no
1161	Seizures, GDD, microcephaly	*ATP6V0A1*	>2	matchmaking (cohort), since published		yes

1199	small stature, microcephaly, FTT, intellectual disability, and seizures	*SLIT1*	0			no
1200	DD, severe eczema, small size	*NAA15*	>2	matchmaking (cohort)	PMID: 29656860	yes
1211	GDD, hypotonia, unilateral hydronephrosis	*SEPT11*	1	matchmaking (single family)		no
1215	ID, dysmorphism, postnatal microcephaly	*EXOC1*	>2	matchmaking (cohort)		no
1233	Leukocytosis, GDD, adrenal suppression	*ZFC3H1*	0			yes
1245	Dystonia, hearing loss, low vision, seizures	*LONP1*	1	since published	PMID: 31923470	no
1246	Decreased level of consciousness, encephalopathy	*MED20*	0			yes
1254	Developmental Delay, Seizures + Delayed Gross Motor Development	*ACO2*	0			no
1265	Leukoencephalopathy, Cataracts, Ataxia +DD	*KEAP1*	0			no
1470	Intellectual Disability Syndrome NYD	*AGO2*	>2	matchmaking (cohort)	PMID: 33199684	no
1486	global developmental delay, ID, developmental regression, hypotonia,	*CRLS1*	>2	matchmaking (cohort) and functional	PMID: 35147173	no
1489	global developmental delay, autism, ataxia, hypotonia, laryngomalacia	*ZNF865*	>2	matchmaking (cohort)		no
1514	muscle weakness, motor delay, gait abnormality	*SNAPC3*	0			no
1522	Polymicrogyria, Intractable seizures, Distal arthrogryposis	*KIF21A*	>2	matchmaking (cohort)		yes
1563	GDD, bilateral Duane syndrome, dysmorphic, deformational plagio/brachycephaly/torticollis	*SMAD5*	0			yes
1566	Congenital bilat inguninal hernia, developmental hip dysplasia, severe hypotonia, GDD, seizure	*MTA1*	0			yes
1583	DD, dolichocehpaly, thin CC and nonspecific white matter changes	*ZNF292*	>2	since published	PMID: 31723249	yes

1594	Extreme macrocephaly, distinctive features, DD, rapid weight gain	*DGKQ*	0			yes
1600	Childhood Osteoporosis and vertebral fractures	*USP34*	0			no
1601	Dysmorphic, Learning Issues, abnormal hand movements, anxiety	*AGO2*	>2	matchmaking (cohort)	PMID: 33199684	yes
1622	GDD, Hypotonia, Dysmorphic Facial Features, FTT, Self Injurious	*ABHD16A*	>2	matchmaking (cohort)	PMID: 34587489	no
1623	GDD, Nystagmus,hypotonia,undescended testes	*IQSEC1*	0			yes
1625	Hypotonia, Muscle weakness, Developmental Motor Disorder, Dysmorphic features	*MYO18A*	0			no
1647	ID, dysmorphic features, myoclonic seizures, autism	*THAP11*	0			no
1667	GDD, dystonia, ataxia, gaze paralysis, orofacial dyskinesia	*GPR156*	0			no
1694	Spastic quadraparesis, polymicrogyria, contractures, failture to thrive, microcephaly	*NRG1*	0			no
1716	short stature, learning disability	*RICTOR*	>2			no
1724	ID, optic atrophy-microcephaly	*MTSS1L*	>2	matchmaking (cohort)		yes
1725	Coarctation of the aorta, undescended testes, arrhythmia and speech delay	*ARID3B*	0			no
1766	ataxia and pontine glioma	*CSMD3*	0			no
1837	Developmental delay with regression and ID	*SCAI*	1	matchmaking (single family) and functional		yes
1846	Autism, cognitive impairment, hypotonia, joint hypermobility, myopathy	*CAPZB*	0			yes
1853	Mild ID, developmental coordination disorder, ADHD, astigmatism	*SEPHS1*	>2	matchmaking (cohort)		no
1866	Failure to thrive, distinctive facies, short stature, microcephaly, facial asymmetry, mild speech delay	*WDR13*	0			no

1869	Bilateral cystic dilation of the renal pelvis, multiple small renal cysts	*WNT9B*	>2	matchmaking (cohort) and segregation	PMID: 34145744	yes
1891	Nodular heterotopia, heart malformation, large primum ASD, FTT, mild GDD	*ZFHX3*	>2	matchmaking (cohort)		no
1954	GDD, hypomyelination, spastic quadriplegia, and seizures	*GAL3ST1*	0			no
1973	Severe GDD, ASD, seizures, abnormal hyperkinetic involuntary movements, generalized hypotonia	*CAMK4*	0			yes
2051	Seizures, cortical visual impairment, hearing impairment, GDD, immunodeficiency	*KIAA0391*	>2	matchmaking (cohort) and functional		yes
2058	Intellectual disability, Short stature, Behavioural issues, Oligodontia, Dysmorphic	*KIAA1033 (WASHC4)*	0			no
1783	Ptosis and myopathy with type 1 fiber prominence and myofiber size variation	*TSPAN6*	0			no
1789	Short stature, dysmorphism, hypotonia, epilepsy, DD	*EIF1AX*	>2	matchmaking (cohort)		no
1893	Moderate ID, psychiatric issues, long facies with broad forehead, mild hypertelorism, long tubular nose	*PUM2*	0			no
1907	Febrile seizures, complex partial seizures, ID, ADHD	*PHACTR1*	0			yes

Chapter 3: Examining how knowledge users have refined this knowledge base to knowledge tools

3.1. Preface

In Chapter 2 we learned that the knowledge base used in exome sequencing and genome sequencing (ES/GS) for rare genetic disease (RGD) diagnostic care is changing at a pace that has diagnostic implications. This creates a challenge for knowledge users that are relying on that knowledge base to provide clinical care. The concept of knowledge creation in the Knowledge-to-Action (KTA) framework is depicted as a funnel that includes the three major forms of knowledge that can be used in healthcare (Graham et al., 2006). Within the funnel, knowledge is refined into more useful forms: from knowledge inquiries (e.g., primary studies), to knowledge syntheses (e.g., systematic reviews with meta-analyses where appropriate), and finally the creation of knowledge tools and products (e.g., clinical guidance documents). A better understanding of how knowledge users have interacted with the evolving knowledge base related to ES/GS can inform how we optimize clinical care related to ES/GS in the future.

In this Chapter, I investigated Objective 2 of my dissertation "To examine how knowledge users have refined this ever-evolving knowledge base into clinical guidance." I did this by examining how one group of knowledge users, genetics healthcare professionals, has made use of knowledge related to ES/GS in knowledge tools, namely clinical guidance documents. We selected genetics healthcare professionals, including medical geneticists, genetic counsellors, and laboratory geneticists, because they have been at the forefront of implementation of ES/GS testing since its introduction as a research method in 2010, tasked with conducting this testing and providing clinical care to the patients receiving it. Here, I conducted a scoping review and critical appraisal of the clinical guidance documents related to ES/GS for

RGD diagnostic care produced by organizations representing genetics healthcare professionals.

Specifically, my sub-objectives for this Article were:

I. To describe the content (e.g., topics within recommendations) of clinical guidance documents related to the use of clinical exome and genome sequencing for the investigation of RGDs;

II. To examine the methodological quality (as per the AGREE II instrument) of these guidance documents and the processes by which they were generated; and,

III. To examine the methods and frequency by which these clinical guidance documents have been or will be updated based on new scientific evidence.

3.2. Statement of permission for use of copyrighted material

The following chapter consists of the manuscript entitled "Exome and genome sequencing for rare genetic disease diagnosis: A scoping review and critical appraisal of clinical guidance documents produced genetics professional organizations". This manuscript is in press at the journal *Genetics in Medicine*. As such, an embargo will be applied at time of final submission.

3.3. Contribution

Taila Hartley led the study design, developed the protocol for the scoping review, contributed to the design of the search strategy, did the grey literature search, was one of two reviewers who screened the documents, did all data extractions which were validated by a second author, was one of two critical appraisers using AGREE II, conducted all data analysis and interpretation of findings, and drafted the manuscript. The specific contributions of all co-authors are available in the section Author Contributions at the end of the Main Manuscript (Section 3.4).

3.4. Main manuscript

Exome and genome sequencing for rare genetic disease diagnosis: A scoping review and critical appraisal of clinical guidance documents produced by genetics professional organizations

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ABSTRACT

BACKGROUND: Exome and genome sequencing have rapidly transitioned from research methods to widely used clinical tests for diagnosing rare genetic diseases. We sought to synthesize the topics covered and appraise the development processes of clinical guidance documents generated by genetics professional organizations.

METHODS: We conducted a scoping review of guidance documents published since 2010, systematically identified in peer-reviewed and grey literature, using established methods and reporting guidelines. We coded verbatim recommendations by topic using content analysis and critically appraised documents using the Appraisal of Guidelines Research and Evaluation (AGREE) II tool.

RESULTS: We identified 30 guidance documents produced by eight organizations (2012-2022), yielding 611 recommendations covering 21 topics. The most common topic related to findings beyond the primary testing indication. Mean AGREE II scores were low across all six quality domains; scores for items related to rigour of development were among the lowest. More recently published documents generally received higher scores.

CONCLUSION: Guidance documents included a broad range of recommendations, but were of low quality, particularly in their rigour of development. Developers should consider using tools such as AGREE II and basing recommendations on living knowledge syntheses to improve guidance development in this evolving space.

INTRODUCTION

Over the past 12 years, exome sequencing and genome sequencing (ES/GS) technologies have transitioned from a research method used to identify new etiologies of rare genetic diseases (RGDs) (Ng et al., 2010) to an implemented diagnostic test available to millions of patients with suspected RGDs through healthcare systems around the world (Birney et al., 2017). These technologies have produced a paradigm shift in the field of Medical Genetics, ushering in an era of genomic discovery and providing an opportunity to shorten long diagnostic odysseys in the clinic (Bamshad et al., 2019; Boycott et al., 2019). This rapid pace of translation from research to clinical practice is unique amongst applications of genomic technologies related to precision medicine (Burke & Korngiebel, 2015) and has resulted in a significant challenge for healthcare system decision-makers, including clinicians and payers, responsible for developing optimal, evidence-based clinical approaches using ES/GS testing in the context of a naïve and rapidly evolving evidence base. Genetics healthcare professionals, including medical geneticists, laboratory geneticists, and genetic counselors, have been at the forefront of this challenge, tasked with identifying which families might benefit from ES/GS, running these tests, deciding what might be reported back to families (including findings unrelated to the primary indication for testing, hereon referred to as additional findings), and contextualizing these results. To support responsible and consistent practice amongst healthcare professionals within their membership, several organizations have generated guidance documents regarding the clinical implementation of ES/GS.

No study has systematically examined the guidance documents developed by organizations representing genetics professionals related to the use of ES/GS in RGD diagnosis. Therefore, this scoping review aimed to examine the question: *What is the nature and range of clinical guidance documents written by professional organizations representing genetics professionals for the use of ES/GS in RGD diagnostic care?* We were particularly interested in understanding which organizations have produced guidance

documents, the topics covered by recommendations, and the methodological quality of the documents. This understanding is imperative to inform future responses, including future iterations of available guidance documents, by identifying areas in need of new research to address gaps in the content of current guidance, identifying areas where the methods to develop guidance documents can be improved, and informing responses to future applications of genomics in medicine as a rapidly evolving area of science with substantial implications for clinical practice.

MATERIALS AND METHODS

Study criteria and search strategy

We designed this scoping review by following the 9 stage methodology from the Joanna Briggs Institute (The Joanna Briggs Institute, 2020) to identify and select the documents, extract relevant content, and synthesize the findings. We registered our protocol prospectively on the Open Science Framework (https://doi.org/10.17605/OSF.IO/HZWRC) and used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Scoping Review extension checklist (PRISMA-ScR) to guide reporting. (Tricco et al., 2018)

The search strategy for peer-reviewed literature was designed and conducted by a librarian experienced in systematic reviews (M.S.), and peer-reviewed by a second librarian using the PRESS guideline (**Supplemental Materials and Methods**) (McGowan et al., 2016). To identify guidance documents, we searched MEDLINE including Epub Ahead of Print, In-Process & Other Non-Indexed Citations (1946-March 1, 2022) and Embase (1980-March 1, 2022) using the Ovid Interface, and we searched TRIPDatabase. Searches were conducted March 2, 2022 and were language-inclusive but were limited to publications since 2010 to increase relevance, given that the first published research application of ES/GS for RGD diagnosis was in 2010 (Ng et al., 2010). We utilized two search strategies for grey

literature (**Supplemental Materials and Methods**). The first was a manual query of the websites of 84 national or international organizations representing genetics professionals from Organization for Economic Cooperation and Development (OECD)-participating countries, in February and March 2022. Next, we reviewed 23 international clinical practice guideline databases as guided by the Canadian Agency for Drugs and Technologies in Health tool for searching health-related grey literature, 'Grey Matters' (Canadian Agency for Drugs and Technologies in Health (CADTH), 2018) We also scanned reference lists of included documents for additional guidance documents.

Duplicates were removed from records retrieved by the electronic and grey literature search and final citations were uploaded to Covidence for two stages of screening. Using pre-specified inclusion/exclusion criteria (see below), two reviewers (T.H., M.G.) first independently screened the title and abstract of each record, and then independently screened full-text articles for citations that passed the first screen. Conflicts were resolved through consensus at both stages of screening.

We included documents that met the following predetermined eligibility criteria: (1) clinical guidance documents, (2) related to the use of ES/GS for RGD diagnosis within the healthcare system, (3) written by professional organizations in OECD-participating countries representing medical geneticists, laboratory geneticists, and/or genetic counsellors, (4) published between 2010 and March 2022. Documents related to opportunistic screening, referring to the secondary use of ES/GS data generated for the primary purpose of diagnostic testing in order to screen for other unrelated diseases, were eligible. We included all clinical guidance documents, regardless of whether they had been updated later, in order to ensure capture of the full range of documents and methodological processes over time**.** The search included all languages, but data extraction and methodological appraisal was limited to English documents due to a lack of expertise and resources for translation from other languages.

We excluded documents in which ES/GS for RGD diagnosis was not the primary focus of the recommendations (e.g., clinical recommendations related to diagnostic workups for particular RGDs or the application of ES/GS for heterogeneous indications within which RGD might be a minor sub-grouping). Similarly, we excluded documents related to the use of ES/GS for programmatic screening, where all members of a target population are systematically invited for a screening service.

Data extraction and synthesis of recommendations

We read each guidance document in order of publication date to ensure the context of the document and recommendations were understood. Data were extracted into two custom-built Excel spreadsheets (forms, see **Supplemental Materials and Methods**), a document-level sheet which included characteristics of the guidance documents, and a recommendation-level sheet to collect each of the verbatim recommendations (defined as a statement that recommends a course of action in clinical practice). We only extracted recommendations related to ES/GS testing for RGD diagnosis. All recommendations were extracted by a genetic counsellor (T.H.) and verified by another author (S.L.).

We used content analysis to identify and describe the topics covered by the recommendations (Hsieh & Shannon, 2005). Initial codes were identified using the sub-headings from the documents. These were refined to a final list of topic codes in an iterative process, merging and redefining codes addressing similar topics as data collection proceeded. A single recommendation could relate to multiple topics and was coded accordingly (i.e., with all applicable topics).

Methodological appraisal

We appraised all included documents using the Appraisal of Guidelines for Research and Evaluation (AGREE II) instrument, an internationally-developed, valid (Brouwers et al., 2020), and reliable (Brouwers, Kho, Browman, Burgers, Cluzeau, Feder, Fervers, Graham, Hanna, et al., 2010) tool designed to assess the methodological rigour of clinical practice guidelines (CPG)s, which are defined as 'systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances' (Brouwers, Kho, Browman, Burgers, Cluzeau, Feder, Fervers, Graham, Grimshaw, et al., 2010). AGREE II contains 23 items related to guideline quality, categorized in six domains. Each item is scored on a 7-point Likert scale; a score of 1 indicates that an item is not addressed and a score of 7 reflects a perfectly executed and described element as per the AGREE II training document (AGREE Next Steps Consortium, 2013). Scores of 2-6 are assigned when AGREE II criteria are partially met; higher scores indicate greater alignment with the considerations described in the AGREE II training document (AGREE Next Steps Consortium, 2013), Each domain assesses a single dimension of guideline quality, comprising: scope and purpose; stakeholder involvement; rigour of development; clarity of presentation; applicability; editorial independence.

AGREE II assessments were undertaken independently by two genetic counsellors (T.H., M.G.) with input from the last author (B.P.). We entered numerical scores and justifying notes into a custom built Excel spreadsheet (see **Supplemental Materials and Methods**). Four, one-hour meetings were held to derive final scores by consensus, to encourage conversation about the strengths and weaknesses of the documents. We examined AGREE II scores summarized by the 6 domains and for each of the 23 individual items. Domain scores were calculated by adding all item scores in a domain, and expressing as a percentage of the maximum possible score for that domain (AGREE Next Steps Consortium, 2013). We calculated the means and standard deviations of total domain scores, and median and interquartile range (IQR) for individual item scores.

RESULTS

Selected clinical guidance documents

From 2,616 records retrieved by the search, 30 clinical guidance documents were eligible for inclusion (**Figure 3.1**).

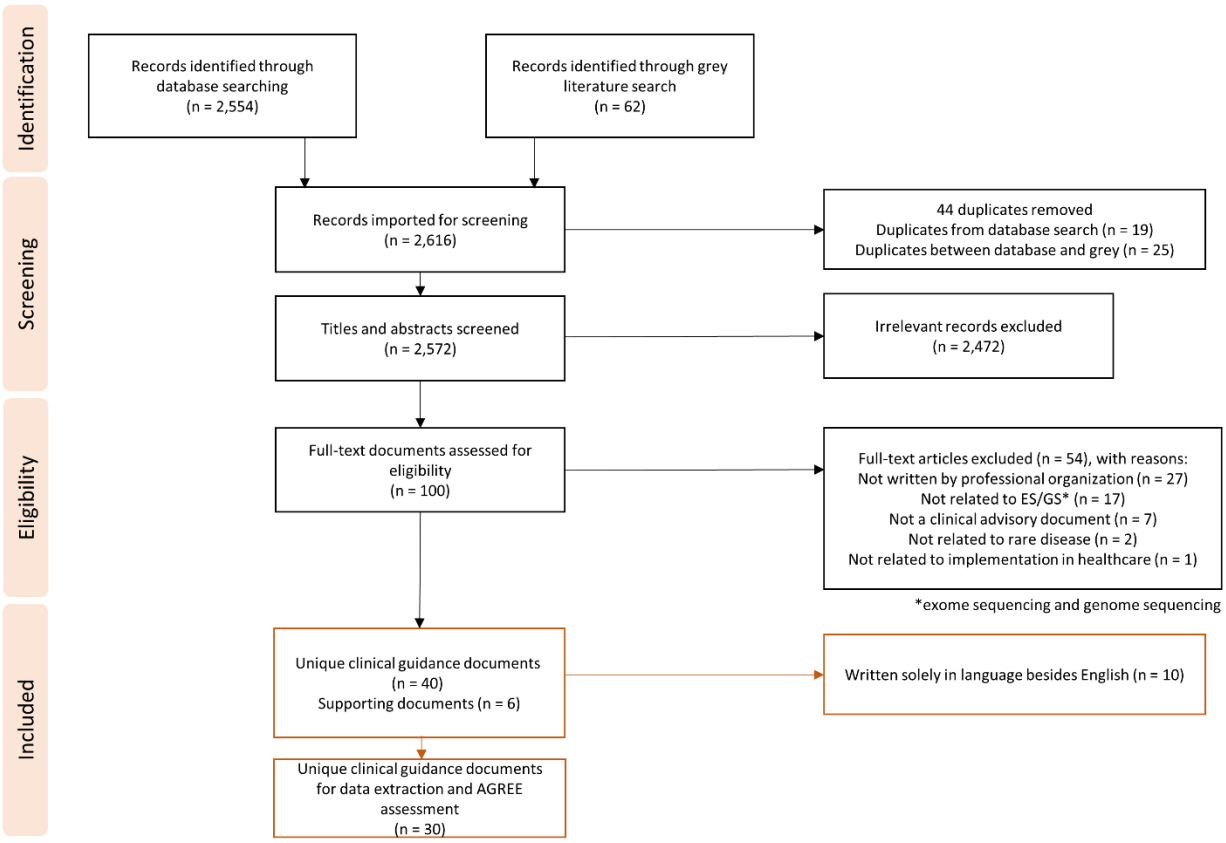
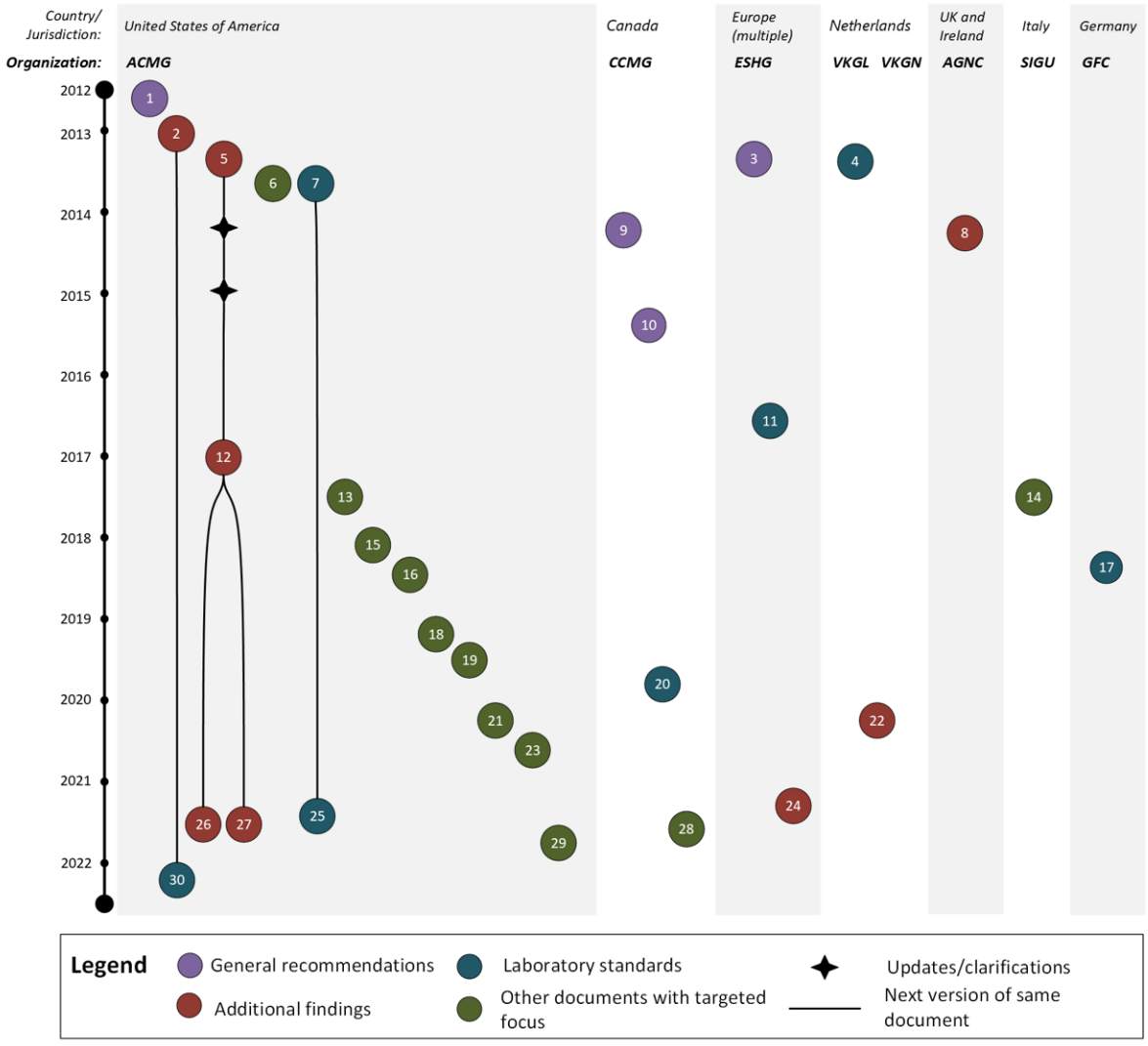


Figure 3.1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of document selection process. Numbers of records at each step of the review process are indicated.

Figure 3.2 depicts a timeline over which these documents were published and includes details of developer jurisdiction and the focus/subject matter of the documents. More than half (18/30) of the

documents were produced by the American College of Medical Genetics and Genomics (ACMG) (ACMG Board of Directors, 2012, 2013, 2017; Bush, Bartoshesky, et al., 2018; Bush, Beck, et al., 2018; David et al., 2019; Deignan et al., 2020; Deignan et al., 2019; Gonzales et al., 2022; Green et al., 2013; Kalia et al., 2017; Manickam et al., 2021; Miller, Lee, Chung, et al., 2021; Miller, Lee, Gordon, et al., 2021; Monaghan et al., 2020; Rehder et al., 2021; Rehder et al., 2013; Rehm et al., 2013), four were associated with the Canadian College of Medical Geneticists (CCMG) (Boycott et al., 2015; Hume et al., 2019; Lazier et al., 2022; Zawati et al., 2014), three with the European Society of Human Genetics (de Wert et al., 2021; Matthijs et al., 2016; van El et al., 2013), two with the Dutch Society for Clinical Genetic Laboratory Diagnostics (VKGL) [one in collaboration with the Association of Clinical Genetics Netherlands (VKGN)] (Stemkens, 2020; Weiss et al., 2013), and one each with the Association for Genetic Nurses of the United Kingdom and Ireland (Middleton et al., 2014), the German Society of Human Genetics (GFH) (Bauer et al., 2018), and the Italian Society of Human Genetics (SIGU) (Borghesi et al., 2017). Four types of documents were identified based on their subject matter (**Figure 3.2**): (i) those that provided general and introductory recommendations related to the use of ES/GS, targeted at a range of audiences; (ii) those that provided clinical and/or laboratory guidance specific to additional findings; (iii) those focused on technical standards for laboratories; and (iv) those that provided focused guidance related to a specific clinical scenario. Three of the eight organizations (i.e., the ACMG, CCMG, and ESHG) first published general recommendations related to the use of ES/GS prior to more targeted documents. Five of the 30 documents (16%) were newer versions of previously published documents.



Identifier	Title	Reference
1	Policy Statement - Points to Consider in the Clinical Application of Genomic Sequencing	15
2	American College of Medical Genetics and Genomics: standards and guidelines for documenting suspected consanguinity as an incidental finding of genomic testing	16
3	Whole-genome sequencing in health care	37
4	Best Practice Guidelines for the Use of Next-Generation Sequencing Applications in Genome Diagnostics: A National Collaborative Study of Dutch Genome Diagnostics Laboratories	40
5	ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing	17
6	Points to consider for informed consent for genome/exome sequencing	18
7	ACMG clinical laboratory standards for next-generation sequencing	19
8	Position statement on opportunistic genomic screening from the Association of Genetic Nurses and Counsellors (UK and Ireland)	42
9	Reporting results from whole-genome and whole-exome sequencing in clinical practice: a proposal for Canada?	33
10	The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists	34
11	Guidelines for diagnostic next-generation sequencing	38
12	Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics	20
13	Laboratory and clinical genomic data sharing is crucial to improving genetic health care: a position statement of the American College of Medical Genetics and Genomics	21
14	Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant	44
15	Professional responsibilities regarding the provision, publication, and dissemination of patient phenotypes in the context of clinical genetic and genomic testing: Points to consider-A statement of the American College of Medical Genetics and Genomics (AC	22
16	Pediatric clinical exome/genome sequencing and the engagement process: Encouraging active conversation with the older child and adolescent: Points to consider - A statement of the American College of Medical Genetics and Genomics (ACMG)	23
17	S1 guideline: Molecular genetic diagnostic testing using high-throughput screening of the germline, such as next-generation sequencing	43
18	Patient re-contact after revision of genomic test results: points to consider A statement of the American College of Medical Genetics and Genomics (ACMG)	24
19	Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG)	25
20	CCMG practice guideline: laboratory guidelines for next-generation sequencing	35
21	The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG)	26
22	Dutch national consensus-based guideline for the disclosure of incidental findings during clinical genetic diagnostic testing	41
23	Points to consider when assessing relationships (or suspecting misattributed relationships) during family-based clinical genomic testing: a statement of the American College of Medical Genetics and Genomics (ACMG)	27
24	Opportunistic genomic screening. Recommendations of the European Society of Human Genetics	39
25	Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG)	28
26	ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG)	29
27	Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG)	30
28	Clinical application of fetal genome-wide sequencing during pregnancy: position statement of the Canadian College of Medical Geneticists	36
29	Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG)	31
30	Interpretation and reporting of large regions of homozygosity and suspected consanguinity/uniparental disomy, 2021 revision: A technical standard of the American College of Medical Genetics and Genomics (ACMG)	32

Figure 3.2. Schematic of clinical guidance document timeline, jurisdiction, and types of subject matter. Thirty clinical guidance documents affiliated with eight genetics professional organizations were identified, the first of which was published in 2012. Four types of documents were identified, those that provided general recommendation, those that provided clinical and/or laboratory guidance specific to additional findings outside of the indication for ES/GS testing, those that provided technical standards for laboratories, and other documents with targeted focuses. Five documents were newer versions of previous documents.

Topic areas covered by recommendations

We extracted 611 unique recommendations from the 30 documents. The mean number of recommendations in each document was 20, but this ranged from 1 to 92. We identified 21 unique topics (**Table 3.1**): three related to the pre-testing period in the clinic, seven related to clinical laboratory procedures, four specific to the post-testing period, and seven related to general topics. The extracted recommendations and associations with one or more topics are available in **Supplemental Table 3.1**.

The most commonly observed pre-test topic related to counselling, consenting, or assenting families to receive ES/GS (13%). Other pre-test recommendation topics pertained to the indications for which ES/GS should be considered, including the clinical assessment related to such and the selection of appropriate ES/GS testing strategy (6%), and the types of professionals and expertise needed to offer, collect consent for, and order ES/GS (1%).

The most common recommendation topics in the testing period related to how laboratories should set up (18%), conduct (16%), and report (22%) ES/GS testing. The following two most common testing topics were related to communication between the clinical laboratories and healthcare providers, patients, and the public. These recommendations highlighted protocols, policies, and documents that laboratories should have available (9%), and opportunities for direct communication between the clinical care teams and the testing laboratory before, during, or after testing (7%). The final two topics in the testing period related to laboratory data storage practices (3%) and the expertise needed to run this test (2%).

Table 3.1. Topics amongst 611 recommendations extracted from 30 clinical guidance documents related to exome and genome sequencing (ES/GS) for rare genetic disease (RGD) diagnosis

	Recommendation topics	Number of recommendations addressing topic	Percentage of recommendations addressing topic
Pre-testing	*Counselling, consenting, and assenting families*	79	13%
	Pre-test clinical assessment, appropriate indications, and selection of ES/GS test	38	6%
	Types of professionals and expertise needed to offer, consent to, and order ES/GS	7	1%
Testing	*Results reporting*	136	22%
	Test development, validation, tracking	111	18%
	Sequencing and analysis	99	16%
	Laboratory protocol, policy, document development	56	9%
	Communication between clinical care teams and testing laboratories	41	7%
	Data storage and internal databases	18	3%
	Types of professionals and expertise needed to conduct this test	14	2%
Post-testing	*Reanalysis, reinterpretation, re-evaluation of ES/GS data*	43	7%
	Clinical interpretation, return of results, and clinical care	42	7%
	Data sharing	23	4%
	Types of professionals and expertise needed to interpret ES/GS results	5	1%
General	*Additional findings*	158	26%
	Considerations for family members	37	6%
	Future research, guideline, and framework development	31	5%
	Ethical and legal considerations for implementation	12	2%
	Navigation of the clinical and research boundary in ES/GS	11	2%
	Healthcare system impact, costs	9	1%
	Genomic education	7	1%

Abbreviations: ES/GS = exome sequencing and genome sequencing

In the post-test period, recommendation topics included immediate interpretation, return of results, and ensuing clinical care for patients (7%), and the expertise needed for such interpretation (1%). The most frequent post-test topic, however, was related to the reanalysis of genomic data, reinterpretation of data using standardized variant assessment criteria, and re-evaluation of data in the context of patient phenotype at a later date (7%). Another topic covered by post-test recommendations related to data sharing between laboratories and external groups (4%), and primarily consisted of recommendations for sharing genomic data with public databases to aid in future interpretations.

The most common topic considered by recommendations overall related to additional findings from ES/GS unrelated to the primary indication. This topic was addressed in 158 (26%) recommendations. Other general topics included considerations for family members of patients receiving ES/GS testing (6%), advice for navigating the complex grey area in genomic medicine between clinical care and research (2%), ethical/legal considerations for implementation (2%), considerations related to healthcare system impacts and costs (1%), and targets for genomic education (1%).

Thirty one (5%) of the recommendations highlighted areas for future research, guideline, and framework development. More than half of these stated knowledge gaps related to additional findings (21/31, 68%), including the need for robust evidence addressing whether the benefits of opportunistic screening outweigh the potential harms in terms of over-diagnosis (particularly for under-represented populations), unnecessary treatment, and the possibility of current or future psychological harm for all age groups and their extended families (de Wert et al., 2021; Matthijs et al., 2016; Middleton et al., 2014; van El et al., 2013). These recommendations also conveyed a need to generate data regarding the resource implications of additional findings, with capacity building for clinicians, clinical laboratories, and healthcare systems (de Wert et al., 2021; Miller, Lee, Gordon, et al., 2021). Beyond additional

findings, recommendations highlighted the importance of gathering more patient perspectives and further data sharing from laboratories and healthcare systems to move beyond preliminary advice and develop best practices related to all aspects of ES/GS testing at local, national, and international levels (van El et al., 2013). This includes the need for further evidence and guidance regarding the utility of ES/GS for specific circumstances [fetuses or specific clinical indications for example (Monaghan et al., 2020)]. Guidance documents also specified that data sharing to support research into the etiologies of RGDs should continue (Bush, Beck, et al., 2018).

Critical appraisal

The full set of consensus-derived AGREE II scores are provided in **Supplemental Table 3.2**. Collectively, documents scored low in all domains, with mean domain scores ranging from 17% (SD=22%) in Domain 3: Rigour of Development to 52% (SD=15%) in Domain 4: Clarity of Presentation (**Figure 3.3**). When we stratified documents into three publication time periods (2012-2015, 2016-2019, and 2020-2022), scores improved over time in all domains except Domain 6: Editorial Independence (**Figure 3.3**).

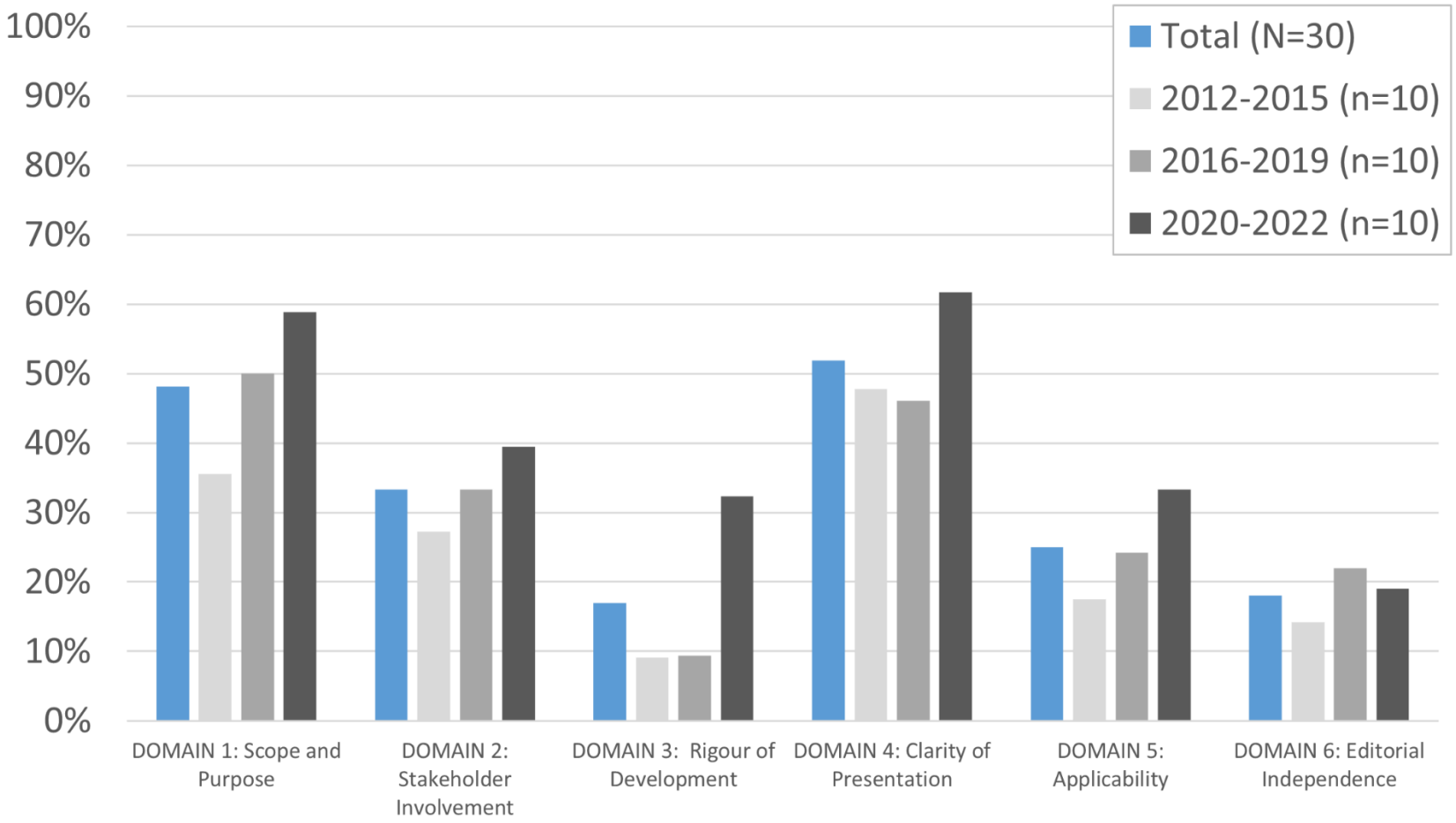


Figure 3.3. Clinical guidance document consensus scores by AGREE II domain. Bar chart of mean total domain scores (%) presented for all documents (Total, N=30) and stratified by publication time periods: the first guidance documents published (2012-2015), a middle time period (2016-2019), and the most recent guidance documents (2020-2022). Each time period included 10 documents. The most recent group of documents had the highest mean total score for all but one domain (Domain 6: Editorial independence).

The two items with the highest median scores were within Domain 4: Clarity of Presentation, and included 'key recommendations are easily identifiable' (item 17, median 5) and 'the recommendations are specific and unambiguous' (item 15, median 4.5) (**Figure 3.4**). The next highest median scores (4) were two items from Domain 1: Scope and Purpose ['the overall objective(s) of the advisory document is (are) specifically described' (item 1) and 'the population (patients, public, etc.) to whom the advisory document is meant to apply is specifically described' (item 3)] and one item from Domain 2: Stakeholder Involvement ['the target users of the advisory document are clearly defined' (item 6)]. Nine items had median scores of 1, meaning that the item was not reported in at least half of the documents. Five of these items were within Domain 3: Rigour of Development and included the items 'systematic methods were used to search for evidence' (item 7), 'the criteria for selecting the evidence are clearly described' (item 8), 'the strengths and limitations of the body of evidence are clearly described' (item 9), 'the methods for formulating the recommendations are clearly described' (item 10), and 'a procedure for updating the advisory document is provided' (item 14). Two items were within Domain 5: Applicability; 'the potential resource implications of applying the recommendations have been considered' (item 20) and 'the advisory document presents monitoring and/or auditing criteria' (item 21). The other two items were 'the views and preferences of the target population (patients, public, etc.) have been sought' (item 5, Domain 2) and 'the views of the funding body have not influenced the content of the advisory document' (item 22, Domain 6). The strengths of these documents were therefore related to the clarity of writing and clear purposes and audiences for which they were written. The main weaknesses were related to the rigour of the methods that organizations used/reported to translate evidence into recommendations.

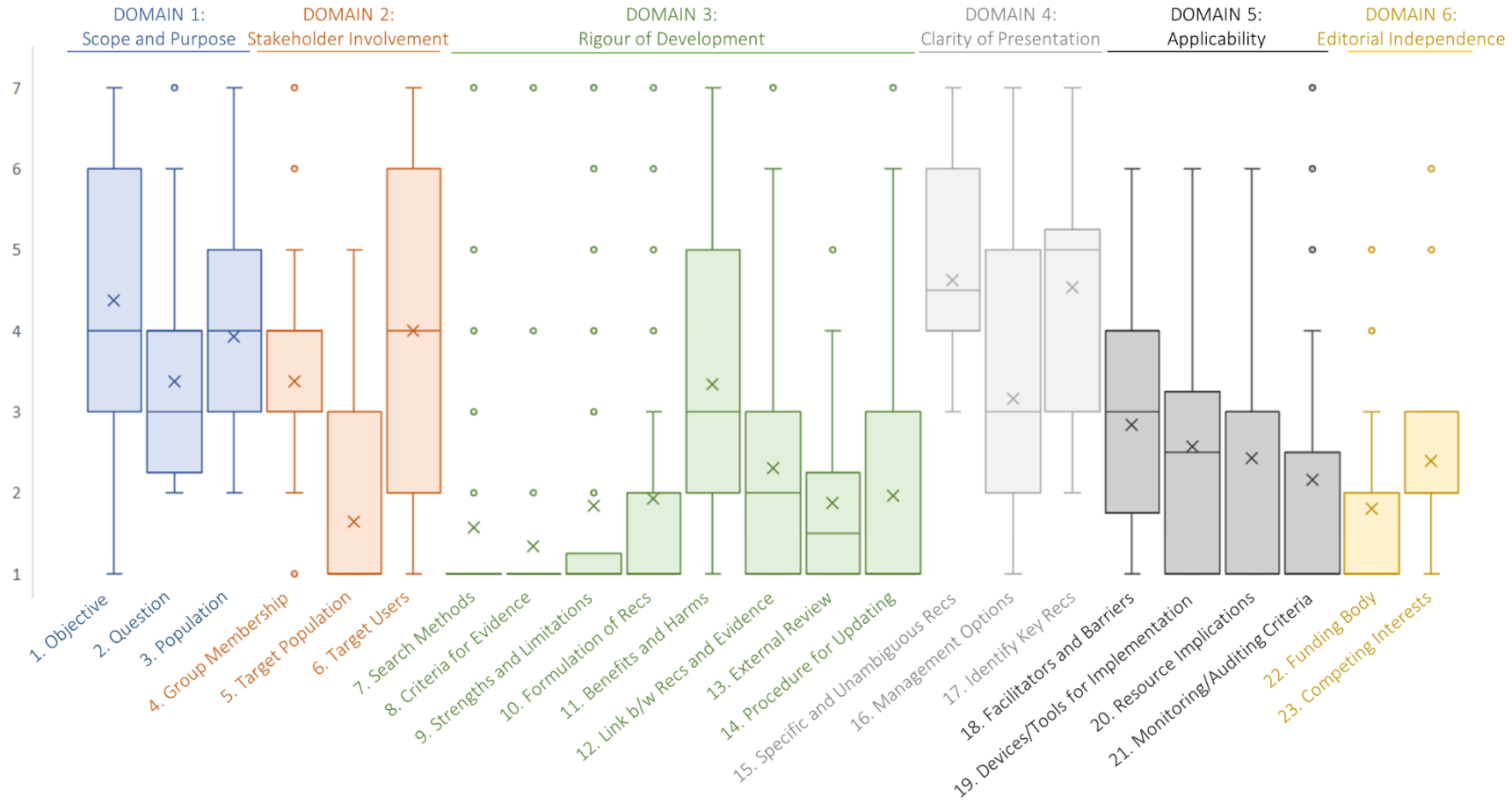


Figure 3.4. Clinical guidance document consensus scores by AGREE II appraisal item. Boxplot of individual item scores, each rated on a 7-point Likert scale, for the 23 items of the AGREE II tool. Denoted are the interquartile range (IQR, box), the median (line), the mean (X), the range of data (whiskers), and outlier observations (dots) defined as more than 1.5 times the IQR greater than the third quartile or less than the first quartile. Highest median scores were observed in items 1, 3, 6, 15, 17. Lowest median scores were observed in items 5, 7, 8, 9, 14, 20, 21, and 22.

DISCUSSION

This review is the first synthesis of guidance documents by organizations representing genetics professionals related to the use of ES/GS for RGD diagnostics. We identified 30 guidance documents written by eight organizations, although the majority of documents (18/30, 60%) were written by the ACMG. From these, we extracted the verbatim recommendations, described a broad range of topics covered by the recommendations, and appraised the methodological processes used in their development to highlight areas in need of improvement.

In total, we extracted 611 recommendations covering 21 topics. The topics we identified highlight unique aspects of ES/GS testing, including the increased likelihood of identifying additional findings, the potential need to test additional family members, the complexity of results and need for follow-up investigations, how to navigate the clinical/research boundary in genomic medicine, and the need for data sharing to enable better interpretation of ES/GS data in the future. The full set of recommendations represents a broad set of topics, with multiple organizations providing guidance on each given topic. We have made these recommendations available in **Supplemental Table 3.1** to enable knowledge users to see all unique recommendations within a particular document, by a particular organization, or related to a particular topic. Important caveats include the fact that new guidance documents have already been published by these organizations (Deans et al., 2022), recommendations will likely evolve over time, and the recommendations identified were not vetted for their level of evidence. This table has other potential applications, including as a tool to train computational strategies for identifying clinical recommendations related to genetics [e.g., similar to Hussain et al. (2018)].

Regarding methodological quality, overall, documents scored low in all domains of AGREE II. While organizations did not claim to be developing 'clinical practice guidelines', often labeling documents as 'consensus statements', 'points to consider', or 'policy statements', we offer this analysis to advance evidence-informed practice in genomic medicine by recognising areas of strength and identifying areas in need of improvement. The highest mean domain scores were within the domains for 'clarity of presentation' and 'scope of purpose', which is consistent with systematic reviews of guidance documents on other topics (Armstrong et al., 2016; Font-Gonzalez et al., 2016; Hayawi et al., 2018; Vale et al., 2021). The lowest mean domain scores were within the domains for 'editorial independence' and 'rigour of development'. Within the latter, in five of eight items the median domain score was one, indicating that at least half of the documents did not have robust processes of development in place or did not report their processes adequately. This relates to how evidence was sought and used in the development of recommendations, and aligns, as a relative weakness, with guidance documents in other areas (Vale et al., 2021). We stress the importance of explicitly communicating the methods used in making recommendations so that health professionals understand the strengths and limitations of the evidence that underpins a clinical recommendation they are considering implementing. We also stress the importance of conducting and reporting robust evidence syntheses, regardless of whether a developer thinks there is adequate high-quality evidence available. A systematic search that finds evidence lacking may be used, for example, as rationale for guidance developers to rely on expert consensus to make recommendations. Given that the majority of recommendations are not currently underpinned by robust knowledge syntheses and knowledge continues to evolve, organizations should consider developing 'living systematic reviews' to inform guidance, in which search strategies are repeated at regular intervals (for example, every 12 months) and sequential analyses and publications are conducted based on *a priori* protocols. This strategy has been applied in other rapidly evolving areas (Elliott et al., 2018; Fragkou et al., 2022; Iannizzi et al., 2023). Further, the development and reporting of

a priori protocols for updating evidence would enable organizations to notify their memberships of anticipated timelines for updated guidance documents.

Four other specific items had median scores of one. These included the consideration of potential resource implications of applying the recommendations, the provision of monitoring or auditing criteria, the description of relevant funding sources for guideline development, and the inclusion of views and preferences of the target population. Developers should consider these elements in future guidance. Of particular note, 22 of the guidance documents (73%) did not report the incorporation of the views and preferences of patients or their family members with lived experience of RGDs. This is an important observation given that the ultimate purpose of clinical guidance is to improve patient outcomes, implying a need for developers to understand the outcomes that are of highest priority to patients (Montori et al., 2013). Burke et al. (2019) highlighted the controversy related to the first ACMG guidance on additional findings (Green et al., 2013) as an example of the importance of incorporating all relevant perspectives in the development process. Here, a guidance document was published, but was met with immediate pushback (Holtzman, 2013; Townsend A, 2013; Wolf SM, 2013) resulting in the release of a clarification (American College of Medical Genetics & Genomics, 2013) and then formal updating of recommendations (ACMG Board of Directors, 2015) (**Figure 3.2**) after a survey of the ACMG's membership (Scheuner et al., 2015). Thus, it is particularly important that varied perspectives be included throughout the process of guidance development, and all the more critical to do so when the evidentiary base for recommendations is not robust (making decisions more preference sensitive).

The LawSeq project has made similar recommendations for improving guidance documents related to genomic testing that are produced prior to sufficient evidence for more definitive clinical practice guidelines, including the need to specify uncertainties in making recommendations and inclusion of

patients in the development process (Burke et al., 2019). As highlighted by the topics we identified in recommendations related to future research, guidance developers recognize a need for further development of recommendations for best practices, informed by the evolving evidence base. It is likely that the quality of guidance documents will increase in the future as a natural transition from preliminary guidance to definitive best practices occurs (Brouwers, Kho, Browman, Burgers, Cluzeau, Feder, Fervers, Graham, Grimshaw, et al., 2010; Vale et al., 2021). We did see some improvement over time in almost all domains (Figure 3A) and some specific recent guidance documents received high AGREE II scores across a number of items and domains, including rigour of development (Manickam et al., 2021). We encourage developers to use AGREE II (Brouwers, Kho, Browman, Burgers, Cluzeau, Feder, Fervers, Graham, Grimshaw, et al., 2010) or another instrument such as Institute of Medicine (IOM) standards (Institute of Medicine Committee on Standards for Developing Trustworthy Clinical Practice Guidelines, 2011) to guide the development of robust guidance documents. We also recommend that healthcare practitioners and payers consider improving their guideline appraisal skills, using these same tools, in order to better understand the strengths and limitations of individual guidance documents they may consider implementing.

Our study had limitations. Although our search strategy was comprehensive, it is possible that additional guidance documents that would be eligible for our review exist outside of this search. Our grey literature search identified another 10 documents that might be of interest but because of language constraints we were not able to review them. In addition, it is somewhat subjective to extract recommendations from guidance documents; we may have missed or misinterpreted recommendations but attempted to mitigate this risk by having a second author verify extracted data. Further, while the AGREE II instrument assesses the development process, it does not assess the quality of the specific recommendations within a guidance document, which was not part of our study objectives. For

practitioners interested in evaluating the quality of individual recommendations, AGREE-REX is a tool that is separate and complementary to AGREE II (Brouwers et al., 2020), which can be used to examine whether recommendations are credible and implementable within a specific context.

Conclusion

Our scoping review identified 30 documents written over a 10-year period by eight organizations related to the use of ES/GS for the diagnosis of RGDs. Professional organizations have made a broad range of recommendations; we extracted 611 recommendations and identified 21 unique topics that these recommendations covered. We identified several knowledge gaps to be addressed in future work. Most prominently, organizations developing guidance recognized the need for research related to the benefits and harms of opportunistic screening for additional findings beyond the primary indication for testing; additional findings was also the most commonly addressed topic in the recommendations within the guidance we reviewed, highlighting this as a priority in the field. Our appraisal of documents identified significant weaknesses in the methodological processes, or reporting of such, that should be addressed as the community works towards new versions of current guidance, guidance documents related to new topics, and definitive CPGs. In particular, guidance developers should prioritize improving methodological rigour with respect to the identification and synthesis of evidence to inform recommendations, for which developers might consider *a priori* protocols for living systematic reviews. Developers should also consider partnering with patients in guideline co-development, consider the resource implications of their recommendations, develop monitoring criteria when making recommendations, and report funding details.

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AUTHOR CONTRIBUTIONS

Conceptualization: T.H., K.M.B., B.P.; Document Identification: T.H., M.G.; Data Extraction: T.H., S.L.; Critical Appraisal: T.H., M.G., B.P.; Formal Analysis: T.H.; Funding Acquisition: K.M.B., Methodology: T.H., K.M.B., B.P., I.G.; Writing: T.H., B.P.; Writing – review and editing: T.H., B.P., M.G., M.S., I.G., R.H., K.M.B., S.L.; Supervision: I.G., R.H.

CONFLICT OF INTERESTS

The authors declare no competing interests.

3.5. References

ACMG Board of Directors. (2017). Laboratory and clinical genomic data sharing is crucial to improving genetic health care: a position statement of the American College of Medical Genetics and Genomics. *Genet Med*, *19*(7), 721-722. https://doi.org/10.1038/gim.2016.196

ACMG Board of Directors. (2012). Policy Statement - Points to Consider in the Clinical Application of Genomic Sequencing. *Genet Med*, *14*, 759-761.

ACMG Board of Directors. (2013). Points to consider for informed consent for genome/exome sequencing. *Genet Med*, *15*(9), 748-749. https://doi.org/10.1038/gim.2013.94

ACMG Board of Directors. (2015). ACMG policy statement: Updated recommendations regarding analysis and reporting of secondary findings in clinical genome-scale sequencing. *Genet Med*, *17*(1), 68-69.

AGREE Next Steps Consortium. (2013). The AGREE II Instrument [Electronic version]. Retrieved March 1, 2022, from https://www.agreetrust.org/

American College of Medical Genetics & Genomics. (2013). Incidental findings in clinical genomics: A clarification. *Genet Med*, *15*(8), 664-666. https://doi.org/10.1038/gim.2013.82

Armstrong, J. J., Rodrigues, I. B., Wasiuta, T., & MacDermid, J. C. (2016). Quality assessment of osteoporosis clinical practice guidelines for physical activity and safe movement: An AGREE II appraisal. *Arch Osteoporos*, *11*, 6. https://doi.org/10.1007/s11657-016-0260-9

Bamshad, M. J., Nickerson, D. A., & Chong, J. X. (2019). Mendelian gene discovery: Fast and furious with no end in sight. *Am J Hum Genet*, *105*(3), 448-455. https://doi.org/10.1016/j.ajhg.2019.07.011

Bauer, P., Wildhardt, G., Gläser, D., Müller-Reible, C., J. Bolz, H., Klein, H.-G., Finckh, U., & Hehr, U. (2018). German guidelines for molecular genetic diagnostic testing using high-throughput technology, such as next-generation sequencing. *OBM Genetics*, *2*(2), 1-1. https://doi.org/10.21926/obm.genet.1802019

Birney, E., Vamathevan, J., & Goodhand, P. (2017). Genomics in healthcare: GA4GH looks to 2022. *bioRxiv.* https://doi.org/10.1101/203554

Borghesi, A., Mencarelli, M. A., Memo, L., Ferrero, G. B., Bartuli, A., Genuardi, M., Stronati, M., Villani, A., Renieri, A., Corsello, G., & their respective Scientific, S. (2017). Intersociety policy statement on the use of whole-exome sequencing in the critically ill newborn infant. *Ital J Pediatr*, *43*(1), 100. https://doi.org/10.1186/s13052-017-0418-0

Boycott, K., Hartley, T., Adam, S., Bernier, F., Chong, K., Fernandez, B. A., Friedman, J. M., Geraghty, M. T., Hume, S., Knoppers, B. M., Laberge, A. M., Majewski, J., Mendoza-Londono, R., Meyn, M. S., Michaud, J. L., Nelson, T. N., Richer, J., Sadikovic, B., Skidmore, D. L., . . . Canadian College of Medical Geneticists. (2015). The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet*, *52*(7), 431-437. https://doi.org/10.1136/jmedgenet-2015-103144

Boycott, K. M., Hartley, T., Biesecker, L. G., Gibbs, R. A., Innes, A. M., Riess, O., Belmont, J., Dunwoodie, S. L., Jojic, N., Lassmann, T., Mackay, D., Temple, I. K., Visel, A., & Baynam, G. (2019). A diagnosis for all rare genetic diseases: The horizon and the next frontiers. *Cell*, *177*(1), 32-37. https://doi.org/10.1016/j.cell.2019.02.040

Brouwers, M. C., Kho, M. E., Browman, G. P., Burgers, J. S., Cluzeau, F., Feder, G., Fervers, B., Graham, I. D., Grimshaw, J., Hanna, S. E., Littlejohns, P., Makarski, J., Zitzelsberger, L., & AGREE Next Steps Consortium. (2010). AGREE II: Advancing guideline development, reporting and evaluation in health care. *CMAJ*, *182*(18), E839-842. https://doi.org/10.1503/cmaj.090449

Brouwers, M. C., Kho, M. E., Browman, G. P., Burgers, J. S., Cluzeau, F., Feder, G., Fervers, B., Graham, I. D., Hanna, S. E., Makarski, J., & AGREE Next Steps Consortium (2010). Development of the AGREE II, part 1: performance, usefulness and areas for improvement. *CMAJ*, *182*(10), 1045-1052. https://doi.org/10.1503/cmaj.091714

Brouwers, M. C., Spithoff, K., Kerkvliet, K., Alonso-Coello, P., Burgers, J., Cluzeau, F., Fervers, B., Graham, I., Grimshaw, J., Hanna, S., Kastner, M., Kho, M., Qaseem, A., Straus, S., & Florez, I. D. (2020). Development and validation of a tool to assess the quality of clinical practice guideline recommendations. *JAMA Netw Open*, *3*(5), e205535. https://doi.org/10.1001/jamanetworkopen.2020.5535

Burke, W., Clayton, E. W., Wolf, S. M., Berry, S. A., Evans, B. J., Evans, J. P., Hall, R., Korngiebel, D., Laberge, A. M., LeRoy, B. S., & McGuire, A. L. (2019). Improving recommendations for genomic medicine: building an evolutionary process from clinical practice advisory documents to guidelines. *Genet Med*, *21*(11), 2431-2438. https://doi.org/10.1038/s41436-019-0549-3

Burke, W., & Korngiebel, D. M. (2015). Closing the gap between knowledge and clinical application: challenges for genomic translation. *PLoS Genet*, *11*(2), e1004978. https://doi.org/10.1371/journal.pgen.1004978

Bush, L. W., Bartoshesky, L. E., David, K. L., Wilfond, B., Williams, J. L., Holm, I. A., & ACMG SELI committee. (2018). Pediatric clinical exome/genome sequencing and the engagement process: encouraging active conversation with the older child and adolescent: points to consider-a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *20*(7), 692-694. https://doi.org/10.1038/gim.2018.36

Bush, L. W., Beck, A. E., Biesecker, L. G., Evans, J. P., Hamosh, A., Holm, I. A., Martin, C. L., Richards, C. S., & Rehm, H. L. (2018). Professional responsibilities regarding the provision, publication, and dissemination of patient phenotypes in the context of clinical genetic and genomic testing: points to consider-a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *20*(2), 169-171. https://doi.org/10.1038/gim.2017.242

Canadian Agency for Drugs and Technologies in Health (CADTH). (2018). *Grey Matters: A Practical Tool For Searching Health-Related Grey Literature*. https://www.cadth.ca/resources/finding-evidence

David, K. L., Best, R. G., Brenman, L. M., Bush, L., Deignan, J. L., Flannery, D., Hoffman, J. D., Holm, I., Miller, D. T., O'Leary, J., Pyeritz, R. E., & ACMG Social Ethical Legal Issues Committee (2019). Patient re-contact after revision of genomic test results: points to consider-a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *21*(4), 769-771. https://doi.org/10.1038/s41436-018-0391-z

de Wert, G., Dondorp, W., Clarke, A., Dequeker, E. M. C., Cordier, C., Deans, Z., van El, C. G., Fellmann, F., Hastings, R., Hentze, S., Howard, H., Macek, M., Mendes, A., Patch, C., Rial-Sebbag, E., Stefansdottir, V., Cornel, M. C., Forzano, F., & European Society of Human Genetics. (2021). Opportunistic genomic screening. Recommendations of the European Society of Human Genetics. *Eur J Hum Genet*, *29*(3), 365-377. https://doi.org/10.1038/s41431-020-00758-w

Deans, Z.C., Ahn, J., Carreira, I.M., Dequeker, E., Henderson, M., Lovrecic, L., Õunap, K., Tabiner, M., Treacy, R., & van Asperen, C.J. (2022). Recommendations for reporting results of diagnostic genomic testing. *Eur J Hum Genet*, *30*(9), 1011-1016. https://doi.org/doi: 10.1038/s41431-022-01091-0

Deignan, J. L., Chao, E., Gannon, J. L., Greely, H. T., Hagman, K. D. F., Mao, R., Topper, S., on behalf of the ACMG Laboratory Quality Assurance Committee. (2020). Points to consider when assessing relationships (or suspecting misattributed relationships) during family-based clinical genomic testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *22*(8), 1285-1287. https://doi.org/10.1038/s41436-020-0821-6

Deignan, J. L., Chung, W. K., Kearney, H. M., Monaghan, K. G., Rehder, C. W., Chao, E. C., on behalf of the ACMG Laboratory Quality Assurance Committee (2019). Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *21*(6), 1267-1270. https://doi.org/10.1038/s41436-019-0478-1

Elliott, J., DeJean, D., Clifford, T., Coyle, D., Potter, B., Skidmore, B., Alexander, C., Repetski, A. E., McCoy, B., & Wells, G. A. (2018). Cannabis for pediatric epilepsy: protocol for a living systematic review. *Syst Rev*, *7*(1), 95. https://doi.org/10.1186/s13643-018-0761-2

Font-Gonzalez, A., Mulder, R. L., Loeffen, E. A., Byrne, J., van Dulmen-den Broeder, E., van den Heuvel-Eibrink, M. M., Hudson, M. M., Kenney, L. B., Levine, J. M., Tissing, W. J., van de Wetering, M. D., Kremer, L. C., & PanCare, L. C. (2016). Fertility preservation in children, adolescents, and young adults with cancer: Quality of clinical practice guidelines and variations in recommendations. *Cancer*, *122*(14), 2216-2223. https://doi.org/10.1002/cncr.30047

Fragkou, P. C., Moschopoulos, C. D., Dimopoulou, D., Ong, D. S. Y., Dimopoulou, K., Nelson, P. P., Schweitzer, V. A., Janocha, H., Karofylakis, E., Papathanasiou, K. A., Tsiordras, S., De Angelis, G., Thölken, C., Sanguinetti, M., Chung, H. R., & Skevaki, C. (2022). Performance of point-of care molecular and antigen-based tests for SARS-CoV-2: a living systematic review and meta-analysis. *Clin Microbiol Infect*. https://doi.org/10.1016/j.cmi.2022.10.028

Genetic Testing Advisory Committee. (2016). *Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario*. Retrieved March 30, 2023, from http://www.health.gov.on.ca/en/pro/programs/gtac/reports.aspx

Gonzales, P. R., Andersen, E. F., Brown, T. R., Horner, V. L., Horwitz, J., Rehder, C. W., Rudy, N. L., Robin, N. H., Thorland, E. C., on behalf of the ACMG Laboratory Quality Assurance Committee. (2022). Interpretation and reporting of large regions of homozygosity and suspected consanguinity/uniparental disomy, 2021 revision: A technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *24*(2), 255-261. https://doi.org/10.1016/j.gim.2021.10.004

Graham, I. D., Logan, J., Harrison, M. B., Straus, S. E., Tetroe, J., Caswell, W., & Robinson, N. (2006). Lost in knowledge translation: Time for a map? *J Contin Educ Health Prof*, *26*(1), 13-24. https://doi.org/10.1002/chp.47

Green, R. C., Berg, J. S., Grody, W. W., Kalia, S. S., Korf, B. R., Martin, C. L., McGuire, A. L., Nussbaum, R. L., O'Daniel, J. M., Ormond, K. E., Rehm, H. L., Watson, M. S., Williams, M. S., Biesecker, L. G., American College of Medical Genetics and Genomics. (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*, *15*(7), 565-574. https://doi.org/10.1038/gim.2013.73

Hayawi, L. M., Graham, I. D., Tugwell, P., & Yousef Abdelrazeq, S. (2018). Screening for osteoporosis: A systematic assessment of the quality and content of clinical practice guidelines, using the AGREE II instrument and the IOM Standards for Trustworthy Guidelines. *PLoS One*, *13*(12), e0208251. https://doi.org/10.1371/journal.pone.0208251

Holtzman, N. A. (2013). ACMG recommendations on incidental findings are flawed scientifically and ethically. *Genet Med*, *15*(9), 750-751.

Hsieh, H.-F., & Shannon, S. E. (2005). Three approaches to qualitative content analysis. *Qual Health Res*, *15*(9), 1277-1288. https://doi.org/10.1177/1049732305276687

Hume, S., Nelson, T. N., Speevak, M., McCready, E., Agatep, R., Feilotter, H., Parboosingh, J., Stavropoulos, D. J., Taylor, S., Stockley, T. L., & Canadian College of Medical Geneticists. (2019). CCMG practice guideline: Laboratory guidelines for next-generation sequencing. *J Med Genet*, *56*(12), 792-800. https://doi.org/10.1136/jmedgenet-2019-106152

Hussain, M., Hussain, J., Sadiq, M., Hassan, A., Sungyoung, L. (2018). *Recommendation Statements Identification in Clinical Practice Guidelines Using Heuristic Patterns* 2018 19th IEEE/ACIS International Conference on Software Engineering, Artificial Intelligence, Networking and Parallel/Distributed Computing (SNPD), Busan, Korea (South).

Iannizzi, C., Chai, K. L., Piechotta, V., Valk, S. J., Kimber, C., Monsef, I., Wood, E. M., Lamikanra, A. A., Roberts, D. J., McQuilten, Z., So-Osman, C., Jindal, A., Cryns, N., Estcourt, L. J., Kreuzberger, N., & Skoetz, N. (2023). Convalescent plasma for people with COVID-19: A living systematic review. *Cochrane Database Syst Rev*, *2*(2), Cd013600. https://doi.org/10.1002/14651858.CD013600.pub5

Institute of Medicine Committee on Standards for Developing Trustworthy Clinical Practice Guidelines. (2011). *Clinical Practice Guidelines We Can Trust* (M. M. Graham R, Wolman DM, Greenfield S, Steinberg E, Ed.) Retrieved March 30, 2023, from https://www.ncbi.nlm.nih.gov/books/NBK209539/

Kalia, S. S., Adelman, K., Bale, S. J., Chung, W. K., Eng, C., Evans, J. P., Herman, G. E., Hufnagel, S. B., Klein, T. E., Korf, B. R., McKelvey, K. D., Ormond, K. E., Richards, C. S., Vlangos, C. N., Watson, M., Martin, C. L., & Miller, D. T. (2017). Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): A policy statement of the American College of Medical Genetics and Genomics. *Genet Med*, *19*(2), 249-255. https://doi.org/10.1038/gim.2016.190

Lazier, J., Hartley, T., Brock, J. A., Caluseriu, O., Chitayat, D., Laberge, A. M., Langlois, S., Lauzon, J., Nelson, T. N., Parboosingh, J., Stavropoulos, D. J., Boycott, K., Armour, C. M., & Canadian College of Medical Geneticists. (2022). Clinical application of fetal genome-wide sequencing during pregnancy: position statement of the Canadian College of Medical Geneticists. *J Med Genet*, *59*(10), 931-937. https://doi.org/10.1136/jmedgenet-2021-107897

Manickam, K., McClain, M. R., Demmer, L. A., Biswas, S., Kearney, H. M., Malinowski, J., Massingham, L. J., Miller, D., Yu, T. W., Hisama, F. M., & ACMG Board of Directors. (2021). Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an

evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(11), 2029-2037. https://doi.org/10.1038/s41436-021-01242-6

Matthijs, G., Souche, E., Alders, M., Corveleyn, A., Eck, S., Feenstra, I., Race, V., Sistermans, E., Sturm, M., Weiss, M., Yntema, H., Bakker, E., Scheffer, H., Bauer, P., EuroGentest, & European Society of Human Genetics. (2016). Guidelines for diagnostic next-generation sequencing. *Eur J Hum Genet*, *24*(1), 2-5. https://doi.org/10.1038/ejhg.2015.226

McGowan, J., Sampson, M., Salzwedel, D. M., Cogo, E., Foerster, V., & Lefebvre, C. (2016). PRESS peer review of electronic search strategies: 2015 guideline statement. *J Clin Epidemiol*, *75*, 40-46. https://doi.org/10.1016/j.jclinepi.2016.01.021

Middleton, A., Patch, C., Wiggins, J., Barnes, K., Crawford, G., Benjamin, C., Bruce, A., & Association of Genetic Nurses and Counsellors in the United Kingston and Ireland. (2014). Position statement on opportunistic genomic screening from the Association of Genetic Nurses and Counsellors (UK and Ireland). *Eur J Hum Genet*, *22*(8), 955-956. https://doi.org/10.1038/ejhg.2013.301

Miller, D. T., Lee, K., Chung, W. K., Gordon, A. S., Herman, G. E., Klein, T. E., Stewart, D. R., Amendola, L. M., Adelman, K., Bale, S. J., Gollob, M. H., Harrison, S. M., Hershberger, R. E., McKelvey, K., Richards, C. S., Vlangos, C. N., Watson, M. S., Martin, C. L., & ACMG Secondary Findings Working Group (2021). ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1381-1390. https://doi.org/10.1038/s41436-021-01172-3

Miller, D. T., Lee, K., Gordon, A. S., Amendola, L. M., Adelman, K., Bale, S. J., Chung, W. K., Gollob, M. H., Harrison, S. M., Herman, G. E., Hershberger, R. E., Klein, T. E., McKelvey, K., Richards, C. S., Vlangos, C. N., Stewart, D. R., Watson, M. S., Martin, C. L., & ACMG Seconday Findings Working Group. (2021). Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical

Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1391-1398. https://doi.org/10.1038/s41436-021-01171-4

Monaghan, K. G., Leach, N. T., Pekarek, D., Prasad, P., Rose, N. C., & ACMG Professional Practice Guidelines Committee. (2020). The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *22*(4), 675-680. https://doi.org/10.1038/s41436-019-0731-7

Montori, V. M., Brito, J. P., & Murad, M. H. (2013). The optimal practice of evidence-based medicine: Incorporating patient preferences in practice guidelines. *JAMA*, *310*(23), 2503-2504. https://doi.org/10.1001/jama.2013.281422

Ng, S. B., Buckingham, K. J., Lee, C., Bigham, A. W., Tabor, H. K., Dent, K. M., Huff, C. D., Shannon, P. T., Jabs, E. W., Nickerson, D. A., Shendure, J., & Bamshad, M. J. (2010). Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet*, *42*(1), 30-35. https://doi.org/10.1038/ng.499

Rehder, C., Bean, L. J. H., Bick, D., Chao, E., Chung, W., Das, S., O'Daniel, J., Rehm, H., Shashi, V., Vincent, L. M., & ACMG Laboratory Quality Assurance Committee. (2021). Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: A technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1399-1415. https://doi.org/10.1038/s41436-021-01139-4

Rehder, C. W., David, K. L., Hirsch, B., Toriello, H. V., Wilson, C. M., & Kearney, H. M. (2013). American College of Medical Genetics and Genomics: Standards and guidelines for documenting suspected consanguinity as an incidental finding of genomic testing. *Genet Med*, *15*(2), 150-152. https://doi.org/10.1038/gim.2012.169

Rehm, H. L., Bale, S. J., Bayrak-Toydemir, P., Berg, J. S., Brown, K. K., Deignan, J. L., Friez, M. J., Funke, B. H., Hegde, M. R., Lyon, E., for the Working Group of the American College of Medical Genetics & Genomics Laboratory Quality Assurance Committee. (2013). ACMG clinical laboratory standards

for next-generation sequencing. *Genet Med*, *15*(9), 733-747. https://doi.org/10.1038/gim.2013.92

Scheuner, M. T., Peredo, J., Benkendorf, J., Bowdish, B., Feldman, G., Fleisher, L., Mulvihill, J. J., Watson, M., Herman, G. E., & Evans, J. (2015). Reporting genomic secondary findings: ACMG members weigh in. *Genet Med*, *17*(1), 27-35. https://doi.org/doi: 10.1038/gim.2014.165

Stemkens, D., Bruggenwirth, H.T., van der Crabben, S., Elting, M.W., Feenstra, I., van Gassen, K., Gerrits, M., Giesbertz, N., Govaerts, L.C.P., Kerstjens-Frederikse, W.S., van der Kold, L.E., Kriek, M., Motazacker, M.M., Sikkema-Raddatz, B., Ruivenkamp, C., Scmidt, M.K., van der Schoot, V., Sinke, R.J., Waisfisz, Q., Yntema, H.G. (2020). Dutch national consensus-based guideline for the disclosure of incidental findings during clinical genetic diagnostic testing. Retrieved March 30, 2023, from https://www.vkgl.nl/images/links/Richtlijnen_en_protocollen/Dutch_national_consensus-based_guideline_for_the_disclosure_of_incidental_findings_during_clinical_genetic_diagnostic_testing_version2.pdf

The Joanna Briggs Institute. (2020). *Joanna Briggs Institute's Reviewer's Manual*. Retrieved March 30, 2023, from https://reviewersmanual.joannabriggs.org/

Townsend A, A. S., Birch PH, Friedman JM. (2013). Paternalism and the ACMG recommendations on genomic incidental findings: Patients seen but not heard. *Genet Med*, *15*(9), 751-752. https://doi.org/doi: 10.1038/gim.2013.105.

Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K. K., Colquhoun, H., Levac, D., Moher, D., Peters, M. D. J., Horsley, T., Weeks, L., Hempel, S., Akl, E. A., Chang, C., McGowan, J., Stewart, L., Hartling, L., Aldcroft, A., Wilson, M. G., Garritty, C., . . . Straus, S. E. (2018). PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. *Ann Intern Med*, *169*(7), 467-473. https://doi.org/10.7326/M18-0850

Vale, S. L., Lobb, M., Netting, M. J., Murray, K., Clifford, R., Campbell, D. E., & Salter, S. M. (2021). A systematic review of infant feeding food allergy prevention guidelines - can we AGREE? *World Allergy Organ J*, *14*(6), 100550. https://doi.org/10.1016/j.waojou.2021.100550

van El, C. G., Cornel, M. C., Borry, P., Hastings, R. J., Fellmann, F., Hodgson, S. V., Howard, H. C., Cambon-Thomsen, A., Knoppers, B. M., Meijers-Heijboer, H., Scheffer, H., Tranebjaerg, L., Dondorp, W., de Wert, G. M., on behalf of the ESHG Public and Professional Policy Committee. (2013). Whole-genome sequencing in health care: recommendations of the European Society of Human Genetics. *Eur J Hum Genet*, *21*(6), 580-584. https://doi.org/10.1038/ejhg.2013.46

Weiss, M. M., Van der Zwaag, B., Jongbloed, J. D., Vogel, M. J., Bruggenwirth, H. T., Lekanne Deprez, R. H., Mook, O., Ruivenkamp, C. A., van Slegtenhorst, M. A., van den Wijngaard, A., Waisfisz, Q., Nelen, M. R., & van der Stoep, N. (2013). Best practice guidelines for the use of next-generation sequencing applications in genome diagnostics: A national collaborative study of Dutch genome diagnostic laboratories. *Hum Mutat*, *34*(10), 1313-1321. https://doi.org/10.1002/humu.22368

Wolf SM, A. G., Elias S. (2013). Point-counterpoint. Patient autonomy and incidental findings in clinical genomics. *Science*, *340*(6136), 1049-1050.

Zawati, M. H., Parry, D., Thorogood, A., Nguyen, M. T., Boycott, K. M., Rosenblatt, D., & Knoppers, B. M. (2014). Reporting results from whole-genome and whole-exome sequencing in clinical practice: a proposal for Canada? *J Med Genet*, *51*(1), 68-70. https://doi.org/10.1136/jmedgenet-2013-101934

3.6. Supplemental materials

Supplemental Materials and Methods

Electronic search strategies

Note: Searches were conducted using an Ovid multi-database search and duplicate records were removed online giving preference to MEDLINE, then Embase, with no field preference. Lines 1-4 are optimized for MEDLINE and the main question constructs are broken out in separate lines for clarity. Lines 5-9 are optimized for Embase. The next lines isolate the records to the database the search was designed for, combine those sets and then remove duplicate records and finally, isolate the records from each database again so each can be downloaded and imported into the citation manager using a database-specific import filter.

1 exp Whole Genome Sequencing/ or High-Throughput Nucleotide Sequencing/ or ((whole or complete or genom* or exome or massively parallel) adj2 sequenc*).ti,ab,kf. or ((ES/GS or WGS or ES or GS or NGS or WES or next generation) and (rare or exome or genome)).ti,ab,kf.

2 (working group* or committee or task force or guideline* or advisory or society or college or statement).ti. or ((clinical or practice* or consensus or position or policy) adj2 (guideline* or statement or advisory)).mp. or "Practice Guidelines as Topic"/ or Practice Guideline.pt.

3 1 and 2

4 limit 3 to yr="2010 -Current"

5 exp Whole Genome Sequencing/ or High Throughput Sequencing/ or ((whole or complete or genom* or exome or massively parallel) adj2 sequenc*).ti,ab,kw. or ((ES/GS or WGS or ES or GS or NGS or WES or next generation) and (rare or exome or genome)).ti,ab,kw.

6 (working group* or committee or task force or guideline* or advisory or society or college or statement).ti. or ((clinical or practice* or consensus or position or policy) adj2 (guideline* or statement or advisory)).mp. or exp Practice Guideline/

7 5 and 6

8 limit 7 to yr="2010 -Current"

9 limit 8 to embase

10 4 use medall

11 9 use emczd

12 10 or 11

13 12 Remove duplicates

14 13 use medall

15 13 use emczd

TRIPDatabase

(exome OR genome) AND sequencing

Limit to Guidelines

Limit to 2010-2022

Five publications used to test the electronic search strategy:

1. Boycott KM, et al. The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists, J Med Genet. 2015;52(7):431-7. (PMID: 25951830)
2. Manickam K, McClain MR, et al. 2021. Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). Genet Med [published online ahead of print July 1, 2021]. doi: 10.1038/s41436-021-01242-6. (PMID: 34211152)
3. van El CG, Cornel MC, et al. 2013. Whole-genome sequencing in health care. Recommendations of the European Society of Human Genetics. Eur J Hum Genet. 2013;21(6):580-4. (PMID: 23676617)
4. Kalia SS, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics (ACMG). Genet Med. 2017;19(2):249-255. (PMID: 27854360)

5. Monaghan KG, et al. The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). 2020;22(4):675-80. (PMID: 31911674)

Grey literature search strategies

Search terms: exome, genome, genome-wide sequencing, genomic sequencing and next-generation sequencing

Genetics Professional Organizations

Outcome	Organization	Website
searched; nothing found	Australasian Society of Genetic Counsellors, special interest group of the Human Genetics Society of Australasia	https://www.hgsa.org.au/asgc
searched; results found	Human Genetics Society of Australasia	https://www.hgsa.org.au/
searched; results may be of peripheral interest	Austrian Society of Human Genetics	http://www.oegh.at/
searched; nothing found	Belgian Society of Human Genetics (BeSHG)	https://beshg.be/
searched; nothing found	Genetic Counselors of Belgium - GCBe	https://beshg.be/workgroups/genetic-counselors
searched; nothing found	Canadian Association of Genetic Counsellors	https://www.cagc-accg.ca/
searched; results found	Canadian College of Medical Geneticists	https://www.ccmg-ccgm.org/
searched; nothing found	Genetic Counsellors of Ontario	https://www.gcontario.org/

Unable to find a website	Colombian Society of Genetics	unable to find a website
searched; results may be of peripheral interest	Czech Society of Medical Genetics [Společnost lékařské genetiky a genomiky České lékařské společnosti Jana Evangelisty Purkyně, z. s., translates to "Society of Medical Genetics and Genomics - Czech Medical Society of Jan Evangelista Purkyně, z. S."]	https://slg.cz/
searched; results found	Danish Society of Medical Genetics	https://dsmg.dk/
searched; results found	British Society for Genetic Medicine	https://bsgm.org.uk/
searched; nothing found	Clinical Genetics Society	https://www.clingensoc.org/
searched; nothing found	Estonian Society of Human Genetics	https://estshg.ut.ee/
searched; nothing found	Estonian Society of Medical Genetics	https://www.kliinikum.ee/emgs/et/
searched; nothing found	European Board of Medical Genetics	https://www.ebmg.eu/413.0.html
searched; results found	European Society of Human Genetics (ESHG)	https://www.eshg.org/index.php?id=home
searched; nothing found	Finnish Society of Medical Genetics	https://www.slgy.info/pub/
searched; nothing found	French Association of Genetic Counsellors (AFCG) - Association Francaise des conseillers en genetique	https://af-cg.fr
searched; results may be of peripheral interest	ANPGM (Association Nationale des Praticiens de Génétique Moléculaire)	https://anpgm.fr
searched; nothing found	Association Francophone de Genetique Clinique	https://af-gc.fr/
searched; nothing found	French Federation of Human Genetics	http://ffgh.net/
searched; nothing found	French Society of Human Genetics (SFGH, Société Française de Génétique Humaine)	http://ffgh.net/index.php/sfgh--societe-francaise-de-genetique-humaine
searched; nothing found	Societe Francaise de Genetique	http://www.sfgenetique.org/

searched; results may be of peripheral interest	German Society of Human Genetics	https://www.gfhev.de/
searched; nothing found	German Genetics Society	https://www.gfgenetik.com/
searched; nothing found	Hellenic Association of Medical Geneticists	https://www.sige.gr
unable to find a website	Hungarian Society of Human Genetics and Genomics	Website is under construction: http://www.szbk.u-szeged.hu/
searched; nothing found	Association of the Hungarian Human Geneticists	Website listed on EuroGenTest "http://www.humangenetika.hu/"
searched; nothing found	Icelandic Human Genetics Society	https://www.mannis.is
searched; nothing found	International Genetic Epidemiology Society	https://www.geneticepi.org/
searched; results found	International Society of Nurses in Genetics	https://www.isong.org
searched; nothing found	Society for the Study of Inborn Errors of Metabolism	https://www.ssiem.org/home/welcome.asp
searched; nothing found	International Federation of Human Genetics Societies	https://ifhgs.org
searched; nothing found	Irish Society of Human Genetics	https://ishg.ie/
searched; nothing found	Genetics Society of Israel	https://www.genetics-il.org
searched; nothing found	Israeli Society of Medical Genetics	https://cdn.doctorsonly.co.il
searched; results found	Italian Society of Human Genetics	https://sigu.net
searched; nothing found	Japanese Society for Genetic Counseling	https://www.jsgc.jp
searched; nothing found	Japanese Association of Certified Genetic Counselors	Japanese Association of Certified Genetic Counselors. - JACGC - - Home | Facebook
searched; nothing found	Japanese Society of Human Genetics	https://jshg.jp/
searched; results found	The Japanese Society for Gene Diagnosis and Therapy	http://www.gene-dt.jp/index.html

searched; nothing found	Japanese Board of Genetic Counseling	https://plaza.umin.ac.jp/~GC/
searched; nothing found	Japanese Board of Medical Genetics and Genomics, Clinical Genetics	https://www.jbmg.jp
searched; nothing found	Asia Pacific Society of Human Genetics	https://www.apshg.info/index.html
searched; nothing found	Korean Society of Genetic Diagnostics	https://ksgd.org
searched; nothing found	Korean Society of Medical Genetics and Genomics	https://kcmg.or.kr
searched; nothing found	The Genetics Society of Korea	https://icgsk.kgenetics.or.kr
unable to find a website	Latin American Society of Genetics (RELAGH in spanish and portuguese)	Not found
searched; nothing found	Latin American Network of Human Genetics	https://www.latingen.org
searched; nothing found	Colombian Society of Human Genetics (CSHG) - ASOCIACIÓN COLOMBIANA DE GENÉTICA HUMANA	https://www.acgh.com.co
searched; nothing found	ASOCIACIÓN COLOMBIANA DE MÉDICOS GENETISTAS - Colombian Society of Medical Geneticists	https://acmgen.org/
searched; nothing found	Latvian Association of Human Genetics	Latvian Biomedical Research and Study Centre - LAHG (lu.lv)
unable to find a website	Latvian Society of Medical Genetics	Not found
unable to find a website	Lithuanian Association of Genetics	Not found
searched; nothing found	Lithuanian Society of Human Genetics	http://www.geneticahumana.lt/
unable to find a website	Luxembourg Genetics Association	Not found
searched; nothing found	Mexican Association of Human Genetics	https://www.amgh.org.mx/index.php
unable to find a website	Mexican Society of Genomic Medicine	https://www.inmegen.gob.mx/
searched; results may be of peripheral interest	Dutch Society for Laboratory Specialist Clinical Genetics	https://www.vkgl.nl
searched; results found	Dutch Society of Clinical Genetics	https://www.vkgn.org
searched; nothing found	Dutch Society of Human Genetics	https://www.NVHG.nl

searched; nothing found	Norwegian Society of Human Genetics (NSHG) - Norsk Selskap for Human Genetikk	http://www.nshg.no/
searched; nothing found	Norwegian Society of Medical Genetics	https://www.legeforeningen.no/foreningsledd/fagmed/norsk-forening-for-medisinsk-genetikk/
searched; results found	Polish Society of Human Genetics	https://http://ptgc.pl/
searched; nothing found	Portuguese Society of Human Genetics	https://www.spgh.net
searched; nothing found	Slovak Society of Medical Genetics	https://www.sslg.sk
unable to find a website	Slovenian Association of Medical Genetics	https://www.zmg-szd.si
unable to find a website	Slovenian Society of Human Genetics	https://www.mf.uni-lj.siSSHG
searched; nothing found	Spanish Association of Human Genetics	https://aegh.org/
searched; results found	Swedish Society of Medical Genetics and Genomics (SFMG)	https://sfmg.se
unable to find a website	Swiss Association of Genetic Counsellors	Not found
searched; results found	Swiss Society of Medical Genetics	https://sgmg.ch/
searched; nothing found	Turkish Society of Medical Genetics	https://www.tibbigenetik.org.tr
searched; results found	British Society for Genetic Medicine	https://bsgm.org.uk/
searched; nothing found	Association of Genetic Nurses and Counsellors	https://www.agnc.org.uk
searched; nothing found	American Board of Medical Genetic and Genomics	http://www.abmgg.org/
searched; results found	American College of Medical Genetics and Genomics	https://www.acmg.net
searched; nothing found	Genetics Society of America	https://www.genetics-gsa.org

searched; nothing found	American Board of Genetic Counseling	https://www.abgc.net
searched; results may be of peripheral interest	American Society of Human Genetics	https://www.ashg.org
searched; results found	National Society of Genetic Counselors	https://www.nsgc.org
searched; nothing found	Transnational Alliance for Genetic Counseling	https://sc.edu/study/colleges_schools/medicine/centers_and_institutes_new/transnational_alliance_for_genetic_counseling/index.php
searched; nothing found	East Asia Union of Human Genetics Societies (EAUHGS)	https://www.eauhgs.jp/

Clinical Practice Guideline Database

Outcome	Database	Website
searched; nothing found	Alberta Medical Association	https://actt.albertadoctors.org/CPGs
searched; nothing found	British Columbia Ministry of Health	https://www2.gov.bc.ca/gov/content/health/practitioner-professional-resources/bc-guidelines
searched; results found	Canadian Medical Association	https://www.cma.ca/En/Pages/clinical-practice-guidelines.aspx
searched; nothing found	Canadian Partnership Against Cancer	https://www.partnershipagainstcancer.ca/work-with-us/procurement/procurement-bid/cancer-guidelines-database/
searched; nothing found	Canadian Standards Association	https://www.csagroup.org/store/
searched; nothing found	The College of Physicians and Surgeons of Ontario	https://www.cpso.on.ca/Physicians/Your-Practice/Quality-Management/CPGs-Other-Guidelines
searched; nothing found	Ontario Association of Medical Laboratories	https://oaml.com/guidelines/

searched; nothing found	Public Health Agency of Canada	http://www.phac-aspc.gc.ca/dpg-eng.php
searched; nothing found	Registered Nurses' association of Ontario	https://rnao.ca/bpq
Unable to find website	University of Ottawa School of Rehabilitation	Website not functioning – http://www.health.uottawa.ca/rehabguidelines/en/search.php
searched; nothing found	Winnipeg Regional Health Authority	http://www.wrha.mb.ca/professionals/ebpt/
searched; nothing found	Academy of Medicine of Malaysia	http://www.acadmed.org.my/index.cfm?&menuid=67
searched; results found	Aetna, Inc.	http://www.aetna.com/healthcare-professionals/policies-guidelines/medical_clinical_policy_bulletins.html
searched; nothing found	American Association of Clinical Chemistry	https://www.aacc.org/science-and-research/practice-guidelines
searched; nothing found	Best Practice Advocacy Centre New Zealand	https://www.bpac.org.nz/default.aspx
searched; results may be of peripheral interest	Centers for Disease Control and Prevention (CDC)	https://phgkb.cdc.gov/PHGKB/phgHome.action?action=home
searched; nothing found	The Regulation and Quality Improvement Authority (RQIA)	https://rqia.org.uk/what-we-do/rqia-clinical-audit-programme/guidelines; https://www.rqia.org.uk/what-we-do/rqia-s-funding-programme/guidelines/
searched; nothing found	Haute Autorite de Sante (HAS)	https://www.has-sante.fr/
searched; nothing found	Institute for clinical Systems Improvement (ICSI)	https://www.icsi.org/guideline/
searched; nothing found	ECRI Institute	https://guidelines.ecri.org/

searched; nothing found	National Health and Medical Research Council (NHMRC)	http://www.clinicalguidelines.gov.au
searched; nothing found	National Institute for Health and Care Excellence (NICE)	http://www.nice.org.uk/guidance
searched; nothing found	Scottish Intercollegiate Guidelines Network (SIGN)	https://www.sign.ac.uk/
Searched; nothing found	Guideline International Network (GIN) *Note: not part of CADTH Grey Matters*	http://g-i-n.net
Unable to find website	National Guideline Clearinghouse *Note: not part of CADTH Grey Matters*	Website no longer funded (as per Agency for Healthcare Research and Quality)

Data collection forms

Document-level data collection form (Excel sheet 1)

Data	Specific variable extracted
General characteristics	Covidence ID Title Year, Month First author (last name, initial) Country of First Author Journal (if relevant, or 'grey') Professional organization Professionals represented Countries represented 'Type' of document
Relationship to other documents	New version of previous document (yes/no)

Recommendation-level data collection form (Excel sheet 2)

Data	Specific variable extracted
General characteristics (copied from above)	Covidence ID Title Year, Month First author (last name, initial) Professional organization Journal (if relevant, or 'grey')
Recommendations	Descriptor of recommendation type (heading/sub-heading from guidance document) Recommendation number (if available) Recommendation details (extracted verbatim recommendation)

AGREE II scoring data collection form (Excel sheet 3)

Data	Specific variable extracted
General characteristics (copied from above)	Covidence ID Title Year, Month First author (last name, initial) Professional organization Journal (if relevant, or 'grey') Other related documents to be taken into consideration
Domain 1: Scope and Purpose	Item 1. The overall objective(s) of the advisory document is (are) specifically described (score, notes) Item 2. The health question(s) covered by the advisory document is (are) specifically described (score, notes) Item 3. The population (patients, public, etc.) to whom the advisory document is meant to apply is specifically described (score, notes)
Domain 2: Stakeholder Involvement	Item 4. The advisory document development group includes individuals from all the relevant professional groups (score, notes)

	Item 5. The views and preferences of the target population (patients, public, etc.) have been sought (score, notes) Item 6. The target users of the advisory document are clearly defined (score, notes)
Domain 3. Rigour of Development	Item 7. Systematic methods were used to search for evidence (score, notes) Item 8. The criteria for selecting the evidence are clearly described (score, notes) Item 9. The strengths and limitations of the body of evidence are clearly described (score, notes) Item 10. The methods for formulating the recommendations are clearly described (score, notes) Item 11. The health benefits, side effects, and risks have been considered in formulating the recommendations (score, notes) Item 12. There is an explicit link between the recommendations and the supporting evidence (score, notes) Item 13. The advisory document has been externally reviewed by experts prior to its publication (score, notes) Item 14. A procedure for updating the advisory document is provided (score, notes)
Domain 4. Clarity of Presentation	Item 15. The recommendations are specific and unambiguous (score, notes) Item 16. The different options for management of the condition or health issue are clearly presented (score, notes) Item 17. Key recommendations are easily identifiable (score, notes)
Domain 5. Applicability	Item 18. The advisory document describes facilitators and barriers to its application (score, notes) Item 19. The advisory document provides advice and/or tolls on how the recommendations can be put into practice (score, notes) Item 20. The potential resource implications of applying the recommendations have been considered (score, notes) Item 21. The advisory document presents monitoring and/or auditing criteria (score, notes)
Domain 6. Editorial Independence	Item 22. The views of the funding body have not influenced the content of the advisory document (score, notes)

	Item 23. Competing interests of advisory document development group members have been recorded and addressed (score, notes)

Supplemental Tables

Supplemental Table 3.1. Extracted recommendations and related topics from clinical guidance documents related to ES/GS is available as a supplemental file to this thesis.

Supplemental Table 3.2. AGREE II consensus-derived scores for clinical guidance documents related to ES/GS is available as a supplemental file to this thesis.

Chapter 4: Examining two phases of the action cycle as part of an integrated KT study

4.1. Preface

In Chapter 3 we learned that organizations representing genetics healthcare professionals have produced clinical guidance documents with a broad set of clinical recommendations, in terms of both number and related topics. However, these documents are of low quality as per AGREE II, particularly as it relates to the stated rigour of the methods by which the knowledge base was utilized in forming recommendations. In this Chapter, we move on to the second concept of the Knowledge-to-Action (KTA) conceptual framework (Graham et al., 2006), the Action Cycle, in order to examine the application of knowledge related to ES/GS in one study setting.

While a number of jurisdictions have now implemented ES/GS into their healthcare systems (Stark et al. 2019), it isn't clear whether scientific evidence of diagnostic utility is translating into clinically-valid diagnoses for patients when conducted within the healthcare system. This is important for decision-makers to understand to ensure there is return on their investments, particularly in systems with limited resources like Canada's publicly-funded healthcare system. The system consists of ten provincial and three territorial health insurance plans, with the federal government providing partial funding to the provinces and territories and setting national standards through the Canada Health Act (Government of Canada, 2023). Provinces and territories are responsible for decisions regarding resource allocation, including the availability of genetic tests, like ES/GS, within each region.

Amongst all Canadian provincial/territorial public healthcare plans, the Ontario Ministry of Health (MOH) has been one of the earliest adopters of ES/GS. While the Ontario MOH implementation process

has not been explicitly guided by the KTA framework, it has mimicked the phases of the Action Cycle. In 2015, the Canadian College of Medical Geneticists (CCMG) published a clinical guidance document related to the use of ES/GS in the diagnosis of RGDs [*KTA step: Knowledge Tool; included in Chapter 3*] (Boycott et al., 2015). Soon after, the MOH developed a formal working group to translate the CCMG recommendations into a set of inclusion and exclusion criteria for publicly-funded clinical ES/GS [*KTA step: Adapt Knowledge to Local Context*] (Genetic Testing Advisory Committee, 2016). A formal and robust evaluative process was recommended by the working group with the implementation of the eligibility criteria to inform future decisions regarding how ES/GS should be offered within the province of Ontario. Based on the recommendations of the working group, the MOH partnered with the Care4Rare Canada Consortium, as part of an integrated KT (iKT) process. During iKT, knowledge users (in this case the MOH) are actively engaged in the entire research process, including shaping the research questions, methods, data collection, and interpretation of findings. As part of this collaboration, ES was made available, as a publicly-funded test, for eligible Ontario patients, and a province-wide research study was conducted in parallel to collect outcomes of such testing. Both together and separately, Care4Rare and the MOH held meetings in early 2018 with representatives from Genetics Clinics across Ontario to assess the barriers to clinical implementation of the eligibility criteria for clinical ES/GS and corresponding monitoring of its use and outcome evaluation [*KTA process: Assess Barriers to Knowledge Use*]. To facilitate approvals for testing, the MOH implemented a physician attestation form that became available in December of 2018 [*KTA process: Implement Intervention*]. To ensure consistency in procedures for this observational research study, Research Ethics Board approval for clinical genetics sites across the province was coordinated by a central study team at the Children's Hospital of Eastern Ontario through the Clinical Trials Ontario system (approvals available in Appendix 1).

Here, we investigated the third objective of my thesis "To examine whether implementation of ES/GS based on this knowledge base results in diagnoses for families with RGDs." We prospectively examined, as part of the larger Care4Rare initiative, the diagnostic utility of publicly-funded ES testing, performed in laboratories outside of Canada, for families from Ontario. Specifically, my sub-objectives for this Article were:

I. To monitor the application of provincial eligibility criteria, including corresponding patient phenotypes;
II. To describe the range of molecular results interpreted and reported by clinical laboratories (including variant classifications and genes reported);
III. To determine the proportion of laboratory interpretations associated with clinically-valid results, as determined by the ordering physicians; and,
IV. To identify cases for which ES was required to achieve a diagnosis compared to those for whom conventional tests would have achieved a diagnosis.

4.2. Statement of permission for use of copyrighted material

The following chapter consists of the manuscript entitled "Evaluation of the diagnostic accuracy of exome sequencing and its impact on diagnostic thinking for rare disease patients in a publicly-funded healthcare system: a prospective cohort study". This manuscript has been accepted pending minor revisions at the journal *Genetics in Medicine*. As such, an embargo will be applied at time of final submission.

4.3. Contribution

Taila Hartley contributed to the study design and protocol, led the submission to the research ethics board, contributed to the piloting of the data collection form and training of sub-sites, participated in

the data auditing process for the full cohort data set and took primary responsibility for auditing data related to this study, conducted all data analysis and interpretation of findings, and drafted the manuscript.

4.4. Main manuscript

Evaluation of the diagnostic accuracy of exome sequencing and its impact on diagnostic thinking for rare disease patients in a publicly-funded healthcare system: a prospective cohort study

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[12] University of Alberta, Edmonton, Alberta, Canada

ABSTRACT

Purpose: To evaluate the diagnostic utility of publicly-funded clinical exome sequencing (ES) for patients with suspected rare genetic diseases (RGDs).

Methods: We prospectively enrolled 297 probands who met eligibility criteria and received ES across five sites in Ontario, Canada, and extracted data from medical records and clinician surveys. Using the Fryback and Thornbury Efficacy Framework, we assessed diagnostic accuracy by examining laboratory interpretation of results and assessed diagnostic thinking by examining the clinical interpretation of results and whether clinical-molecular diagnoses would have been achieved via alternative hypothetical molecular tests.

Results: Laboratories reported 105 molecular diagnoses and 165 uncertain results in known and novel genes. Of these, clinicians interpreted 102 of 105 (97%) molecular diagnoses and 6 of 165 (4%) uncertain results as clinical-molecular diagnoses. The 108 clinical-molecular diagnoses were in 104 families (35% diagnostic yield). Each eligibility criteria resulted in diagnostic yields of 30-40%, and higher yields were achieved when >2 eligibility criteria were met (up to 45%). Hypothetical tests would have identified 61% of clinical-molecular diagnoses.

Conclusion: We demonstrate robustness in eligibility criteria and high clinical validity of laboratory results from ES testing. The importance of ES was highlighted by the potential 40% of patients that would have gone undiagnosed without this test.

Keywords: exome sequencing, implementation science, clinical validity, clinical-molecular diagnoses, rare disease, healthcare system

INTRODUCTION

The introduction of genomic technologies, like exome sequencing (ES), has created an opportunity to shorten the uncertain and emotionally taxing "diagnostic odyssey" experienced by families with suspected rare genetic diseases (RGDs) (Carmichael et al., 2015; Michelson, 2018; Sawyer et al., 2016). A meta-analysis has demonstrated a diagnostic yield of 36% for both clinical and research-based ES in the context of a broad range of RGD indications (Clark et al., 2018). However, studies have typically been retrospective analyses on convenience samples of patients or large cohorts with unspecified phenotypes reported by diagnostic laboratories. Despite few randomized-controlled trials, systematic reviews, and health technology assessments, healthcare systems in various jurisdictions have moved to implement ES as a clinical test (Birney et al., 2017; Phillips et al., 2021). How jurisdictions optimize this test's implementation will depend on their healthcare system and context. However, sharing empirical evidence of the utility of this test and policy processes that support its use can guide healthcare systems in these implementation efforts (Phillips et al., 2020).

The province of Ontario, Canada, has a publicly-funded health insurance plan for approximately 15 million residents. This program is government-run by the Ministry of Health (MOH) and funds medically-necessary hospital and physician services, including specialist consultations and approved laboratory tests, all of which are of no cost to patients at the point of care. After ES became available through commercial diagnostic laboratories, Ontario physicians could access publicly-funded testing for their patients through the MOH's Out-of-Country/Out-of-Province Prior Approval Program for Diagnostic Laboratory Testing (Ontario Ministry of Health, 2018). In 2018, the MOH standardized clinical eligibility criteria for publicly-funded ES for patients with suspected RGD (**Table 4.1**) (Genetic Testing Advisory Committee, 2016). These criteria were derived from factors that increase the suspicion of a monogenic etiology diagnosable by ES (Boycott et al., 2015; Genetic Testing Advisory Committee, 2016). In this

process, ordering clinicians (primarily Medical Geneticists) must attest to the criteria, confirm their expertise (defined as the ability to determine if ES is the appropriate test, counsel families, and interpret results) and confirm that results could alter medical management. When MOH developed these criteria, the evidence base related to ES was limited. It was therefore deemed imperative to introduce this testing alongside an evaluative process to ensure the appropriate use of ES in the context of finite healthcare resources. As a starting point, a provincial benchmark of 30% diagnostic yield for ES for RGD diagnosis was set[9] based on early, peer-reviewed primary literature (from 2013 and 2014) (Lee et al., 2014; Soden SE, 2014; Yang et al., 2013; Yang et al., 2014).

Table 4.1. Clinical presentation eligibility criteria for publicly-funded ES in Ontario (must meet 2 or more)

Criteria	*Description*
1	Moderate to severe developmental or functional impairment
2	Multisystem involvement
3	Progressive clinical course
4	A differential diagnosis that includes ≥ 2 well defined conditions requiring evaluation by multiple targeted gene panels
5	A suspected severe genetic syndrome for which multiple family members are also affected, or where parents are consanguineous

To evaluate the implementation of routine ES, the MOH engaged with Care4Rare Canada, a pan-Canadian research consortium devoted to enhancing diagnostic care for patients with RGDs (Boycott, Hartley, et al., 2022). Together, we designed a multi-site prospective cohort study to evaluate the implementation of ES based on the six-level, hierarchical, Fryback and Thornbury efficacy framework (Fryback & Thornbury, 1991). Our study was launched alongside the implementation of the provincial eligibility criteria in April 2018 (Hayeems et al., 2022). As part of this broader initiative to evaluate ES, our objective here is to report on its diagnostic utility (diagnostic accuracy and impact on clinician diagnostic thinking) in a prospective cohort of patients with undiagnosed suspected RGDs who had

publicly-funded ES conducted at clinical laboratories outside of Canada. We hypothesized ES would be an effective test for this cohort of patients and that the proportion of families who receive clinical-molecular diagnoses would meet the provincial benchmark of 30% for ES testing. We also hypothesized that this efficacy would be similar amongst biological sexes but potentially differ based on clinical presentation.

MATERIALS AND METHODS

We conducted this study with approval from the Clinical Trials Ontario Streamlined Research Review System (CTO-1577, Appendix 1) as part of the Care4Rare-SOLVE study (Boycott, Hartley, et al., 2022). We have published the complete protocol for evaluating publicly-funded ES elsewhere (Hayeems et al., 2022). We report our findings adhering to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (von Elm et al., 2007) (**Supplemental Table 4.1**) and the Sex and Gender Equity in Research (SAGER) (Heidari et al., 2016) guidelines.

Settings and Sample

We conducted this prospective observational study at five clinics in Ontario (the Medical Genetics programs at the Children's Hospital of Eastern Ontario, Hospital for Sick Children, London Health Sciences Centre, Kingston Health Sciences Center and the Neuromuscular and Neurometabolic program at McMaster Children's Hospital). These sites were selected since they accounted for most OOC/OOP applications requesting ES (MOH personal communication, 2017). Any patient receiving publicly-funded clinical ES at a participating site was eligible for inclusion and was identified by their ordering clinician during their routine clinical care. Sample size for this analysis was limited to those participants who received testing from April 1, 2018 to March 31, 2021. All families consented to participation prior to

enrollment. If multiple affected family members were enrolled, we included just one proband (the first to present to genetics) in this analysis.

Data collection

We sought to examine the diagnostic utility of ES testing as defined by two levels of the Fryback and Thornbury framework. Level 2, diagnostic accuracy efficacy, is defined as the ability of ES to predict whether a patient has or does not have a particular disease. We examined this by collecting the laboratory results from ES testing, including, but not limited to, the number of molecular diagnoses. Level 3, diagnostic thinking efficacy, relates to whether the test helps clinicians establish a specific diagnosis. We operationalized this in two ways; by asking clinicians whether they interpreted molecular results to be an actual clinical-molecular diagnosis (e.g., a clinically valid result) and by examining whether hypothetical alternative molecular tests might have identified the diagnoses.

We trained study personnel to extract relevant data points from medical records and to enter these data into Genomics4RD, a Care4Rare research database (Driver et al., 2022).

After ES was ordered, but prior to receiving results, study personnel completed a pre-test data collection form. Pertinent data points included the eligibility criteria (**Table 4.1**) and clinical characteristics (including mutually-exclusive phenotypic category [i.e., syndromic intellectual disability (ID) or developmental delay (DD), isolated severe ID, multiple congenital anomalies (MCA) without ID/DD, multisystem disease without ID/DD, or single system disease without ID/DD]. We also collected the hypothetical care pathways to assess level 3 diagnostic thinking efficacy, which we prompted with the question “If ES was not available to order for this patient today, what diagnostic tests would you order at this point in time?” Clinicians could select “none” or one or more of a series of 84 types of tests,

including two types of molecular testing (i.e., single gene, multi-gene panel), and a range of other cytogenetic, biochemical, imaging, and physiological tests (Hayeems et al., 2022). For this analysis, we focused on the selected molecular tests. If clinicians selected a single gene test, they were asked to provide the gene name, and if they selected a gene panel, they were asked to provide the name of the panel and how many genes it included.

After results were returned, personnel completed a post-test data collection form. We used data from the laboratory's clinical report to assess diagnostic accuracy efficacy (level 2). This included testing details (e.g., laboratory name, testing strategy) and molecular results, with variant details and classification as per American College of Medical Genetics and Genomics (ACMG) criteria (Richards et al., 2015). To assess diagnostic thinking efficacy (level 3), ordering clinicians were asked to interpret the laboratory results as diagnostic (provide a complete explanation of phenotype), partially-diagnostic (explain only a portion of the presenting phenotype), potentially-diagnostic (variants that remain uncertain but could provide an explanation of phenotype), or non-diagnostic (does not explain phenotype).

We accessed the total number of probands approved for ES over the same time period via a data request to the Laboratories and Diagnostics Branch at the Ontario MOH.

Analysis

All data were exported from Genomics4RD into Excel and audited by study personnel, including a certified genetic counselor (GC, T.H.). We requested missing data from clinical care teams, except for hypothetical care pathways, which we only requested from clinicians prior to result receipt from the clinical laboratories. We excluded probands without hypothetical care pathways from that particular

analysis. In cases where the molecular results and clinical interpretation did not align (i.e., molecular diagnoses interpreted as non-diagnostic or variants of uncertain significance interpreted as diagnostic), we contacted clinical teams to determine if this discrepancy was a data collection error or true discordance between the molecular results and clinical interpretation.

The frequency of diagnostic and partially-diagnostic results was tallied and stratified based on phenotypic category, clinical presentation criteria, and sequencing strategy. We employed chi-square tests of independence and goodness of fit to determine whether there were statistical associations between the diagnostic results and case characteristics.

For those families with clinical-molecular diagnoses from ES, we examined the relevant company's website for each hypothetical molecular test to determine if the most recent iteration of the test included the relevant gene (queried September 6, 2022). If the testing laboratory was not specified, we used the most commonly ordered similar test as a reference point for this assessment.

RESULTS

Proband characteristics, eligibility for testing, and testing details

The five recruiting hospitals applied for approximately 70% (1611/2299) of ES in the province of Ontario from April 2018 to March 2021 (**Supplemental Table 4.2**). We prospectively enrolled 297 probands in this cohort (18% of total potentially eligible families): 149 from the Children's Hospital of Eastern Ontario, 75 from the Hospital for Sick Children, 41 from McMaster Children's Hospital, 21 from London Health Sciences Centre, and 11 from the Kingston Health Sciences Center. Patient characteristics and testing details are summarized in **Table 4.2**. There was an equal distribution of biologically male and

female probands. Most of the cohort had a pediatric-onset disease (96%) and syndromic ID/DD phenotype (69%). Parental consanguinity was noted for 28 probands (9% of cohort).

Table 4.2. Proband characteristics and testing details (N=297)

Proband Characteristics	No. Probands (%)
Sex assigned at birth	
Male	156 (53)
Female	141 (47)
Age of onset	
Congenital/Infantile (onset ≤1 year old)	221 (74)
Childhood (onset 2-18 years old)	66 (22)
Adult (onset >18 years old)	8 (3)
Unknown	2 (1)
Parental consanguinity reported	28 (9)
Phenotype	
Syndromic ID/DD	206 (69)
MCA without ID/DD	14 (5)
Multisystem	46 (15)
Single System	19 (6)
Isolated severe ID	12 (4)
Clinical presentation criteria (≥2 per patient)	
1. Moderate to severe impairment	242 (81)
2. Multisystem	211 (71)
3. Progressive	82 (28)
4. Differential ≥ 2 conditions	240 (81)
5. Multiple family members or consanguinity	46 (15)
Clinical laboratory	
GeneDx	286 (96)
Prevention Genetics	6 (2)
Blueprint Genetics	2 (1)
Medical Neurogenetics	2 (1)
Centogene/LifeLabs	1 (<1)
Sequencing strategy	
Singleton	20 (7)
Duo	32 (11)
Trio	235 (79)
Quads or quints	10 (3)

Abbreviations: ID, Intellectual Disability; DD, Developmental Delay; MCA, Multiple Congenital Anomalies

The most common clinical presentation criteria, each selected in >80%, were "moderate to severe developmental or functional impairment" and "a differential diagnosis that includes ≥ 2 well-defined conditions requiring evaluation by multiple targeted gene panels" (**Table 4.2**). Over half of the cohort met more than the minimum two criteria (60%), and seven probands (2%) met all five criteria. Clinical testing was performed at five laboratories (**Table 4.2**), with one provider, GeneDx, performing most tests (97%).

Molecular results related to the primary indication for testing (Level 2, Diagnostic Accuracy)

Within the clinical laboratory reports for the 297 probands, 263 results (i.e., one or more variants within a gene) were reported (**Figure 4.1**). The mean number of results per report was 0.89 (SD = 0.79) and ranged from 0 to 4. Ninety-nine probands (33%) received *positive reports* with one or more molecular diagnoses [i.e., one or more pathogenic (P) or likely pathogenic (LP) variants with the zygosity that fits a known disease with potential phenotypic overlap with the proband]. Six probands (2%) received two molecular diagnoses, resulting in 105 molecular diagnoses within the cohort (**Figure 4.1, orange**). These positive reports also included uncertain results: 25 variants of uncertain significance (VUSs) in known disease genes and two variants in genes of uncertain significance (GUSs). *Uncertain reports* were issued to 102 probands (34%) which included VUSs or single LP/P variants in genes associated with autosomal recessive disease (112, 38%) and/or a GUS (26, 9%). Ninety-six probands (33%) received *negative reports* with no molecular results related to their primary indication for testing. In total, molecular results were reported in 207 unique genes with known disease-gene associations and 28 GUS.

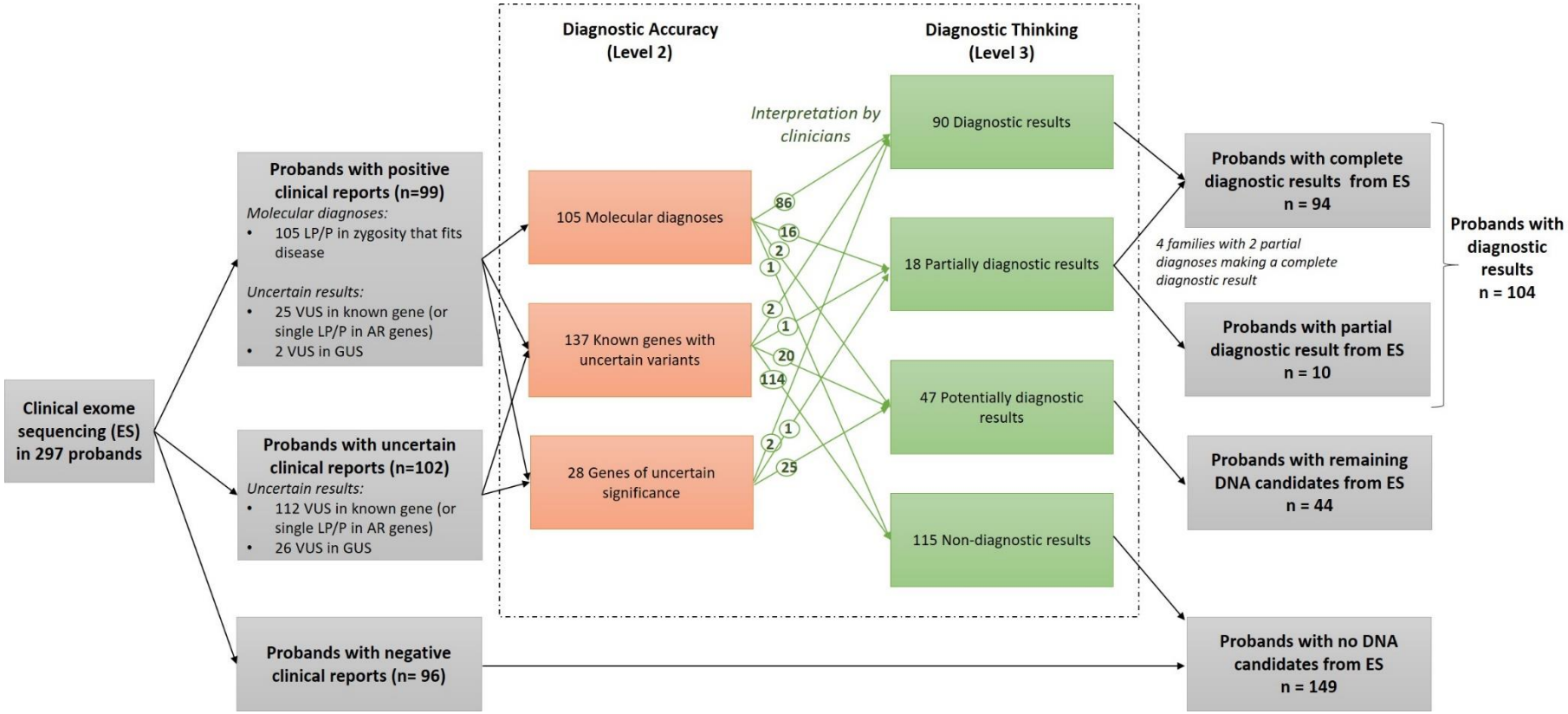

Figure 4.1. Diagnostic outcomes from the clinical ES of 297 probands from Ontario. Grey boxes represent results reported per proband (N=297), colored boxes within the hashed square are reported per molecular result (N=270), defined as one or more DNA variants in a particular gene in a proband. Probands received either a *positive clinical report* that included one or more molecular diagnosis with or without other uncertain variants, an *uncertain clinical report* with only variants of uncertain significance in either known genes or genes of uncertain significance, or a *negative clinical report* with no molecular results reported. The breakdown of these molecular results is shown in orange and represents the diagnostic accuracy (Level 2) of ES as per the Fryback and Thornbury efficacy framework. These molecular results were then interpreted by the ordering clinicians as a representation of their diagnostic thinking (Level 3) and shown in green. Overall, publicly-funded ES resulted in diagnoses explaining the complete phenotype of 94 probands (probands with complete diagnostic results); in four probands their complete diagnoses were based on two partially-diagnostic clinical-molecular diagnoses that accounted for their full presentation. An additional 10 probands received partial diagnoses, 44 probands have remaining DNA candidates, and 149 probands received no DNA candidates for their disease.

Clinical interpretation of molecular results (Level 3, Diagnostic Thinking)

We asked the ordering clinicians to interpret the molecular results (**Figure 4.1, green**). Of the 105 molecular diagnoses (in 99 probands), clinicians considered 86 of the results diagnostic and 16 results partially diagnostic; therefore, clinicians interpreted 97% (102/105) of the molecular results as clinically valid "clinical-molecular diagnoses". Three molecular diagnoses were not interpreted as clinical-molecular diagnoses. In two cases, the clinicians downgraded the molecular diagnosis to potentially diagnostic because their proband's phenotype did not fit the phenotype described in the literature (*ATP1A3* and *PROK2*). In the third case, the clinician interpreted a molecular diagnosis in *OTC* as non-diagnostic after the variant segregated in unaffected male family members.

For the six probands with two molecular diagnoses, clinicians interpreted the results from four as multiple clinical-molecular diagnoses, in which two diseases accounted for their proband's complete phenotype. The two other cases included the *PROK2* and *OTC* variants reported above; these were interpreted as potentially-diagnostic and non-diagnostic, leaving one remaining diagnostic result.

Of the 137 uncertain results in known disease genes, clinicians considered two results diagnostic and one partially-diagnostic. In all three cases, the clinicians upgraded to a clinical-molecular diagnosis based on the specificity of the phenotypes. Twenty of the other uncertain results were considered potentially-diagnostic. Therefore, clinicians interpreted 17% (23/137) of the VUSs to be in genes that could fit some portion of the proband's phenotype, and the remainder (114/137, 83%) were classified as non-diagnostic.

Of the 28 molecular results in GUSs, clinicians interpreted two as diagnostic soon after receiving the laboratory result. In both cases (*AGO2, CLDN5*), the clinicians used the Matchmaker Exchange[22] to

identify other specialists following probands with this new RGD. In another case, a clinician interpreted a variant in *ARR3* as partially-diagnostic based on additional published literature available soon after the report was issued and appropriate segregation in the family. The remainder require additional evidence to resolve and are, therefore, potentially-diagnostic.

Overall, clinicians identified 108 clinical-molecular diagnoses (**Supplemental Table 4.3**), of which 90 were diagnostic and 18 were partially diagnostic (**Figure 4.1**). These included 96 unique genes, with multiple diagnoses made in 10 genes (three diagnoses each in *CACNA1A* and *FLG*, and two diagnoses in *MED13L, ANKRD11, ADNP, ASXL3, TUBA1A, FOXG1, KCNQ2,* and *SHANK3*). Of the 96 unique genes, six (6%) did not have publications linking them to Mendelian disease five years ago (e.g., prior to 2018). Four probands received two partial diagnoses that comprised a complete explanation for their phenotype (1% of cohort, 4% of those diagnosed). Therefore, of the 297 probands, 104 (35%) received one or more clinical-molecular diagnoses; 94 probands (32%) received diagnostic results that accounted for their complete clinical presentation, and ten probands (3%) received diagnostic results that accounted for part of their clinical presentation.

Relationship between diagnoses and biological sex, phenotype, and testing criteria

We stratified the diagnostic results made in 104 probands by various test ordering criteria, including biological sex at birth, phenotypic category, sequencing strategy, and the type and number of clinical presentation criteria reported by the ordering clinicians (**Table 4.3**). The proband's biological sex can affect diagnostic yield given the higher propensity to X-linked molecular diagnoses in males and the variable penetrance of some RGDs based on biological sex. We did not observe a difference in diagnostic yield based on biological sex. We did observe differences in the proportions of probands that received diagnostic results from ES based on their phenotypic category [$\chi^2(4,N=297)=11.8$, p=.02], with the

highest yields in probands with ID/DD. Many sub-groupings based on the sequencing strategy were small because of the preponderance of trios, however, the lowest yield was in singletons (20%). Diagnostic yields related to the non-mutually exclusive MOH clinical presentation criteria were similar and ranged from 33-40%. The proportion of patients receiving a diagnostic result increased as the number clinical presentation criteria met increased; from 25% among those who met two criteria, to 41% among those who met three, to 45% among those who met four or five criteria [χ^2 (2,N=297)=9.7, p=.01].

Impact of ES on intensity of diagnostic investigations (Level 3 continued, Diagnostic thinking)

Of the 104 probands that received one or more clinical-molecular diagnoses, we collected hypothetical pathways for 97 (representing 101 clinical-molecular diagnoses). Nineteen of those pathways did not specify a particular molecular test and, therefore, would have missed the diagnoses (20%) without ES. For the other 78 probands, 121 molecular tests were selected (**Supplemental Table 4.4**). This included 34 types of multi-gene panels and seven unique single-gene tests; the most commonly selected molecular test was a large ID panel of >1000 genes (43 probands). Within these 78 probands there were 82 clinical-molecular diagnoses; 62 (75%) would likely have been captured by at least one of the selected molecular tests. Therefore, had all molecular testing within the hypothetical pathway been pursued, 62 of the 101 (61%) clinical-molecular diagnoses might have been identified in the absence of ES.

Table 4.3. Stratification of probands with diagnostic results (clinical-molecular diagnoses) by biological sex, phenotype, sequencing strategy, and Ontario criteria

Criteria	*No. Probands*	*No. Probands with diagnostic result†*	*Diagnostic yield (%)*
Total cohort	**297**	**104**	**35%**
Biological sex			
Male	156	53	34%
Female	141	51	36%
Phenotypic category (only 1 per proband)			
Syndromic ID/DD	206	83	40%
Isolated severe ID	12	6	50%
MCA without ID/DD	14	2	14%
Multisystem without ID/DD	46	9	20%
Single System without ID/DD	19	4	21%
Sequencing strategy			
Singleton	20	4	20%
Duo	32	13	41%
Trio	235	84	36%
Quad or Quint	10	3	30%
Clinical presentation criteria (≥2 per patient)			
Moderate to severe impairment	242	96	40%
Multisystem	211	78	37%
Progressive	82	29	35%
Differential ≥ 2 conditions	240	85	35%
Multiple family members or consanguinity	46	15	33%
Number of clinical presentation criteria (≥2 per patient)			
Probands with 2 clinical presentation criteria selected	118	29	25%
Probands with 3 clinical presentation criteria selected	137	56	41%
Probands with 4 or 5 clinical presentation criteria selected	42	19	45%

†*complete or partially diagnostic. Abbreviations: ID, Intellectual Disability; DD, Developmental Delay; MCA, Multiple Congenital Anomalies*

DISCUSSION

To assist the Ontario MOH in evaluating the diagnostic utility of publicly-funded clinical ES, we prospectively recruited 297 probands who received ES through laboratories outside of Canada, collected the molecular results, asked the ordering clinicians to interpret those results to identify clinical-molecular diagnoses, and compared those findings to hypothetical care pathways. In doing so, we examined the diagnostic accuracy of ES and how it impacts clinician diagnostic thinking.

With respect to the diagnostic accuracy of laboratory results, 99 of 297 probands (33%) received one or more molecular diagnoses. This proportion is higher than reported in publications of large cohorts with non-specific phenotypes who received clinical testing by reference laboratories (~25%) (Lee et al., 2014; Retterer et al., 2016; Smith et al., 2019; Soden SE, 2014; Yang et al., 2013; Yang et al., 2014) and similar to diagnostic rates reported in clinical and research cohorts (36%; 95% CI 0.33-0.40) (Clark et al., 2018).

A clinical laboratory's classification of pathogenicity for particular DNA variants in a gene is not equivalent to diagnosing a proband with the associated disease. We, therefore, asked ordering clinicians to interpret the 105 molecular diagnoses and 165 uncertain results in known genes and GUSs identified by laboratories. In doing so, the ordering clinicians identified 108 clinical-molecular diagnoses. The vast majority of molecular diagnoses (97%, 102/105) were clinically valid. This proportion aligns with ACMG standards for variant reporting, in which variants are categorized as likely-pathogenic or pathogenic if evidence suggests a probability of pathogenicity greater than 90% or 99% (Richards et al., 2015). The other six clinical-molecular diagnoses were identified in VUSs in known genes (n=3) and GUSs (n=3). Clinicians reported using additional phenotypic information, family history information, additional testing, and specialist consultations through data sharing to upgrade these results. The clinicians downgraded three of 105 (3%) molecular diagnoses and upgraded six of 165 (4%) non-diagnostic

molecular results. To the best of our knowledge, results such as these for probands with RGDs have not been reported before, but the approach is similar to that described by Biesecker, Nussbaum and Rehm (2018). If replicated in future studies, this might indicate that using diagnostic yields from molecular laboratories (level 2 efficacy) is a reasonable proxy for the intensive data collection required for clinical interpretation data (level 3 efficacy).

Overall, 104 (35%) probands received one or more clinical-molecular diagnoses. As hypothesized, the diagnostic yield met the 30% benchmark for provincial ES testing. MOH developed these criteria as a first iteration that could be adapted over time and were designed based on factors believed to increase the appropriate use of ES (Genetic Testing Advisory Committee, 2016). We found that these criteria had a diagnostic yield greater than 30% and that as the number of eligibility criteria applicable to a family increased, the corresponding yield increased (from 25% to 45%). The consistency in yield among individual criteria and additive effect on yield of criteria in combination may indicate that number of criteria can predict the likelihood of achieving a diagnostic result. The number of probands in our cohort was too small to assess the utility of ES for particular combinations of eligibility criteria. In addition, we were inadequately powered to understand the value of different sequencing strategies (i.e. singleton, duo, trio). The trend in our data align with other studies, however, which show an increased diagnostic yield for trios compared to singletons (Clark et al., 2018).

We found that clinicians use ES instead of a broad range of molecular tests. These results indicate that ES represents a streamlined approach in the context of a broad differential diagnosis. We found that the hypothetical molecular tests would have missed almost 40% of the clinical-molecular diagnoses, allowing us to speculate, in the absence of a non-ES comparator group, that there is an immediate diagnostic impact in introducing ES compared to standard-of-care in Ontario. Importantly, in a study

conducted in Australia that performed ES in parallel to standard-of-care and collected the planned diagnostic investigations at enrollment, it was found that less than half of the proposed molecular tests (13 of 37) were pursued, indicating that the decision to pursue the considered testing is complicated by the concern that ordering many panels may cost as much or more than ES and a concern that the conventional test may not contain all genes of interest for a particular family (Stark et al., 2016). This hesitancy to pursue hypothetical testing has implications for the analysis reported here. These hypothetical tests may have never been pursued, been ordered sequentially, or entirely different tests may have been ordered if ES was not available at the time.

Immediately after testing, 64% of this cohort had no genetic diagnosis. However, 47 probands (16% of the cohort) had a candidate (VUS or GUS) that could become diagnostic with re-interpretation at a later date. These findings highlight additional potential diagnostic value for ES data. We have shown that translational research methods can increase the number of diagnoses identified from genomic data within Ontario patients (Hartley et al., 2022), highlighting the value of identifying GUS and the importance of policy development and reimbursement for clinical ES reanalysis. For other undiagnosed probands, a negative clinical ES may have aided clinicians in ruling out a genetic diagnosis or in establishing a different, non-genetic diagnosis. It is a limitation of our study that we did not ask clinicians for their impressions on the utility of negative results in refining their diagnostic thinking (level 3).

Our study had other limitations. Data entry errors are possible. While we aimed to capture the majority of patients receiving clinical ES, our design was limited by historic test ordering data and enrollment was limited to those clinics and clinicians with interest and available resources to implement our study; this introduces a selection bias. As mentioned, we enrolled 18% of potentially-eligible participants. This capture rate is an underestimate because (i) not all families approved for testing pursue it, (ii) the start

of recruitment varied between sites, and (iii) not all ordering clinicians from a given hospital were involved in our study. We speculate that the COVID-19 pandemic compromised our capture rate; whereas some sites quickly implemented virtual care and verbal consent procedures, others were never able to and relied on cumbersome alternative ways to collect consent. If we focus on a period within which all sites were actively recruiting and using similar protocols (April 2019 to March 2020), 28% of families approved for sequencing at participating hospitals were enrolled (**Supplemental 4.2**). Unfortunately, we do not know how many families were invited and declined to participate. Since April 2021, most post-natal ES has been done locally as a publicly-funded service through the Genome-wide Sequencing Ontario (www.GSOntario.ca) pilot program, enabling the future evaluation of laboratory-based variables from a larger, unselected cohort of patients from Ontario.

Our study provides evidence of the utility of ES in identifying molecular diagnoses (level 2) and guiding the clinician's diagnostic thinking (level 3). We show that implementing a standardized set of eligibility criteria to guide the use of clinical ES met the provincial benchmark for the proportion of patients receiving clinical-molecular diagnoses. The clinical utility and cost-consequences on the healthcare system of ES results will be the subject of future publications from Care4Rare SOLVE. Further, these results may act as a starting point for discussions regarding sustainability and scaling of provincial criteria and recommendations related to testing, including calibration of proband eligibility for testing in the context of finite resources within a publicly-funded healthcare system.

DATA AVAILABILITY

All data supporting this study has been collected using the Care Pathway data collection surveys in Genomics4RD, the official database for Care4Rare Canada, and is available upon controlled-access

request and approval. Many of the candidate genes and corresponding phenotypes are available through the PhenomeCentral node of the Matchmaker Exchange.

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AUTHOR CONTRIBUTIONS

Conceptualization: T.H., K.M.B., R.H., B.P., I.G.; Data curation: T.H., M.A., K.F., M.G.; Formal Analysis: T.H.; Funding Acquisition: K.M.B., R.H., D.M., Methodology: T.H., K.M.B., R.H., D.M., I.G., B.P.; Investigation: T.H., M.A., K.F., A.W.-B., L.M., S.K.M., L.B., A.Y.H., J.D.A., A.C., E.W., E.S., G.U.E., S.R., D.A., D.D., M.T., S.L.S., C.C., G.L., K.A., J.L., R.M.-L., J.J.D., T.B.B., C.M.A., P.T., G.C., L.D., M.C., L.B., J.R., C.B.-P., P.K., D.C., J.W.-C., G.G., V.M.S., C.C., A.R., R.B.A., G.Y., H.G., V.M., S.M.-A., A.G., A.R.D., A.M., R.W., N.K., M.C., S.A.Q., A.Y.S., M.I.-F., R.C., A.S., C.I., M.P., L.C.; Writing – Original Draft: T.H., R.H., K.M.B.; Writing – review and editing: D.M., M.A., K.F., M.G., E.M.P., I.D.G., B.P., A.W.-B., L.M., S.K.M., L.B., A.Y.H., J.D.A., A.C., E.W., E.S., G.U.E., S.R., D.A., D.D., M.T., S.L.S., C.C., G.L., K.A., J.L., R.M.-L., J.J.D., T.B.B., C.M.A., P.T., G.C., L.D., M.C., L.B., J.R., C.B.-P., P.K., D.C., J.W.-C., G.G., V.M.S., C.C., A.R., R.B.A., G.Y., H.G., V.M., S.M.-A., A.G., A.R.D., A.M., R.W., N.K., M.C., S.A.Q., A.Y.S., M.I.-F., R.C., A.S., C.I., M.P., L.C.; Supervision: I.G., R.H., B.P., K.M.B.

ETHICS DECLARATION

Written informed consent was obtained for use of medical chart data with approval from the Clinical Trials Ontario Streamlined Research Review System (CTO-1577).

CONFLICT OF INTEREST

The authors declare no competing interests.

4.5. References

Biesecker, L. G., Nussbaum, R. L., & Rehm, H. L. (2018). Distinguishing variant pathogenicity from genetic diagnosis: How to know whether a variant causes a condition. *JAMA*, *320*(18), 1929-1930. https://doi.org/10.1001/jama.2018.14900

Birney, E., Vamathevan, J., & Goodhand, P. (2017). Genomics in healthcare: GA4GH looks to 2022. *bioRxiv*. https://doi.org/10.1101/203554

Boycott, K., Hartley, T., Adam, S., Bernier, F., Chong, K., Fernandez, B. A., Friedman, J. M., Geraghty, M. T., Hume, S., Knoppers, B. M., Laberge, A. M., Majewski, J., Mendoza-Londono, R., Meyn, M. S., Michaud, J. L., Nelson, T. N., Richer, J., Sadikovic, B., Skidmore, D. L., . . . Canadian College of Medical Geneticists. (2015). The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position statement of the Canadian College of Medical Geneticists. *J Med Genet*, *52*(7), 431-437. https://doi.org/10.1136/jmedgenet-2015-103144

Boycott, K. M., Azzariti, D. R., Hamosh, A., & Rehm, H. L. (2022). Seven years since the launch of the Matchmaker Exchange: The evolution of genomic matchmaking. *Hum Mutat*, *43*(6), 659-667. https://doi.org/10.1002/humu.24373

Boycott, K. M., Hartley, T., Kernohan, K. D., Dyment, D. A., Howley, H., Innes, A. M., Bernier, F. P., Brudno, M., & Care4Rare Canada, C. (2022). Care4Rare Canada: Outcomes from a decade of network science for rare disease gene discovery. *Am J Hum Genet*, *109*(11), 1947-1959. https://doi.org/10.1016/j.ajhg.2022.10.002

Carmichael, N., Tsipis, J., Windmueller, G., Mandel, L., & Estrella, E. (2015). "Is it going to hurt?": The impact of the diagnostic odyssey on children and their families. *J Genet Couns*, *24*(2), 325-335. https://doi.org/DOI 10.1007/s10897-014-9773-9

Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M., Dimmock, D., & Kingsmore, S. F. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal

microarray in children with suspected genetic diseases. *NPJ Genom Med*, *3*, 16. https://doi.org/10.1038/s41525-018-0053-8

Driver, H. G., Hartley, T., Price, E. M., Turinsky, A. L., Buske, O. J., Osmond, M., Ramani, A. K., Kirby, E., Kernohan, K. D., Couse, M., Elrick, H., Lu, K., Mashouri, P., Mohan, A., So, D., Klamann, C., Le, H., Herscovich, A., Marshall, C. R., . . . Boycott, K. M. (2022). Genomics4RD: An integrated platform to share Canadian deep-phenotype and multiomic data for international rare disease gene discovery. *Hum Mutat*, *43*(6), 800-811. https://doi.org/10.1002/humu.24354

Fryback, D., & Thornbury, J. (1991). The efficacy of diagnostic imaging. *Med Decis Making*, *11*(2), 88-94.

Genetic Testing Advisory Committee. (2016). *Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario*. http://www.health.gov.on.ca/en/pro/programs/gtac/reports.aspx

Government of Canada (2023) *Canada Health Act.* Retrieved March 30, 2023, from https://www.canada.ca/en/health-canada/services/health-care-system/canada-health-care-system-medicare/canada-health-act.html

Graham, I. D., Logan, J., Harrison, M. B., Straus, S. E., Tetroe, J., Caswell, W., & Robinson, N. (2006). Lost in knowledge translation: Time for a map? *J Contin Educ Health Prof*, *26*(1), 13-24. https://doi.org/10.1002/chp.47

Hartley, T., Soubry, E., Acker, M., Osmond, M., Couse, M., Gillespie, M., Ito, Y., Marshall, A. E., Lemire, G., Huang, L., Chisholm, C., Eaton, A. E., Price, E. M., Dowling, J. J., Ramani, A. K., Mendoza-Londono, R., Costain, G., Axford, M. M., Szuto, A., . . . Boycott, K. M. (2022). Bridging clinical care and research in Ontario, Canada: Maximizing diagnoses from reanalysis of clinical exome sequencing data. *Clin Genet*. https://doi.org/10.1111/cge.14262

Hayeems, R. Z., Bernier, F., Boycott, K. M., Hartley, T., Michaels-Igbokwe, C., & Marshall, D. A. (2022). Positioning whole exome sequencing in the diagnostic pathway for rare disease to optimise

utility: A protocol for an observational cohort study and an economic evaluation. *BMJ Open*, *12*(10), e061468. https://doi.org/10.1136/bmjopen-2022-061468

Heidari, S., Babor, T. F., De Castro, P., & Curno, M. (2016). Sex and gender equity in research: Rationale for the SAGER guidelines and recommended use. *Res Integr Peer Rev*, *1*, 2.

Lee, H., Deignan, J. L., Dorrani, N., Strom, S. P., Kantarci, S., Quintero-Rivera, F., Das, K., Toy, T., Harry, B., Yourshaw, M., Fox, M., Fogel, B. L., Martinez-Agosto, J. A., Wong, D. A., Chang, V. Y., Shieh, P. B., Palmer, C. G., Dipple, K. M., Grody, W. W., . . . Nelson, S. F. (2014). Clinical exome sequencing for genetic identification of rare Mendelian disorders. *JAMA*, *312*(18), 1880-1887. https://doi.org/10.1001/jama.2014.14604

Michelson, J. (2018). My diagnostic odyssey - a call to expand access to genomic testing for the next generation. *Hastings Cent Rep*, *48*, S32-S34. https://doi.org/ https://doi.org/10.1002/hast.882.

Ontario Ministry of Health. (2018). *OHIP Out of Country Prior Approval Program*. Retrieved November 6 2022, from https://www.health.gov.on.ca/en/public/programs/ohip/outofcountry/prior_approval.aspx#1

Phillips, K. A., Douglas, M. P., & Marshall, D. A. (2020). Expanding use of clinical genome sequencing and the need for more data on implementation. *JAMA*, *324*(20), 2029-2030. https://doi.org/10.1001/jama.2020.19933

Phillips, K. A., Douglas, M. P., Wordsworth, S., Buchanan, J., & Marshall, D. A. (2021). Availability and funding of clinical genomic sequencing globally. *BMJ Glob Health*, *6*(2). https://doi.org/10.1136/bmjgh-2020-004415

Retterer, K., Juusola, J., Cho, M. T., Vitazka, P., Millan, F., Gibellini, F., Vertino-Bell, A., Smaoui, N., Neidich, J., Monaghan, K. G., McKnight, D., Bai, R., Suchy, S., Friedman, B., Tahiliani, J., Pineda-Alvarez, D., Richard, G., Brandt, T., Haverfield, E., . . . Bale, S. (2016). Clinical application of

whole-exome sequencing across clinical indications. *Genet Med*, *18*(7), 696-704. https://doi.org/10.1038/gim.2015.148

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, *17*(5), 405-424. https://doi.org/10.1038/gim.2015.30

Sawyer, S. L., Hartley, T., Dyment, D. A., Beaulieu, C. L., Schwartzentruber, J., Smith, A., Bedford, H. M., Bernard, G., Bernier, F. P., Brais, B., Bulman, D. E., Warman Chardon, J., Chitayat, D., Deladoey, J., Fernandez, B. A., Frosk, P., Geraghty, M. T., Gerull, B., Gibson, W., . . . Boycott, K. M. (2016). Utility of whole-exome sequencing for those near the end of the diagnostic odyssey: Time to address gaps in care. *Clin Genet*, *89*(3), 275-284. https://doi.org/10.1111/cge.12654

Smith, E. D., Blanco, K., Sajan, S. A., Hunter, J. M., Shinde, D. N., Wayburn, B., Rossi, M., Huang, J., Stevens, C. A., Muss, C., Alcaraz, W., Hagman, K. D. F., Tang, S., & Radtke, K. (2019). A retrospective review of multiple findings in diagnostic exome sequencing: Half are distinct and half are overlapping diagnoses. *Genet Med*, *21*(10), 2199-2207. https://doi.org/10.1038/s41436-019-0477-2

Soden S.E., Saunders, C.J., Willig, L.K., Farrow, E.G., Smith, L.D., Petrikin, J.E., LePichon, J.B., Miller, N.A., Thiffault, I., Dinwiddie, D.L., Twist, G., Noll, A., Heese, B.A., Zellmer, L., Atherton, A.M., Abdelmoity, A.T., Safina, N., Nyp, S.S., Zuccarelli, B., . . . Kingsmore, S.F. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med*, *6*(265), 265ra168. https://doi.org/doi: 10.1126/scitranslmed.3010076

Stark, Z., Dolman, L., Manolio, T. A., Ozenberger, B., Hill, S. L., Caulfied, M. J., Levy, Y., Glazer, D., Wilson, J., Lawler, M., Boughtwood, T., Braithwaite, J., Goodhand, P., Birney, E., & North, K. N. (2019). Integrating genomics into healthcare: A global responsibility. *Am J Hum Genet*, *104*(1), 13-20. https://doi.org/10.1016/j.ajhg.2018.11.014

Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., McGillivray, G., Downie, L., Ekert, P. G., Theda, C., James, P. A., Yaplito-Lee, J., Ryan, M. M., . . . White, S. M. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet Med*, *18*(11), 1090-1096. https://doi.org/10.1038/gim.2016.1

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., Vandenbroucke, J. P., & Initiative., S. (2007). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Lancet*, *370*(9596), 1453-1457. https://doi.org/10.1016/S0140-6736(07)61602-X

Yang, Y., Muzny, D. M., Reid, J. G., Bainbridge, M. N., Willis, A., Ward, P. A., Braxton, A., Beuten, J., Xia, F., Niu, Z., Hardison, M., Person, R., Bekheirnia, M. R., Leduc, M. S., Kirby, A., Pham, P., Scull, J., Wang, M., Ding, Y., . . . Eng, C. M. (2013). Clinical whole-exome sequencing for the diagnosis of Mendelian disorders. *N Engl J Med*, *369*(16), 1502-1511. https://doi.org/10.1056/NEJMoa1306555

Yang, Y., Muzny, D. M., Xia, F., Niu, Z., Person, R., Ding, Y., Ward, P., Braxton, A., Wang, M., Buhay, C., Veeraraghavan, N., Hawes, A., Chiang, T., Leduc, M., Beuten, J., Zhang, J., He, W., Scull, J., Willis, A., . . . Eng, C. M. (2014). Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*, *312*(18), 1870-1879. https://doi.org/10.1001/jama.2014.14601

4.6. Supplemental information

Supplemental Table 4.1. STROBE Statement—Checklist of items that should be included in reports of cohort studies [von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. 2007;370(9596):1453-1457. doi:10.1016/S0140-6736(07)61602-X]

	Item No	Recommendation	Details
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Done
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Done
Introduction			
Background/ rationale	2	Explain the scientific background and rationale for the investigation being reported	Done
Objectives	3	State specific objectives, including any prespecified hypotheses	Done
Methods			
Study design	4	Present key elements of study design early in the paper	Done
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Done
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Done
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Diagnostic criteria defined
Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Done
Bias	9	Describe any efforts to address potential sources of bias	None

Study size	10	Explain how the study size was arrived at	Done
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Done
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	Done
Results			
Participants	13	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Done for potentially eligible and analysed, other details not possible
		(b) Give reasons for non-participation at each stage	Not possible, added as limitation
		(c) Consider use of a flow diagram	
Descriptive data	14	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Done
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	
Outcome data	15	Report numbers of outcome events or summary measures over time	Done

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Done for unadjusted estimates
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Done
Discussion			
Key results	18	Summarise key results with reference to study objectives	Done
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Done
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Done
Generalizability	21	Discuss the generalizability (external validity) of the study results	Done
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Done

Supplemental Table 4.2. Total number of approved publicly-funded ES over recruitment period and capture rate												
Time period:	**April 2018-March 2019**			**April 2019-March 2020**			**April 2020-March 2021**			**Total time period**		
Region of ordering provider	**Number Approved**	**Number Recruited**	**Capture Rate**	**Number Approved**	**Number Recruited**	**Capture Rate**	**Number Approved**	**Number Recruited**	**Capture Rate**	**Total Approved**	**Total Recruited**	**Capture rate**
Toronto Region	189	3	2%	181	45	25%	143	27	19%	513	75	15%
East Region	189	26	14%	181	87	48%	177	47	27%	547	160	29%
West Region	179	7	4%	216	31	14%	156	24	15%	551	62	11%
Total across recruiting sites	**557**	**36**	**6%**	**578**	**163**	**28%**	**476**	**98**	**21%**	**1611**	**297**	**18%**
Total across province	802	36	4%	813	163	20%	684	98	14%	2299	297	13%

Supplemental Table 4.3. List of 108 clinical-molecular diagnoses identified in 104 probands receiving publicly-funded ES									
Study Identifier	***Primary molecular result***	***Clinical Interpretation***	***Gene***	***DNA Variant(s)***	***ACMG Classification***	***Zygosity***	***OMIM Disease-Gene Association***	***OMIM Identifier***	***OMIM Inheritance***
CH4002	Positive	diagnostic	ALDH3A2	arr[GRCh37] 17p11.2(19436246_19584983)x1, NM_000382.2:c.835T>A p.(Tyr279Asn)	Pathogenic, Pathogenic	Heterozygous, Heterozygous	Sjogren-Larsson syndrome	270200	AR
CH4007	Positive	diagnostic	PACS2	NM_001100913.2:c.625G>A p.(Glu209Lys)	Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 66	618067	AD
CH4012	Positive	diagnostic	MAN1B1	NM_016219.4:c.1075G>T p.(Gly359Ter)	Pathogenic	Homozygous	Rafiq syndrome	614202	AR
CH4015	Positive, multiple	diagnostic based on 2 partial diagnoses	WAC	NM_016628.4:c.1747-2A>G	Pathogenic	Heterozygous	Desanto-Shinawi syndrome	616708	XLR
			G6PD	NM_001360016.2:c.563C>T p.(Ser188Phe)	Pathogenic	Hemizygous	Hemolytic anemia, G6PD deficient (favism)	300908	XLD
CH4018	Positive	diagnostic	SLC2A1	NM_006516.2:c.654dup p.(Asn219GlnfsTer18)	Pathogenic	Heterozygous	GLUT1 deficiency syndrome 1, infantile onset, severe	606777	AD,AR
CH4020	Positive	diagnostic	PURA	NM_005859.5:c.112_116dup p.(Gly41AlafsTer39)	Likely Pathogenic	Heterozygous	Neurodevelopmental disorder with neonatal respiratory insufficiency, hypotonia, and feeding difficulties	616158	AD
CH4021	Uncertain novel	diagnostic	AGO2	NM_012154.3:c.2252G>A p.(Cys751Tyr)	GUS	Heterozygous	Lessel-Kreienkamp syndrome	619149	AD
SK4003	Positive	diagnostic	NIPBL	NM_133433.4:c.5582 G>T p.(Gly1861Val)	Pathogenic	Heterozygous	Cornelia de Lange syndrome 1	122470	AD
SK4004	Positive	diagnostic	MED13L	Partial Gene Deletion	Pathogenic	Heterozygous	Impaired intellectual development and distinctive facial features with or	616789	AD

							without cardiac defects		
SK4007	Positive	diagnostic	WDR45	NM_001029896.3:c.686_687del p.(Leu229ArgfsTer8)	Pathogenic	Heterozygous	Neurodegeneration with brain iron accumulation 5	300894	XLD
SK4008	Positive, multiple	diagnostic based on 2 partial diagnoses	ANKRD11	NM_013275.5:c.3860_3861del p.(Glu1287GlyfsTer4)	Pathogenic	Heterozygous	KBG syndrome	148050	AD
			FLG	NM_002016.1:c.2282_2285del (p.Ser761fsCyfsTer36)	Pathogenic	Heterozygous	Ichthyosis vulgaris	146700	AD, AR
SK4011	Positive	diagnostic	CACNA1A	NM_001127222.1:c.1060C>T p.(Leu354Phe)	Likely Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 42	617106	AD
HA4005	VUS	diagnostic	RTN4IP1	NM_032730.5:c.712C>T p.(Fln238Ter), NM_032730.5:c.426+5G>T	Pathogenic, VUS	Heterozygous , Heterozygous	Optic atrophy 10 with or without ataxia, mental retardation, and seizures	616732	AR
HA4003	Positive	diagnostic	GATAD2B	NM_020699.4:c.5A>T p.(Asp2Val)	Likely Pathogenic	Heterozygous	GAND syndrome	615074	AD
HA4006	Positive	diagnostic	KIF1A	NM_001244008.2:c.939G>C p.(Trp313Cys)	Pathogenic	Heterozygous	Nescav syndrome	614255	AD
HA4004	Positive	diagnostic	BRAF	NM_004333.6:c.2135C>A p.(Ala712Asp)	Pathogenic	Heterozygous	Noonan syndome 7	613706	AD
HA4007	Uncertain novel	diagnostic	CLDN5	NM_001363066.2:c.121G>A p.(Val41Met)	GUS	Heterozygous	None yet described		
HA4010	Positive	diagnostic	MECP2	NM_004992.3:c.473C>T p.(Thr158Met)	Pathogenic	Heterozygous	Rett syndrome	312750	XLD
HA4008	Positive	diagnostic	PPP2R5D	NM_006245.4:c.592G>A p.(Glu198Lys)	Pathogenic	Heterozygous	Intellectual developmental disorder, autosomal dominant 35	616355	AD
CH4028	Positive	diagnostic	KAT6B	NM_012330.3:c.1061+1G>A	Pathogenic	Heterozygous	Genitopatellar syndrome	606170	AD
CH4032	VUS	partially-diagnostic	GH1	NM_000515.3:c.-185T>C	VUS	Homozygous	Growth hormone deficiency, isolated, type IB	612781	AR

CH4034	Positive	diagnostic	AP4M1	NM_004722.3:c.916C>T p.(Arg306Ter), NM_004722.3:c.1137+1G>T	Pathogenic, Pathogenic	Heterozygous, Heterozygous	Spastic paraplegia 50, autosomal recessive	612936	AR
CH4037	Positive	diagnostic	ASXL3	NM_030632.1:c.3330_3333dup p.(Ala1112LeufsTer15)	Pathogenic	Heterozygous	Bainbridge-Ropers syndrome	615485	AD
HA4011	Positive	diagnostic	CHD8	NM_001170629.2:c.4371-1G>A	Pathogenic	Heterozygous	Intellectual developmental disorder with autism and macrocephaly	615032	AD
SK4013	Positive	diagnostic	MRPS34	NM_023936.1:c.94C>T p.(Gln32Ter), NM_023936.1:c.37G>A p.(Glu13Lys)	Pathogenic, Likely Pathogenic	Heterozygous, Heterozygous	Combined oxidative phosphorylation deficiency 32	617664	AR
SK4014	Positive	diagnostic	OFD1	NM_003611.2:c.629A>C p.(Gln210Pro)	Pathogenic	Heterozygous	Orofaciodigital syndrome 1	311200	XLD
SK4015	Positive	diagnostic	ATP1A2	NM_000702.3:c.2936C>T p.(Pro979Leu)	Pathogenic	Heterozygous	Familial hemiplegic migraine	602481	AD
SK4021	Positive	diagnostic	GABBR2	NM_005458.7:c.1724C>A p.(Thr575Asn)	Pathogenic	Heterozygous	Neurodevelopmental disorder with poor language and loss of hand skills	617903	AD
HA4012	Positive	diagnostic	STXBP1	NM_001032221.6:c.416C>T p.(Pro138Leu)	Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 4	612164	AD
SK4024	Positive	diagnostic	CDK13	NM_003718.4:c.2255A>G p.(Glu752Gly)	Likely Pathogenic	Heterozygous	Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder	617360	AD
HA4014	Positive	diagnostic	ADNP	NM_001282531.3:c.56_57del p.(Val19fsTer5)	Pathogenic	Heterozygous	Helsmoortel-van der Aa syndrome	615873	AD
SK4025	Positive	diagnostic	PDHA1	NM_000284.3:c.506C>T p.(Ala169Val)	Pathogenic	Heterozygous	Pyruvate dehydrogenase E1-alpha deficiency	312170	XLD
SK4026	Positive	diagnostic	EP300	NM_001429:Partial Gene Deletion	Pathogenic	Heterozygous	Rubinstein-Taybi syndrome 2	613684	AD

SK4028	Positive	diagnostic	DGAT1	NM_012079.5:c.629_631del p.(Ser210del), NM_012079.5:c.751+2T>C	Pathogenic, Pathogenic	Heterozygous , Heterozygous	Diarrhea 7, protein-losing enteropathy type	615863	AR
SK4034	Positive	diagnostic	INPP5K	NM_016532.3:c.67G>A (p.Val23Met)	Likely Pathogenic	Homozygous	Muscular dystrophy, congenital, with cataracts and intellectual disability	617404	AR
SK4023	Positive	partially-diagnostic	GANAB	NM_198335.3:c.2575C>T p.(Gln859Ter)	Pathogenic	Heterozygous	Polycystic kidney disease 3	600666	AD
CH4054	Positive	diagnostic	TUBA1A	NM_006009.2:c.1181A>G p.(Lys394Arg)	Pathogenic	Heterozygous	Lissencephaly 3	611603	AD
HA4016	Positive	diagnostic	CASK	NM_001367721.1:c.2032C>T p.(Gln678Ter)	Pathogenic	Heterozygous	Intellectual developmental disorder and microcephaly with pontine and cerebellar hypoplasia	300749	XLD
CH4058	Positive	diagnostic	DLG4	NM_001365.3:c.1054C>T p.(Arg352Ter)	Pathogenic	Heterozygous	Intellectual developmental disorder, autosomal dominant 62	618793	AD
CH4060	Positive	diagnostic	INSR	NM_000208.2:c.438C>G p.(Ile146Met)	Pathogenic	Homozygous	Rabson-Mendenhall syndrome	262190	AR
CH4059	Positive	diagnostic	CTNNB1	NM_001904.3:c.367C>T p.(Gln123Ter)	Pathogenic	Heterozygous	Neurodevelopmental disorder with spastic diplegia and visual defects	615075	AD
HA4013	Positive	diagnostic	SLC1A4	NM_003038.5:c.527G>A p.(Arg176Lys)	Likely Pathogenic	Homozygous	Spastic tetraplegia, thin corpus callosum, and progressive microcephaly	616657	AR
CH4066	Positive	diagnostic	EFTUD2	NM_004247.3:c.2642_2643del p.(Phe881TrpfsTer3)	Pathogenic	Heterozygous	Mandibulofacial dysostosis, Guion-Almeida type	610536	AD

CH4068	Positive	diagnostic	SCN2A	NM_021007.2:c.1706C>T p.(Pro569Leu)	Likely Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 11	613721	AD
CH4071	Positive	diagnostic	EBF3	NM_001005463.2:c.1183C>T p.(Arg395Ter)	Pathogenic	Heterozygous	Hypotonia, ataxia, and delayed development syndrome	617330	AD
SK4035	Positive	diagnostic	ARX	NM_139058.2:c.1105G>A p.(Glu369Lys)	Likely Pathogenic	Hemizygous	Developmental and epileptic encephalopathy 1	308350	XLR
SK4037	Positive	diagnostic	FOXG1	NM_005249.5:c.95dup p.(Asn32LysfsTer89)	Pathogenic	Heterozygous	Rett syndrome, congenital variant	613454	AD
CH4075	Uncertain novel	partially-diagnostic	ARR3	NM_004312.2:c.298C>T (p.Arg100Ter)	VUS	Heterozygous	Myopia, X-linked, female-limited	301010	XL
KG4003	Positive	diagnostic	ATM	NM_000051.4:c.6049_6052del, NM_000051.4:c.7638_7646del	Pathogenic, Pathogenic	Heterozygous, Heterozygous	Ataxia-telangiectasia	208900	AR
KG4004	Positive	diagnostic	CDH2	NM_001792.5:c.1880A>G p.(Asp627Gly)	Pathogenic	Heterozygous	Agenesis of corpus callosum, cardiac, ocular, and genital syndrome	618929	AD
SK4040	Positive	diagnostic	UBA1	NM_003334.4:c.1660C>T p.(Pro554Ser)	Likely Pathogenic	Hemizygous	Spinal muscular atrophy, X-linked 2, infantile	301830	XLR
SK4044	VUS	diagnostic	POMGNT2	NM_032806.5:c.1089G>T p.(Glu363Asp)	VUS	Homozygous	Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 8	614830	AR
CH4084	Positive	diagnostic	NDUFB3	NM_002491.2:c.64T>C p.(Trp22Arg)	Pathogenic	Homozygous	Mitochondrial complex I deficiency, nuclear type 25	618246	AR
CH4085	Positive, multiple	diagnostic based on 2 partial diagnoses	EED	NM_003797.4:c.581A>G p.(Asn194Ser)	Pathogenic	Heterozygous	Cohen-Gibson syndrome	617561	AD
			CACNA1A	NM_001127222.1:c.400-1G>A	Pathogenic	Heterozygous	Episodic ataxia, type 2	108500	AD

CH4086	Positive	diagnostic	FOXG1	NM_005249.3:c.460dup p.(Glu154GlyfsTer301)	Pathogenic	Heterozygous	Rett syndrome, congenital variant	613454	AD
CH4087	Positive	diagnostic	ARID1B	NM_020732.3:c.4870C>T p.(Arg1624Ter)	Pathogenic	Heterozygous	Coffin-Siris syndrome 1	135900	AD
CH4090	Positive	diagnostic	SPG11	NM_025137.3:c.782C>A p.(Ser261Ter)	Pathogenic	Homozygous	Spastic paraplegia 11, autosomal recessive	604360	AR
LD4007	Positive	diagnostic	ADNP	NM_001282531.3:c.361_362del p.(Leu121fsTer5)	Likely Pathogenic	Heterozygous	Helsmoortel-van der Aa syndrome	615873	AD
CH4098	Positive	partially-diagnostic	FBN1	NM_000138.4:c.5636G>A p.(Gly1879Asp)	Likely Pathogenic	Heterozygous	Marfan syndrome	154700	AD
LD4008	Positive	diagnostic	DYNC1H1	NM_001376.5:c.5884C>T p.(Arg1962Cys)	Pathogenic	Heterozygous	Intellectual developmental disorder, autosomal dominant 13	614563	AD
LD4012	Positive	diagnostic	IFIH1	NM_022168.4:c.995G>C p.(Gly332Ala)	Likely Pathogenic	Heterozygous	Aicardi-Goutieres syndrome 7|Singleton-Merten syndrome 1	615846, 182250	AD
HA4022	Positive	diagnostic	ANKRD11	NM_013275.6:c.6413dup p.(Pro2139fsAlafsTer8)	Pathogenic	Heterozygous	KBG syndrome	148050	AD
SK4052	Positive	diagnostic	GRIA3	NM_000828.4:c.1957G>A p.(Ala653Thr)	Pathogenic	Hemizygous	Intellectual developmental disorder, X-linked, syndromic, Wu type	300699	XLR
CH4103	Positive	diagnostic	MICU1	NM_006077.3 c.1078-1G>C	Pathogenic	Homozygous	Myopathy with extrapyramidal signs	615673	AR
SK4051	Positive	diagnostic	MAG	NM_002361.3:c.719del p.(Pro240ArgfsTer2)	Pathogenic	Homozygous	Spastic paraplegia 75, autosomal recessive	616680	AR
SK4054	Positive	diagnostic	MORC2	NM_001303256.2:c.79G>A p.(Glu27Lys)	Pathogenic	Heterozygous	Charcot-Marie-Tooth Disease Type 2Z	616688	AD
LD4015	Positive	diagnostic	CDC42	NM_001791.4:c.191A>G p.(Tyr64Cys)	Pathogenic	Heterozygous	Takenouchi-Kosaki syndrome	616737	AD
SK4056	Positive	diagnostic	GNB1	NM_002074.4:c.233A>G p.(Lys78Arg)	Pathogenic	Heterozygous	Intellectual developmental	616973	AD

							disorder, autosomal dominant 42		
SK4057	Positive	partially-diagnostic	FLG	NM_002016.1:c.2282_2285del p.(Ser761CysfsTer36), NM_002016.1:c.7339C>T p.(Arg2447Ter)	Pathogenic, Pathogenic	Heterozygous, Heterozygous	Ichthyosis vulgaris	146700	AD, AR
HA4025	Positive	diagnostic	CACNA1A	NM_001127222.2:c.4930G>A p.(Ser1640Asn)	Likely Pathogenic	Heterozygous	Episodic ataxia, type 2	108500	AD
CH4112	Positive	diagnostic	DPF2	NM_006268.4:c.1045G>A p.(Asp349Asn)	Pathogenic	Heterozygous	Coffin-Siris syndrome 7	618027	AD
HA4028	Positive	diagnostic	FOXP1	NM_001349338.3:c.996del p.(Leu333TrpfsTer19)	Pathogenic	Heterozygous	Intellectual developmental disorder with language impairment with or without autistic features	613670	AD
HA4030	Positive	partially-diagnostic	FLG	NM_002016.2:c.9740C>A p.(Ser3247Ter)	Pathogenic	Heterozygous	Ichthyosis vulgaris	146700	AD, AR
CH4113	Positive	diagnostic	MEIS2	NM_170674.4:c.573_575delins10 p.(Glu191AspfsTer13)	Pathogenic	Heterozygous	Cleft palate, cardiac defects, and mental retardation	600987	AD
CH4116	Positive	diagnostic	MED13L	NM_015335.4:c.5645G>A p.(Trp1882Ter)	Pathogenic	Heterozygous	Impaired intellectual development and distinctive facial features with or without cardiac defects	616789	AD
HA4032	Positive	diagnostic	TUBA1A	NM_006009.4:c.1225G>A p.(Val409Ile)	Pathogenic	Heterozygous	Lissencephaly 3	611603	AD
SK4061	Positive	diagnostic	PNPO	NM_018129.3:c.364-1G>A	Pathogenic	Homozygous	Pyridoxamine 5'-phosphate oxidase deficiency	610090	AR
SK4062	Positive	diagnostic	KCNQ2	NM_172107.3:c.944G>C p.(Gly315Ala)	Likely Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 7	613720	AD

SK4063	Positive	diagnostic	PPP3CA	NM_000944.4:c.841C>A p.(His281Asn)	Likely Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 91	617711	AD
CH4119	Positive	diagnostic	DEAF1	NM_021008.2:c.634G>A p.(Gly212Ser)	Pathogenic	Heterozygous	Vulto-van Silfout-de Vries syndrome	615828	AD
HA4033	Positive	diagnostic	SNAP29	NM_004782.4:c.487dup (p.Ser163LysfsTer6)	Pathogenic	Homozygous	Cerebral dysgenesis, neuropathy, ichthyosis, and palmoplantar keratoderma syndrome	609528	AR
SK4069	Positive, multiple	diagnostic	CDKL5	NM_003159.2:c.380A>G p.(His127Arg)	Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 2	300672	XLD
SK4070	Positive	diagnostic	SHANK3	NM_033517.1:c.3439C>T p.(Gln1147Ter)	Pathogenic	Heterozygous	Phelan-McDermid syndrome	606232	AD
SK4072	Positive	diagnostic	SHANK3	NM_033517.1:c.4422G>A p.(Trp1474Ter)	Pathogenic	Heterozygous	Phelan-McDermid syndrome	606232	AD
SK4073	Positive	diagnostic	KCNQ2	NM_172107.2:c.523G>A p.(Val175Met)	Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 7	613720	AD
HA4034	Positive	diagnostic	MED12	NM_005120.3:c.3662T>C p.(Phe1221Ser)	Likely Pathogenic	Heterozygous	MED12-Related Disorder	Various	XLR
HA4036	Positive	diagnostic	FBXO11	NM_001190274.2:c.807T>A p.(Tyr269Ter)	Pathogenic	Heterozygous	Intellectual developmental disorder with dysmorphic facies and behavioral abnormalities	618089	AD
LD4017	Positive	diagnostic	DNM1	NM_004408.4:c.134G>A p.(Ser45Asn)	Pathogenic	Heterozygous	Developmental and epileptic encephalopathy 31	616346	AD
SK4075	Positive	partially-diagnostic	WT1	NM_024426.4:c.1339+1G>A	Pathogenic	Heterozygous	WT1-Related Disorder	Various	AD
LD4018	Positive	diagnostic	MEFV	NM_000243.3:c.2084A>G p.(Lys695Arg)., NM_000243.3:c.2177T>C p.(Val726Ala)	Pathogenic, Pathogenic	Heterozygous, Heterozygous	Familial Mediterranean fever, AR	249100	AR

CH4130	Positive	diagnostic	SPART	NM_015087.4:c.696del p.(Phe232LeufsTer2)	Pathogenic	Homozygous	Troyer syndrome	275900	AR
CH4133	Positive	diagnostic	NR2F1	NM_005654.4:c.667G>A p.(Ala223Thr)	Likely Pathogenic	Heterozygous	Bosch-Boonstra-Schaaf optic atrophy syndrome	615722	AD
CH4134	Positive	partially-diagnostic	IMPG2	NM_016247.3:c.3229dup p.(Cys1077LeufsTer2)	Pathogenic	Heterozygous	Macular dystrophy, vitelliform, 5	616152	AD
CH4135	Positive, multiple	diagnostic based on 2 partial diagnoses	ASXL3	NM_030632.1:c.1083del p.(Lys361AsnfsTer10)	Pathogenic	Heterozygous	Bainbridge-Ropers syndrome	615485	AD
			CSNK2A1	NM_001895.3:c.451_452delinsAA p.(Gly151Lys)	Likely Pathogenic	Heterozygous	Okur-Chung neurodevelopmental syndrome	617062	AD
CH4143	Positive	diagnostic	NCKAP1	NM_205842.2:c.2802_2803insGTTA p.(Ser935ValfsTer13)	Pathogenic	Heterozygous	*No disease yet in OMIM*		
HA4038	Positive	diagnostic	HPRT1	NM_000194.3:c.28-2A>T	Pathogenic	Hemizygous	Lesch-Nyhan syndrome	300322	XLR
HA4039	Positive	diagnostic	ITPR1	NM_001378452.1:c.748T>C p.(Phe250Leu)	Pathogenic	Heterozygous	Spinocerebellar ataxia 29, congenital nonprogressive	117360	AD
LD4020	Positive	partially-diagnostic	COL1A1	NM_000088.4:c.2032G>A p.(Glu678Lys)	Likely Pathogenic	Heterozygous	Ehlers-Danlos syndrome, arthrochalasia type, 1	130060	AD
HA4041	Positive	diagnostic	MAP2K1	NM_002755.4:c.199G>A p.(Asp67Asn)	Pathogenic	Heterozygous	Cardiofaciocutaneous syndrome 3	615279	AD
CH4158	Positive	diagnostic	KDM5C	NM_004187.2:c.3880C>T p.(Gln1294Ter)	Pathogenic	Hemizygous	Intellectual developmental disorder, X-linked syndromic, Claes-Jensen type	300534	XLR
CH4153	Positive	diagnostic	OCRL	NM_000276.3:c.982G>C p.(Ala328Pro)	Likely Pathogenic	Heterozygous	Dent disease 2|Lowe syndrome	300555, 309000	XLR
CH4159	Positive	partially-diagnostic	CRYAA	NM_000394.3:c.347G>A p.(Arg116His)	Pathogenic	Heterozygous	Cataract 9, multiple types	604219	AD, AR
CH4163	Positive	diagnostic	ACTL6A	NM_178042.3:c.1096C>T p.(Gln366Ter)	Likely Pathogenic	Heterozygous	*No disease yet in OMIM*		

SK4079	Positive	diagnostic	NAA15	NM_057175.3:c.1645C>T (p.Arg549Ter)	Pathogenic	Heterozygous	Intellectual developmental disorder, autosomal dominant 50, with behavioral abnormalities	617787	AD

Supplemental Table 4.4. Probands with diagnostic results and corresponding molecular tests from hypothetical care pathways

Study Identifier	*Gene*	*Captured by Hypothetical test*	*Molecular Testing in Hypothetical Care Pathway*	*Number of Molecular Tests*
CH4002	ALDH3A2	Yes, GeneDx ID and Single Gene	Intellectual Disability (105 genes), Ichthyosis (39 genes), Single gene: ALDH3A2 del/dup	3
CH4007	PACS2	Yes, GeneDx ID	Intellectual Disability Panel (>1000)	1
CH4012	MAN1B1	Yes, GeneDx ID	Intellectual Disability Panel (>1000), Connective tissue disorders with bone involvement panel (43 genes)	2
CH4015	WAC	Yes, GeneDx ID	Intellectual Disability Panel (>1000), Pediatric Tumor Panel (27 genes)	2
	G6PD	No		2
CH4018	SLC2A1	Yes, GeneDx Epilepsy	Ataxia Panel (7 genes), Comprehensive Epilepsy Panel (87 Genes)	2
CH4020	PURA	Yes, GeneDx ID	Intellectual Disability Panel (>1000)	1
CH4021	AGO2	No	Intellectual Disability Panel	1
SK4003	NIPBL	No		0
SK4004	MED13L	No	Single gene: MECP2	1
SK4007	WDR45	Not provided		N/A
SK4008	ANKRD11	Yes, GeneDx Epilepsy	Seizure Panel, Single gene: MECP2	2
	FLG	No		
SK4011	CACNA1A	No	Single gene: FMR1	1
HA4005	RTN4IP1	No		0
HA4003	GATAD2B	Yes, GeneDx ID	Intellectual Disability Panel	1
HA4006	KIF1A	Yes, GeneDx ID	Intellectual Disability Panel	1
HA4004	BRAF	Yes, GeneDx ID	Intellectual Disability Panel	1
HA4007	CLDN5	No		0
HA4010	MECP2	No		
HA4008	PPP2R5D	Yes, GeneDx ID	Intellectual Disability Panel	1
CH4028	KAT6B	No	Noonan / RAS panel (21 genes)	1
CH4032	GH1	No	Intellectual Disability Panel (>1000)	1
CH4034	AP4M1	Yes, GeneDx ID	Intellectual Disability Panel (>1000), HSP Panel (50 genes), Microcephaly panel (880 genes)	3
CH4037	ASXL3	Yes, GeneDx ID	Intellectual Disability Panel (>1000)	1
HA4011	CHD8	Yes, BluePrint Macrocephaly	Macrocephaly Panel	1
SK4013	MRPS34	No		0
SK4014	OFD1	Yes, Single gene	Single gene: OFD1	1

SK4015	ATP1A2	No	Obesity Panel	1
SK4021	GABBR2	No		0
HA4012	STXBP1	Yes, GeneDx ID	Intellectual Disability Panel	1
SK4024	CDK13	No		0
HA4014	ADNP	Yes, GeneDx ID	>300 Gene Custom ID Panel	1
SK4025	PDHA1	Yes, GeneDx ID	ID/Developmental Delay >1000 genes, Seizure Panel	2
SK4026	EP300	Yes, GeneDx ID	Short stature panel (75 genes), ID/Developmental Delay >1000 genes	2
SK4028	DGAT1	Yes, Prevention Neonatal Crisis Panel	Failure To Thrive Panel	1
SK4034	INPP5K	Yes, BluePrint Congenital Muscular Dystrophy	Congenital muscular dystrophy panel, Single gene: LMNA	2
SK4023	GANAB	No	Connective tissue disorders with bone involvement panel (43 genes)	1
CH4054	TUBA1A	Yes, Prevention Brain Malformation	Brain malformations panel (103 genes)	1
HA4016	CASK	Yes, GeneDx ID	Intellectual Disability Panel	1
CH4058	DLG4	No		0
CH4060	INSR	Yes, GeneDx ID	Autism/ID Panel (1000+ genes)	1
CH4059	CTNNB1	Yes, GeneDx ID	Intellectual Disability Panel (>1000)	1
HA4013	SLC1A4	No		0
CH4066	EFTUD2	Yes, GeneDx ID	Comprehensive Intellectual Disability Panel, Short Stature Panel	2
CH4068	SCN2A	Yes, GeneDx ID	Comprehensive Intellectual Disability Panel, Overgrowth Panel	2
CH4071	EBF3	Yes, GeneDx ID	ID/Autism Panel	1
SK4035	ARX	No		0
SK4037	FOXG1	Yes, GeneDx ID	Microcephaly Panel, ID Panel	2
CH4075	ARR3	No	Comprehensive Growth Disorders/Skeletal Dysplasias and Disorders Panel (374 genes), Collagenopathy Panel (4 genes), Primary Immunodeficiency Panel (298 genes)	3
KG4003	ATM	Yes, BluePrint Ataxia	Comprehensive ataxia panel	1
KG4004	CDH2	Yes, GeneDx ID	ID Panel, Macrocephaly Panel	2
SK4040	UBA1	Yes, BluePrint Arthrogryposis	BluePrint Arthrogryposis Congenita panel (78 genes)	1

SK4044	POMGNT2	Yes, BluePrint Congenital Muscular Dystrophy	Congenital Muscular Dystrophy Panel	1
CH4084	NDUFB3	No		0
CH4085	EED CACNA1A	Yes, GeneDx ID Yes, GeneDx ID	Episodic Ataxia Panel (5 genes), Intellectual Disability Panel (>1000)	2
CH4086	FOXG1	Yes, GeneDx ID	Intellectual Disability Panel (>1000), Microcephaly panel (880 genes)	2
CH4087	ARID1B	Yes, GeneDx ID	Hypotonia Panel (28 genes), Intellectual Disability Panel (>1000)	2
CH4090	SPG11	Yes, GeneDx ID	ID Panel (>1000 genes), Hereditary Spastic Paraplegia (50 genes) Single gene: AMT and GLDC	4
LD4007	ADNP	Yes, Prevention IDEA	Prevention Intellectual Disability, Epilepsy, Autism (IDEA) panel (>2000 genes)	1
CH4098	FBN1	Yes, Sick Kids CTD	Vascular connective tissue disorders, thoracic aortic aneurysms, and aortic dissections panel (11 genes), Comprehensive Epilepsy Panel (127 Genes)	2
LD4008	DYNC1H1	No, Plain Insight	Plain Insight Panel	1
LD4012	IFIH1	Yes, BluePrint Immunodeficiency	Skin panel, Immune/autoinflammatory panel	2
HA4022	ANKRD11	Yes, GeneDx ID	ID Panel	1
SK4052	GRIA3	No		0
CH4103	MICU1	Yes, BluePrint Myopathy	Myopathy panel, Single gene: DMD sequencing	2
SK4051	MAG	No, BluePrint Ataxia	Ataxia Panel (117 Genes)	1
SK4054	MORC2	No		0
LD4015	CDC42	Yes, GeneDx ID	ID Panel	1
SK4056	GNB1	Not provided		N/A
SK4057	FLG	No		0
HA4025	CACNA1A	Yes, GeneDx ID	ID Panel	1
CH4112	DPF2	No, BluePrint Ectodermal Dysplasia	Common and Non-Syndromic Hearing Loss Panel (53 genes), Ectodermal Dysplasia Panel	2
HA4028	FOXP1	Yes, GeneDx ID	ID Panel	1
HA4030	FLG	No, GeneDx ID	ID Panel	1
CH4113	MEIS2	No		0
CH4116	MED13L	Yes, GeneDx ID	Autism/ID Panel (1000+ genes)	1
HA4032	TUBA1A	No		0

SK4061	PNPO	Yes, GeneDx Epilepsy	Comprehensive Epilepsy Panel (87 Genes)	1
SK4062	KCNQ2	Yes, GeneDx Epilepsy	Comprehensive Epilepsy Panel (87 Genes)	1
SK4063	PPP3CA	No		0
CH4119	DEAF1	Yes, GeneDx ID	Autism/ID Panel (1000+ genes), Comprehensive Epilepsy Panel (87 Genes), Cortical Malformation Panel (61 genes)	3
HA4033	SNAP29	No		0
SK4069	CDKL5	Not provided		N/A
SK4070	SHANK3	Not provided		N/A
SK4072	SHANK3	No		0
SK4073	KCNQ2	Not provided		N/A
HA4034	MED12	Yes, GeneDx ID	ID Panel	1
HA4036	FBXO11	Yes, GeneDx ID	ID Panel	1
LD4017	DNM1	Yes, Prevention IDEA	Prevention Intellectual Disability, Epilepsy, Autism (IDEA) panel (>2000 genes), Seizure Panel	2
SK4075	WT1	Not provided		N/A
LD4018	MEFV	No, Sick Kids CTD	Connective Tissue Panel	1
CH4130	SPART	Yes, GeneDx ID and SickKids HSP	Autism/ID Panel (1000+ genes), HSP Panel (50 genes)	2
CH4133	NR2F1	Yes, GeneDx Epilepsy	Comprehensive Epilepsy Panel (over 200 genes), Ataxia exome panel (565 genes)	2
CH4134	IMPG2	No, GeneDx Epilepsy	Comprehensive Epilepsy Panel (over 200 genes), Comprehensive Dementia Panel (25 genes)	2
CH4135	ASXL3 CSNK2A1	Yes, GeneDx ID Yes, GeneDx ID	Autism/ID Panel (over 1000 genes), Comprehensive Epilepsy Panel (over 200 genes)	2
CH4143	NCKAP1	No	Autism/ID Panel (over 1000 genes), Updated HSP Panel (50 genes), Charcot Marie Tooth Panel (34 genes)	3
HA4038	HPRT1	No		
HA4039	ITPR1	Yes, BluePrint Ataxia	Ataxia Panel, Spinocerebellar Ataxia Repeat Expansion Panel (7 genes)	2
LD4020	COL1A1	Yes, Prevention IDEA	Seizure Panel, Connective tissue disorders with bone involvement panel (43 genes), PreventionGenetics IDEA panel	3
HA4041	MAP2K1	Yes, GeneDx ID	ID Panel	1
CH4158	KDM5C	No	Craniosynostosis Panel Plus Analysis (37 genes)	1

CH4153	OCRL	Yes, GeneDx ID	Autism/ID Panel (1000+ genes), Comprehensive Epilepsy Panel (over 200 genes), Congenital cataracts (38 genes)	3
CH4159	CRYAA	Yes, BluePrint Cataract	Updated HSP Panel (50 genes), Congenital cataract panel (38 genes)	2
CH4163	ACTL6A	No	Autism/ID Panel (1000+ genes)	1
SK4079	NAA15	Not provided		N/A

Chapter 5: Integrated Discussion

5.1. Introduction

This final chapter synthesizes and integrates the findings from the three articles to present the overall contribution of this dissertation. First, I review the purpose of this thesis and summarize each of the studies, including their objectives, methods, and main findings. Next, I provide a synthesis of the key findings as they relate to the Knowledge-to-Action (KTA) Framework and discuss how this thesis contributes to our greater understanding of knowledge translation processes related to, and relevant considerations to optimize, exome sequencing and genome sequencing (ES/GS) testing. I then discuss the implications of this work for various knowledge users and its contribution to the field of population health. Finally, I examine the strengths and limitations of this body of work and provide suggestions for future research, ending with an overall conclusion.

5.2. Purpose of thesis and how it is addressed

The aim of this dissertation was to examine the rapidly evolving scientific evidence base related to ES/GS testing and how it has been translated into diagnostic care for families with rare genetic diseases (RGDs) to inform clinical practice, policy, and research in the future. To do so, I delineated three overall objectives for my thesis that included:

1. To examine how the knowledge base related to ES/GS is changing over time and its diagnostic implications;
2. To examine how knowledge users have refined this ever-evolving knowledge base into clinical guidance; and,
3. To examine whether implementation of ES/GS based on this knowledge base results in diagnoses for families with RGDs.

To address these overall objectives, I designed, in collaboration with my supervisors and advisory committee, three studies guided by the KTA conceptual framework (**Figure 5.1**). Two of the studies were designed to generate more general evidence related to Knowledge Creation, the funnel at the center of the framework, and the third examined two phases of the Action Cycle in one particular study setting.

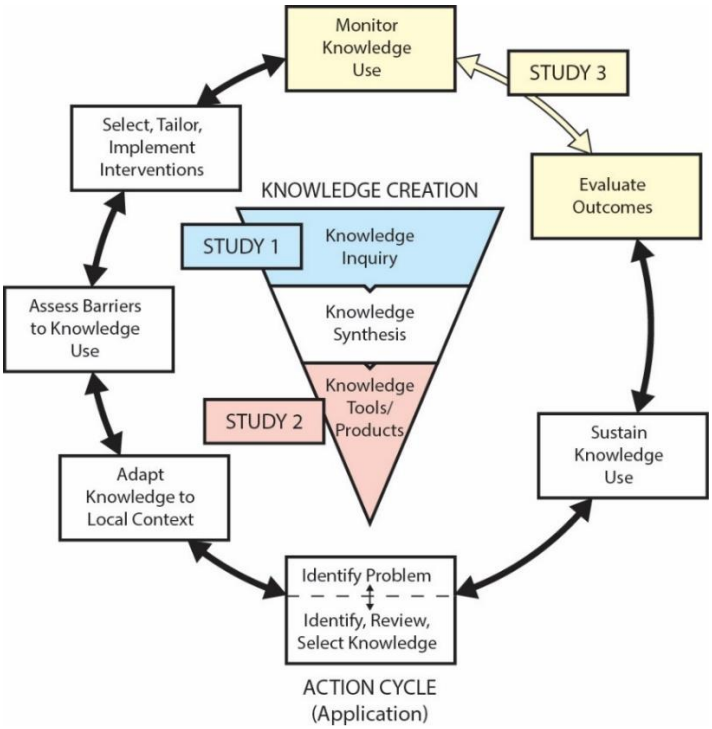

Figure 5.1. Components of the KTA framework that are targeted by the three articles within this dissertation (adapted with permission from Graham et al., 2006).

5.2.1. Summary of Article 1 (Chapter 2)

Article 1 is a proof-of-principle retrospective study designed to produce evidence related to Objective 1 of my thesis (i.e., to examine how the knowledge base related to ES/GS is changing over time and its diagnostic implications). Here, we examined the extent to which evolving knowledge regarding the causes of RGDs [i.e., new knowledge inquiry] impacts RGD diagnosis. This study was designed to investigate the implications of new genomic knowledge, including diagnostic implications and implications for knowledge users, such as clinicians and policymakers, working in healthcare systems

interested in adopting ES/GS. A summary of the sub-objectives, methods, and main findings for Article 1 is presented in **Table 5.1.**

Table 5.1. Summary of sub-objectives, methods, and main findings from Article 1 (Chapter 2), entitled "Bridging clinical care and research in Ontario, Canada: Maximizing diagnoses from reanalysis of clinical exome sequencing data"

Sub-objectives	Methods	Main findings
i. To determine if the reanalysis of non-diagnostic clinical exome sequencing data, using Care4Rare's infrastructure, resulted in additional diagnoses; ii. To examine how improvement in genomic knowledge, in comparison to other aspects of the Care4Rare infrastructure (a new bioinformatics pipeline, the incorporation of the clinical care team in analysis) has contributed to the diagnostic yield that results from reanalysis; and, iii. To examine whether the amount of time since initial analysis influences the proportion of diagnoses that can be made from reanalysis, and hence identify any implications for knowledge user decisions about the frequency of incorporation of new genomic knowledge into clinical practice.	Retrospective cohort study examining Ontario families who received non-diagnostic, publicly-funded, exome sequencing performed in clinical laboratories outside of Canada. In partnership with 8 sites, we recruited 287 families with suspected rare genetic diseases tested between 2014 and 2020. Data from 7 laboratories was reanalyzed with the referring clinicians. We evaluated the diagnostic yield from a 'clinical reanalysis,' which focused on genes with curated disease-gene associations (e.g., known genes) relevant to the proband's primary indication for testing. This analysis could be implemented in several settings (e.g., clinical laboratories or by clinicians themselves). We then applied a 'translational research reanalysis' protocol that might be considered for future implementation as part of standard clinical care. We examined what factors contributed to new diagnoses.	Reanalysis of non-diagnostic exome sequencing data resulted in diagnoses for 37 families (13% of the cohort) (**sub-objective i**). Reanalysis of established clinically-associated genes identified diagnoses in 13 families (5%). Of these, new genomic knowledge was the greatest contributor to new diagnoses in known genes (9 diagnoses) (**sub-objective ii**). Other factors included the use of different bioinformatics and the inclusion of clinical geneticists to provide detailed phenotypes. A translational research reanalysis identified candidates in genes of uncertain significance (GUSs) in 61 families (21%). Results in 24 families (8% of cohort) were upgraded to diagnoses based on the use of data sharing to identify two or more additional families with the same new RGD. Families who had testing prior to 2019 were more likely to receive a diagnosis than those who had testing in 2019 or later. There was a trend towards a higher proportion of families receiving diagnoses in clinically-relevant genes when reanalysis was performed after two years compared to less than two years (**sub-objective iii**).

This study generated evidence that the reanalysis of non-diagnostic ES data resulted in additional diagnoses (**sub-objective i**). A 'clinical reanalysis', which restricted the analysis to genes with known disease-gene associations and could be implemented in a number of settings (e.g., clinical laboratories or ordering clinicians), found new diagnoses in 5% of families. New genomic knowledge was the greatest contributor to these diagnoses, but there was also contribution from differing analytical processes (including the use of different bioinformatics and inclusion of the ordering clinicians) (**sub-objective ii**). Care4Rare's 'translational research reanalysis', which expanded the analysis to include genes of uncertain significance (GUSs), identified candidates in an additional 21% of the cohort; 40% of these candidates (24/61) have been upgraded to diagnoses based on additional evidence that they represent a new RGD not yet described in the primary literature. The amount of time since initial analysis appeared to increase the proportion of diagnoses made in established clinically-associated genes through reanalysis (**sub-objective iii**). The proportion of families with candidates in GUSs remained high at all time points, indicating that there remains significant potential for new genomic knowledge in this cohort, and that new genomic knowledge will continue to contribute to diagnoses identified by 'clinical reanalysis' in the future (**sub-objective ii**).

5.2.2. Summary of Article 2 (Chapter 3)

Article 2 is a scoping review designed to produce evidence related to Objective 2 of my thesis (i.e., to examine how knowledge users have refined this ever-evolving knowledge base into clinical recommendations). Here, we targeted the knowledge tools (clinical guidance documents) generated by a particular knowledge user community (organizations representing genetics healthcare professionals) to examine how the evolving knowledge base related to ES/GS is being used and refined. A summary of the sub-objectives, methods, and main findings for Article 2 are presented in **Table 5.2.**

Table 5.2. Summary of sub-objectives, methods, and main findings from Article 2 (Chapter 3), entitled "Exome and genome sequencing for rare genetic disease diagnosis: A scoping review and critical appraisal of clinical guidance documents by genetics professional organizations"

Sub-objectives	Methods	Main findings
i. To describe the content (e.g., topics within recommendations) of clinical guidance documents related to the use of clinical ES/GS for the investigation of RGDs; ii. To examine the methodological quality (as per the AGREE II instrument) of these guidance documents and the processes by which they were generated; and, iii. To examine the methods and frequency by which these clinical guidance documents have been or will be updated based on new scientific evidence.	This scoping review was designed using Joanna Briggs Institute (JBI) methodology and followed the PRISMA-Scoping Review extension to guide reporting. Both peer-reviewed and grey literature sources were searched for documents published between 2010 and 2022. Recommendations were extracted verbatim and coded using iterative content analysis to identify unique topics. All documents were critically appraised by two individuals using the AGREE II appraisal instrument. Scores were reached by consensus. Mean total domain scores were used to examine document quality over time. Median individual item scores were used to identify specific areas to be targeted for improvement by guideline developers.	We identified 30 clinical guidance documents produced by eight organizations. Five were newer versions of previous documents. We extracted 611 recommendation statements and found 21 topics within the recommendations, related to a broad range of aspects of testing (**sub-objective i**). The topics with the most associated recommendations related to additional findings beyond the primary indication for testing (26% of recommendations), and technical recommendations for how laboratories should validate (18%), conduct (16%) and report results (22%) for the test. Documents scored low in all domains of AGREE II, but showed some improvement over time in five of six domains. Nine individual AGREE II items had median scores of one (indicating the item was not addressed at all), five of which related to the rigour of processes used in translating the body of evidence into recommendations (**sub-objective ii**). This included the item 'a procedure for updating the guideline is provided'; 19/30 documents did not include a statement that the guideline would be updated. Only two documents provided explicit time intervals and methodology for updates (**sub-objective iii**). Other items with median scores of one included seeking the perspectives of patients, including monitoring criteria, and stating funding sources (**sub-objective ii**).

This study was the first knowledge synthesis of clinical guidance documents produced by organizations representing genetics professionals related to the use of ES/GS in RGD diagnosis. We identified 30 clinical guidance documents, produced from 2012 to 2022, by eight organizations representing genetics healthcare professionals (clinical geneticists, clinical laboratory geneticists, and genetic counsellors) in Organisation for Economic Co-operation and Development (OECD) countries. We extracted 611 recommendation statements and conducted an iterative content analysis that identified 21 topics within the recommendations made by these documents (**sub-objective i**). We appraised the development processes using the Appraisal of Guidelines Research and Evaluation (AGREE) II instrument (Brouwers et al., 2010), and determined that they were of low quality in all domains (**sub-objective ii**). By examining the individual AGREE II items we were able to highlight areas in need of particular improvement for guidance documents (**sub-objective ii**). Just five of the documents were newer versions of previous documents and more than half of the documents did not include statements explicitly stating that the documents would be updated based on the evolving knowledge base (**sub-objective iii**).

5.2.3. Summary of Article 3 (Chapter 4)

Article 3 is a prospective cohort study designed to produce evidence related to Objective 3 of my thesis (i.e., to examine whether implementation of ES/GS based on this knowledge base results in diagnoses for families with RGDs). Here, as part of a larger integrated KT study, we examined a phase of the Action Cycle in the Ontario healthcare system, namely, monitoring and evaluating the outcomes from implementation of a piece of instrumental knowledge (e.g., eligibility criteria) to support routine publicly-funded clinical ES testing. A summary of the sub-objectives, methods, and main findings for Article 3 are presented in **Table 5.3.**

Table 5.3. Summary of sub-objectives, methods, and main findings from Article 3 (Chapter 4), entitled "Evaluation of the diagnostic accuracy of exome sequencing and its impact on diagnostic thinking for rare disease patients in a publicly-funded healthcare system: a prospective cohort study"

Sub-objectives	Methods	Main findings
i. To monitor the application of provincial eligibility criteria, including corresponding patient phenotypes; ii. To describe the range of molecular results interpreted and reported by clinical laboratories (including variant classifications and genes reported); iii. To determine the proportion of laboratory interpretations associated with clinically-valid results, as determined by the ordering physicians; and, iv. To identify cases for which ES was required to achieve a diagnosis compared to those for whom conventional tests would have achieved a diagnosis.	Prospective cohort study examining Ontario families who received publicly-funded exome sequencing performed at clinical laboratories outside of Canada. In partnership with five sites, we enrolled 297 probands with suspected RGD receiving testing between 2018 and 2021 performed at laboratories outside of Canada. We extracted data from medical records and clinician surveys. We assessed diagnostic accuracy, the ability of ES to predict whether a patient has or does not have a particular disease, by examining the molecular results reported by laboratories. We assessed diagnostic thinking efficacy, whether the test helps clinicians establish a specific diagnosis, by asking ordering physicians to interpret the molecular findings. We also compared diagnostic results to hypothetical care pathways collected prior to testing, which specified molecular tests that would be ordered in lieu of ES.	All 297 probands had two or more eligibility criteria selected. The majority of probands were affected with pediatric-onset, syndromic ID/DD, for which trio ES was ordered with unaffected parents (**sub-objective i**). Laboratories reported 105 molecular diagnoses, 137 uncertain results in known genes, and 28 results in genes of uncertain significance (GUSs). Molecular results were reported in 235 unique genes (**sub-objective ii**). 102/105 (97%) molecular diagnoses were interpreted as diagnostic, and therefore considered clinically valid. 3/137 (4%) uncertain results in known genes and 3/28 (10%) uncertain results in GUS were interpreted as diagnostic. In total, 104 families (35%) received clinical-molecular diagnoses (**sub-objective iii**). 61% of clinical-molecular diagnoses would have been identified by the hypothetical molecular tests (**sub-objective iv**). The eligibility criteria each resulted in diagnostic yields of 30-40%, and in greater combinations resulted in higher diagnostic yields.

In this study, we sought to examine whether implementation of ES/GS based on the knowledge base results in diagnoses for families with RGDs, by monitoring and evaluating the implementation of ES/GS

in one study setting. We prospectively enrolled 297 families that received publicly-funded clinical ES testing in the province of Ontario, monitored the application of a set of provincial eligibility criteria, and evaluated diagnostic utility guided by the Fryback and Thornbury Efficacy Framework (Fryback & Thornbury, 1991). In doing so, we found that participating ordering clinicians were able to apply the eligibility criteria (all probands had two or more criteria selected) and the corresponding phenotypes resulted primarily in patients with pediatric-onset, syndromic ID/DD, for which trio ES was ordered with unaffected parents (**sub-objective i**). Using clinical ES, laboratories were able to identify and report molecular findings in 235 unique genes; this included DNA variants classified as molecular diagnoses, uncertain results in clinically-relevant genes, and uncertain results in GUSs (**sub-objective ii**). Ordering physicians interpreted the vast majority of molecular diagnoses (102/105, 97%) as clinically-valid, clinical-molecular diagnoses. Ordering physicians also interpreted 4% (6/165) of uncertain results as clinical-molecular diagnoses (**sub-objective iii**). The molecular tests within hypothetical care pathways might have identified 61% of the clinical-molecular diagnoses (**sub-objective iv**). Overall, 35% (104/297) of families with suspected RGD received clinical-molecular diagnoses from publicly-funded clinical ES in Ontario.

5.2.4. Synthesis – Knowledge to Action Framework

Each of the three articles was designed to target a particular aspect of the KTA framework, but KTA was never meant to be a stagnant framework with explicit phases that are independent from one another. Because the phenomenon of translating evidence to practice is complex, dynamic, and iterative, the boundaries for Knowledge Creation, the Action Cycle, and their internal steps are fluid and permeable (Graham et al., 2006). Here, within this dissertation, we too see that these articles provide deeper insight into KT processes together rather than individually and thus draw on evidence from all three

Articles to address the original objectives and propose considerations for optimizing ES/GS testing (summarized in **Table 5.4**).

Table 5.4. Summary of main findings from dissertation related to the Knowledge-to-Action (KTA) Framework and considerations for optimized exome sequencing and genome sequencing (ES/GS)

Main findings related to KTA	Mapped considerations for optimized ES/GS
Knowledge Creation: Knowledge Inquiry • The knowledge base related to ES/GS diagnosis has two components that are changing and have diagnostic implications: new genomic knowledge, and evolving analytical processes (including technological advancement and clinical expertise) (Chapters 2, 3, 4).	
• New genomic knowledge accounted for the majority of new diagnoses identified by research reanalysis (Chapter 2) and will have diagnostic implications for years to come (Chapters 2, 4).	1. Genomic knowledge is evolving at a rate that has diagnostic implications for ES/GS
• Evolving analytical approaches, including different bioinformatics or the inclusion of additional expertise, identified primarily new candidates in known disease genes (Chapters 2, 4).	2. Analytical processes are evolving at a rate that has diagnostic implications for ES/GS
• Reanalysis and translational research processes have diagnostic potential but need additional studies and clinical recommendations. (Chapters 2, 3, 4).	3. The optimization of diagnostic yield from ES/GS will require the continued close, reciprocal interaction between clinical care and research
Knowledge Creation: Knowledge Tools/Products • 30 guidance documents related to the use of ES/GS for RGD diagnosis were produced by eight genetics professional organizations between May 2012 and March 2022 (Chapter 3).	
• Guidance documents by genetics professional organizations were of low quality; however, quality across 5/6 domains improved over time (Chapter 3). • Few knowledge syntheses were used as the basis of clinical recommendations in guidance documents by genetics professional organizations (Chapter 3).	4. The majority of guidance documents available to healthcare practitioners are of low methodological quality, particularly with respect to whether and how they synthesize existing evidence and use it to inform recommendations

• Guidance documents included 611 recommendations related to 21 topics, with most topics addressed by multiple organizations. This included a number of recommendations related to knowledge gaps in need of future research (Chapter 3).	5. There are a number of critical knowledge gaps in need of further evidence, particularly related to additional findings and their implications for patients/families and healthcare systems.
Action Cycle: Monitor Knowledge Use • 30 ordering clinicians (primarily medical geneticists) implemented a set of provincial eligibility criteria with fidelity to the instructions (minimum of 2 criteria selected and majority of families run as trios) (Chapter 4).	6. Healthcare practitioners in a single exemplar jurisdiction can apply a set of eligibility criteria related to ES/GS testing
Action Cycle: Evaluate Outcomes Use of the MOH eligibility criteria: • Identified a broad range of molecular results (Chapter 4); • Was effective in impacting diagnostic thinking for ordering clinicians, both in terms of providing them with clinical-molecular diagnoses as well as altering the use of diagnostic tests (Chapter 4).	7. Implementation of ES/GS appears to have diagnostic efficacy
Action Cycle: Sustain Knowledge Use • Given the knowledge base is rapidly evolving (Chapters 2, 3, 4), current guidance documents are of low quality (Chapter 3), and studies evaluating implementation can be difficult to compare, decision-makers should consider continuous evaluation of implementation to ensure continued value based on outcomes important to themselves, healthcare practitioners, and most importantly patients.	8. Continuous evaluation is necessary to determine if clinical use of ES/GS is worthy of sustaining, spreading, or scaling

Objective 1 - Knowledge Inquiry - To examine how the knowledge base related to ES/GS is changing over time

The first objective of this thesis was "to examine how the knowledge base related to ES/GS is changing over time." This objective was pursued primarily by examining the reanalysis of non-diagnostic ES data in a single cohort (Article 1), but we are also informed by the recommendations identified in Article 2 and the molecular results in Article 3. We found that new diagnoses following non-diagnostic testing

were made based on two changing aspects of the knowledge base: increasing genomic knowledge related to the etiology of RGDs, and evolving analytical processes at laboratories. Both of these developments contribute to the identification of new DNA candidates.

Impact of an increasing genomic knowledge base

The primary source of evidence used in identifying new diagnoses was the **genomic knowledge base**, including new peer-reviewed primary studies related to the etiologies of RGDs, often curated by the Online Mendelian Inheritance in Man (OMIM) database (www.omim.org), and newly curated genomic variants in public databases such as ClinVar (Harrison et al., 2016). In Article 1, we found that new genomic knowledge contributed to new diagnoses in 9 of 287 families (4%) with reanalysis after a mean of 22 months (range 1 to 73 months) following the initial analysis. These findings included new disease-gene associations, variant-disease associations, and phenotype expansions. This source of evidence is a well-known contributor to the advancing knowledge base related to ES/GS and is the predominant factor for diagnoses in 93% of studies examining reanalysis of genomic data reported to date (Tan et al., 2020).

We can anticipate this impact from an evolving genomic knowledge base to continue for some time. In Article 1 we observed high proportions of families with candidates in GUSs at all time points from initial testing. Similarly, clinical laboratories reported candidates in GUSs in 28 families (9% of the cohort) in Article 3. This indicates that there is still significant work to do to understand the full catalogue of genes responsible for RGDs. This is supported by recent estimates that there remain 6,000 to 13,000 Mendelian diseases left to be delineated (Bamshad et al., 2019). It appears that genetics organizations recognize this continued gap in our understanding of the etiologies of rare disease. Within Article 2, we identified a number of recommendations related to data sharing from clinical laboratories to generate

this evidence (ACMG Board of Directors, 2012; Boycott et al., 2015; Hume et al., 2019; Matthijs et al., 2016; Rehder et al., 2021; Rehm et al., 2013; Weiss et al., 2013) and the continued need to publish research related to the genetic etiology of diseases (Bush et al., 2018).

Impact of evolving analytical processes at laboratories

Beyond new genomic knowledge, we saw that evolving **analytical processes at laboratories** also contributed to identifying new diagnoses. This included technological improvements that increased the sensitivity of the test to particular types of DNA variants. This was observed in both Article 1 and Article 3. In Article 1, we identified four diagnoses that were missed by the clinical laboratories, all of which involved genes with well-established disease-gene relationships at the time of initial testing. Similarly, in Article 3, we observed clinical laboratories reporting molecular results with DNA variation that would have been missed by the Care4Rare bioinformatics pipeline (e.g., copy number variants and mitochondrial variants). Similar findings have been observed in the literature in which two groups, using the same sequencing data, identify different candidates (Eldomery et al., 2017; Shashi et al., 2019). This finding is important because it alludes to the fact that laboratories are continuing to adapt and develop their technologies and protocols. Evidence of this adaptation is perhaps observed in Article 1, whereby missed diagnoses were only observed in patients who had testing prior to 2018, potentially indicating that laboratories have adapted their pipelines to catch these previous misses. In the case of Eldomery et al. (2017) a clinical laboratory established a separate research arm to establish new bioinformatics practices that could then be transitioned to clinical care after validation.

Another adaptation to analytical processes we identified that increased the sensitivity of genomic testing was the inclusion of the ordering clinician's clinical expertise in the analysis process. The contribution of clinician expertise was highlighted in Article 1. We found that the inclusion of clinicians

in the analysis of the ES data, to provide additional related phenotypic information, resulted in the identification of additional candidates in known disease genes that the initial laboratory could have found: 14/30 (47%) variants of uncertain significance (VUS) in known genes were not on the original laboratory report despite being in well-established disease-genes at the time of initial interpretation. There appears to be a disconnect between the clinicians and the laboratories, whereby laboratories are not receiving the amount of information needed for optimal ES analysis. A meta-analysis that examined clinical ES/GS supports this observation: it found that hospital-based interpretation, where deeper phenotypic information was available for analysis, had a significantly higher diagnostic yield than testing done at reference laboratories (Clark et al., 2018). Our results indicate that best practices for the incorporation of patient phenotype in genomic analysis have either not been established or not fully implemented.

Further complicating matters, we see that clinical expertise is also evolving over time. We found that the inclusion of clinicians could actually help resolve uncertain findings in GUSs prior to peer-reviewed publication and curation into the genomic knowledge base. This was highlighted in Article 3, in which we observed two instances where variants in GUSs were interpreted as diagnostic shortly after receiving the uncertain laboratory report. In both cases the referring clinician interpreted the variants as diagnostic following data sharing (i.e., patient matchmaking) and expert consultation to determine if multiple families harbouring deleterious-appearing variants in the same gene likely exhibited the same undescribed RGD. These diagnoses were made prior to formal publication of a new disease-gene association in the literature. The diagnostic implications of these results, therefore, are reliant on more than the genomic knowledge base (which did not yet include reference these disease-gene associations) and the technological processes of the laboratories (which reported them as uncertain results); they were also reliant on the expertise of multiple clinicians providing care to several patients around the

world. In both cases, the ordering clinicians contributed to peer-reviewed publications linking these genes to new RGDs (Deshwar et al., 2022; Lessel et al., 2020), one of which (*AGO2*) has been curated by OMIM as a new disease-gene association (https://www.omim.org/entry/619149). This grey area, between clinical care and interdisciplinary research, has been previously described in genomic medicine (Long et al., 2021) and is acknowledged in Article 2 by the genetics professional organizations in the number of recommendations made related to the topic "navigation of the clinical and research boundary".

Considerations for optimization of ES/GS technologies for RGD diagnosis

The optimization of ES/GS technologies within healthcare systems for RGD diagnosis therefore needs to include consideration for the evolving genomic knowledge base and maturation of technological processes and clinician expertise. These articles provide evidence that: 1) genomic knowledge is evolving at a rate that has diagnostic implications for ES/GS, 2) analytical processes are evolving at a rate that has diagnostic implications for ES/GS, and 3) the optimization of diagnostic yield from ES/GS will require the continued close, reciprocal interaction between clinical care and research.

Objective 2 - Knowledge Tools/Products - To examine how knowledge users have refined this ever-evolving knowledge base into clinical guidance

The second objective of this thesis was to "examine how knowledge users have refined this ever-evolving knowledge base into clinical guidance." Within the KTA framework, Knowledge Creation is depicted as a funnel in which knowledge is gradually refined to more useful forms. For example, from the multitude of primary studies to knowledge syntheses and then to more applicable knowledge tools and products such as practice guidelines. In Article 2, we generated evidence related to this process by conducting a synthesis of the knowledge tools produced by one knowledge user group: we reviewed

clinical guidance documents generated by organizations representing genetics healthcare professionals related to ES/GS testing for RGD patients. In doing so, we gained insight into how this knowledge user selected knowledge from this knowledge base and highlight implications for future guidance.

We found that the first guidance documents produced by genetics professional organizations were published shortly after the introduction of clinical ES/GS testing; the first laboratory to offer clinical ES/GS did so in 2011 (Baylor Genetics, 2023), and the first guidance document on this topic was published by the American College of Medical Geneticists (ACMG) in 2012 (ACMG Board of Directors, 2012). This is an important observation because it indicates that these first documents may have been in response to, rather than guiding or prompting, the first implementation of this new technology. Over the next decade, we identified another 29 published guidance documents, just five of which were newer versions of previous documents. Together, these 30 documents included over 611 recommendation statements, from which we identified 21 unique topics.

Based on the Appraisal of Guidelines for Research and Evaluation (AGREE) II instrument, we found the documents overall were of poor quality; the mean domain score (N=30) was less than 60% of the possible maximum in all six domains of quality. Most predominantly, we identified low AGREE II scores in the items related to Domain 3: Rigour of Development, particularly as it related to the use of systematic methods to search for evidence, the use of criteria for selecting that evidence, describing the strengths and limitations of that evidence, and methods for formulating the recommendations. While it is possible that these documents were created using rigorous methods that were not clearly described, we believe that in most cases rigorous knowledge syntheses were not conducted. To the best of our knowledge, the first peer-reviewed, published knowledge synthesis related to ES/GS testing for RGD diagnosis was published in 2018 (Clark et al., 2018). When generating recommendations for their

memberships, organizations representing genetics professionals utilized experiential knowledge (expert opinion), often combined with selective and non-systematic reference to existing evidence. Notably, the first set of recommendations made by the ACMG in 2012 provided no references to primary literature, insinuating that recommendations may have been made by experiential knowledge alone (ACMG Board of Directors, 2012). Many of these guidance documents are therefore not evidence-based given they did not use established, rigorous systematic methods to identify, evaluate, and synthesize existing studies as the basis of their recommendations. This phenomenon of relying on experiential knowledge and selective evidence summaries may be changing, however. In two guidance documents, by two different organizations, published in 2021, we see reference to systematic knowledge syntheses (Lazier et al., 2022; Manickam et al., 2021), one of which was commissioned to produce that particular set of recommendations (Malinowski et al., 2020). This may indicate that organizations representing genetics healthcare professionals have begun using more rigorous approaches to synthesize the primary literature prior to the creation of clinical guidance documents, aligned with the refining of knowledge as depicted by the Knowledge Creation funnel in KTA.

Synthesis of these guidance documents determined that all 23 items of AGREE II could be improved; the highest median score was 5 on a scale from 1 (the item was not addressed at all) to 7 (the guideline perfectly addressed the item). As such, we recommended that guidance developers use appraisal tools like AGREE II to guide their methods when developing future guidelines. There were nine items where guidance developers received particularly poor scores (**Table 5.5**). Each of these items had median scores of 1, indicating that at least half of guidance documents did not address the particular item at all.

Table 5.5. Nine AGREE II appraisal items with median scores of 1 from Article 2 (N = 30)		
Domain	**Item**	**Item Description**
Domain 2: Stakeholder Involvement	5	The views and preferences of the target population (patients, public, etc.) have been sought
Domain 3: Rigour of Development	7	Systematic methods were used to search for evidence
Domain 3: Rigour of Development	8	The criteria for selecting the evidence are clearly described
Domain 3: Rigour of Development	9	The strengths and limitations of the body of evidence are clearly described
Domain 3: Rigour of Development	10	The methods for formulating the recommendations are clearly described
Domain 3: Rigour of Development	14	A procedure for updating the advisory document is provided
Domain 5: Applicability	20	The potential resource implications of applying the recommendations have been considered
Domain 5: Applicability	21	The advisory document presents monitoring and/or auditing criteria
Domain 6: Editorial Independence	22	The views of the funding body have not influenced the content of the advisory document

Given the majority of recommendations about the use of ES/GS for RGD diagnosis are not currently based on systematic knowledge syntheses, that five of nine domains with median scores of 1 were related to the quality domain Rigour of Development, and that our study findings related to Objective 1 have confirmed that the knowledge base related to ES/GS is evolving at a pace that has diagnostic implications, we propose that guidance developers should consider the use of living systematic reviews (LSR) to support future guidance development. In LSRs, *a priori* protocols are established and new evidence is sought after a predetermined length of time (Elliott et al., 2017). LSRs are becoming increasingly popular in areas of evolving science and became a dominant method in addressing the tsunami of evidence related to the COVID-19 pandemic (Au & Cheung, 2022; Fragkou et al., 2022; Iannizzi et al., 2023), another rapidly-evolving space in which new evidence had immediate, important implications for clinical practice. The use of a well-developed protocol for a LSR could potentially improve scores related to items 7 (systematic methods were used to search for evidence), 8 (the criteria

for selecting the evidence are clearly described), 9 (the strengths and limitations of the body of evidence are clearly described), and 14 (a procedure for updating the advisory document is provided). Further, the variables collected and reviewed as part of the LSR could potentially be interpreted as the monitoring and/or auditing criteria important for making recommendations (item 21).

In Article 2, we identified a key stakeholder whose views have been thus far largely excluded from recommendations, those of the patients receiving this testing and/or their families. While the range and types of recommendations identified in Article 2 have given us some understanding of which patients might be most commonly receiving this testing, Articles 1 and 3 have also shed some light on who these families are in our particular study setting, the province of Ontario. We found that the vast majority of those included in the patient cohorts receiving ES/GS in Ontario have pediatric-onset disease involving syndromic intellectual disability or developmental delay and testing strategies often included unaffected parents in their sequencing (e.g., a trio analysis). Future guidance should ensure the perspectives of patients and families in this population are included.

Article 2 also identified a number of knowledge gaps that should be addressed in future research and guidance documents. The predominant area for future research highlighted by guidance documents was related to additional findings and the need for a better understanding regarding their frequency and impact for both families and healthcare systems. We reiterate it here given it appears to be a priority for organizations; over 25% of recommendations related to this topic.

Considerations for optimization of ES/GS technologies for RGD diagnosis

The optimization of ES/GS technologies within healthcare systems for RGD diagnosis should include consideration for how the knowledge base has been incorporated into knowledge tools. These articles

provide evidence that: 1) the majority of guidance documents available to healthcare practitioners are of low methodological quality, particularly with respect to whether and how they synthesize existing evidence and use it to inform recommendations, and 2) there are a number critical knowledge gaps in need of further evidence generation, particularly related to additional findings.

Objective 3 – Action Cycle - To examine whether implementation of ES/GS based on this knowledge base results in diagnoses for families with RGDs

The final objective of this dissertation was 'to examine whether the implementation of ES/GS based on this knowledge base results in diagnoses for families with RGDs'. Here, we narrowed our focus to examine the Action Cycle in one jurisdiction as part of a larger integrated KT study with the Ministry of Health (MOH) in Ontario. By partnering with the MOH, we aimed to produce research findings and considerations that are relevant to this particular end user. Accordingly, I make some observations and considerations in this section of my thesis that are Ontario-specific to provide implications that are particularly relevant to this context and population.

Prior to the involvement of Care4Rare Canada, a working group for the MOH called the Genetic Testing Advisory Committee (GTAC) (Ontario Ministry of Health and Long-Term Care, 2018) had adapted a clinical guidance document by the Canadian College of Medical Genetics (CCMG) into a jurisdiction-specific policy, which included a set of five clinical presentation eligibility criteria for ES/GS testing for the diagnosis of RGDs (Genetic Testing Advisory Committee, 2016). In addition to the eligibility criteria, the policy included a recommendation that families with sporadic disease be sequenced as trios (i.e., affected probands and their unaffected parents). The MOH eligibility criteria were made available to ordering clinicians in early 2018 and Care4Rare launched a prospective multi-site research study in parallel, in an effort to evaluate the effectiveness of ES/GS testing for Ontario patients. Article 3 focused on evaluating this testing based on two levels of efficacy as per the Fryback and Thornbury framework

(Fryback & Thornbury, 1991), the diagnostic accuracy of results and how they impact the diagnostic thinking of ordering clinicians.

Monitor Knowledge Use

Article 3 provided evidence that the ordering clinicians involved in this study (26 clinical geneticists and 4 neurologists) understood the MOH policy requirements; two or more eligibility criteria were selected for all 297 families and the majority of sporadic families received trio sequencing. This indicates that the knowledge related to recommendations about the clinical appropriateness of families and sequencing strategy had diffused through the target decision-maker group involved in this study. Within the cohort we identified a range of presentations, in terms or phenotypes (all five mutually-exclusive phenotypic categories were represented) and ages of presentation (from fetal onset to age 64). We also found that ES was being used in lieu of a broad number of other diagnostic molecular tests. While it is difficult to assess the appropriateness of ES testing for each patient given this wide variability, the majority of families exhibited a pediatric-onset, syndromic intellectual disability (ID) or developmental delay (DD) for which ES testing has been re-iterated as an appropriate indication for testing in a recently published guidance document, based on a systematic review (Malinowski et al., 2020), and appraised as part of Article 2 (**Table 5.6**) (Manickam et al., 2021).

Table 5.6. AGREE II Appraisal of Manickam et al. (2021)

Domain	Total Domain Score
Domain 1: Scope and Purpose	94%
Domain 2: Stakeholder Involvement	72%
Domain 3: Rigour of Development	77%
Domain 4: Clarity of Presentation	89%
Domain 5: Applicability	71%
Domain 6: Editorial Independence	42%

Evaluate Outcomes

Guided by the Fryback and Thornbury Efficacy Framework (Fryback & Thornbury, 1991), we found that the use of the MOH eligibility criteria identified a broad range of molecular results (Level 2 efficacy) and was effective in impacting diagnostic thinking (Level 3 efficacy) for ordering clinicians, both in terms of providing them with clinical-molecular diagnoses as well as altering the use of diagnostic tests. The proportion of families with clinical-molecular diagnoses following testing was 35%, meeting a provincial benchmark that was set based on a non-systematic review of the early primary literature related to testing (Genetic Testing Advisory Committee, 2016).

ES/GS testing has also been found to provide diagnoses for patients with suspected RGD in other publicly-funded healthcare systems; albeit results are hard to compare given differences in eligibility criteria, technologies, and reporting practices. For example, the single-payer National Health Service in England provides publicly-funded genome sequencing for patients with suspected rare diseases classified into 15 domains, each with pre-specified enrolment criteria (Turro et al., 2020). Partial or fully diagnostic results were issued to 1,138 of 7,065 (16.1%) tested patients, but they noted significant differences in yields depending on the phenotypic domain (ranging from 0% to 54%) (Turro et al., 2020). A number of other countries with at least some government-funded healthcare, including Australia, Brazil, China, Denmark, Estonia, Finland, France, the Netherlands, Qatar, Saudi Arabia, Sweden, Turkey, Japan, and the United States of America, have national genomic-medicine initiatives and are working towards implementation of ES/GS for patients with suspected RGD (Stark, Dolman, et al., 2019). While few countries have published the results of these initiatives, Australian Genomics has published a number of studies as part of their 'rare-disease flagship projects' (Gaff et al., 2017). These studies indicate that ES testing increased diagnostic yield and improved short-term patient management and longer-term patient and family outcomes while decreasing costs for the healthcare system (Stark et al.,

2018; Stark et al., 2017; Stark, Schofield, et al., 2019; Stark et al., 2016; Tan et al., 2017). There, diagnostic yields have hovered between 50 and 60%, but they have actively tried to implement ES as a first tier test in acutely sick children with suspected monogenic disorders, yielding a population receiving testing that is not directly comparable to Ontario.

Sustain, Spread, and Scale Knowledge Use

While we did not directly address this step of the Action Cycle in Ontario, our studies raise further questions that should be answered related to decisions to sustain the current program for ES/GS testing in the province of Ontario, spread the program by adapting it to other organizational contexts, or scale the program by expanding the health intervention in some way.

For example, prior to deciding whether to **sustain** the current intervention related to ES/GS in Ontario specifically, the Ministry of Health might want to ask:

1) Whether we also observe effectiveness for this testing as it relates to clinical utility and costs;
2) Whether our findings related to diagnostic utility are replicable in a less selected cohort with broader provincial representation (from both patients and clinicians); and,
3) Whether a more comprehensive evaluation of implementation identifies any important areas for improvement. For example, by using the Reach, Effectiveness, Adoption, Implementation, and Maintenance (RE-AIM) framework (Glasgow et al., 1999), the MOH might evaluate how to improve equity in the patient population receiving testing, identify unanticipated harms, or gain granularity in how clinicians are adopting the eligibility criteria and if they are doing so inappropriately.

Given how complex ES/GS testing is, a decision to **spread** this program to other organizational contexts, should ensure equivalence in clinical care. For example, the MOH might wish to allow other sub-specialties, like pediatrics, to order ES/GS testing. Prior to doing so, they should examine if those providers believe that ordering this testing is within their purview, have the expertise to do so, and are also able to implement the set of clinical eligibility criteria in a manner that maintains this diagnostic efficacy. Similarly, equivalence should be done to show patient care is not compromised in spreading this intervention.

Finally, additional research is necessary prior to **scaling** the intervention further. Our studies show that there might be utility in expanding this intervention to include reanalysis of ES/GS data at a later date, but the timing of such is still unclear. Another consideration for scaling would be related to the clinical eligibility criteria for testing, which were developed as a first iteration of criteria that could be adapted over time (Genetic Testing Advisory Committee, 2016). Article 3 found that each of the criteria had a diagnostic yield greater than 30%. The MOH might consider adapting and evaluating the requirements for testing such that the number of required criteria are different (e.g., one or more rather than two or more), the criteria themselves are different (e.g., mild impairment rather than moderate impairment), or new criteria are included (e.g., the inclusion of specific clinical phenotypes known to be likely caused by genetic etiologies).

One thing is clear, however: given that *the knowledge base related to ES/GS is rapidly evolving*, that a key knowledge user community has produced *low quality clinical guidance documents*, and that we have observed *diagnostic efficacy but in a selected population*, *continued evaluation in different settings and contexts will be necessary for years to come*.

Considerations for optimization of ES/GS technologies for RGD diagnosis

The optimization of ES/GS technologies within healthcare systems for RGD diagnosis needs to consider the potential efficacy of ES/GS for patients with RGDs. These articles provide evidence that: 1) healthcare practitioners in a single exemplar jurisdiction can apply a set of eligibility criteria related to ES/GS testing, 2) despite there being few good quality guidance documents, ES/GS appears to have diagnostic efficacy, and 3) given the evolving knowledge base, continuous evaluation is necessary to determine if clinical use of ES/GS is worthy of sustaining, spreading, or scaling.

Summary of the considerations for optimized ES/GS testing

1. Genomic knowledge is evolving at a rate that has diagnostic implications for ES/GS.
2. Analytical processes are evolving at a rate that have diagnostic implications for ES/GS.
3. The optimization of diagnostic yield from ES/GS will require the continued close, reciprocal interaction between clinical care and research.
4. The majority of guidance documents available to healthcare practitioners are of low methodological quality, particularly with respect to whether and how they synthesize existing evidence and use it to inform recommendations.
5. There are a number critical knowledge gaps in need of further evidence, particularly related to additional findings.
6. Healthcare practitioners in a single exemplar jurisdiction can apply a set of eligibility criteria related to ES/GS testing.
7. Implementation of ES/GS appears to have diagnostic efficacy.
8. Continuous evaluation is necessary to determine if clinical use of ES/GS is worthy of sustaining, spreading, or scaling.

5.3. Implications of considerations for knowledge users

5.3.1. Implications related to the evolving knowledge base

Genomic knowledge and analytical processes are evolving at rates that have diagnostic implications for ES/GS (Considerations 1 and 2) and this indicates that there may be opportunity for new processes such as reanalysis and other translational research to improve diagnostic yields (Consideration 3). This has implications for **healthcare practitioners working in clinical laboratories or ordering ES/GS**, **patients, guidance developers**, **payers, and researchers**.

For **healthcare practitioners working in clinical laboratories**, the evolving genomic knowledge base presents both opportunities and challenges for gene and variant interpretation. Articles 1 and 3 identified multiple cases in which new disease-gene associations resulted in clinically-valid molecular diagnoses. Laboratories might therefore strive to optimize the sensitivity of their test for clinically-valid molecular results in both established clinically-associated genes and GUSs, while limiting uncertainty. To contextualize results for ordering clinicians, laboratories might consider reporting results based on frameworks for evaluating the levels of evidence related to disease-gene relationships, like that developed by the ClinGen network (Strande et al., 2017). With respect to variant interpretation, Article 1 identified multiple cases in which new variant-specific information (e.g., variant-disease associations) provided additional evidence for pathogenicity, and therefore supports suggestions that using the most up-to-date genomic knowledge can aid in the interpretation of DNA variants (Deignan et al., 2019). The findings in Article 1 similarly support recommendations that clinical laboratories contribute their own data to advance genomic knowledge; whether by sharing specific genomic variation (e.g., in databases such as ClinVar), new potential disease-gene associations (e.g., in databases connected to the Matchmaker Exchange), or full genomic datasets for secondary research purposes (e.g., as was done with Care4Rare in Article 1). Analytical processes at laboratories will continue to mature and the

communication and sharing of laboratory methods will be important in optimizing this testing for all patients. This includes sharing methodological details within the clinical reports sent to ordering clinicians, as well as communication between laboratories to advance methods more broadly.

For **healthcare practitioners ordering ES/GS**, the observations presented here have both pre- and post-test implications. Given the rapid pace of change in analytic technologies, clinicians should acquaint themselves to a particular clinical laboratory's processes to understand what analytical processes are employed, particularly in understanding the types of genetic variation being tested and assessing the appropriateness of such in relation to the clinical presentation of their patient. When feasible, ordering clinicians might consider aiding in the genomic analysis of their patient's data. Barring this, our observations indicate there may be utility in providing deep and comprehensive phenotypic data (including phenotypes that may not appear to be related to the particular RGD) to laboratories conducting testing. In the post-test period, the greatest benefit of the increasing knowledge base will be for patients that do not yet have a genetic diagnosis for their RGD. For families with uncertain DNA variants in genes with known disease-gene associations that fit the phenotype of the patient, re-interpretation at a later date may yield additional evidence to support or discredit pathogenicity. For families with candidate DNA variants in GUSs, ordering clinicians might consider data sharing or laboratory studies. For families without a compelling DNA candidate, there may be utility in reanalysis to identify a new candidate at a later date.

For **patients**, these studies have highlighted the potential uncertainty that can come with genomic results; but that this uncertainty may resolve with time. This aligns with recommendations that families should be counselled appropriately about uncertainty, the limitations of the current genomic knowledge base, and the potential opportunity for diagnoses at a later date. Patients might also be provided with

opportunities to participate in, and provide feedback on, secondary research related to their genomic data and other translational research processes.

Guideline developers should ensure the evolving knowledge base is considered when developing clinical guidance. Procedures for updating guidance documents should be explicitly stated. The data presented here also indicate that genetics healthcare professionals have not established or implemented best practices in phenotyping, analytical processes related to ES/GS testing, or reanalysis of genomic data, therefore, updated guidance might be considered.

For **payers**, these observations provide evidence that reanalysis of existing non-diagnostic ES data in light of an evolving knowledge base (including new genomic knowledge, methodological advancements, and with improved integration of clinician expertise) can result in additional diagnoses, and that policies regarding such may be of interest to healthcare systems.

For **researchers**, there remain a number of unanswered questions related to the optimal integration of reanalysis and its cost-effectiveness at various time periods and under different testing conditions (for example by sequencing strategy or depending on patient phenotype). Analytical sensitivity of exome sequencing continues to improve, and studies and tools to improve the detection of genomic variants intractable by exome sequencing (new types of mutations like non-coding variants or structural variants) will be more important as laboratories transition to genome sequencing as the costs of sequencing decrease. Our studies also indicate that there remains significant potential for gene discovery in patients with suspected RGD who met eligibility criteria for ES in the province of Ontario.

5.3.2. Implications related to knowledge refining

Thus far, knowledge refinement by organizations representing genetics professionals into guidance documents has been of low methodological quality, particularly as it relates to synthesis of knowledge to inform recommendations (Consideration 4), and there remains a number of knowledge gaps (Consideration 5).

For **healthcare practitioners** considering implementation of the clinical recommendations included in these guidance documents, it is important to learn how to appraise guidance documents and their recommendations using tools like AGREE II, AGREE-REX, and/or other tools like the Institute of Medicine clinical practice guideline (CPG) tool. When implementing recommendations, healthcare practitioners and clinical laboratories might consider collaborating with researchers to examine the action cycle within their own practice.

For **patients,** thus far their perspectives have been largely omitted from clinical guidance documents related to ES/GS. As the field moves towards establishing best practices, it is vital that families with known and suspected RGDs be included in guidance development.

For **guidance developers,** all domains of quality for guidance documents could be improved. Using tools such as those developed by the AGREE consortium can help improve quality of guidance documents overall (Brouwers et al., 2020). Guidance developers should include additional perspectives in the development of future guidance; in particular, methodologists and patients. We recommend incorporating rigorous knowledge syntheses and considering the use of living systematic reviews to account for the rapidly evolving knowledge base related to ES/GS. Further, groups might consider

providing training opportunities related to guidance development to improve future guidance development and improve appraisal skills within their membership.

For **payers,** guidance documents are one of several tools that might be used in making policy decisions related to genomic medicine. It is important to critically appraise the guidance that is used in making policy decisions and evaluate any subsequent policy implementation.

For **researchers,** Article 2 identified a number of knowledge gaps to be addressed with future research. In particular, organizations noted a need for studies related to additional findings. As mentioned above, researchers should consider collaborating with healthcare practitioners implementing clinical recommendations, from guidance documents, to monitor and evaluate their use.

5.3.3. Implications related to the action cycle

Our examination of the action cycle in one study setting, Ontario, indicated that healthcare practitioners (primarily clinical geneticists) were able to apply a set of prescribed clinical eligibility criteria (Consideration 6) and that the implementation was effective in identifying diagnoses and altering diagnostic thinking (Consideration 7). Our study was limited, however, to focusing primarily on the efficacy of testing and provided limited monitoring of the implementation of ES/GS. Given this, and all of the previous considerations identified, continuous monitoring and evaluation of ES/GS is necessary to determine if knowledge related to ES/GS is worthy of sustaining, scaling, or spreading (Consideration 8).

For **healthcare practitioners and researchers**, these considerations indicate how imperative it is to contribute to research, whether by initiating their own studies or participating when given the opportunity. Despite the naïve evidence base, ES/GS testing is resulting in diagnoses within the Ontario

healthcare system, but further studies are necessary to ensure a fulsome understanding of how ES/GS testing has been implemented and its benefits and harms. Additional studies are needed to optimize its use to ensure all appropriate patients are receiving the best test possible as defined by patients, their healthcare practitioners, and the payers.

For **patients,** our studies have shown that ES/GS has the potential to identify diagnoses for families within the Ontario healthcare system. Patients should be provided with opportunities to be made aware of the availability for this testing and access to the providers available with expertise to order such testing when appropriate.

For **guidance developers,** our evidence suggests that the adoption of ES/GS testing has resulted in diagnoses for patients from Ontario, the majority of whom presented with indications considered appropriate for testing. It will be important that studies, like Article 1 and Article 3, are synthesized with other studies and clinical guidance is updated and modified as appropriate.

For **payers,** particularly in Ontario, these results indicate that implementation of ES/GS is performing as anticipated at the time of eligibility criteria development. Additional studies are forthcoming from this collaboration, which will help in understanding the clinical management and costing implications of this testing. Payers should continue engaging with researchers and integrate ongoing evaluation opportunities related to decisions to sustain, scale, or spread ES/GS testing.

5.4. Contribution to population health

While individually each RGD is rare, patients affected by RGDs are collectively common and experience shared challenges in the provision of their healthcare, making them a worthy target for a population

health lens. This thesis acknowledges and tries to address the widespread inequity in diagnostic care by focusing on the provision of a single, new diagnostic technology, ES/GS, with the potential to provide timely and accurate genetic diagnoses for thousands of unique RGDs. Three articles were generated using interdisciplinary thinking, weaving methodologies from a range of fields including natural sciences, medicine, epidemiology, implementation science, and policy. By considering these disciplines together, I generated evidence that improves our understanding of the knowledge base related to ES/GS, the clinical guidance that has been developed based on that knowledge base, and its diagnostic efficacy within a particular healthcare system. This dissertation demonstrates one of the first applications of the KTA conceptual framework to the field of genomic medicine. Based on evidence from these articles, I produce novel insight into the knowledge translation processes and highlight considerations for optimizing this test, by way of maximizing diagnoses, minimizing uncertainty, improving clinical guidance, advancing our understanding of diagnostic efficacy, and identifying knowledge gaps.

5.5. Strengths and limitations of this thesis

In this thesis, we examined how knowledge related to ES/GS technologies has been translated into clinical care, guided by the KTA conceptual framework. The particular strengths and limitations of each individual study have been included within the relevant chapters, the focus here is on the strengths and limitations across the dissertation.

The execution of these three studies required the collaboration of 112 unique co-authors with varied expertise across healthcare (medical and laboratory genetics, neurology, genetic counselling) and disciplines of science (epidemiology, health economics, computational science and bioinformatics, natural sciences, library and information science). The inclusion of these varied perspectives, in

combination with the collaboration with the Ontario MOH, will make results more relevant for various knowledge users.

Articles 1 and 3 included results from 532 unique probands who received clinical ES in the province of Ontario (52 families were involved in both studies). Testing was ordered by 59 different ordering providers. The consistency in characteristics between the two cohorts (both in terms of distribution of phenotypes and biological sex) indicates that these results may be a true reflection of the landscape of phenotypes receiving clinical ES in this jurisdiction.

Of course, these Articles also have limitations. First, it is important to note that there is diverse terminology related to "exome sequencing and genome sequencing (ES/GS)". Other terms used to refer to the sequencing of disease-related genes across the human genome for diagnostic testing purposes include "genomic sequencing", "genome-wide sequencing", "clinome sequencing", as well as others, each of which might have subtle differences in their meaning. The diverse terminology might have impacted our searches for relevant literature and impede our ability to contextualize our findings. We made efforts in our search strategies, particularly as it related to Article 2, to be as language inclusive as possible.

Second, while we attempted to generate evidence related to KT more generally, Articles 1 and 3 within the dissertation relied on data collected primarily within one study setting, the hospitals within the province of Ontario that actively collaborate with Care4Rare Canada. As such, our studies may have limited external generalizability to the greater province and other jurisdictions. We are encouraged, however, by the consistency in ratios of biological sexes, ages-of-onset, and phenotypes between Articles 1 and 3, and their consistency with phenotypes of probands deemed appropriate for clinical

ES/GS in guidance documents (e.g., pediatric-onset of syndromic ID) (Boycott et al., 2015; Manickam et al., 2021).

Third, both Articles 1 and 3 relied on healthcare practitioners to identify, offer, consent, and enroll families into our cohorts. This may have introduced a selection bias which can affect the external validity and generalizability to the greater Ontario population. For example, healthcare practitioners may have only been motivated to recruit families they consider at a higher likelihood of a genetic condition, thus inflating our diagnostic yields for both initial ES/GS testing (Article 3) and reanalysis (Article 1). We encouraged clinicians to recruit all eligible participants and provided support whenever possible in the form of research assistant time.

5.6. Future Research

Based on the results of this dissertation and the limitations described in Section 5.5., I propose several areas for future research.

1. *Knowledge synthesis:* As the knowledge base related to ES/GS matures, there is opportunity in the development of protocols for living systematic reviews that can be used in future iterations of clinical guidance documents.
2. *Guideline development and education related to critical appraisal*: The currently available clinical guidance documents related ES/GS are of low quality, and as such there is opportunity for education related to clinical guidance document development and critical appraisal.
3. *Implementation studies*: Our evaluation of diagnostic utility for ES/GS testing in Ontario was limited by our recruitment methods, which may have introduced a selection bias. A better study design would have been integrated with the laboratories and hospitals, as part of a quality improvement initiative, to enable a comprehensive analysis of all patients receiving testing. The

introduction of the Genome-wide Sequencing Ontario program has provided the opportunity to examine some of the variables from Article 3 for all receiving testing, as well as examine other important evaluation questions.

4. *Research about the effectiveness of translational research approaches*: There are a number of translational research approaches that may improve the diagnostic yield of ES/GS testing, but their effectiveness is not yet understood. This includes approaches that are currently implemented inconsistently amongst laboratories, including reanalysis of non-diagnostic genomic data and the reporting of GUSs, as well as secondary approaches that may help resolve uncertain results, such as data sharing practices and other technologies (for example, the addition of RNA sequencing following ES/GS to assess the functional consequence of DNA variants that may impact mRNA splicing).

5.7. Conclusions

The pace of implementation of ES/GS into healthcare systems has been rapid. In just over a decade, ES/GS has transitioned from a research method to an implemented test for patients with suspected RGDs worldwide, and healthcare systems are challenged to optimize its use within their own jurisdictions. This thesis sought to better understand how knowledge related to ES/GS is changing, being refined, and being applied, to produce considerations for healthcare systems on how it might be optimized. The three original articles presented here were each designed to produce evidence related to a particular aspect of the KTA framework. First, we examined the evolving knowledge base and determined that evidence related to the etiologies of RGDs and analytical processes are progressing at a pace that has diagnostic implications. Next, we found that one knowledge user, organizations representing genetics professionals, has produced a broad range of clinical recommendations, but the quality of these guidance documents is low. Regardless, in our final study, we found that the

introduction of ES/GS into the Ontario healthcare system has resulted in clinically-valid diagnoses for Ontario patients that have met internal benchmarks for diagnostic yield. Together, these studies have informed considerations for the optimization of ES/GS testing and offer a foundation for future studies related to knowledge translation. The results of these studies contribute to the growing knowledge base available for knowledge users to further optimize diagnostic outcomes for families with suspected RGDs receiving ES through their healthcare systems; whether it be via fine-tuning policies within healthcare systems that dictate who might receive this testing, improving the clinical recommendations that guide the clinicians in their diagnostic approaches for their patients, or identifying the needs for future research and policymaking to optimize ES/GS testing. Ultimately, these findings will enable better diagnostic care for families with RGDs.

5.8. References

ACMG Board of Directors. (2012). Policy Statement - Points to Consider in the Clinical Application of Genomic Sequencing. *Genet Med*, *14*, 759-761.

Au, W. Y., & Cheung, P. P. (2022). Effectiveness of heterologous and homologous covid-19 vaccine regimens: Living systematic review with network meta-analysis. *BMJ*, *377*, e069989. https://doi.org/10.1136/bmj-2022-069989

Bamshad, M. J., Nickerson, D. A., & Chong, J. X. (2019). Mendelian gene discovery: Fast and furious with no end in sight. *Am J Hum Genet*, *105*(3), 448-455. https://doi.org/10.1016/j.ajhg.2019.07.011

Baylor Genetics. (2023). *Our History*. Retrieved January 26, 2023 from https://www.baylorgenetics.com/history/

Boycott, K., Hartley, T., Adam, S., Bernier, F., Chong, K., Fernandez, B. A., Friedman, J. M., Geraghty, M. T., Hume, S., Knoppers, B. M., Laberge, A. M., Majewski, J., Mendoza-Londono, R., Meyn, M. S., Michaud, J. L., Nelson, T. N., Richer, J., Sadikovic, B., Skidmore, D. L., . . . Canadian College of Medical Geneticists. (2015). The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet*, *52*(7), 431-437. https://doi.org/10.1136/jmedgenet-2015-103144

Brouwers, M. C., Kho, M. E., Browman, G. P., Burgers, J. S., Cluzeau, F., Feder, G., Fervers, B., Graham, I. D., Grimshaw, J., Hanna, S. E., Littlejohns, P., Makarski, J., Zitzelsberger, L., & AGREE Next Steps Consortium. (2010). AGREE II: advancing guideline development, reporting and evaluation in health care. *CMAJ*, *182*(18), E839-842. https://doi.org/10.1503/cmaj.090449

Brouwers, M. C., Spithoff, K., Lavis, J., Kho, M. E., Makarski, J., & Florez, I. D. (2020). What to do with all the AGREEs? The AGREE portfolio of tools to support the guideline enterprise. *J Clin Epidemiol*, *125*, 191-197. https://doi.org/10.1016/j.jclinepi.2020.05.025

Bush, L. W., Beck, A. E., Biesecker, L. G., Evans, J. P., Hamosh, A., Holm, I. A., Martin, C. L., Richards, C. S., & Rehm, H. L. (2018). Professional responsibilities regarding the provision, publication, and dissemination of patient phenotypes in the context of clinical genetic and genomic testing: points to consider-a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *20*(2), 169-171. https://doi.org/10.1038/gim.2017.242

Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M., Dimmock, D., & Kingsmore, S. F. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*, *3*, 16. https://doi.org/10.1038/s41525-018-0053-8

Deignan, J. L., Chung, W. K., Kearney, H. M., Monaghan, K. G., Rehder, C. W., Chao, E. C., & ACMG Laboratory Quality Assurance Committee (2019). Points to consider in the reevaluation and reanalysis of genomic test results: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *21*(6), 1267-1270. https://doi.org/10.1038/s41436-019-0478-1

Deshwar, A. R., Cytrynbaum, C., Murthy, H., Zon, J., Chitayat, D., Volpatti, J., Newbury-Ecob, R., Ellard, S., Lango Allen, H., Yu, E. P., Noche, R., Walker, S., Scherer, S. W., Mahida, S., Elitt, C. M., Nicolas, G., Goldenberg, A., Saugier-Veber, P., Lecoquierre, F., . . . Weksberg, R. (2022). Variants in CLDN5 cause a syndrome characterized by seizures, microcephaly and brain calcifications. *Brain*. https://doi.org/10.1093/brain/awac461

Eldomery, M. K., Coban-Akdemir, Z., Harel, T., Rosenfeld, J. A., Gambin, T., Stray-Pedersen, A., Kury, S., Mercier, S., Lessel, D., Denecke, J., Wiszniewski, W., Penney, S., Liu, P., Bi, W., Lalani, S. R., Schaaf, C. P., Wangler, M. F., Bacino, C. A., Lewis, R. A., . . . Lupski, J. R. (2017). Lessons learned from additional research analyses of unsolved clinical exome cases. *Genome Med*, *9*(1), 26. https://doi.org/10.1186/s13073-017-0412-6

Elliott, J. H., Synnot, A., Turner, T., Simmonds, M., Akl, E. A., McDonald, S., Salanti, G., Meerpohl, J., MacLehose, H., Hilton, J., Tovey, D., Shemilt, I., & Thomas, J. (2017). Living systematic review: 1. Introduction-the why, what, when, and how. *J Clin Epidemiol*, *91*, 23-30. https://doi.org/10.1016/j.jclinepi.2017.08.010

Fragkou, P. C., Moschopoulos, C. D., Dimopoulou, D., Ong, D. S. Y., Dimopoulou, K., Nelson, P. P., Schweitzer, V. A., Janocha, H., Karofylakis, E., Papathanasiou, K. A., Tsiordras, S., De Angelis, G., Thölken, C., Sanguinetti, M., Chung, H. R., & Skevaki, C. (2022). Performance of point-of care molecular and antigen-based tests for SARS-CoV-2: A living systematic review and meta-analysis. *Clin Microbiol Infect*. https://doi.org/10.1016/j.cmi.2022.10.028

Fryback, D., & Thornbury, J. (1991). The efficacy of diagnostic imaging. *Med Decis Making*, *11*(2), 88-94.

Gaff, C. L., Winship, I. M., Forrest, S. M., Hansen, D. P., Clark, J., Waring, P. M., & Sinclair, A. H. (2017) Preparing for genomic medicine: a real world demonstration of health system change. *npj Genomic Medicine*, *2*(16), doi:10.1038/s41525-017-0017-4

Genetic Testing Advisory Committee. (2016). *Use of Genome-Wide Sequencing for Undiagnosed Rare Genetic Diseases in Ontario*. Retrieved March 30, 2023, from http://www.health.gov.on.ca/en/pro/programs/gtac/reports.aspx

Glasgow, R. E., Vogt, T. M., & Boles, S. M. (1999). Evaluating the public health impact of health promotion interventions: The RE-AIM framework. *Am J Public Health*, *89*(9), 1322-1327. https://doi.org/10.2105/ajph.89.9.1322

Graham, I. D., Logan, J., Harrison, M. B., Straus, S. E., Tetroe, J., Caswell, W., & Robinson, N. (2006). Lost in knowledge translation: Time for a map? *J Contin Educ Health Prof*, *26*(1), 13-24. https://doi.org/10.1002/chp.47

Harrison, S. M., Riggs, E. R., Maglott, D. R., Lee, J. M., Azzariti, D. R., Niehaus, A., Ramos, E. M., Martin, C. L., Landrum, M. J., & Rehm, H. L. (2016). Using ClinVar as a resource to support variant

interpretation. *Curr Protoc Hum Genet*, *89*, 8 16 11-18 16 23. https://doi.org/10.1002/0471142905.hg0816s89

Hume, S., Nelson, T. N., Speevak, M., McCready, E., Agatep, R., Feilotter, H., Parboosingh, J., Stavropoulos, D. J., Taylor, S., Stockley, T. L., & Canadian College of Medical Geneticists. (2019). CCMG practice guideline: Laboratory guidelines for next-generation sequencing. *J Med Genet*, *56*(12), 792-800. https://doi.org/10.1136/jmedgenet-2019-106152

Iannizzi, C., Chai, K. L., Piechotta, V., Valk, S. J., Kimber, C., Monsef, I., Wood, E. M., Lamikanra, A. A., Roberts, D. J., McQuilten, Z., So-Osman, C., Jindal, A., Cryns, N., Estcourt, L. J., Kreuzberger, N., & Skoetz, N. (2023). Convalescent plasma for people with COVID-19: A living systematic review. *Cochrane Database Syst Rev*, *2*(2), Cd013600. https://doi.org/10.1002/14651858.CD013600.pub5

Lazier, J., Hartley, T., Brock, J. A., Caluseriu, O., Chitayat, D., Laberge, A. M., Langlois, S., Lauzon, J., Nelson, T. N., Parboosingh, J., Stavropoulos, D. J., Boycott, K., Armour, C. M., & Canadian College of Medical Geneticists. (2022). Clinical application of fetal genome-wide sequencing during pregnancy: position statement of the Canadian College of Medical Geneticists. *J Med Genet*, *59*(10), 931-937. https://doi.org/10.1136/jmedgenet-2021-107897

Lessel, D., Zeitler, D. M., Reijnders, M. R. F., Kazantsev, A., Hassani Nia, F., Bartholomäus, A., Martens, V., Bruckmann, A., Graus, V., McConkie-Rosell, A., McDonald, M., Lozic, B., Tan, E. S., Gerkes, E., Johannsen, J., Denecke, J., Telegrafi, A., Zonneveld-Huijssoon, E., Lemmink, H. H., . . . Kreienkamp, H. J. (2020). Germline AGO2 mutations impair RNA interference and human neurological development. *Nat Commun*, *11*(1), 5797. https://doi.org/10.1038/s41467-020-19572-5

Long, J. C., Gul, H., McPherson, E., Best, S., Augustsson, H., Churruca, K., Ellis, L. A., & Braithwaite, J. (2021). A dynamic systems view of clinical genomics: a rich picture of the landscape in Australia

using a complexity science lens. *BMC Med Genomics*, *14*(1), 63. https://doi.org/10.1186/s12920-021-00910-5

Malinowski, J., Miller, D. T., Demmer, L., Gannon, J., Pereira, E. M., Schroeder, M. C., Scheuner, M. T., Tsai, A. C., Hickey, S. E., Shen, J., on behalf of the ACMG Professional Practice and Guidelines Committee. (2020). Systematic evidence-based review: Outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. *Genet Med*, *22*(6), 986-1004. https://doi.org/10.1038/s41436-020-0771-z

Manickam, K., McClain, M. R., Demmer, L. A., Biswas, S., Kearney, H. M., Malinowski, J., Massingham, L. J., Miller, D., Yu, T. W., Hisama, F. M., & Directors, A. B. o. (2021). Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: An evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(11), 2029-2037. https://doi.org/10.1038/s41436-021-01242-6

Matthijs, G., Souche, E., Alders, M., Corveleyn, A., Eck, S., Feenstra, I., Race, V., Sistermans, E., Sturm, M., Weiss, M., Yntema, H., Bakker, E., Scheffer, H., Bauer, P., EuroGentest, & European Society of Human Genetics. (2016). Guidelines for diagnostic next-generation sequencing. *Eur J Hum Genet*, *24*(1), 2-5. https://doi.org/10.1038/ejhg.2015.226

Ontario Ministry of Health and Long-Term Care. (2018). *Genetic Testing Advisory Committee*. Retrieved January 27, 2023, from https://www.health.gov.on.ca/en/pro/programs/gtac/

Rehder, C., Bean, L. J. H., Bick, D., Chao, E., Chung, W., Das, S., O'Daniel, J., Rehm, H., Shashi, V., Vincent, L. M., & ACMG Laboratory Quality Assurance Committee. (2021). Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1399-1415. https://doi.org/10.1038/s41436-021-01139-4

Rehm, H. L., Bale, S. J., Bayrak-Toydemir, P., Berg, J. S., Brown, K. K., Deignan, J. L., Friez, M. J., Funke, B. H., Hegde, M. R., Lyon, E., Working Group of the American College of Medical Genetics & Genomics Laboratory Quality Assurance Committee. (2013). ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*, *15*(9), 733-747. https://doi.org/10.1038/gim.2013.92

Shashi, V., Schoch, K., Spillmann, R., Cope, H., Tan, Q. K., Walley, N., Pena, L., McConkie-Rosell, A., Jiang, Y. H., Stong, N., Need, A. C., & Goldstein, D. B. (2019). A comprehensive iterative approach is highly effective in diagnosing individuals who are exome negative. *Genet Med*, *21*(1), 161-172. https://doi.org/10.1038/s41436-018-0044-2

Stark, Z., Dolman, L., Manolio, T. A., Ozenberger, B., Hill, S. L., Caulfied, M. J., Levy, Y., Glazer, D., Wilson, J., Lawler, M., Boughtwood, T., Braithwaite, J., Goodhand, P., Birney, E., & North, K. N. (2019). Integrating genomics into healthcare: A global responsibility. *Am J Hum Genet*, *104*(1), 13-20. https://doi.org/10.1016/j.ajhg.2018.11.014

Stark, Z., Lunke, S., Brett, G. R., Tan, N. B., Stapleton, R., Kumble, S., Yeung, A., Phelan, D. G., Chong, B., Fanjul-Fernandez, M., Marum, J. E., Hunter, M., Jarmolowicz, A., Prawer, Y., Riseley, J. R., Regan, M., Elliott, J., Martyn, M., Best, S., . . . White, S. M. (2018). Meeting the challenges of implementing rapid genomic testing in acute pediatric care. *Genet Med*, *20*(12), 1554-1563. https://doi.org/10.1038/gim.2018.37

Stark, Z., Schofield, D., Alam, K., Wilson, W., Mupfeki, N., Macciocca, I., Shrestha, R., White, S. M., & Gaff, C. (2017). Prospective comparison of the cost-effectiveness of clinical whole-exome sequencing with that of usual care overwhelmingly supports early use and reimbursement. *Genet Med*, *19*(8), 867-874. https://doi.org/10.1038/gim.2016.221

Stark, Z., Schofield, D., Martyn, M., Rynehart, L., Shrestha, R., Alam, K., Lunke, S., Tan, T. Y., Gaff, C. L., & White, S. M. (2019). Does genomic sequencing early in the diagnostic trajectory make a

difference? A follow-up study of clinical outcomes and cost-effectiveness. *Genet Med*, *21*(1), 173-180. https://doi.org/10.1038/s41436-018-0006-8

Stark, Z., Tan, T. Y., Chong, B., Brett, G. R., Yap, P., Walsh, M., Yeung, A., Peters, H., Mordaunt, D., Cowie, S., Amor, D. J., Savarirayan, R., McGillivray, G., Downie, L., Ekert, P. G., Theda, C., James, P. A., Yaplito-Lee, J., Ryan, M. M., . . . White, S. M. (2016). A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. *Genet Med*, *18*(11), 1090-1096. https://doi.org/10.1038/gim.2016.1

Strande, N. T., Riggs, E. R., Buchanan, A. H., Ceyhan-Birsoy, O., DiStefano, M., Dwight, S. S., Goldstein, J., Ghosh, R., Seifert, B. A., Sneddon, T. P., Wright, M. W., Milko, L. V., Cherry, J. M., Giovanni, M. A., Murray, M. F., O'Daniel, J. M., Ramos, E. M., Santani, A. B., Scott, A. F., . . . Berg, J. S. (2017). Evaluating the clinical validity of gene-disease associations: An evidence-based framework developed by the Clinical Genome Resource. *Am J Hum Genet*, *100*(6), 895-906. https://doi.org/10.1016/j.ajhg.2017.04.015

Tan, N. B., Stapleton, R., Stark, Z., Delatycki, M. B., Yeung, A., Hunter, M. F., Amor, D. J., Brown, N. J., Stutterd, C. A., McGillivray, G., Yap, P., Regan, M., Chong, B., Fanjul-Fernandez, M., Marum, J., Phelan, D., Pais, L. S., White, S. M., Lunke, S., & Tan, T. Y. (2020). Evaluating systematic reanalysis of clinical genomic data in rare disease from single center experience and literature review. *Molec Genet Genomic Med*, *8*, e1508 https://doi.org/10.1002/mgg3.1508

Tan, T. Y., Dillon, O. J., Stark, Z., Schofield, D., Alam, K., Shrestha, R., Chong, B., Phelan, D., Brett, G. R., Creed, E., Jarmolowicz, A., Yap, P., Walsh, M., Downie, L., Amor, D. J., Savarirayan, R., McGillivray, G., Yeung, A., Peters, H., . . . White, S. M. (2017). Diagnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. *JAMA Pediatr*, *171*(9), 855-862. https://doi.org/10.1001/jamapediatrics.2017.1755

Turro, E., Astle, W. J., Megy, K., Graf, S., Greene, D., Shamardina, O., Allen, H. L., Sanchis-Juan, A., Frontini, M., Thys, C., Stephens, J., Mapeta, R., Burren, O. S., Downes, K., Haimel, M., Tuna, S., Deevi, S. V. V., Aitman, T. J., Bennett, D. L., . . . Ouwehand, W. H. (2020). Whole-genome sequencing of patients with rare diseases in a national health system. *Nature*, *583*(7814), 96-102. https://doi.org/10.1038/s41586-020-2434-2

Weiss, M. M., Van der Zwaag, B., Jongbloed, J. D., Vogel, M. J., Bruggenwirth, H. T., Lekanne Deprez, R. H., Mook, O., Ruivenkamp, C. A., van Slegtenhorst, M. A., van den Wijngaard, A., Waisfisz, Q., Nelen, M. R., & van der Stoep, N. (2013). Best practice guidelines for the use of next-generation sequencing applications in genome diagnostics: a national collaborative study of Dutch genome diagnostic laboratories. *Hum Mutat*, *34*(10), 1313-1321. https://doi.org/10.1002/humu.22368

Appendix 1 – Research Ethics Board Approvals

RESEARCH INSTITUTE
INSTITUT DE RECHERCHE

Date: 8 August 2018

To: Dr. Kym Boycott, Children's Hospital of Eastern Ontario

CTO Project ID: 1577

Study Title: Care4Rare Canada: Harnessing multi-omics to deliver innovative diagnostic care for rare genetic diseases in Canada (C4R-SOLVE)

Sponsor Study ID: N/A

Application Type: Observational Provincial Initial Application

Review Type: Full Board

Meeting Date: 04/Jul/2018 12:00

Date Approval Issued: 08/Aug/2018 11:29

Approval Expiry Date: 15/Jul/2019

Dear Dr. Kym Boycott,

Thank you for submitting the above referenced study on behalf of all Ontario centres through the Clinical Trials Ontario Streamlined Research Ethics Review System. The Children's Hospital of Eastern Ontario Research Ethics Board has reviewed the study and granted initial provincial approval as of the date noted above.

Provincial documents approved:

Document Name	Document Date	Document Version
Care_Pathway_Data Elements_CTO Submit	18/Jun/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_Phase 1_Children's Assent_Version 1_Aug 3 2018_clean	03/Aug/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_Phase 1_Optional_Version 1_Aug 3 2018_clean	03/Aug/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_PHASE 1_Version 1_Aug 3 2018_clean	03/Aug/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_Phase 1_Very Young Children Assent_Version 1_Aug 3 2018_clean	03/Aug/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_Phase 2_Children's Assent_Version 1_Aug 3 2018_clean	03/Aug/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_PHASE 2_Version 1_Aug 3 2018_clean	03/Aug/2018	1
Protocol 1577 CTO Boycott_C4R-SOLVE_Phase 2_Very Young Children Assent_Version 1_Aug 3 2018_clean	03/Aug/2018	1
vF_C4RSOLVE_Minimal Risk Protocol_Version 1_June 18 2018	18/Jun/2018	1

Provincial documents acknowledged:

Document Name	Document Date	Document Version
7.ICES		
Response Letter - Protocol 1577 CTO Boycott August 3 2018	03/Aug/2018	1

The membership requested that all future annual renewal reports be submitted for delegated review.

Note: Provincial REB approval does not confer ethics approval for participating centres. Each participating centre, including that of the Provincial Applicant, must submit the "Centre Initial Application" and receive approval from this REB prior to the conduct of the study at that centre. All other required institutional approvals must also be obtained prior to the conduct of the study.

No deviations from, or changes to, the protocol should be initiated without prior written approval from Children's Hospital of Eastern Ontario Research Ethics Board, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial (such as a change in telephone number).

REB members involved in the research project do not participate in the review, discussion or decision.

Children's Hospital of Eastern Ontario Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Children's Hospital of Eastern Ontario Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Dr. Richard Carpentier

Chair, CHEO REB

CHEO

Date: 5 July 2019

To: Dr. Kym Boycott, Children's Hospital of Eastern Ontario

Participating Centres:

Contact Full Name	Contact Centre
Dr Mark Tarnopolsky	McMaster Children's Hospital
Dr Jodi Warman	Ottawa Hospital Research Institute
Dr. Sarah Sawyer	Health Sciences North/Horizon Santé-Nord
Dr. Kym Boycott	Children's Hospital of Eastern Ontario
Dr. Marjan Nezarati	North York General Hospital
Dr. Roberto Mendoza-Londono	The Hospital of Sick Children - Division of Clinical and Metabolic Genetics
Dr Lauren Badalato	Kingston General Hospital - Division of Medical Genetics
Dr. Tugce Balci	London Health Sciences Centre, Victoria Hospital
Dr Karen Chong	Mount Sinai Hospital - The Prenatal Diagnosis and Medical Genetics Program

CTO Project ID: 1577

Study Title: Care4Rare Canada: Harnessing multi-omics to deliver innovative diagnostic care for rare genetic diseases in Canada (C4R-SOLVE)

Sponsor Study ID: N/A

Application Type: Observational Provincial Continuing Review Form

Review Type: Delegated

Date Approval Issued: 05/Jul/2019

Approval Expiry Date: 15/Jul/2020

Dear Provincial Applicant,

The Children's Hospital of Eastern Ontario Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Children's Hospital of Eastern Ontario Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Children's Hospital of Eastern Ontario Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Dr. Richard Carpentier

Chair, CHEO Research Ethics Board

CHEO

Date: 16 June 2020

To: Dr. Kym Boycott, Children's Hospital of Eastern Ontario

CTO Project ID: 1577

Study Title: Care4Rare Canada: Harnessing multi-omics to deliver innovative diagnostic care for rare genetic diseases in Canada (C4R-SOLVE)

Application Type: Observational Centre Continuing Review Form

Review Type: Delegated

Date Approved:16/Jun/2020

Study Approval Expiry Date: 15/Jul/2021

Dear Principal Investigator,

The Children's Hospital of Eastern Ontario Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Children's Hospital of Eastern Ontario Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Children's Hospital of Eastern Ontario Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Dr. Cecile Bensimon

Chair, CHEO Research Ethics Board

Date: 18 June 2021

To: Dr. Kym Boycott, Children's Hospital of Eastern Ontario

CTO Project ID: 1577

Study Title: Care4Rare Canada: Harnessing multi-omics to deliver innovative diagnostic care for rare genetic diseases in Canada (C4R-SOLVE)

Sponsor Study ID: N/A

Application Type: Observational Centre Continuing Review Form

Review Type: Delegated

Date Approved:18/Jun/2021

Study Approval Expiry Date: 15/Jul/2022

Dear Principal Investigator,

The Children's Hospital of Eastern Ontario Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

Current Approved Protocol:

Document Type	Document Name	Document Date	Document Version
Protocol	vF_C4RSOLVE_Minimal Risk Protocol_Version 2_April 21 2020_clean_FINAL	21/Apr/2020	2

REB members involved in the research project do not participate in the review, discussion or decision.

Children's Hospital of Eastern Ontario Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Children's Hospital of Eastern Ontario Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.

Sincerely,

Dr. Cecile Bensimon

Chair, CHEO Research Ethics Board

Date: 28 June 2022

To: Dr. Kym Boycott, Children's Hospital of Eastern Ontario

CTO Project ID: 1577

Study Title: Care4Rare Canada: Harnessing multi-omics to deliver innovative diagnostic care for rare genetic diseases in Canada (C4R-SOLVE)

Sponsor Study ID: N/A

Application Type: Observational Centre Continuing Review Form

Review Type: Delegated

Date Approval Issued:28/Jun/2022

Study Approval Expiry Date: 15/Jul/2023

Dear Principal Investigator,

The Children's Hospital of Eastern Ontario Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

Current Approved Protocol:

Document Type	Document Name	Document Date	Document Version
Protocol	vF_C4RSOLVE_Minimal Risk Protocol_Version 3_May 16 2022_Clean.	16/May/2022	3

REB members involved in the research project do not participate in the review, discussion or decision.

Children's Hospital of Eastern Ontario Research Ethics Board operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. Children's Hospital of Eastern Ontario Research Ethics Board is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP).

Please do not hesitate to contact us if you have any questions.