



Review

Utility of whole-exome sequencing for those near the end of the diagnostic odyssey: time to address gaps in care

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An accurate diagnosis is an integral component of patient care for children with rare genetic disease. Recent advances in sequencing, in particular whole-exome sequencing (WES), are identifying the genetic basis of disease for 25–40% of patients. The diagnostic rate is probably influenced by when in the diagnostic process WES is used. The Finding Of Rare Disease GENes (FORGE) Canada project was a nation-wide effort to identify mutations for childhood-onset disorders using WES. Most children enrolled in the FORGE project were toward the end of the diagnostic odyssey. The two primary outcomes of FORGE were novel gene discovery and the identification of mutations in genes known to cause disease. In the latter instance, WES identified mutations in known disease genes for 105 of 362 families studied (29%), thereby informing the impact of WES in the setting of the diagnostic odyssey. Our analysis of this dataset showed that these known disease genes were not identified prior to WES enrollment for two key reasons: genetic heterogeneity associated with a clinical diagnosis and atypical presentation of known, clinically recognized diseases. What is becoming increasingly clear is that WES will be paradigm altering for patients and families with rare genetic diseases.

Conflict of interest

None declared.

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Rare genetic diseases affect at least 1 in 50 individuals (1, <http://orphanet.net>). The total number of these diseases is estimated to be 6000–7000 (2, <http://www.omim.org>) and while each is individually rare, together these genetic conditions contribute significantly to morbidity, mortality, and healthcare costs. Estimates suggest that up to 50% of patients with a rare genetic disease never receive a diagnosis (3). Patients without a diagnosis often embark on a diagnostic odyssey that includes multiple specialist consultations, imaging studies [e.g. magnetic resonance imaging (MRI), skeletal survey], invasive investigations (e.g. skin and muscle biopsy), and other laboratory and genetic tests. The diagnostic odyssey is by definition a slow, costly venture, and for many is ultimately disappointing when a diagnosis is not reached (4). A survey of patients with rare diseases demonstrated that for 25% of participants the time to diagnosis was extensive, ranging from 5 to 30 years, and during that time 40% received an incorrect diagnosis (5). As many as a third of these patients with rare diseases incurred inappropriate care for their eventual diagnosis, including those who underwent unnecessary surgical procedures (5). Providing a molecularly confirmed diagnosis, in a timely manner, for children and adults with rare diseases shortens the diagnostic odyssey, improves disease management, including targeted treatments and surveillance for later-onset comorbidities for a subset of patients, and informs genetic counseling with respect to recurrence risks and prenatal diagnosis options for families.

Traditional diagnostic assessment in clinical genetics

Currently, an individual with a suspected rare genetic disease may have genetic testing performed on a gene-by-gene basis or by a gene-panel (simultaneous analysis of two or more candidate genes) approach. Single gene testing is traditionally performed by Sanger sequencing; the gold standard that has been used for molecular diagnosis for two decades. This is often performed sequentially; if no mutations are identified in a proposed candidate gene, additional candidate gene(s) may subsequently be selected for testing by the physician. The sequencing of a single gene may take several weeks to months to complete and costs anywhere from one to a few thousand US dollars in a commercial diagnostic laboratory. Genetic testing performed using a gene-panel approach is an advantageous strategy when more than one gene can cause the clinical presentation. Though the data is limited, the rate of molecular diagnosis for patients with rare diseases using traditional, comprehensive clinical genetics evaluation and targeted genetic testing (including karyotypes, chromosomal

microarray analysis, single gene tests, gene-panel tests, and specialized genetic biochemical laboratory tests) was 46% in one recent study that analyzed a cohort of 500 unselected consecutive patients referred to the genetics department at an American tertiary medical center (3). Of those diagnosed, 72% were diagnosed on their first visit, implying that subsequent genetic testing had diminishing returns using these traditional approaches and that next-generation sequencing (NGS) (including WES and WGS) may be economically beneficial following the first clinical visit.

Impact of next-generation sequencing on rare disease diagnosis

The advent of NGS technologies has created a paradigm shift in our approach to both the discovery of new disease genes and the timely diagnosis of genetic disease. Whole-genome sequencing (WGS) refers to the sequencing of nearly the entire human genome simultaneously. WGS data can typically be generated in less than a month for approximately \$2000 on the latest platforms. However, the assembly of the genome is computationally laborious and most of the non-coding sequence is difficult to interpret. Whole-exome sequencing (WES) can be completed in a similar timeframe and interrogates approximately 95% of the coding region of the genome, comprising ~20,000 genes. Diseases that were previously intractable to gene identification given their rarity, clinical and genetic heterogeneity, and paucity of multiplex families have shown tremendous discovery success using WES. As a result, the pace of novel disease gene discoveries has increased dramatically over the last few years (6). However, it quickly became apparent that WES was also very useful for identifying mutations in known genes in these research patients (7). In one of the first studies to examine the success rate of WES in a clinical setting, a probable genetic diagnosis was established in 6 of the 12 (50%) patients recruited with a broad range of phenotypic presentations (8). A large-scale research study of pediatric-onset neurodevelopmental disease identified a known disease-causing gene in 8% of patients who had the most relevant genes excluded prior to WES (9). A retrospective review of 28 families in the FORGE Canada research study with cerebellar ataxia showed a diagnostic success rate of 46% using WES (10), and a review of 9 families in FORGE with severe and early-onset epilepsy demonstrated a diagnosis in 7, possibly 8, families (11). Similarly, there is literature to support the role of WES in patients with moderate-severe intellectual disability or neurodevelopmental disorders, with success rates ranging from 16 to 45% (12–15). Soden et al. undertook

rapid WGS for patients with high-acuity illness, defined as symptomatic at or shortly after birth requiring admittance to the neonatal or pediatric intensive care units (15). An impressive 73% (11 of 15) of families with acutely ill children were diagnosed using this approach, which may be a reflection of both the likelihood of a monogenic presentation with such an early and severe presentation as well as decreased prior testing. The success of WES and WGS in providing diagnoses to patients in a research setting is enabling the translation of this technology to the clinic, with the expectation of increasing the diagnostic yield over traditional genetic testing approaches and thereby providing answers to families who had previously gone without a molecular diagnosis.

Over the past 2–3 years, several large studies have demonstrated the diagnostic utility of WES for a large number of patients analyzed in clinical diagnostic laboratories (16–18). The first of these was reported by the Baylor College of Medical Genetics Laboratory which demonstrated a 25% molecular diagnostic rate for 250 probands with varying indications (although 80% had a neurologic phenotype) (16). Interestingly, when they increased the number of patients to 2000, the diagnostic rate remained 25% (17). The Lee et al study of 814 patients who underwent WES in a clinical diagnostic laboratory reported a similar diagnostic rate of 22–31% (18). This range probably reflects a number of factors including analysis strategy [affected singleton versus trio (proband and parents)], family history, type of disorder, age at presentation, and acuity of the clinical presentation, which speaks to the likelihood the disorder is caused by a single gene. Lastly, where in the diagnostic evaluation the WES was utilized also likely plays a role in the success rate.

In the setting of limited resources, the question of singleton vs trio sequencing is an important one. The analysis of the largest reported cohort of 2000 patients was primarily as singleton WES (17). Approximately 400 patients in the cohort of 814 underwent trio analysis and this was associated with the higher diagnostic rate of 31% (18). The higher diagnostic rate for trios is perhaps not surprising given that *de novo* mutations are only readily detected by this approach and appear to be enriched in neurodevelopmental cohorts, a common indication for referral to these clinical laboratories. However, the reported diagnostic rates of 22–25% for singleton analysis speaks to the considerable diagnostic utility of WES even in this sequencing paradigm (16–18). In addition, other approaches may be just as successful. Analysis of sibling pairs and one parent for autosomal recessive conditions thought to be secondary to compound heterozygous mutations will enable rapid analysis which includes phasing of the variants to determine if the variants are in *cis* or *trans*. For consanguineous families, analysis of three family members is probably not necessary, although the addition of a second affected sibling is useful for decreasing shared variants when the inbreeding coefficient is high. Considerations regarding study design must also include cost and the urgency of the diagnosis, which may be related to possible treatment or management implications that might be missed or delayed if

secondary analysis of parental data is required to reach a conclusive answer.

There is also now evidence that supports the diagnostic advantage of WES over single-gene and panel-based approaches for several referral indications (19). In the study by Neveling et al. the diagnostic rate of patients who received traditional Sanger-based testing based on candidate genes in 2011 was compared with patients who instead underwent WES the following year with a filtered analysis of large gene panels based on the indication for referral. The diagnostic rates were higher for patients after WES for clinical presentations such as blindness (25% vs 52%), deafness (10% vs 44%), movement disorders (5% vs 20%) and mitochondrial disorders (11% vs 15%) (19). This success of WES over traditional approaches may reflect extensive genetic heterogeneity for some of these clinical presentations and thus the number of genes assayed using the respective approaches. For disease presentations, where a limited number of genes are known to cause the disorder, targeted panel NGS approaches may provide a more comprehensive assessment.

The range of diagnostic success is also influenced by when in the diagnostic evaluation WES occurs. For the large studies reported to date (16–18), such detailed information is not available; however, it is very likely that at a minimum first line testing (karyotype, chromosomal microarray, some single gene testing as indicated) had been completed for these patients prior to WES. Whereas Shashi et al. have hypothesized that use of WES and WGS may be economically beneficial when used early in the diagnostic evaluation of a patient (3), it has not yet been determined how the diagnostic rate will be influenced by the timing at which WES is performed. The recent completion of Canada's nation-wide FORGE (*Finding Of Rare disease Genes*) project allowed us to retrospectively investigate the diagnostic utility of WES after standard-of-care genetic testing in a cohort of children from Canada with unexplained rare diseases.

Evaluation of the utility of WES for patients near the end of the diagnostic odyssey; FORGE Canada Consortium

We investigated the diagnostic yield of WES for a cohort of patients who had already received standard-of-care genetics evaluation and diagnostic testing in Canada and were suspected to have a genetic disorder by the referring physician. Initial evaluation included single gene testing, occasional small gene panels, and chromosome microarray. In addition, we examined why these patients were not diagnosed after standard-of-care assessment. To do this we performed a retrospective study of the patients enrolled in the FORGE Canada project who received a molecular diagnosis in a known disease gene. FORGE was a 2-year, pan-Canadian initiative to rapidly identify novel genes responsible for a wide spectrum of pediatric-onset disorders present in the Canadian population (20). The project ascertained >500 children from 362 families with rare diseases from across Canada and utilized WES to provide a definitive molecular diagnosis

Whole-exome sequencing for rare disease diagnosis

WES data generation and analysis

Patients underwent WES at one of three centralized Genome Canada Science and Technology Innovation Centres (STICs): McGill University and Genome Quebec Innovation Centre (Montreal), The Centre for Applied Genomics (Toronto), or The Genome Sciences Centre (Vancouver), with over half of the samples run at the McGill site. Exome target enrichment was performed with the Agilent SureSelect 50 Mb (V3) All Exon Kit; samples were sequenced on the Illumina HiSeq 2000 platform, multiplexing three samples per lane. After removal of duplicate reads, the mean coverage of coding sequence regions ranged from $\times 70$ to $\times 200$. Alignment and variant annotation were performed by the FORGE informatics team at each STIC, using comparable analytical pipelines with publicly available tools and custom scripts as described previously (20). Annotations used included variant functional effect, gene OMIM associations, and metrics for missense single-nucleotide-variant pathogenicity (e.g. SIFT (<http://sift.jcvi.org>), Poly-Phen (<http://genetics.bwh.harvard.edu/pph2>), Mutation Taster (<http://www.mutationtaster.org/>)). Filtering of variants was performed with our internal exome database to identify rare variants ($<1\%$). Potentially deleterious rare variants in known disease genes associated with a phenotype similar to the patient were communicated to the referring physician for genotype-phenotype evaluation. A definitive outcome for a patient occurred when the variant under consideration was in a gene previously known to cause disease (known gene) and the referring clinician provided feedback that this gene explained the affected individual's phenotype, rendering the patient 'solved'. Genetic diagnoses were confirmed in clinically certified molecular diagnostic laboratories before the results were used to change patient management. We then reviewed why WES successfully identified these mutations in known genes for patients who had already had a standard-of-care diagnostic assessment.

Diagnostic rate; mutations in known disease genes

Of 362 families submitted to FORGE for WES, we identified disease-causing mutations in known genes for 105 families (29%). The diseases studied were enriched for neurodevelopmental phenotypes and dysmorphic syndromes (Table 1). The success rate ranged from 12% (immunological disorders) to 44% (ciliopathies). When we considered the sequencing and data analysis strategy for each family who had undergone WES, the diagnostic rate in known disease genes ranged from 23% to 34%; 23% of 207 singletons, 32% of 72 sibling pairs, and 34% of 76 families who were either consanguineous or from isolated populations.

Contributors to the diagnostic odyssey

The most common contributing factor for patients not receiving a molecular diagnosis prior to WES was that their disorder was associated with significant genetic heterogeneity (Table 2), which we defined as a disorder

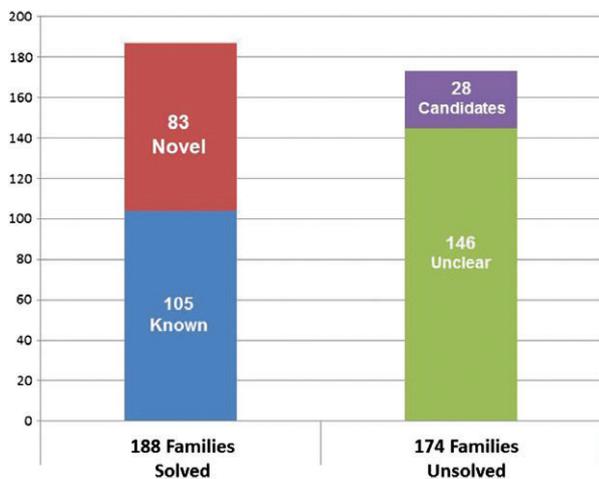


Fig. 1. Outcomes of the 362 families studied using whole-exome sequencing (WES) as part of the Finding Of Rare Disease GENes (FORGE) Canada research study. Overall, 188 of the 362 (51.9%) families received a definitive molecular diagnosis, either in a gene previously known to cause a similar disease (known) or in a gene that had not been previously associated with the family's disease (novel). Of the 174 (48.1%) of families that remain unsolved, 28 have single-surviving candidates for which we are gathering additional evidence to support pathogenicity.

in either a known disease-causing gene (105 families) or in a gene that represented a novel discovery (83 families) (Fig. 1); the FORGE experience with novel gene discovery has been reviewed elsewhere (20). The study was approved by Research Ethics Boards from all participating sites and informed consent was obtained from all families. Our consent process informed families that we would not systematically look at WES data for incidental findings, but that if we observed a convincing mutation in a gene that was deemed to be medically actionable in childhood we would return the result to the family.

Patient selection

Patients enrolled in the FORGE project were ascertained by physicians, mainly Medical Geneticists, from 21 participating academic centers across the country. In almost all patients, appropriate clinical and molecular investigations had been completed prior to enrollment to exclude known causes of the patient's clinical presentation (standard-of-care for the respective province, including chromosomal microarray and targeted gene testing when available). Access to clinical testing of specific single genes or panels of genes is dependent on the tests available within a province and varies considerably (e.g. few to 200 tests). There is also marked variability across Canada as to the resources available to fund out-of-country genetic testing. However, the vast majority of patients had already experienced a protracted diagnostic odyssey (several years to more than two decades) prior to entering FORGE. Patients were accepted either with a recognized clinical diagnosis (e.g. Dubowitz syndrome, etc.) or with a description of their clinical presentation if the diagnosis was unknown (e.g. microcephaly, short stature, sparse hair, intellectual disability).

Table 1. Broad phenotypes and associated diagnostic rates in known disease genes of the families studied using WES in FORGE

Broad phenotype	Total families (N = 362)	Families with known genes (N = 105)	Diagnostic rate (%)
Neurodevelopmental	98	31	31.6
Dysmorphic syndromes	80	18	22.5
Ocular	40	11	27.5
Metabolic	31	12	38.7
Neuromuscular	30	7	23.3
Ciliopathy	27	12	44.4
Congenital malformation syndromes	19	4	21.1
Immunological	17	2	11.8
Other (isolated cardiac, endocrinology, skeletal dysplasia, connective tissue disorders, mental illness, lung disorder)	20	8	40.0

FORGE, Finding Of Rare Disease GEnes; WES, whole-exome sequencing.

Table 2. Explanations for families who received a molecular diagnosis in FORGE using WES who had not been diagnosed during standard-of-care clinical genetics evaluation

Explanation	Number of families (N = 105)
Genetic heterogeneity	49
Atypical presentation	26
Missed by another method	9
Gene identified while in the pipeline	9
Extremely rare condition	5
Conflation	4
Limited access to testing	3

FORGE, Finding Of Rare Disease GEnes; WES, whole-exome sequencing.

with more than three associated genes. It is a fiscal reality of our provincially funded healthcare systems that extensive gene-by-gene interrogation for a genetically heterogeneous condition is prohibitively expensive and thus generally not available. As a result, only the most common genes for a particular rare disease presentation tended to be evaluated prior to study enrollment. For example, several patients with a clinical diagnosis of Joubert syndrome were included in this study. There are currently over 20 genes known to be associated with Joubert syndrome (21). Prior to inclusion in this study, each patient had several of the more common genes associated with Joubert syndrome excluded, and for each of these patients a different, but previously known, Joubert syndrome-associated gene was identified by WES. Given the vast number of clinical presentations associated with significant genetic heterogeneity, WES has remarkable advantages by providing a comprehensive approach.

The next most common factor contributing to the diagnostic odyssey resolved by WES was that of phenotypic expansion (Table 2). There were 26 patients with an atypical presentation of a known disorder, such that the clinical diagnosis was unrecognizable to the clinician to inform genetic testing of the correct (as identified by WES) gene. This speaks to the challenges inherent in the diagnosis of rare diseases. The progressive nature of many rare diseases means that the clinician will often

evaluate a patient early in the course of disease when ‘tell-tale’ features may not yet be present (e.g. neonate in the NICU), making an early and accurate diagnosis difficult. The large number of atypical diagnoses also highlights how limited our understanding is of the phenotypic spectrum for many rare diseases. For example, mandibulofacial dysostosis and microcephaly (MFD) syndrome was identified independently three times in the FORGE study. The initial cohort of patients with the classic presentation led to the discovery of the *EFTUD2* gene as causative (22) and this was followed by the identification of patients on the mild and severe ends of this emerging syndrome. This suggests that we will not truly appreciate the variability of any rare disease until unbiased approaches, such as WES, have enabled the identification of the complete clinical spectrum.

In nine families a disease-causing mutation was identified by WES that had been missed by another method of testing (Table 2). Two patients had undergone Sanger sequencing of the candidate gene in clinically accredited laboratories and the mutations had not been reported. In one patient the quality of the sequencing was poor, thus the mutation was not reported as it was presumed to be a sequencing error. In the other patient, the clinical lab re-designed the test after communication regarding the WES result, and was subsequently able to identify the mutation. Most laboratories quote an error rate of approximately 1%, and our cases likely reflect this value. In two patients only common mutations in the gene were assessed by the clinical laboratory and were negative; the mutations identified by WES were not included in this targeted clinical test. Four patients had prior testing performed in a research laboratory and the results were conveyed as unrevealing. In one family the gene had been ruled out in some members of the family with a similar presentation but not in the patient who underwent WES. The affected members in this family were reasonably assumed to have the same underlying genetic basis for their pulmonary fibrosis; however, the findings by WES were in keeping with genetic heterogeneity in a large family with mutations in more than one gene causing the same phenotypic presentation. For another nine patients (Table 2) the causative gene was unknown at the outset of FORGE but was published while the project

was underway, thus there was no clinical testing available prior to inclusion in this study.

Five families were diagnosed with conditions that are exceedingly rare (<1/1,000,000) (Table 2), and the literature relatively sparse, such that most subspecialist physicians would not be expected to encounter a patient with that particular diagnosis in their career. For example, methylmalonate semialdehyde deficiency had only been molecularly diagnosed in two published individuals prior to our identification of mutations in the *ALDH6A1* gene in a patient in this study (23).

In four individuals without a clinical diagnosis prior to WES, the patients were thought to have a novel syndrome due to a mutation in a single gene; however, they were in fact found to have a conflated clinical presentation comprising two or more known rare diseases (Table 2). This scenario has been reported previously by Yang et al. in which 4.6% (23 of 504) of those who received a molecular diagnosis had blended clinical presentations resulting from two single gene defects (17). Data analysis is challenging for these types of patients as the phenotype has to be dissected and attributed to mutations in different genes. For example, two siblings from consanguineous parents presented with severe congenital neutropenia and oculocutaneous albinism, and a novel disorder was suspected. WES identified homozygous mutations in *G6PC3*, which is known to cause severe congenital neutropenia type 4, in both siblings, and one sibling had homozygous mutations in *SLC45A2* causing oculocutaneous albinism type 4 (OCA4). The second sibling had one mutation in *SLC45A2*, and manifested some features of OCA4 (24). We expect that the subset of undiagnosed patients who are revealed by WES to have two distinct rare diseases will only rise as analysis of this data continues to improve over time.

Finally, for three patients (Table 2) limited availability of clinical testing anywhere in the world for ultra-rare conditions at the time of the study meant that WES through FORGE was the best option for these clinicians to identify a molecular diagnosis; with the added potential for novel gene discovery if no pathogenic variant was found in the candidate gene of interest.

Impact on patient management

Rare diseases represent a valuable model for the implementation of personalized health. In addition to a clear diagnosis informing prognosis, recurrence risks, and providing prenatal options, there were six of the 105 families (26%) in this study whose medical management changed dramatically subsequent to a diagnosis through WES; three had their therapy adjusted and three had specific therapy initiated. For example, a child that was originally thought to have infantile myofibromatosis (IM) based on clinical suspicion and pathological findings was undergoing low-dose chemotherapy to reduce the size of the myofibromas. WES was performed to identify the gene for IM, but instead identified the common causative mutation for fibrodysplasia ossificans progressiva (FOP) in *ACVRI* (25). Patients with FOP develop heterotopic ossifications secondary to mild trauma, such

as intramuscular injections, contributing to poor outcomes. Once the diagnosis was clarified all interventions other than those that were life-saving were stopped. In another family, two sisters with epilepsy and intellectual disability were diagnosed in their early 30s with autosomal recessive cerebral folate transport deficiency. Treatment for this rare disease with folinic acid has shown very encouraging outcomes if started early in the disease (26). Although the white matter damage was already extensive, folinic acid therapy initiated after diagnosis appears to be reducing seizure frequency and improving the quality of life for these patients. By providing a timely diagnosis we can halt the diagnostic odyssey and for a small number of conditions, prevent long term end-organ damage when treatment options are available, the benefits of which cannot be overstated.

Clinical translation

Success rates for WES to provide a molecular diagnosis for patients have ranged from 22% to 46% in several large studies now summarizing outcomes for almost 3000 patients (15–18). It is clear that this is a diagnostically useful test. The wide-spread clinical translation of genome-wide NGS approaches to enable personalized medicine for rare diseases requires guidance regarding clinical indications for testing using this approach; when it should occur in the diagnostic evaluation, how to approach incidental findings, and how these complex results should be reported to primary care clinicians and patients/families. Position statements and best practice guidelines are now available for many jurisdictions to address these issues (27–30). Both the American College of Medical Genetics and Genomics (ACMG) (27; <https://www.acmg.net>) and the Canadian College of Medical Geneticists (CCMG) (28; <http://www.ccmg-ccgm.org>) recommend the consideration of clinical genome-wide sequencing for affected individuals in which either phenotype or family history raises suspicion of a monogenic rare disease. The ACMG and CCMG both recognize that this technology may be a more practical approach than standard-of-care genetic testing in certain scenarios, and both advocate for its use when it might be more efficient (with regards to both time and cost) than alternative approaches. The ACMG further recommends consideration of the use of this technology for the investigation of a fetus with a probably monogenic disorder and specific genetic tests have failed to arrive at a diagnosis (27).

Owing to the nature of genome-wide sequencing in which genes unrelated to the primary indication of a patient are sequenced, careful consideration must be given to incidental findings. The ACMG (29), the European Society of Human Genetics (ESHG) (30), and most recently the CCMG (28), have all provided perspectives on how such findings might be handled in their jurisdictions. The ACMG has generated a list of genes that it recommends should be screened for deleterious mutations in clinical exome or genome sequencing studies, irrespective of the defined clinical phenotype of the proband (29). Adults would have the right to decline

this information, but mutations that are medically actionable in childhood would be reported back to families. In contrast, the European guidelines present these as unsolicited findings, and do not advocate actively searching the data for deleterious variants in genes that are not associated with the primary reason for genetic testing (30). Similarly, the CCMG does not endorse the intentional clinical analysis of disease genes unrelated to the primary indication (28), but if one is inadvertently identified recommends that competent adults be given the option prior to testing to receive such findings. In children, incidental results that reveal a risk for a highly penetrant condition that is medically actionable during childhood should be reported back (28).

The optimal timing for WES in the diagnostic process is still unclear, whether it should be at the first appointment if clinically indicated (e.g. disease presentation associated with significant genetic heterogeneity), second appointment after initial tests normal (e.g. first tier of genes ruled out), or towards the end of the diagnostic odyssey (e.g. extensive, possibly invasive tests have occurred). The reported literature shows diagnostic utility in all of these scenarios and the optimal timing is probably dependent on the particular patient and clinical circumstances. The data from FORGE Canada, which was focused on diagnostic outcomes from patients that were towards the end of the diagnostic odyssey, demonstrates yields as high as 29% for this patient group. These data also show that patients with genetically heterogeneous diseases, those with unknown disorders which may be due to an atypical presentation, and those with ultra-rare diseases, will benefit immensely from relatively non-biased clinical testing methods. The irony is that these patients with atypical and ultra-rare diseases are not diagnosed as such until these technologies are used and mutations are identified, which underscores our current limited understanding of many phenotypic presentations of rare disease. Thus, improved phenotyping, reporting of atypical patients, and sharing of rare variants through databases is essential to improve the diagnosis and management going forward. Indeed, both our data and that of Soden et al. show that a significant proportion, 6% and 8% respectively, of patients will have therapies adjusted or initiated based on molecular results, highlighting the importance of providing every child with a diagnosis such that treatable conditions and windows of opportunity are not missed (15).

It is very likely that in the years to come, clinical genomic sequencing (a process used to determine the sequence of most, if not all, clinically significant genes and its associated interpretation, including bioinformatic analysis and clinical genotype–phenotype correlation) will be performed on an ever increasing number of patients with undiagnosed rare diseases. From the data accrued to date, we know that approximately 70% of those who undergo WES will remain without a molecular diagnosis in a known disease gene. We strongly believe that as clinical translation strategies are implemented in various jurisdictions, it is of utmost importance that the approach includes research opportunities for patients and families to consent to having coded

genome-wide and phenotypic data deposited and stored in national/international databases to assist in interpretation and solving the unsolved patients.

Solving the unsolved

There are a variety of reasons why up to 70% of patients might be unsolved after WES, including incomplete coverage of the exome and genetic mutations elusive to the technology itself [e.g. non-coding variants, trinucleotide repeats, copy number variants, chromosome rearrangements, oligogenic inheritance, etc.; summarized in (31)]. From our experience, however, there are a proportion of these cases in which the disease-causing variant is in fact within the WES data but for a variety of reasons (not enough information on the variant, variant acting on gene function through novel molecular mechanism, unclear inheritance, etc.) there is insufficient evidence to support a definitive diagnosis. For some of these cases, performing WES on additional family members, including affected relatives or unaffected parents, may be useful. Analysis may also benefit from the use of large-scale population variant control databases (such as ExAC at the Broad Institute; <http://exac.broadinstitute.org>); greatly reducing the number of rare variants identified and requiring interpretation.

In addition, there may be value in pursuing additional genomic technologies for those families that remain elusive to diagnosis. In a recent study by Gilissen et al., 50 patients with severe intellectual disability underwent trio WGS following uninformative WES and microarray analysis (32). To their surprise, 21 of the 50 (42%) received diagnoses in known ID genes through analysis for *de novo* and recessive coding mutations [both single nucleotide variants (SNVs) and copy number variants (CNVs)]. It is unclear if the SNV coding mutations that were identified (13 diagnoses) had been captured or missed in the previously uninformative WES data (32). It is well recognized that WGS offers a more complete coverage of the protein-coding portion of the genome, but in theory one can expect the majority of these variants will be identified as technology to both capture and sequence the exome improves. Of interest, a total of eight *de novo* structural variants (five deletions, a tandem duplication, an inter-chromosomal duplication and one complex inversion/duplication/deletion event), previously undetected by diagnostic microarray analysis, were identified, underlining that even as WES improves a proportion of cases will benefit from WGS.

In addition to diagnosing families with pathogenic mutations in known disease genes, the FORGE Canada project successfully demonstrated that a significant proportion of unexplained patients have pathogenic mutations in novel disease genes. An additional 23% of families studied in FORGE were shown to have pathogenic mutations in novel genes validated by cohort analysis and/or functional studies (reviewed in 20). Despite extensive efforts, this means that 48%, or 173 families, were not diagnosed by WES in FORGE, highlighting a need for both reanalysis of data for variants in recently identified disease-causing genes and

large-scale data sharing to solve more of these intractable families.

In 28 families studied in the FORGE project, single-candidate genes were identified but little is known about the function of the gene, rendering cellular characterization and functional studies challenging. For such cases, finding just a single additional unrelated case with a deleterious-appearing variant in the same gene and overlapping phenotype may provide sufficient evidence to causally implicate the gene or support further functional investigation. However, significant collaborative infrastructure, both within Canada and internationally, to enable sharing of phenotypic and genotypic datasets from patients with undiagnosed rare diseases will be needed to maximize the use of the data generated from such genome-wide approaches, which up until now have been confined to the institutions that generated and/or analyzed the data. International efforts have made available a standard set of computer readable terms to describe the phenotypic features of a patient with a rare disease [e.g. Human Phenotype Ontology, HPO (33)]. Clinical interface software, such as Phenotips (34; <https://phenotips.org>), have been developed to facilitate deep phenotyping for a patient using HPO terms in less than five minutes and are now being widely used in rare disease research projects such as the successor to FORGE, Care4Rare (<http://care4rare.ca/>). The associated web-based match-making tool, PhenomeCentral (<http://phenomecentral.org>), shows contributors information about other phenotypically similar patients with deleterious-appearing variants in the same candidate gene, and allows these researchers to contact each other when a match is made. Other similar ‘matchmaking’ databases have been developed, such as GeneMatcher (35, <http://www.genematcher.com>), which connects investigators based on their interest in the same gene, and DECIPHER (36; <http://www.decipher.sanger.ac.uk>), which allows investigators to search coded phenotypic and genotypic data to identify possible matches. Clinicians and scientists involved in these data sharing initiatives recognize the value in ever larger datasets to enable the identification of second families and thus MatchmakerExchange (<http://matchmakerexchange.org>) was launched this year to provide a more systematic approach to large-scale data sharing for rare disease gene discovery through a federated network connecting databases through a common application programming interface (API). Thus far, the API connects PhenomeCentral, GeneMatcher and DECIPHER and thereby avoids the need for the same data to be entered into multiple databases. Such international sharing efforts will advance our knowledge of disease genes and their associated phenotypic spectrum, ultimately improving diagnostic accuracy.

Future perspectives

In the next decade we will witness a paradigm shift in the way we care for patients with rare genetic diseases, addressing a significant gap in the management of individuals with rare diseases. The diagnostic journey

for patients will include clinical genomic sequencing, which in the not too distant future will be WGS as costs decrease with new platforms. The accessibility of WGS-based diagnostics for patients will be central to understanding the complete compendium of human genetic pathology. Strategies and infrastructure should be put in place to facilitate discovery in clinical settings, and to further mine large phenotypic and genomic datasets for disease mechanisms. As we understand the etiology of more rare diseases, it is probable that we will increasingly implicate known genes and that the approach to the completion of the complete Mendelian dataset will be asymptotic. There will be a subset of rare diseases that are due to non-coding mutations in regulatory regions that may be more readily identified with the imminent use of WGS and RNA sequencing, and to implicate and understand this type of variation will require large-scale data sharing at an impressive level. Ultimately, such datasets will be instrumental in identifying modifiers of disease, providing insight into phenotypic variability, prognosis, and for a subset of diseases, identifying drug targets. Finally, NGS technologies are providing significant opportunities to implement personalized health strategies including prevention or early detection of disease, improved health maintenance, and development of tailored therapy for patients with rare genetic diseases.

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