Leading Edge

**Commentary**

**A Diagnosis for All Rare Genetic Diseases: The Horizon and the Next Frontiers**

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The introduction of exome sequencing in the clinic has sparked tremendous optimism for the future of rare disease diagnosis and there is exciting opportunity to further leverage these advances. To provide diagnostic clarity to all of these patients, however, there is a critical need for the field to develop and implement strategies to understand the mechanisms underlying all rare diseases and translate these to clinical care.

# **INTRODUCTION**

Hundreds of millions of lives are affected by an estimated 10,000 unique genetically-determined diseases. Individually each disease affects a relatively small number of people, leading to their common label as rare genetic diseases (RDs); however, collectively they represent an important public health opportunity. The vast majority of these patients experience long and grueling diagnostic odysseys and lack treatment. In 2011, recognition of both the longstanding inequity in care and the great opportunity for tractability due to technical developments led to the founding of the International Rare Diseases Research Consortium (IRDiRC), which aims to advance global cooperation amongst numerous stakeholders (Dawkins et al., 2018). The vision of IRDiRC is to enable all people living with a RD to receive an accurate diagnosis, care, and available therapy within 1 year of coming to medical attention (Austin et al., 2018). Achieving an accurate and timely molecular diagnosis will largely depend on progress in the discovery of the genes and genetic mechanisms associated with RDs. While the exact number of RDs is debated (Hartley et al., 2018), it is estimated that thousands of RD genes and disease mechanisms remain undiscovered. Over the past eight years, exome sequencing (ES) in both research and clinical settings has been a powerful tool for discovering new disease genes for RDs that were intractable to previous approaches. Most advances have been for highly recognizable clinical presentations associated with early age of onset and significant morbidity and mortality, and caused by highly penetrant (typically protein-coding) variants (Boycott et al., 2017). The diagnostic utility of ES has translated beautifully into the clinic, with a diagnostic yield in the range of 25-30% amongst large and heterogeneous RD cohorts (Clark et al., 2018). Here, we discuss the continued importance of ES in both the clinic and the research environment, the next wave of technologies on the horizon, and the next frontiers for RD discovery, moving towards the ultimate goal of diagnostic clarity for each and every family affected by a RD.

# **ACHIEVING A DIAGNOSIS FOR ALL**

## **The ‘here and now’: The continued role of exome sequencing**

The application of ES with RD patients represents a remarkable achievement in diagnostics with a diagnostic yield far higher than other genetic tests (Clark et al., 2018). Nonetheless, in >70% of patients in whom there was a high degree of pre-test suspicion for a monogenic RD, ES provides no molecular diagnosis. For the benefit of RD patients, it is imperative that we drive this diagnostic yield to as close to 100% as possible. While the theoretical yield of ES is unknown, in patient populations with specific presentations and a high degree of certainty that there is a genetic cause to the RD, the yield of the coding genome is likely well over 50% (Boycott et. al., 2014; Shamseldin et al., 2017). Indeed, there remains substantial diagnostic potential in existing ES data. For starters, evidence is emerging that re-analysis of negative clinical ES data just one to three years later increases diagnostic yield by 10% (Wenger et al., 2017). This is because at initial analysis there was insufficient evidence for candidate variant or gene causality, but this evidence emerges upon reanalysis in light of the annual curation of >10,000 disease variants [ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>)] and 250 novel disease-gene associations [OMIM (<https://www.omim.org/>) and Orphanet (<https://www.orpha.net/consor/cgi-bin/index.php>)]. Even higher diagnostic yields can be achieved through reanalysis in collaboration with the referring physician, with estimates as high as 12% (Salmon et al., 2018). Collaboration with research laboratories can provide additional increases (Eldomery et al., 2017), boosted by the application of novel computational tools, sequencing of additional family members, and gene discovery efforts. These strategies have been bolstered by platforms that share genotype and phenotype information to identify patients with overlapping phenotypes and candidate genes, an approach called matchmaking (reviewed in Philippakis et al. 2015; [www.matchmakerexchange.org](http://www.matchmakerexchange.org)). While technical limitations of ES are well-recognized, in the last decade capture kits have continued to improve their coverage of the coding genome, with additional gene features such as promoters and intron flanking regions. In addition, computational tools continue to improve and facilitate the identification of variation. Given cost and other practical considerations, ES will continue to play a major role in RD variant diagnosis and discovery.

Globally, thousands of clinical exomes are performed weekly but, unfortunately, the majority of these data are inaccessible for discovery and matchmaking. To realize the theoretical maximal diagnostic yield of ES will require a globally coordinated paradigm shift; every patient must have the opportunity to be a research patient. More international and less restrictive data-sharing is critical to drive variant interpretation, better control datasets, and development of computational tools. This will enable identification of RDs that are understood at a genetic level, while RDs that require further research can be studied as part of an ‘exome negative’ clinical infrastructure. Importantly, aggregation of such data will contribute to the development of large datasets that can be used for as yet undefined purposes as we explore new mechanisms for RD.

## **The ‘horizon’ in RD diagnosis: The next wave of technologies to reveal RD mechanisms**

Regardless of the ultimate capability of ES to provide diagnoses for RD patients, some disease mechanisms are difficult or impossible to detect using this approach (Table 1). For example, mosaicism of a pathogenic variant would not be routinely identified by current analytical approaches. Challenges for detection of mosaicism include: the distribution of the causative genomic variation which can be non-random and can exclude the most often sampled tissue (blood) for genetic testing; the changing level of mosaicism over time; the difficulty in distinguishing pathogenic from benign or unrelated mosaic variation (signal-to-noise); and the high sequencing cost for the depth and breadth of coverage needed to detect low level mosaic variants. New data analysis tools are emerging to screen for mosaicism in unsolved exome datasets, and approaches that facilitate very deep sequencing of targeted regions in a cost-effective manner will improve detection of mosaicism in the near-term.

Some pathogenic genomic variants are missed entirely by ES. Genome sequencing (GS) (short-read sequencing) out-performs ES for indels (small insertions-deletions), CNVs, (copy number variations) and chromosomal rearrangements, while long-read GS promises further improvements in detection of rearrangements and the ability to identify RDs secondary to pathogenic repeat expansions. GS also provides the opportunity to identify regulatory variants that lie outside the exome, such as in promoters, enhancers, deep intronic regions, or distant-acting regulatory sequences located in intergenic regions; though interpretation of such variants and proof of causality are challenging. Such advantages of GS are the basis for promoting this approach over ES, and while robust head-to-head comparisons of the two approaches are still lacking, we hypothesize that GS will increase the diagnostic yield of a genome-wide clinical test by at least 10% in the near-term. As clinical GS data accumulate and understanding of intronic and intergenic variation improves, this yield will significantly increase over the years.

Several emerging technologies offer value as adjunct diagnostic tools by providing an approach to assess the functional significance of variants. For example, transcriptome sequencing can evaluate the functional consequences of variants that may affect splicing or gene regulation (e.g., decreased, increased, or monoallelic gene expression). This approach has been suggested to increase the diagnostic yield by 10-35% in known genes for certain clinical indications. Although promising, its broad applicability for RDs is unknown given challenges around the availability of relevant tissues, including those at critical stages of development. Similarly, methylation arrays are providing functional insight into imprinting disorders, which are caused by alterations of the expressed copy number of imprinted genes, through epigenetic error, uniparental disomy, or CNVs/SNVs (single nucleotide variants) of the regulatory DNA or the expressed allele (Soellner et al., 2017). More than one hundred human germline imprinted genes distributed across the genome have been identified, and it is likely that more remain to be found. In addition, arrays can detect specific DNA methylation epi-signatures for RDs associated with chromatin dysregulation; these syndrome-specific biomarkers complement standard clinical diagnostics (Aref-Eshghi et al., 2018). The true prevalence and phenotypic spectra of imprinting disorders will only be determined by the coordinated implementation of genomic and epigenomic technologies and recognition that the right family member to analyze might not be the affected individual. Similarly, atypical inheritance patterns should be considered when analyzing genomic data of unsolved RD patients, this will require even more sophisticated approaches to data analysis that will identify such mechanisms in a diagnostic setting (Table 1). Finally, for all of these new technologies, diagnostic standards will need to be established before clinical implementation to facilitate diagnostic clarity for as many patients as possible.

## **The ‘next frontiers’ in RD discovery: Building out from Mendelian inheritance**

Despite the excitement around GS, few RD discoveries have been made outside of the protein-coding regions of the genome. Comprehensive analysis of the noncoding genome on a broader scale represents a significant frontier (Table 1). The opportunity lies in the interpretation of noncoding variation, which is exponentially more difficult given the unresolved complexity of how noncoding DNA regulates gene expression, lack of adequate control datasets, and computational tools to predict variant impact, and the fact that each of these noncoding variants is likely affecting only a single patient or family resulting in a high benchmark to establish pathogenicity. While confirming predicted splicing abnormalities is relatively straightforward as highlighted in the previous section, estimating the impact of mechanisms such as long-range DNA regulation, aberrant DNA modifications (such as methylation), pathogenic alterations to non-coding RNA, and post-transcriptional and post-translational dysregulation on RD will require significantly greater understanding of the genome and significant development of functional analytical approaches. Initial successes will likely center on the use of large families with linkage data to narrow the search space and a focus on noncoding *de novo* alterations in parent-affected child studies.

Alongside monogenic RDs, we will face the challenge of RDs of complex etiology, with a primary genetic driver but clinical presentations that are contextualized by additional factors and represents another significant frontier of study. The relative impact of genetic and environmental components on RDs will depend on the underlying mechanism of interaction (signal transduction pathway, unfolded protein response, epigenetic modifications, etc.) and in the case of embryogenesis, when during development the impact is elicited and which developing organs/tissues are most vulnerable to perturbation at a given time. Environmental exposures may be pre- or post-natal, and the challenge will be to capture such exposure information based on history as well as the data from the metabolome, the latter of which is dynamic. These studies require integration of epidemiological and genomic data in exposed and non-exposed populations. Experimental support for such complex mechanisms will require the use of functional assays and model organisms to validate findings (Shi et al., 2017).

By comparison, the investigation of digenic, oligogenic, and polygenic inheritance models may seem relatively straightforward, but one should not be deceived and this represents yet another frontier. To perform such analyses and collect the evidence required for the statistical certainty needed to support an RD mechanism, a massive amount of harmonized phenotypic, genotypic, and family history data will be required. The establishment of such datasets reinforces the need to offer research access and broad data sharing to all RD patients and their families.

# **THE UNSOLVED RD COHORT: THE WAY FORWARD**

Our ability to diagnose all RDs is limited by our incomplete understanding of the full mutational spectrum associated with all RDs and the sheer number of unique RDs that have yet to be defined. The way forward is readily recognized as multi-faceted and will likely focus on specific subsets of patients from the unsolved RD cohort (Figure 1); each subset has significant utility for exploring RD mechanisms and optimizing approaches for clinical translation of novel diagnostic tests. Patients in the unsolved RD cohort can be considered in four groups and while the approaches used to uncover the genetic mechanism for the respective RDs may be similar between groups, the knowledge gained for each will be unique.

## **Patients with no causative variant after an appropriate, highly sensitive test**

Patients in this group have had the appropriate genetic test that is highly sensitive for that particular RD, but remain without a molecular diagnosis (e.g., single gene disorders such as cystic fibrosis and neurofibromatosis type 1). In all likelihood, the causative variant(s) in these patients is/are not detected by the current testing methodologies, and therefore this subset of patients represent a remarkable opportunity to explore novel diagnostic approaches, including new technologies and computational tools, to more comprehensively assess the spectrum of possible genetic causes of a given disease. The insights delivered will be directly relevant to these patients, whilst also optimizing patient sampling, computational tools, and diagnostic algorithms based on emerging technologies. More broadly, such knowledge will contribute significantly to the mechanistic spectrum of other unsolved RDs. This type of exploratory focus represents a shift in the types of studies that our community ‘values’ and both funders and publishers will need to recognize the intermediate importance and long-term impacts of the resulting insights.

## **Patients with no identified causative variant in the context of genetic heterogeneity**

Patients in this group have a clinically recognizable presentation associated with genetic heterogeneity (e.g., hereditary spastic paraplegia, myopathy, and retinitis pigmentosa) but negative results for the appropriate testing and analysis, including most of the relevant disease genes. These patients have either a pathogenic variant in one of the known disease genes that was not detected using the current testing approach, or a yet-to-be-discovered disease-associated gene. To diagnose these patients, we will need large datasets of patients that include detailed phenotypic and genomic information for data comparison and novel technologies and computational tools to identify cryptic variants. Besides GS, transcriptomic, metabolomic, epigenomic, and proteomic data may be necessary to identify the underlying genetic causes, particularly for simplex patients in which family-based analyses are not possible. This patient group represents an opportunity similar to the subset described above but is also enriched for novel disease gene discovery and likely represents one of the largest populations in the unsolved cohort.

## **Patients with an unsolved but recognizable syndrome**

Patients in this group have a clinical diagnosis based on similarity to a previously described syndrome for which the underlying etiology is unknown (e.g., PHACE and Hallermann-Streiff syndromes, and VACTERL association). With the efforts of the clinical and scientific communities and the increased use of ES, we now understand the genetic basis of most of the frequent and recognizable human malformation syndromes. However, some well-established syndromes (defined as reported in >10 unrelated patients and curated by OMIM and Orphanet) remain without an understood molecular etiology despite intensive investigation. Examples of such unsolved syndromes have been recently reviewed (Boycott et. al., 2018) in a Special issue on Unsolved Recognizable Patterns of Human Malformation: Challenges and Opportunities in the American Journal of Medical Genetics (<https://onlinelibrary.wiley.com/journal/15524876>). Possible explanations for their current intractability include genetic and phenotypic heterogeneity, mosaicism, epigenetics, gene-environment interactions, and other non-Mendelian contributions. The way forward for this group of disorders will require the use of emerging and new technologies, global cooperation, and data sharing.

## **Patients with syndromes without a name**

The fourth group of patients present with a constellation of clinical symptoms and signs that are not recognizable as a previously described syndrome or condition, which may have non-specific clinical features, and fit into none of the above groups. Part of the challenge in the diagnosis of these patients is that the full extent of the clinical presentation may not yet have become manifest (as occurs in the evaluation of ill newborns). These patients are most suitable for genome-wide sequencing approaches and, for the foreseeable future, should undergo ES or GS as a first line test followed by detailed genotypic and phenotypic data-sharing for matchmaking purposes. Their diagnoses will likely include early-presentations of recognized RDs, expanded phenotypes of previously recognized RDs, and novel RDs associated with new genes that will only be identified once RD datasets contain sufficient genotypic and phenotypic data to provide statistical confidence that an accurate diagnosis has been made and/or following validation in model organisms.

# **CONCLUSIONS**

We face a grand opportunity in precision public health; to understand the cause of each and every RD and provide a diagnosis for each individual RD patient. Clinical ES is transforming molecular diagnosis and will continue to have a remarkable impact on this area of medicine. For the patients that remain unsolved after genetic testing, the future remains optimistic. A large number of emerging technologies are on the horizon and will play an important role in RD diagnostics in the near-term. Computational approaches that focus on large-scale data integration across patients and within the single patient (“systems diagnostics”), and from healthy individuals, will enable the next frontiers of RD discovery. As we work toward our goal of diagnostic clarity for all, we will gain important insights into the RD genome and the attendant knowledge about human biology that this will bring. Importantly, there are some cross-cutting requisites for the clinical and research community to enable this important work and reach not just the current horizons of RD diagnostics, but the next frontiers as well. To start, we need to provide all RD patients the ability to access clinical genome-wide testing and participate in research. At the health systems level, we must implement the timely, prioritized, and sustainable clinical integration of proven innovative diagnostic approaches; this will scale the input side of the equation, serving patient need and fueling translational research discovery. In facilitating research participation, we must include those that we do not typically consider, collecting data for those with molecular diagnoses, and the clinically diagnosed but causative-variant negative patients, and support the necessary infrastructure. Going forward, we need to address the fundamental lack of RD researchers that study complex mechanisms by enabling an emerging new generation of scientists in this area with adequate funding and by contributing to comprehensive RD datasets that will provide the foundation for their work. Most critically, we must recognize that the future of RD diagnostics will depend on the international RD community working as one team towards an ambitious and important joint goal. We need to overcome a mindset limited to individual patients, individual researchers, individual genetic mechanisms, and even individual consortia, countries, or continents. The vision of IRDiRC, for each RD patient to receive a diagnosis within 1 year, is achievable only if we collectively take up this grand opportunity on a global scale.

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# **CONFLICTS OF INTEREST**

J.W.B. is a full time employee of Illumina, Inc. L.G.B. is a member of the Illumina Medical Ethics committee, receives royalties from Genentech, and receives honoraria from Cold Spring Harbor Press. The remaining authors have no conflict of interest to declare.

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## **Figure 1. Clinical Groupings of the Unsolved RD Cohort.**

## The unsolved cohort of patients can be considered in four groups, each will require a multi-faceted approach and will give us different insights into the incredible landscape of mechanisms underlying RD.

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| **Table 1. Mechanisms of RD that are currently intractable to exome sequencing and analysis** |
| **Mechanism** | **Description** | **Approaches** |
| **General Mechanism**  | **Perspective for Solutions\*** | **Mechanism Subcategory** |
| Mosaicism | Horizon | Tissue-specific mosaicism | Mosaic manifestations of Mendelian disorders; Disorders that manifest only as mosaicism  | Deep sequencing of multiple tissues |
| Genomic alterations | Horizon | Small insertions/deletions  | Small structural changes missed by ES and microarray (<50bp) | GS |
| Large insertions/deletions | Larger structural changes missed by ES and microarray (>50bp) | GS |
| Chromosomal rearrangements | Inversions/translocations; Multiple deletions/duplications | GS |
| Repeat expansions | Triplet and other expansions | Long-read GS |
| Transposable Elements (Retrotransposons) | Genomic sequences that copy and paste into locations throughout the genome (such as Mobile Element Insertions)  | Novel approaches to data analysis |
| Gene regulation  | Horizon | Splicing mutations | Synonymous or splice site or intronic mutations | GS, RNASeq |
| Imprinting | Altered parent-of-origin specific expression pattern | Methylation arrays |
| Next Frontier | Regulatory DNA mutations | Promoter, enhancer, and other regulatory mutations | GS, RNASeq, High-C, Prediction Tools |
| Non-coding RNA mutations | Intronic, intergenic, and antisense RNAs (e.g., microRNAs, snoRNAs) | Novel approaches to data analysis |
| Mutations that alter post-transcriptional or post-translational modifications  | Altered RNA or protein modifications that impact stability or catalytic function | Novel approaches to data analysis |
| Complex inheritance | Horizon | Unusual or less common inheritance patterns | Sex-limited expression; paradoxical inheritance; necessary but not sufficient CNVs; uniparental disomy | Novel approaches to data analysis |
| Next Frontier | Genetic modifiers | Allele from one gene reduces or exacerbates the penetrance or expressivity of phenotype associated with another gene | Novel approaches to data analysis; Validation in model organisms |
| Gene-environment interaction | Rare susceptibility allele combined with environmental trigger | Environmental exposure data capture; Validation in model organisms; Metabolomics |
| Maternal effects | Mutation in the mother results in altered fetal environment | Environmental exposure data capture; Validation in model organisms |
| Digenic, oligogenic, or polygenic | Interaction of two or more genes | Novel approaches to data analysis; Validation in model organisms |

\*Horizon: near-term (within 5 years); Frontier: longer-term (5 years and beyond)