"The molecule’s the thing:" the promise of molecular modeling and dynamic simulations in aiding the prioritization and interpretation of genomic testing results [version 1; referees: 3 approved with reservations]

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Abstract
Clinical genomics is now a reality and lies at the heart of individualized medicine efforts. The success of these approaches is evidenced by the increasing volume of publications that report causal links between genomic variants and disease. In spite of early success, clinical genomics currently faces significant challenges in establishing the relevance of the majority of variants identified by next generation sequencing tests. Indeed, the majority of mutations identified are harbored by proteins whose functions remain elusive. Herein we describe the current scenario in genomic testing and in particular the burden of variants of unknown significance (VUSs). We highlight a role for molecular modeling and molecular dynamic simulations as tools that can significantly increase the yield of information to aid in the evaluation of pathogenicity. Though the application of these methodologies to the interpretation of variants identified by genomic testing is not yet widespread, we predict that an increase in their use will significantly benefit the mission of clinical genomics for individualized medicine.

Keywords
Individualized Medicine, Clinical Genomics, Diagnostic Odyssey, Oncology, Molecular Modeling, Molecular Dynamics, Variants of Unknown Significance

This article is included in the Rare diseases collection.
In under a decade, sequencing of a human genome moved from a three billion dollar, multi institutional effort, to a common research assay. Now, 16 years after completion of the draft sequence, genomic testing is an increasingly prevalent component of clinical testing. Oncology, hematology and the diagnosis of rare genetic disorders (diagnostic odyssey) have particularly benefited from the increased genetic testing resolution afforded by modern sequencing technologies. The commercialization of clinical assays to profile patients’ somatic or germline genomes is creating the potential for higher resolution diagnoses and individualized treatment options. Stories of the resounding success of such efforts have been widely and justifiably publicized, and have now captured imaginations well beyond the laboratory or clinic; even so far as the White House and US Congress. The President’s Precision Medicine Initiative has now been signed into existence and guarantees expanded exploration within this burgeoning area of health sciences research.

For the researchers and clinicians on the ground of this nascent field, enthusiasm is high but it is tempered with frustration due to the as-yet high rate of cases for which genomic testing remains insufficiently informative (around 75% for diagnostic odyssey)\(^1,2\). While the explosion of genomics research has increased our understanding of the human genome and the wide-ranging functions it encodes, most regions of the genome remain uncharacterized and poorly understood. The majority of genomic sequence variants detected in clinical genomic testing go unreported or are classified as variants of unknown significance (VUSs) due to the lack of understanding of their potential to affect normal physiological function.

A recurring scene for many of the clinicians, genetic counselors and researchers who are adopting and exploring this new arena is the convening of large, multidisciplinary teams of clinical and research staff to pore over lengthy lists of genomic sequence variants, exchange professional opinion and debate next steps. However, in the end, these involved efforts often fail to establish variants with highly confident causal or mechanistic relationships with the disease phenotype. In oncology, the knowledge gained for treatment selection is often compelling, but with little prior evidence, most are difficult to actualize. For example, identification of a novel missense mutation in the functional domain of a known, druggable oncogene might logically appear to be a therapeutic target, but no information may exist linking the mutation to drug efficacy. It is this disconnect between novel genotypes identified and their link to the patient’s phenotypes which thwarts our ability to further improve clinical decision making. Thus, there is a significant need to overcome this critical challenge to expand the success of genomic testing in individualized medicine.

Wet-lab assays designed to ascertain functional relevance of specific mutations are a highly desired solution, but they remain cumbersome, time-consuming and cost prohibitive in the great majority of cases. They are therefore misaligned with the high throughput nature of modern genomics. Meanwhile, computational methods of predicting the pathogenicity of genomic sequence alterations exist and are both amenable to high-throughput predictions and widely applied in the field\(^3\). These algorithms utilize varied information including evolutionary conservation, genomic position or basic protein-level structural information to assign probabilistic scores or categorical predictions of pathogenicity. Their accuracy varies widely, with alternative tools often producing conflicting predictions for the same variant. Furthermore, the predictions often lack contextualization in terms of biological effect. Algorithmic categorization of a variant as pathogenic offers little insight into the nature of its phenotypic effect and less indication still of whether it is relevant clinically or how to act upon it. Many researchers continue to work to improve these methods, but the burden of VUS interpretation persists and the need for a means to address this burden and facilitate clinical interpretation is widely felt within the field.

The aforementioned computational tools largely used leverage genome centric information. While there is much proven value to these data, the biological reality comprises many additional layers of complexity. Mutations affect atomic-level biophysical changes that may alter the structure and biochemical function of the genome’s protein products. While we detect variants in DNA, we typically interpret their effect by inferring or confirming their deleterious effect on encoded proteins (Figure 1). Even the effects of regulatory variants are typically interpreted as altering the probability of a protein to be expressed or spliced into a given functional form. Thus ideally, any method used to assess pathogenicity of genomic variants should possess the ability to look beyond genomic annotation to a functional context, at an atomic resolution. This increased resolution is likely to yield information that can add context to mutations, better identify the mechanism of pathogenicity, and combine with existing knowledge to facilitate in clinical decision making.

Motivated by the conditions described, we have begun to apply molecular modeling and dynamic simulation techniques in the interpretation of genomic variants identified by next generation sequencing. These methodologies abandon the practice of regarding mutations as occurring in linear strings of nucleotides or amino acids and instead offer a three dimensional, dynamic view, at an atomic resolution. They involve the computational generation, optimization and verification of a protein model, often based on homology to an experimentally determined protein structure\(^4,5\). Ab initio modeling is also possible, albeit with lower confidence. The model itself contains information about the linear amino acid sequence of the protein, along with the relative spatial coordinates of its atoms. Precise mathematical and biophysical parameters in the form of a force field are applied to the model to calculate energetic characteristics of the system. The methods are often mature and under reasonable conditions can be expected to produce a model with error comparable to that of a typical structure solved by nuclear magnetic resonance spectroscopy.

Molecular modeling allows us to visualize the manner in which proteins are folded to create a functional structure and to accurately simulate how this is disrupted by mutational events. Because we can visualize atomic bonds, mutations which disrupt inter-molecular interactions - for example between an enzyme and its substrate - can also be modeled. To illustrate their function, many enzymes are analogized to hand tools. One example likens proteases and scissors; if a variant inhibits the closing motion of the scissors about their fulcrum, the blades cannot function; if a variant blocks entry
Figure 1. The HIV-1 protease as a model system for computational biophysics. 

HIV-1 protease has become a model system because of its disease relevance, the availability of mutational and drug binding data, and for its tractable size and molecular stability. The protein’s function is to cleave HIV peptides into the functional proteins of the infectious HIV virion. A) Ligand binding residues are spatially separated. The functional protein dimer is shown with a pharmacologic inhibitor bound to the active site. Residues that are within 3.5Å of the inhibitor are highlighted in tan. The primary sequence is colored identically to the three-dimensional structure to indicate relative positioning of residues. It is apparent that the ligand binding portion of the protease consists of residues that are non-adjacent within the primary sequence, illustrating an advantage of modelling over linear sequence analysis. B) Drug resistance mutations tend to occur in residues within the active site. Many mutations have been characterized that are associated with resistance to inhibitor drugs. While the sites of these mutations are also disjoint in sequence, nearly all of them fall into the same set of drug binding residues, indicating how modeling can enable prediction of a mutation’s effect. C) Structural effects of mutations beyond the active site. Drug-resistance mutations in non-ligand-binding residues have been shown, using computational experiments, to impact the flexibility of the protein and therefore alter drug binding. Computational modeling has characterized the flexibility of the protein in multiple mutated states, illustrating the potential to predict the functional effect of mutations beyond an active site. D) Dynamics of unbound ligand. Computational studies have the advantage of being able to simulate conditions that are difficult to assay experimentally, such as the dynamics of ligand-free forms in atomic detail.
laboratory experiments, computational calculations and simulations are most interpretable when they are well designed, test a specific hypothesis, contain positive and negative controls, connect the data generated to the pre-existent knowledge in the field, and allow drawing further functional inferences. When all these conditions are met, the three dimensional and dynamic representation of the modeled mutations may add a significant value to the interpretation of a genomic test’s finding.

Of course, molecular modeling methods have limitations. The generation of a reliable model is often dependent on the pre-existence of experimentally determined homologous structures and even where these exist, they may provide only partial information. A model is by nature an approximation of reality. Nonetheless, current techniques have achieved suitable accuracies such that they are frequently used in applications including drug design, virtual screening, protein engineering and site-directed mutagenesis. With this in mind, their relatively slow uptake in the clinical setting is somewhat surprising. This fact may simply reflect the relative nascent of clinical genomics and the tendency of specialists to seek out fields in which their specialty is already known and accepted.

We encourage those working within or in proximity to the clinical genomics setting to engage in or promote increased exploration of these methods in their work. Our initial experiences of applying such techniques at the clinical-research boundary of genomics-driven oncology, hematology and diagnostic odyssey have been encouraging and have begun to inform decision-making. We are observing clear initial benefits in regard to variant prioritization, interpretation and validation, with several initial publications in preparation to highlight the value obtained. Of course, not everyone will possess the necessary scientific knowledge or technical skills to deploy these methods directly, but we propose that inter or intra-institutional collaborations may enable those lacking the requisite expertise to identify and access appropriate resources. Furthermore, several freely available online solutions exist and enable a researcher or clinician to experiment with core modeling methods in the absence of extensive technical expertise. Such independent or collaborative exploration will open the door to deeper understanding of biological mutations and have the potential to inform clinical thinking.

In summary, genomic testing is assuming an increasingly prominent role in the identification, prioritization, and interpretation of disease-associated genomic variants. While a few of these variants are known to be pathogenic, knowledge of the deleterious effects of the majority remain elusive. Laboratory methods remain gold-standards for functional characterization, but are generally incompatible with large-scale characterization of variant effects. Predictive algorithms are in some instances successfully applied to differentiate pathogenic variants from variants of unknown significance. However, these methods largely ignore measures of protein structure, energies, molecular bonds, intermolecular interactions, post-translational modification effects, protein aggregation, and stability. Conversely, this information lies at the heart of molecular modeling and dynamic simulation, which collectively equip us more fully to grapple with interpreting the effects of VUSs. We strongly believe that these methodologies constitute the future frontiers in forging the analytical pipeline of interpreting the results of genomic testing for individualized medicine and advocate their increased deployment within variant characterization efforts.

Author contributions
EWK & GRO conceived the manuscript. GRO wrote the manuscript. MTZ generated figures and wrote the manuscript. RAU wrote the manuscript. All authors proof-read and approved the manuscript and have been involved in the modeling and interpretation of genomic variants in diagnostic odyssey, hematology and oncology studies within Mayo Clinic’s Center for Individualized Medicine.

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No competing interests were disclosed.

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References


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In this article Oliver et al. highlight the importance of analytical methods such as “molecular modeling and molecular dynamic simulations” as tools that can significantly increase the interpretation of the functional impact of mutations/polymorphisms currently classified as variants of unknown significance (VUSs).

While the topic of molecular dynamics simulation for VUSs is worth discussing, the article could expand on

- the current limitation of these methods
- the complexity of these nucleotide changes in e.g. coding and non-coding genomic regions
- integration with other high throughput technologies and thus increase challenges for multimodal effects in such simulations
- HIV is given as an example, however other clinical examples, their associated specificity and limitations should be provided to make this article relevant for a wider audience.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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The authors of this article “highlight a role for molecular modeling and molecular dynamic simulations as tools that can significantly increase the yield of information to aid in the evaluation of pathogenicity.”

Molecular dynamics simulations are indeed excellent tools to study protein and nucleic acid structures, protein-ligand, protein-protein and protein-nucleic acid interactions. These structural changes and interactions are fundamental in molecular biology and thus molecular simulations are an excellent tool to
increase our understanding of these biological processes. In particular the proposed method may help the understanding of coding variants associated with amino acid substitution. In addition, RNA structure can be modeled, which can be informative about some micro-RNA variants.

However, these methods are not without limitations and this paper does not state these limitations explicitly. Pathogenic effects come in all strength and complexities. Most often pathogenicity is not associated with a single variant, but with a combination of several variants and the effect of a particular variant depends on the genotypes of other genes. Most likely, variants will stay of unknown significance unless we develop a system level understanding of biology. We may reach a point that we can simulate viral functions at molecular level, but it will not be applied to human variants any time soon.

Molecular modelling has some limitations and requires expert knowledge to apply it properly.

While I agree that molecular modelling is an excellent tool to understand molecular biology, we need to develop a system level of understanding of the connection between molecular biology and genetics before we can apply molecular simulations. Currently, molecular modeling can make practical contribution to understand variants associated with strong effects or the molecular basis of some Mendelian disorders.

**Competing Interests:** No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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In this article the Authors seek ways on how to better interpret the functional impact of mutations/polymorphisms currently classified as variants of unknown significance (VUSs). To tackle such a problem, the authors propose “molecular modeling and molecular dynamic simulations as tools that can significantly increase the yield of information to aid in the evaluation of pathogenicity.” Expectations are that this “will significantly benefit the mission of clinical genomics for individualized medicine.”

The idea is good and deserves to be published. Most of all, it deserves to be extensively discussed.

**Points to be discussed**
As much as the idea is good, it would benefit from better highlighting its (current) limitations.

1. *Ab initio* molecular modeling is a difficult enterprise. It can rarely be performed to some extent of accuracy by automatized engines/softwares and often reaches good structure prediction in around 60% of cases.

Several large-scale, tentatively whole-coding genome, protein crystallization initiatives have been
Several large-scale, tentatively whole-coding genome, protein crystallization initiatives have been undertaken. Discussion of the state of the art of these initiatives may provide metrics on what could be expected from real-life application of the Authors’ proposal, and how this is expected to evolve in the near future.

2. Most mutations/polymorphisms are detected in non-coding regions of DNA. This is hardly surprising given that non-coding DNA represents approximately 97% of the total content of a mammalian cell. The authors mention mutations in non coding regions, but this deserves being more extensively discussed in the publication.

Suggestions

Discussion of the points above and of the suggestions below, may possibly lead to extend the proposed approach to additional layers of prediction.

Algorithms are there that do recognise promoter, enhancer and super-enhancer regions with increasing accuracy. Clearly, allocating a mutation/polymorphism in one such region would increase its investigational and possibly medical value.

Additional regions of interest are CpG islands upstream or, less reliably, within coding regions.

Additional regions of interest are anchoring sites of DNA loops to distinct nuclear/nuclear membrane regions.

Additional regions of interest are those hosting the increasing family of non-coding RNA (NCRNA), including long NCRNA and miRNA, that have been shown to play distinct regulatory functions and may have roles in cancer development.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Discuss this Article

Version 1

Author Response 13 Jun 2016

Gavin Oliver, Mayo Clinic in Rochester, USA

We wish to thank each of the reviewers for their thorough review of our article and their varied insightful comments and suggestions. We have considered all of these and both edited and expanded our argument to address them. Firstly, we better define the current norms within clinical genomics testing specifically, in order to better orient the reader and set the scenario for our opinions. Furthermore, the article now more clearly defines our argument in the context of the wider medical field, and better defines the scope of our suggested uses of protein modeling. We acknowledge the many, diverse approaches that likely represent future components of clinical testing and define our position in relation to these.
Ultimately we feel that the comments received enable our new version to present our position with greater clarity and a more pointed argument, which is better tailored to a diversity of expert readers.

**Competing Interests:** No competing interests were disclosed.

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