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**Value of predictive bioinformatics in inherited metabolic diseases**

Timson DJ. Predictive bioinformatics in inherited metabolic diseases

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**Abstract**

Typically, inherited metabolic diseases arise from point mutations in genes encoding metabolic enzymes. Although some of these mutations directly affect amino acid residues in the active sites of these enzymes, the majority do not. It is now well accepted that the majority of these disease-associated mutations exert their effects through alteration of protein stability, which causes a reduction in enzymatic activity. This finding suggests a way to predict the severity of newly discovered mutations. *In silico* prediction of the effects of amino acid sequence alterations on protein stability often correlates with disease severity. However, no stability prediction tool is perfect and, in general, better results are obtained if the predictions from a variety of tools are combined and then interpreted. In addition to predicted alterations to stability, the degree of conservation of a particular residue can also be a factor which needs to be taken into account: alterations to highly conserved residues are more likely to be associated with severe forms of the disease. The approach has been successfully applied in a variety of inherited metabolic diseases, but further improvements are necessary to enable robust translation into clinically useful tools.

**Key words:** Genetic disease; Metabolism; *In silico* method; Protein stability; Disease-associated mutation

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**Core tip:** Bioinformatics and other *in silico* methods are increasingly being used to predict the severity of disease-associated mutations in inherited metabolic diseases. In general, severity correlates with altered protein stability and the best predictions occur when a variety of tools are applied.

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**INTRODUCTION**

Inherited metabolic diseases result from mutations in the genes encoding enzymes involved in intermediary metabolism. Well characterised examples include galactosemia, lysosomal storage diseases and phenylketonuria (PKU). Typically these diseases manifest with effects at the whole organism level, despite their origins at the metabolic pathway level. Physical and cognitive disabilities are associated with many inherited metabolic diseases. While individual diseases are generally rare, the cumulative effect of many of these diseases has a significant effect on societies and economies[1-4]. Furthermore, the burdens on patients, their families and their immediate communities can be devastating since many of these diseases result in progressive deterioration of the patient resulting, in some cases, in death in childhood or early adulthood. Very few of these diseases have effective therapies (*i.e*., treatments which restore normal, or near-normal, functioning to the patient). One barrier to the development of therapies is the rareness of the diseases: there is limited incentive to the development of drugs or other treatments which would only be applicable to a small number of patients worldwide[2,5,6]. Where therapies do exist, they tend to be extremely expensive, often exceeding US$100000 per patient per year (for example, see[7,8]).

Biochemical studies on the underlying molecular pathology of a range of inherited metabolic diseases have revealed some common themes. In particular, mutations associated with these diseases often causes changes which destabilise the corresponding protein (for examples, see[9-15]). Very few disease-associated mutations directly affect the residues in the active site of the enzyme; the majority affect residues elsewhere in the protein. A common molecular mechanism of disease causation is that the altered amino acid residue causes a global reduction in the enzyme’s stability resulting in reduced catalytic activity[16]. The loss of stability can also be associated with reduced affinity for essential cofactors or increased aggregation of the partially folded protein. It is, of course, the loss of enzymatic activity which commonly leads to disease, for example by reducing the amount of product made or causing a build-up of toxic intermediates. In other cases the accumulation of aggregated protein results in a breakdown of cellular homeostasis. Nevertheless, partial protein misfolding lies at the base of these problems and is the fundamental cause of the disease in these cases.

It is also apparent that, in many inherited metabolic diseases, there is a range of possible symptoms. This is particularly stark in diseases like type III galactosemia and mevalonate kinase deficiency. In these diseases the manifestations of diseases range from near-normal physiology with some alterations in blood chemistry to highly disabling, life-threatening conditions[17,18]. The experience of each patient will be determined by his/her genetic background, lifestyle and environment. Critical elements include the patient’s diet, activity levels and access to good quality medical care. However, the most important factor in determining the severity of symptoms is normally the exact mutation(s) that the patient has. Most inherited metabolic diseases are not caused by one, single mutation. The majority have many possible mutations which are associated with the disease. For example, there are almost 250 mutations in galactose 1-phosphate uridylyltransferase (GALT) which are associated with type I galactosemia[19,20]. Since these mutations alter different amino acid residues, it follows that they will have different effects on the protein. Some will have relatively minor effects on the protein’s overall structure and stability whereas others may render the protein essentially non-functional.

Novel, disease-associated mutations continue to be discovered. Indeed, with the decreasing price of whole exome sequencing, we should expect that the rate of discovery of novel mutations will increase in the next few years[21,22]. In some inherited metabolic diseases problems are apparent within a few days of birth; however, in other cases, babies are born with near normal physiology but progressively decline over the following years. Given the range of possible severities associated with some inherited metabolic diseases it is a challenge to physicians and scientists to predict the likely symptoms of an individual patient and to plan treatment accordingly. This is particularly the case for newly discovered disease-associated mutations.

**THE CHALLENGE OF PREDICTION**

The link between protein stability and severity of disease suggests a way in which predictions might be made. There are a variety of software packages, many freely available online, which claim to predict the stability of proteins (Table 1). In theory if a range of disease-associated variant proteins are compared to the wild-type in one of these packages then the greater the predicted instability, the more severe the disease is likely to be. In practice the situation is more complex. No prediction software is 100% accurate and different packages can give different results for the same variant. This problem can be overcome by using a variety of different programs and aggregating the results together to obtain a consensus. However, problems still remain. In some diseases, both decreases and increases in stability can be associated with disease (for examples see[9,23]). Indeed, it appears that as well as an optimum structure for activity, proteins also require an optimal degree of flexibility and stability. Protein flexibility is inextricably linked to ligand binding and catalysis[24]. Thus, increased stability can lead to a more rigidified, less flexible protein which is less able to bind substrates and catalyse the reaction.

While protein stability is undoubtedly a key factor in disease causation, there are other factors which need to be considered. Alterations in key residues involved in binding substrates or in the chemistry of catalysis will lead to direct loss of activity. A failure to interact with cellular chaperones may impede folding. Disruptions to other protein-protein interactions may also affect the enzyme’s function. Residues involved in catalysis and protein-protein interactions are generally well conserved through evolution. Therefore, we might expect that mutations which alter highly conserved residues might also lead to more severe forms of disease. Therefore, many predictions incorporate measures of sequence conservation and propensity to interact with cellular chaperones (Table 1). Overall, it is accepted that the best predictions will result from using a variety of different software packages which address different aspects of the protein’s structure and function[25-27]. Furthermore, any output requires intelligent and critical analysis by the users.

**APPLICATION TO INHERITED METABOLIC DISEASES**

These approaches have been employed in a number of inherited metabolic diseases (Table 2). Typically, a set of known mutations and their associated protein variants are identified from the literature and classified according to their association with different severities of the disease. Other information from the literature is required - most importantly an experimental demonstration that protein misfolding is an important factor in disease causation. Using the known variants, a range of prediction tools are applied and the combination which best predicts the known outcomes are then selected. This can then be applied to uncharacterised mutations or to polymorphisms identified through genome and exome sequencing projects. In general, the severity of disease correlates with the predicted loss of stability of the protein. The degree of conservation of the residue(s) affected is also important in some conditions (Table 2). Most studies employ a range of different prediction tools and aggregate results together to make informed predictions (for example see[28]).

**CONCLUSION AND FUTURE PERSPECTIVES**

To date, no prediction protocol has achieved complete accuracy and it is unlikely that physicians would be confident to rely on them to guide treatment of their patients. In addition, the prediction protocols published so far mostly require extensive bioinformatics analysis using a number of different tools on separate websites. Ideally these would be integrated into a single web-based package which enabled the user to submit a novel variant and receive a prediction. This would require the software to submit the new variant to the various online tools and integrate the responses into a single prediction. This, in turn, requires the software tools to remain “live” and at the same, stable url – something which cannot be guaranteed when each tool is provided by different organisations. One solution would be to use the source code for the prediction tools directly, but this is not always freely available.

Nevertheless carefully designed prediction methods can already provide some guidance in the assessment of novel mutations and have also had some success in classifying mutations identified through genome and exome sequencing projects. Interestingly, these tend to suggest that there are a number of mutations in the human population which were not previously associated with disease. In the case of type III galactosemia, several of these were predicted to be associated with severe forms of the disease. Further experimental testing will be required to see if these predictions are valid. In conclusion, the best predictions will be made when a variety of tools are used and where they are supported by strong experimental and clinical studies. It is highly unlikely that bioinformatics predictions will completely supplant experimental studies; however, with continuing improvements in the tools used they have the potential to be a useful tool for both clinicians and scientists who work on inherited metabolic diseases.

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**Table 1 Examples of freely available, online tools for predicting the properties of variant proteins**

|  |  |  |  |
| --- | --- | --- | --- |
| Category | Name | Weblink | Reference(s) |
| Structural analysis | YASARA energy minimisation | www.yasara.org/minimizationserver.htm | [29] |
|  | LS-SNP | ls-snp.icm.jhu.edu/ls-snp-pdb/main | [30] |
|  | GETAREA | curie.utmb.edu/getarea.html | [31] |
| Stability prediction | I-Mutant 3.0 | gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi | [32,33] |
|  | mCSM | bleoberis.bioc.cam.ac.uk/mcsm/ | [34] |
|  | SDM score | mordred.bioc.cam.ac.uk/~sdm/sdm.php | [35,36] |
|  | Mupro | mupro.proteomics.ics.uci.edu | [37] |
|  | iStable | predictor.nchu.edu.tw/iStable/ | [38] |
|  | PredictSNP 1.0 | loschmidt.chemi.muni.cz/predictsnp/ | [39] |
|  | Meta-SNP | snps.biofold.org/meta-snp/ | [40] |
|  | KD4V | decrypthon.igbmc.fr/kd4v | [41] |
|  | Fold-X | foldx.crg.es | [42] |
|  | PoPMuSiC | dezyme.com/ | [43] |
|  | CUPSAT | cupsat.tu-bs.de | [44,45] |
|  | GETAREA | curie.utmb.edu/getarea.html | [31] |
| Binding affinity changes | BeAtMuSiC | babylone.ulb.ac.be/beatmusic) | [46] |
| Aggregation tendency, amyloid formation and chaperone binding | TANGO | tango.crg.es/ | [47] |
|  | WALTZ | www.switchlab.org/bioinformatics/waltz | [48] |
|  | LIMBO | www.switchlab.org/bioinformatics/limbo | [49] |
| Sequence conservation | Clustal Omega | www.ebi.ac.uk/Tools/msa/clustalo/ | [50] |
|  | Scorecons | www.ebi.ac.uk/thornton-srv/databases/cgi-bin/valdar/scorecons\_server.pl | [51] |
|  | SIFT | sift.jcvi.org/ | [52] |
|  | PROVEAN | provean.jcvi.org/index.php | [53] |
|  | LS-SNP | ls-snp.icm.jhu.edu/ls-snp-pdb/ | [30] |
|  | SNPs and GO | snps.biofold.org/snps-and-go/pages/help.html | [54] |
|  | PANTHER | www.pantherdb.org/tools/csnpScoreForm.jsp | [55] |
|  | GenMAPP | www.genmapp.org | [56] |
|  | PolyPhen 2 | genetics.bwh.harvard.edu/pph2/ | [57,58] |
|  | nsSNP Analyzer | snpanalyzer.uthsc.edu | [59] |
|  | FI mutation assessor | mutationassessor.org/v1 | [60] |
|  | YALE MU2A | krauthammerlab.med.yale.edu/mu2a | [61] |

**Table 2 Examples of bioinformatics based predictions of the severity of variants associated with inherited metabolic diseases**

|  |  |  |  |
| --- | --- | --- | --- |
| Disease | Protein | Comments | Ref. |
| Alkaptonuria | Homogentisate 1,2-dioxygenase | Combining a variety of computational approaches gave rise to the most accurate predictions | [62] |
| Apparent mineralocorticoid excess | 11βHSD2 | The predicted degree of structural change in the enzyme correlates with disease severity | [63] |
| Fabry Disease | GLA | A purpose built program designed to detect protein instability outperformed existing, generic tools | [64] |
| Fabry Disease | GLA | A purpose built web interface allows prediction of a patient’s responsiveness to pharmacological chaperone therapy | [65] |
| Gaucher Disease | GBA | Slightly different results were obtained with different programs; however, 22 out of 47 variants were predicted to be harmful by all seven programs used | [28] |
| Glucose 6-phosphate dehydrogenase deficiency | G6PDH | A combination of prediction tools suggested that protein stability is an important factor in this disease; novel potentially disease-associated variants were identified | [66] |
| Hyperargininemia | ARG1 | Mutations affect residues in the active site, or protein stability, or quaternary structure | [67] |
| Maturity-onset diabetes of the young, type 2 (MODY 2) | GCK | Variations which decrease protein stability and/or occur in highly conserved regions of the protein are associated with disease | [68] |
| Niemann-Pick Disease type C | NPC1 and NPC2 | The majority of disease-associated variants were predicted to be less stable than wild-type | [69] |
| Phenylketonuria | PAH | Protein stability predicted to be most important factor in disease causation | [10] |
| Pyruvate kinase deficiency | PK1 and PK2 | A combination of prediction tools suggested that protein stability is an important factor in this disease; novel potentially disease-associated variants were identified | [66] |
| Type I galactosemia | GALT | Main predicted effect is the loss of stability of GALT | [70] |
| Type III galactosemia | GALE | Effects on protein stability and degree of sequence conservation combined were required for good predictions | [71] |

11βHSD2: 11β-hydroxysteroid dehydrogenase type 2; GLA: α-galactosidase A; GBA: Glucocerebrosidase; G6PDH: Glucose 6-phosphate dehydrogenase; ARG1: Arginase 1; GCK: Glucokinase; PAH: Phenylalanine hydroxylase; PK1 and PK2: Pyruvate kinase isoforms 1 and 2; GALT: Galactose 1-phosphate uridylyltransferase; GALE: UDP-galactose 4’-epimerase.