HPMV: Human protein mutation viewer — relating sequence mutations to protein sequence architecture and function changes

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Next-generation sequencing advances are rapidly expanding the number of human mutations to be analyzed for causative roles in genetic disorders. Our Human Protein Mutation Viewer (HPMV) is intended to explore the biomolecular mechanistic significance of non-synonymous human mutations in protein-coding genomic regions. The tool helps to assess whether protein mutations affect the occurrence of sequence-architectural features (globular domains, targeting signals, post-translational modification sites, etc.). As input, HPMV accepts protein mutations — as UniProt accessions with mutations (e.g. HGVS nomenclature), genome coordinates, or FASTA sequences. As output, HPMV provides an interactive cartoon showing the mutations in relation to elements of the sequence architecture. A large variety of protein sequence architectural features were selected for their particular relevance to mutation interpretation. Clicking a sequence feature in the cartoon expands a tree view of additional

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information including multiple sequence alignments of conserved domains and a simple 3D viewer mapping the mutation to known PDB structures, if available. The cartoon is also correlated with a multiple sequence alignment of similar sequences from other organisms. In cases where a mutation is likely to have a straightforward interpretation (e.g. a point mutation disrupting a well-understood targeting signal), this interpretation is suggested. The interactive cartoon can be downloaded as standalone viewer in Java jar format to be saved and viewed later with only a standard Java runtime environment. The HPMV website is: <http://hpmv.bii.a-star.edu.sg/>.

Keywords: Human protein mutations; protein sequence architecture; protein function; genomic variation; clinical genome sequencing.

1. Introduction

Genome sequencing is rapidly becoming a routine tool for investigating medical disorders with an underlying genetic basis.¹⁻³ Next-generation sequencing is poised to revolutionize the investigation of rare genetic disorders caused by individual mutations and, generally, the influence of specific mutations and gene variants on the fidelity of physiological functions and on pathogenesis.

The dramatic decrease in sequencing prices, coupled with technologies that allow sequencing from saliva and other easy-to-collect samples, make it feasible now to sequence affected individuals as well as their unaffected family members. In combination with lists of common variants from resources such as the 1000 genomes project⁴ and PhyloTree,⁵ the number of possible variants can be reduced to a level where identification of a specific causative mutation is often possible.

Traditional genome-wide association studies connect phenotypic properties and disease states with genomic features just at the statistical level; yet, there is a wide range of problems and one might suggest that it will not be possible to find significant links between many genomic aberrations and any phenotype since the predictive power of these approaches is limited.⁶⁻⁹ It is not just the issue of largeness of the analyzed subpopulations and, in some cases, even involving whole mankind might be insufficient. But for example, when the mechanistic networks involving the genes considered are complicated, their effect might be enhancing or suppressing depending on the variants of other genes participating in the system of pathways and structural associates.

Mechanistic insights into pathogenesis provide rational arguments for potential cures and, therefore, it becomes most desirable to deduce hints that indicate how protein-coding gene mutations alter gene function and modulate biomolecular pathways and subcellular structures. In the present concept of protein function,^{10,11} a protein sequence consists of several segments (of either globular or non-globular nature), each carrying specific structural and molecular functional properties and the overall function of the protein is a synthesis of the segments' functions. In turn, the entirety of these segments, the so-called sequence architecture, determines the range of cellular and phenotypic functions that a protein can execute.

Non-synonymous mutations alter the protein sequence and, thus, can change the molecular function of the respective segment. This effect might even include the

disappearance or *de novo* emergence of the respective sequence architectural element as it is well known for targeting signals or post-translational modifications (PTMs).¹² Our Human Protein Mutation Viewer (HPMV) is a tool that is designed to map reported mutations onto sequence architectural features and to explore how they might affect protein function via influencing the respective segment's properties.

There is already quite a variety of databases and programs available that support researchers in analyzing human mutations. For many specific sequence variants of interest, general and specialized databases provide valuable curated information. These include general sequence-oriented databases such as Ensembl,¹³ UniProt,¹⁴ NCBI RefSeq,¹⁵ the wwPDB,¹⁶ Pfam,¹⁷ CDD,¹⁸ and ProTherm¹⁹ on the one hand and disease-gene and mutation-focused databases including OMIM,²⁰ ClinVar,²¹ HGMD,²² dbSNP,²³ and MITOMAP²⁴ on the other.

Analyses attempting to relate mutations to biomolecular mechanisms are quite time consuming and there is a pressing need for software to facilitate this process. Tools such as ANNOVAR,²⁵ SeattleSeq Annotation,²⁶ and Ensembl's Variant Effect Predictor²⁷ are helpful for the first annotational step of lists of mutations as they link mutations with gene annotational features. Another class of tools including MutationTaster²⁸ and Mutation@A Glance²⁹ assemble useful information from existing databases for assessing the severity of a single mutation. Similarly, Distributed Annotation System (DAS) viewers (Dasty3,^{29,30} DASher,³¹ SPICE,³² and the CBS DAS Viewer³³) conveniently collate graphical representations from databases and servers.

Programs exemplified by SIFT,^{34,35} GERP,³⁶ and PolyPhen³⁷ quantitate the likelihood that mutations affecting the coding sequence might be deleterious for protein function but without assessing possible mechanisms. Generally, these tools have a greater chance for correct prediction in regions with 3D structure since mutations that affect the required hydrophobic pattern or conserved functional residues are most likely deleterious for function. In many types of non-globular segments, it is not that clear. For mutations within 3D structures of proteins, 3D structure analysis tools such as SDM³⁸ and FoldX³⁹ can be used to assess their impact if the 3D structure of the globular domain or a homologue of the sequence studied is available.

HPMV is intended to be used downstream of tools like ANNOVAR, etc. in the analysis pipeline. When the initial stages of sequence data processing reach the level of recognizing non-synonymous mutations within protein-coding genes, the stage for applying HPMV has come. By conveniently mapping human mutations to a large variety of protein sequence architectural features and suggesting interpretations, our HPMV fills the gap between initial variant filtering and specialized analysis of individual variants. No other tool available directly compares wild-type and mutant protein sequences and assesses the effect on the existence of diverse sequence-architectural features. These features were chosen for their particular relevance to mutation interpretation based on extensive experience in computational protein function prediction and include both characteristics of globular domains and 3D structures as well as a variety of non-globular segments (Table 1). The tool is inspired by the computational and graphical user interface framework

Table 1. Sequence architectural features processed by HPMV.

Feature	Algorithm/Database	References
Natural Variants	UniProt	UniProt Consortium ¹⁴
Coiled-Coil	IMP-COIL (modified)	Lupas <i>et al.</i> ⁴³
Transmembrane	Phobius	Käll <i>et al.</i> ⁴⁴
Signal Peptide	SignalP-3.0	Bendtsen <i>et al.</i> ⁴⁵
Signal Peptide	Phobius	Käll <i>et al.</i> ⁴⁴
Signal Peptide	UniProt	UniProt Consortium ¹⁴
Transit Peptide	UniProt	UniProt Consortium ¹⁴
Peroxisomal Targeting	PeroPS/PTS1 (metazoan)	Neuberger <i>et al.</i> ^{46,47}
Short Sequence Motif	UniProt	UniProt Consortium ¹⁴
GPI Anchor	BigPI3.2 (metazoa)	Eisenhaber <i>et al.</i> ^{48–51}
N-Myristoylation	MyrPS/NMT	Maurer-Stroh <i>et al.</i> ^{52,53}
Farnesylation	PrePS-FT	Maurer-Stroh <i>et al.</i> ^{54,55}
Geranylgeranylation	PrePS-GGT1	Maurer-Stroh <i>et al.</i> ^{54,55}
Rab Geranylgeranylation	PrePS-GGT2	Maurer-Stroh <i>et al.</i> ^{54,55}
Modified Residue	UniProt	UniProt Consortium ¹⁴
Low Complexity	SEG (windows 12&25)	Wootton ⁵⁶
Disorder	IUPred (long disorder)	Dosztányi <i>et al.</i> ⁵⁷
Charge runs	SAPS (charge clusters)	Brendel <i>et al.</i> ⁵⁸
Pfam Domains	HMMER3 against Pfam	Finn <i>et al.</i> , ¹⁷ Eddy ⁵⁹
PDB Structures	Ssearch against PDB	Berman <i>et al.</i> , ¹⁶ Farrar, ⁶⁰ Pearson and Lipman ⁶¹

of the ANNOTATOR suite^{40,41} and the ANNIE tool⁴² which are also publicly available. These software tools pioneered the compact graphical, cartoon-based presentation of diverse structural and functional predictions for query protein sequences. Additionally, HPMV uses the ANNOTATOR as backend engine for the sequence-based predictions and annotations. With two examples, we show how mutations can affect the occurrence and/or the properties of sequence architectural features and how such findings open a mechanistic way towards explaining the phenotypic effect of mutations.

Notably, the analysis of the biomolecular mechanistic implications of non-synonymous mutations is complicated. Currently, it cannot be automated well since it involves many diverse manual interventions depending on the nature of the affected protein sequence segment and it also requires consultations of the specific scientific literature about related biological and medical observations. We think that having the mutations mapped to sequence architectural features and illustrating the findings with intuitively designed cartoons allows quick screening of larger sets of mutations and selecting the few that deserve more detailed attention.

2. Results

2.1. HPMV functionality — input options

The most basic input to HPMV is human protein mutations. To specify the protein mutations directly, each input line starts with a (UniProt) protein accession code

Table 2. Mutation formats recognized by HPMV.

Mutation	Examples
Point	A123L p.A123L p.Ala123Leu
Nonsense	A123* p.A123* p.Ala123Ter
Deletion (Single)	p.A123del p.Ala123del
Deletion (Region)	p.A123_C124del p.Ala123_Cys124del
Duplication (Single)	p.A123dup p.Ala123dup
Duplication (Region)	p.A123_C124dup p.Ala123_Cys124dup
Insertion (Sequence)	p.A123_C124insQSK p.Ala123_Cys124insGlnSerLys
Insertion (Length)	p.A123_C124ins3 p.Ala123_Cys124ins3
Deletion with Insertion (Single)	p.A123delinsWV p.Ala123delinsTrpVal
Deletion with Insertion (Region)	p.A123_C124delinsW p.Ala123_Cys124delinsTrp
Frameshift (Plain)	p.A123fs p.Ala123fs
Frameshift (Alternate)	p.A123Gfs p.Ala123Glyfs
Frameshift (with Stop)	p.A123Gfs*26 p.Ala123GlyfsTer23
Extension Upstream	p.M1ext-5 p.Met1ext-5
Extension Downstream	p.*110Gext*17 p.Ter110GlnextTer17

followed by a set of mutation specifiers in the same input line (i.e. one or several mutations for the same protein). Several protein sequences (each being one input line) can be processed in one go. Essentially all of the HGVS mutation nomenclature⁶² is accepted. An example list of possible mutation formats is provided in Table 2. But HPMV also processes arbitrary labels where the first (or only) number in the label is interpreted as the sequence position. An example of accepted input lines are:

```
sp|Q5VW32|BROX_HUMAN p.K283E p.Cys408Tyr p.Leu175_His225del
or
Q9C000 M77?
```

HPMV also maps genome coordinates to UniProt entries (including alternate isoforms) on a best-effort basis — for example, when the corresponding Ensembl and UniProt records do not have major discrepancies. The mappings are parsed from the relevant UniProt and Ensembl¹³ data files. HPMV reads genome coordinates in a format of one genome coordinate per line and allows the user to specify column order and choose from a variety of token separators accommodating, for example, VCF format.

Other websites can also link directly to HPMV cartoons with a simple URL, for example,

```
http://hpmv.bii.a-star.edu.sg/SeqLink?acc=P05067&mut=V123L.
```

Also, for proteins not included in the UniProt human proteome, such as proteins from other organisms, the sequence features for the cartoon can be calculated on-the-fly just from the FASTA sequence.

2.2. HPMV functionality — output: the sequence architecture cartoon

HPMV’s main output is an interactive cartoon — provided as both an interactive (JavaScript) web page and as a standalone (Java) application. A screenshot of the

Fig. 1. Interactive cartoon for protein PDE6B along with the L854V mutation. The feature tree has been expanded to show information for the predicted C-terminal prenylation that may be disrupted by the L854V mutation. The full name of PDE6B is “Rod cGMP-specific 3',5'-cyclic phosphodiesterase subunit beta” (UniProt: sp|P35913|PDE6B.HUMAN).

interactive web page is shown in Fig. 1. Clicking on a particular sequence feature in the cartoon image at the top of the page will provide additional information in an expandable tree format at the bottom of the page. For sequences that contain conserved globular domains (e.g. from Pfam), a multiple sequence alignment for the domain can be displayed. For sequences that have a mapping to known 3D structures, a simple wire frame view of the structure can also be displayed showing the location of mutation(s) on the structure. Directly below the cartoon image is a multiple sequence alignment of similar sequences from other organisms. In cases, where a mutation is likely to have straightforward interpretation, this interpretation is suggested in a highlighted text box just above the expandable feature tree.

Designing a graphical representation of sequence features that occur along a sequence presents significant challenges. On one hand, laying out everything in a single band can become very crowded causing essential information to become buried under other elements. On the other hand, giving each type of sequence feature its own band can result in an image that is too large for many uses. By designing the HPMV

Table 3. Color code for functional effect of mutations.

Functional effect	Color	Meaning
LIKELY	Red	...is likely to affect...
MORE LIKELY	Orange	...could potentially affect...
LESS LIKELY	Cyan	...is less likely to affect...
UNLIKELY	Blue	...is unlikely to affect...
UNCERTAIN	Green	...has uncertain/unknown effect...
NONE	Black	...has not been interpreted...

cartoon around a carefully chosen set of six bands (described below), we hope to have found an optimal medium.

Briefly, the first band, “Mutation”, shows the user specified mutations of interest — the difference between the query protein and the wildtype reference protein. The color code suggests the likelihood to affect the respective functional feature (Table 3).

The second band, “Variants”, displays natural variants (red: specific annotation — usually disease variant; blue: dbSNP variant — without additional annotation; green: no information available).

The third band, “Targ&Mod”, visualizes certain non-globular segments such as targeting signals, PTMs, and coiled-coil and transmembrane segments. The fourth band, “NonGlob”, shows other non-globular features relating to sequence composition including charge clusters, low complexity regions, and regions predicted to be disordered. The fifth band, globular “Domains”, indicates matches within the sequence to known conserved domains. Finally, the sixth band, “3DStruct”, shows any PDB structures with significant sequence similarity to the query sequence (highest E-value being overlaid on top).

In the case of multi-sequence input, the user is first shown the cartoons generated for all sequences in one view (Fig. 2). Then, additional information is accessible by opening separate windows for each sequence of interest that can be navigated as in the case of single protein input (see also Supplementary Figs. S1 and S2 provided as additional files).

The cartoon image can be exported in PNG format. A key feature of this export is that the width/horizontal-resolution of the image can be specified in terms of pixels per residue. This allows cartoons for sequences of different length to all be printed with the same scale.

2.3. HPMV functionality — output: the sequence feature tree

Details for the sequence features shown in the top cartoon image can be accessed by expanding the Feature Tree in the lower panel (Fig. 1). The first branching level of the tree corresponds to the six bands in the cartoon image. The next branching level shows sequence features within that band followed by a branch level for the individual algorithms that predicted the feature. The final branch level shows details of

Fig. 2. Interactive cartoon for multiple protein input each having several mutations. This screenshot shows the primary output, the cartoons, for the input lines “sp|P04156|PRIO_HUMAN L9R A117E S230L” and “sp|P12931|SRC_HUMAN G2R Y530F”. The detailed feature tree can be seen individually for each protein by opening a new window. The respective views are captured in the Supplementary Figs. S1 and S2 provided as additional files.

the algorithm predictions, such as scores, and any additional information associated with the function prediction. The feature tree can be sequentially opened and closed but also be fully opened and closed by a one-button click.

For conserved domains, clicking the “MSA” button will show a multiple sequence alignment (MSA) for that domain. To note, there is no automated jump between the sequence coordinate in the cartoon and in the MSA (this is a browser version issue; so, HPMV is compatible with many older versions). The problem is caused by gaps/insertions in the alignment in comparison with the query sequence. By setting the width of the cartoon to “full-sequence”, approximate alignment can be achieved.

For 3D structures, clicking the “3D” button will show a simple rotatable wireframe representation of the PDB structure — with labels for the positions of any mutations that happen to overlap the structure.

An interpretation of the effect of the mutation is suggested as part of the feature tree. The comments hint on the emergence or disappearance of a feature as a result of the mutation as well as possible changes of scores. In the case of mutations hitting globular domains with known structures, the structure visualization together with the MSA can be used to judge whether the mutation affects the hydrophobic pattern typical for the fold or any of the conserved functional sequence regions.

2.4. HPMV functionality — output: alignment and sequence conservation

The conservation MSA for the middle panel of the interactive cartoon was constructed by using ggsearch^{60,61} to find the best global alignment match (where it existed) in each proteome from a diverse range of organisms. Then, these sequences were aligned with mafft-ginsi.⁶³ All the MSAs can be colored according to sequence conservation (blue or heat map) or the standard Clustal color scheme.

2.5. HPMV functionality — output: sequence feature prediction

The algorithms used for predicting the cartoon's sequence features are listed in Table 1. They are either developed within our research division (for example, for lipid PTMs⁵⁰) or they are contributed by the academic community. The specific prediction scores from the algorithms can be accessed by expanding the tree view of the sequence feature information. The computations are run on a cluster managed by the PBS/torque cluster management system. For proteins that are part of the UniProt human proteome, sequence features and conservation MSAs are precalculated.

For conserved domains and PDB structures, redundancy is a major challenge. For the conserved domains, using only Pfam domains¹⁷ rather than all domains in CDD was judged to be the best solution. For PDB structures, redundancy was reduced by using a filtering scheme inspired by PISCES.⁶⁴

HPMV also imports certain features from the UniProt annotations as listed in Table 1. UniProt gives the position of the features relative to the canonical isoforms so HPMV performs its own mapping to also show the correct locations of the features on the alternate isoforms.

2.6. HPMV implementation: HTML4-compliant webserver version and the standalone Java application

Most of the protein features that HPMV shows and analyzes are obtained from running feature prediction algorithms on the protein as well as on mutant sequences. The protein features for the (wild type) UniProt sequence are pre-calculated and, for interpretations, the feature prediction algorithms are run again for the mutant sequence when it is submitted to the HPMV website by a user. Protein function is likely to be affected if the mutation affects whether the protein feature is predicted to occur in the wild type versus the mutant sequence.

The HPMV server is one of BII's WWW functionalities backed by 512 core compute cluster. When a user submits mutation to the web server, a compute client is submitted to the job queue in the cluster. The compute client runs the prediction algorithms, parses them and packages them up as Java objects, connects back to the web server, and sends back the Java objects using the java serializable protocol. The HPMV web server combines the mutation predictions with its precalculated features for the wild type sequence (stored in custom indexed data files on a solid-state

drive) — and then provides the interpreted data to the user as its interactive cartoon. Ideally, as HPMV becomes widely used, the biological database updates will be synchronized with UniProt releases (essentially, every four weeks). But, for our own use, we will need to update the database at least every six months.

In addition to the HTML4-compliant webserver version, the interactive cartoon is also available as a standalone Java application. The interactive cartoon can contact the HPMV web server for data interactively but it can also be embedded with the data for a user-specified set of protein mutations (using Java serialization and jar file technologies). This embedding allows the interactive cartoons to be saved and viewed later using only the standard Java Runtime Environment. The standalone Java application has been verified to run correctly on the three major operating systems: Windows, MacOS, and Linux.

The standalone application also has some extra interactive features for displaying the MSAs. The conservation MSA scrolling is coupled to the gray bar at the top of the cartoon image — showing where the displayed alignment falls relative to the cartoon. Selecting a particular sequence in the MSA shows the numbering for that sequence. Sliders provide more compact and convenient views of the MSA. The vertical slider incrementally removes redundant sequences from the MSA. The horizontal slider incrementally compresses large gaps in the MSA.

2.7. HPMV application: Variant L854V in protein PDE6B

HPMV includes a set of feature prediction algorithms that were developed previously within our research group. The following examples highlight the utility of these algorithms for human mutation interpretation. The mutation shown in Fig. 1, L854V in protein PDE6B, is known as a cause of Retinitis Pigmentosa 40 (RP40).⁶⁵ It maps into the non-globular segment (840–854) responsible for S-geranylgeranylation. This mutation is predicted by the published PrePS webserver⁵⁴ to alter an S-geranylgeranyl PTM motif by switching its specificity to S-farnesylation instead (the corresponding interactive cartoon can be found on the HPMV website as both a web page link and a downloadable jar file). The specific lipid anchor type may play a role in proper localization and complex formation of PDE6B at the membrane.⁶⁶

2.8. HPMV application: Variant Δ S26 in protein XRP2

The single residue deletion Δ S6 maps into the non-globular segment 1-17 of XRP2 (sp|O75695|) responsible for N-terminal myristoylation (Fig. 3). This mutation is predicted by the published MyrPS/NMT webserver as abolishing myristoylation. It is thought that the failure to myristoylate the protein at Gly-2 will lead to weak interaction with intracellular membranes and to inefficient palmitoylation at Cys-3 (see the HPMV website for a downloadable jar file or link to the corresponding interactive cartoon). Thus, improper subcellular localization⁶⁷ appears to be the biomolecular mechanism that causes Retinitis Pigmentosa 2 (RP2) in this case.^{67–70}

Fig. 3. Interactive cartoon for protein XRP2 along with the variant Δ S26. The feature tree has been expanded to show information for the predicted N-terminal myristoylation that is disrupted by the Δ S26 mutation. The protein XRP2 (O75695) acts as a GTPase-activating protein (GAP) involved in intracellular protein trafficking and protein localization. Thus, the lipid anchor is important for correct functioning.

3. Discussion

HPMV is intended to fill a niche between initial coarse filtering of the full list of variants generated by next-generation sequencing and detailed manual analysis of one or two variants of particular interest. The idea is that an investigator can submit a few dozen variants to HPMV and by a quick and easy inspection of each cartoon arrive at a short list of one or two variants of particular interest for further study.

As general biological databases become increasingly detailed, there is an emerging need to present the most relevant information for a particular task. By focusing specifically on the task of human mutation interpretation and by leveraging our extensive expertise in computational protein function prediction, HPMV is able to present a selection of sequence features where the likely impact of a mutation can be assessed with only a quick glance at the cartoon. These sequence feature prediction algorithms are run uniformly across all sequences insuring a high level of consistency.

In the future, we intend to continue to enrich the sequence architectural features and interpretations that are provided by HPMV. High on our list are a more

complete set of transmembrane predictors,^{71–74} the concept of simple and complex⁷⁵ as well as enrichment with many more short motifs known to have functional significance (such as Huf-Zinc⁷⁶). We also intend to develop, with guidance from our users, capabilities that allow HPMV to be more easily integrated into clinical genetics workflows.

4. Conclusions

The HPMV is an efficient software tool to relate human protein sequence mutations to sequence architectural features and to study their influence on mechanistic, segment-specific functional properties of proteins.

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