In silico prediction of the effects of mutations in the human mevalonate kinase gene: towards a predictive framework for mevalonate kinase deficiency

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ABSTRACT

Mevalonate kinase (MVK) catalyses the phosphorylation of mevalonate. Deficiency of MVK is associated with two rare periodic fever syndromes, mevalonic aciduria (MA), a severe form and hyper-Immunoglobulin-D syndrome (HIDS), a milder form. An in silico approach was used to analyse the physicochemical and structural effects of 47 disease-associated variants of MVK. A further 20 variants, which are present in human genome databases, were also analysed. Variants associated with MA are clustered into a "hotspot" consisting of residues 8-35 and 234-338 and tended to result in a prediction of severely reduced protein stability. Four of the uncharacterised variants, p.H24P, p.G198R, p. R253W and p.G335S were likely to be associated with MA. This method could be used as the basis for initial predictions of severity when new MVK variants are discovered.

Keywords: periodic fever syndrome; mevalonic aciduria; hyper-Immunoglobulin-D syndrome; HIDS; protein stability; disease prediction

INTRODUCTION

Mevalonate kinase (MVK) deficiency is associated with a wide range of systemic diseases ranging from the most severe form, mevalonic aciduria (MA; OMIM#610377) to a milder form, hyper-immunoglobulin D syndrome (HIDS; OMIM#60920) (Drenth et al. 1999, Haas & Hoffmann 2006, Houten et al. 2000b). These rare autosomal recessive disorders are caused by a mutation in the MVK gene, which is found on the long arm of chromosome 12 (12q24) (Haas & Hoffmann 2006, Houten et al. 2000b, Houten et al. 2000a). Mevalonate kinase (EC 2.7.1.36) is an essential enzyme in the cholesterol pathway (Buhaescu & Izzedine 2007). It is a dimeric protein that catalyses the ATP-dependent phosphorylation of mevalonate to 5-phosphomevalonate (Potter & Miziorko 1997). This step follows the one catalysed by the highly regulated enzyme and statin target, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.34). Although there is no effective treatment for MVK deficiency, preliminary evidence has shown that Simvastatin, an HMG-CoA reductase inhibitor, may be useful in treating inflammatory attacks in HIDS (Simon et al. 2004).

MVK is part of the GHMP family of kinases (Bork et al. 1993, Timson 2007). Each subunit has two domains with the active site located in a cleft at the domain interface (Yang et al. 2002, Fu et al. 2002, Sgraja et al. 2007, Fu et al. 2008). The enzyme is dimeric. Thus, mutations causing amino acid changes at the dimer interface may disrupt dimer formation leading to an inactive or unstable protein (Fu et al. 2002). The crystal structure of rat mevalonate kinase in complex with MgATP revealed that an Mg^{2+} ion is co-ordinated by both β- and γ-phosphates of ATP and side chains of Glu-193 and Ser-146 (Fu et al. 2002). Asp204 was found to make a salt bridge with Lys-13, which interacts with the γ-phosphate (Fu et al. 2002). Lys-13 affects the pK_a of the C5 hydroxyl of mevalonate, while Asp-204 abstracts the proton from this hydroxyl. The resulting penta-coordinated γ-phosphoryl group may be stabilized by Mg^{2+}, Lys-13, and Glu-193 (Fu et al. 2002). The structure of the human MVK enzyme is very similar to the rat one (rmsd 0.81 Å) and the mechanism of reaction is expected to be identical with Asp-204 acting as a base in the active site (Fu et al. 2008).

MA and HIDS are rare autosomal recessive disorders characterised by recurrent periodic fevers and generalised inflammation (Haas & Hoffmann 2006, Frenkel et al. 2000). Typical symptoms of the severe form, MA, can include psychomotor retardation, ataxia, failure to thrive, dysmorphic features with recurrent episodes of fevers, lymphadenopathy and rashes (Frenkel et al. 2000, van der Burgh et al. 2013). These symptoms typically present in infancy and patients usually die in childhood (Frenkel et al. 2000). Suffers of MA can be confirmed based on their biochemical, clinical and genetic data (Drenth et al. 1999). The residual activity of MVK in MA sufferers is below 0.5%, leading to a characteristic build-up of mevalonic acid (Prietsch et al. 2003).

HIDS is a milder phenotype of MVK deficiency with more documented cases (Haas & Hoffmann 2006, Frenkel et al. 2000, van der Burgh et al. 2013, Grose 2005). Symptoms usually present in the first year of life and include recurrent lifelong episodes of fever, rashes, abdominal pain and lymphadenopathy (Haas & Hoffmann 2006, Frenkel et al. 2000, McDermott & Frenkel 2001, Haas et al. 2001, Hoffman et al. 2001). A high level of IgD and IgA accompanies these symptoms and is often used as a diagnostic confirmation of HIDS (Drenth et al. 1999, Houten et al. 2000b, Buhaescu & Izzedine 2007). Moreover the residual activity of MVK in HIDS is around 1-20% (Houten et al. 2000b). HIDS can be distinguished from MA by the lack of abnormal levels of mevalonic acid present as well as the lack of neurological manifestations which feature in MA (Hoffman et al. 2001). Furthermore unlike individuals with MA, people suffering with HIDS do not experience any symptoms between fever episodes and typically have a normal life expectancy (Haas & Hoffmann 2006).

The most frequent mutation associated with HIDS is c.1129G>A (rs28934897) resulting in an amino acid change from valine to isoleucine at position 377 (p.V377I) (Cuisset et al. 2001). This is found in approximately 80% of HIDS suffers and has never been reported in MA (Cuisset et al. 2001). MVK deficiency is particularly common in northern Europe (especially the The Netherlands and France) but cases have been reported worldwide (Prietsch et al. 2003). Approximately 80 mutations have been reported to cause mevalonate kinase deficiency (Haas & Hoffmann 2006).

Overlapping phenotypes of MA and HIDS have been observed in an intermediate form, although few cases have been documented (Hoffman et al. 2001). However it is important to note that the relationship between mutation and reported phenotype may not always be straightforward. Phenotypes are classified based on individual clinical observation and can be influenced by environmental factors; for example, the symptoms of MVK deficiency appear to be amplified by increased temperature (Houten et al. 2002, Tricarico et al. 2013).

It is not known precisely how each disease-associated mutation alters the enzyme structure or how this contributes to the different levels of severity or pathology. It is assumed that the residual activity of MVK determines whether or not a patient has MA or HIDS, therefore a mutation associated with the severe form must disrupt the enzyme's structure and function significantly to decrease its activity below 0.5%. At the biochemical level this is likely to result from effects on protein folding and stability. A bioinformatics approach was taken to understand the functional and structural contribution of molecular alterations in MVK and how these correlate to the associated severity. This was then applied to variants of MVK whose disease association is currently unknown. The approaches can be broadly arranged into three approaches: sequence and evolutionary conservation based methods, protein sequence and structure-based methods, and supervised learning methods. Similar approaches have been successful in the investigation of type I galactosemia (galactose 1-phosphate uridylyltransferase deficiency), type III galactosemia (UDP-galactose 4'-epimerase deficiency), hyperargininemia (arginase 1 deficiency) and apparent mineralocorticoid excess (11β-hydroxysteroid dehydrogenase type 2 deficiency) to provide the basis for predicting the severity of newly discovered mutations (Carvalho et al. 2012, Manning et al. 2010, Facchiano & Marabotti 2010, d'Acierno et al. 2009, d'Acierno et al. 2014, McCorvie & Timson 2013).

MATERIALS AND METHODS

Datasets, sequence information and data analysis

Literature searches were used to identify disease-associated variants. Where available, the clinical symptoms, observed residual activity of the MVK variant, any biochemical analysis (including any experimental conditions), environmental factors and the genetic background were noted. Characterised variants and uncharacterised missense mutations from exome sequencing were identified from the databases: NCBI dbSNP (http://www. ncbi.nlm.nih.gov/SNP/), UniProt (http://www.uniprot. org), and Infevers (http://fmf.igh.cnrs.fr/ISSAID/ infevers/). Excel (Microsoft) and GraphPad Prism 5.0 (GraphPad Software) were used to record scores, perform statistical analysis and obtain graphical representations of the results.

Secondary and tertiary structural investigations

Structural studies were based on the crystal structure of human MVK (PDB: 2R3V) (Fu et al. 2008). Structures were visualised using PyMol (http://www.pymol.com) and were computationally solvated and energy minimized using YASARA (http://www.yasara.org) (Krieger et al. 2009). Sequence variations were introduced in silico using the Mutate function in PyMol and these variant forms of the protein were minimised using YASARA. Thus, all structural comparisons were made using minimised structures. Each variant's structure was further analysed using LS-SNP/PDB (http://ls-snp.icm.jhu.edu/ls-snp-pdb/main). This tool provided solvent accessibility scores, variant's position at domain interface, location in the 3D structure, if it is exposed or buried, the secondary structure where the variant is found and a 3D representation of the variant in the structure. The GETAREA server (http://curie.utmb.edu/ getarea.html) was used to determine the change in surface and buried atoms from the variant compared to the wild type (Fraczkiewicz & Braun 1998).

Prediction of the physicochemical effect of each missense mutation on MVK structure, stability and function

Bioinformatics tools were used to investigate a range of physicochemical effects of each variant on MVK's structure and function. The following tools were used to estimate the change in protein stability: I-Mutant 3.0 Binary and Ternary classification (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) (Capriotti et al. 2005, Capriotti et al. 2006); mCSM (http://bleoberis.bioc.cam.ac.uk/mcsm/) (Pires et al. 2014); SDM score (http://mordred.bioc.cam.ac.uk/~sdm/sdm.php) (Worth et al. 2011, Worth et al. 2007); Mupro (http://mupro.proteomics.ics.uci.edu/) (Cheng et al. 2006); iStable score (http://predictor.nchu.edu.tw/iStable/) (Chen et al. 2013); PredictSNP 1.0 (http://loschmidt.chemi.muni.cz/predictsnp/) (Bendl et al. 2014); Meta-SNP (http://snps.biofold.org/meta-snp/) (Capriotti et al. 2013); KD4V score (http://decrypthon.igbmc.fr/kd4v) (Luu et al. 2012).

The tools used to estimate the change in free energy resulting from the point mutations (all calculated at pH 7.5, 30 °C) were: I-Mutant 3.0 DDG, SVM3 and SVM2 values (Capriotti et al. 2006); Fold-X DDG value (http://foldx.crg.es) (Schymkowitz et al. 2005); PoPMuSiC 2.1 (http://dezyme.com/) (Dehouck et al. 2011); CUPSAT DDG ((http://cupsat.tu-bs.de) (Parthiban et al. 2006, Parthiban et al. 2007); iStable DDG (Chen et al. 2013); GETAREA energy change (http://curie.utmb.edu/getarea.html) (Fraczkiewicz & Braun 1998).

The tools used to predict change in binding affinity were: mCSM Protein-Protein affinity prediction (Pires et al. 2014); BeAtMuSiC binding affinity prediction and score (http://babylone.ulb.ac.be/beatmusic) (Dehouck et al. 2013). The tools used predict the change in solvent accessibility were: PoPMuSiC 2.1 (Dehouck et al. 2011); CUPSAT RSA ((http://cupsat.tu-bs.de) (Parthiban et al. 2007); SDM score (Worth et al. 2011, Worth et al. 2007); BeAtMuSiC (Dehouck et al. 2013).

In addition, further physicochemical analysis was carried out using SNP effect 4.0 server (http://snpeffect.switchlab.org) (De Baets et al. 2012) which uses four programs to predict the biochemical effects of missense mutations; TANGO (http://tango.crg.es/) (Fernandez-Escamilla et al. 2004) which predicts the tendency to aggregate; Fold-X (Schymkowitz et al. 2005) which predicts changes in stability, WALTZ (http://www.switchlab.org/bioinformatics/waltz) (Maurer-Stroh et al. 2010) which predicts the tendency to form amyloids and LIMBO (http://www.switchlab.org/bioinformatics/limbo) (Van Durme et al. 2009) which predicts the ability to bind chaperones. A estimation of the change in hydrogen bond satisfaction was also carried out using SDM (Worth et al. 2011, Worth et al. 2007). The KD4V server (Luu et al. 2012) provided additional scores in the change in size, charge polarity, modifications and hydrophobicity. Change in hydrophobicity scores were calculated using data from (Black & Mould 1991).

Multiple sequence alignment and evolutionary conservation based methods

Multiple sequence alignment was carried out using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers et al. 2011) from sequences gathered from UniProt database (http://www.uniprot.org). Only those classified as MVK and with “reviewed” status were used. A total of 19 sequences were obtained, which included one from yeast, four from vertebrates, one from slime mould, one from plants, one from Archaea and eleven from eubacteria. The resulting alignment (Supplementary Figure S1) was used with the Scorecons server (https://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/valdar/scorecons_server.pl) based on the Valdar01 score (Valdar 2002). The alignment was also used to calculate the degree of tolerance of each amino acid alteration using the SIFT server (http://sift.jcvi.org/) (Kumar et al. 2009) and the PROVEAN server (http://provean.jcvi.org/index.php) (Choi et al. 2012), both of which are based on updated versions of Ensembl gene annotation (GRCh37 Ensembl 66) and NCBI dbSNP database (Build 137).

Additional investigations of the tolerance to change at positions which are altered in the disease-associated variants was carried out using the tools: LS-SNP/PDB (http://ls-snp.icm.jhu.edu/ls-snp-pdb/) (Ryan et al. 2009); SNPs&GO (http://snps.biofold.org/snps-and-go/pages/help.html) (Calabrese et al. 2009); PhD-SNP (http://snps.biofold.org/phd-snp/phd-snp.html) (Capriotti et al. 2006); PANTHER (http://www.pantherdb.org/tools/csnpScoreForm.jsp) (Brunham et al. 2005); GenMAPP (http://www.genmapp.org) (Salomonis et al. 2007); PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei et al. 2010, Adzhubei et al. 2013); nsSNP Analyzer (http://snpanalyzer.uthsc.edu) (Bao et al. 2005); FI mutation assessor (http://mutationassessor.org/v1) (Reva et al. 2011); KD4V (Luu et al. 2012); YALE MU2A (http://krauthammerlab.med.yale.edu/mu2a) (Garla et al. 2011).

Supervised learning methods for combined overall predictions of each variant severity

Supervised learning methods that include a variety of bioinformatics tools to compare variants and provide an overall prediction on the effect of the variant on the protein's structure, function and pathology were used. Programs that provided this overall score, predicting if the mutation was disease causing, damaging, destabilizing or deleterious were: I-Mutant 3.0 Prediction of Disease (Capriotti et al. 2006); SIFT (Kumar et al. 2009); PROVEAN prediction (Choi et al. 2012); PolyPhen 2 prediction (Adzhubei et al. 2013); mCSM prediction (Pires et al. 2014); SNAP prediction (https://www.rostlab.org/services /snap/) (Bromberg & Rost 2007); PANTHER (Brunham et al. 2005); PhD-SNP predictor (Capriotti et al. 2006); nsSNP Analyzer (Bao et al. 2005); Mupro prediction (Cheng et al. 2006); PoPMuSiC 2.1 (Dehouck et al. 2011); CUPSAT (Parthiban et al. 2007); iStable (Chen et al. 2013); PredictSNP (Bendl et al. 2014); MAPP (Salomonis et al. 2007); Meta-SNP (Capriotti et al. 2013); FI mutation assessor (Reva et al. 2011); BeAtMuSiC (Dehouck et al. 2013).

RESULTS AND DISCUSSION

MVK alterations associated with MA tend to located in the central cleft, near the active site

Literature and database searches identified forty-seven MVK mutations for which there was sufficient biochemical and clinical information to be included in this study. Four of these were associated with the intermediate severity MVK deficiency, twelve with the severe form (MA) and the remainder with the mild form (HIDS). The location of the changes in the protein sequence showed that there was a relationship between the position of the variant and associated severity, with severe and intermediate mutations only occurring between amino acid positions 8-35 and 234-338 (Figure 1). These two sequences are located around the inside of the protein's cleft close to the active site and around the domain interface. Four of the variations associated with the severe form (p.L264F, p.L265P, p.I268H and p.N301T) occur in α-helices at the dimer interface. To our knowledge there is no published information on the effect of these (or other) mutations on the ability of MVK to dimerise; nor is there any information on whether, or not, dimerization is required for full activity of human MVK. However, MVK from some bacteria is monomeric, suggesting that the oligomeric state may have limited impact on activity (Hedl & Rodwell 2004, Voynova et al. 2004). The alterations associated with HIDS were more widespread throughout the MVK structure (Figure 1). The position of the variants in the 3D structure from LS-SNP/PDB showed that the majority of variants associated with severe and intermediate forms of the disease affected buried residues, while the variants associated with HIDS affected a mixture of buried, intermediate and exposed residues.

Computational analysis tends to place the MVK variants into two groups

All the results from the physicochemical analysis of the disease-associated and uncharacterised variants are given in Supplementary Table S1. There was a tendency to predict decreasing structural

stability with increasing severity of the disease-associated variants. The programs that gave this trend with were: I-Mutant DDG, solvent accessibility, FOLD-X, mCSM stability, Mupro, PoPMuSiC 2.1, BeAtMuSiC, CUPSAT (RSA), iStable DDG, GETAREA, SDM, BETAMUSIC, and KD4V. The change in hydrophobicity tended to increase with severity (data not shown). In the majority of cases, however, there was no statistical significance ($p>0.05$) when the three groups (mild, intermediate and severe) were compared. In general, alterations at more highly conserved residues were more likely to result in severe forms of MVK deficiency. This result was supported by predictions from: POLYPHEN-2, SAM, SCORECONS, PHD-SNP, SNP&GO, PANTHER, MAPP, FI-mutant and 4D4V WT residue representation. However, like the physiochemical scores, there was no significant difference between the scores when comparing the results from the three groups with the majority of the programs (data not shown).

Supervised learning methods predict effects on MVK stability using a combination of methods

Programs that used supervised learning methods for an overall prediction were used to predict each variant's effect on MVK's stability. It should be noted that this approach is based on the assumption that all the prediction tools are equally good. However, the percentage score provides an overall consensus which has been found to be more accurate than only using one or two tools (Zhao et al, 2014). The results in Supplementary Table S2 are based on 28 prediction tools and show that, in general, the percentage of tools predicting a reduction in MVK stability slightly increases with increasing severity. For each group the percentage predicting a decrease ranged from: 28.6-89.3 (mild), 53.6-89.3 (intermediate) and 64.3-92.9 (severe). Although there is an increasing trend, the discrimination between groups was not sufficient to make meaningful predictions about variants of unknown severity.

The intermediate form was not well detected by these methods

Across the three different prediction methods, the intermediate group was not well discriminated. In general it showed results similar to, or greater than, the severe group (Supplementary Table S1). This suggests that either these methods are not sufficiently discriminating or that the intermediate group would be better considered as part of the severe group. Combining these two groups enabled discrimination between the combined intermediate/severe group and the mild group on the basis of the physicochemical scores (Figure 2). Therefore, in developing a predictive framework, these two groups were considered together.

Towards a predictive framework: four key postulates

Based on the data (summarised in Supplementary Table S2), four postulates were made:

1. The decrease in enzyme activity is correlated with severity of disease. In the majority of cases, this decrease results from improper folding of the protein.
2. Changes to highly conserved residues are more likely to result in improper folding.
3. Intermediate mutations should be treated with the severe group due to their similarity to this group.
4. For a severe or intermediate phenotype to occur, the mutation is likely to cause a change in the "hotspot" region, identified from structural analysis, i.e. around residues 8-35 and 234-338 in the protein sequence.

Prediction of the likely clinical severity associated with uncharacterised variants

There are a number of variants which have been identified through genome and exome sequencing projects, but have not yet been characterised in terms of their associated disease severity. Applying the postulates above, four of these variants are predicted to be associated with the intermediate or severe form (MA). These are p.H24P, p.G198R, p.R253W and p.G335S (Supplementary Table S3). Three of these uncharacterised variants were predicted to cause either the mild form (HIDS) or no disease and the rest were predicted to be associated with HIDS (Supplementary Table S3). Experimental testing will be necessary to confirm these predictions. Molecular dynamics simulations may be valuable in explaining why disease-associated variants affect protein stability and would also be useful in investigating the properties of these uncharacterised variants. It is further hypothesised that the MVK variants which are predicted to be associated with MA will have very low activities and stabilities in vitro. It is also expected that cells homozygous for the corresponding mutations are likely to be deficient in the mevalonate pathway and will accumulate mevalonate.

Conclusions

The analyses presented here suggest that point mutations in the MVK gene which result in changes to the protein coding sequence subtly alter the structure and stability of MVK, thus affecting it activity. These effects are greater when the change occurs in a well-conserved residue. Variants associated with MA generally result in changes to the highly conserved "hotspot" region and also result in greater changes to stability and other biophysical parameters. In addition to providing a predictive framework, these results also suggest that pharmacological interventions to stabilise the MVK protein ("small molecule chaperone therapy") may be possible in the treatment of MA and the alleviation of the feverish episodes in HIDS.

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Figure legends

Figure1: Structure of human mevalonate kinase showing the location of changes resulting from disease-associated mutations. (a) The dimeric structure of human MVK showing the N- and C-termini and active site cleft of one of the subunits. (b) The sequence of human MVK (GenBank: NP_001107657) showing residues which when altered are associated with mild (green), intermediate (magenta) and severe (red) forms of MVK deficiency. (c) These residues mapped onto the three dimensional structure of a single MVK subunit (left). Each residue associated with disease is shown as a space-filled model. Those associated with the severe form (MA) are coloured red. Magenta indicates residues which are altered in the intermediate form and green residues altered in the mild form (HIDS). The "hot-spot" which includes all the alterations associated with MA is coloured blue in the right hand figure. Parts (a) and (c) were produced using PyMol and (PDB: 2R3V) (Fu et al. 2008).

Figure2: When combined, the intermediate and severe groups of variants show a statistically significant difference from the mild variants in the mean physicochemical score (p=0.038).

Supporting Information

Supplementary FigureS1: A sequence alignment generated in Clustal Omega (see Materials and Methods) of 19 verified MVK protein sequences. This alignment was used in analysis of sequence

Supplementary TableS2: The percentage of predictions from 28 prediction tools (see Materials and Methods) for MVK variants predicting a decrease, increase or neutral change in stability. Whether or not the residue occurs in the structural "hotspot" is also tabulated.

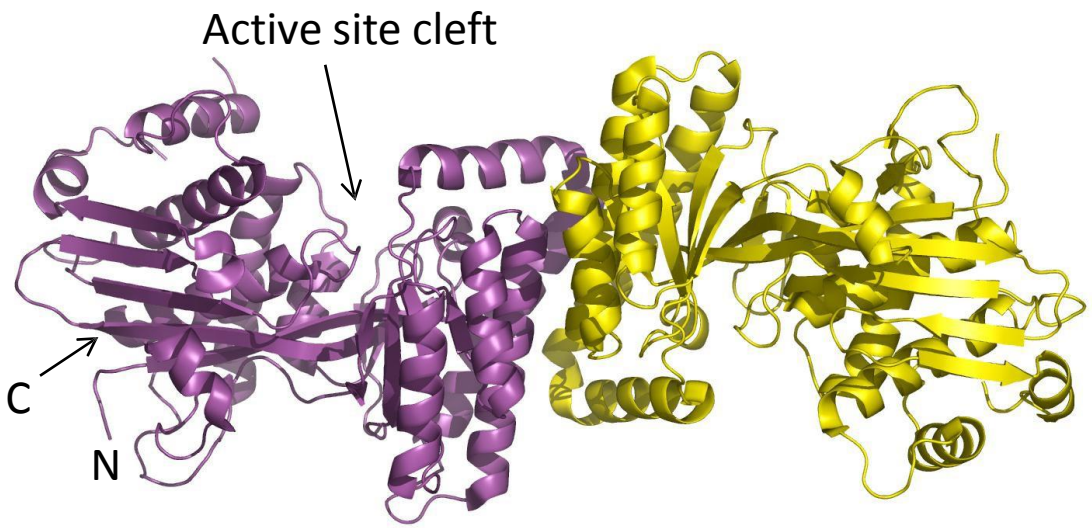
conservation.

Supplementary TableS1: The complete dataset from the various prediction tools used in this study.

Supplementary TableS3: Predicted severities associated with currently uncharacterised variants. Variants were predicted to be intermediate/severe (I/S), mild (M) and mild/neutral (M/N) based on whether, or not, the altered residue was in the "hotspot" and whether the variant was predicted to be substantially less stable than wild-type.

Supplementary TableS2: The percentage of predictions from 28 prediction tools (see Materials and Methods) for MVK variants predicting a decrease, increase or neutral change in stability. Whether or not the residue occurs in the structural "hotspot" is also tabulated.

(a)



(b)

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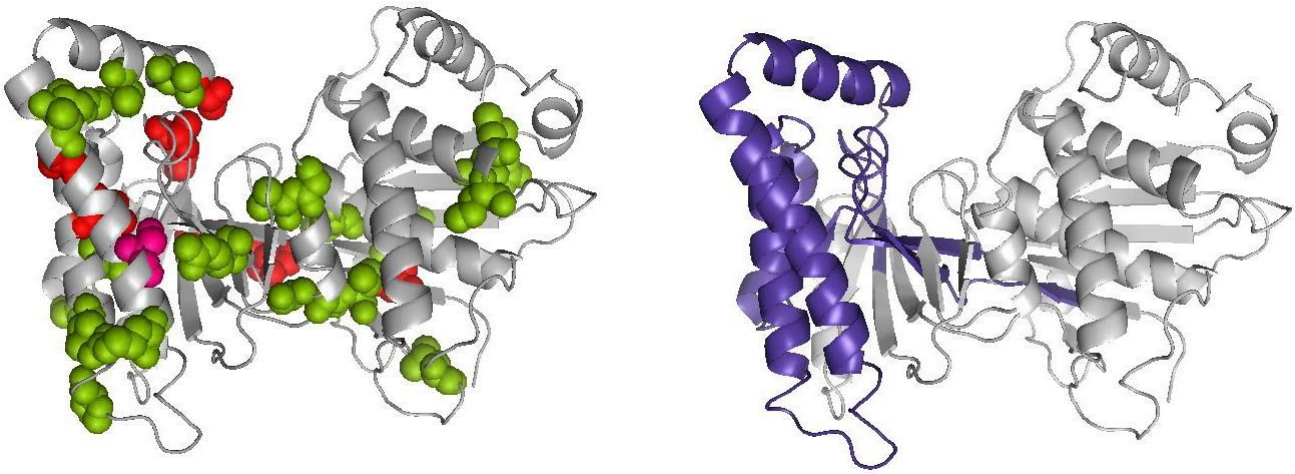
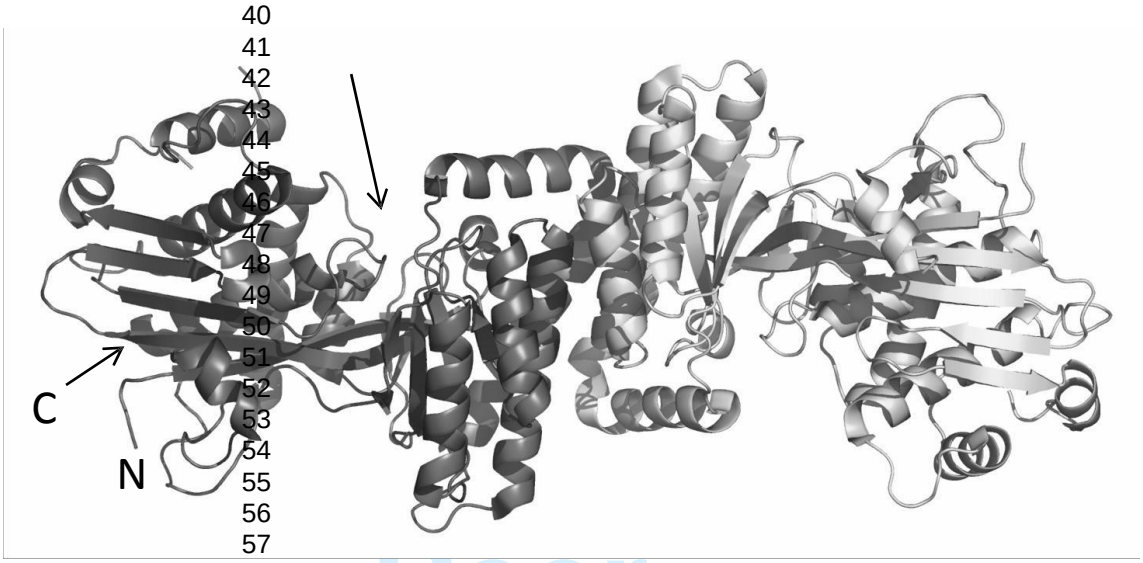
  1 MLSEVLLVSA PGKVILHGEH AVVHGKVALA VSLNLRTFLR LQPHSNGKVD LSLPNIGIKR
 61 AWDVARLQSL DTSFLEQGDV TTPTSEQVEK LKEVAGLPDD CAVTERLAVL AFLYLYLSIC
121 RKQRALPSLD IVVWSELPPG AGLGSSAAYS VCLAAALLTV CEEIPNPLKD GDCVNRWTKE
181 DLELINKWAF QGERMIHGNP SGVDNAVSTW GGALRYHQGK ISSLKRSPAL QILLTNTKVP
241 RNTRALVAGV RNRLLKFPEI VAPLLTSIDA ISLECERVLG EMGEAPAPEQ YLVLEELIDM
301 NQHHLNALGV GHASLDQLCQ VTRARGLHSK LTGAGGGGCG ITLLKPGLEQ PEVEATKQAL
361 TSCGFDCLET SIGAPGVSIH SATSLDSRVQ QALDGL
```

(c)



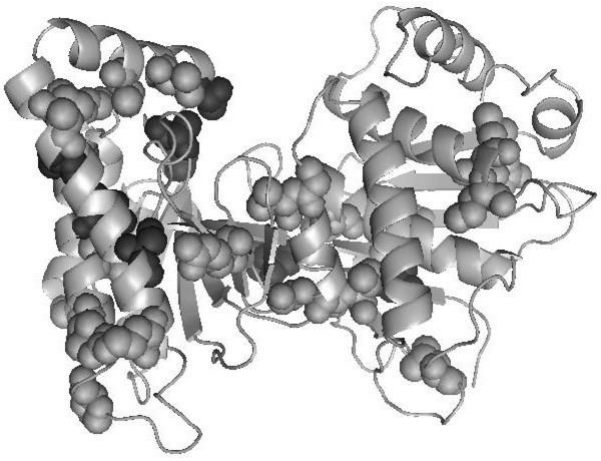
(a)

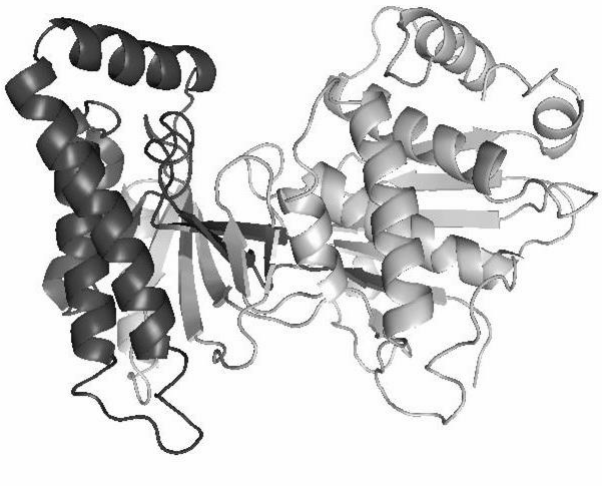


(b)

```
  1 MLSEVLLVSA PGKVILHGEH AVVHGKVALA VSLNLRTFLR LQPHSNGKVD LSLPNIGIKR
 61 AWDVARLQSL DTSFLEQGDV TTPTSEQVEK LKEVAGLPDD CAVTERLAVL AFLYLYLSIC
121 RKQRALPSLD IVVWSELPPG AGLGSSAAYS VCLAAALLTV CEEIPNPLKD GDCVNRWTKE
181 DLELINKWAF QGERMIHGNP SGVDNAVSTW GGALRYHQGK ISSLKRSPAL QILLTNTKVP
241 RNTRALVAGV RNRLLKFPEI VAPLLTSIDA ISLECERVLG EMGEAPAPEQ YLVLEELIDM
301 NQHHLNALGV GHASLDQLCQ VTRARGLHSK LTGAGGGGCG ITLLKPGLEQ PEVEATKQAL
361 TSCGFDCLET SIGAPGVSIH SATSLDSRVQ QALDGL
```

(c)





Active site
cleft

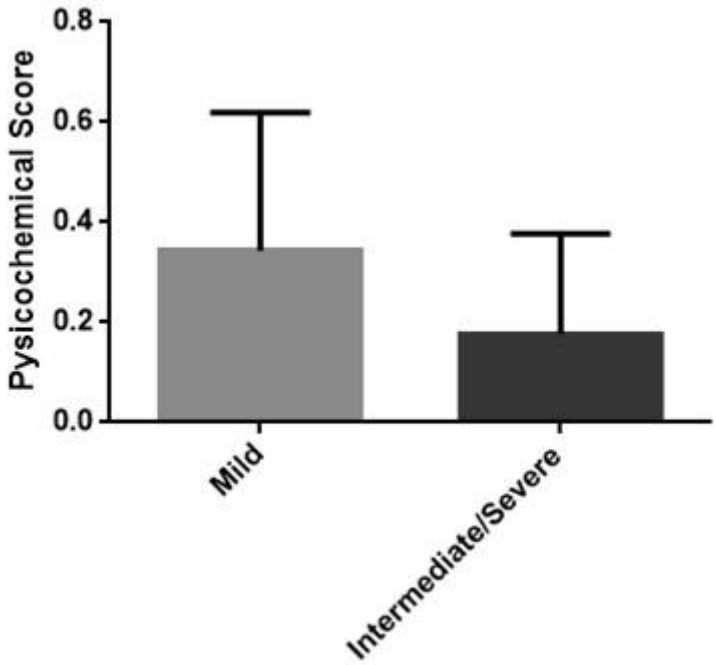

Pysicochemical Score
0.8
0.6
0.4
0.2
0.0
Mild
Intermediate/Severe

Table 4. Showing the results from all bioinformatic and structural analysis on known and uncatagorised varinats of MK

Point Mutati	Hydrophobic	I-Mutant 3.0	I-Mutant 3.0(	I-Mutant 3.0	I-Mutant 3.0	SIFT score	SIFT predictio	PROVEAN PR	PROVEAN pr	PolyPhen-2 s	PolyPhen-2 p	SAM conserv	Solvent acces	Location in 3	Secondary st	Domain Inter	phylopConsS	blosum45(Ya	Panther_Pde	LogR.E-value	pfam_eval_m	SCORECONS	FOLDX (stabil
HIDS																							
V377I	-0.118	-0.58	4 (Decrease)	3 (Large decr	6 (neutral)	0.149	Tolerated	-0.561	Neutral	0.331	Benign	0.3	0	Buried	Bend	N	-	-	-	-	-	0.548	-0.04
G309S	0.142	-0.91	5 (Decrease)	1 (Neutral)	5 (disease)	0	Damaging	-5.217	Deleterious	1	Probably dam	0.32	0.61	Exposed	Turn	Y	4.255	-2	0.83724	0.84724	0.00006	0.912	3.31
R215Q	-0.251	-0.85	8 (Decrease)	3 (Large decr	4 (disease)	0.109	Tolerated	-1.252	Neutral	0.925	Possibly dam	0.28	0.22	Intermediate	Beta Ladder	N	-	-	-	-	-	0.348	0.56
H20Q	-0.086	-0.17	3 (Decrease)	3 (Neutral)	5 (disease)	0	Damaging	-7.199	Deleterious	1	Probably dam	0.69	0.03	Buried	Turn	N	-	-	-	-	-	1	1.63
H20N	-0.071	-0.47	1 (Decrease)	2 (Neutral)	3 (disease)	0	Damaging	-6.304	Deleterious	1	Probably dam	0.69	0.03	Buried	Turn	N	-	-	-	-	-	1	2.37
L39P	0.232	-1.71	5 (Decrease)	4 (Large decr	6 (disease)	0.007	Damaging	-4.4	Deleterious	1	Probably dam	0.05	0.01	Buried	Beta Ladder	N	-	-	-	-	-	0.728	8.83
V132I	-0.118	-0.48	5 (Decrease)	4 (Large decr	7 (neutral)	0.301	Tolerated	-0.454	Neutral	0.035	Benign	0.29	0.07	Buried	Beta Ladder	N	-	-	-	-	-	0.399	1.54
A148T	0.166	-0.61	7 (Decrease)	2 (Large decr	3 (neutral)	0.003	Damaging	-3.21	Deleterious	1	Probably dam	0.73	0	Buried	Helix	N	4.559	1	0.19768	-0.38535	1.40E-20	0.796	1.61
P167L	-0.232	-0.9	8 (Decrease)	1 (Large decr	5 (disease)	0.559	Tolerated	-4.53	Deleterious	1	Probably dam	0.02	0.13	Intermediate	Turn	N	-	-	-	-	-	0.069	6.42
G171R	0.501	-0.56	5 (Decrease)	0 (Large decr	3 (disease)	0.15	Tolerated	-1.488	Neutral	0.298	Benign	0.04	0.76	Exposed	Turn	N	1.85	-2	0.10391	0.644612	1.50E-19	0.084	3.39
G202R	0.501	-0.56	4 (Decrease)	5 (Neutral)	7 (disease)	0	Damaging	-7.733	Deleterious	1	Probably dam	0.46	0.07	Buried	Bend	N	4.494	-2	0.92565	0.899885	2.70E-19	0.801	1.8
G211E	0.458	-0.6	2 (Decrease)	2 (Neutral)	7 (disease)	0	Damaging	-7.656	Deleterious	1	Probably dam	0.04	0.04	Buried	Coil	N	-	-	-	-	-	0.903	18.12
V250I	-0.118	-0.88	9 (Decrease)	6 (Large decr	6 (neutral)	0	Damaging	-0.93	Neutral	1	Probably dam	0.1	0	Buried	Helix	N	-	-	-	-	-	1	0.56
L265R	0.943	-1.65	8 (Decrease)	5 (Large decr	6 (disease)	0.002	Damaging	-4.657	Deleterious	1	Probably dam	0.24	0.05	Buried	Helix	N	3.211	-2	0.65837	-	-	0.631	4.65
G326R	0.501	-0.33	2 (Decrease)	5 (Neutral)	5 (disease)	0.17	Tolerated	-3.893	Deleterious	1	Probably dam	0.02	0.46	Exposed	Turn	N	4.255	-2	0.15811	0.608793	0.000026	0.622	2.14
G376V	-0.324	-0.43	5 (Decrease)	0 (Neutral)	2 (disease)	0	Damaging	-7.628	Deleterious	1	Probably dam	0.05	0	Buried	Coil	N	4.517	-	-	-	-	0.921	20.24
S135L	-0.584	-0.14	6 (Decrease)	3 (Neutral)	5 (disease)	0.001	Damaging	-5.499	Deleterious	1	Probably dam	0.05	0.13	Intermediate	Beta Ladder	N	-	-	-	-	-	0.909	-0.97
L117P	0.221	-1.49	5 (Decrease)	4 (Large decr	7 (disease)	0.084	Tolerated	-4.242	Deleterious	1	Probably dam-	-			Helix	-						0.393	9.66
I119M	0.205	-1.51	7 (Decrease)	4 (Large decr	0 (neutral)	0.003	Damaging	-2.225	Neutral	0.959	Probably dam	0.03	0.03	Buried	Helix	N	-	-	-	-	-	0.172	0.82
Y116H	0.715	-1.3	7 (Decrease)	3 (Large decr	6 (disease)	0.008	Damaging	-4.216	Deleterious	1	Probably dam	0.1	0	Buried	Helix	N	2.948	2	0.52761	-	-	0.46	3.73
G211A	-0.115	-0.91	5 (Decrease)	0 (Large decr	5 (disease)	0	Damaging	-5.689	Deleterious	1	Probably dam	0.04	0.04	Buried	Coil	N	2.704	-2	0.94854	0.866461	2.50E-19	0.903	7.6
V203A	0.209	-1.67	9 (Decrease)	5 (Large decr	0 (neutral)	0.002	Damaging	-3.8	Deleterious	1	Probably dam	0.78	0.11	Intermediate	Helix	N	3.735	-3	0.81093	2.012589	3.50E-18	0.712	0.96
L246P	0.221	-1.79	6 (Decrease)	6 (Large decr	7 (disease)	0.001	Damaging	-6.033	Deleterious	1	Probably dam	0.11	0.19	Intermediate	Helix	N	-	-	-	-	-	0.831	2.73
V261A	0.209	-1.7	9 (Decrease)	5 (Large decr	4 (neutral)	0.008	Damaging	-2.505	Deleterious	0.827	Possibly dam	-			Helix	-	-	-	-	-	-	0.526	2.1
A262P	-0.095	-0.06	6 (Increase)	3 (Large decr	6 (disease)	0.129	Tolerated	-1.212	Neutral	0.71	Possibly dam	-			Helix	-	-	-	-	-	-	0.436	3.55
R277C	-0.68	-0.8	4 (Decrease)	1 (Neutral)	5 (disease)	0.015	Damaging	-2.863	Deleterious	1	Probably dam	0.1	0.51	Exposed	Helix	N	2.099	-3	0.67461	-	-	0.507	1.01
R277H	-0.165	-1.22	9 (Decrease)	3 (Large decr	4 (disease)	0.069	Tolerated	-1.978	Neutral	0.231	Benign	0.1	0.51	Exposed	Helix	N	2.099	-	-	-	-	0.507	0.93
P288L	-0.232	-0.14	2 (Decrease)	6 (Large incre	1 (disease)	0.387	Tolerated	-1.875	Neutral	0.001	Benign	0.04	0.72	Exposed	Helix	N	-	-	-	-	-	0.236	1.37
V293M	0.087	-0.72	5 (Decrease)	1 (Neutral)	2 (neutral)	0.057	Tolerated	-0.975	Neutral	0.998	Probably dam	0.02	0.11	Intermediate	Helix	N	0.035	1	0.2051	0.685182	6.40E-06	0.394	0.13
I298T	0.493	-1.94	8 (Decrease)	2 (Large decr	5 (disease)	0.007	Damaging	-2.984	Deleterious	0.962	Probably dam-	-			Helix	-	-	-	-	-	-	0.725	2.39
V310L	-0.118	-0.85	7 (Decrease)	1 (Neutral)	1 (disease)	0	Damaging	-2.675	Deleterious	1	Probably dam	0.38	0.01	Buried	Coil	N	2.755	1	0.57634	0.339948	0.000014	0.874	-0.3
Average	0.08054839	-0.9009677				0.07164516		-3.7381613		0.84764516		0.22481481	0.17925926				3.10828571	-1.0833333	0.5520075	0.72437333	1.1822E-05	0.62409677	3.64
INTERMEDIATE																							
I268T	0.493	-2.19	9 (Decrease)	6 (Large decr	3 (disease)	0.003	Damaging	-3.863	Deleterious	0.888	Possibly dam	0.06	0.01	Buried	Helix	Y	3.211	-	-	-	-	0.65	1.26
H20P	-0.546	0.41	6 (Increase)	2 (Large incre	5 (disease)	0	Damaging	-8.956	Deleterious	1	Probably dam	0.69	0.03	Buried	Turn	N	-	-	-	-	-	1	1.62
S272F	-0.641	0.2	1 (Increase)	3 (Neutral)	6 (disease)	0.002	Damaging	-4.273	Deleterious	0.998	Probably dam	0.16	0	Buried	Helix	N	-	-	-	-	-	0.517	5.2
R241C	-0.68	-1.05	5 (Decrease)	1 (Neutral)	5 (disease)	0	Damaging	-7.267	Deleterious	1	Probably dam--	None	-	-		-	-	-	-	-	-	0.632	-0.05
Average	-0.3435	-0.6575				0.00125		-6.08975		0.9715		0.30333333	0.01333333				3.211					0.69975	2.0075
MA																							
A334T	0.166	-0.26	2 (Increase)	0 (Large decr	5 (disease)	0.002	Damaging	-3.431	Deleterious	1	Probably dam	0.93	0.09	Intermediate	Bend	N	4.255	1	0.58355	1.49485	0.0002	1	1.15
T243I	-0.493	-0.93	9 (Decrease)	4 (Large decr	1 (neutral)	0	Damaging	-5.6	Deleterious	0.996	Probably dam	0.14	0.52	Exposed	Helix	N	-	-	-	-	-	1	-0.73
N301T	-0.214	0	1 (Decrease)	1 (Neutral)	3 (disease)	0.003	Damaging	-5	Deleterious	1	Probably dam	0.05	0	Buried	Helix	N	3.469	-2	0.85882	2.14321	0.00089	0.915	-0.58
L264F	-0.057	-1.14	7 (Decrease)	5 (Large decr	3 (disease)	0.005	Damaging	-2.494	Neutral	0.985	Probably dam	0.1	0.01	Buried	Helix	Y	-	-	-	-	-	0.731	9.02
L265P	0.232	-1.56	6 (Decrease)	5 (Large decr	5 (disease)	0.003	Damaging	-5.387	Deleterious	1	Probably dam	0.24	0.05	Buried	Helix	N	-	-	-	-	-	0.631	7.34
I268H	0.778	-2.27	9 (Decrease)	7 (Large decr	4 (disease)	0	Damaging	-5.832	Deleterious	0.999	Probably dam	0.06	0.01	Buried	Helix	Y	3.211	-	-	-	-	0.65	6.71
R241H	0.451	-1.4	9 (Decrease)	4 (Large decr	6 (disease)	0	Damaging	-4.533	Deleterious	1	Probably dam-	-			None	-	-	-	-	-	-	0.631	-0.06
V310M	0.087	-0.57	2 (Decrease)	3 (Neutral)	4 (disease)	0	Damaging	-2.625	Deleterious	1	Probably dam	0.38	0.01	Buried	Coil	N	2.755	1	0.79025	-0.09861	5.1E-0.6	0.874	0.94
V8M	0.087	-1.35	8 (Decrease)	3 (Large decr	1 (neutral)	0.003	Damaging	2.131	Neutral	0.99	Probably dam	0.05	0	Buried	Beta Ladder	N	3.014	1	-	-	-	0.638	-0.57
L35S	0.584	-2.31	9 (Decrease)	6 (Large decr	6 (disease)	0	Damaging	-5.157	Deleterious	1	Probably dam	0.16	0.08	Buried	Beta Ladder	N	3.906	-3	-	-	-	0.797	3.08
L234P	0.232	-1.66	5 (Decrease)	6 (Large decr	5 (disease)	0.001	Damaging	-5.899	Deleterious	1	Probably dam	0.36	0.04	Buried	Beta Ladder	N	-	-	-	-	-	0.623	9.38
G338D	0.473	-0.45	2 (Increase)	3 (Neutral)	7 (disease)	0	Damaging	-6.125	Deleterious	1	Probably dam-	-			Bend	-	-	-	-	-	-	1	3.35
Average	0.19383333	-1.1583333				0.00141667		-4.1626667		0.9975		0.247	0.081				3.435	-0.4	0.74420667	1.17981667	0.000545	0.79083333	3.2525
UNKNOWNS																							
S52N	0.123	-0.77	4(Decrease)	3(Neutral)	3 (neutral)	1	Tolerated	1.102	Neutral	0	Benign	0.25	0.06	Buried	Beta Ladder	N	-	-	-	-	-	0.484	-0.51
T356M	-0.288	0.13	1 (Increase)	0(Large decre	4 (neutral)	0.134	Tolerated	0.132	Neutral	0.621	Possibly dam	0.23	0.01	Buried	Helix	N	-	-	-	-	-	0.436	-0.17
H24P	-0.546	0.52	6 (Increase)	2(Large decre	5 (disease)	0.003	Damaging	-7.697	Deleterious	0.998	Probably dam	0.39	0.26	Intermediate	3/10 Helix	N	-	-	-	-	-	0.675	0.64
R124W	-0.878	-0.46	5 (Decrease)	0(Large decre	6 (disease)	0.017	Damaging	-2.525	Deleterious	0.952	Possibly dam	-	-		-	-	-	-	-	-	-	0.29	0.07
I56V	0.118	-1.12	8(Decrease)	6(Large decre	6 (neutral)	0.355	Tolerated	-0.629	Neutral	0	Benign	0.14	0.47	Exposed	Turn	N	-	-	-	-	-	0.627	1.17
R60W	-0.878	-0.42	6(Decrease)	1(Neutral)	6 (disease)	0.084	Tolerated	-1.889	Neutral	0.059	Benign	0.04	0.23	Intermediate	Beta Ladder	N	-	-	-	-	-	0.076	-0.81
V160L	-0.118	-0.94	8(Decrease)	2(Large decre	3 (neutral)	0.655	Tolerated	-0.082	Neutral	0	Benign	0.09	0.25	Intermediate	Helix	N	-	-	-	-	-	0.418	0.59
E162K	-0.24	-0.7	9(Decrease)	0(Large decre	4 (disease)	0.513	Tolerated	-0.777	Neutral	0.824	Possibly dam	0.07	0.8	Exposed	Turn	N	-	-	-	-	-	0.455	-0.1
N175S	-0.123	-0.35	6(Decrease)	0 (Neutral)	1 (neutral)	1	Tolerated	0.88	Neutral	0	Benign	0.01	0.29	Intermediate	Beta Ladder	N	-	-	-	-	-	0.067	-0.4
G198R	0.501	-0.54	3(Decrease)	5(Neutral)	7 (disease)	0	Damaging	-7.733	Deleterious	1	Probably dam	0.3	0.07	Buried	Bend	Y	-	-	-	-	-	1	2.41
S227L	-0.584	0.13	1 (Increase)	2(Neutral)	4 (disease)	0.663	Tolerated	0.905	Neutral	0.002	Benign	0.02	0.08	Buried	Coil	N	-	-	-	-	-	0.083	-0.15
D269H	-0.137	-0.59	5(Decrease)	1(Large decre	6 (disease)	0.041	Damaging	-3.573	Deleterious	0.167	Benign	-	-		Helix	-	-	-	-	-	-	0.669	3.2
N252S	-0.123	-0.53	7(Decrease)	3(Large decre	3 (disease)	0.387	Tolerated	-0.171	Neutral	0	Benign	0.43	0.45	Exposed	Helix	Y	-	-	-	-	-	0.423	-0.13
R253W	-0.878	-0.56	8(Decrease)	0(Large decre	6 (disease)	0.001	Damaging	-0.6057	Deleterious	1	Probably dam	0.03	0.13	Intermediate	Helix	Y	-	-	-	-	-	0.477	1.7
I292F	-0.057	-1.16	6(Decrease)	1(Large decre	1 (disease)	0.05	Damaging	-0.778	Neutral	0.367	Benign	0.1	0.45	Exposed	Helix	Y	-	-	-	-	-	0.442	-0.1
G335S	0.142	-0.6	4(Increase)	1(Neutral)	6 (disease)	0	Damaging	-5.2	Deleterious	1	Probably dam	0.59	0	Buried	Coil	N	-	-	-	-	-	1	10.96
V353E	0.782	-0.8	2(Decrease)	3(Large decre	0 (disease)	0.001	Damaging	-4.339	Deleterious	1	Probably dam	0.03	0.02	Buried	Helix	N	-	-	-	-	-	0.146	2.65
S384P	-0.352	-0.16	3(Increase)	4 (Neutral)	4 (disease)	0.145	Tolerated	-1.452	Neutral	0.007	Benign	0.01	0.28	Intermediate	Coil	N	-	-	-	-	-	0.182	-0.7
V5A	0.209	-1.78	9(Decrease)	7 (Large decr	7 (neutral)	0.31	Tolerated	0.337	Neutral	0	Benign	0.01	0.22	Intermediate	Bend	N	-	-	-	-	-	0.246	0.77
A10V	-0.209	0.21	4(Increase)	0(Large decre	2 (disease)	0	Damaging	-3.589	Deleterious	1	Probably dam-	-	-		Beta Ladder	-	-	-	-	-	-	0.929	3.44

Point Mutati	mCSM protei	mCSM predic	mCSM Protei	mCSM Protei	mCSM Protei	mCSM Protei	SNAP	PANTHER sco	PANTHER pre	SNP&GO Nali	PhD-SNP scor	PhD-SNP pre	nsSNPAnalyz	nsSNPAnalyz	SDM score	SDM predicti	Mupro (Kcal/	Mupro predi	PoPMuSiC (D	PoPMuSiC (R	PoPMuSiC pr	CUPSAT (DDG	CUPSAT(RSA)	CUPSAT pred	iStable score	iStable DDG	iStable predic	PredictSNP	PredictSNP p	MAPP	MAPP predic	Meta-SNP sc	Meta-SNP pr
V377I	-0.871	Destabilizing	-1.467	Destabilizing	-3.393	Destabilizing	1 (Neutral)	0.251	Neutral	105	0.58	Neutral	0.65	Neutral	-0.71	Slightly desta	-0.177581	Decrease	0.72	0.3	Decrease	-2.08	0	Decrease	0.628483	-0.116418	Decrease	0.83	Neutral	0.72	Neutral	0.167	Neutral
G309S	-0.978	Destabilizing	0.029	Stabilizing	-0.343	Destabilizing	4 (Non-neutr	0.732	Disease	173	0.88	Deleterious	0.63	Deleterious	-2.47	Highly destab	-0.9021354	Decrease	1.52	52.78	Decrease	0.05	64.8	Increase	0.698159	-0.948321	Decrease	0.76	Deleterious	0.65	Neutral	0.813	Disease
R215Q	-1.001	Destabilizing	-0.178	Destabilizing	-3.609	Destabilizing	0 (Non-neutr	0.362	Neutral	177	0.55	Neutral	0.65	Neutral	1.45	Stabilizing an	-1	Decrease	0.96	24.12	Decrease	-2.76	24.02	Decrease	0.649136	-0.703575	Decrease	0.65	Neutral	0.78	Neutral	0.362	Neutral
H20Q	-1.334	Destabilizing	-1.203	Destabilizing	-3.642	Destabilizing	5 (Non-neutr-	-		167	0.86	Deleterious	0.65	Neutral	-2.29	Highly destab	-0.0836759	Decrease	1.23	2.72	Decrease	-0.76	2.75	Decrease	0.672123	-0.682095	Decrease	0.87	Deleterious	0.57	Deleterious	0.789	Disease
H20N	-1.721	Destabilizing	-1.276	Destabilizing	-3.573	Destabilizing	5 (Non-neutr-	-		167	0.86	Deleterious	0.65	Neutral	-3.27	Highly destab	0.37948357	Increase	1.44	2.72	Decrease	-1.92	2.75	Decrease	0.579595	1.05766	Increase	0.87	Deleterious	0.87	Deleterious	0.829	Disease
L39P	-1.7	Destabilizing	-0.337	Destabilizing	-0.171	Destabilizing	0 (Neutral)	-		159	0.88	Deleterious	0.63	Deleterious	-4.04	Highly destab-	-		-	-		-	-		-	-		0.76	Deleterious	0.63	Deleterious	0.749	Disease
V132I	-0.836	Destabilizing	-1.144	Destabilizing	0.052	Stabilizing	1 (Neutral)	0.298	Neutral	168	0.78	Neutral	-		-0.26	Neutral and n	-1	Decrease	0.81	5.32	Decrease	0.76	6.51	Increase	0.631072	-0.272555	Decrease	0.83	Neutral	0.66	Neutral	0.13	Neutral
A148T	-1.296	Destabilizing	-1.31	Destabilizing	-0.166	Destabilizing	1 (Non-neutr	0.338	Neutral	177	0.45	Neutral	0.65	Neutral	-3.04	Highly destab	-1	Decrease	1.28	0	Decrease	-4.13	0	Decrease	0.836157	-0.745989	Decrease	0.51	Deleterious	0.59	Deleterious	0.406	Neutral
P167L	-0.617	Destabilizing	-0.462	Destabilizing	-0.636	Destabilizing	0 (Non-neutr	0.626	Disease	123	0.68	Deleterious	0.65	Neutral	1.97	Stabilizing an	-0.1054934	Decrease	0.98	12.43	Decrease	-0.2	12.68	Decrease	0.745859	-0.598328	Decrease	0.55	Deleterious	0.59	Deleterious	0.492	Neutral
G171R	-0.197	Destabilizing	0.052	Stabilizing	-0.101	Destabilizing	2 (Non-neutr	0.104	Neutral	112	0.68	Neutral	-		-0.77	Slightly desta	-0.8906818	Decrease	0.77	64.13	Decrease	0.34	81.32	Increase	0.610999	-0.418897	Decrease	0.75	Neutral	-	-	0.309	Neutral
G202R	-0.913	Destabilizing	-1.472	Destabilizing	0.291	Stabilizing	5 (Non-neutr	0.926	Disease	182	0.86	Deleterious	0.63	Deleterious	1.22	Stabilizing an	0.26824473	Increase	1.8	6.24	Decrease	-6.13	7.62	Decrease	0.528534	-1.63173	Decrease	0.87	Deleterious	0.88	Deleterious	0.79	Disease
G211E	-2.086	Destabilizing	-0.524	Destabilizing	-0.385	Destabilizing	5 (Non-neutr	0.949	Disease	182	0.88	Deleterious	0.63	Deleterious	-3.71	Highly destab	0.03062227	Increase	3.79	3.97	Decrease	-2.8	3.81	Decrease	0.58004	0.907633	Increase	0.87	Deleterious	0.88	Deleterious	0.845	Disease
V250I	-0.602	Destabilizing	-1.16	Destabilizing	-3.258	Destabilizing	3 (Non-neutr	0.499	Neutral	177	0.73	Deleterious	0.65	Neutral	-0.1	Neutral and n	0.05185944	Increase	0.86	0.46	Decrease	-1.63	0	Decrease	0.528332	-0.293082	Decrease	0.72	Deleterious	0.63	Neutral	0.714	Disease
L265R	-1.741	Destabilizing	-1.299	Destabilizing	-0.341	Destabilizing	4 (Non-neutr	0.658	Disease	172	0.86	Deleterious	0.63	Deleterious	-3.25	Highly destab	-0.7146279	Decrease	1.58	3.11	Decrease	-0.07	4.37	Decrease	0.781894	-2.11921	Decrease	0.87	Deleterious	0.88	Deleterious	0.78	Disease
G326R	-0.803	Destabilizing	-1.202	Destabilizing	-3.262	Destabilizing	3 (Non-neutr	0.158	Neutral	172	0.86	Deleterious	0.63	Deleterious	-1.53	Destabilizing	0.48878028	Increase	1.27	32.92	Decrease	-4.3	49.56	Decrease	0.548191	-0.473804	Decrease	0.61	Deleterious	0.64	Neutral	0.6	Disease
G376V	-0.532	Destabilizing	-1.03	Destabilizing	-2.83	Destabilizing	4 (Non-neutr	0.994	Disease	110	0.88	Deleterious	0.63	Deleterious	1.04	Stabilizing an	0.87302453	Increase	1.57	0	Decrease	-9.17	0	Decrease	0.602486	-0.430549	Decrease	0.87	Deleterious	0.76	Deleterious	0.871	Disease
S135L	-0.063	Destabilizing	-0.479	Destabilizing	-0.318	Destabilizing	4 (Non-neutr	0.707	Disease	176	0.88	Deleterious	0.63	Deleterious	2.84	Highly stabili	1	Increase	0.46	15.6	Decrease	1.55	14.51	Increase	0.521752	0.157841	Increase	0.87	Deleterious	0.76	Deleterious	0.76	Disease
L117P	-1.353	Destabilizing	-0.796	Destabilizing	-0.001	Destabilizing	2 (Neutral)	0.74	Disease	145	0.88	Deleterious	0.63	Deleterious	-4.85	Highly destab-	-		-	-		-	-		-	-		0.61	Deleterious	0.62	Deleterious	0.767	Disease
I119M	-0.628	Destabilizing	-1.562	Destabilizing	0.052	Stabilizing	5 (Neutral)	0.299	Neutral	147	0.55	Neutral	0.65	Neutral	-0.3	Neutral and n-	-		-	-		-	-		-	-		0.61	Deleterious	0.57	Deleterious	0.493	Neutral
Y116H	-0.795	Destabilizing	-0.564	Destabilizing	0.332	Stabilizing	1 (Non-neutr	0.528	Disease	145	0.73	Deleterious	0.63	Deleterious	-0.95	Slightly desta	-1	Decrease	2.78	0	Decrease	-5.97	0	Decrease	0.772159	-1.58913	Decrease	0.87	Deleterious	0.77	Deleterious	0.698	Disease
G211A	-0.843	Destabilizing	-0.035	Destabilizing	-0.313	Destabilizing	4 (Non-neutr	0.891	Disease	182	0.88	Deleterious	0.63	Deleterious	-2.19	Highly destab	-0.0794474	Decrease	2.5	3.97	Decrease	-3.88	3.81	Decrease	0.686492	-0.686381	Decrease	0.87	Deleterious	0.77	Deleterious	0.769	Disease
V203A	-1.62	Destabilizing	-0.895	Destabilizing	-0.274	Destabilizing	0 (Neutral)	0.527	Disease	182	0.59	Deleterious	0.65	Neutral	-0.81	Slightly desta	-1	Decrease	1.87	7.14	Decrease	-1.61	9.76	Decrease	0.790729	-1.22263	Decrease	0.61	Deleterious	0.78	Deleterious	0.461	Neutral
L246P	-1.457	Destabilizing	-0.576	Destabilizing	-2.587	Destabilizing	3 (Non-neutr	0.88	Disease	180	0.88	Deleterious	0.63	Deleterious	-3.7	Highly destab	-1	Decrease	3.33	16.31	Decrease	-3.33	16.93	Decrease	0.730036	-1.72421	Decrease	0.87	Deleterious	0.81	Deleterious	0.755	Disease
V261A	1.697	Destabilizing	-0.667	Destabilizing	-0.717	Destabilizing	2 (Neutral)	0.649	Disease	174	0.68	Neutral	-		-1.53	Destabilizing	-1	Decrease	2.16	0.61	Decrease	-0.41	0.65	Decrease	0.759876	-1.34816	Decrease	0.65	Neutral	0.46	Deleterious	0.162	Neutral
A262P	-0.42	Destabilizing	-1.192	Destabilizing	-0.468	Destabilizing	5 (Neutral)	0.458	Neutral	172	0.82	Deleterious	0.65	Neutral	-2.22	Highly destab	-0.5774476	Decrease	1.43	38.1	Decrease	0.37	40.83	Increase	0.629401	-0.70117	Decrease	0.6	Neutral	0.46	Deleterious	0.511	Disease
R277C	-0.371	Destabilizing	-0.181	Destabilizing	2.021	Stabilizing	1 (Neutral)	0.675	Disease	171	0.66	Neutral	0.65	Neutral	-0.21	Neutral and n	-0.6846569	Decrease	1.17	47.17	Decrease	0.85	55.46	Increase	0.667106	-0.57655	Decrease	0.61	Deleterious	0.51	Deleterious	0.425	Neutral
R277H	-1.139	Destabilizing	0.31	Stabilizing	0.308	Stabilizing	7 (Neutral)	0.346	Neutral	171	0.83	Neutral	0.65	Neutral	-0.49	Neutral and n	-0.5495372	Decrease	0.47	47.17	Decrease	-0.4	55.46	Decrease	0.7562	-0.505408	Decrease	0.83	Neutral	0.72	Neutral	0.166	Neutral
P288L	-0.332	Destabilizing	-0.653	Destabilizing	-0.774	Destabilizing	1 (Neutral)	0.219	Neutral	142	0.78	Neutral	0.65	Neutral	0.54	Slightly stabil	0.28645925	Increase	0.42	66.42	Decrease	0.2	69.06	Increase	0.523442	0.247829	Increase	0.83	Neutral	0.63	Neutral	0.154	Neutral
V293M	-0.397	Destabilizing	-1.301	Destabilizing	-0.051	Destabilizing	4 (Neutral)	0.205	Neutral	155	0.78	Neutral	0.65	Neutral	0.98	Slightly stabil	-0.5732895	Decrease	0.67	8.97	Decrease	-0.95	9.76	Decrease	0.832125	-0.325591	Decrease	0.74	Neutral	0.76	Neutral	0.192	Neutral
I298T	-3	Destabilizing	-0.944	Destabilizing	-1.209	Destabilizing	0 (Non-neutr	0.36	Neutral	171	0.58	Neutral	-		-4.03	Highly destab	-1	Decrease	1.96	3.18	Decrease	-3.9	3.24	Decrease	-3.9	3.24	Decrease	0.61	Deleterious	0.51	Deleterious	0.432	Neutral
V310L	-0.701	Destabilizing	-0.868	Destabilizing	-2.744	Destabilizing	5 (Non-neutr	0.576	Disease	173	0.88	Deleterious	0.65	Neutral	0.73	Slightly stabil	-0.1842745	Decrease	0.56	0.91	Decrease	0.51	1.3	Increase	0.736531	-0.188765	Decrease	0.87	Deleterious	0.62	Deleterious	0.667	Disease
Average	-0.9241935		-0.7866452		-1.0358065			0.53410714		161.580645	0.76354839		0.65		-1.1596774		-0.3622991		1.44142857	16.6703571		-1.5433333	22.1883333		0.50453246	-0.4532709		0.74967742		0.68666667		0.55025806	
I268T	-2.975	Destabilizing	-0.516	Destabilizing	-0.422	Destabilizing	2 (Non-neutr	0.681	Disease	176	0.68	Deleterious	0.65	Neutral	-4.03	Highly destab	-1	Decrease	2.3	0.41	Decrease	-0.61	1.08	Decrease	0.80173	-2.56071	Decrease	0.87	Deleterious	0.57	Deleterious	0.736	Disease
H20P	-0.79	Destabilizing	-1.35	Destabilizing	-3.415	Destabilizing	4 (Non-neutr -	-		167	0.88	Deleterious	0.65	Neutral	-2.03	Highly destab	-0.1762532	Decrease	1.97	2.72	Decrease	-5.45	2.75	Decrease	0.566879	-0.974564	Decrease	0.87	Deleterious	0.84	Deleterious	0.859	Disease
S272F	-0.794	Destabilizing	-0.881	Destabilizing	-4.893	Destabilizing	0 (Non-neutr	0.854	Disease	173	0.61	Deleterious	0.65	Neutral	3.03	Highly stabili	0.25059202	Increase	-0.28	0	Increase	3.68	0	Increase	0.767788	0.685235	Increase	0.87	Deleterious	0.59	Deleterious	0.567	Disease
R241C	-0.824	Destabilizing	-0.635	Destabilizing	-1.315	Destabilizing	4 (Non-neutr	0.945	Disease	178	0.88	Deleterious	0.65	Neutral	1.55	Stabilizing an	-0.3632834	Decrease	0.3	24.71	Decrease	-0.12	27.51	Decrease	0.558955	-0.198894	Decrease	0.87	Deleterious	0.91	Deleterious	0.818	Disease
Average	-1.34575		-0.8455		-2.51125			0.82666667		173.5	0.7625		0.65		-0.37		-0.3222361		1.0725	6.96		-0.625	7.835		0.673838	-0.7622333		0.87		0.7275		0.745	
A334T	-1.3	Destabilizing	-0.127	Destabilizing	-0.552	Destabilizing	3 (Non-neutr	0.639	Disease	171	0.82	Deleterious	0.65	Neutral	-0.1	Neutral and n	-0.5027285	Decrease	0.57	10.37	Decrease	-3.62	9.07	Decrease	0.608033	-0.58267	Decrease	0.87	Deleterious	0.84	Deleterious	0.726	Disease
T243I	-0.197	Destabilizing	-0.284	Destabilizing	-1.005	Destabilizing	4 (Non-neutr	0.913	Disease	179	0.88	Deleterious	0.65	Neutral	2.79	Highly stabili	-0.3441432	Decrease	0.19	42.46	Decrease	1.38	53.35	Increase	0.541203	0.182661	Increase	0.87	Deleterious	0.82	Deleterious	0.849	Disease
N301T	-1.757	Destabilizing	-1.127	Destabilizing	-0.877	Destabilizing	4 (Non-neutr	0.685	Disease	173	0.88	Deleterious	0.63	Deleterious	1.1	Stabilizing an	-0.7445981	Decrease	0.02	0	Decrease	-2.57	0	Decrease	0.783536	-0.133177	Decrease	0.87	Deleterious	0.59	Deleterious	0.802	Disease
L264F	-1.498	Destabilizing	-1.213	Destabilizing	-0.459	Destabilizing	1 (Non-neutr	0.543	Disease	170	0.66	Neutral	0.63	Deleterious	-0.29	Neutral and n	-1	Decrease	1.15	0.39	Decrease	0.51	0.55	Increase	0.787553	-0.757825	Decrease	0.6	Neutral	0.57	Deleterious	0.444	Neutral
L265P	-1.417	Destabilizing	-0.972	Destabilizing	-0.832	Destabilizing	4 (Non-neutr	0.733	Disease	172	0.88	Deleterious	0.63	Deleterious	-4.85	Highly destab	-1	Decrease	3.61	3.11	Decrease	-0.26	4.37	Decrease	0.800747	-2.13331	Decrease	0.87	Deleterious	0.86	Deleterious	0.796	Disease
I268H	-2.444	Destabilizing	-0.287	Destabilizing	-0.286	Destabilizing	5 (Non-neutr	0.905	Disease	176	0.73	Deleterious	0.65	Neutral	-1.98	Destabilizing	-1	Decrease	1.61	0.41	Decrease	12	1.08	Increase	0.692282	-2.16412	Decrease	0.87	Deleterious	0.75	Deleterious	0.772	Disease
R241H	-1.481	Destabilizing	-0.07	Destabilizing	-1.152	Destabilizing	0 (Neutral)	0.796	Disease	178	0.88	Deleterious	0.65	Neutral	0.28	Neutral and n	-0.6639918	Decrease	0.1	24.71	Decrease	-0.27	27.51	Decrease	0.691215	-0.219187	Decrease	0.76	Deleterious	0.75	Deleterious	0.629	Disease
V310M	-0.66	Destabilizing	-0.804	Destabilizing	-2.675	Destabilizing	4 (Non-neutr	0.79	Disease	173	0.88	Deleterious	0.65	Neutral	0.79	Slightly stabil	-0.6666272	Decrease	1.02	0.91	Decrease	-0.18	1.3	Decrease	0.787094	-0.481615	Decrease	0.87	Deleterious	0.75	Deleterious	0.841	Disease
V8M	-1.106	Destabilizing	-1.27	Destabilizing	-3.673	Destabilizing	2 (Non-neutr -	-		113	0.59	Deleterious	0.65	Neutral	-0.88	Slightly desta	-0.370241	Decrease	1.76	0.15	Decrease	-1.84	0	Decrease	0.624005	-1.60117	Decrease	0.87	Deleterious	0.57	Deleterious	0.282	Neutral
L35S	-2.72	Destabilizing	0.289	Stabilizing	-0.242	Destabilizing	1 (Neutral) -	-		163	0.88	Deleterious	0.63	Deleterious	-3.04	Highly destab	-1	Decrease	2.1	8.54	Decrease	-2.26	7.1	Decrease	0.871305	-1.53066	Decrease	0.76	Deleterious	0.59	Deleterious	0.677	Disease
L234P	-1.736	Destabilizing	-0.784	Destabilizing	3.177	Stabilizing	1 (Neutral)	0.937	Disease	179	0.88	Deleterious	0.63	Deleterious	-4.04	Highly destab	-1	Decrease	2.92	2.85	Decrease	-2.86	3.82	Decrease	0.752253	-1.34593	Decrease	0.87	Deleterious	0.84	Deleterious	0.797	Disease
G338D	-2.417	Destabilizing	-0.83	Destabilizing	-0.748	Destabilizing	6 (Non-neutr	0.746	Disease	170	0.88	Deleterious	0.63	Deleterious	-3.17	Highly destab	-0.0109465	Decrease	2.05	6.24	Decrease	1.06	7.62	Increase	0.597625	-1.26047	Decrease	0.87	Deleterious	0.88	Deleterious	0.843	Disease
Average	-1.5610833		-0.62325		-0.777			0.7687		168.083333	0.82		0.64		-1.1158333		-0.6919397		1.425	8.345		-0.8091667	9.6475		0.71140425	-1.0022894		0.82916667		0.73416667		0.70483333	
S52N	-0.991	Destabilizing	-0.258	Destabilizing	-0.055	Destabilizing	0 (Neutral) -	-		157	0.83	Neutral	-		-0.27	Neutral and n	-0.8182978	Decrease	0.64	15.22	Decrease	0.55	6.83	Increase	0.707038	-0.694075	Decrease	0.83	Neutral	0.85	Neutral	0.122	Neutral
T356M	0.119	Stabilizing	-0.752	Destabilizing	0.159	Stabilizing	1 (Neutral)	0.194	Neutral	128	0.89	Neutral	-		1.77	Stabilizing an	-0.5213254	Decrease	-0.16	0.49	Increase	-0.08	1.44	Increase	0.590477	0.125546	Increase	0.83	Neutral	0.64	Neutral	0.107	Neutral
H24P	0.299	Stabilizing	-1.223	Destabilizing	-2.6	Destabilizing	4 (Non-neutr -	-		167	0.86	Deleterious	0.65	Neutral	-2.72	Highly destab	-0.017423	Decrease	2.56	23.58	Decrease	-0.97	25.84	Decrease	0.668177	-0.876095	Decrease	0.87	Deleterious	0.84	Deleterious	0.788	Disease
R124W	-0.555	Destabilizing	-0.172	Destabilizing	0.13	Stabilizing	0 (Non-neutr	0.816	Disease	133	0.58	Neutral	0.65	Neutral	1.44	Stabilizing an	-0.1441623	Decrease	0.29	97.75	Decrease	-0.5	114.14	Decrease	0.784403	-0.263238	Decrease	0.63	Neutral	0.65	Neutral	0.603	Disease
I56V	-0.533	Destabilizing	-0.664	Destabilizing	-0.464	Destabilizing	3 (Neutral) -	-		152	0.66	Neutral	-		-0.74	Slightly desta	-0.4200442	Decrease	0.23	42.82	Decrease	-0.95	42.7	Decrease	0.824867	-0.0065668	Decrease	0.83	Neutral	0.71	Neutral	0.199	Neutral
R60W	-0.304	Destabilizing	-0.678	Destabilizing	-0.4	Destabilizing	1 (Neutral) -	-		141	0.59	Deleterious	-		5.18	Highly destab	-0.9310868	Decrease	-0.63	27.93	Increase	-0.54	24.89	Decrease	0.51972	0.186462	Increase	0.63	Neutral	0.64	Neutral	0.426	Neutral
V160L	-0.552	Destabilizing	-0.369	Destabilizing	-0.198	Destabilizing	7 (Neutral)	0.238	Neutral	153	0.83	Neutral	-		1.1	Stabilizing an	0.46820498	Increase	0.29	18.84	Decrease	-1.6	22.77	Decrease	0.596361	-0.530036	Decrease	0.83	Neutral	0.85	Neutral	0.065	Neutral
E162K	0.358	Stabilizing	0.501	Stabilizing	-0.045	Destabilizing	5 (Neutral)	0.204	Neutral	151	0.66	Neutral	0.65	Neutral	0.43	Neutral and n	-0.0953345	Decrease	-0.21	83.92	Increase	1.05	89.3	Increase	0.550978	0.319054	Increase	0.83	Neutral	0.79	Neutral	0.337	Neutral
N175S	-1.076	Destabilizing	-0.376	Destabilizing	-0.86	Destabilizing	7 (Neutral)	0.225	Neutral	133	0.89	Neutral	-		0.28	Neutral and n	-0.2025741	Decrease	0.96	24.47	Decrease	2.64	30.74	Increase	0.815999	-0.187528	Decrease	0.83	Neutral	0.77	Neutral	0.116	Neutral
G198R	-1.081	Destabilizing	-1.579	Destabilizing	0.138	Stabilizing	6 (Non-neutr	0.903	Disease	182	0.88	Deleterious	0.63	Deleterious	-1.53	Destabilizing	0.67816948	Increase	0.73	22.42	Decrease	-2.32	7.62	Decrease	0.521893	0.187331	Increase	0.87	Deleterious	0.77	Deleterious	0.878	Disease
S227L	-0.082	Destabilizing	-0.097	Destabilizing	-0.349	Destabilizing	6 (Neutral)	0.202	Neutral	171	0.66	Neutral	-		1.86	Stabilizing an	1	Increase	0.2	10.59	Decrease	-1.38	8.53	Decrease	0.612998	0.101624	Increase	0.83	Neutral	0.78	Neutral	0.148	Neutral
D269H	-1.246	Destabilizing	0.305	Stabilizing	-2.607	Destabilizing	3 (Neutral)	0.782	Disease	176	0.51	Neutral	0.65	Neutral	2.01	Highly stabili	-0.3899455	Decrease	0.14	17.64	Decrease	-1.15	20.12	Decrease	0.779503	-0.110538	Decrease	0.61	Deleterious	0.43	Deleterious	0.621	Disease
N252S	-0.538	Destabilizing	-0.02	Destabilizing	-1.757	Destabilizing	5 (Neutral)	0.184	Neutral	173	0.83	Neutral	-		0.89	Slightly stabil	-0.1876977	Decrease	0.11	46.83	Decrease	1.39	48.5	Increase	0.793589	-0.412245	Decrease	0.83	Neutral	0.74	Neutral	0.161	Neutral
R253W	-0.755	Destabilizing	-1.022	Destabilizing	-0.703	Destabilizing	2 (Non-neutr	0.931	Disease	170	0.88	Deleterious	0.63	Deleterious	2.48	Highly stabili	0.52233862	Increase	-0.08	13.38	Increase	-0.02	13.54	Decrease	0.634444	0.190483	Increase	0.76	Deleterious	0.56	Deleterious	0.743	Disease
I292F	-1.249	Destabilizing	-0.445	Destabilizing	-3.247	Destabilizing	2 (Neutral)	0.462	Neutral	151	0.83	Neutral	0.65	Neutral	-0.72	Slightly desta	-0.1371759	Decrease	0.55	36.77	Decrease	1.33	40.42	Increase	0.84915	-0.83596	Decrease	0.75	Neutral	0.7	Neutral	0.23	Neutral
G335S	-1.3	Destabilizing	-0.306	Destabilizing	-0.591	Destabilizing	6 (Non-neutr	0.908	Disease	171	0.77	Deleterious	0.63	Deleterious	-4.27	Highly destab	-0.2713381	Decrease	1.59	0.28	Decrease	-4.89	0	Decrease	0.618651	-0.539751	Decrease	0.87	Deleterious	0.57	Deleterious	0.858	Disease
V353E	-2.756	Destabilizing	-1.584	Destabilizing	-0.576	Destabilizing	0 (Non-neutr	0.156	Neutral	132	0.77	Deleterious	0.65	Neutral	-2.68	Highly destab	-0.5524886	Decrease	1.79	3.34	Decrease	-4.42	1.95	Decrease	0.789789	-1.54786	Decrease	0.76	Deleterious	0.91	Deleterious	0.397	Neutral
S384P	-0.421	Destabilizing	-0.41	Destabilizing	-0.682	Destabilizing	6 (Neutral) -	-		32	0.72	Neutral	0.65	Neutral	0.19	Neutral and n	0.23085791	Increase	1.57	30.05	Decrease	-1.63	30.72	Decrease	0.557808	1.4426	Increase	0.84	Neutral	0.63	Neutral	0.373	Neutral
V5A	-1.189	Destabilizing	-0.549	Destabilizing	-0.442	Destabilizing	8 (Neutral) -	-		42	0.89	Neutral	0.65	Neutral	0.08	Neutral and n	-1	Decrease	1.62	21.58	Decrease	-0.32	20.17	Decrease	0.809748	-2.19831	Decrease	0.83	Neutral	0.72	Neutral	0.067	Neutral
A10V	-0.626	Destabilizing	-1.205	Destabilizing	-3.496	Destabilizing	3 (Non-neutr -	-		155	0.88	Deleterious	0.65	Neutral	1.02	Stabilizing an	-0.2205198	Decrease	0.28	0	Decrease	0.49	0	Increase	0.565601	0.840707	Increase	0.87	Deleterious	0.57	Deleterious	0.692	Disease

Point Mutati	GETAREA (ch	GETAREA (ch	SDM change	Sidechain hy	BeAtMuSiC b	Binding affini	BeAtMuSiC S	BeAtMuSiC S	FI-mutant ass	FI prediction	Fathamm sco	KD4V size ch	KD4V charge	KD4V polarit	KD4V hydrop	KD4V modific	KD4V numbe	KD4V WT res	SNP effect
V377I	1.74	14	0	No Hbonds	-0.48	Increase	0.31%	0.30%	0.2	Neutral	2.11	Increase	Unchanged	Unchanged	Unchanged	11	1	67	-
G309S	18.07	4	-0.4	No Hbonds	1.07	Decrease	53.92%	53.92%	3.57	High	1.95	Increase	Unchanged	Increase	Unchanged	22	1	88	Based on FoldX, the mutation reduces the protein stability
R215Q	-30.24	10	24.1	No Hbonds t	0.09	Decrease	24.22%	24.22%	1.7	Low	2.27	Decrease	Decrease	Unchanged	Increase	23	1	36	Based on FoldX, the mutation slightly reduces the protein stability
H20Q	-3.69	4	-2	Saturated to	-0.09	Increase	2.97%	2.96%	3.75	High	1.4	Decrease	Decrease	Unchanged	Unchanged	25	3	90	Based on FoldX, the mutation reduces the protein stability
H20N	-3.69	4	-1.9	Saturated to	0.06	Decrease	2.97%	2.96%	4.09	High	1.41	Decrease	Decrease	Unchanged	Decrease	29	3	90	Based on FoldX, the mutation reduces the protein stability
L39P	-1.39	20	-1.3	No Hbonds	2.25	Decrease	0.26%	0.26%	3.36	Median	2.1	Decrease	Unchanged	Unchanged	Decrease	68	1	44	Based on FoldX, the mutation severely reduces the protein stability
V132I	27.72	2	-2.7	No Hbonds	-0.36	Increase	6.08%	6.08%	1.33	Low	2.27	Increase	Unchanged	Unchanged	Unchanged	11	1	23	Based on FoldX, the mutation reduces the protein stability
A148T	1.35	8	-0.6	No Hbonds	0	-	0%	0%	2.6	Median	1.27	Increase	Unchanged	Increase	Decrease	14	1	71	Based on FoldX, the mutation reduces the protein stability
P167L	-8.56	14	0.8	No Hbonds	-0.46	Increase	14.31%	14.31%	2.03	Median	2.28	Increase	Unchanged	Unchanged	Increase	68	1	38	Based on FoldX, the mutation severely reduces the protein stability
G171R	-166.18	14	-17.4	No Hbonds	0.69	Decrease	68.67%	68.67%	1.2	Low	2.33	Increase	Increase	Increase	Decrease	61	1	28	Based on FoldX, the mutation reduces the protein stability
G202R	10.21	24	-21.4	No Hbonds	0.4	Decrease	6.25%	6.24%	2.93	Median	1.18	Increase	Increase	Increase	Decrease	61	1	88	Based on FoldX, the mutation reduces the protein stability
G211E	1.26	10	-14.3	No Hbonds t	1.82	Decrease	5.68%	5.68%	2.84	Median	1.25	Increase	Increase	Increase	Decrease	22	2	90	Based on FoldX, the mutation severely reduces the protein stability
V250I	1.81	8	-1.4	No Hbonds	-0.37	Increase	0.61%	0.61%	3.31	Median	1.97	Increase	Unchanged	Unchanged	Unchanged	11	1	92	Based on FoldX, the mutation slightly reduces the protein stability
L265R	-1.66	6	-13.5	No Hbonds	0.19	Decrease	3.37%	3.37%	3.08	Median	0.37	Increase	Increase	Increase	Decrease	58	2	51	Based on FoldX, the mutation reduces the protein stability
G326R	-157.68	14	-19.1	No Hbonds t	-0.04	Increase	31.79%	31.78%	-	-	-1.26	Increase	Increase	Increase	Decrease	61	1	63	Based on FoldX, the mutation reduces the protein stability
G376V	11.79	10	-0.2	No Hbonds	0.56	Decrease	0%	0%	2.96	Median	1.78	Increase	Unchanged	Unchanged	Increase	44	1	88	Based on FoldX, the mutation severely reduces the protein stability
S135L	33.16	24	9.8	No Hbonds	-0.56	Increase	15.03%	15.02%	2.99	Median	2.14	Increase	Unchanged	Decrease	Increase	45	1	86	Based on FoldX, the mutation slightly enhances the protein stability
L117P	-24.64	28	-8.4	No Hbonds	0.45	Decrease	4.15%	4.14%	3.05	Median	2.25	Decrease	Unchanged	Unchanged	Decrease	68	0	38	Based on FoldX, the mutation severely reduces the protein stability
I119M	-2.74	0	0.7	No Hbonds	-0.4	Increase	1.38%	1.38%	3.12	Median	2.19	Increase	Unchanged	Unchanged	Unchanged	7	1	40	Based on FoldX, the mutation slightly reduces the protein stability
Y116H	-0.86	22	0.1	No Hbonds	1.05	Decrease	0%	0%	3.21	Median	1.29	Unchanged	Increase	Unchanged	Decrease	49	1	42	Based on FoldX, the mutation reduces the protein stability and based on TANGO the mutation decreases the aggregation tendency of the protein
G211A	1.12	2	-2.4	No Hbonds	0.78	Decrease	5.68%	5.68%	1.6	Low	2.29	Unchanged	Unchanged	Unchanged	Increase	14	2	90	Based on FoldX, the mutation severely reduces the protein stability
V203A	-13.79	4	-8.3	No Hbonds	0.57	Decrease	8.21%	8.21%	1.6	Low	2.29	Decrease	Unchanged	Unchanged	Unchanged	33	1	40	Based on FoldX, the mutation slightly reduces the protein stability
L246P	6.62	6	4	No Hbonds	1.61	Decrease	15.80%	15.79%	3.24	Median	2.05	Decrease	Unchanged	Unchanged	Decrease	68	1	73	Based on FoldX, the mutation reduces the protein stability
V261A	-5.57	40	-2.5	No Hbonds	0.59	Decrease	3.65%	0.91%	2.55	Median	2.27	Decrease	Unchanged	Unchanged	Unchanged	33	0	32	Based on FoldX, the mutation reduces the protein stability
A262P	7.11	10	25.2	No Hbonds	0.38	Decrease	38.10%	38.10%	2.05	Median	2.25	Increase	Unchanged	Unchanged	Decrease	15	0	25	Based on FoldX, the mutation reduces the protein stability
R277C	24.8	10	-22.8	No Hbonds t	0.71	Decrease	47.46%	47.46%	2.47	Median	2.01	Decrease	Decrease	Decrease	Increase	77	2	30	Based on FoldX, the mutation reduces the protein stability
R277H	24.8	10	-23.5	No Hbonds t	0.18	Decrease	47.46%	47.46%	2.47	Median	2.04	Decrease	Unchanged	Unchanged	Increase	14	2	30	Based on FoldX, the mutation slightly reduces the protein stability
P288L	-7.46	4	5.8	No Hbonds	0.3	Decrease	69.15%	69.14%	1.39	Low	2.28	Increase	Unchanged	Unchanged	Increase	68	1	32	Based on FoldX, the mutation reduces the protein stability
V293M	14.52	2	-16.6	No Hbonds	0.02	Decrease	24.62%	10.33%	1.75	Low	2.26	Increase	Unchanged	Unchanged	Unchanged	16	1	28	-
I298T	-33.42	12	1.1	No Hbonds	0.57	Decrease	4.14%	4.14%	2.96	Median	2.12	Decrease	Unchanged	Increase	Decrease	29	0	32	Based on FoldX, the mutation reduces the protein stability
V310L	7.95	20	-0.8	No Hbonds	-0.54	Increase	1.22%	1.22%	2.63	Median	1.71	Increase	Unchanged	Unchanged	Unchanged	21	1	90	-
Average	-8.6303226	11.6129032	-3.5451613		0.35612903		16.37%	15.82%	2.5025		1.81032258					36.9677419	1.16129032	56.6129032	
I268T	-0.59	6	-0.6	No Hbonds	1.03	Decrease	0.55%	0.55%	2.87	Median	2.18	Decrease	Unchanged	Increase	Decrease	29	1	67	Based on FoldX, the mutation reduces the protein stability
H20P	-57.06	6	-9.6	Saturated to	0.42	Decrease	2.97%	2.96%	4.09	High	1.4	Decrease	Decrease	Decrease	Decrease	57	3	90	Based on FoldX, the mutation reduces the protein stability
S272F	0.31	18	0	No Hbonds	-1.57	Increase	0%	0%	3.13	Median	2.29	Increase	Unchanged	Decrease	Increase	63	1	51	Based on FoldX, the mutation severely reduces the protein stability
R241C	-112.24	10	-19	Unsaturated	-0.35	Increase	25.59%	25.59%	3.22	Median	1.97	Decrease	Decrease	Decrease	Increase	77	0	65	-
Average	-42.395	10	-7.3		-0.1175		7.28%	7.28%	3.3275		1.96					56.5	1.25	68.25	
A334T	2.66	4	7	No Hbonds	-0.43	Increase	9.32%	9.31%	3.38	Median	0.89	Increase	Unchanged	Increase	Decrease	14	1	86	Based on FoldX, the mutation reduces the protein stability
T243I	2.09	4	-2.5	No Hbonds	0.02	Decrease	46.23%	46.23%	3.32	Median	1.63	Increase	Unchanged	Decrease	Increase	29	1	92	Based on FoldX, the mutation slightly enhances the protein stability
N301T	-4.2	12	0	Unsaturated	-1.31	Increase	0%	0%	2.56	Median	2.04	Decrease	Unchanged	Unchanged	Increase	24	1	86	Based on FoldX, the mutation slightly enhances the protein stability
L264F	-16.66	24	-25.1	No Hbonds	0.39	Decrease	15.80%	0.78%	1.93	Median	2.18	Increase	Unchanged	Unchanged	Unchanged	28	1	25	Based on FoldX, the mutation severely reduces the protein stability
L265P	-24.17	14	-3.5	No Hbonds	1.83	Decrease	3.37%	3.37%	3.06	Median	1.98	Decrease	Unchanged	Unchanged	Decrease	68	2	51	Based on FoldX, the mutation severely reduces the protein stability
I268H	-8.11	6	0	No Hbonds t	0.35	Decrease	0.55%	0.55%	-	-	2.12	Increase	Unchanged	Increase	Decrease	42	1	67	Based on FoldX, the mutation severely reduces the protein stability
R241H	-112.24	10	-18.8	Unsaturated	-0.6	Increase	25.59%	25.59%	3.22	Median	2.09	Decrease	Unchanged	Unchanged	Increase	14	0	65	-
V310M	8.18	8	0.8	No Hbonds	-0.16	Increase	1.22%	1.22%	3.88	High	1.6	Increase	Unchanged	Unchanged	Unchanged	16	1	90	Based on FoldX, the mutation slightly reduces the protein stability
V8M	0.22	4	-0.1	No Hbonds	0.37	Decrease	0%	0%	2.94	Median	2.01	Increase	Unchanged	Unchanged	Unchanged	16	1	61	Based on FoldX, the mutation slightly enhances the protein stability
L35S	-59	14	13.4	No Hbonds	0.75	Decrease	8.03%	8.03%	3.26	Median	1.97	Decrease	Unchanged	Increase	Decrease	45	1	80	Based on FoldX, the mutation reduces the protein stability
L234P	-44.29	26	-12	No Hbonds	0.74	Decrease	2.85%	2.85%	3.1	Median	1.8	Decrease	Unchanged	Unchanged	Decrease	68	1	59	Based on FoldX, the mutation severely reduces the protein stability
G338D	7.94	16	-4.6	No Hbonds	0.56	Decrease	6.81%	6.81%	2.89	Median	-0.1	Increase	Increase	Increase	Decrease	28	0	92	Based on FoldX, the mutation reduces the protein stability
Average	-20.631667	11.8333333	-3.7833333		0.20916667		9.98%	8.73%	3.04909091		1.68416667					32.6666667	0.91666667	71.1666667	
S52N	10.21	4	-2.1	No Hbonds t	-0.34	Increase	11.56%	11.56%	-1.07	Neutral	2.29	Increase	Unchanged	Unchanged	Decrease	15	1	13	Based on FoldX the mutation slightly enhances the protein stability
T356M	0	2	-1.6	Saturated to	-1.17	Increase	0.99%	0.98%	0.9	Low	2.31	Increase	Unchanged	Decrease	Increase	32	1	13	-
H24P	-80.06	6	-0.1	No Hbonds	1.33	Decrease	23.21%	23.21%	3.34	Median	2.49	Decrease	Decrease	Decrease	Decrease	57	1	48	Based on FoldX the mutation slightly reduces the protein stability
R124W	-21.56	6	1.7	Saturated	0.37	Decrease	100%	100%	1.52	Low	2.23	Unchanged	Decrease	Decrease	Increase	54	0	30	-
I56V	-6.62	2	15.4	No Hbonds	-0.11	Increase	40.34%	40.33%	1.42	Low	2.24	Decrease	Unchanged	Unchanged	Unchanged	11	1	44	Based on FoldX the mutation reduces the protein stability
R60W	-40.15	20	29.2	Saturated	-1.11	Increase	24.22%	24.22%	2.21	Median	2.3	Unchanged	Decrease	Decrease	Increase	54	0	25	Based on FoldX the mutation slightly enhances the protein stability
V160L	-11.89	2	0.8	No Hbonds	-0.59	Increase	22.50%	22.49%	-	-	2.71	Increase	Unchanged	Unchanged	Unchanged	21	0	13	Based on FoldX the mutation slightly reduces the protein stability
E162K	-94.16	2	-6	Saturated	-0.01	Increase	87.01%	87%	1.19	Low	2.31	Increase	Opposite	Unchanged	Unchanged	27	0	28	Based on TANGO the mutation increases the aggregationtendency of the protein
N175S	3.42	4	-9	Unsaturated	-0.08	Increase	33.84%	33.84%	-	-	2.35	Decrease	Unchanged	Unchanged	Increase	15	1	11	-
G198R	-257.76	14	-19.3	No Hbonds	0.68	Decrease	60.73%	60.73%	3.29	Median	1.52	Increase	Increase	Increase	Decrease	61	0	98	Based on FoldX the mutation reduces the protein stability
S227L	11.79	10	2.2	No Hbonds	-0.6	Increase	15.02%	15.02%	-	-	2	Increase	Unchanged	Decrease	Increase	45	0	13	-
D269H	11.39	11	-2.9	No Hbonds	-0.57	Increase	17.96%	17.96%	2	Median	2.23	Increase	Opposite	Unchanged	Increase	40	0	48	Based on FoldX the mutation reduces the protein stability
N252S	15.36	4	22.1	Unsaturated	-0.08	Increase	58.61%	58.61%	-	-	2.33	Decrease	Unchanged	Unchanged	Increase	15	1	11	-
R253W	-52.44	16	-13	Unsaturated	-1.74	Increase	40.63%	13.48%	2.89	Median	2.17	Unchanged	Decrease	Decrease	Increase	54	1	50	Based on FoldX the mutation reduces the protein stability
I292F	-17.03	6	-14.8	No Hbonds	-0.32	Increase	38.33%	33.66%	1.49	Low	2.27	Increase	Unchanged	Unchanged	Unchanged	28	1	19	-
G335S	-0.01	4	-0.2	No Hbonds t	0.19	Decrease	0%	0%	4.07	High	-1.62	Increase	Unchanged	Increase	Unchanged	22	1	92	Based on FoldX the mutation severely reduces the protein stability
V353E	5.12	4	-1.8	No Hbonds t	0.63	Decrease	4.86%	4.86%	1.75	Low	1.12	Increase	Increase	Increase	Decrease	43	0	42	Based on FoldX the mutation reduces the protein stability
S384P	0.68	4	16.1	No Hbonds	0.9	Decrease	28.51%	28.51%	0.55	Neutral	2.32	Increase	Unchanged	Decrease	Decrease	23	2	26	Based on FoldX the mutation slightly enhances the protein stability
V5A	-24.17	4	-8.8	No Hbonds	0.54	Decrease	22.19%	22.19%	-	-	2.33	Decrease	Unchanged	Unchanged	Unchanged	33	1	23	Based on FoldX the mutation slightly reduces the protein stability and based on TANGO the mutation decreases the aggregation tendency of your protein
A10V	0	4	0	No Hbonds	-0.8	Increase	0%	0%	3.39	Median	1.48	Increase	Unchanged	Unchanged	Unchanged	33	0	80	Based on FoldX the mutation reduces the protein stability