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Research article

Structural and functional impact of missense mutations in TPMT: An integrated computational approach

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ABSTRACT

Background: Thiopurine S-methyltransferase (TPMT) detoxifies thiopurine drugs which are used for treatment of various diseases including inflammatory bowel disease (IBD), and hematological malignancies. Individual variation in TPMT activity results from mutations in *TPMT* gene. In this study, the effects of all the known missense mutations in TPMT enzyme were studied at the sequence and structural level

Methods: A broad set of bioinformatic tools was used to assess all the known missense mutations affecting enzyme activity. The effects of these mutations on protein stability, aggregation propensity, and residue interaction network were analyzed.

Results: Our results indicate that the missense mutations have diverse effects on TPMT structure and function. Stability and aggregation propensities are affected by various mutations. Several mutations also affect residues in ligand binding site.

Conclusions: In vitro study of missense mutation is laborious and time-consuming. However, computational methods can be used to obtain information about effects of missense mutations on protein structure. In this study, the effects of most of the mutations on enzyme activity could be explained by computational methods. Thus, the present approach can be used for understanding the protein structure-function relationships.

1. Introduction

TPMT is a cytoplasmic enzyme catalyzes the S-methylation of thiopurine drugs used for treatment of inflammatory bowel disease (IBD), hematological malignancies, and as immunosuppressant after organ transplantation. 6-Mercaptopurine, 6-thioguanine and azathiopurine are the drugs which their metabolism is affected by the low and intermediate TPMT enzyme activity (Wang et al., 2010).

TPMT gene is located on chromosome 6 (6p22.3) and contains 10 exons (Szumlanski et al., 1996). TPMT enzyme contains 8 helices and 9 sheets shown by letters (A–H) and number (1–9), respectively (Fig. 1) (Wu et al., 2007; Stivala et al., 2011). Genetic polymorphisms in TPMT enzyme result in decreased or absence of enzymatic activity in individuals carrying these alleles (Weinshilboum, 2001). To date, 43 alleles of TPMT designed from *1 to *37 are identified in human population (Appell et al., 2013). TPMT*1 is the wild type form of the enzyme which has the normal activity. Reduced enzyme activity was observed in individuals carrying a normal allele and a defective allele (Relling et al., 2013).

Despite the introduction of TPMT as a drug metabolizer for approximately half a century, there is no information about the natural substrate of this enzyme. To date, four substrates of TPMT in the metabolic pathway of thiopurine drugs are identified (Zaza et al., 2010). 6-Mercaptopurine (6MP), 6-thioguanine (6-TG), thioguanine monophosphate (6-TGMP) and thioinosine monophosphate (6TIMP) are identified as methyl acceptor and S-adenosyl-L-methionine (SAM) as methyl donor (Ujiie et al., 2008; Peng et al., 2008). Considerable progress has been made in understanding the effects of several TPMT alleles on protein activity using heterologous expression systems (Ujiie et al., 2008; Garat et al., 2008; Salavaggione et al., 2005; Hamdan-Khalil et al., 2005). However, there are only a few reports illustrating the effects of most common mutations on TPMT enzyme structure using in vitro and computational approaches (Wu et al., 2007; Garat et al.,

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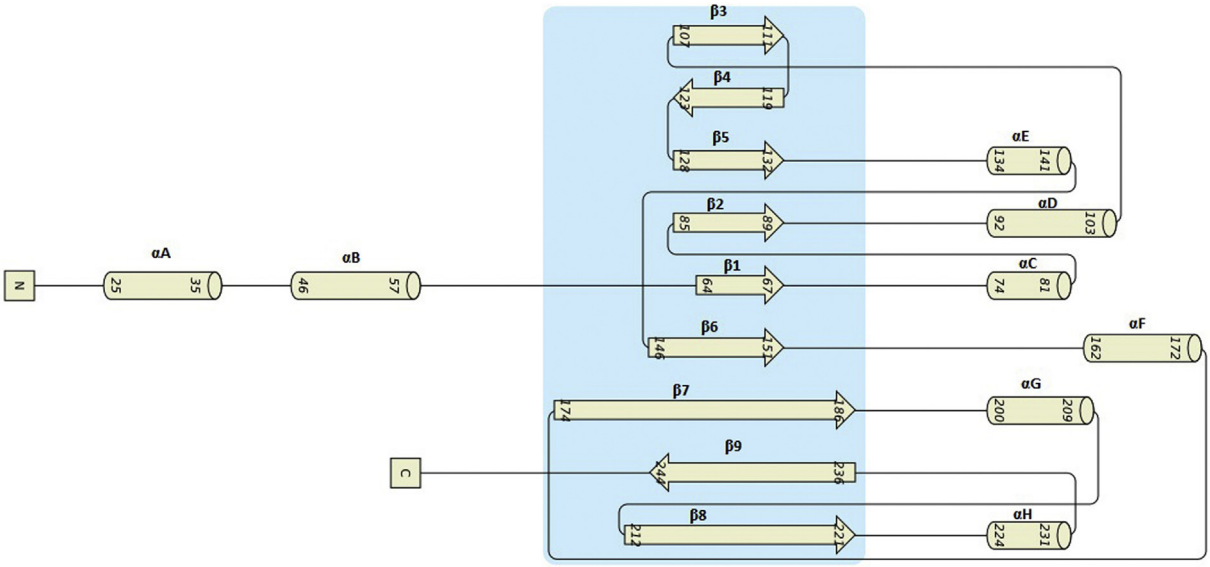

Fig. 1. Schematic representation of TPMT enzyme. Residue numbers indicate the range of each secondary structure. Cylinders are helices (letters from A–H) and broad arrows (numbers from 1 to 9) are strands (image was obtained from http://munk.csse.unimelb.edu.au/pro-origami/(Stivala et al., 2011) using 2 h11 chain A as input).

2008; Wennerstrand et al., 2012; Rutherford and Daggett, 2008). Bioinformatic analysis of point mutations is used routinely by researchers and clinicians to advise the pathogenecity of mutations in combination with other evidence in a variety of diseases (Heineman et al., 2015; Nouri et al., 2014; Keivani et al., 2015). There are different approaches used by researchers, but the most reliable results can be obtained by applying an extensive set of prediction programs. Most of the programs accept both sequence and structure as input; however, using structure information when one is available yields a more complete prediction. For example, structural and functional effects of missense mutations in GJB2 gene was predicted by several in silico tools using both sequence and structure information of the human Connexin 26. Researchers can use the results obtained by this study in their in vitro studies to provide further insights into molecular mechanisms of deafness (Yilmaz, 2015). In the present study, we used bioinformatic tools to study the effects of TPMT variants on protein structure. With the exception of M1V and M1T which result in lack of TPMT translation, all the missense mutation identified in TPMT were modeled onto the corresponding structure and their effects on protein structure were evaluated. In addition, sequence-based analysis of TPMT was used for illustration of the effects of TPMT variants on protein aggregation.

2. Methods

2.1. Collection of missense mutations and protein information

Information about TPMT alleles was obtained from http://www.imh.liu.se/tpmtalleles/tabell-over-tpmt-alleler?l=en (Appell et al., 2013). The effects of mutations on enzyme activity were retrieved from the PharmGKB (Whirl-Carrillo et al., 2012) and the literature (Ujiie et al., 2008; Salavaggione et al., 2005).

2.2. Amino acid conservation

Polyview (http://polyview.cchmc.org/) (Porollo et al., 2004) was used for scoring conserved residues in TPMT protein. Polyview is a web server program for annotation and 2D visualization of protein structures which represent information such as conserved residues and residue relative solvent accessibility. For multiple sequence alignment visualization, sequence homologues to human TPMT protein were obtained from NCBI http://www.ncbi.nlm.nih.gov/ Multiple sequence alignment was performed by Clustal W omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) (Sievers et al., 2011) and then visualized by Jalview (Waterhouse et al., 2009). Jalview is available as a Java applet for on-line use and a desktop application for visualization and annotation of multiple sequence alignments.

2.3. Effects of nsSNPs on protein aggregation and stability

AMYLPRED2 (http://aias.biol.uoa.gr/AMYLPRED2/) (Tsolis et al., 2013) was used to detect the effects of mutation on TPMT aggregation. AMYLPRED2 integrates 11 algorithms for prediction of aggregation prone regions in proteins. Effect of mutations on chaperone binding was investigated using LIMBO (http://limbo.switchlab.org/limbo-analysis) (Van Durme et al., 2009). LIMBO algorithm is developed based on information from peptide binding experiments using the prokaryotic heat-shock protein DnaK which is a representative of the ubiquitous Hsp70 family. These molecular chaperones are specialized in the binding to exposed hydrophobic regions in unfolded polypeptides which result in protein quality control. Stability changes of protein upon amino acid substitutions were predicted by four programs including, SDM (http://mordred.bioc.cam.ac.uk/~sdm/sdm.php) (Worth et al., 2011), I-Mutant2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html) (Capriotti et al., 2005) I-Mutant3.0 (http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi) (Capriotti et al., 2008) and FoldX (Guerois et al., 2002). FoldX predictions were obtained from SNPeffect 4.0 (http://snpeffect.switchlab.org/), an online predition tool using several bioinformatic tools to predict the effects of coding SNPs on protein structure (De Baets et al., 2012). For all these programs protein structure was used as input and default parameters were not changed.

2.4. Structural analysis

Effects of mutations on protein structure were studied by determination of clashing sides and Residueinteraction network (RIN) changes upon mutations. Crystal structure of the TPMT (PDB 2h11) was used for structural analysis. Mutated structures were