## ORIGINAL ARTICLE *IN-SILICO* ASSESSMENT OF COMMON β-GLOBIN GENE MUTATIONS FOUND IN KHYBER PAKHTUNKHWA, PAKISTAN

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Background: β-thalassaemia manifests a spectrum of clinical phenotypes, ranging from mild subclinical disease to severe transfusion-dependent anaemia. This remarkable diversity of disease patterns is not completely explained, but various disease modifying factors have been identified and categorized in to primary, secondary and tertiary modifiers. Nearly 300 mutations in β-globin genes (HBB) have been reported so far. In our previous study we identified six mutations; (Cd 5 (-CT), FR 8-9(+G), FR 16(-C), FR 41-42(-TTCT), Cd 30(G>A) and Cd 15(G>A) to be the most frequent ones in Khyber Pakhtunkhwa province of Pakistan. It is aimed to observe if bioinformatics tools could be used to construct the structure of globin chains carrying these six common mutations. Methodology: Using a computational approach, the sequences of mutated HBB were hypothetically constructed, protein structures were formulated and analysed for homology, and post-translational modifications. In mutations where protein structure formation is halted in vivo, stop codons from the DNA sequence of each of the mutational variant were exclude to allow further analysis. Results: These mutants exhibited variable post-translational modification pattern with little effect on overall structure. Mutations at critical sequences in HBB that do not allow further translation of HBB in vivo and did not stop computer modelling from developing protein structure in-silico. Conclusion: Computational analysis for constructing mutant proteins does not take into account some of the critical checkpoints present in the cell. Studies using computational analysis should be followed by rigorous in vivo validation.

**Keywords:** Thalassaemia, β-globin genes, Post-translational Modification, Computational Genetic Polymorphic Analysis

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#### **INTRODUCTION**

Thalassaemias are a group of congenital disorders characterized by genetic mutations in the globin chain genes.<sup>1</sup> The primary defect is quantitative, whereby the mutation(s) results in reduced production or absence of globin chains of haemoglobin molecule. Other less common forms include structural variants or unstable haemoglobins due to genetic mutations. Based on the globin chain gene involved, the disease is categorized into  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta\beta$ -thalassaemia.<sup>2</sup> Of these,  $\beta$ thalassaemia is most prevalent form in Pakistan. With an autosomal recessive pattern, an estimated 10 million people are carriers and approximately 100,000 patients are currently affected by homozygous thalassaemia.<sup>3,4</sup> Like many other hereditary disorders,  $\beta$ - thalassaemics express a spectrum of clinical phenotypes -ranging from mild anaemia to severe transfusion-dependent anaemia. The pattern of diversity is not completely understood, but various modifying factors have been identified and categorized into primary, secondary and tertiary modifiers of disease.5

Nearly 300 mutations in  $\beta$ -globin genes have been reported so far —most of them are point mutations in the coding or regulatory regions of the

gene (http://globin.cse.psu.edu). Many common mutations involve frameshift whereby the subsequent sequence is entirely altered, and the transcription process is halted. Secondary modifiers of the disease include loci that are involved in globin synthesis.<sup>6</sup> Family studies conducted on siblings with identical βglobin gene mutations have been reported to demonstrate variable clinical phenotype. The secondary modifiers include: co-inheritance of aglobin genes which reduces the cellular damage caused by excess  $\alpha$ -chains, and polymorphisms in Xmn1 and BCL11 genes causing a dilutional effect on the  $\alpha$ -chain excess. Tertiary modifiers include genetic variations associated with disease complications and treatment response. These include mutations in iron metabolism genes, and genes involved in immune system resulting in altered response to infections.<sup>7</sup>

As with most genetic diseases, the mutations in  $\beta$ -thalassaemia pool in each geographic region/ ethnic population presents with a different set of common mutations. In Pakistan, a number of common mutations have previously been reported.<sup>8</sup> In our own work (in press), we have reported that (Cd 5(-CT), FR 8-9(+G), FR 16(-C), FR 41-42(-TTCT), Cd 30(G>A) and Cd 15(G>A) are the six most common mutations in patients from Khyber Pakhtunkhwa. The first four of these mutations are frameshift mutations. The last two are splice, and nonsense mutation respectively.

The current study aimed if bioinformatics tools could be used to construct the structure of globin chains carrying these 6 most common mutations. In a hypothetical scenario where most of the selected mutations did not incorporate early stop codons, it was aimed to assess the effect of these mutations on overall protein structure, post translational modifications and subsequent impairment of haemoglobin protein. To that end, the stop codons from the DNA sequence of each of the mutational variant were excluded. It is that mutations constructed reported through bioinformatics tools carry significant differences from HBB chains. The computational analysis failed to account for the molecular checkpoints that exist in vivo and halt further assembly of protein.

# MATERIAL AND METHODS

Primary data (nucleotide and peptide sequences) were retrieved from GenBank and Uniprot data bases (www.ncbi.nlm.nih.gov/, and www.uniprot.org/). For each of the selected six mutations (Cd 5(-CT), FR 8-9(+G), FR 16(-C), FR 41-42(-TTCT), Cd 30(G>A) and Cd 15(G>A), the normal HBB gene sequences were manually mutated and six different sequences were developed. The mutant nucleotide sequences were translated computationally using online ExPASy-Translate tool (http://web.expasy.org/translate/). Sequences were submitted to the online MSA tool Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/) with default parameters. The subsequent outcome of the MSA was subjected to Phylogenetic Tree development using the Tree development (Neighbourjoining) option in Clustal Omega tool.<sup>9</sup> Online servers provided by Center for Biological Sequence Analysis CBS (www.cbs.dtu.dk/services/) were employed for assessment of post-translational modifications. For observing glycosylation, mannosylation, phosphorylation sites in the normal and mutant sequences; NetCGlyc 1.0<sup>10</sup>, NetCorona 1.0<sup>11</sup>, NetGlycate 1.0<sup>12</sup>, NetNGlyc 1.0<sup>13</sup>, NetOGlyc 4.0<sup>14</sup>, NetPhos 3.1<sup>15</sup> servers were used respectively.

For normal HBB, the crystal (experimentally determined) structure was searched and retrieved from Protein DataBank (www.rcsb.org/). This crystal structure was used as template for development of 3d structures for each of the mutant sequence using Swiss Model online protein prediction server (https://swissmodel.expasy.org/).<sup>16</sup> The resultant structures were analyzed by Rampage server for Ramachandran plot in order to evaluate the predicted structure quality.<sup>17</sup> Predicted protein structures were visualized via Biovia Discovery Studio version 4.5.<sup>18</sup> https://wishart.biology.ualberta.ca/SuperPose/ (The

SuperPose server) was used for protein structurestructure alignment for observing effects of mutations on the protein 3d structure.

## RESULTS

The retrieved HBB sequences were DNA (NCBI id NC 000011.10) and protein (UniProt id P68871). The variant sequences and subsequent translated products are shown in Table-1. Homology and phylogenetic analysis revealed that Cd30 was most homologous to the normal HBB in terms of sequences similarity while variants with frameshift mutations, i.e., FR 8-9, FR 16, and FR 41-42 were least homologous. The MSA revealed the Fr 41-42 to be most distant in terms of sequence identity with a unique gap in the alignment. Two distinct gap patterns were observed in the FR mutations (FR 8-9(+G), FR 16(-C), and FR 41-42(-TTCT)) and CD mutations (CD5 (-CT), CD30(G>A), and CD 15(G>A)). Interestingly CD5(-CT) exhibited the both patterns (Figure-1 A). This sequential arrangement resulted in Cd5 (-CT) to at the center of the Phylogenetic Tree (Figure-1 B). These gaps were the early stop codons which we deleted in the transcript and hence appeared as deletions or gaps in the translated sequences. CBS servers observed the submitted sequences and provided a comprehensive overview of the post-translational modifications for HBB normal and mutant sequences. The default cutoff was 0.5 for each of the prediction tool, with greater values suggesting greater probability. No Cmannosylation sites were observed in the sequences using NetCGlyc 1.0 server in neither the protein nor the variants. NetGlycate 1.0 server computed about 3similar glycation sites while 0 N-linked 5 Glycosylation sites in all the submitted sequences while only Cd15(G>A) variant sequence harbored the only predicted O-linked Glycosylation site, computed by NetNGlyc1.0 and NetOGlyc 4.0 servers respectively. About 7-11 similar phosphorylation sites were estimated by NetPhos 3.1 server. The 6 sites observed in the normal sequence were observed in all of the variants with exception of phosphorylation site at position 5 which was absent in Cd5 (-CT), FR 8-9 (+G), FR 16 (-C), and FR 41-42 (-TTCT). Interestingly FR 8-9 (+G) and FR 41-42 (-TTCT) both exhibited two unique phosphorylation sites at position 9 and 12 and 21 and 40 respectively. All PTM sites are shown in Table-1. Crystal structure of human HBB (PDB ID 1A00) was retrieved from PDB database. The superimposed variants modelled using Swiss Model are shown in Figure-2. Each of the variant structure is shown in red while the normal HBB structure is

depicted in yellow. Overall no drastic change was observed in the variant structures while when Ramachandran plots were observed in comparative

manner minor effects were observed in placement and strain on amino acids within the structures.

ATGGTGCATCTGACGTGGGGGGGGGGGCCTGGGTGGTCTGCCTGTGGGGGCAAGGTGACC GGGGATGAGAGTGCTGGGGCGCTGGGCGCCCTGGGGGCAAGGTGACC AGAGGTCCTTTGAGGCCTGGCCGGCCCGGGCGCCCTGGGGGCAAGGTGA TGAGGGCGCCTGGGCCGGCGCGCGCGGCGGCGCGCGCGCG	<b>NF</b> 1 10	Table-1: List of HBB mutations and respective nucleotide and peptide	
<ul> <li>GTGGATGAAGTTGGTGGTGGGGGGCCCGGGCAGGCTCGGTGGGTCACCCTGGACCC</li> <li>DEVGGCTTAGTGGAGGCTGGTGGCTGGTGGCCCGGGGACCCTTGGGCAGGCG</li> <li>DRIKGTFATLSELHCDXLH</li> <li>DRIKGTGGCTGGCTGGTGGCCGGGGGGGGGCACGTTGGCGCAGGGTG</li> <li>CAGGCTGCCTGGGGAGGGGGGGGGGGGGGGGGGGGGGGG</li></ul>	Mutations	Nucleotide sequence	Translated product
AGAGGTTCTTTGAGTCCTTGGGGATCGTCCACTCCACTC			
<ul> <li>AAGGTGAAGGCTCATGGCAAGGAAGGTCCTC</li> <li>CH5</li> <li>GGCCTTTATGGCATGGCTCGCCTCACCTGGACAACCTCAAGGCACTTGCCACTTGCACACCTCAGGACGCTCGGGTGGCTGGC</li></ul>			DEVGGEALGRLLVVYPWTQ
CDS GGTGCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTGCCACAC TGGAGGTGGCTGGTGTGGCCGCAGGGGAGCTCCTGAGGACACCTGAGGGCACCAC WEILERRESAVTALSELHCDKLH VACACGTGGATGGGCGTGGGTGGGCGCGGTGGGCGCAGGGGCCAGGGG WEILERRESAVTALSELHCDKLH VACAGGGAGTGCTGAGGGGGGGGGGGGGGCGCGCGCGGGGCCAGGGG WEILERRESAVTALSELHCDKLH VCCAGGGGTGGGTGAGGCCGTGGGGGGGGGCGCCGCGGGGCGCGCGGCGCGCGC		AGAGGTTCTTTGAGTCCTTTGGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCT	RFFESFGDLSTPDAVMGNPK
TGAGTGGACTGCACTGTGGTGGCACAGGTGCGTGGGGTGGTGGCCACGAGGTCGGGTGGGCAGGGTA CAGGCTGCCTATCAGAAAGTGGTGGGCGTGGTGGCGCAGGCCCCACGAGGTATC ACTAA ATGGTGCATCTGACTCCTGAGGACAAGGTCTGCCGTTAGCCCTGTGGGGCAAGGGTC GGCAGGGATCATGGGTGGTGGGCGCGGGCGGGGCG		AAGGTGAAGGCTCATGGCAAGAAAGTGCTC	VKAHGKKVLGAFSDGLAHL
TGAGTGGAGTGGACTGGTGGGCGGGGTGGGGTGGGGTGG	>CD5	GGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACAC	DNLKGTFATLSELHCDKLH
<ul> <li>CAACGTGCTGGTCTGGTCTGGTCGGCCGCACTGTGGCGAAGGACGTGGCCACGAGGTG</li> <li>KGANALAHKYH</li> <li>ATGTGGATGCATCTGACTCCTGAGGGAGGAGGCTGCGCGCTGGTGGGCAAGGTG</li> <li>WCILLERKSAVTALWGKYN</li> <li>ACGTGGATGAAGTGGGGGGAGGTGCCGCGCTGGTGGGCAAGGTG</li> <li>WCILLERKSAVTALWGKYN</li> <li>CCAAGGTGATGGAGGCCTGGGGAGGTGCCCGCCTGGTGGGGCAAGGTG</li> <li>CCAAGGTGAAGGCCATGGGCAAGAAGTGCTCGGTGCCTTTAGTGATGGCCGCAC</li> <li>CCAAGGTGAAGGCCCTGGGCAAGAAGTGCTCGGTGCGTTAGTGCTGGCC</li> <li>CCAAGGTGAAGGCTCCTGGGCAAGAAGTGCTCGGTGCGTTAGTGCTGGCC</li> <li>CCAAGGTGAAGGCCCTGGGCCACAGAAGTGCTCCGGTGCCTTAGTGATGGCGCGC</li> <li>CCAGGTGGATGCTCCGGGCCACCAGGTGCTTAGTGCTGGCGCC</li> <li>CCAGGTGGATGCTGCGGGCCCACAGGTGCTTAGTGCGGGGAAGTGGCCCCTGGGGAAGTGGCCCCTGGGGGAAGGTGCTTAGGCACGTGGCAGCACGTGCCTAGGAAGTCCCCCCCACAGGTCCTTAGGGAAGGTGCTGCCCCCCCC</li></ul>		TGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGCTCCTGGG	VDPENFRLLGNVLVCVLAH
CAGGCTGCCTATCAGAAAGTGGTGGCTGTGGGCTGATGCCCTGGGCCCACAGTATC GYANALAHKYH ATGGTGCATCTGACTCCTGAGGAAAGGTCTGCCGTTATCGCCTGTGGGGCAAGGT CCCAGAGGTTCTTGAGTCCTTGGGGATCGCCGTGGCGTGGGGTCATCCGCTGGGGGCAAGGT CCCAGAGGTCTTTGAGTCCTTTGGGGATCGTCGCCTTGTGGGGCAAGGTC CCCAGGGACCAAGGCCCGGGCCCGGGCGCGCTGGGGGGCAGGCC CCCAGGGACCCAGGACGTCAGGCCACGGCGCGCTATCGACAGCGC CCCAGGGCCCTGGGCCGGCCGGGGCGCAGGGCGCAGGCG CCGGGCACCCTCAGGGACGCCGGCGCGGGCGCAGGGCGCAGGCG CCGGGGCACGCCTGGGGGGAGGCCGCGGCGGCGGCGCAGGCG CCGGGGCACGCCTGGGGGGAGGCCCGGGCGGGGGCAGGGCA CCGGGCACCCCAAGGGCCCGGCGCGGGGGGGCAGGGCA			
ACTAA  ACTAA  VCILLRRRSAVTALWGKVN AACGTGGACATCTGACTCTGGAGGAGAAGGTCTGCCGTTACTGCCCTTGGGGCAAGGTG ACGTGGATGACAGTTGGTGGGAGGCCCTGGCGGCGCGCTGCTGGTGGTCACCCTTGGCACGCCGAGGCAGGC			
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<pre>&gt;FR 89 CCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAAC 0RFESFGDLSTPDAVMGNP KCGCGTCAGTGCTAGGCCAGAGAGAGTGTCGGCTGGGTGGTCGGCC LINDPENFRLLGNVLVCVL CATCACTTTGGCAAAGAATTCACCCCACCGGTGCGGTGGGTG</pre>			
<ul> <li>&gt;FR 8-9</li> <li>CCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGTGTCCTTTAGTGACGCCTGCT</li> <li>ÉVXAHGKKVLGAFSDGLA</li> <li>CCTAGGTGAACCTCAAGGACCCTTGCCAACGAGCACAGGTGGCTGGTGGCACAGCT</li> <li>LINDEKGTFATISELHCDK</li> <li>CCTGGACAACCTCGAGGAACAGTGCCGCGCAGGTGGCTGGTGGCTGGC</li></ul>			
<ul> <li>SPR 8-9</li> <li>ACCTGGACAACCTCAAGGGCACCTTGCCACACGAGTGACGTGCACTGTGACAAGCT</li> <li>HLDNIKGTFATLSELHCDK</li> <li>ACGTGGATCTGAGAACTCACCCACACGAGCGCCGCGGCTGCTGGTGGTGCCCGCC</li> <li>HLDNIKGTFATLSELHCDK</li> <li>ATGGTGCATCTGAGAACTCACCCACACGAGCGCCGCTGGCGGCGCCCC</li> <li>ATGGTGCATCTGAGACCTCGGCCACAGGAGCTGCGGCGCCCTGGGGGAAGGTGAA</li> <li>ATGGTGCATCTGAGCCCTGGGCAAGAGTCGTCGCGTGCTGGTGGTGCACCCTGGGCAACGCC</li> <li>ATGGTGCATCTGAGGCCCTGGCCAGGCTGCGGTGCGGTG</li></ul>			•
<pre>ACC 100ACAACC TCAAGGCTACTGAGCCAACGGCCGCGGCTGGTGCTGGCCGC GCGGGGCCCCAAAGAATCACCCCACCAGTGCAGGCGCGCGGGCTGCGGCCGCC CACGGGGATCGAGTGGGCGCAACGGCCGCGGGCGGCGGGGCGCCTGGGGCAACGGGCC CGGGAGCAGCCCAAGGGCCGCGGGGGCGCCGGGGCGCCCTGGGGCAACGGGCC CGGGGCCCCTGGGGAAAGGCCCTGGGGCACGGGCGCCGGGCCCC CGGGGCCCCCGGGCCCCCGGGGCCCCGGGCGCCGGGCCGGCGGCCGGCCG CGGGGCCCCGGGCCCCGGGCGCCGGGGCCGCGGGCCGCGGCCG CGGGGCCCCGGGCCCCGGGCGCCGGGGCCGCGGGCCGGCGGCGGCGGCGGCGGCGGCCGGCCG CGGGGCCCCGGGCCCCGGGCGCGCGGGGCGCGGGGCGGC</pre>	>FR 8-9		
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<ul> <li>CTGGTGGGCTAATGCCCTGGCCCACAGTATCACTAA</li> <li>VAGVANLAIIKÝI</li> <li>ATGGTGCATCGACTCCTGAGGAGAAGTCTGCCCGTTGCGCCTGGGGGAAGGTGAA</li> <li>GABDSGEVCRYCPVCKVKVV</li> <li>CGGGATGTAGTGGTGGTGGGGAGGCCTGGGGCAGGCTGCTGTGTGGGCAACCC</li> <li>RFESFGDLSTPDAVMONK</li> <li>CGGGATCCTAGGGAGACTCGTCGGGCACCTGCGGCTGGTGCTGGCCTGC</li> <li>CTGGACAACCTCAAGGGCACCTGGGCACCGTGGGGCTGCCTGC</li></ul>			
ATGGTGCATICTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGAAGGAA		CATCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGG	
>FR16 COTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTGGGCACGTGACGAGGTCACGAGGTGAGGAGGCTCATGGCAAGGAAAGTGCTGGGCAAAGAAGTGCTGGGCGCACGTGAGGCAGGC		CTGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAA	VAGVANALAHKYH
>FR16 COTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTGGGCACGTGACGAGGTCACGAGGTGAGGAGGCTCATGGCAAGGAAAGTGCTGGGCAAAGAAGTGCTGGGCGCACGTGAGGCAGGC		ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGAAGGTGAA	GASDSGEVCRYCPVGKVNV
>FR16 CAGAGGTTCTTTGGGTCCTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCC RAGGTGAAGGCTCATGGCAAGAAAGTCTCGGGCCTCTGGGACAAGCTGC CAGGGACAACCTCAAGGGCACCTTTGCCACACTGAGGAGCTGCTGTGTGTG			DEVGGEALGRLLVVYPWTQ
<pre>&gt;FR16</pre>			RFFESFGDLSTPDAVMGNPK
FR 16 C1GGACAACCTCAAGGGCACCTTGCCACACTGAGGCAGCTGCGCACGTGGCAAGGCTGC DNLKGTFATLSELHCDKLH ACGTGGATCTGAGAACTTCAGGCCCCAGGGCACGTGCGGCTGGTGGTGGGGCCA CTGGGCAACGTGAGAACTTCAGGCCCCCACCAGGCGCCCCTACCAAAGTGGCGGC CGTGTGGCTATGCCCCTGGGCAAGGCTGCCGGTGCTGGGGCAAGGTGA CGTGTGGCAACGTGAGGCAGGCTGGCGGCGCGCGCTATCAGAAAGTGGTGGCG CGTGTGGCCACGTGGGCAGGCTGCGGGCAGGCTGCCGGGCAAGGTGA CGCTTGGCACCCTGGGCAGGCTGCGGGCAGGCTGCCGGCCCACGGCAGGCTGCGGCAAGGTGA CGCTTGGCCACCGGGCAGGCTGCGGGCAGGCTGCCGGGCAAGGTGCAGGCCCAGGGCAAGGTGA CGCTTGGCCACGTGGCCAGGCCGGCGCGCGGCGCGCCCACGGGCAAGGTGCAGGCCCAGGGCAAGGTGA CGCTGGCCAAAGAATTCACCCCACCAGGGCAGGCTGCCGGGCAAGGTGCAGGCGCCA CCAGGGGTGAGCTAGGCAAGGTCGGGCAGGCTGCGGCGCGCCTATCAGAAGGTGGCGGCAGGTGGAGGCCCAGGGCAAGGTGCAGGCCCGGCCAAGGTGCAGGCCCGGCCA CCAGAGGTGAGCACCTCAAGGGCACGCGGCGCCTATCAGAAAGTGGTGGCT CGGGCAACCTCAAGGGCACCTGGGCAAGGTGCGGCGCGCGC			VKAHGKKVLGAFSDGLAHL
ACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGGTGGTCTGTGCGCCAC VPDPENFRLLGNVLVCVLAH TGGTGGCATCGCACGGCCACAGTATCACTAA TGGTGGCATCGCACCGGCCACAGTATCACTAA Child and a strain a	>FR 16		
<ul> <li>TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCT</li> <li>HFGKEFTPPVQAAYQKVVA</li> <li>GGTGGGCTATGGCCCGGCCAAGGTATCACTAA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAGTCTGCCGTTACTGCCCCTGGGGCAAGGTGA</li> <li>GSWWGPQAAAGLPLDPE</li> <li>TGGTGGTGCAGCCTGGGCAGGCTGTGTGGTGCTCACCCTTGGACCCAGAGGTGAG</li> <li>GGACTGCCACTCCTGATGCTGTTATGGGCAACCTCAAGGTGAAGGCTCATGGCAAG</li> <li>GGACTGCCACTCGATGCCTGGCCAGCCTGGGCAAGCTCAAGGGCACCCCACGGA</li> <li>GGTGACCAGCACT</li> <li>GGTGGCGCCCA</li> <li>GTGACCAGCCAT</li> <li>GGTGGGCCACT</li> <li>GGTGGGCAAGTTCACCCCACCAGTGGAAGCTCAGGGCACGCC</li> <li>SFR 41 42</li> <li>TCACTTTGGCAAGAATTCACCCCACCAGTGGCAGGCTGCTGTGGGCCACC</li> <li>GGGGGGGCAAGTTGAGCGCCTGGGCAGGCTGGCCGTTACTGCCCTGGGGCAAGGTGA</li> <li>ACGTGGACAGCCAAGAAATTCACCCCACCACGTGGCCGTTACTGCCCTGGGGCAAGGTGA</li> <li>ACGTGGACGCCCAAGAAATTCACCCCACCAGGGCGCGCGC</li></ul>			
GGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAA       GVANALAHKYH         ATGGTGCATCTGACCCCTGGGCAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA       GASDSGEVCRYCPVGQGER         ACGTGGATGAAG       GSWWGPGQAAGGCLPDPG         TTGGTGGTGAGGCCCTGGCCAGGCTGCTGGTGGTCTACCCTTGGACCAAGGGTGAG       GSDSGEVCRYCPVGQGER         GGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAG       NLKGFKATLSELHCDKLHY         AAAGTGCTCGGT       VESFGDLSTPDAVMGNPK V         GGTGTGGCTAATGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACTGA       NLKGFFATLSELHCDKLHY         DPENFRLLGNVLVCVLAHH       GTGAACAGCTGCACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGGTGGTCT       VANALAHKYH         GTGTGGCCAA       GTGTGGCCAAA       VANALAHKYH       VANALAHKYH         >FR 41 42       TCACTTTGCAAAGAATTCACCCACCAGTGCCAGGCTGCCTATCAGGAAAGTGGTGGCT       GGTGTGGCTAAT       VANALAHKYH       VANALA			
<ul> <li>ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA</li> <li>ACGTGGATGAAG</li> <li>GAGTGGATGAAG</li> <li>GAGTGGAGGACCTGGGCAGGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTGAG</li> <li>GGACTGCTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAG</li> <li>GGACTGCTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGGGAAGGCTCATGGCAAG</li> <li>GGAGCTGCACTGGACTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACGAG</li> <li>GTGACAGCCCCA</li> <li>GTGACTGCACT</li> <li>GGTGGTGGCTGACCCGGGACACCTGAGGACACCTCAGGGCAACGTGCGGCTGGCT</li></ul>			
<ul> <li>ACGTGGATGAAG</li> <li>ACGTGGTGAGGCCCTGGGCAGGCTGGTGGTGGTCTACCCTTGGACAGAGGTGAG</li> <li>TTGGTGGTGAGGCCCTGGCAGGCTGGTGTATGGGCGAGCCCTAGGAGAGGCTCAGGGAAGGCTCATGGCACCCTGGTGGCCCACCTGGATGCTGGTATGGGCAACCTCAAGGGCAAGCCTATGGCAAGGCTAGGGAAGGCGCAGGTGGGGGGCGCGCCTTAGGAAGGCTGCACCTGGGCAAGGCGCCGGCCCACGGGCAGCTGGGCGCAAGGTGGGGGGGCGGGGCGCGCCACGTGGGCGCGCCACGTGGGCGCAAGGTGGGGGGGCGGGGGGGCGCGGCCAAGGTGGGGGGGG</li></ul>			
<ul> <li>TTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCACCCTTGGACCCAGAGGTTGAG</li> <li>VESFGDLSTPDAVMGNPKV TCCTTTGG</li> <li>GGATCTGTCCACTCCGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAG GGATCGTCCACTCGATGGCTGGTCACCTGGACACCCTCAGGGGAAGGCTCATGGCAG GTGACCAGCGCCACGTGGATCCTGGACAACCTCAAGGGCACCCTTGGCCACACGTG GTGACGCCCAC</li> <li>FR 41 42</li> <li>TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTGTGGGGGCAAGGTGA ACGTGGATGAAGTGCTGGTGAGGCCCTGGGCAGGCTGCCTGTGGGGGCAAGGTGA ACGTGGATGAAGTTGGTGGGGAAGGTCGCCGGCAGGCTGCTGGGGGCAAGGTGA ACGTGGATGAAGTTGGTGGGGAAGGTCGCCGGCAGGTGCGCTGGGGCAAGGTGA ACGTGGATGAAGTTCACTAA ATGGTGCCACCTGAGGCACGTGCCGGCAGGCTGCCTGGGGGCAAGGTGA ACGTGGATGAAGTCGCGGCAAGGAAGTCTGCCGGTCGCCTTACGAAAGTGGGGCAC GTGGACCCCAAGGGCACCTTGGCCACGGCGCGCTGCGGTGGGCTGACCCTG GGGATCCTGAGGCACCTTGGCCACAGTGCAGGTGCGCTGGCGGCCACCCT ACTTTGGCAAAGGCACCTCGGGGCAAGGTGCGCGTTACGGCGGCCACCCT ACTTGGCAATGGCCTGGGCCACAGTGCCGGCCGGCCGGCC</li></ul>			
<ul> <li>TCCTTTGG</li> <li>KAHGKKVLGAFSDGLAHLD</li> <li>GGATCTGTCCACTCGGTGGTATGGGCAACCTAAGGTGAAGGCTATGGCAGA</li> <li>AAAGTGCTCGGT</li> <li>GTGACCACTCGGCCACCTGGCACCTGGACAACCTCAAGGCGACGCTGTGCCACCTGGGCAACGTGCGCCACT</li> <li>FGGCTTAGTGGCCGACAGGTCCTGAGAACTTCAGGCCCTGGGCAACGTGCGGCTG</li> <li>TGTGCTGGCCCA</li> <li>FR 41 42</li> <li>TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCGTTACTGGCGCAACGTGGCGCA</li> <li>FGGTGACAAGCTCACAGTGGTGGGGAAGGCTGCCGTTACTGCCCTGTGGGGCAAGGTGA</li> <li>ACGTGGACACGTGACGTGGGGGAAGGCCCTGGGCAGGCTGCTGGTGGGGCAAGGTGA</li> <li>ACGTGGATCGAAGTTGGGGGAAGGCCCTGGGCAGGCTGCCTGTGGGGGCAAGCTGCACCTTGGGACAACCTCAAGGGCCCACGTGGGCAAGGTCGCGGTGCGGGCCAACGTGCACCTGGGCAACCCCAAGGAAGTTGGGGGAAGGCCCAGGCTGCCGGTGCGTGTGGTGGGCCAACCTGAGGAAGTTGGGGGAAGGTCACCCTGGGCAAGCTGCACGTGGGGCAAGGTGCAGGCCCCACCTGGGCAAGGCTGCCGGTGCGGGCCAAGCTGCAGGTGGCCCACC</li> <li>ACGTGGACACCCAAGAAATTCACCCCACCGGGCAAGGTGCAGGCTGCCGGTGCGGGCCAAGGTGCA</li> <li>ACGTGGAAGGCCCAGGGCGCCTGGGCAAGGTGCCGGTGCGCCACCTGGGGCAAGGTGCA</li> <li>ACGTGGAAGGCCCAAGGGCGCCTGGGCAAGGTGCCGGTGCGCCAACCTGGGCAAGGTGCA</li> <li>ACGTGGAAGGCCCAAGGGCGCCTGGGCAAGGTGCCCGTGGGCAAGGTGCA</li> <li>CCAGGGGATCAAGGCCCAAGGAAGTCGCCCGGTCACCTGGGGCAAGGTGC</li> <li>CCTGGACAACCTCAAGGGCACCTTGCCCACTGGGCAAGCTGCCGGCCCACCTGGGCAAGGTGC</li> <li>CCTGGACAACCTCAAGGGCACCTTGCCCACTGGGCAAGCTGCCGGTCACCTGGGCCAAGCTGCGCCCACCTGGGCAAGGTGCCATGGGCAAGGTGCCCTGGGCAAGGTGCCCATGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCCAAGGTGCCCTGGGCCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGCCCTGGGCAAGGTGC</li> <li>CCTGGGACAACCTCAAGGGCACCTGGCCGGCGGCGCGGGGGGGCGCGGGGGGCGCGGGGCGCGGGCGGGG</li></ul>			
<ul> <li>&gt;CBATCGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGGTGAAGGCTCATGGCAAG</li> <li>NLKGTFATLSELHCDKLHY</li> <li>AAAGTGCTCGGT</li> <li>GCCTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTGCCACACTGA</li> <li>GTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGCACCTCAGGGCACCGTGCGGTCG</li> <li>&gt;FR 41 42</li> <li>TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCT</li> <li>GCCTGGCCCACAAGTATCACTAA</li> <li>ATGGTGCATCTGAAGTGCTGGGGAAGGCTGCCGTACGCCGGGCAAGGTGA</li> <li>ACGTGGATAGACTCTGAGGAAGATCGCCCGGCAGGCTGCCTGTGGGGCAAGGTGA</li> <li>ACGTGGATGAAGGTCGCTTGGGGAAGGCCCTGGGCAGGCTGCCTGTGGGCGAAGGTGA</li> <li>ACGTGGATGAAGTCGCCGGAGAAGTCGCCGGGCAGGTGCCTGTGGGGCAAGGTGA</li> <li>ACGTGGATCGAAGACTTCAGGCCCCGGGCAAGGTGCCTTATGGGCAAGCTGCAC</li> <li>GGACAACCTCAAGGGCACCTTGGGCAAGGTGCCTTTAGGGCAAGCTGCAC</li> <li>ATGGTGCAATGCCCTGGAGAAAGTGCTCGCGGTGCCTATCAGAAAGTGGTGGCCG</li> <li>ATGGTGCAATGCCCTGGAGGAAAGTCGCCGGGCCAGGTGCCTATCAGAAAGTGGTGGCCG</li> <li>ATGGTGCAATGCCCTGGGGAAGAAGTCGCCGGGTCGGTCG</li></ul>			
AAAGTGCTCGGT Characterization PENFRILLGNULVCVLAHH GCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACGTGA GTGACCTGGCCCAC GTGACCAGCTGCACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTCGGCCCA GTGACCAGCTGCACGTGGATCCTGAGAACTTCAGGCAGGC			
<ul> <li>&gt;CR11</li> <li>GCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCACACTGA GTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTGTG GTGCGGCGCCA</li> <li>&gt;FR 41 22</li> <li>TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCT GCCCTGGCCCACAAGTATCACTAA</li> <li>ATGGTGCATCTGAGTGCTGGGGAACGTCGCCGGCCAGGCTGCCTGGGGGCAAGGTGA ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCACCCTTGGAC ACGTGGATGAAGTCCTTTGGGGAACGTCGCTGGGCCAGGCTGCTGGTGGCCCACC GGACAACCTCAAGGGCACCTTTGCCACCTCGGGCCAGGCTGCTGGTGGCGCCCACC GTGGATCATGCCCTGGCCAAGAAGTCTGCCGTTACTGCCGTTGTGGCGGCCCACC GTGGATCATGCCCTGGCCAAGAAGTCTGCCGGCCACGTGCGGCTGGGCGCCACCT GGGCTAATGCCCTGGCCAAGAAGTCTGCCGGCCACGTGGGGCAGGGCAGGTGA ACGTGGATGAAGTTCACCCCACGGGCAAGGTGCGCCTATCAGAAAGTGGGGGGCAG TGTGGCTAATGCCCTGGGCAAGAAGTCTGCCGGTACCGTGGGGGCAAGGTGA ACGTGGATGAAGTTGACTCCTGAGGACAAGTCGCCGGCCACGTGGGGCAAGGTGA ACGTGGATGAAGTTGACTCCTGAGGACGCCTGGGCAAGCTGCTGGGGGCAAGGTGA ACGTGGATGAAGTTGGGGGGAGAAGTCTGCCGTTACTGCCGTGTGGGGGCAAGGTGA ACGTGGATGAAGTTGACCCCACAGGAAGTCTGCCGTTACTGCGCTGTGGGGGCAAGGTGA ACGTGGATGAAGTTGGGGGGAGAAGTCTGCCGTGCCCTTATGGGGCAACG CCAGAGGTTCTTTGGGACCCTTGGGACACGTGCTGGGTGGCTGGC</li></ul>			
<ul> <li>&gt;FR 41 42</li> <li>TCACTTTGGCAAGATCACGAGAACTTCAGGAACTTCAGGCAACGTGGGCGGCAAGGTGGA</li> <li>FR 41 42</li> <li>TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCT</li> <li>GGTGTGGCTAAT</li> <li>GGCCCTGGCCCACAAGTATCACTAA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA</li> <li>ACGTGGATGAAGCTCATGGCAGGCTCGCGGTGCCTGTGTGTG</li></ul>			
<ul> <li>&gt;FR 41 21</li> <li>TCACTTGGCAAGAATTCACCCCACGTGCAGGCTGCCTATCAGAAAGTGGTGGCT GGTGTGGCTAAT</li> <li>GCCCTGGCCACAAGTATCACCCAAGAAGTCGCCGGTGCTGTGGGGGCAAGGTGA ACGTGGATGAAGTTGGTGGTGAGGCCCTGGCGGTGCTGGGGGCAAGGTGA ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTAAGTGGGGCAAGAAGTCTGCCGTCACTCCTGATGCGGCCAACCCTA AGGTGAAGGCTCATGGCAAGAAGTGCCGGGCACGTGGTGGTGTACTGGCCACCCT GGACAACCTCAAGGCACCTTTGCCACACTGAGGCGCCTGGGGCCAACGTGGAC GTGGATCCTGAGAACTTCAGGCCCCACGAGGCGCCGCTGGTGGTGGCCGCCATC ACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCGCCGCTGGGGGCAAGGTGA TGTGGCTAATGCCCTGGGCGAAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA ACGTGGATGAAGGTCGTGGGGGAGGCCCTGGGCCAACGTGGGGGCAAGGTGA CCAGAGGTTCTTGGGGGGAGAGGCCCTGGGCCAACGTGGGGGGCAAGGTGA ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA CCAGGGGATGAAGGTGGTGGGGGGGGGG</li></ul>			
FR 41 42 TGTGCTGGCCCA FR 41 42 TCACTTTGGCAAAGAATTCACCCCACAGTGCAGGCTGCCTATCAGAAAGTGGTGGCT GGTGTGGCCTAAT GCCCTGGCCAAAGTATCACTAA ATGGTGCATCTGACTCCTGAGGAGAAAGTCGCCGTTACTGCCCTGTGGGGCAAGGTGA ACGTGGATGAAGTTGGGGGTGAGGCCTGGCCTGGCTGGTGCTATCGGCAAGGTGA ACGTGGATGAAGTTGGGGGTGGCACGGGCCCTGGCCGGCC			VANALAHKYH
FR 41 42 TCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCT GGTGTGGCTAAT GGCCTGGCCCACAAGTATCACTAA ATGGTGCATCTGACCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGA ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTGAGTCCTTGGGGATCTGTCCCGCGCCACCTGATGGCGGCCACCT AGGTGAAGGCTCATGGCAAGAAAGTGCTCGGGCCACGCTGCGTGGCCAAGCTGCACCT GGACAACCTCAAGGGCACCTTTGCCACACTGAGGAGGCTGCTGTGGGGCAAGCTGCACCT GGACAACCTCAAGGGCACCTTGCCACACTGAGGAGGCTGCTGTGGGCCAAGCTGCACCT AGTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCGCTGTGGTGGCGCCACCT ACTTTGGCAAAGATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCGGC CTAGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA CTAGGTGAAGGCTCATGGTGGGGAGGCCCTGGGCAAGCTGCCTGTGGGGCAAGGTGA CTAGGTGAAGACTCCAGGGCCCTGGGCAAGCTGCCGTGCCCTGGCCAAGGTGA CTAGGTGAAGACTCCAGGCACCTTTGGCGACACGTCGGGGCCAGGCTGCACCCTTGGACAAGCTGGCGCCACCTTGGACAAGCTGCAGGCCCTGGCCACCCTGGGCAAGGTGCCTGTATGGGCAACC CTAGGTGAAGACTCAAGGCCCCTGGGCAAGTGCGGCCCGGCCACGTGGCCACCCTGGACAAGGCTGCCTGGCCACCCTGGGCAAGGTGGCCCGGGCAAGTGGGGCCCGGGCACGTGCCTGGCCACCTGGGCAAGGTGGCC CCAGAGGTCCTGAGAACTCAGGCCCCTGGGCAAGGTGGCGCCGCTGGCCGGCC			
<ul> <li>&gt;CD30</li> <li>GGTGTGGCTAAT</li> <li>GCCCTGGCCACAGAGTATCACTAA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGCTGTACTGCCCTGTGGGGGCAAGGTGA</li> <li>ACGTGGATGAAGTTGGTGGTGAGGCCCTGGCCAGGCTGCTGGTGGGTCTACCCTTGGAC</li> <li>CCAGAGGTTGAGTCCTTTGGGGATCATGCCACTCGGCCACCT</li> <li>GGACAACCTCAAGGCACCTTTGCCACCTGGGCAGGTGAGCTGCACTGTGACAAGCTGCAC</li> <li>GTGGATCCTGAGAACTTCAGGCTCCTGGGCAAGGTGCACTGTGGCTGGGCCACCT</li> <li>ACTTTGGCAAAGAATTCACCCCACAGTGCAGGCTGCCTATCAGAAAGTGGTGGCCGG</li> <li>TGTGGCTATGCCCTGGGGAGAGCTGCGCGCACGCTGGCGGGGCAGGGCAGGGCA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAGTCTGCCGCGCAAGGTGCCTGGGGGCAAGGGCG</li> <li>ATGGTGCATCTGAGCCCTGGGGAGAGTCTGCCGCGCAAGGTGCCTGGGGGCAAGGGCA</li> <li>CTAAGGTGAAGGTCCTGGGCAAGAAGTGCTGCGGCAAGGTGCCGGGCAAGGCG</li> <li>CCAGAGGTTCTTTGAGCCCTTGGGGAAGCTCGCGGCCACCCGGCCAAGGTGCCGGCCCACACGTGCGGCAAGGCG</li> <li>CTAAGGTGAAGGTCCTGGGCAAGAAGTGCTCGGGCGCCACCCGGCCAAGGGCAAGGCG</li> <li>CCAGAGGTCCTGAGAACTTCAGGCACCTTTGCCACACGAGGTGCGCTGAGCCGGCCG</li></ul>			
<ul> <li>GCCCTGGCCCACAAGTATCACTAA</li> <li>ATGGTGCATCTGACTCGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA</li> <li>ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTGCTACCCTTGGAC</li> <li>CCAGAAGGTCATGGCCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGGAGCCACCCTA</li> <li>AGGTGAAGGCTCATGGCAAGAAAGTGCTCGGGCACGTGCACTGTGGCCAACCCCTA</li> <li>GGGATCCTGAGAACTTCAGGCCCTGGCCACCTGGGCACGTGGCCTGGCCCACCC</li> <li>GGGATCCTGAGAACTTCAGGCCCCGGCACGGCGCCTTACGAGAAGTGGTGGCCGCGC</li> <li>ACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCCTTACGAAAGTGGTGGCCACC</li> <li>ATGGTGCATCTGAGCACCTTTGGGGAACGTCGCCGTTACTGCCCTGGGGCAAGGTGA</li> <li>MVHLTPEEKSAVTALWGK</li> <li>ACGTGGATGAAGGCTCATGGCGAGGCCCTGGGCCACACTGGGGCCAAGGTGA</li> <li>MVHLTPEEKSAVTALWGK</li> <li>CCAGAGGTGCATGGGGGAGGCCTTGGCGCCACACTGGGCCCTTACGAGAAGTGGCGCGCCAC</li> <li>CTAAGGTGAAGGCCCATGGCAAGAAAGTGCTCGGCGCCCTTAGTGGGCCTGGCCCAAAGCTG</li> <li>CCTGGACAACCTCAAGGGCACCTTTGCCGCACCACTGGGCGCCGCTGGTGGCCTGGCGCCAAGGCCC</li> <li>CCTGGGACAACCTCAAGGCACCTTGCCCACACGGCAGCTGCCCTATCAGAAAGTGGTGGC</li> <li>CCTGGGCAAAGAATTCACCCCACCAGTGCAGGCGCGCGCCTGTGGGGCCAAGGGCC</li> <li>CCTTTGGGCAAAGAATTCACCCCACAGTGCCGGCCGCTGGGTGGCCTGGGGCCAAGGTGG</li> <li>VNLPVEVGGEALGRLLVVYPW</li> <li>CCAGAGGTGAGAGCTCATGGCAAGAAGTGCTCGGCCGGGCGCGCGC</li></ul>	>FR 41 42		
<ul> <li>ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGA</li> <li>ACGTGGATGAAGTTGGTGGTGAGGCCCTGGCCAGCTGCTGGTGGTGCTACCCTTGGAC</li> <li>CCAGAGGTTGAGCCCTTTGGCAAGAAAGTGCTCGGGTCCTGTGTGGCGGCACCCTA</li> <li>AGGTGAACCTCAAGGCACCTTTGCCACACTGAGTGAGCTGCCTGTGTGCGGCCCACC</li> <li>GTGGCTAATGCCCTGGCCACACGTGCCGCCTGGCCTGTGTGGCGGCCACC</li> <li>ACTGTGGCAAGAAGTTCACCCCACAGTGCAGGCTGCCTGTGCGGGGCAAGGTGGC</li> <li>ATGGTGCAATGCCCTGAGGAGAGAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGCA</li> <li>ATGGTGCAATGCCCTGAGGAGAGAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGGC</li> <li>ATGGTGCAATGCCCTGAGGAGAGAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA</li> <li>AVHLTPEEKSAVTALWGK</li> <li>ACGTGGATGAAGTTGGTGGTGGGGGAGGCCCTGGGCAAGCTGCTGTGTGGCGCAAGGTGA</li> <li>CTAAGGTGAAGGCTCATGGCAAGGAGAAGTCTGCCCTGGGGCCCTTACTGGCCACCCTGGACAACCT</li> <li>CCAGAGGTTCTTGAGTCCTTGGGGAACGTCGCGGCCCTTACTGGCAAGCTG</li> <li>CTAAGGTGAAGGCTCATGGCAAGAAGTGCTCGGGCCCCTGGGCCCTGGCCGCCC</li> <li>ATGGTGCATCTGAGAAACTTCAGGCCCCGGGCAACGTGCTGGTGTGTGGCGGCCC</li> <li>CACGTGGATGAAGATTCACCCCCACACGTGGCAGGCTGCCTGTGGGCAAGGTGGC</li> <li>CCAAGGTGCATGGCAAGAATTCACCCCCACACGTGGCAGGCTGCTGGTGGGCAAGGTGGC</li> <li>ATGGTGCATCTGAGACTCTGGGGAGACTGCCCGTTACTGCCCTGTGAGGCAAGGTGG</li> <li>ATGGTGGATGAAGCTCCTGGGGAGAGTCGCCCGGTGCTGGTGGTGGTGGCCGCC</li> <li>ATGGTGATGAAGTTGGTGGAGGCCCTGGGCAGGCTGCTGGTGGGCTAAGGCAAGGTGA</li> <li>VHLTPEEKSAVTALGKVN</li> <li>CCAGAGGTCTTTGGGAGAGGCCCTGGGGCAGCTGCTGGTGGGCAAGGTGA</li> <li>WHLTPEEKSAVTALGKVN</li> <li>CCAGAGGTCTTTGGGGAAGAAGTCGCCGGAGGCTGCTGGTGGGCTAAGGCAAGGTGA</li> <li>VDEVGGEALGRLVVYPWT</li> <li>CCAGAGGTCATGGTGAGGCCCTGGGGGCAGCTGGTGGTGGTGGTGAGGCCAGAGGCG</li> <li>CTAAGGTGAAGGCCCTGGGGAGCTGCTGGGGGCTGCTGGGGCAGGCTGGCT</li></ul>			
<ul> <li>ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTCAGTCCTTTGGGGATCTGTCCCACTCGTGATGCTGTTATGGGCAACCCTA AGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCCCACC GGGACAACCTCAAGGGCACCTTTGCCACTGAGTGAGCTGCACTGTGGTGGCCCACC ACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCGTGTGGTGGCTGGC</li></ul>			
CCAGAGGTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTA AGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCT GGACACCTCAAGGGCACCTTTGCCACACTGAGTGAGGCTGGCCTGGTCGGCCCACC GTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGGTGGTCTGTGGCGCCACC ACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCCGG TGTGGCTAATGCCCTGGCCCACAAGTATCACTAA ATGGTGCATCTGACTCCTGAGGAGAAGTCGCCGTGGCCCGCTGGTGGTCTACCCTTGGGCAACG CCAGAGGTTCTTGAGTCCTTGGGGAAGCCCTGGGCCACGCTGGTGGTCTACCCTTGGGCAACC WTQRFESFGDLSTPDAVM CCAGAGGTCCTTGAGCCCTGGGCAAGGCTGCCGGTGGCCTGTGGCGCGCC GTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCGCTGGTGGCCAAGGCTG CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
AGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCT GGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCAGTGGACAAGCTGCAC GTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTGGTGGTGGCCCACC ACTTTGGCAAAGAATTCACCCACAGTGCAGGCTGCTGGTGGTGGTGGCCCACC ACTTGGCAAAGAATTCACCCACAGTGCAGGTCGCCTATCAGAAAGTGGTGGCC ACGTGGATGAAGTGGTGGGGGAGAGCTCGCCGGTGACTGCCGGTGGTCTACCCTTGGGA CCAGAGGTTCTTTGAGTCCTTGGGGAAAGTCGCCGTGCCGTGGTGGTCACCCTTGGGCAACC CCAGAGGTCCTGAGACGCCCTGGGGAAAGTCGCCGGTGCCTTAGTGGGGCAAGGTGA CCTAAGGTGAAGGCTCATGGCAGAAAGTGCTCGGGCACGGTGCCTTTAGTGAGGCCTGGCCC ATCACTTTGGCAAAGAACTTCAGGCTCCGGGCAAGGTGCACTGTGGGCGGCCC ACGTGGACAACCTCAAGGCACCTTGCCACACGGGCGCGCTGCTGGTGGTCGGCCC ATCACTTTGGCAAAGAATTCACCCCACACGAGGCGCGCCTGCGGCGCC CCCGGGCAAAGTGGCCGGCCCCGGGCAAGGTGCCCTGTGGGGGGGCAAGGTGG CCAGAGGTTCTTGAGCCCTGGGCCACAGGTGCGCGCCCTATCAGAAAGTGGTGGCC CCCGGGATGAAGGCCCCGGGCAAGGTCGCCGCGCGCGGCGGCAAGGTGG ACGTGGGATGAAGTGGGGGGAGAGGCCGCGGCGGCGCGCGGGGGAAGGGGGA ACGTGGATGAAGTGGGGGGGGGG			
<ul> <li>&gt;CD30</li> <li>GGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGCAC GTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTGTGTGGTGGCGCCATC ACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCGCGCCTATCAGAAAGTGGTGGCTGG TGTGGCTAATGCCCTGGCGCACAAGTATCACTAA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTGACGCGTGGTGGTCTACCCTTGGAC ACGTGGATGAAGTTGGTGGGGGAGGCCTGGGCAAGCTGCCGTGGTGGTCTACCCTTGGAC CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAGTGCTCGGTGGCCTTAGTGATGGCCTGGCCC ACCTGGACAACCTCAAGGGCACCTTTGCCACACGTGAGGCGCGCCTGGTGACAAGCTG CACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGGCTGGTCGTGGTGGCGCC ACCTGGGACAACCTCAAGGGCACCTTTGCCACCACGTGGCGCCTACAGAAGTGG CTGGGTGGCTAATGCCCTGGGCCACAGTGCAGGCTGCCTGTGGTGGTGGCGCC ATGGTGGGCTAATGCCCTGGGCAAGGTGCCGTTACTGCCGTGTGGTGGCGCC CCAGAGGTCTTGAGTCCTTGGGGAGAAGTCTGCCGTTACTGCCCTGTGAGGCAAGGTGA ACGTGGATGAAGGTCGTGGGGGGGGGG</li></ul>			
<ul> <li>&gt;CD30</li> <li>ATGGTGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATC ACTTTGGCAAAGAATTCACCCACAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGG TGTGGCTAATGCCCTGAGCACAAGTATCACTAA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGA ACGTGGATGAAGTGGTGGTGGGGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGA CCAGAGGTTCTTTGAGTCCTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAGTGCTCGGTGCTCACCTCTGATGGCGGCCTG CCTGGACAACCTCAAGGGCACCTTTGCCAACTGAGTGAGCTGCACTGTGACAAGCTG CACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTGGTGGCCGGCC</li></ul>			
<ul> <li>ACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGG TGTGGCTAATGCCCTGGCCCACAAGTATCACTAA</li> <li>ATGGTGCATCTGACTCCTGAGGAGAGAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA</li> <li>ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAAGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCCC CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG</li></ul>			
TGTGGCTAATGCCCTGGCCCACAAGTATCACTAA>CD30>CD31ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAAGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCCAGAGGTCCTAGGCAAGGCACCTTTGCCACACTGGTGACGCTGGTGGCCTGGCCCCCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
>CD30 ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGA MVHLTPEEKSAVTALWGK ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGGCAAGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCCAAGGTGA CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
>CD30 ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAAGCTGCTGGTGGTCTACCCTTGGAC VNVDEVGGEALGKLLVVYP CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCGGTGGTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCCC CACGTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG		TGTGGCTAATGCCCTGGCCCACAAGTATCACTAA	
>CD30 ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAAGCTGCTGGTGGTCTACCCTTGGAC VNVDEVGGEALGKLLVVYP CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCGGTGGTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCCC CACGTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG		ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGGCAAGGTGA	MVHLTPEEKSAVTALWGK
<ul> <li>CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC</li> <li>WTQRFFESFGDLSTPDAVM</li> <li>CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCTCA</li> <li>CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG</li></ul>			
>CD30 CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCA CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
<ul> <li>CD30</li> <li>CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG</li></ul>			
CACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCC ATCACTTTGGCAAAGAATTCACCCCACAGTGCAGGCTGCCTATCAGAAAGTGGTGGC TGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAA ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGAGGCAAGGTGA ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTCTTGAGTCCTTTGGGGATCTGTCCACTCGGGCGCTGTGGTGTATGGGCAACC CCAGAGGTTCTTGAGTCCTTGGGGATCTGTCCACTCGGGCGCTTAGTGATGGCCTGGCCCA CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGCCGTTACTGATGGCCTGGCCTAA CCTGGACAACCTCAAGGCACCTTTGCCACACGGGGCGCTGTGGTGTGTGGCCTGGCCCCA CCAGGTGGATCCTGAGAACTTCAGGCCCCGGGCAACGTGCTGGTGCTGGCCGGCC	>CD30		
>Cs 15 ATCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGC CVLAHHFGKEFTPPVQAAY QKVVAGVANALAHKYH ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGAGGCAAGGTGA MVHLTPEEKSAVTALGKVN ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCGGTGCTGTTATGGCCAACC CTAAGGTGAAGGCTCATGGCAAGAAGTGCTCGCTGGTGGCCTTAGTGATGGCCTGGCCCA CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGACGCCTGGGCCAGGCTGGTGTGTGT			
TGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAAQKVVAGVANALAHKYHATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGAGGCAAGGTGAMVHLTPEEKSAVTALGKVNACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGACVDEVGGEALGRLLVVYPWTCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCQRFFESFGDLSTPDAVMGNFCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTAGTGATGGCCTGGCCCACAGCTGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
>Cs 15 ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGAGGCAAGGTGA MVHLTPEEKSAVTALGKVN ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC VDEVGGEALGRLLVVYPWT CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCCTCA KVKAHGKKVLGAFSDGLA CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
>Cs 15 ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC VDEVGGEALGRLLVVYPWT CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCCTCA CTAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCCC CTAGGTGAAGCTCAAGGGCACCTTTGCCACACTGAGTGAG			-
CS 15 CCAGAGGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACC CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCCTCA CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			
Cs 15 CTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCA CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG	>Cs 15		
CS IS CCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAG			-
CACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGGTGGGCTGGTGGTGGTGGCCG ATCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGC AHHFGKEFTPPVQAAYQKV			
ATCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGC AHHFGKEFTPPVQAAYQKV			
TGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAA VAGVANALAHKYH			
		TGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAA	VAGVANALAHKYH

	Post-Translational Sites								
Sequences	netglyca	te-1.0 predic	tion results	# netphos-3.1b prediction results					
	•,•	C	<b>G</b> *4 4	<b>D</b>	Serine/	<b>a</b>	C		
	position 9	Score 0.89	Site nature glycate	Position 5	Threonine T	Context MVHLTPEEK		Kinase p38MAPK	
	18	0.89	glycate	5	T	MVHLTPEEK		unsp	
	67	0.842	glycate	39	T	VYPWTQRFF		PKC	
Normal	121	0.825	glycate	45	S	RFFESFGDL		CKI	
rtormar	145	0.779	glycate	45	S	RFFESFGDL		unsp	
	110	01112	gijeate	50	S	FGDLSTPDA		unsp	
				51	T	GDLSTPDAV	0.529	p38MAPK	
				88	Т	GTFATLSEL	0.624	PKA	
				88	Т	GTFATLSEL	0.586	unsp	
	7	0.796	glycate	37	Т	VYPWTQRFF	0.633	PKC	
	16	0.781	glycate	43	S	RFFESFGDL	0.571	CKI	
	65	0.841	glycate	43	S	RFFESFGDL	0.62	unsp	
CD5	119	0.825	glycate	48	S	FGDLSTPDA	0.987	unsp	
	143	0.779	glycate	49	Т	GDLSTPDAV		p38MAPK	
				86	Т	GTFATLSEL		PKA	
			<u> </u>	86	T	GTFATLSEL		unsp	
	17	0.93	glycate	9	S	LRRRSAVTA		unsp	
	66	0.841	glycate	9	S	LRRRSAVTA		PKA	
	120	0.825	glycate	9	S	LRRRSAVTA		PKG	
	144	0.779	glycate	12	T T	RSAVTALWG		PKC PKC	
Fr 8-9				38 44	S	VYPWTQRFF RFFESFGDL			
FI 8-9				44	S	RFFESFGDL		unsp CKI	
				49	S	FGDLSTPDA		unsp	
				50	T	GDLSTPDAV		p38MAPK	
				87	T	GTFATLSEL		PKA	
				87	T	GTFATLSEL		unsp	
	16	0.719	glycate	37	T	VYPWTQRFF		PKC	
	65	0.841	glycate	43	S	RFFESFGDL		unsp	
	119	0.825	glycate	43	S	RFFESFGDL		CKI	
Fr 16	143	0.779	glycate	48	S	FGDLSTPDA	0.987	unsp	
				49	Т	GDLSTPDAV	0.529	p38MAPK	
				86	Т	GTFATLSEL	0.624	PKA	
				86	Т	GTFATLSEL	0.586	unsp	
	62	0.841	glycate	21	S	GERGSWWGP	0.659	PKA	
	116	0.825	glycate	21	S	GERGSWWGP	0.557	unsp	
	140	0.779	glycate	40	S	PEVESFGDL		unsp	
Fr 41-42				40	S	PEVESFGDL		CKI	
11 11 12				45	S	FGDLSTPDA		unsp	
				46	T	GDLSTPDAV		p38MAPK	
				83	T	GTFATLSEL		PKA	
		0.00		83	T	GTFATLSEL		unsp	
	9	0.89	glycate	5	T	MVHLTPEEK	Score           0.557           0.929           0.633           0.571           0.62           0.987           0.529           0.624           0.586           0.623           0.571           0.624           0.586           0.624           0.586           0.624           0.586           0.987           0.529           0.624           0.586           0.951           0.856           0.633           0.62           0.571           0.987           0.529           0.624           0.586           0.623           0.624           0.529           0.624           0.586           0.627           0.529           0.624           0.529           0.624           0.529           0.624           0.586           0.624	unsp #28MADK	
	18	0.778	glycate	5 39	T	MVHLTPEEK VYPWTQRFF		p38MAPK	
cd30	67 121	0.842 0.825	glycate glycate	45	T	RFFESFGDL		PKC	
	121	0.825	glycate	45	S	RFFESFGDL		unsp CKI	
cuso	173	0.//7	giyeate	50	S	FGDLSTPDA		unsp	
				51	T	GDLSTPDAV		p38MAPK	
				88	T	GTFATLSEL		PKA	
				88	T	GTFATLSEL		unsp	
	9	0.834	glycate	5	T	MVHLTPEEK		unsp	
cs15	66	0.841	glycate	5	T	MVHLTPEEK		p38MAPK	
	120	0.825	glycate	38	Т	VYPWTQRFF		РКС	
	144	0.779	glycate	44	S	RFFESFGDL	0.62	unsp	
				44	S	RFFESFGDL		CKI	
				49	S	FGDLSTPDA	0.987	unsp	
				50	Т	GDLSTPDAV	0.529	p38MAPK	
				87	Т	GTFATLSEL	0.624	PKA	
				87	Т	GTFATLSEL	0.586	unsp	

# Table-2: List of predicted post-translational sites

.)	Multiple Sequence Alignment of normal and mutated HBB gene product	
CLUSTAL O(	1.2.4) multiple sequence alignment	
FR41-42	GASDSGEVCRYCPVGQGERGSWWGPGQAAGGLP-LDPEVESFGDLSTPDAVMG	52
FR-16	GASDSGEVCRYCPVGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMG	55
FR8-9	WCILLRRRSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMG	56
CD5	WCIL-EEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMG	55
Cs15 Normal	MVHLTP-EEKSAVTA-LGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMG MVHLTP-EEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTORFFESFGDLSTPDAVMG	56 57
CD30	MVHLTP-EEKSAVTALWGKVNVDEVGGEALGKLLVVYPWTQKFFESFGDLSTPDAVMG MVHLTP-EEKSAVTALWGKVNVDEVGGEALGKLLVVYPWTQKFFESFGDLSTPDAVMG	57
CDS0	PIVIL IP-EEKSAV IALWGKVNVDEVGGEALGKLLVV IPW IQKFEESFGDLS IPDAVPG	57
FR41-42	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	112
FR-16	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	115
FR8-9	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	116
CD5	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	115
Cs15	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	116
Normal	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	117
CD30	NPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAH	117
FR41-42	HFGKEFTPPVQAAYQKVVAGVANALAHKYH 142	
FR-16 FR8-9	HEGKEETPPVQAAYQKVVAGVANALAHKYH 145	
CD5	HFGKEFTPPVQAAYQKVVAGVANALAHKYH 146	
Cs15	HEGKEFTPPVQAAYQKVVAGVANALAHKYH 145 HEGKEFTPPVQAAYOKVVAGVANALAHKYH 146	
Normal	HEGKEFTPPVQAATQKVVAGVANALAHKTH 146 HEGKEFTPPVQAAYOKVVAGVANALAHKTH 147	
CD30	HFGKEFTPPVQAAYQKVVAGVANALAHKYH 147	
CODO	11 CALEFIEL & QUAL OX & AND AND ALL ALL ALL ALL ALL ALL ALL ALL ALL AL	
(B)		
Phylo	ogenetic Tree	
This is a l	Neighbour-joining tree without distance corrections.	
This is a l	veighbour-joining tree without distance corrections.	
	FR41-42	0 1156
	FR-16-0	
	FR8-9 0.	
	CD5 0.00	0182
	Cs15 0.0	
	CD30 0.0	00641
	Normal C	0.0

Figure-1: HBB Homology and Phylogenetic Analysis. (A) Multiple sequence alignment (B) Phylogenetic tree. Both analyses revealed Cd30 to be more identical and homologous to normal HBB while Fr41-42 was the least identical variant

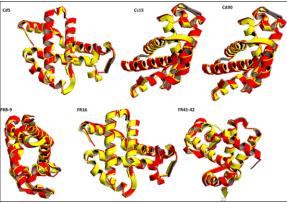


Figure-2: Built 3-D structures of the HBB variants superimposed on the normal structure. Each variant is shown in red while normal HBB is depicted in yellow

#### DISCUSSION

In this study we have focused on the effects of HBB mutations on sequence, post-translational modification, and structure using *in-silico* computational approaches. Hypothetically all the nonsense mutations were assumed to be missense. Moreover, early stop codons from the translated sequences were removed. This strategy relied on prediction tools available in public domain.

Post-translational modifications in general, have been reported to enhance haemoglobin yield without effecting the gene expression levels.<sup>19</sup> Hence, studying such, in our opinion, was a critical factor in

better understanding of haemoglobin disorders. Our selection of HBB mutations was influenced by our previous study (in press) in which prominent HBB genetic polymorphism among various ethnic groups within Khyber Pakhtunkhwa, Pakistan population were reported. The same study observed Fr 8-9 (+G) to be the most frequent or the only mutation in 13 ethnic groups followed by CD 5 (-CT). When considered in reference to geographic distribution, Fr 8-9 (+G) mutation was most common in central regions of Khyber Pakhtunkhwa, i.e., Kurram, Khyber, Peshawar, Charsadah, Mardan, Hangu, Swabi. While distant regions harbored different mutations, i.e., Cd-15 (G>A) in North Waziristan, ISV 1-5/Cap+1 (A-C) in Karak, and Fr 41-42 (-TTCT) in Swat. These reported mutations are in accordance with local and regional reported data.20-23

A unique O linked glycosylation site was observed in CD15 (G>A) variant unlike in the other mutants or the normal protein itself. Generally, glycation increases the overall stability of a glycoprotein.<sup>24</sup> Yet for haemoglobin glycation is considered as diagnostic marker for diabetes.<sup>25</sup> Another key post-translational modification was the varying number of Phosphorvlation sites. Four such unique sites (two in each) in proteins FR8-9(+G) and FR41-42(-TTCT) make them target for unnecessary victim of PTMs. Unlike the other variants where the sites were shifted up or down stream due to indel mutations, these four sites were different in protein sequence. Phosphorylation of eukaryotic initiation factor 2a (eIF2 $\alpha$ ) is reported to enhance fetal haemoglobin production in thalassemia patients.<sup>19</sup> However, the implication of new phosphorylation sites needs to be studied in more details before deducing any implications. In a surprising manner the built variant structures did not exhibit any prominent structural variation in comparison to the normal structure, yet when Ramachandran plots were studied, the effects of the variations were evident in terms of slight strains on the amino acids as observed due to varying sequence.

The current results underscore the utility of computational modelling in hypothesizing the pathogenesis of genetic disorders. Despite its potential, ease of access and low costs, this approach carries a number of weaknesses. Firstly, human body has a complex ecosystem and processes such as haemoglobin function are modulated by numerous cellular and molecular mechanisms, not taken into consideration during in-silico analysis. It is remarkable how protein structures which are halted from production in the body were produced hypothetically. Secondly, lack of understanding of computational biology by clinical scientists makes it difficult to use this in clinical research. Nonetheless, this study highlighted several critical dissimilarities of HBB molecules from mutated

sequences which may help in understanding the pathogenesis of reduced production of HBB in thalassaemia. There is a need for greater collaboration between computational biologists and clinical and lab scientists to utilize the potential of computational biology in understanding, preventing and treating disease.

#### CONCLUSION

HBB genetic polymorphic variants exhibit a varying pattern of post-translational modification sites, i.e., glycation and phosphorylation sites. At least one O linked glycosylation site in CD 15(-CT) and two phosphorylation sites in FR 8-9(+G) and FR 41-42(-TTCT) were predicted. This study is proof-of-principle that thalassaemia genetic mutations not only reduce the amount of globin chain synthesis, but also produce differentially structured proteins, adding to the complexity of the genotype-phenotype relationship. Integration of computational biology and clinical sciences will be required to fully utilize the potential of *in-silico* studies.

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