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Research Paper

Insilico analysis of the MRP1 Protein implicated in Neuroblastoma

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Abstract: ***MRP1 is a uni-directional efflux transporter that confersmultidrug resistanceand mediates the effluxof both endobiotic andxenobiotic organicanions. MRP1 transports many therapeutic and physiological substrates. It has a key role in the onset of drug resistance in several cancers for example lung, breast, prostate, and childhood neuroblastoma. In the present work, the wild type and mutant protein sequence of MRP1 protein were analysed using an insilico approach for studying the functional role of the MRP1 protein in the normal and diseased condition. The variation in the mutant protein was analysed at the structural level to decipher the mode of the disease progression.***

Keywords: ***Multidrug resistance, Neuroblastoma, in silico, MRP1.***

INTRODUCTION

Multidrug resistance protein 1 (MRP1/ABCC1) is atransmembrane protein of the ATP-binding cassette superfamily as exemplified in the Pfam database (1,2). When the expression of the gene is exceptionally high in tumour cells, MRP1 shows resistance to antineoplastic drugs and other xenobiotics (3, 5). MRP1 facilitates the ATP-dependent efflux oforganic anions derived from endogenous metabolites and exogenous xenobiotics which are complexed withthe tripeptide molecule Glutathione (4, 6, 7).

MRP1 is ubiquitously expressed in humans. The mRNA is highly expressed in testis, cardiomyocytes, placenta, prostate, lung,thymus, and kidney, with lower expression is seen in small intestine, colon, brain, and mononuclear cells (11).

MRP1has a molecular weight of 190-kDa. It has a core structure of three membrane-spanning domains (MSDs) and two nucleotide-binding domains (NBDs). The MSDs contain five to six transmembrane segments which forms a pore in thelipid through which solutes are extruded out of the cell (8, 9).

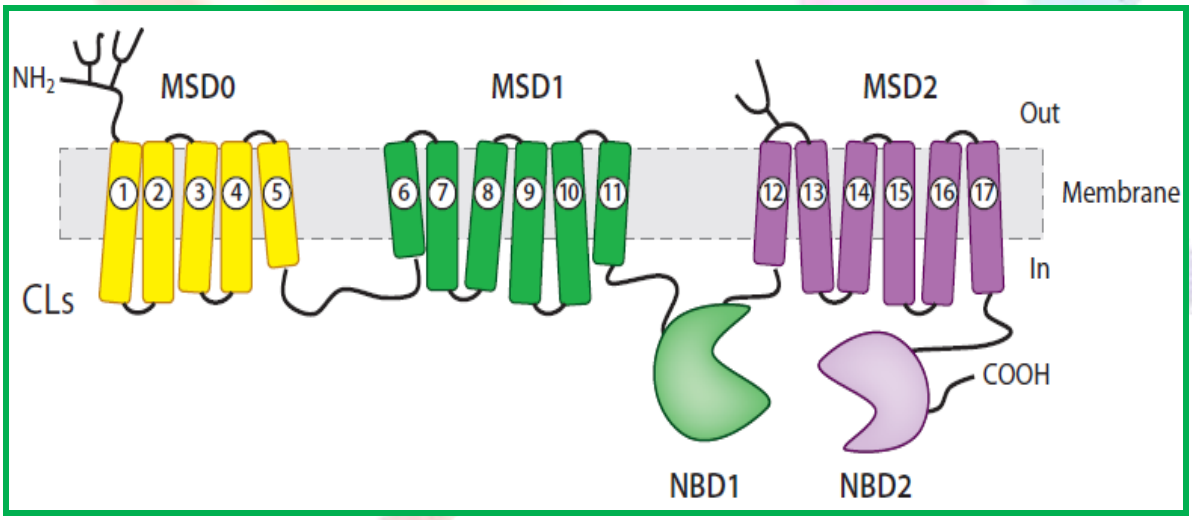

Fig:1 Topology and domain structure of MRP1

The two NBDs of MRP1 coordinate to form a sandwich dimer that comprises two composite nucleotide binding sites, which bind and hydrolyse ATP to provide the energy necessary for the transport process (Fig.1)

The Transmembrane α-helices of the core membrane spanning domains forms the channel for the translocation of substrates. Theylie close to the Nucleotide Binding Domainsby means of the cytoplasmic loops that connect the helical region (11). The loopsfacilitate the juxtaposition of the ATPase activity at the nucleotide binding sites for the process of transport of substances.

MRP1 has a significant role to play in the inflammatory responses (12).

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MULTIDRUG TRANSPORTER PROTEIN1 IN CANCER: -

Drug resistance is a major challenge to cancer treatment and leads to poor diagnosis in the people carrying the mutation. MRP1 plays a great role in the onset of the drug resistance in Neuroblastoma (15). Neuroblastoma leads to solid tumour outside the cranium in children. It is usually seen in the cells of neural crest origin. High copy number of the MycN oncogene is remarkedly a significant manifestation clinically for neuroblastoma. The amplification of MycN is seen in 25% of primary neuroblastomas and thereby leads to rapid tumour progression andcan be manifested clinically.

MycN is related to Myc family that codes for transcription factors. It is involved in the growth and differentiation of neuroblastoma cells. Studies on the MRP1 promoter has suggested that MycN keeps a check onthe expression of MRP1 gene through interaction with a putative E-box element and other cis-acting elements in the MRP1 promoter region. Neuroblastoma cells over expressing MycN have enhanced levels of Multidrug Transporterprotein and eventually become intolerant to well-characterized MRP1 substrate drugs (5, 15).

MRP1 is implicated in cell differentiation and cell death in this disease (10,13).

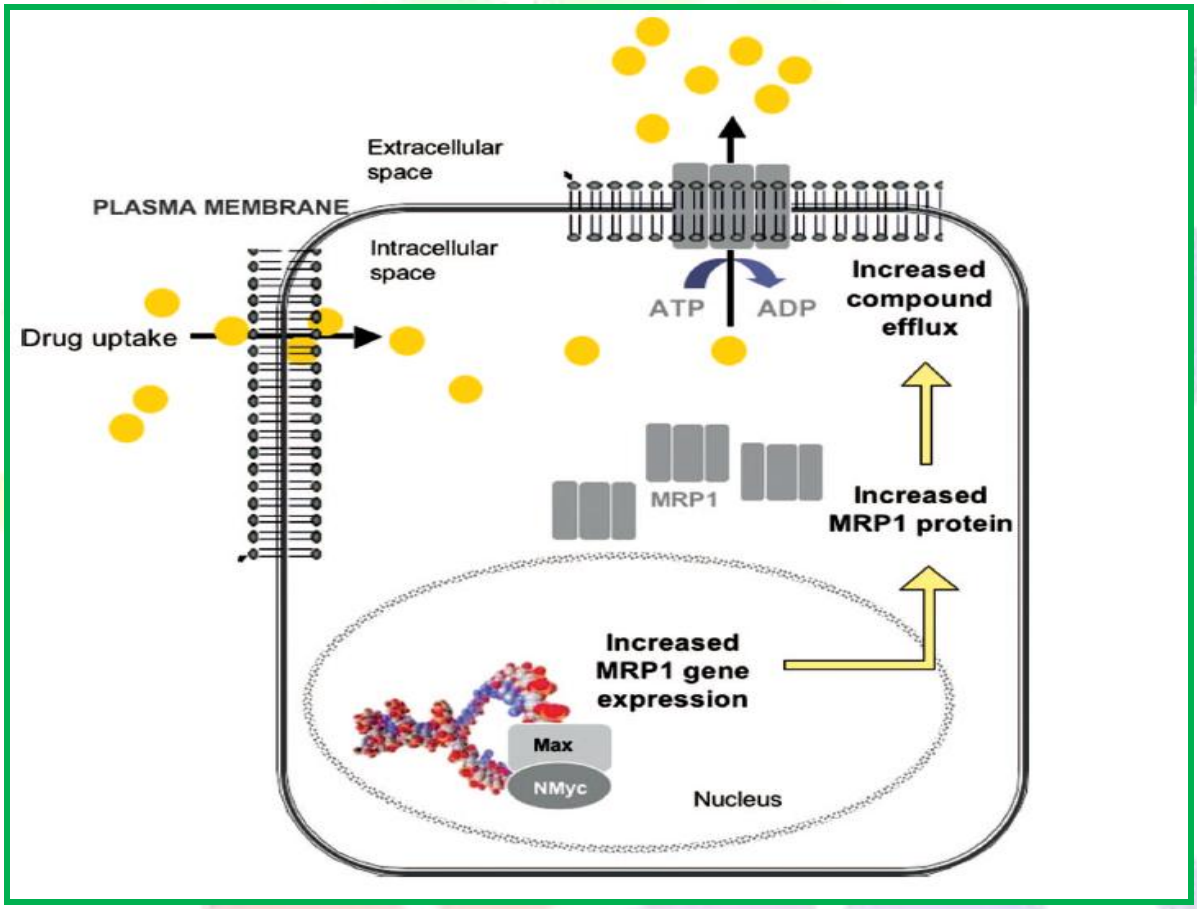

Fig.1.1. MRP1 regulation by MycN and its role in drug resistance.

METHODOLOGY

Sequence retrieval: The wild type (NP_004987) and the mutant (NP_009487.2) MRP1 protein in *Homo sapiens* was extracted from the protein sequence database at NCBI. **Fig.2 and 3.**

CDD: Conserved domains in wild type and diseased MRP1 protein were studied by Conserved Domain Database (www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). **Fig 4, 5.**

Phyre2: Tertiary structure of normal and mutant MRP1was compared to analyse theimpact of mutation on the 3D structure of protein. **Fig 6 and 7**

RESULTS AND DISCUSSIONS

The MRP1 protein sequence of*Homo sapiens* was retrieved from NCBI protein database. The wild type protein has a length of 1531 residues while the protein showing variation has 1472 residues. **Fig 2 and 3.**

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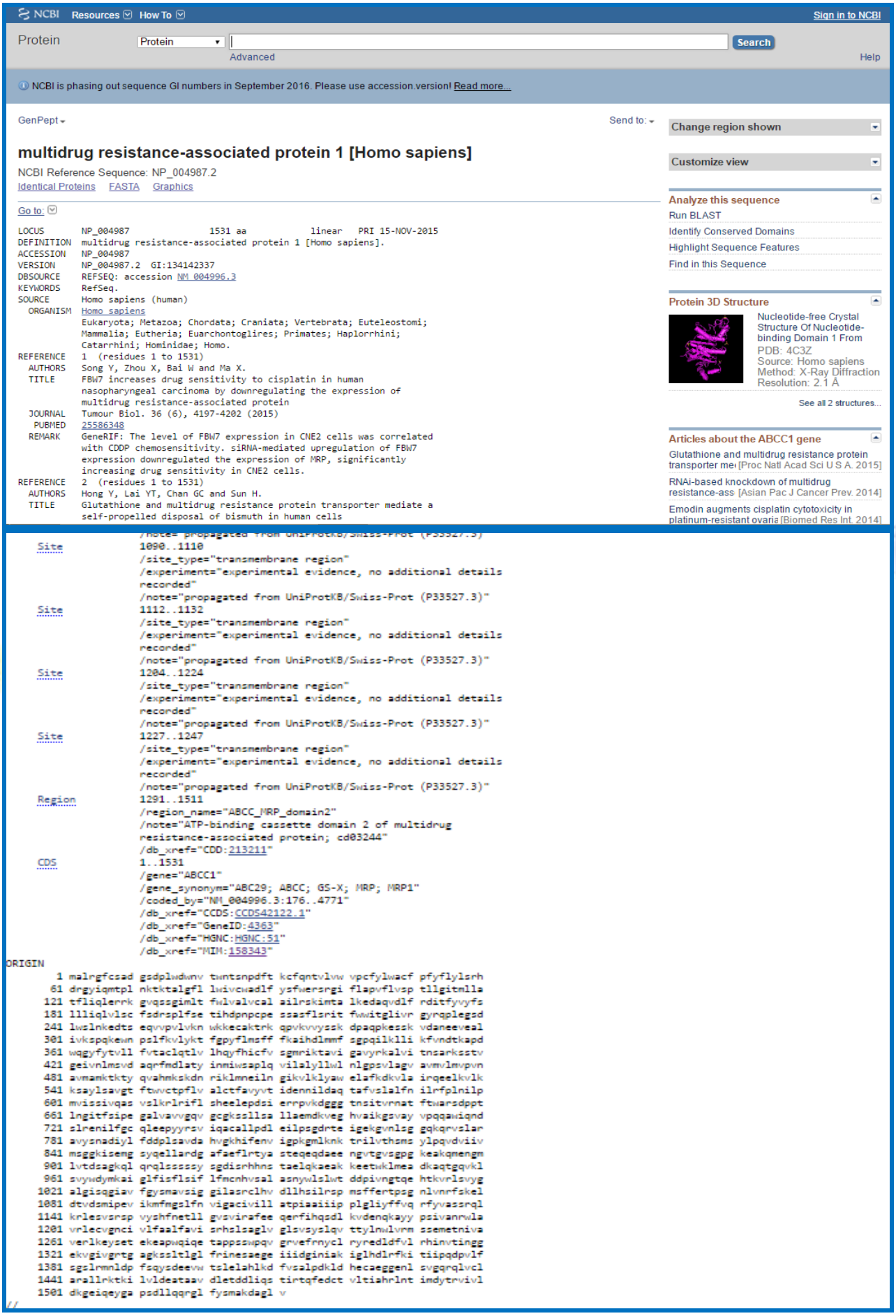

Fig 2: The wild type MRP1 *(Homo sapiens)* protein.

Protein

NCBI is phasing out sequence GI numbers in September 2016. Please use accession.version! Read more...

GenPept

multidrug resistance-associated protein 1 isoform 2 [Homo sapiens]

NCBI Reference Sequence: NP_063915.2

Record removed. NM_019862.2: This RefSeq was permanently suppressed because currently there is no full-length support for the transcript and the protein.

Identical Proteins FASTA Graphics

Go to:

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VERSION     NP_063915.2  GI:134142340
DBSOURCE    REFSEQ: accession NM_019862.2
KEYWORDS    RefSeq.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (residues 1 to 1472)
  AUTHORS   Chen,M., Xue,X., Wang,F., An,Y., Tang,D., Xu,Y., Wang,H., Yuan,Z.,
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  TITLE     Expression and promoter methylation analysis of ATP-binding
            cassette genes in pancreatic cancer
  JOURNAL   Oncol. Rep. 27 (1), 265-269 (2012)
   PUBMED   21956451
  REMARK    GeneRIF: ABCB1/MDR1, ABCC1/MRP1 and ABCG2/BCRP expression
            correlated with pancreatic cancer tumorigenesis and drug resistance
            in a mechanism that is independent of promoter methylation
REFERENCE   2  (residues 1 to 1472)
  AUTHORS   Cho,S., Lu,M., He,X., Ee,P.L., Bhat,U., Schneider,E., Miele,L. and
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       61 drgyiqmtpl nktktalgfl lwivcwadlf ysfwersrgi flapvflvsp tllgitmlla
      121 tfliqlerrk gvqssgimlt fwlvalvcal ailrskimta lkedaqvdlf rditfyvyfs
      181 llliqlvlsc fsdrsplfse tihdpnpcpe ssasflsrit fwwitglivr gyrqplegsd
      241 lwslnkedts eqvvpvlvkn wkkecaktrk qpvkvvyssk dpaqpkessk vdaneeveal
      301 ivkspqkewn pslfkvlykt fgpyflmsff fkaihdlmmf sgpqilklli kfvndtkapd
      361 wqgyfytvll fvtaclqtlv lhqyfhicfv sgmriktavi gavyrkalvi tnsarksstv
      421 geivnlmsvd aqrfmdlaty inmiwsaplq vilalyllwl nlgpsvlagv avmvlmvpvn
      481 avmamktkty qvahmkskdn riklmneiln gikvlklyaw elafkdkvla irqeelkvlk
      541 ksaylsavgt ftwvctpflv alctfavyvt idennildaq tafvslalfn ilrfplnilp
      601 mvissivqas vslkrlrifl sheelepdsi errpvkdggg tnsitvrnat ftwarsdppt
      661 lngitfsipe galvavvgqv gcgkssllsa llaemdkveg hvaikgvnls ggqkqrvsla
      721 ravysnadiy lfddplsavd ahvgkhifen vigpkgmlkn ktrilvthsm sylpqvdvii
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      901 lsvywdymka iglfisflsi flfmcnhvsa lasnywlslw tddpivngtq ehtkvrlsvy
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     1021 ldtvdsmipe vikmfmgslf nvigacivil latpiaaiii pplgliyffv qrfyvassrq
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```

Fig 3: The mutant MRP1 *(Homo sapiens)* protein.

The CDD analysis of wild type MRP1 revealed that it has transmembrane regions and two ATP binding domains. The two domains consist of nucleotide binding sites which are involved inhydrolysing ATP to provide the energy for the transport mechanism. In the domain1 the residues walker B motif, A loop, D loop, H loop contribute to ATP binding and hydrolysis. The Q loop significantly interacts with metal cofactortofacilitate ATP binding and other residues of the loop lead to conformational variationsin nucleotide binding domains (NBD) and transmembrane domains that are involved in ATP dependent transport of solute molecules.

CDD analysis of mutant MRP1 revealed that the Q loop is missing at domain1. Variations in the MRP1 protein results in loss of activity of the wild type MRP1 protein. The mutation at the Q loop region of NBD1 in mutant MRP1results in deletion of Q loop that differs it from normal protein and leads to the formation of truncated protein. Loss of MRP1 protein function or its over expression in tissues might lead to the decrease in the efficacy of drug administration in Neuroblastoma.

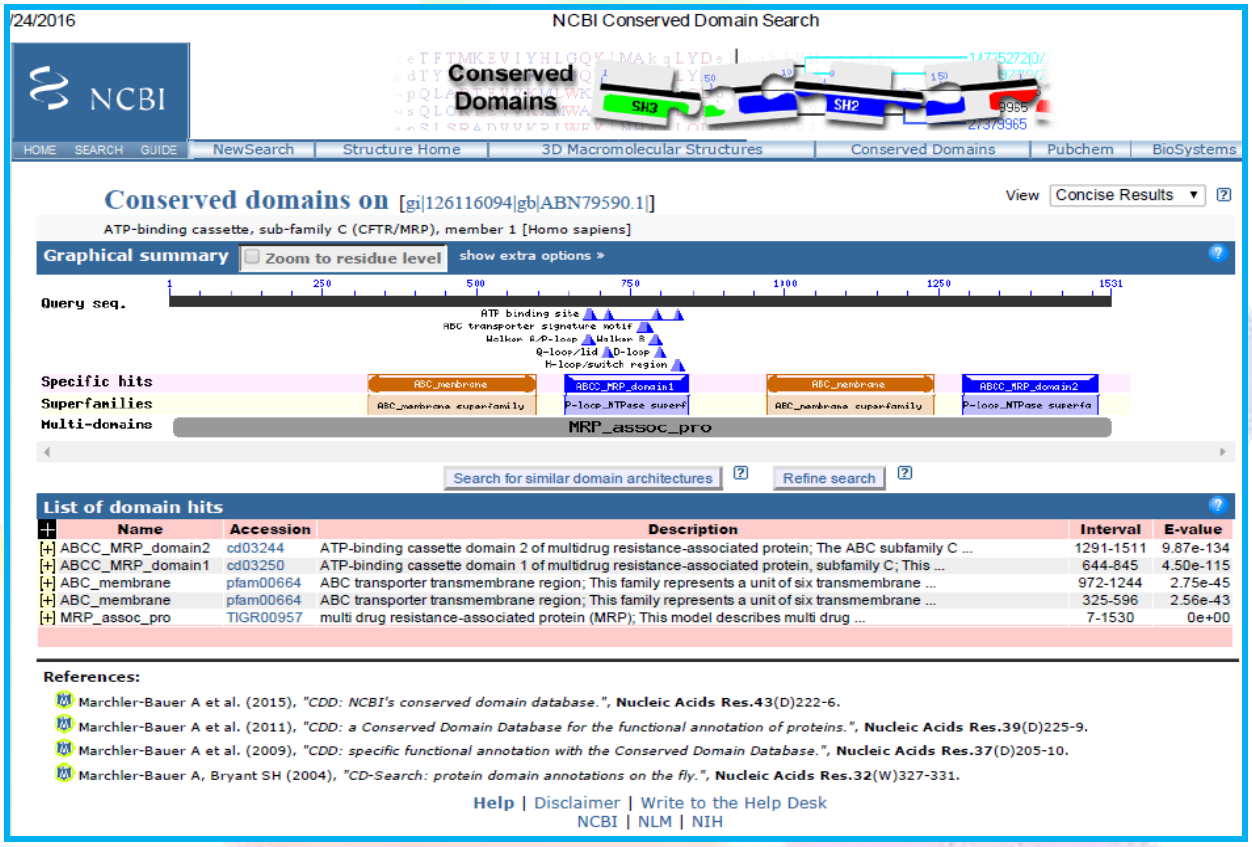

Fig. 4 Conserved domains in wild type MRP1 *Homo sapiens* Protein.

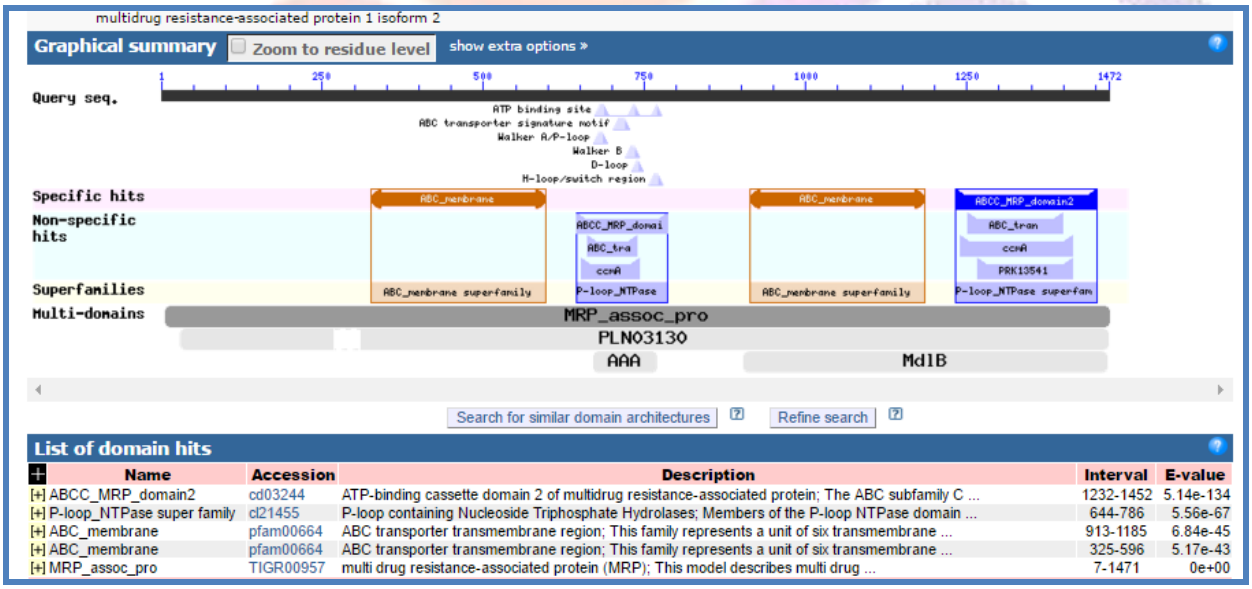

Fig. 5 Conserved domains in mutant MRP1 *Homo sapiens* Protein.

The missing hydrophilic amino acidGlutamine in the Q-loops of MRP1 have a crucial role to play role in Mg·ATP binding, and in inter-domain communications between the NBDs and TMDs.

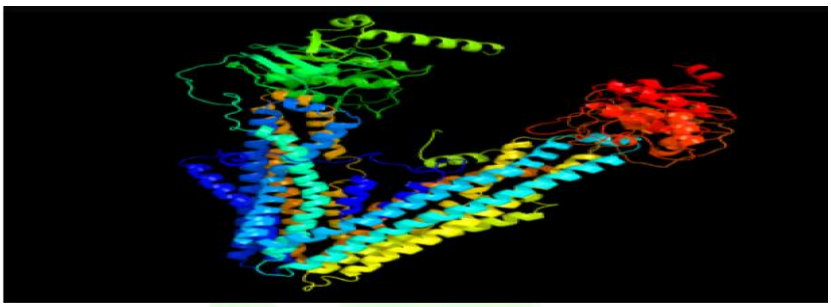

Fig. 6 Tertiary Structure Prediction of MRP1 (Wild type) by Phyre2 and 3D Visualisation by JSmol

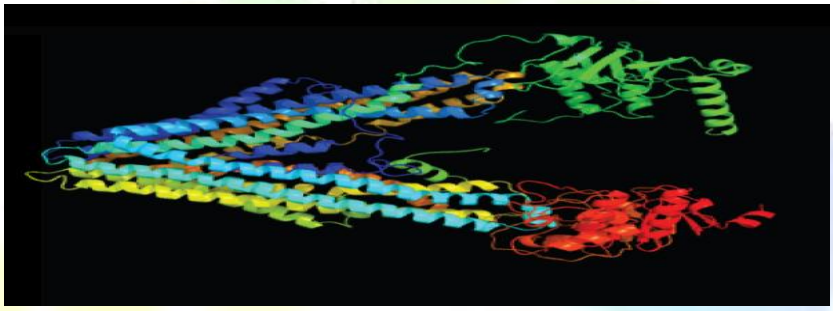

Fig. 7 Tertiary Structure Prediction of MRP1 (mutant) by Phyre2 and 3D Visualisation by JSmol

CONCLUSION

MRP1 has a critical impact on the drug efficacy of different cancers and majorly leads to inflammatory responses.

Domain comparison of the wild type and mutant protein revealed that in ATP binding domain 1the Q loop interacts with metal cofactor to participate in ATP binding and other residues to transmit the conformational changes between NBDs and integral domains, in ATP dependent solute transport. Therefore, the mutation at the Q loop region of NBD1 in the mutant MRP1results in deletion of Q loop that remarkedly differs in the normal protein andthe hence the formation of a shorter protein. Loss of MRP1 protein function or its unusually abnormal expression in tissues might be the causative reasonfor drug resistance behaviour in Neuroblastoma. The missing glutamine residues in the Q-loops of the protein have a significant role to play role in Mg·ATP binding, and in inter-domain communications amongst the Nucleotide binding domains and Transmembrane domains.

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