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Unmasking of two mutations within *XK* gene that could be used as diagnostic markers to predict McLeod Syndrome: using *in silico* analysis

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Abstract McLeod neuroacanthocytosis syndrome (MSL) is a rare X-linked recessive multisystem disorder affecting the peripheral and central nervous systems, red blood cells, and internal organs. The objective of this study was to identify the most deleterious SNPs within *XK* gene and to predict their influence on the functional and structural level by a several *in silico* analysis tools. The raw data of *XK* gene were recovered from dbSNP database and then used to investigate the damaging effect using SIFT, PolyPhen-2, PROVEAN, SNAP2, SNPs&GO, and PHD-SNP. Furthermore; we submitted the common six damaging results from the previous functional analysis tools to I-mutant 3.0, and MUPro respectively to study their effect on structural perspective. The 3D structure of *XK* was predicted by RaptorX and visualized using UCSF Chimera to compare the differences between the native and the mutant amino acids. The functional analysis predicted that two SNPs "rs28933690 and rs145996031" have a deleterious effect at functional level. The structural analysis revealed that, the same two mutations predicted a dramatic decrease of the protein stability, thus proposing that the C294R and Y370D mutations within *XK* could destabilize the amino acid interactions, causing functional abnormalities of XK protein. In this study, the impact of functional mutations in the *XK* gene was investigated through different computational analysis tool, which determined that (C294R & Y370D) are deleterious SNPs that are a potential responsibility for the functional and structural alterations of *XK* protein.

Keywords: X-linked recessive multisystem disorder, nsSNP, *XK* gene, central nervous system, computational analysis

1. Introduction

McLeod syndrome (MSL) is a rare X-linked disorder. Approximately 150 cases have been reported worldwide ("McLeod neuroacanthocytosis syndrome," 2019). Characterized by misshapen red blood cells and progressive degeneration of the basal ganglia disease, which manifest as: movement disorders, cognitive alterations, psychiatric symptoms, and cardiac manifestations. The hematological features are strongly related to abnormalities of different degrees in other organ systems, including neuromuscular involvement (Jung et al 1993). Hematologically, MLS is defined as a specific blood group phenotype that results from the absent expression of the Kx erythrocyte antigen and weakened expression of Kell blood group antigens (Jung et al 1993; Jung et al 2001). The KEL gene encodes kell antigens on the long arm of chromosome 7. The *XK* gene encodes *kx* antigen on the short arm of the X chromosome(Bansal et al 2008).

MSL is caused by mutations of *XK* gene, an X-chromosomal gene of unknown function. But all we know is different *XK* mutations may have different effects on the *XK* gene product and thus may account for the variable phenotype (Danek et al 2001; Frey et al 2015; Ho et al 1994; Jung et al 2003; Klempir et al 2008; Singleton et al 2003; Walker et al 2007). The *XK* gene provides instructions for producing the XK protein, which carries the blood antigen Kx. Some males with MSL also have chronic granulomatous disease (CGD). It is generally believed that patients with non-CGD McLeod may develop anti-Km but not anti-Kx, but that those with CGD McLeod can develop both anti-Km and anti-Kx (Jung et al 2007). But sometimes blood may not always be compatible (Russo et al 2000). McLeod syndrome mimics Huntington's disease; it usually includes progressive movement disorder (e.g., autosomal recessive chorea-acanthocytosis), cognitive impairment, and psychiatric symptoms, eventually associated with seizures, suggesting that the corresponding proteins-*XK* (Hewer et al 2007). Most importantly, given the absence of a cure, it's vital for appropriate genetic counseling, neurological examination, and a cardiologic evaluation for the presence of treatable cardiomyopathy (Jung et al 2007). Unfortunately, no clear correlations between the clinical findings with the genotype of *XK* mutations have yet been unrevealed (Walker at al 2007; Walker et al 2018).

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Single-nucleotide polymorphism (SNP) refers to -base DNA differences among individuals. One of the interests in association studies is the association between SNPs and disease development (Shaw 2013). This study will be a valuable resource for neurologists, hematologists, and clinical geneticists on this rare and debilitating disease. This is the first *in silico* study to classify the possible mutations within *XK* gene for further genetic mapping studies.

2. Materials and Methods

2.1. Data mining

The human *XK* gene data were obtained from the National Center for Biological Information (NCBI) website, and the protein reference sequence was retrieved from the UniProt database ("UniProt: the universal protein knowledgebase" 2017). (Protein ID: 9606).

2.2. Functional analysis

2.2.1. SIFT

We used SIFT to observe the effect of each amino acid substitution on protein function. SIFT predicts damaging SNPs based on the degree of conserved amino acid residues in aligned sequences to the closely related sequences gathered through PSI-BLAST (Sim et al 2012).

2.2.2. PolyPhen-2

PolyPhen-2 stands for polymorphism phenotyping version 2. We used PolyPhen to study the potential effects of each amino acid substitution on the structural and functional properties of *XK* protein by considering physical and comparative approaches (Capriotti and Altman 2011).

2.2.3. PROVEAN

PROVEAN is an online tool that predicts whether an amino acid substitution affects the function of a protein based on the alignment-based score (Choi et al 2012).

2.2.4. SNAP2

SNAP2 is a trained analysis server that distinguishes between effect and neutral SNPs by taking a diversity of elements into interpretation. SNAP2 got an accuracy of 83%. It is considered a substantial upgrade over other approaches (Hecht et al 2015).

2.2.5. SNPs&GO

It is a support vector machine (SVM) grounded on the method to predict the mutations from protein sequences accurately. A probability score higher than 0.5 reveals the disease-related effect of mutation on the parent protein function (Calabrese et al 2009).

2.2.6. PHD-SNP

An online Support Vector Machine (SVM) based classifier is optimized to predict if a given single point protein mutation can be classified as disease-related or a neutral SNP (Capriotti et al 2006).

2.3. Stability analysis

2.3.1. I-Mutant 3.0

Change in protein stability disturbs both protein structure and protein function. I-Mutant is a suite of support vector machine, based predictors integrated into a unique web server. It offers the opportunity to predict the protein stability changes upon single-site mutations. The FASTA format sequence of *XK* protein is used as an input to predict the mutational effect on protein and the stability RI value (reliability index) computed (Capriotti et al 2005).

2.3.2. MUPro

MUPro is a support vector machine-based tool for predicting protein stability changes upon nsSNPs. The energy change value is predicted, and a confidence score between -1 and one for measuring the confidence of the prediction is calculated. A score <0 means the variant decreases the protein stability; conversely, a score >0 means the variant increases the protein stability (Cheng et al 2006).

2.4. Biophysical and visualization analysis

2.4.1. Project HOPE

Project HOPE is a server to predict the biophysical validation of SNPs. The FASTA format sequence of *XK* protein was retrieved from UniProt that used as an input to predict the biophysical validation for our SNPs of interest (project HOPE server 2022).

2.4.2. RaptorX

The 3D structure of human *XK* protein is not available in the Protein Data Bank. Thus, we used RaptorX to generate a 3D structural model for wild-type *XK*. The FASTA format sequence of *XK* protein was retrieved from UniProt; it was then used as an input to predict the 3D structure of *XK* protein (Wang et al 2016).

2.4.3. UCSF Chimera

UCSF Chimera is a multi-use tool for 3D visualization and analysis of molecular structures and related data. The amino acid changes were visualized using a PDB file as input (Pettersen et al 2004).

2.5. ConSurf server

It is a server that proposes evolutionary conservation assessments for proteins of known structure in the PDB. ConSurf runs MSA for similar AA sequences. The conserved regions are Spotify by unique algorisms (Ashkenazy et al 2016).

2.6. GeneMANIA

GeneMANIA was used to know the protein function of unknown proteins. The input was the gene's name (*XK*), while the output was the gene-gene interactions and its function after the mutations occurred (Warde-Farley et al 2010).

2.7. ClinVar

It is a public archive of reported studies of the relationships among human variations and phenotypes, with supporting evidence. We used it to compare our prediction approach with the clinical one (Landrum et al 2018).

2.8. Variant Effect Predictor (VEP)

The Variant Effect Predictor (VEP) is a toolset for annotating mutations in analysis investigations. The SNPs IDs are used as input to predict the Functional consequences of mutations (McLaren et al 2016).

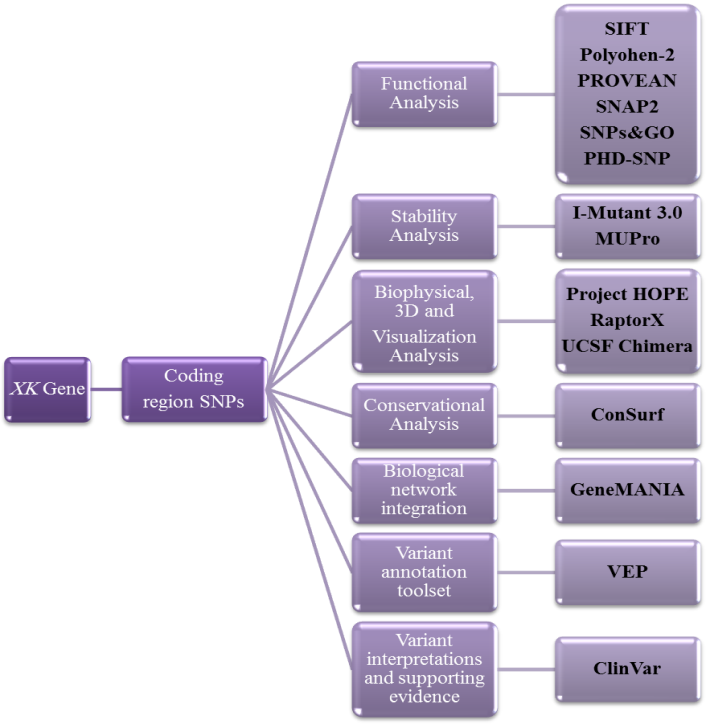

Figure 1 Schematic demonstration of bioinformatics tools for computational analysis of *XK* gene.

3. Results

The total number of SNPs in the coding region that was recovered from NCBI was 104 nsSNPs, which were submitted to SIFT, PolyPhen-2, PROVEAN, and SNAP2 servers, respectively; 35 SNPs were predicted to be deleterious by SIFT server; PolyPhen-2 result shows that 63 were found to be damaging (16 possibly damaging and 48 probably damaging), and 42 were found to be deleterious by PROVEAN, while in the SNAP2 server, our results show that 52 SNPs were predicted to be an effect. In Table 2, we submitted four positive results from SIFT, PolyPhen-2, PROVEAN, and SNAP2 (Table 1) to observe the -causing disease by SNP&GO and PHD-SNP servers. SNP&GO and PHD-SNP servers were used to predict the association of SNPs with the disease. These online tools revealed that 2 and 13 SNPs were predicted to be disease-related SNPs, respectively. We selected the double disease-causing SNPs for further analysis by I-Mutant 3.0, and MUPro results revealed that the protein stability decreased, destabilizing the amino acid interaction (Table 3). VEP reported regulatory consequences for many variants (Table 4).

Table 1 Damaging nsSNPs associated variations predicted by various software.

dbSNP rs#	SUB	SIFT prediction	Score	POLYPHEN prediction	Score	Prediction (cutoff= -2.5)	PROVEAN score	SNAP2 prediction	Score
rs868965185	P4Q	DAMAGING	0.00	probably damaging	1.000	Deleterious	-4	Effect	19
rs371386605	L24Q	DAMAGING	0.01	probably damaging	0.993	Deleterious	-2.928	Effect	41
rs868964451	Y28D	DAMAGING	0.00	possibly damaging	0.954	Deleterious	-7.007	Effect	89
rs782374462	W36R	DAMAGING	0.00	probably damaging	0.999	Deleterious	-9.566	Effect	92
rs782030330	W36C	DAMAGING	0.00	probably damaging	1.000	Deleterious	-8.861	Effect	54
rs781973539	L41S	DAMAGING	0.01	possibly damaging	0.947	Deleterious	-3.141	Effect	38
rs1226579221	L50P	DAMAGING	0.01	probably damaging	1.000	Deleterious	-3.911	Effect	83
rs1366754946	R60H	DAMAGING	0.02	probably damaging	1.000	Deleterious	-2.82	Effect	40
rs1171547000	H73Q	DAMAGING	0.00	probably damaging	0.997	Deleterious	-5.938	Effect	70
rs1411240743	P150S	DAMAGING	0.00	probably damaging	1.000	Deleterious	-7.603	Effect	79
rs868947024	Q151K	DAMAGING	0.00	probably damaging	1.000	Deleterious	-3.801	Effect	80
rs1205585478	D197N	DAMAGING	0.00	probably damaging	1.000	Deleterious	-4.267	Effect	29
rs1414126215	S261F	DAMAGING	0.01	probably damaging	0.993	Deleterious	-3.07	Effect	40
rs28933690	C294R	DAMAGING	0.00	probably damaging	1.000	Deleterious	-9.433	Effect	97
rs782058104	L331F	DAMAGING	0.04	probably damaging	1.000	Deleterious	-3.161	Effect	43
rs782654569	I357T	DAMAGING	0.01	possibly damaging	0.739	Deleterious	-2.538	Effect	40
rs781812995	L367F	DAMAGING	0.02	probably damaging	0.998	Deleterious	-2.947	Effect	52
rs145996031	Y370D	DAMAGING	0.00	probably damaging	1.000	Deleterious	-8.304	Effect	86
rs782069779	H374Q	DAMAGING	0.01	probably damaging	1.000	Deleterious	-7.311	Effect	78
rs139942937	L379F	DAMAGING	0.02	probably damaging	1.000	Deleterious	-2.789	Effect	57

Table 2 Disease effect nsSNPs associated variations predicted by SNPs&GO and PhD-SNP tools.

dbSNP rs#	SNPs&GO prediction	RI	Score	PhD-SNP prediction	RI	Score
rs28933690	Disease	5	0.762	Disease	9	0.931
rs145996031	Disease	2	0.596	Disease	7	0.829

Table 3 Stability analysis predicted by I-Mutant 3.0 & MUPro.

dbSNP rs#	Amino Acid change	SVM2 Prediction Effect	RI	DDG Value	MUPro Predation	Score
rs28933690	C294R	Decrease	2	-0.28	Decrease	-1.3063699
rs145996031	Y370D	Decrease	1	-0.9	Decrease	-0.98561027

The 3D protein structure analysis enables mapping of amino acid substitutions and, therefore, RaptorX was used to make a 3D structure model for *XK* protein (Figure 2) support and matches the results acquired from different computational tools, UCSF Chimera and project HOPE serves this purpose (Figure 3 and 4), show the differences between native and mutant amino acids, in the green and red boxes the schematic structures of the native amino acids (in the left side), and the mutant

ones (in the right side); The backbone, which is the same for each amino acid, is colored red and the side chain, unique for each amino acid is colored black, the 3D wide type residues colored green and mutant ones colored red. In contrast, the protein is colored dark gray.

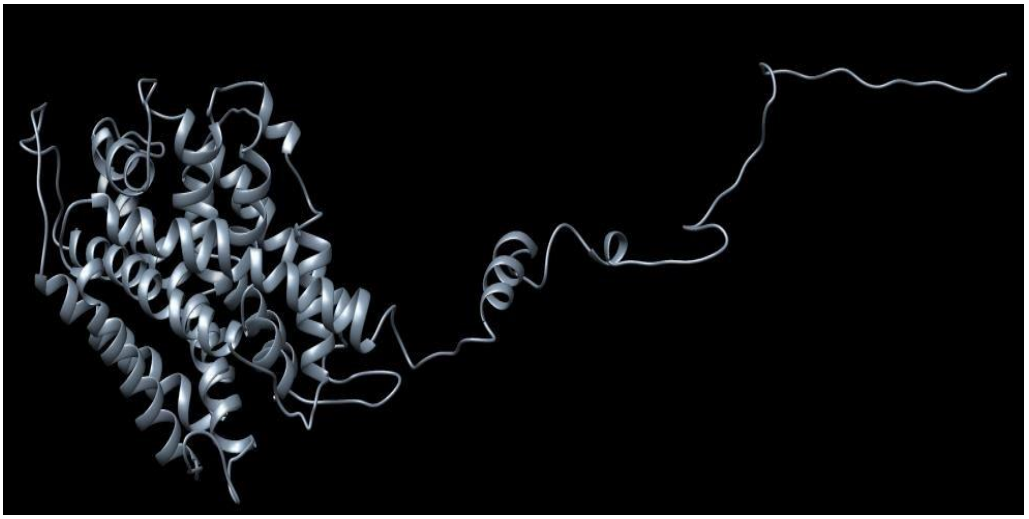

Figure 2 The 3D structure of *XK* protein model was generated using RaptorX.

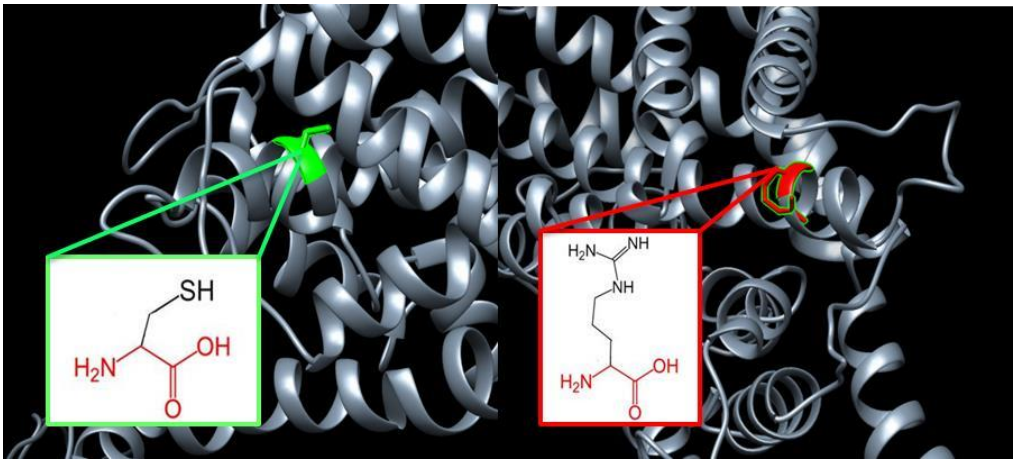

Figure 3 (C294R): change in the amino acid Cysteine into Arginine at position 294.

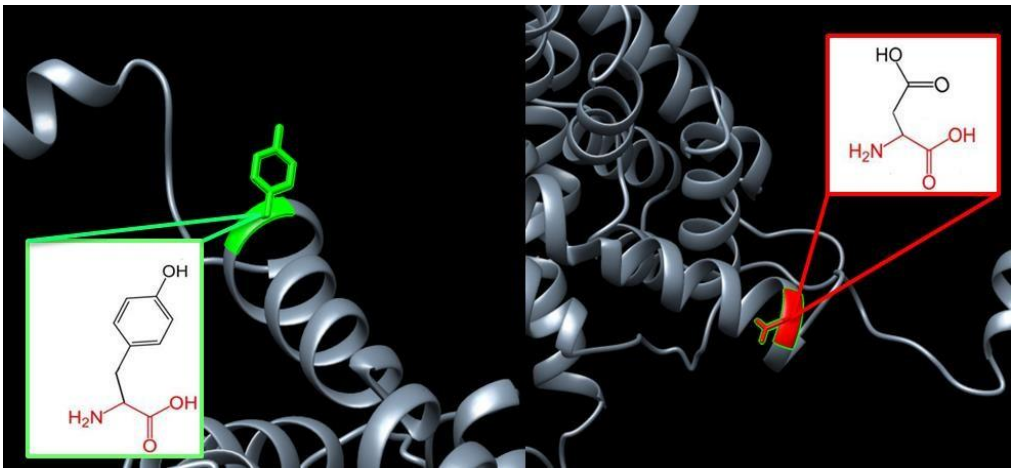

Figure 4 (Y370D): change in the amino acid Tyrosine into Aspartate at position 370.

In Figure 3, the wild-type residue was buried in the core of the protein, while the mutant residue is bigger and probably will not fit. The mutant residue is more hydrophobic than the wild-type residue; this will cause a possible loss of external

interactions. In Figure 4, there is a difference in charge between the wild-type and mutant amino acid; this mutation loses the charge of the wild-type residue, which can cause a loss of interactions with other molecules.

We also used ConSurf to flag the SNPs that are sited at highly conserved amino acid positions, which tends to be more damaging than SNPs that are sited at non-preserved positions. Our ConSurf analysis revealed that (C294R and Y370D) mutations were found in the highly conserved site and are expected to possibly impact *XK* protein (Figure 5).

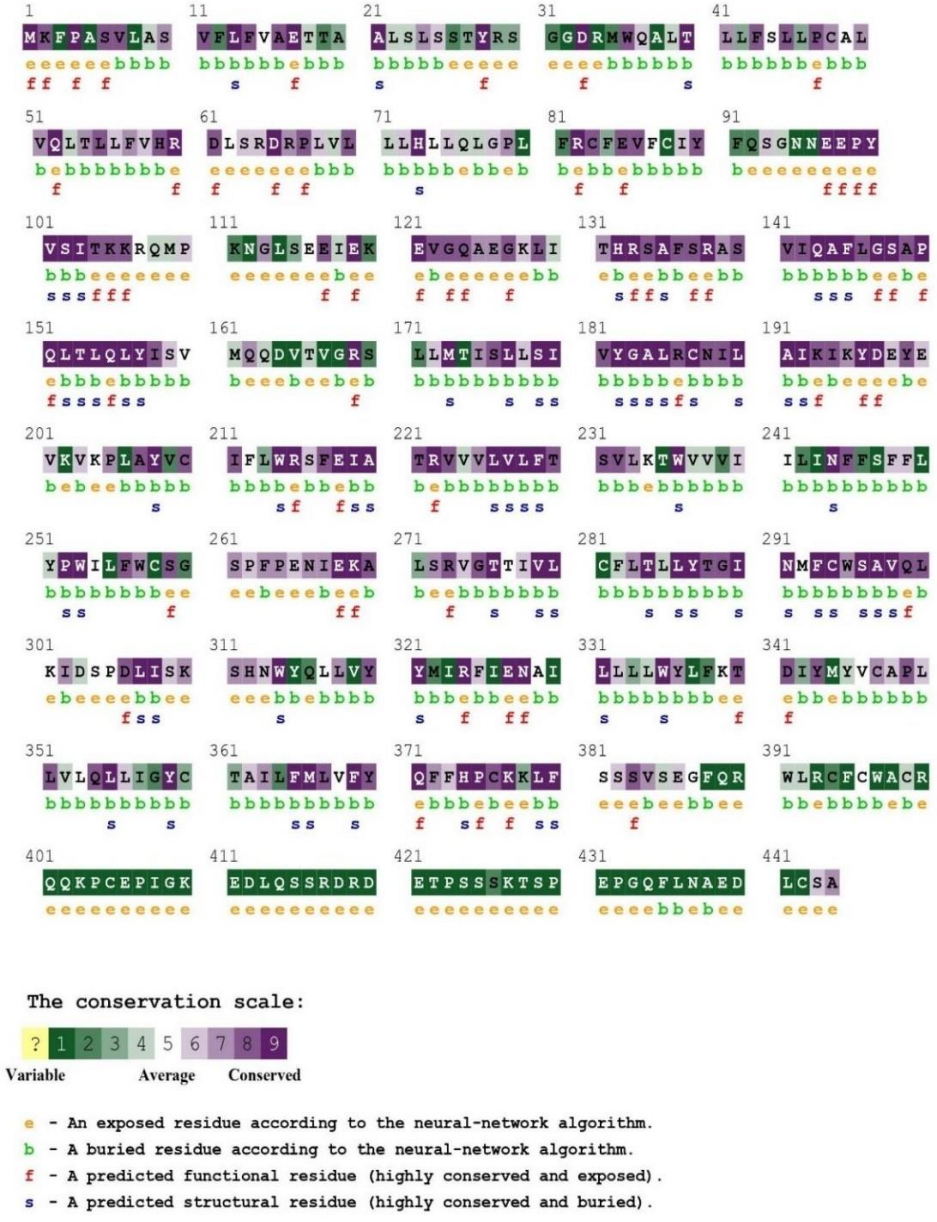

Figure 5 The conserved amino acids across species in XK protein were determined using ConSurf.

Interestingly, GeneMANIA could not predict *XK* gene function after the mutations. The genes co-expressed with, share similar protein domains, or participate to achieve similar functions were illustrated by GeneMANIA and shown in (Table 5 and Figure 6).

Table 4 Variants consequences, transcripts, and regulatory features by VEP tool.

Uploaded variation	Amino acids	Protein position	Consequence	IMPACT	SYMBOL	Feature type	BIOTYPE
rs28933690	C/R	294	missense variant	MODERATE	*XK*	Transcript	Protein coding
rs28933690	-	-	intron variant	MODIFIER	*AF241726.2*	Transcript	Protein coding
rs145996031	Y/D	370	missense variant	MODERATE	*XK*	Transcript	Protein coding
rs145996031	-	-	intron variant	MODIFIER	*AF241726.2*	Transcript	Protein coding

Table 5 The *XK* gene functions and its appearance in the network and genome.

Function	FDR*	Genes in network	Genes in genome
cAMP catabolic process	0.703371	2	11
cyclic nucleotide catabolic process	0.703371	2	12
cyclic-nucleotide phosphodiesterase activity	0.812113	2	18
3',5'-cyclic-nucleotide phosphodiesterase activity	0.812113	2	18

***FDR:** false discovery rate is greater than or equal to the probability that this is a false positive

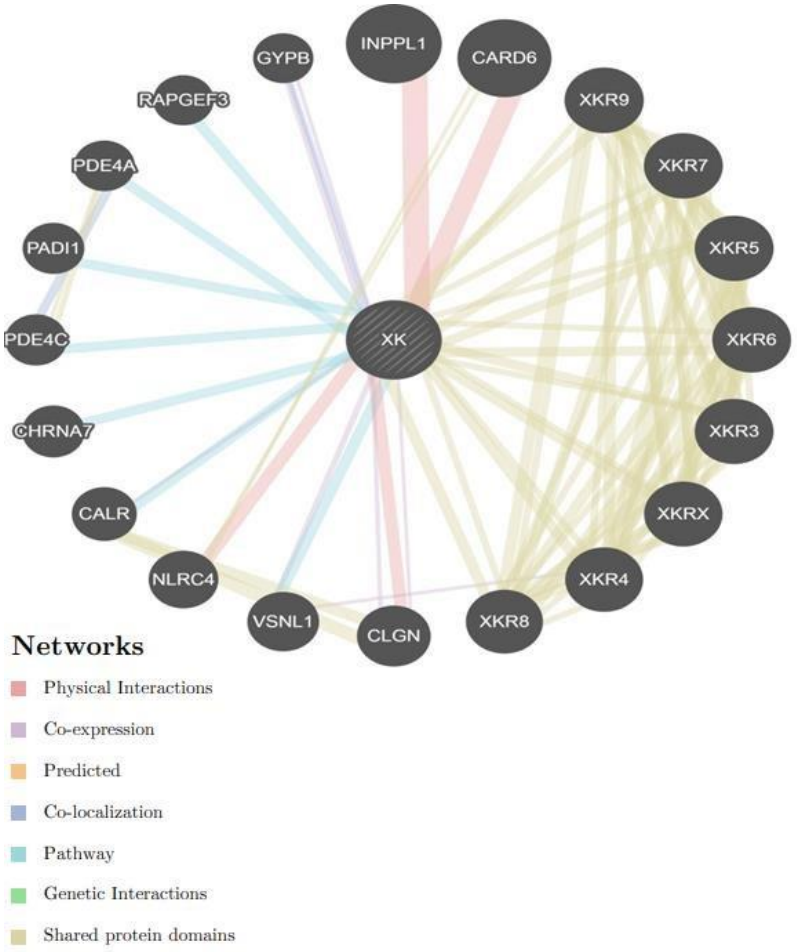

Figure 6 Network Interaction between *XK* and its associated genes.

4. Discussion

Functional studies can gain from a preliminary multi-step approach, which revealed two deleterious SNPs that are potentially responsible for the functional and structural modifications of XK protein by using bioinformatics tools. The methods used were based on different aspects and parameters describing the pathogenicity and provided pieces of evidence on the molecular level about the effect of mutations. The differences in prediction capabilities refer to the fact that every prediction algorithm uses different sets of sequences and alignments (Figure 1).

Single-nucleotide polymorphism (SNP) association studies have become crucial in revealing the genetic correlations of genomic variants with complex diseases. *In silico* analysis has been done for many disorders for cancer-related genes and other disorders (e.g., Huntington's disease) (George et al 2008). It was not accurate to predict the pathogenic effect of SNPs using a single method. Therefore, multiple methods were used to compare and rely on predicted results.

Also, we used ClinVar to compare our results that had been found by the computational approach with the clinical one, in (C294R) SNP, our result matches with Danek's result (Danek et al 2001), which was found to be pathogenic; while the other SNP (Y370D), is registered in ClinVar as "benign" ("National Center for Biotechnology Information". ClinVar; [VCV000695682.2], Jan 29, 2020) which disagree with our result.

Moreover, we used the VEP tool to find out the Functional consequences of these two variants; the predicted variants' consequences are shown in (Table 4); VEP reported regulatory consequences for many variants, including two variants within

a coding region and two variants within intron; in general, mutations within a coding region affect the protein function, while intronic mutations can disrupt transcription regulatory motifs and non-coding RNA genes (Vaz-Drago et al 2017). The limitation of this study is that it focuses on the coding region using different computational analysis tools; non-coding SNPs are likely to affect the level of gene expression (Ramirez-Bello and Jimenez-Morales 2017).

This study is the first *in silico* analysis of *XK* gene based on functional and structural analysis, while all previous studies (Gassner et al 2017; Ho et al 1992) were based on in vivo and in vitro analysis. There is an extended phenotypic overlap between McLeod syndrome, Huntington's disease, and chorea-acanthocytosis (Cardoso 2014; Danek et al 2001; Gantenbein et al 2011; Peikert et al 2018; Shah et al 2013; Zhang et al 2013), this may help to achieve a better should be understating of those diseases through our findings.

This work revealed two pathological SNPs with a potential functional and structural impact and may be used as diagnostic markers for McLeod neuroacanthocytosis syndrome. These findings can be used as a platform to develop large-scale studies in the future. Finally, some appreciations of wet lab techniques are suggested to support our findings.

5. Conclusions

In this study, the impact of functional mutations in the *XK* gene was investigated through different computational analysis tools, which determined that (C294R and Y370D) are the most deleterious SNPs that have a potential responsibility for the functional and structural alterations of *XK* protein. Therefore, it can be used as a diagnostic marker to predict McLeod neuroacanthocytosis syndrome.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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