Unmasking of novel mutations within *XK* gene that could be used as Diagnostic Markers to Predict McLeod syndrome: Using in silico analysis

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Abstract:

Background: McLeod neuroacanthocytosis syndrome is a rare X-linked recessive multisystem disorder affecting the peripheral and central nervous systems, red blood cells, and internal organs.

Methods: We carried out in silico analysis of structural effect of each SNP using different bioinformatics tools to predict substitution influence on protein structural and functional level.

Result: 2 novel mutations out of 104 nsSNPs that are found to be deleterious effect on the *XK* structure and function. **Conclusion:** The present study provided a novel insight into the understanding of McLeod syndrome, SNPs occurring in coding and non-coding regions, may lead to RNA alterations and should be systematically verified. Functional studies can gain from a preliminary multi-step approach, such as the one proposed here; we prioritize SNPs for further genetic mapping studies. This will be a valuable resource for neurologists, hematologists, and clinical geneticists on this rare and debilitating disease.

Keywords: McLeod neuroacanthocytosis syndrome, X-linked recessive, central nervous systems, red blood cells, in silico analysis, *XK* gene, neurologists, hematologists, clinical geneticists.

Introduction:

McLeod syndrome (MSL) is one subtype of rare X-linked recessive neuroacanthocytosis syndromes, approximately 150 cases have been reported worldwide.(1) Characterized by misshapen red blood cells and progressive degeneration of the basal ganglia disease including: movement disorders, cognitive alterations, and psychiatric symptoms and cardiac manifestations. Neuromuscular symptoms leading to weakness and atrophy of different degrees.(2-6) Hematologically, MSL is defined as a specific blood group phenotype that results from absent expression of the Kx erythrocyte antigen and weakened expression of Kell blood group antigens.(3, 7) Kell antigens are encoded by the KEL gene on the long arm of chromosome 7. Kx antigen is encoded by the XK gene on the short arm of the X chromosome.(8)

McLeod syndrome 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 upon the *XK* gene product and thus may account for the variable phenotype. (9-15) The *XK* gene provides instructions for producing the XK protein, which carries the blood antigen Kx. A variety of *XK* mutations has been reported but no clear phenotype-genotype correlation has been found, especially for the point mutations affecting splicing sites.(16-19) Some males with the 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.(5, 8, 20-23) but sometimes blood may not always be compatible.(24)

McLeod syndrome mimics Huntington disease; usually include progressive movement disorder (e.g: autosomal recessive chorea-acanthocytosis), cognitive impairment, and psychiatric symptoms, eventually associated with seizures, suggests that the corresponding proteins-XK.(4, 6, 9, 25-30) Most importantly, given the absence of cure, it's vital for appropriate genetic counseling, neurological examination and a cardiologic evaluation for the presence of a treatable cardiomyopathy.(5, 26, 31) Unfortunately, no clear correlations of the clinical findings with the genotype of *XK* mutations have yet been unrevealed.(32, 33)

Single-nucleotide polymorphism (SNPs) refers to single base differences in DNA among individuals. One of the interests in association studies is the association between SNPs and disease development.(34) This is the first in silico study which aim prioritize the possible mutations in *XK* gene located in coding regions and 3'UTR, to propose a modeled structure for the mutant protein that potentially affects its function. We prioritize our novel mutated SNPs for further genetic mapping studies. This will be a valuable resource for neurologists, hematologists, and clinical geneticists on this rare and debilitating disease.(35, 36)

2. Materials and Methods:

Data mining:

The data on human *XK* gene was collected from National Center for Biological Information (NCBI) web site.(37) The SNP information (protein accession number and SNP ID) of the *XK* gene was retrieved from the NCBI dbSNP (<http://www.ncbi.nlm.nih.gov/snp/>) and the protein sequence was collected from Uniprot database (protein ID: 9606).(38)

SIFT:

We used SIFT to observe the effect of A.A. substitution on protein function. SIFT predicts damaging SNPs on the basis of the degree of conserved amino A.A. residues in aligned sequences to the closely related sequences, gathered through PSI-BLAST.(39) It's available at (<http://sift.jcvi.org/>).

PolyPhen:

PolyPhen (version 2) stands for polymorphism phenotyping version 2. We used PolyPhen to study probable impacts of A.A. substitution on structural and functional properties of the protein by considering physical and comparative approaches.(40) It's available at (<http://genetics.bwh.harvard.edu/pp2/>).

Provean:

Provean is an online tool that predicts whether an amino acid substitution has an impact on the biological function of a protein grounded on the alignment-based score. The score measures the change in sequence similarity of a query sequence to a protein sequence homolog between without and with an amino acid variation of the query sequence. If the PROVEAN score ≤ -2.5 , the protein variant is predicted to have a "deleterious" effect, while if the PROVEAN score is > -2.5 , the variant is predicted to have a "neutral" effect.(41) It is available at (<https://roslab.org/services/snap2web/>).

SNAP2:

Functional effects of mutations are predicted with SNAP2. SNAP2 is a trained classifier that is based on a machine learning device called "neural network". It distinguishes between effect and neutral variants/non-synonymous SNPs by taking a variety of sequence and variant features into account. The most important input signal for the prediction is the evolutionary information taken from an automatically generated multiple sequence alignment. Also structural features such as predicted secondary structure and solvent accessibility are considered. If available also annotation (i.e. known functional residues, pattern, regions) of the sequence or close homologs

are pulled in. In a cross-validation over 100,000 experimentally annotated variants, SNAP2 reached sustained two-state accuracy (effect/neutral) of 82% (at anAUC of 0.9). In our hands this constitutes an important and significant improvement over other methods.(42) It is available at (<https://roslab.org/services/snap2web/>).

SNPs&GO:

Single Nucleotide Polymorphism Database (SNPs) & Gene Ontology (GO) is a support vector machine (SVM) based on the method to accurately predict the disease related mutations from protein sequence. FASTA sequence of whole protein is considered to be an input option and output will be the prediction results based on the discrimination among disease related and neutral variations of protein sequence. The probability score higher than 0.5 reveals the disease related effect of mutation on the parent protein function.(43) it's available at (<https://roslab.org/services/snap2web/>).

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 as a neutral polymorphism. It's available at: (<http://snps.biofold.org/phd-snp/phdsnp.html>).

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 in a unique web server. It offers the opportunity to predict the protein stability changes upon single-site mutations. From the protein structure or sequence. The FASTA sequence of protein retrieved from UniProt is used as an input to predict the mutational effect on protein and stability RI value (reliability index) computed.(44) It's available at (<http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi>).

MUpro:

MUpro is a support vector machine-based tool for the prediction of protein stability changes upon nonsynonymous SNPs. The value of the energy change is predicted, and a confidence score between -1 and 1 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.(45) It's available at (<http://mupro.proteomics.ics.uci.edu/>).

GeneMANIA:

We submitted genes and selected from a list of data sets that they wish to query. GeneMANIA approach to know protein function prediction integrate multiple genomics and proteomics data

sources to make inferences about the function of unknown proteins.(46) It is available at (<http://www.genemania.org/>)

Structural Analysis:

Developing 3D structure of mutant STAT3 gene:

The 3D structure of human Signal transducer and activator of transcription 3 (STAT3) protein is not available in the Protein Data Bank. Hence, we used RaptorX to generate a 3D structural model for wild-type STAT3. RaptorX is a web server predicting structure property of a protein sequence without using any templates. It outperforms other servers, especially for proteins without close homologs in PDB or with very sparse sequence profile.(47) It is available at (<http://raptorx.uchicago.edu/>).

Modeling Amino Acid Substitution:

UCSF Chimera is a highly extensible program for interactive visualization and analysis of molecular structures and related data, including density maps, supramolecular assemblies, sequence alignments, docking results, conformational analysis Chimera (version 1.8).(48) It's available at (<http://www.cgl.ucsf.edu/chimera/>).

Identification of DNA polymorphisms in miRNAs and miRNA target sites by PolymiRTS Database 3.0:

We submitted SNPs located within the 3'-UTRs then the PolymiRTS checked if the SNP variants could alter putative miRNA target sites focusing on mutations that alter sequence complementarity to miRNA seed regions possibly leading to McLeod syndrome.(49) It's available at (<http://compbio.uthsc.edu/miRSNP/>).

Figure 1: Diagrammatic representation of *XK* gene in silico work flow.

3. Results:

Table (1): Damaging or Deleterious or effect nsSNPs associated variations predicted by various softwares:

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	1.000	Deleterious	-3.911	Effect	83

rs1366754946	R60H	DAMAGING	0.02	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 softwares:

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

*RI: Reliability Index

Table (3): stability analysis predicted by I-Mutant version 3.0 and MUPro (also Show the 2 novel mutations):

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

Table (4): The *XK* gene functions and its appearance in network and genome:

Function	FDR	Genes in network	Genes in genome
cAMP catabolic process	0.703371188	2	11
cyclic nucleotide catabolic process	0.703371188	2	12
cyclic-nucleotide phosphodiesterase activity	0.812112981	2	18
3',5'-cyclic-nucleotide phosphodiesterase activity	0.812112981	2	18

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

Table (5) The gene co-expressed, share domain and Interaction with *XK* gene network:

Gene 1	Gene 2	Weight	Network group
GYPB	XK	0.0223527	Co-expression
VSNL1	XKR4	0.0111655	Co-expression
VSNL1	XK	0.0230949	Co-expression
CLGN	XK	0.0080287	Co-expression
CLGN	XK	0.0162705	Co-expression
CALR	XK	0.0155188	Co-expression
GYPB	XK	0.0144295	Co-expression
PDE4A	PDE4C	0.033181	Co-localization
GYPB	XK	0.0142386	Co-localization
VSNL1	XK	0.1165764	Pathway
CALR	XK	0.1165764	Pathway
CHRNA7	XK	0.1165764	Pathway
PDE4C	XK	0.1165764	Pathway
PADI1	XK	0.1165764	Pathway
PDE4A	XK	0.1165764	Pathway
RAPGEF3	XK	0.1165764	Pathway
INPPL1	XK	0.2043565	Physical Interactions
CARD6	XK	0.380832	Physical Interactions
NLRC4	XK	0.2288567	Physical Interactions
CLGN	XK	0.4778901	Physical Interactions
XKR9	XK	0.125	Shared protein domains
XKR7	XK	0.125	Shared protein domains
XKR7	XKR9	0.125	Shared protein domains
XKR5	XK	0.125	Shared protein domains

XKR5	XKR9	0.125	Shared protein domains
XKR5	XKR7	0.125	Shared protein domains
XKR6	XK	0.125	Shared protein domains
XKR6	XKR9	0.125	Shared protein domains
XKR6	XKR7	0.125	Shared protein domains
XKR6	XKR5	0.125	Shared protein domains
XKR3	XK	0.125	Shared protein domains
XKR3	XKR9	0.125	Shared protein domains
XKR3	XKR7	0.125	Shared protein domains
XKR3	XKR5	0.125	Shared protein domains
XKR3	XKR6	0.125	Shared protein domains
XKRX	XK	0.125	Shared protein domains
XKRX	XKR9	0.125	Shared protein domains
XKRX	XKR7	0.125	Shared protein domains
XKRX	XKR5	0.125	Shared protein domains
XKRX	XKR6	0.125	Shared protein domains
XKRX	XKR3	0.125	Shared protein domains
XKR4	XK	0.125	Shared protein domains
XKR4	XKR9	0.125	Shared protein domains
XKR4	XKR7	0.125	Shared protein domains
XKR4	XKR5	0.125	Shared protein domains
XKR4	XKR6	0.125	Shared protein domains
XKR4	XKR3	0.125	Shared protein domains
XKR4	XKRX	0.125	Shared protein domains
XKR8	XK	0.125	Shared protein domains
XKR8	XKR9	0.125	Shared protein domains
XKR8	XKR7	0.125	Shared protein domains
XKR8	XKR5	0.125	Shared protein domains
XKR8	XKR6	0.125	Shared protein domains
XKR8	XKR3	0.125	Shared protein domains
XKR8	XKRX	0.125	Shared protein domains
XKR8	XKR4	0.125	Shared protein domains
NLRC4	CARD6	0.0228792	Shared protein domains
CALR	CLGN	0.1553349	Shared protein domains
PDE4A	PDE4C	0.0508035	Shared protein domains
XKR9	XK	0.125	Shared protein domains
XKR7	XK	0.125	Shared protein domains
XKR7	XKR9	0.125	Shared protein domains
XKR5	XK	0.125	Shared protein domains
XKR5	XKR9	0.125	Shared protein domains
XKR5	XKR7	0.125	Shared protein domains

XKR6	XK	0.125	Shared protein domains
XKR6	XKR9	0.125	Shared protein domains
XKR6	XKR7	0.125	Shared protein domains
XKR6	XKR5	0.125	Shared protein domains
XKR3	XK	0.125	Shared protein domains
XKR3	XKR9	0.125	Shared protein domains
XKR3	XKR7	0.125	Shared protein domains
XKR3	XKR5	0.125	Shared protein domains
XKR3	XKR6	0.125	Shared protein domains
XKRX	XK	0.125	Shared protein domains
XKRX	XKR9	0.125	Shared protein domains
XKRX	XKR7	0.125	Shared protein domains
XKRX	XKR5	0.125	Shared protein domains
XKRX	XKR6	0.125	Shared protein domains
XKRX	XKR3	0.125	Shared protein domains
XKR4	XK	0.125	Shared protein domains
XKR4	XKR9	0.125	Shared protein domains
XKR4	XKR7	0.125	Shared protein domains
XKR4	XKR5	0.125	Shared protein domains
XKR4	XKR6	0.125	Shared protein domains
XKR4	XKR3	0.125	Shared protein domains
XKR4	XKRX	0.125	Shared protein domains
XKR8	XK	0.125	Shared protein domains
XKR8	XKR9	0.125	Shared protein domains
XKR8	XKR7	0.125	Shared protein domains
XKR8	XKR5	0.125	Shared protein domains
XKR8	XKR6	0.125	Shared protein domains
XKR8	XKR3	0.125	Shared protein domains
XKR8	XKRX	0.125	Shared protein domains
XKR8	XKR4	0.125	Shared protein domains
NLRC4	CARD6	0.0459523	Shared protein domains
CALR	CLGN	0.3333333	Shared protein domains
PDE4A	PDE4C	0.0549344	Shared protein domains

Table (6) SNPs and INDELs in miRNA target sites in XK gene:

Location	dbSNP ID	miR ID	Conservation	miRSite	Function Class	context+ score change
37587804	rs2230148	hsa-miR-4312	<u>2</u>	gggaACAAGGCag	D	-0.404
		hsa-miR-642b-5p	<u>2</u>	GGGAACAaggcag	D	-0.164
		hsa-miR-3135a	<u>4</u>	gggaaCTAGGCag	C	-0.168
37587850	rs189111996	hsa-miR-142-5p	<u>5</u>	tCTTTATAgagct	D	0.013
		hsa-miR-340-5p	<u>5</u>	tCTTTATAgagct	D	-0.008
		hsa-miR-5590-3p	<u>5</u>	tCTTTATAgagct	D	0.033
		hsa-miR-3607-3p	<u>5</u>	tcTTTACAGAgct	C	-0.117
		hsa-miR-3686	<u>5</u>	tctTTACAGAgct	C	-0.106
37588205	rs193010807	hsa-miR-4290	<u>3</u>	gGGAGGGCgttgc	D	-0.218
		hsa-miR-4687-5p	<u>7</u>	ggGAGGGCTgttc	D	-0.181
		hsa-miR-6856-3p	<u>7</u>	gggaGGGCTGTtc	D	-0.213
		hsa-miR-132-3p	<u>11</u>	gggagGACTGTTc	C	-0.076
		hsa-miR-1910-5p	<u>7</u>	gggAGGACTGttc	C	-0.156
		hsa-miR-212-3p	<u>11</u>	gggagGACTGTTc	C	-0.067
		hsa-miR-6749-3p	<u>2</u>	GGGAGGActgttc	C	-0.201
37588300	rs41297338	hsa-miR-4524a-3p	<u>2</u>	catgCTGTCTCta	D	-0.126
		hsa-miR-6758-5p	<u>2</u>	catgctCTCTCTA	C	-0.038
		hsa-miR-6856-5p	<u>2</u>	catgctCTCTCTA	C	-0.044
37588561	rs185326501					
		hsa-miR-101-5p	<u>2</u>	ccttgGATAACTt	C	-0.041
37588581	rs28998784	hsa-miR-4263	<u>5</u>	ttTTAGAAAaccc	D	0.064
		hsa-miR-4799-5p	<u>5</u>	tTTTAGAAAaccc	D	0.07
		hsa-miR-576-5p	<u>5</u>	ttTTAGAAAaccc	D	0.072
		hsa-miR-3685	<u>6</u>	tttTAGGAAAccc	C	0.004
		hsa-miR-384	<u>6</u>	tttTAGGAAAccc	C	0.013
		hsa-miR-5009-3p	<u>5</u>	tTTTAGGAaacc	C	0.004
37588605	rs200936215	hsa-miR-1262	<u>4</u>	cTCACCCAgttca	D	-0.087
		hsa-miR-203b-3p	<u>7</u>	ctcaccCAGTTCA	D	-0.003
		hsa-miR-4701-3p	<u>4</u>	cTCACCCAgttca	D	-0.124
		hsa-miR-6736-5p	<u>4</u>	cTCACCCAgttca	D	-0.152
		hsa-miR-6776-5p	<u>5</u>	ctCACCCAgttca	D	-0.129
		hsa-miR-4680-5p	<u>7</u>	ctcaccGAGTTCA	C	-0.016
		hsa-miR-598-5p	<u>4</u>	cTCACCGAgttca	C	-0.167
37588618	rs189953054	hsa-miR-1284	<u>2</u>	ggTGTATAGggaa	D	-0.025
		hsa-miR-454-5p	<u>2</u>	ggtgtATAGGGAA	D	-0.085
		hsa-miR-4789-5p	<u>2</u>	gGTGTATAGggaa	D	0.044
		hsa-miR-6883-3p	<u>2</u>	ggtgtATAGGGAA	D	-0.206

		hsa-miR-339-5p	<u>2</u>	ggtgtACAGGGAa	C	-0.09
		hsa-miR-486-5p	<u>2</u>	ggtGTACAGGgaa	C	-0.153
		hsa-miR-670-5p	<u>2</u>	ggtgtaCAGGGAA	C	-0.092
37588652	<u>rs17146124</u>	hsa-miR-26a-1-3p	<u>0</u>	gGAATAGAgtttt	N	-0.009
		hsa-miR-26a-2-3p	<u>0</u>	gGAATAGAgtttt	N	-0.009
		hsa-miR-618	<u>2</u>	ggaaTAGAGTTt	D	-0.108
		hsa-miR-6731-3p	<u>0</u>	GGAATAGAgtttt	N	-0.132
		hsa-miR-5582-3p	<u>3</u>	ggaataAAGTTTT	C	0.057
37588772	<u>rs181428605</u>					
		hsa-miR-4798-3p	<u>4</u>	cataatCGTGAGT	C	-0.216
37588773	<u>rs142405495</u>	hsa-miR-6750-3p	<u>4</u>	ataattGTGAGTT	D	-0.153
37588819	<u>rs150893835</u>					
		hsa-miR-135a-3p	<u>2</u>	caCCCTATAatgt	C	-0.067
		hsa-miR-4528	<u>2</u>	caccTATAATGt	C	0.091
37588858	<u>rs140233842</u>	hsa-miR-1178-5p	<u>2</u>	GACCCTAtttgtt	D	-0.094
		hsa-miR-1182	<u>2</u>	GACCCTAtttgtt	D	-0.095
		hsa-miR-7-1-3p	<u>8</u>	gaccctATTTGTT	D	0.065
		hsa-miR-7-2-3p	<u>8</u>	gaccctATTTGTT	D	0.065
		hsa-miR-495-3p	<u>8</u>	gaccctGTTTGTT	C	0.064
		hsa-miR-5688	<u>8</u>	gaccctGTTTGTT	C	0.064
37588914	<u>rs184091517</u>					
		hsa-miR-586	<u>0</u>	aAATGCATAgatc	O	-0.01
37589069	<u>rs188429260</u>	hsa-miR-454-5p	<u>2</u>	gaATAGGGAtcca	D	-0.018
		hsa-miR-6883-3p	<u>2</u>	gaATAGGGAtcca	D	-0.009
37589088	<u>rs180937360</u>	hsa-miR-135b-3p	<u>3</u>	tgtaaCCTACAA	D	-0.034
		hsa-miR-154-5p	<u>3</u>	tgtTAACCTAcaa	D	0.035
		hsa-miR-302c-5p	<u>3</u>	TGTTAAActacaa	C	0.125
		hsa-miR-4703-3p	<u>2</u>	tgtaAACTACAa	C	0.027
37589098	<u>rs144198509</u>	hsa-miR-6082	<u>2</u>	caaaACGTATTta	D	-0.075
		hsa-miR-19a-5p	<u>2</u>	CAAAACAatattta	C	0.083
		hsa-miR-19b-1-5p	<u>2</u>	CAAAACAatattta	C	0.079
		hsa-miR-19b-2-5p	<u>2</u>	CAAAACAatattta	C	0.079
		hsa-miR-2052	<u>2</u>	CAAAACAatattta	C	0.08
		hsa-miR-4307	<u>2</u>	cAAAACATAttta	C	0.19
37589113	<u>rs147778350</u>	hsa-miR-106a-3p	<u>3</u>	tggeCATTGCAct	D	0.053
		hsa-miR-130a-3p	<u>3</u>	tggccaTTGCACT	D	0.002
		hsa-miR-130b-3p	<u>3</u>	tggccaTTGCACT	D	0.002
		hsa-miR-301a-3p	<u>3</u>	tggccaTTGCACT	D	0.011
		hsa-miR-301b	<u>3</u>	tggccaTTGCACT	D	0.011

		hsa-miR-3666	<u>3</u>	tggccaTTGCACT	D	-0.036
		hsa-miR-4295	<u>4</u>	tggccaTTGCACT	D	0.011
		hsa-miR-454-3p	<u>4</u>	tggccaTTGCACT	D	0.002
		hsa-miR-143-5p	<u>3</u>	tggccACTGCACt	C	0.005
		hsa-miR-3677-5p	<u>3</u>	tGGCCACTgcact	C	-0.106
		hsa-miR-5693	<u>3</u>	tgGCCACTGcact	C	-0.078
		hsa-miR-6499-3p	<u>3</u>	tggeCACTGCAct	C	0.02
37589183	<u>rs17146127</u>	hsa-miR-1914-5p	<u>2</u>	aCACAGGAgaaaa	D	-0.01
		hsa-miR-1976	<u>2</u>	acaCAGGAGAAAA	D	0.002
		hsa-miR-4279	<u>2</u>	acacAGGAGAAaa	D	0.009
		hsa-miR-629-3p	<u>2</u>	acacaGGAGAAAa	D	0.037
		hsa-miR-3182	<u>2</u>	acACAGAAGAAAA	C	0.061
		hsa-miR-4659a-3p	<u>2</u>	acacAGAAGAAAa	C	0.102
		hsa-miR-4659b-3p	<u>2</u>	acacAGAAGAAAa	C	0.102
		hsa-miR-6875-3p	<u>2</u>	acacaGAAGAAAa	C	0.099
37589189	<u>rs56296545</u>	hsa-miR-1250-3p	<u>3</u>	gaGAAAATGcaat	D	0.117
		hsa-miR-33a-5p	<u>5</u>	gagaaAATGCAAt	D	0.039
		hsa-miR-33b-5p	<u>5</u>	gagaaAATGCAAt	D	0.039
		hsa-miR-106a-3p	<u>5</u>	gagaaATTGCAAt	C	0.042
37589241	<u>rs185849854</u>	hsa-miR-1277-5p	<u>2</u>	cagtATATATTct	D	0.205
		hsa-miR-5011-5p	<u>2</u>	cagTATATATtct	D	0.219
37589319	<u>rs6610594</u>	hsa-miR-2467-5p	<u>4</u>	attataGAGCCTC	D	-0.131
		hsa-miR-6822-3p	<u>3</u>	attaTAGAGCCtc	D	-0.114
		hsa-miR-3188	<u>4</u>	attataAAGCCTC	C	-0.064
		hsa-miR-374a-5p	<u>1</u>	ATTATAAagcctc	C	0.167
		hsa-miR-374b-5p	<u>1</u>	ATTATAAagcctc	C	0.164
37589516	<u>rs78116169</u>	hsa-miR-1178-3p	<u>2</u>	taGTGAGCAtata	D	-0.139
37589550	<u>rs28998785</u>	hsa-miR-4781-3p	<u>4</u>	CAACATAtacttc	D	0.058
		hsa-miR-4666a-5p	<u>4</u>	caACATGTActtc	C	0.016
		hsa-miR-5585-5p	<u>4</u>	caacatGTACTTC	C	-0.014
37589600	<u>rs28998786</u>	hsa-miR-6082	<u>1</u>	cacaatCGTATTA	N	-0.005
		hsa-miR-374c-5p	<u>1</u>	cacaatTGTATTA	C	0.054
		hsa-miR-4666a-3p	<u>1</u>	cacaATTGTATta	C	0.071
		hsa-miR-655-3p	<u>1</u>	cacaatTGTATTA	C	0.054
		hsa-miR-7849-3p	<u>1</u>	caCAATTGTAtta	C	0.047
37589619	<u>rs149075680</u>	hsa-miR-1305	<u>7</u>	acacccTTGAAAA	D	0.135
		hsa-miR-498	<u>5</u>	acaccCTTGAAAA	D	0.076

		hsa-miR-561-5p	<u>5</u>	acacCCTTGAAaa	D	-0.014
		hsa-miR-5096	<u>7</u>	acacccGTGAAAA	C	0.078
37589679	<u>rs28998787</u>	hsa-miR-4476	<u>0</u>	ttgtttCTTCCTA	C	0.023
		hsa-miR-6876-5p	<u>0</u>	ttgtttCTTCCTA	C	0.016
		hsa-miR-3148	<u>0</u>	ttgtTTTTCCta	N	0.058
		hsa-miR-4496	<u>0</u>	ttgtttTTTCCTA	N	0.05
37589858	<u>rs192298353</u>	hsa-miR-210-5p	<u>1</u>	gtAGGGGCAatag	N	-0.046
		hsa-miR-4749-3p	<u>1</u>	gtAGGGGCAatag	N	-0.053
		hsa-miR-4725-5p	<u>1</u>	gtAGGGTCAatag	C	-0.01
		hsa-miR-504-5p	<u>1</u>	gtAGGGTCAatag	C	-0.017
37589882	<u>rs67287347</u>	hsa-miR-125a-3p	<u>5</u>	CACCTGAaatgcc	O	-0.012
		hsa-miR-205-3p	<u>6</u>	cacCTGAAATgcc	O	0.183
		hsa-miR-3934-5p	<u>5</u>	CACCTGAaatgcc	O	-0.007
		hsa-miR-764	<u>5</u>	CACCTGAaatgcc	O	-0.007
		hsa-miR-506-5p	<u>5</u>	caCCTGAATgcc	O	0.105
37589949	<u>rs181939360</u>	hsa-miR-3135a	<u>3</u>	CTAGGCAtgtag	D	-0.021
37590096	<u>rs35312956</u>	hsa-miR-3122	<u>2</u>	gtgaTCCCAAaccaa	O	0.035
		hsa-miR-3913-5p	<u>2</u>	gtgaTCCCAAaccaa	O	0.025
		hsa-miR-450a-1-3p	<u>2</u>	gtgaTCCCAAaccaa	O	0.03
		hsa-miR-450b-3p	<u>2</u>	gtGATCCCAAaccaa	O	-0.144
		hsa-miR-4713-3p	<u>2</u>	gtGATCCCAAaccaa	O	-0.068
		hsa-miR-5087	<u>14</u>	gtgatccCAAACCAa	O	0.023
		hsa-miR-5089-5p	<u>2</u>	gtgATCCCAAaccaa	O	-0.003
		hsa-miR-638	<u>2</u>	gtGATCCCAAaccaa	O	-0.073
		hsa-miR-6513-5p	<u>2</u>	gtgATCCCAAaccaa	O	0.043
		hsa-miR-6728-5p	<u>2</u>	gtgATCCCAAaccaa	O	-0.005
		hsa-miR-6740-5p	<u>5</u>	gtgatCCCAAACcaa	O	-0.052
		hsa-miR-769-3p	<u>2</u>	gtGATCCCAAaccaa	O	-0.133

		hsa-miR-887-5p	<u>2</u>	gtgaTCCCCAAAccaa	O	0.036
		hsa-miR-2110	<u>2</u>	gtgaTCCCCAAaaccac	O	-0.033
		hsa-miR-3150a-3p	<u>2</u>	gtgaTCCCCAAaaccac	O	-0.034
		hsa-miR-4260	<u>5</u>	gtgatCCCCAAAccaa	O	-0.035
		hsa-miR-4450	<u>2</u>	gtgATCCCCAaaaccac	O	-0.085
		hsa-miR-450a-2-3p	<u>2</u>	gtgaTCCCCAAAcca a	O	-0.108
		hsa-miR-5572	<u>5</u>	gtgatCCCCAAAccaa	O	-0.032
		hsa-miR-6763-5p	<u>2</u>	gtgaTCCCCAAaaccac	O	-0.028
		hsa-miR-6810-5p	<u>2</u>	gtgaTCCCCAAaaccac	O	-0.052
		hsa-miR-6857-5p	<u>2</u>	gtgATCCCCAaaacca a	O	-0.105
37590136	<u>rs187127162</u>	hsa-miR-27a-5p	<u>8</u>	ccaAAGCCCActt	D	-0.028
		hsa-miR-4417	<u>2</u>	ccaaAGCCCACtt	D	-0.128
		hsa-miR-203b-5p	<u>2</u>	ccaaaGACCACTt	C	-0.094
		hsa-miR-6718-5p	<u>2</u>	ccaaaGACCACTt	C	-0.113
		hsa-miR-9-5p	<u>7</u>	CCAAAGAccactt	C	0.063
37590399	<u>rs190486991</u>	hsa-miR-5087	<u>5</u>	ataaaACAAACCA	D	-0.143
		hsa-miR-8068	<u>4</u>	ataaAACAACca	D	0.003
37590476	<u>rs6610596</u>	hsa-miR-29a-3p	<u>1</u>	tctTGGTGCTttg	N	-0.254
		hsa-miR-29b-3p	<u>1</u>	tctTGGTGCTttg	N	-0.254
		hsa-miR-29c-3p	<u>1</u>	tctTGGTGCTttg	N	-0.254
		hsa-miR-330-3p	<u>9</u>	tcttgTGCTTTG	D	-0.027
		hsa-miR-6835-3p	<u>10</u>	tcttgTGCTTTg	D	-0.014
		hsa-miR-5695	<u>1</u>	tCTTGAGccttg	C	-0.176
37590608	<u>rs28998788</u>					
		hsa-miR-4799-5p	<u>6</u>	TTTAGAAAttctc	C	0.035
37590758	<u>rs182629937</u>	hsa-miR-130a-5p	<u>6</u>	taatagATGTGAA	D	-0.066
		hsa-miR-23a-3p	<u>6</u>	taatagATGTGAA	D	-0.069
		hsa-miR-23b-3p	<u>6</u>	taatagATGTGAA	D	-0.066
		hsa-miR-23c	<u>6</u>	taatagATGTGAA	D	-0.066
		hsa-miR-1228-3p	<u>5</u>	taatAGGTGTGAa	C	-0.322
		hsa-miR-377-3p	<u>6</u>	taatagGTGTGAA	C	-0.111

37590772	<u>rs5963263</u>	hsa-miR-1305	<u>7</u>	atgctTTGAAAAt	D	0.04
		hsa-miR-330-3p	<u>7</u>	aTGCTTTGAaaat	D	-0.239
		hsa-miR-4422	<u>7</u>	ATGCTTTgaaaat	D	-0.101
		hsa-miR-607	<u>7</u>	atgcTTTGAaAat	D	0.031
37590866	<u>rs6610597</u>	hsa-miR-627-3p	<u>9</u>	tGAAAAGAAataa	D	-0.013
		hsa-miR-335-3p	<u>2</u>	TGAAAAAaaataa	C	0.031
37590909	<u>rs59479974</u>	hsa-miR-153-3p	<u>1</u>	aTATGCAAatctt	C	-0.047
		hsa-miR-3680-3p	<u>1</u>	atATGCAAAtctt	C	-0.066
		hsa-miR-448	<u>1</u>	ATATGCAAatctt	C	-0.128
37590944	<u>rs138184834</u>	hsa-miR-4710	<u>6</u>	ttctCTCACCAAct	C	-0.1
		hsa-miR-4792	<u>6</u>	ttctCTCACCAAct	C	-0.114
		hsa-miR-7855-5p	<u>6</u>	ttctCTCACCAAct	C	-0.173
37591065	<u>rs17312163</u>	hsa-miR-302b-5p	<u>7</u>	ttttcGTTAAAGg	C	-0.08
		hsa-miR-302d-5p	<u>7</u>	ttttcGTTAAAGg	C	-0.071

***D:** The derived allele disrupts a conserved miRNA site (ancestral allele with support ≥ 2).

***C:** The derived allele creates a new miRNA site.

***O:** The ancestral allele cannot be determined.

Figure 2: (C294R): change in the amino acid Cysteine into Arginine at position 294.

Figure 3: (Y370D): change in the amino acid Tyrosine into Aspartate at position 370.

Figure 4: Interaction between *XK* and its related genes.

Discussion:

2 novel mutations have been found *that* effect on the stability and function of the *XK* gene using bioinformatics tools. The methods used were based on different aspects and parameters describing the pathogenicity and provide clues on the molecular level about the effect of mutations. 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 disease).(50-52)

It was not easy to predict the pathogenic effect of SNPs using single method. Therefore, multiple methods were used to compare and rely on the results predicted. In this study we used different in silico prediction algorithms: SIFT, PolyPhen-2, Provean, SNAP2, SNP&GO, PHD-SNP, P-mut, I-Mutant 3.0 and MUPro (figure 1). This study identified the total number of nsSNP in Homo sapiens located in coding region of *XK* gene, were investigate in dbSNP/NCBI Database. Out of 363 there are 104 nsSNPs, were submitted to SIFT server, PolyPhen-2 server, Provean sever and SNAP2 respectively, 35 SNPs were predicted to be deleterious in SIFT server. In PolyPhen-2 server, the result showed that 63 were found to be damaging (16 possibly damaging and 48 probably damaging showed deleterious). In Provean server our result showed that 42 SNPs were predicted to be deleterious. While in SNAP2 server the result showed that 52 SNPs were predicted to be Effect. The differences in prediction capabilities refer to the fact that every prediction algorithm uses different sets of sequences and alignments. In table (2) we were submitted four positive results from SIFT, PolyPhen-2, Provean and SNAP2 (table 1) to observe the disease causing one by SNP&GO and PHD-SNP servers. In SNP&GO and PHD-SNP servers, were used to predict the association of SNPs with disease. According to SNPs&GO and PHD-SNP revealed that, 2 and 13 SNPs were predicted to be disease related SNPs respectively. We selected the double disease related SNPs only in 2 softwares for further analysis by I-Mutant 3.0 ,Table (3) .While I-Mutant and MUPro results revealed that the protein stability decreased which destabilize the amino acid interaction.(table3)

SNPs in 3'UTR of *XK* gene were submitted as batch to PolymiRTS server. The output showed that among 448 SNPs in 3'UTR region of *XK* gene, about 42 SNPs were predicted, among these 42 SNPs, 156 alleles disrupted a conserved miRNA site and 69 derived alleles created a new site of miRNA. As an example (rs200936215) SNP contained (D) allele had 5 miRSite as target binding site can be disrupts a conserved miRNA and (C) alleles had 70 miRSite disrupts a conserved miRNA site, while 27 ancestral alleles (O) cannot be determined in the all predicted 42 SNPs. Table (6) below demonstrates the SNPs predicted by Polymirt to induce disruption or formation of mirRNA binding site.

UCSF Chimera was used to visualize and analysis of molecular structures for the two deleterious nsSNPs: (rs28933690) (C294R) and (rs145996031) (Y370D). (Figures 2&3)

Interestingly, GeneMANIA could not predict *XK* gene function after the mutations. The genes co-expressed with, share similar protein domain, or participate to achieve similar function were illustrated by GeneMANIA and shown in figure 4, Tables (4&5).

We also found that (rs28933690) (C294R) pathogenic, which is matched with the clinical result that we retrieved from dbSNPs/NCBI database .also we Retrieved (rs145996031) (Y370D) as untested we found to be damaging. Interestingly we found that, (rs145996031) (Y370D) novel SNP may also cause L1 syndrome (53).

Our study is the first in silico analysis of *XK gene* which was based on functional and structural analysis while previous studies (20, 54) based on in vivo and in vitro analysis. There is an extended phenotypic overlap between McLeod syndrome, Huntington disease and chorea-acanthocytosis, (9, 25, 29, 55-57) this overlapping may help to achieve better understating for those diseases by our findings.

This study revealed 2 Novel Pathological mutations have a potential functional impact and may thus be used as diagnostic markers for McLeod neuroacanthocytosis syndrome. Constitute possible candidates for further genetic epidemiological studies with a special consideration of the large heterogeneity of *XK* SNPs among the different populations.

Conclusion:

A total of 2 novel nsSNPs were predicted to be responsible for the structural and functional modifications of *XK* protein These findings contribute to the understanding of the physiology of *XK* protein, and the pathogenetic mechanisms of acanthocytosis, myopathy, and striatal neurodegeneration in McLeod syndrome and in other associated rare neurodegenerative diseases. Also can be used for pharmacogenomics studies. Finally some appreciations of wet lab techniques are suggested to support our in silico analysis results.

Conflict of interest:

The authors declare that they have no competing interests. The authors declare that there is no conflict of interest regarding the publication of this paper.

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