

DETECTION OF A NOVEL MUTATION IN THE PROTEOGLYCAN-4 GENE CAUSING CAMPTODACTYLY ARTHROPATHY COXA-VARA PERICARDITIS (CACP)

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Abstract

Background: The Camptodactyly Arthropathy Coxa-Vara Pericarditis (CACP) syndrome is a rare autosomal recessive condition characterized by camptodactyly, non-inflammatory arthropathy, coxa-vara abnormality development, and pericarditis. This study investigates the molecular screening of CACP within autosomal recessive inheritance patterns. **Methods:** A large consanguineous family from Tehsil Domel district Bannu, Khyber Pakhtunkhwa province, Pakistan, was sampled. deoxy ribo nucleic Acid (DNA) extraction was performed on family subjects, followed by whole exome sequencing, Sanger sequencing and relevant online software analysis to identify the mutated gene. Additionally, computational analysis of mutated and unmutated proteoglycan 4 (PRG4) gene structures was conducted to assess protein structure. **Results:** Investigation of whole exome sequencing data highlighted the role and expression of genes involved in limb deformities, joint inflammation, synovial malformation and cardiac abnormalities. Sanger sequencing revealed a homozygous frame shift 4 base pair (4bp) deletion mutation (c.3007_3010delGAAA, p.Glu1003fs) in the PRG4 gene on chromosome 1q25eq31. Clinical features of family subjects included camptodactyly, osteoporosis, joint swelling and pain, synovial hyperplasia, and hip joint deformity without pericardial inflammation. **Conclusion:** The present findings not only expands the variant spectrum of PRG4 but also be helpful in genetic counseling, prenatal diagnosis, and bringing awareness regarding the prevention of the disease among Pakistani population. Furthermore, the 3-dimensional models of the mutated and non mutated PRG4 was designed, refined and validated by computational analysis. The present study will reveal to avoid the cousin marriages and can lead to inherit the homozygous sequence variant state in the coming generations causing more sever clinical features.

INTRODUCTION

Camptodactyly-arthropathy-coxa-vara-pericarditis (CACP) is a rare autosomal recessive disorder described by camptodactyly, non-inflammatory arthropathy, coxa-vara and pericarditis. This syndrome was first explained in 1986.¹ Genetic cause of the disease was identified in 1999.² Some patients with CACP also have hereditary cataracts.³ The accurate incidence of CACP disorder is unknown and more prevalent in those families where cousin marriages are high such as Egypt, United Arab Emirates and Saudi Arabia.^{4,5} Patients with CACP have a typical form of articular features that characterize most common rheumatic syndromes. These patients are generally identified and cured as juvenile idiopathic arthritis (JIA).⁶ The patients' joints in CACP are normal at the time of birth, but with passage of time they cause painless hyperplasia of synoviocytes leading to camptodactyly and arthropathy that indicates early joint contracture. It appears in 90% of CACP.^{7,8}

To date, 39 pathogenic mutations have been described in *PRG4* gene responsible for CACP disorder (Table 1). Careful review of the literature showed that the previously reported variants in the gene cause a premature stop codon, leading to the absence of operating lubricin protein.^{9,10} *PRG4* gene translates into the lubricin protein. It is present in huge quantity in synovial liquid in joints. The protein is released by joints, fibroblasts and chondrocytes cells which acts as lubricant for borderline and defends the cartilage from friction during wound. Lubricin is present in

different parts of the body like tendon surface, liver, kidney, muscles and pericardial and pleural cavities. In these cavities, the protein acts against adhesive function. Lack of this lubricin protein is mostly liable for CACP syndrome in human.¹¹ Absence of this protein also causes synovial hyperplasia, which has harmful effects on joint cartilage by reducing the beneficial and deleterious materials exchange. Tissue restoration and tendon covering calcification may lead to Camptodactyly.¹² For proper managing, cautious evaluation and initial identification of CACP disorder is valuable. Pathological examinations like erythrocytic sedimentation rate (ESR), C reactive protein (CRP) and complete blood count (CBC) are generally normal in this disorder, while 30% patients of CACP are recorded with pericarditis. In both infant and adults radiological results like ultrasound and MRI are helpful techniques in evaluating synovitis of metacarpophalangeal and wrist joints.¹³ The current study analyzed a consanguineous family segregating syndromic CACP in an autosomal recessive pattern of inheritance. Moreover, the 3-dimensional models of the mutated and non mutated *PRG4* was designed, distinguished and validated by computational analysis.

MATERIALS & METHODS

Study approval, family enrollment and DNA extraction

The present study was approved from the Institutional Review Board (IRB) of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan with reference number is

(PMAS-AAUR/D.FoS/269). The ethical committee has been scrutinized the present research and for completion of documentary requirements and has been found satisfactory. The competent authority has been approved the present research by providing the consent letters signed from the guardian of the family member. Attached consent letter and ethical certificate.

A large consanguineous family was sampled from Tehsil Domel district Bannu, Khyber Pakhtunkhwa province of Pakistan. Written informed consent form was signed from the patients and control members of the family. The photograph and radiological images of the patients were done at the near hospital. 7 ml of peripheral blood was collected from both control (IV-1, V-1, V-5, VI-7, VI-12 and VI-17) and patients (VI-8, VI-13 and VI-19) of the family. DNA was extracted using DNA kit (Sigma- Aldrich, Cat 2010-1, St. Louis, USA) and manual (phenol chloroform) method.

Whole exome and Sanger sequencing

Whole exome sequencing of the family member (V-3) was analyzed in order to find the novelty of gene and their mutation. Sequencing was done using an Illumina HiSeq 2000 sequencer. The Burrows-Wheeler Aligner (BWA) Enrichment application of Base Space (Illumina Inc., 5200 Illumina Way San Diego, California) was used to assess the reads. Configuration was carried out using BWA15, and genomic analysis was used to recognize the causal variations. Illumina variation Studio v2.2 was used to annotate each and every called variation. Exome data was filtered for

homozygous mutations due to the autosomal recessive inheritance, and Moon analysis (<http://www.diploid.com/moon>) was utilized for filtering based on Human Phenotype Ontology (HPO) terms. Gene function, vertebral column expression, rib development, and pathogenicity of the mutation were taken into consideration while highlighting variations. Using OMIM and PubMed, the phenotypes of the chosen genes for the disease were compared to the phenotypes of the afflicted family members. Sanger sequencing was used to recognize the pathogenic mutation in each family member, both afflicted and unaffected. Using the software programmer's primer 3 and primer blast, the variant-specific primers were created. The pathogenicity of the variations was evaluated using the <http://www.clcbio.com> link and ACMG criteria.

Computational analysis

Prediction of mutated and un-mutated structures of PRG4

The mutated and non mutated nucleotide sequences of *PRG4* exon 7 obtained by sequencing were used to design the 3-dimensional models using Swiss Model.¹⁴ Firstly, the nucleotide sequences were translated into amino acid sequences via ExPASy-Translate tool followed by the homology modeling based on Alpha Fold v2 method.¹⁵

Refinement and verification of predicted models

Subsequently, the selected models were refined by Galaxy Web

server(<https://galaxy.seoklab.org> to improve their Mol Probtity, clash, Rama favored score and RMSD values.¹⁶ This software expected results in the form of 5 refined models for each of the input protein. Afterwards, the best refined models were validated by PROCHECK and ERRAT programs available on UCLA-DOE LAB - SAVES v6.0 (<https://saves.mbi.ucla.edu>).

Molecular Dynamics Simulations

The molecular dynamics simulations (MDS) for both proteins were assessed by internal coordinate 's normal mode analysis server (iMODS). This software is easily accessible at

imods.iqfr.csic.es and helps in the evaluation of biological macromolecules.¹⁷ The results are obtained in the form of graphs.

RESULTS

Medical description

In the pedigree the parents are normal, while 3 patients of syndromic CACP segregating autosomal recessive manner. The patients VI-8, VI-19, VI-13 and VI-18 were suffered with camptodactyly, osteoporosis, swelling, joints pain, synovial hyperplasia, pericarditis, redness and hotness of joints respectively (Figure 1 a&b).

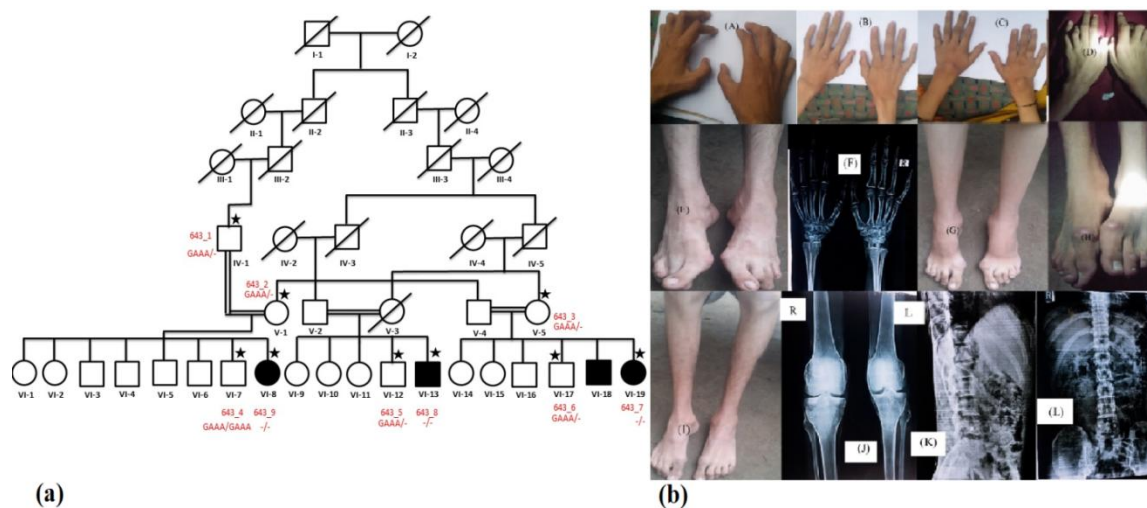


Fig 1: (a) Six generation pedigree of the family segregating consanguineous autosomal recessive CACP syndrome. Males are represented by square and females are represented by circle. Affected members are represented by filled symbol, while unaffected members are represented by unfilled ones. Crossed symbols represent dead members of the family. Double lines between individuals indicate consanguinity. Roman digits indicate

generation of the individuals with in pedigree. Star symbol indicates the members with blood samples available for genetic base analysis. Affected member VI-13 has been used for WES. The members IV-1, V-1, V-5, VI-7, VI-12 and VI-17 are healthy indicated heterozygous carrier GAAA and VI-8, VI-13, VI-19 are affected members with 4 bp deletion. Among these VI-8 and VI-19 are female with age of 21 and 23 years, while VI-13 and VI-18 are male

with age of 25 and 33 years respectively. The disease has diagnosed by local physciane and treated from the local hospital Khalifa Gul Nawaz Hopspital (KGN) district Bannu and Lady Reading Hospital district Peshawer. (b) The individual A (VI-13) showing deep camptodactyly in fingers of the hand and E (VI-13) is their toes of the legs reveals camptodactyly containing joint swelling and inflammation with morning time pain. Proband B (VI-18) show joint swelling and camptodactyly in the fifth fingers of both hands. F and J is their x ray indicating clear joint swelling and inflammation in fingers and knee joints, while I (VI-18) is their toes showing camptodactyly. Proband C (VI-8) is also showing camptodactyly in their phalanges and G (VI-8) is their toes of the legs. Proband D (VI-19) show severe camptodactyly in their phalanges and H (VI-18) is their toes of the legs. K and L is their x ray showing weakness in their vertebral column and ribs.

Sanger sequencing assessment and percentile of familes

The DNA of the patient (VI-13) was used in Whole exome sequencing (WES) to search the pathogenic mutation responsible for CACP disorder by moon software. Investigation was created upon the role and expression of genes elaborate in the limbs deformities, joints inflammation, synovial malformation and

cardiac abnormalities. On the basis of role and function of *PRG4* gene in limbs and joints bone expression, abnormal pathogenicity and maintenance, a deletion variant (c.3007_3010delGAAA, p.Glu1003fs) in the said gene was expected to be the strongest candidate. This mutation showed the exact segregation in rest members of the family by Sanger sequencing. This described mutation was absent in the DNA of the control members of the family. This pathogenic and novelty of the *PRG4* gene mutation (c.3007_3010delGAAA, p.Glu1003fs) lead to truncated protein was established by applying online bioinformatics tools (<http://www.clcbio.com>). The ACMG guidelines interpreted the recognized mutation to be pathogenic. Sanger sequencing validated homozygous mutation 4 bp deletions in patients of CACP disorder. Parents of the affected individuals were heterozygous while normal siblings were either wild type or heterozygous for the identified variant. Turkey had a greater frequency of CACP (16%), whereas the Netherlands, China, Algeria, and Egypt had low prevalence rates of 1%. It was also reported on the occurrence by gender. Cousin marriages account for 73.30% of disorder, while non-cousin marriages account for 26.60% (Figure 2 a&b).

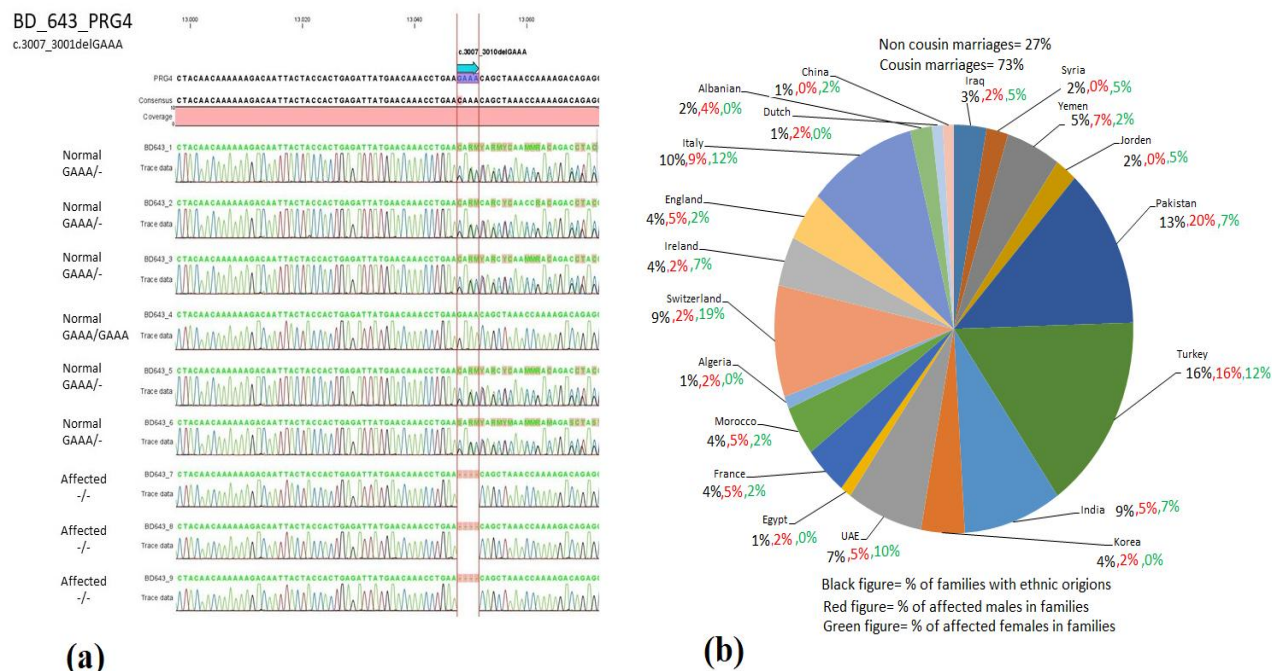


Fig 2: (a) Sanger sequencing indicated homozygous mutation of 4 bp deletion in patient of PRG4 gene represented by arrow, while normal and carrier are heterozygous GAAA bp. **(b)** Percentile of families with ethnic origins.

Table 1: To date 39 mutations reported in PRG4 gene indicating exons with ethnic origins

S. No	Mutations	Exon	Ethnic origin	References
	DNA base	Protein base		
01	c.1194delC	p.Thr399Profs*513	7	Turkey [5]
	c.3917_3934del	p.Arg1306_Ser1311del	11	
	c.3276_3277delAA	p.Lys1093Glufs*2	7	
	c.4101C>G	p.Tyr1367*	12	
	c.1192delC	p.Thr399Profs*513	7	
	c.1911delT	p.Glu638Argfs*274	7	
	c.2215A>T	p.Lys739*	7	
	c.1910_1911delCT	p.Pro637Argfs*9	7	
	c.2837_2838delAA	p.Lys947Asnfs*30	7	
	c.849delA	p.Val284Leufs*30	7	
02	c.4101C>G	p.Tyr1367*	11	Iraq [5]
03	c.1320dupC	(p.P440fsX197	7	UAE [3]

04	c.2754_2758delGACAA	p.Lys918Asnfs*10	7	Italy	[8]
	c.3636p3A4G	p.Hys1168Phefs*9	10		
	c.3894_3898delGGTTA	p.Val1299Glufs*5	11	Albanian	
	c.1982_1983delCT	p.Pro661Argfs*17	7		
	c.3648C4A	p.Tyr1216*	9	Dutch	
	c.2153delA	p.Lys718Argfs*194	6		
05	c.3755_3756insT	p.Lys1253fs*1	9	China	[18]
06	c.2645_2646delGA	p.Asp882LysfsX5	6	India	[19]
	c.2883_2886delAAGA	p.glu961glufsX39	7		
07	c.2866_2817delAA	P.Lys939fsX38	7	Pakistan	[20]
08	c.3139_3140delAA	p.Lys1047AspfsX33	7	UAE	[4]
	c.4078A>T	p.Arg1360X	12		
	c.923_924delAA	p.Lys308ArgfsX11	7		
	c.3125_3128delGAGT	p.Val1043GlufsX12	7		
	c.3276_3277delAA	p.Lys1093GlufsX2	7		
09	c.884_885delAG	~~~~~	6	India	[12]
10	c.1128-2733	~~~~~	7	Turkey	[7]
	c.538C>T	(p.Arg180Trp).	7	Turkey	[21]
	c.1194delC	(p.Thr399ProfsTer513	6		
	c.1194del	(p.Thr399fs)	6		
11	c.2805del5;	~~~~~	7	Jorden	[4]
	c.3023del2,	~~~~~	7		
	c.3240del7;	~~~~~	7		
	c.3690del5,,	~~~~~	10	Ireland	[4]
	c.4190CC → AG	~~~~~	13		
12	c.2831_2832insT	(p.Thr944IlefsTer34)	6	Caucasian	[22]
	c.3892C > T	~~~~~	7		
13	c.3007_3010delGAAA	p.Glu1003fs	7	Pakistan	Present study

Table	2.		Ethnic																
	Present																		
	Iraq		1	2	3	4	+	+	,	,	+	+	+	+	+	,	,	,	,
			1	2	3	4	+	+	+	+	+	+	+	+	+	+	+	+	+

[illegible]

S.no	Patient	Clinical Features	Syria	Jordan	Yemen	Pakistan	Turkey
1	Male		0	4	0	9	12
2	Female		2	1	2	1	5
3	Total		2	5	2	10	17
4	---	Swelling & stiffness of joints	+	+	-	+	+
5	---	Camptodactyly	+	-	+	+	+
6	---	Coxa vera	-	+	+	+	+
7	---	Hips pain	+	+	+	-	+
8	---	Osteoporosis	-	-	-	+	+
9	---	Pericarditis	-	-	+	-	+
10	---	Synovial hyperplasia	-	-	+	-	-
11	---	Redness & hotness of joints	-	+	-	-	-
12	---	Osteopenia	-	-	-	-	-
13	---	Lumbago	-	-	-	-	+
14	---	Lordosis	-	-	-	-	+
15	---	Respiratory infection	-	-	-	+	+
16	---	Flexion deformity of fingers & toes	-	-	-	+	-
17	---	Non inflammatory arthropathy of	-	-	-	+	-

Prediction of mutated and un-mutated structures of *PRG4*

For non mutated *PRG4* exon 7, three models (UM1-3) were predicted by the homology modeling using Swiss Model. The model 1

(UM1) had greatest 95.22% of sequence identity and highest coverage (0.98) with the SMB domain-containing protein (UniProtKB id: G1S8R9). In case of mutated *PRG4* exon 7, two models (M1 and M2) were predicted and

the model 1 was selected for further analysis. It is because, model 1 (M1) had 80.95% of sequence identity with the proteoglycan 4 (UniProtKB id: Q92954). The figure c indicated the structure of UM1b can be more easily deformed than changed protein model M1a. Afterwards, the covariance plot was also predicted which represents the motion of pair of residues in the form of colors. The red color

indicate the correlated motion, blue color represent the anti-correlated motion while the white color indicate the uncorrelated motion of pair of residues. The figure c elucidates the covariance matrix of UM1b and M1a and predicted that both protein structures are stable as most of the color is red and blue (Figure 3 a,b&c).

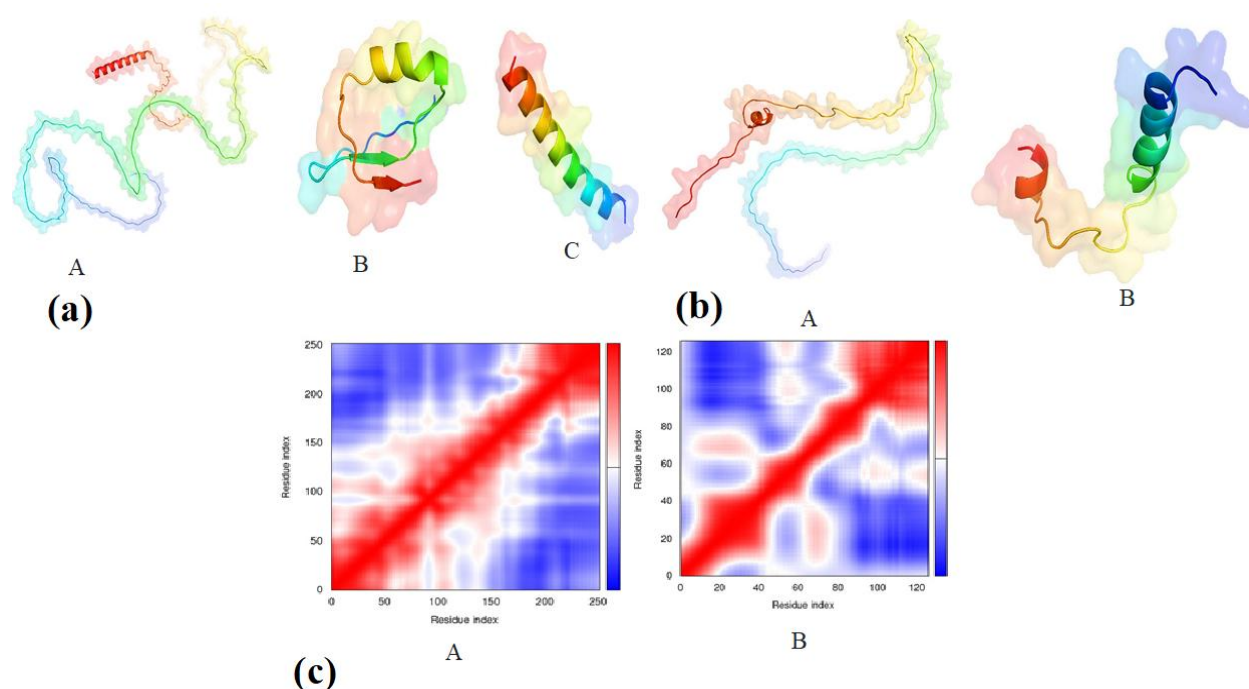


Fig 3: (a): All the predicted models of un-mutated nucleotide sequence of *PRG4*. (A) UM1 (B) UM2 (C) UM3. (b): All the predicted models of mutated nucleotide sequence of *PRG4*. (A) M1 (B) M2. (c): Covariance Plot for UM1b and M1a. (A) The most of the region in the graph is red in color, which is indicating the correlated motion of pair of residues hence representing the stability of protein. The blue color is showing anti-correlated motion of

residues while white is representing the uncorrelated motion. (B) The M1a is also stable because a major proportion of the plot is also red in color and showing correlated motion of pair of residues.

Refinement and verification of predicted models

The designated models for both the forms of *PRG4*, were refined by Galaxy Web server. Among the five models (UM1a-e) predicted for un-mutated protein, UM1b was selected

because it has the highest value of Rama favored score that is 94.0 (Table 3). In addition, the clash score, Mol Probity and RMSD values were 0.7, 1.134 and 1.177. Contrarily, M1a

was preferred from the five refined models for mutated protein (M1a-e) because it has 1.0 clash score, 1.242 Mol Probity, 1.178 RMSD and 92.8 Rama favored score (Table 4).

Table 3: The characteristic features of UM1 and its refined models.

Model	Rama favored	Clash score	MolProbity	RMSD
UM1	54.6	4.0	2.962	0.000
UM1a	92.0	1.2	1.324	1.154
UM1b	94.0	0.7	1.134	1.177
UM1c	91.0	0.5	1.182	1.215
UM1d	92.0	1.5	1.370	1.187
UM1e	91.2	1.2	1.352	1.169

Table 4: The characteristic features of M1 and its refined models.

Model	Rama favored	Clash score	MolProbity	RMSD
M1	33.6	11.2	3.440	0.000
M1a	92.8	1.0	1.242	1.178
M1b	89.6	1.9	1.680	1.251
M1c	88.8	1.9	1.701	1.232
M1d	88.8	0.0	1.246	1.273
M1e	88.0	1.0	1.385	1.301

Molecular dynamics simulations

Afterwards, the quality of UM1b and M1a were validated as shown in figure a. According to ERRAT prediction, the overall model quality factor of UM1b was 95.833%, which indicates the high resolution of predicted structure. Whereas, the M1a has a resolution between 2.5 and 3 Å because it's overall model quality factor is 90.909%. Additionally, the Ramachandran plot for both the predicted structures was also studied. For UM1b, 89.4% (195 amino acid residues) resides in the most favored region indicated by red color in the plot. On the other hand, 6.4% of the amino acid residues lie in the additional allowed region (yellow color). Additionally, just 0.9%

residues are present in generously allowed region (pale yellow) while 3.2% residues exist in the disallowed region (white). In case of M1a, 101 amino acid residues lay in the most favored region (87.1%); whereas, 9.5% residues are present in the additional allowed region. Similarly, two residues exists in the generously allowed region (1.7%) and 2 residues lie in the disallowed region (1.7%) (Figure 10D). The MDS for both models were conducted via iMODS. The deformability potential for UM1b residues is approximately 0.8 (Figure 11A); similarly, the deformability potential of M1a amino acid residues was also nearly 0.8 figure b. However, the peaks for the UM1b have abrupt variations as compared to

the deformability plot of M1a. Moreover, the energy for deforming the structure is also predicted in the form of eigenvalue. The ease to deform the structure is directly associated

with the least eigenvalue. According to figure b, the eigenvalue of UM1b is less ($2.214160\text{e-}09$) than the eigenvalue of M1a ($1.485349\text{e-}08$) (Figure 4 a&b

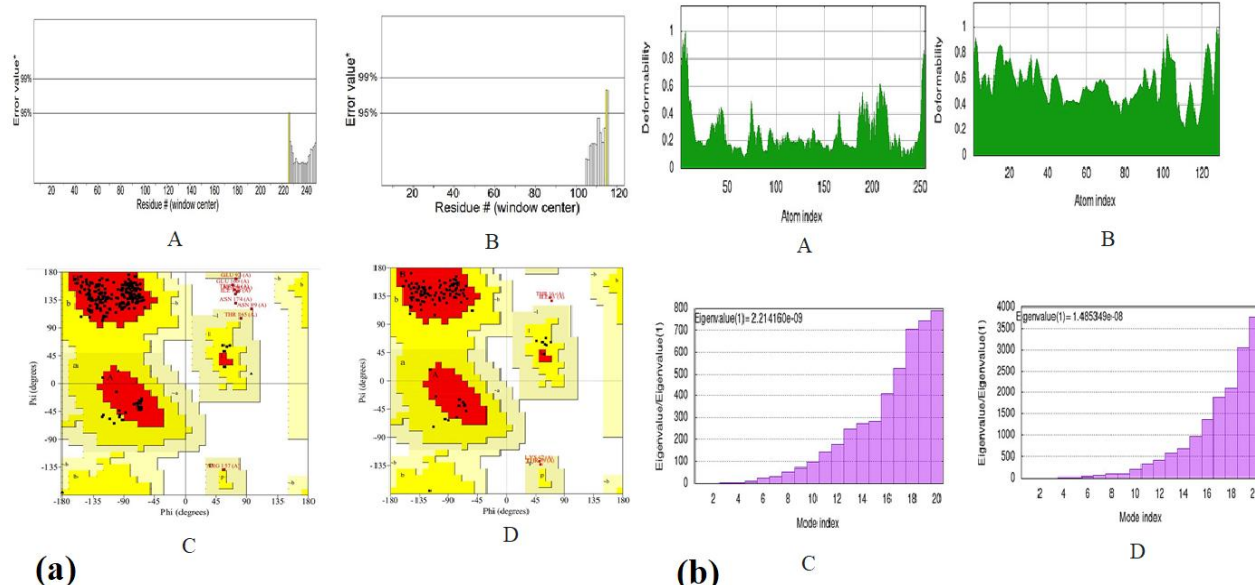


Fig 4: (a) Validation of refined tertiary structures of UM1 and M1. (A) It indicates the overall model quality factor of UM1b. (B) It represents the overall quality factor of M1a. (C) Ramachandran plot for UM1b illustrating that most of the amino acid residues lie in the most favored region of the plot (red color). (D) It elucidates that refined model of mutated protein M1a is quite stable as most of its residues are present in the red region of the plot (the most favored region). (b) Molecular dynamics simulations of UM1b and M1a. (A) The peaks in the plot changes abruptly and has a higher value of deformation (0.8). (B) The variation in the peaks in low but the overall deformation is 0.8. (C) The eigenvalue is lower, so it will be easy to deform UM1b. (D) It has a higher eigenvalue than M1a; therefore, it is somewhat stable than UM1b.

DISCUSSION

CACP syndrome is a rare inherited abnormality of childhood, which is defined by camptodactyly, non-inflammatory arthropathy, developing coxa-vara abnormality and pericarditis. In the present research, we have examined a Pakistani consanguineous family segregating syndromic CACP disorder in autosomal recessive pattern of inheritance. WES followed by Sanger sequencing

recognized a novel homozygous 4-bp deletion mutation (c.3007_3010del GAAA) leading to a frameshift variant and premature stop codon (p.Glu1003fs). Previously [20] reported a novel homozygous frameshift mutation (c.2816_2817delAA) (p.Lys939fsX38) in a family affected with CACP in Pakistan. A secreted mucin protein termed lubricin, which has 1404 amino acids and is connected with the megakaryocyte stimulating gene

proteoglycan 4, is thought to be a key component of synovial joints.²³ It resides in the cartilage side and synovial fluid as well as being secreted by synoviocytes and superficial area chondrocytes, acting as a lubricant for articulating joints.^{24, 25} The role of lubricin in joints comprises protection of the cartilage side against protein and cell adhesion buildup, inhibition of synovial cell formation, and lubricating media at the cartilage side.²⁶ Somatomedin B (SMB) and hemopexin-like (PEX) regions are two examples of the several protein sections that make up lubricin.^{26, 27} Current research on synovial fluid in CACP syndrome patients have shown that the absence of lubricin in the liquid would make it less able to dissolve the energy effect on joints that occurs during movement.²⁸

To date thirty nine mutations are documented from different ethnic origin indicating different exons,²⁹ showed in table (1). It is expected that all mutations will start to a premature stop codon, causing in the lack of operating lubricin protein.^{9, 10} The consanguineous family of the United Arab Emirates (UAE) described homozygous novel frame shift mutation (c.1320dupC) (p.P440fsX197) responsible for the CACP syndrome which targets the mucin protein like domain. In chine population reported novel frame shift mutation in exon 9 (c.3755_3756insT) (p.Lys1253fs*1) responsible for CACP syndrome.¹⁸ A novel homozygous 4 base pair deletion in exon 6 (c.2883_2886delAAGA; p.Glu961glufsX39) are described in CACP syndrome from the

adjacent country of India.³⁰ The *PRG4* homozygous gene, null mice formed with anomalous morphological variations like abnormal endochondral growing plates and altered cartilage side.²⁶ Moreover, those mice confirmed camptodactyly and developing synovial hyperplasia, which ultimately deteriorate the joint function.²⁶ The biological functions of *PRG4* gene are emphasis of deep research, because of its probable usage as a bio therapeutic level in arthritic disorders³¹ proved that lubricin protein, which is the result of *PRG4* gene and acts as grease in the mouse joints of knee. It is also confirmed that declined in grease of ankle joints of altered animals is indicating the lack of lubricin protein and causes irregular alterations within tendon sheath and other muscular tissue.²⁶ The protein models of mutated and un-mutated *PRG4* also elucidated the truncation in the main chain. Both of these protein models were refined after modeling to develop their quality. The M1a, mutated protein model of *PRG4*, has 87.1% of their amino acid residues in most favorable region according to Ramachandran plot. On the other hand, UM1b, un-mutated protein model of *PRG4*, has 89.4% of amino acid residues in the most favored region of Ramachandran plot.

Conclusion

In conclusion, we have recognized a homozygous frame shift mutation (c.3007_3010delGAAA, p.Glu1003fs) mapped on chromosome 1q25eq31 in *PRG4* gene segregating syndromic CACP in autosomal

recessive manner monitored by WES and Sanger sequencing. Affected member VI-13 has been used for WES. The members IV-1, V-1, V-5, VI-7, VI-12 and VI-17 are healthy indicated heterozygous carrier GAAA and VI-8, VI-13, VI-19 are affected with 4 bp deletion. Additionally, the 3-dimensional models of the mutated and non mutated *PRG4* was designed, refined and validated by computational analysis. The present study will reveal to avoid the consanguineous marriages and can lead to inherit the homozygous sequence variant state in the coming generations causing more severe clinical features.

Acknowledgment

The authors would like to thank the family members who participated in this study.

Ethical statement

The studies involving human participants were reviewed and approved from Institutional Review Board (IRB) of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan (PMAS-AAUR/D.FoS/269).

Informed consent

Informed consent was obtained from all participants

Data Availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further, inquiries can be directed to the corresponding authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors contributions

The following authors have made contribution to the manuscript as under:

Data curation: F. Khan.

Clinical Description: A. Khan.

Sequencing analysis: B. Ullah.

DNA Extraction: A. M. U. H. Shah.

Computational analysis: H. A. Hameed.

Pedigree analysis: I. Ullah.

Edited the manuscript: A. Arshad.

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