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RESEARCH ARTICLE
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PCT, CPT, S-TREM1 and Neopetrin as inflammatory biomarkers Detection, DNA sequencing, 3D Protein and recording a new gene in cancer children with Respiratory tract infection

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ABSTRACT

The aim of this study was to determine molecular detection of inflammatory biomarkers (PCT, CPT, S-TREM1 and Neopetrin) by using DNA sequencing, 3D Protein to give acknowledge about the roles of these biomarkers among cancer's patients with Respiratory tract Basrah province. DNA sequencing of PCT, CPT, S-TREM1, and Neopetrin has shown that there is a convergence between studied PCT and that of the Gen Bank database (NCBI) with identity 91/96 (95%) in forward PCT. A case-control study included 100 confirmed cancer's patients with Respiratory infection and 100 children as a control group. There was a convergence between studied CPT isolated and that of the Gen Bank database (NCBI) with identity 186/190(98%) in forward CPT that had been recorded four mutations appeared in (GGG to GGC), (GTA to GTC), (GGT to GGG) and (CC-to CCC). The forward CPT shown an identity144/145(99%) when compared with Gen Bank database (NCBI) one mutations reported as (CC-to CCC), (TCT to TCG), (GT-to GTG) and (TG-to TGG) in isolated s-trem1 that had been recorded. The reverse CPT had an identity 344/349 (99%), which is similar to that of the Gen Bank data. There was a convergence between studied Neopeterin isolated and that of the GenBank database (NCBI) with identity 166/167(99%) in forward Neopetrin that had been recorded one mutation appeared in (TGA to TG-). The reverse s-trem1 shown an identity 327/328(99) when compared with Gen Bank database that found one mutations was, (CGC to CG-). Neopetrin shown an identity 166/169(98%) when compared with Gen Bank database (NCBI) three mutations reported as (GGT to GGG), (TG- to TGT), (GA to GA-) and (TGA to TG-). No previous studies about the same this mutation in biomarkers. The present study sequencing of PCT, CPT, S-TREM1 and Neopetrin that showed partial a convergence between study PCT isolated and that of the GenBank database (NCBI) with identity 47/47(100%) PCT. A four mutations appeared as (S to P), (Y to S), (Ito GAB) and (R to Q). There convergence between study S-TREM1 isolated and that of the GenBank database (NCBI) with identity 71/74(96%) in S- TREM1 had a three mutations appeared as (M to I), (I to G) and (E to D). No previous studies interested studied in 3D protein with biomarkers. Neopetrin isolated and N-methylenyl methionine isolated showed high convergence between the two, with identity 103/104 (99%) and 99% convergence.

Keywords: *Biomarkers, genetic, cancer, RIT, protein, genes, DNA sequencing*

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INTRODUCTION

Respiratory tract infections (RTIs) are the most frequent infectious illnesses in the world and the second largest cause of mortality in children under the age of five [1]. The clinical spectrum varies from asymptomatic or moderate infection to severe or deadly illness, with severity determined by the interplay of three factors: the causal agent, the environment, and the host, these infections are often acute, with a quick clinical onset spanning from hours to days after infection with a variety of symptoms including fever, cough, sore throat, coryza, shortness of breath, wheezing, and/or trouble breathin [2]. In healthy children, LRTIs are typically self-limiting, provided proper supportive care and antibiotics or antiviral medications are started early in the infection. LRTI are more likely to have serious consequences in immunocompromised children, who have weakened immune systems [3]. Normal healthy people defend themselves against invading microorganisms via a variety of interconnected but distinct systems, such as physical barriers, circulating molecules, and cells, as well as their soluble mediators [4]. The wide variety of respiratory specimens and accessibility to certain anatomical respiratory structures are some of the challenges to the differential diagnosis of RTI [5]. At the heart of the dilemma, the question remains: "what is the fastest way to come to the correct diagnosis?" Physicians now are becoming more and more interested in the Use of biomarkers since there is no "gold standard" which is both sensitive and specific enough to help them reach the "correct" diagnosis [6]. An ideal biomarker for bacterial infections would help with early detection, prediction of illness course and prognosis, and treatment decision-making (e.g., antibiotic stewardship). [7] . Some of the biomarkers that are approaching as a complement in the diagnosis of pneumonia include C-reactive protein, leukocyte count, immunoglobulins, and proinflammatory cytokines. This Research mainly focuses on procalcitonin, and Soluble Triggering receptor expressed on myeloid cells-1 (TREM-1), calprotectin and Neopterin. Other biomarkers still being studied for their likely use in pneumonia; these include copeptin, cortisol, endotoxin, pro adrenomedullin, among others [8].

The study aimed to determine molecular detection of PCT, CPT, S-TREM1 and Neopetrin in cancer patient with respiratory tract infection.

MATERIALS AND METHODS

Respiratory tract infection in cancer patient's cases-control study was designed to collected in Basrah province from cancer's patient's in Al Basrah Specialized Teaching Hospital for children during November 2021 to July 2022. All cases were diagnosing and approve by Pulmonologist according to clinical criteria patient's in Al Basrah Specialized Teaching Hospital for children.

TABLE 1: The kits used in the study

Item	Description and Company	Country
DNA Extraction Kit	TRAN, Catalog No. EE121	China
PCR Kit	BioLabs, Catalog No. M0486	England

TABLE 2: show Biomarkers primer.

Target gene	Primer sequences (5'-'3)	Amplicon size	References
PCT	F 5′- GGAGAGCAGCCCAGCAGACCC -3′ R 3′- GTTGGCATTCTGGGGCATGCTAA - 5′	1267bp	De Werra ,et al., 1997
Calprotectin	F5′ - TGCCGTCTACAGGGATGAC -3′ R 3′ TCTGCAGGTACATGTCCAGG -5'	250bp	New design by this study and tested in NCBI

S-TREM1	F 5′ CATTCGGACGCGCAGTAAAC-3′ R 3′GGAGGCCTCAAGAACCTCAT- 5′	381bp	New design by this study and tested in NCBI
Neopterin	F5 ′ -TCCATGACATAGACCCTGCC - 3′ R 3′ -AGAGAGTGGTGCAGGGAAAA -5′	215bp	New design by this study and tested in NCBI

DNA Sequencing

The sequence of the nucleotide of biomarkers genes was known in two samples cases and one sample control, three samples have sequenced through PCR-sequences by Macro-Gene Company in the Korea. Nucleotides substitutions have determined by comparing the data obtained from gene bank publish which is available at NCBI (https://www.ncbi.nlm.nih.gov).

Alignment and Identity

The process of lining up two or more proteins (or nucleic acid) sequences to assess the similarity of their amino acids. Then determine identity in sequence alignment.

Phylogenetic tree

A phylogenic tree of based in the (PCT, CaL, TREM and Neopterin) genes Molecular phylogenetic is the branch of phylogeny that analyses hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationships. However, phylogeny estimated from a single gene should be treated with caution. The phylogenetic tree derived from (PCT, CaL, TREM, and NeoPterin) genes respectively sequences 2 samples with different sequences available at NCBI. As mentioned in Figures (2-80), (2-81), (2-82), and Figure (2-83) respectively was done by the (omega, 7) program.

3D Protein Structure

Was done by comparison with preserved protein in Blast, NCBI and drawing by NCBI.

Statistical analysis

Statistical analysis was carried out by using SPSS (VER.23) and the student's chi-square was applied to find out the statistical differences between all variables. probability less than 0.05 is significant ($P<0.05$).

RESULTS

DNA sequencing

DNA sequence analysis of procalcitonin (PCT) gene

Three samples have been sequenced through PCR sequences by Macrogen Company/ Korea. Nucleotides substitutions have determined by comparing the data obtained the from gene bank publish which is available at NCBI (https://www.ncbi.nlm.nih.gov) and the results were registered in NCBI under accession numbers (LC731316) which is available on this link (https://www.ncbi.nlm.nih.gov/nuccore/LC731316.1/).

PCT Forward primer sequence for sample

PCT Forward Primer Alignment and Identity for sample (1)

The Sequence ID:AH002628.2 Length:(1734) Number of Matches: 1,Range 1:685 to 777 Gen Bank Graphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
147 bits(79)	4e-30	91/96(95%)	3/96(3%)	Plus/Plus

FIGURE 2: Alignment statistics for Sample (1) PCT Forward primer

PCT Revers primer sequence for sample (1)
PCT Reverse Primer Alignment and Identity for sample (1)

Sequence ID: AH002628.2Length: 1734Number of Matches: 1
Range 1:1045 to 878 Gen Bank Graphics

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
289 bits (156)	8e-73	165/169(98%)	2/169(1%)	Plus/Minus

FIGURE (2-17): Alignment statistics for Sample (1) PCT gene Reverse primer.

PCT alignment and mutations observation for sample (1)

Table (2) was shown the most common types of mutations in the PCT forward and reverse gene, sequence in this study.

TABLE 2-13: Type mutation of in the PCT gene

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
1 F	Transition	715	GAA> GAG	Glutamic acide > Glutamic acide	Silent mutation	(PCT) gene
	Addition	718	GG-> GGG	No functional protein > Glycine	The protein made by the gene may not function properly	
	Addition	721	CC-> CCG	No functional protein > Proline	The protein made by the gene may not function properly	
	Tranversion	735	CAG> CAC	Glutamine> Histidine	Silent mutation	
1R	Addition	1040	GC-> GCA	No functional protein > Alanine	Silent mutation	
	Deletion	1034	ACA> AC-	Threonine > No functional protein	Defect protein	
	Tranversion	946	AGG> AGC	Arginine > Serine	The protein made by the gene may not function properly	
	Tranversion	894	TGG> TGC	Tryptophan > cysteine	The protein made by the gene may not function properly	

(PCT) Revers primer for sample (2)

(PCT) Reverse Primer Alignment and Identity for sample (2)

The Sequence ID: AH002628.2 Length: 1734Number of Matches: 1, Range 1: 878 to 1047 GenBank Graphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
279 bits (151)	5e-70	164/170(96%)	1/170(0%)	Plus/Minus

FIGURE (2-27): Alignment statistics for Sample (2) (PCT) gene Reverse primer

Preprocalcitonin (PCT) alignment and mutations observation for sample (2) Table (3-20) was shown the most common types of mutations in the (PCT) reverse gene, sequence in this study

TABLE (2-28): Type mutation of in the (PCT) gene sequence

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
2R	Transion	1044	AGT>AGC	No functional protein > Serine	The protein made by the gene may not function properly	gene (PCT)
	Transversion	1041	CGC>CGA	Araginine> Araginine	Silent Mutation	
	Transversion	1037	TGG>TGA	Tryptophan > Stop codon	Missense mutation	
	Deletion	1034	ACA>AC-	Threonine > No functional protein	No amino acid created	
	Transversion	946	AGG>AGC	Arginine > Serine	The protein made by the gene may not function properly	
	Transversion	895	TGG>TGC	Tryptophan > Cysteine		

DNA sequence analysis of (CPT) gene

Three samples have sequenced through PCR-sequences by Macrogen Company/ Korea. Nucleotides substitutions have determined by comparing the data obtained from gene bank publish which is available at NCBI (https://www.ncbi.nlm.nih.gov) and the results were registered in NCBI under accession numbers (LC731317)which is available on this link (https://www.ncbi.nlm.nih.gov/nuccore/LC731317.1/) .

CPT gene Forward primer for sample (1)

Calcium binding protein Forward Primer Alignment and Identity for sample (1)

The Sequence ID: NM_002964.5 Length: 408 Number of Matches: 1

GenBank Graphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
327 bits(177)	5e-85	186/190(98%)	1/190(0%)	Plus/Plus

Query 183
aaaaaGGGTGCAGACGTCTGGTTCAAAGAGTTGGATATCAACACTGATGGGGCAGTTA
AC 242
|||||||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct 197
AAAAAGGGTGCAGACGTCTGGTTCAAAGAGTTGGATATCAACACTGATGGTGCAGT
TAAC 256
Query 243
TTCCAGGAGTTCCTCATTCTGGTGATAAAGATGGGCGTGGCAGCCCACaaaaaaaGCCA
T 302
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 257
TTCCAGGAGTTCCTCATTCTGGTGATAAAGATGGGCGTGGCAGCCCACAAAAAAAG
CCAT 316
Query 303
GAAGAAAGCCACAAAGAGTAGCTGAGTTACTGGGCCCAGAGGCTGGCCCCCTGGAC
ATGT 362
|||||||||||||||||||||||||||||||||||||||| ||||||||||||
Sbjct 317
GAAGAAAGCCACAAAGAGTAGCTGAGTTACTGGGCCCAGAGGCTGGGCCCCTGGAC
ATGT 376
Query 363 CCCCTGCAGA 372
|| ||||||
Sbjct 377 ACC-TGCAGA 385

FIGURE (3-29): Alignment statistics for Sample (1) (CPT) gene Forward primer.

(CPT) Revers primer for sample (1).
(CPT) gene Reverse primer Alignment and Identity for sample (1)
The Sequence ID: NM_002964.5Length: 408 Number of Matches: 2 GenBank Graphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
261 bits(141)	5e-65	144/145(99%)	1/145(0%)	Plus/Minus

Query 22
AGCTACTCTTTGTGGCTTTCTTCATGGCttttttGTGGGCTGCCACGCCCCATCTTTAT 81
||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct 339 AGCTACTCTTTGTGGCTTTCTTCATGGCTTTTTTTGTGGGCTGCCACGCCC-
ATCTTTAT 281
Query 82
CACCAGAATGAGGAACTCCTGGAAGTTAACTGCACCATCAGTGTTGATATCCAACTC
TTT 141
|||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct 280
CACCAGAATGAGGAACTCCTGGAAGTTAACTGCACCATCAGTGTTGATATCCAACTC
TTT 221
Query 142 GAACCAGACGTCTGCACCCTTTTTC 166
|||||||||||||||||||||
Sbjct 220 GAACCAGACGTCTGCACCCTTTTTC 196

FIGURE (2-31): Alignment statistics for Sample (1) (CPT) gene Reverse primer

Calcium binding protein (CPT) gene alignment and mutations observation for sample (1)

Table (2-32) was shown the most common types of mutations in the calcium binding protein gene (CPT) forward and reverse gene, sequence in this study.

TABLE (2-21): Type mutation of in the calcium binding protein gene (CPT) gene sequence

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
1F	Transion	247	GGT > GGG	Glycine > Glycine	Silent Mutation	gene Cal
	Transversion	363	GGG > GGC	Glycine > Glycine	Silent Mutation	
	Transversion	377	GTA>GTC	Valine>Valine	Silent Mutation	
	Addition	380	CC- >CCC	> No functional protein > Proline	The protein made by the gene may not function properly	
1R	Addition	288	CC- >CCC	> No functional protein > Proline	The protein made by the gene may not function properly	

(CPT) Forward primer for sample (2)

Calprotectin Forward Primer Alignment and Identity for sample (2)

The Sequence
ID: NM_002964.5Length: 408Number of Matches: 1
GenBank Graphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
315 bits(170)	4e-81	184/191(96%)	0/191(0%)	Plus/Plus

```
Query 181
GAAAAGGGGTGCAGACGTCTGGTTCAAAGAGTTGGATATCAACACTGATGGGGCAG
TTAA 240
      ||||| |||||||||||||||||||||||||||||||||||||||||||| |||||||
Sbjct 196
GAAAAAGGGTGCAGACGTCTGGTTCAAAGAGTTGGATATCAACACTGATGGTGCAG
TTAA 255
Query 241
CTTCCAGGAGTTCCTCATTCTGGTGATAAAGAGGGGCGTGGCAGCCCACaaaaaaaGCC
A 300
      |||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct 256
CTTCCAGGAGTTCCTCATTCTGGTGATAAAGATGGGCGTGGCAGCCCACAAAAAAA
GCCA 315
Query 301
TGAAGAAAGCCACAAAGAGAAGCTGAGTTACTGGGCCCAGAGGCTGGCCCCTTGGA
CATG 360
      |||||||||||||||||| |||||||||||||||||||||| ||| |||||||
Sbjct 316
TGAAGAAAGCCACAAAGAGTAGCTGAGTTACTGGGCCCAGAGGCTGGGCCCCTGGA
CATG 375
Query 361 TCCCTGCAGAA 371
      | |||||||||
Sbjct 376 TACCTGCAGAA 386
```

FIGURE : Alignment statistics for Sample (2) calcium binding protein gene (Cal) Forward primer

(CPT) Revers primer for sample (2)

The Sequence ID: NM_002964.5Length: 408Number of Matches: 2
GenBank Graphics.

Alignment statistics for match #1

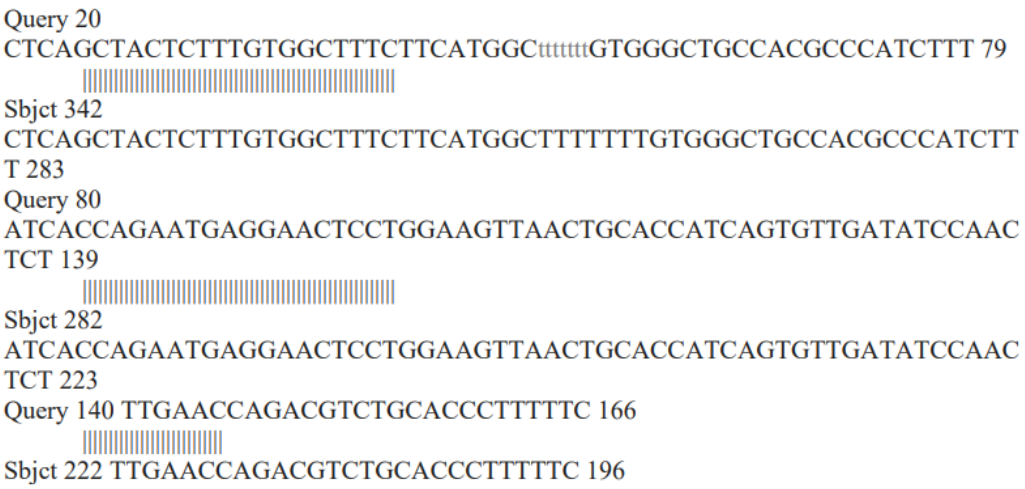

FIGURE (2-44): Alignment statistics for Sample (2) calcium binding protein gene (Cal) Reverse primer

Calcium binding protein gene (Cal) alignment and mutations observation for sample (2)

Table (2-23) was shown the most common types of mutations in the calcium binding protein gene forward and reverse gene, sequence in this study.

TABLE (2-23): Type mutation of in the calcium binding protein gene sequence

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
2F	Transition	201	AAA>AAG	> lysine lysine	Silent Mutation	Gene (Cal)
	Transversion	248	GGT>GGG	Glysine > Glysine	Silent Mutation	
	Transversion	288	GAT>GAG	Aspartic acid > Glutamic acid	The protein made by the gene may not function properly	
	Transversion	335	AGT>AGA	Serine > Arginine	The protein made by the gene may not function properly	
	Transversion	367	GGG>GGC	Glysine >Glysine	Silent Mutation	
	Transition	363	CCC>CCT	Proline> Proline	Silent Mutation	
2R	Transversion	377	GTA>GTC	Valine > Valine	Silent Mutation	

S-TREM1 Forward primer sequence for sample (1)

DNA sequence analysis of (TREM1) gene

Three samples have sequenced through PCR-sequences by Macrogen Company/ Korea. Nucleotides substitutions have determined by comparing the data obtained from gene bank publish which is available at NCBI (https://www.ncbi.nlm.nih.gov) and the results were registered in NCBI under accession

numbers (LC731320) which is available on this link (https://www.ncbi.nlm.nih.gov/nuccore/LC731320.1/).

TREM1 Forward Primer Alignment and Identity for sample (1)

The Sequence ID: NG_029525.2 Length: 26335 Number of Matches: 1

Range 1: 8844 to 9190 Gen Bank Graphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
616 bits(333)	9e-172	344/349(99%)	2/349(0%)	Plus/Minus

```
Query 4
GGTGAGTCGTCTAGTCCGATCCTCCCCACTTGGACTGGATGGGAATTCTTTGAAGGC
CTC 63
      ||| |||| ||||||  ||||||||||||||||||||||||||||||||||||||
Sbjct 9190 GGT-
AGTCTTCTAGTATGATCCTCCCCACTTGGACTGGATGGGAATTCTTTGAAGGCCTC
9132
Query 64
TCTGTGCATGCCAGGGTCTTGGGCATCTCTCCGTCCCTTATTATCTGCCAAGCTTTCT
GG 123
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9131
TCTGTGCATGCCAGGGTCTTGGGCATCTCTCCGTCCCTTATTATCTGCCAAGCTTTCT
GG 9072

Query 124
CTGCTGGCAAACTTCTCTAGCGTGTAGTCACATTTCACATCCAGGGTCTGCCCCTCTT
TC 183
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9071
CTGCTGGCAAACTTCTCTAGCGTGTAGTCACATTTCACATCCAGGGTCTGCCCCTCTT
TC 9012
Query 184
AGTTCATACTTTTCCTCAGTTAATTTAGTTGCAGCTCGGAGTTCTATAAGCAGGGAA
AGA 243
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9011
AGTTCATACTTTTCCTCAGTTAATTTAGTTGCAGCTCGGAGTTCTATAAGCAGGGAA
AGA 8952
Query 244
GAATGGGTTCTGTGAGGAATTATTTTTCTCTCTCTGAGTGGGGAAAAAGGAGAGGAG
CCC 303
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 8951
GAATGGGTTCTGTGAGGAATTATTTTTCTCTCTCTGAGTGGGGAAAAAGGAGAGGAG
CCC 8892
Query 304   CCTGTTTTTCTTGTCCAACCACCCATATTTCAGATGAGGTTCTTGGAGG
352
      ||||||||||||||||||||||||||||||||||||| |||
Sbjct 8891 CCTGTTTTTCTTGTCCAACCACCCATATTTCAGATGAGGTTCTTG-AGG
8844
```

FIGURE : Alignment statistics for Sample (1) triggering receptor expressed on myeloid cells 1 (TREM1) forward primer

TREM1 Revers primer sequence for sample (1) The Sequence ID: NG_029525.2Length: 26335Number of Matches: 1

Range 1: 8887 to 9214 GenBankGraphics >NG_029525.2:c9214-8887 Homo sapiens triggering receptor expressed on myeloid cells 1 (TREM1), Ref SeqGene on chromosome 6

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
599 bits(324)	9e-167	327/328(99%)	1/328(0%)	Plus/Plus

```
Query 21
ACAGGGGGCTCCTCTCCTTTTTCCCCACTCAGAGAGAGAAAAATAATTCCTCACAGA
ACC  80
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 8887
ACAGGGGGCTCCTCTCCTTTTTCCCCACTCAGAGAGAGAAAAATAATTCCTCACAGA
ACC  8946

Query 81
CATTCTCTTTCCCTGCTTATAGAACTCCGAGCTGCAACTAAATTAACTGAGGAAAAG
TAT  140
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 8947
CATTCTCTTTCCCTGCTTATAGAACTCCGAGCTGCAACTAAATTAACTGAGGAAAAG
TAT  9006
Query 141
GAACTGAAAGAGGGGCAGACCCTGGATGTGAAATGTGACTACACGCTAGAGAAGTT
TGCC  200
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9007
GAACTGAAAGAGGGGCAGACCCTGGATGTGAAATGTGACTACACGCTAGAGAAGTT
TGCC  9066
Query 201
AGCAGCCAGAAAGCTTGGCAGATAATAAGGGACGGAGAGATGCCCAAGACCCTGG
CATGC  260
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9067
AGCAGCCAGAAAGCTTGGCAGATAATAAGGGACGGAGAGATGCCCAAGACCCTGG
CATGC  9126
Query 261
ACAGAGAGGCCTTCAAAGAATTCCCATCCAGTCCAAGTGGGGAGGATCATACTAGA
AGAC  320
        ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9127
ACAGAGAGGCCTTCAAAGAATTCCCATCCAGTCCAAGTGGGGAGGATCATACTAGA
AGAC  9186
Query 321  TACCATGATCATGGTTTACTGCG-GTCC  347
        ||||||||||||||||||| ||||
Sbjct 9187 TACCATGATCATGGTTTACTGCGCGTCC  9214
```

FIGURE : Alignment statistics for Sample (1) triggering receptor expressed on myeloid cells 1 (TREM1) Reverse primer.

TREM1 alignment and mutations observation for sample (1)

Table (2-24) was shown the most common types of mutations in the Table (2-24): Type mutation in the TREM1 gene sequence.

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
1F	Addition	9187	GT- > GTG	No functional protein>Valine	The protein made by the gene may not function properly	gene (TREM)
	Transversion	9182	TCT > TCG	Serine > Serine	Silent Mutation	
	Transversion	9175	GTA > GTC	Valine > Valine	Silent Mutation	
	Transition	9174	TAT > TCC	Tyrosine > Serine	The protein made by the gene may not function properly	
	Addition	8847	TG->TGG	No functional protein > Tryptophan	The protein made by the gene may not function properly	
1R	Deletion	9210	CGC > CG-	Arginine > No functional protein	No amino acid created	

S- TREM1 Forward primer for sample (2)

The Sequence ID: NG_029525.2Length: 26335Number of Matches: 1
Range 1: 8839 to 9181GenBankGraphics

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
623 bits (337)	6e-174	342/344(99%)	1/344(0%)	Plus/Minus

```
Query 17
CTAGCTGTGATCCTCCCCACTTGGACTGGATGGGAATTCTTTGAAGGCCTCTCTGTGC
AT 76
      |||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9181 CTAG-
TATGATCCTCCCCACTTGGACTGGATGGGAATTCTTTGAAGGCCTCTCTGTGCAT
9123
Query 77
GCCAGGGTCTTGGGCATCTCTCCGTCCCTTATTATCTGCCAAGCTTTCTGGCTGCTGG
CA 136
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9122
GCCAGGGTCTTGGGCATCTCTCCGTCCCTTATTATCTGCCAAGCTTTCTGGCTGCTGG
CA 9063
Query 137
AACTTCTCTAGCGTGTAGTCACATTTCACATCCAGGGTCTGCCCCTCTTTCAGTTCAT
AC 196
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9062
AACTTCTCTAGCGTGTAGTCACATTTCACATCCAGGGTCTGCCCCTCTTTCAGTTCAT
AC 9003
Query 197
TTTTCCTCAGTTAATTTAGTTGCAGCTCGGAGTTCTATAAGCAGGGAAAGAGAATGG
GTT 256
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9002
TTTTCCTCAGTTAATTTAGTTGCAGCTCGGAGTTCTATAAGCAGGGAAAGAGAATGG
GTT 8943
Query 257
CTGTGAGGAATTATTTTTCTCTCTCTGAGTGGGGAAAAAGGAGAGGAGCCCCCTGTT
TTT 316
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Sbjct 8942
CTGTGAGGAATTATTTTTCTCTCTCTGAGTGGGGAAAAAGGAGAGGAGCCCCCTGTT
TTT 8883
Query 317 CTTGTCCAACCACCCATATTTCAGATGAGGTTCTTGAGGCCTCC 360
||||||||||||||||||||||||||||||||||||||||
Sbjct 8882 CTTGTCCAACCACCCATATTTCAGATGAGGTTCTTGAGGCCTCC 8839

FIGURE: Alignment statistics for Sample (2) triggering receptor expressed on myeloid cells 1(TREM1) Forward primer

(S- TREM1) Reverse primer for sample (2)
The Sequence ID: NG_029525.2Length: 26335Number of Matches: 1
Range 1: 8888 to 9219GenBankGraphics

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
614 bits(332)	4e-171	332/332(100%)	0/332(0%)	Plus/Plus

Query 25
CAGGGGGCTCCTCTCCTTTTTCCCCACTCAGAGAGAGAAAAATAATTCCTCACAGAA
CCC 84
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 8888
CAGGGGGCTCCTCTCCTTTTTCCCCACTCAGAGAGAGAAAAATAATTCCTCACAGAA
CCC 8947
Query 85
ATTCTCTTTCCCTGCTTATAGAACTCCGAGCTGCAACTAAATTAACTGAGGAAAAGT
ATG 144
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 8948
ATTCTCTTTCCCTGCTTATAGAACTCCGAGCTGCAACTAAATTAACTGAGGAAAAGT
ATG 9007
Query 145
AACTGAAAGAGGGGCAGACCCTGGATGTGAAATGTGACTACACGCTAGAGAAGTTT
GCCA 204
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9008
AACTGAAAGAGGGGCAGACCCTGGATGTGAAATGTGACTACACGCTAGAGAAGTTT
GCCA 9067
Query 205
GCAGCCAGAAAGCTTGGCAGATAATAAGGGACGGAGAGATGCCCAAGACCCTGGC
ATGCA 264
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9068
GCAGCCAGAAAGCTTGGCAGATAATAAGGGACGGAGAGATGCCCAAGACCCTGGC
ATGCA 9127

Query 265
CAGAGAGGCCTTCAAAGAATTCCCATCCAGTCCAAGTGGGGAGGATCATACTAGAA
GACT 324
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 9128
CAGAGAGGCCTTCAAAGAATTCCCATCCAGTCCAAGTGGGGAGGATCATACTAGAA
GACT 9187

Query 325 ACCATGATCATGGTTTACTGCGCGTCCGAATG 356
||||||||||||||||||||||||||||||
Sbjct 9188 ACCATGATCATGGTTTACTGCGCGTCCGAATG 9219

FIGURE : Alignment statistics for Sample (2) triggering receptor expressed on myeloid cells 1 (TREM1) Reverse primer

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TREM1alignment and mutations observation for sample (2)

Table (3-26) was shown the most common types of mutations in the Forward and Reverse TREM1 gene, sequence in this study.

TABLE (2-26): Type mutation of in the TREM1 gene sequence

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
2F	Addition	9177	AG->AGC	No functional protein > Serine	The protein made by the gene may not function properly	gene (TREM)
	Addition and transition	9175	-TA> CTG	No functional protein > Lucien	The protein made by the gene may not function properly	

DNA sequence analysis of (Pterin) gene

Three samples have sequenced through PCR-sequences by Macrogen Company/ Korea. Nucleotides substitutions have determined by comparing the data obtained from gene bank publish which is available at NCBI (https://www.ncbi.nlm.nih.gov) and the results were registered in NCBI under accession numbers (LC731321) which is available on this link (https://www.ncbi.nlm.nih.gov/nuccore/LC731321.1/).

Neopterin Forward primer sequence for sample (1)

Sequence ID: NM_001289797.2Length: 781Number of Matches: 1Range 1: 378 to 544GenBankGraphics

Alignment statistics for match #1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
302 bits(163)	1e-77	166/167(99%)	1/167(0%)	Plus/Plus

Query 22 GGGTG-
CTGAACTGGGAGTCCAGGGAGGGAGCTGAGGAGCCCTTACCCTCCCACCACTCC 80
||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 378
GGGTGACTGAACTGGGAGTCCAGGGAGGGAGCTGAGGAGCCCTTACCCTCCCACCA
CTCC 437

Query 81
CCTCCCAAGACCCAGCCGCCGCCGTTGAGGGCTGAGTCCTTGCTGTGGGATGTGCCA
GTG 140
||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 438
CCTCCCAAGACCCAGCCGCCGCCGTTGAGGGCTGAGTCCTTGCTGTGGGATGTGCCA
GTG 497
Query 141 TCCCCACCAACACCAGGAATTTAGACCTTTTCCCTGCACCACTCTCT 187
||||||||||||||||||||||||||||||||||||||||||||
Sbjct 498 TCCCCACCAACACCAGGAATTTAGACCTTTTCCCTGCACCACTCTCT 544

FIGURE : Alignment statistics for Sample (1) Neopterin gene Forward primer

Neopterin Revers primer for sample (1)

The Sequence ID: AF082858.1Length: 829Number of Matches: 1

Range 1: 377 to 544 GenBankGraphics

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
294 bits(159)	2e-75	166/169(98%)	2/169(1%)	Plus/Minus

FIGURE : Alignment statistics for Sample (1) Neopterin gene Reverse primer

Neopterin alignment and mutations observation for sample (1)

Table (2-21) was shown the most common types of mutations in the Neopterin forward and reverse gene, sequence in this study.

TABLE (21): Type mutation of in the Neopterin gene sequence

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
1F	Deletion	383	TGA> TG-	Stop codon > No functional protein	No amino acid created	Pterin gene
1R	Deletion	540	TG-> TGT	No functional protein > Cysteine	The protein made by the gene may not function properly	
	Transition	478	GGT>GGG	Glycine > Glycine	Silent mutation	
	Deletion	402	GAA>GA-	Glutamic acid > No functional protein	No amino acid created	

Neopterin Forward primer for sample (2)

Neopterin Forward Primer Alignment and Identity for sample (2)

Sequence ID: NM_001289797.2Length: 781Number of Matches: 1

Range 1: 383 to 544GenBankGraphics

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
292 bits (158)	9e-75	161/162(99%)	1/162(0%)	Plus/Plus

```
Query 31   ACTG-
ACTGGGAGTCCAGGGAGGGAGCTGAGGAGCCCTTACCCTCCCACCACTCCCCTCC 89
           |||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 383
ACTGAACTGGGAGTCCAGGGAGGGAGCTGAGGAGCCCTTACCCTCCCACCACTCCC
CTCC 442

Query 90
CAAGACCCAGCCGCCGCCGTTGAGGGCTGAGTCCTTGCTGTGGGATGTGCCAGTGTC
CCC 149
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 443
CAAGACCCAGCCGCCGCCGTTGAGGGCTGAGTCCTTGCTGTGGGATGTGCCAGTGTC
CCC 502

Query 150 ACCAACACCAGGAATTTAGACCTTTTCCCTGCACCACTCTCT 191
           |||||||||||||||||||||||||||||||||||
Sbjct 503 ACCAACACCAGGAATTTAGACCTTTTCCCTGCACCACTCTCT 544
```

FIGURE : Alignment statistics for Sample (2) Neopterin gene Forward primer

Neopterin Revers primer for sample (2)

The Sequence ID: NM_001289797.2Length: 781Number of Matches: 1

Range 1: 330 to 496GenBankGraphics

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
292 bits(158)	8e-75	165/168(98%)	2/168(1%)	Plus/Minus

```
Query 20   ACTGAGC-
CATCCCACAGCAAGGACTCAGCCCTCAACGGCGGCGGCTGGGTCTTGGGAGG 78
           |||| || ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 496 ACTG-
GCACATCCCACAGCAAGGACTCAGCCCTCAACGGCGGCGGCTGGGTCTTGGGAGG
438
Query 79
GGAGTGGTGGGAGGGTAAGGGCTCCTCAGCTCCCTCCCTGGACTCCCAGTTCAGTCA
CCC 138
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 437
GGAGTGGTGGGAGGGTAAGGGCTCCTCAGCTCCCTCCCTGGACTCCCAGTTCAGTCA
CCC 378
Query 139 TTTCCCCCGGAAGAATTCAAAGAGGAAGGGCAGGGTCTATGTCATGGA
186
           ||||||||||||||||||||||||||||||||||||||||
Sbjct 377 CTTCCCCCGGAAGAATTCAAAGAGGAAGGGCAGGGTCTATGTCATGGA 330
```

FIGURE : Alignment statistics for Sample (2) Neopterin gene Reverse primer

Neopterin alignment and mutations observation for sample (2)

Table (2-22) was shown the most common types of mutations in the Neopterin forward and reverse gene, sequence in this study.

TABLE (2-22): Type mutation of in the Neopterin gene sequence

No of sample	Type of substitution	Location	Nucleotide change	Amino acid change	Predicted effect	Source
2F	Deletion	387	TGA>TG-	Stop codon > No functional protein	No amino acid created	Pterin gene
2R	Addition	492	TG->TGA	No functional protein > Cysteine	The protein made by the gene may not function properly	
	Deletion	489	GCA>GC-	Alanine > No functional protein	No amino acid created	
	Transition	377	CCC>CCT	Proline>Proline	Silent mutation	

Phylogenic tree of based in the (PCT, CaL, TREM and Pterin)

A phylogenic tree of based in the (PCT, CaL, TREM and Pterin) genes Molecular phylogenetic is the branch of phylogeny that analyses hereditary molecular differences, mainly in DNA sequences, to gain information on an organism's evolutionary relationships. However, phylogeny estimated from a single gene should be treated with caution. The phylogenetic tree derived from (PCT, CaL, TREM and Pterin) genes respectively sequences 2 samples with different sequences available at NCBI. As mentioned in Figure (2-80), (2-81), (2-82) and Figure (2-83) respectively.

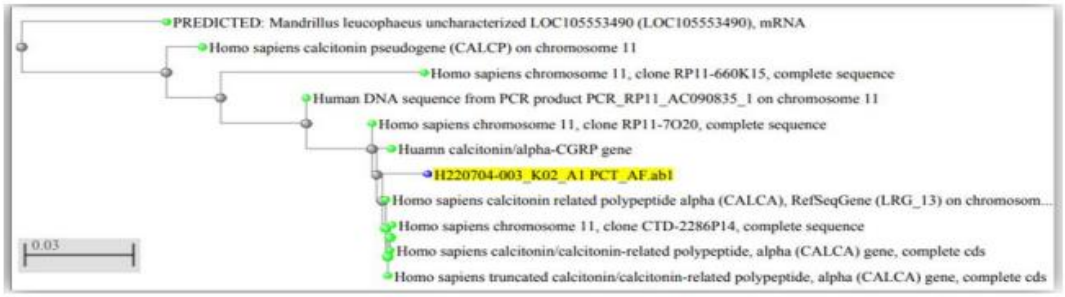

FIG (2-80): Phylogenetic tree of (PCT) gene sequence analysis.

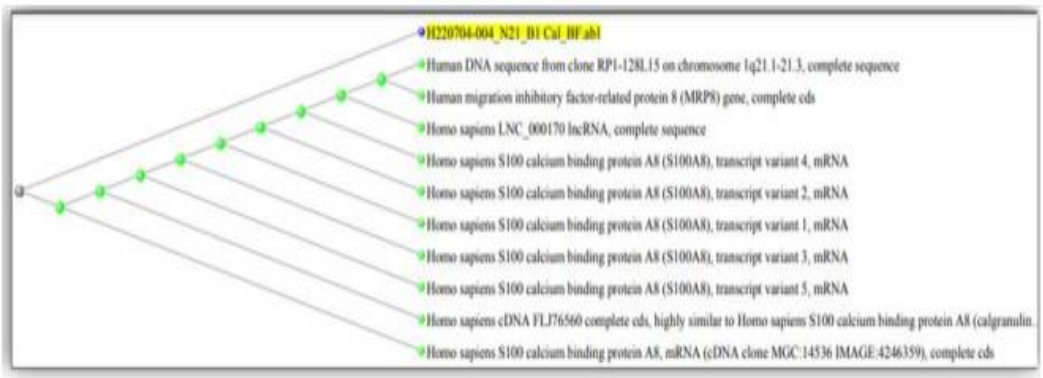

FIG (2-81): Phylogenetic tree based on Cal gene sequence analysis.

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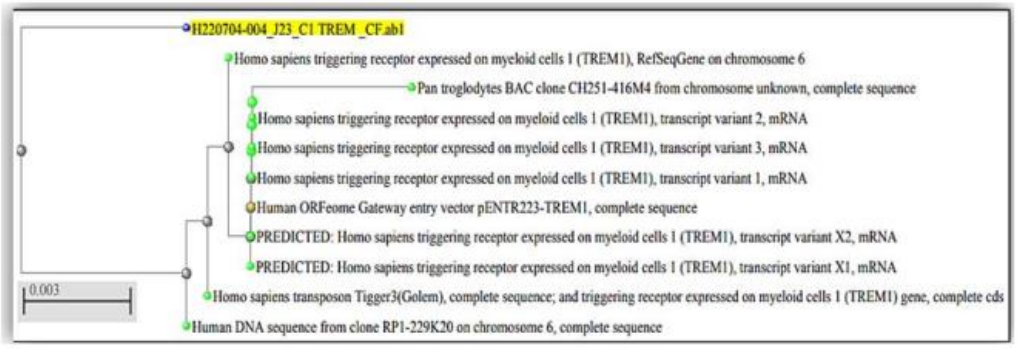

FIG (2-82): Phylogenetic tree based on TREM gene sequence analysis.

FIG (2-83): Phylogenetic tree based on Petrin gene sequence analysis.

3D Protein Structure

3D Protein Structure for PCT

Sequence ID: CAA26189.1Length: 93Number of Matches: 1

Alignment statistics for match #1						
Score	**Expect**	**Metho**	**Identities**	**Positives**	**Gaps**	**Frame**
104 bits (259)	5e-23	Compositional matrix adjust.	47/47(100%)	47/47(100%)	0/47(0%)	-1

Query 181 SLDSPRSKRCGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAPGKKRDM 41
Sbjct 28 74

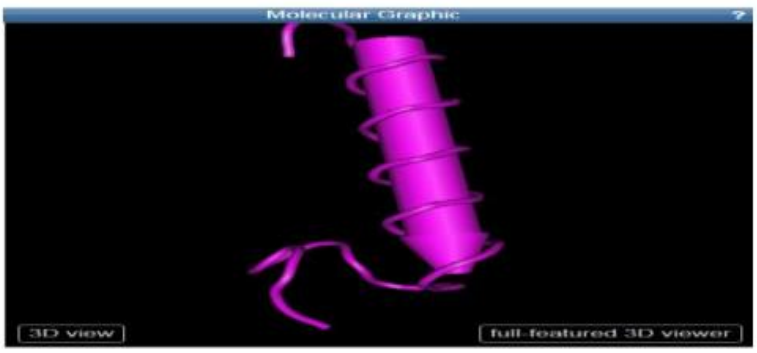

FIGURE (2-56): Crystal structure of PCT complete

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3D Protein Structure for calprotectin (S100A8/S100A9) [Homo sapiens]
Sequence ID: 1XK4_ALength: 93NumberMatches: 1

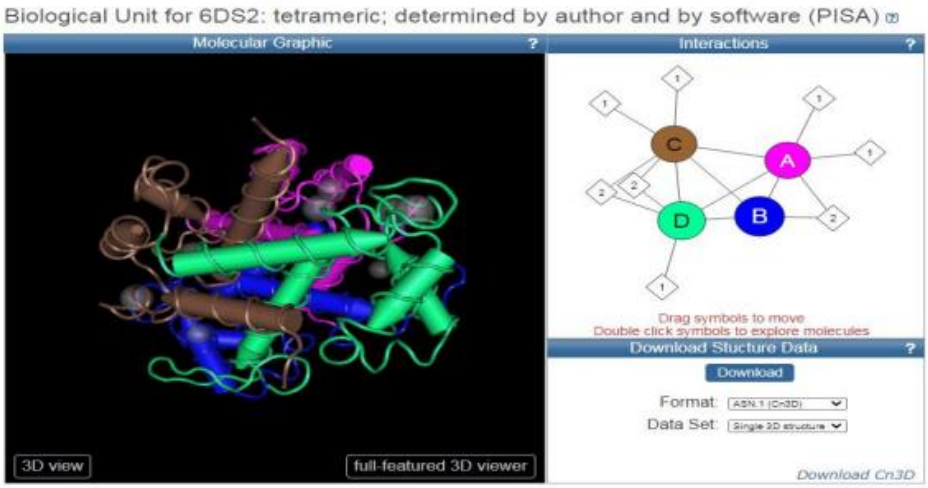
Alignment statistics for match #1						
Score	**Expect**	**Method**	**Identities**	**Positives**	**Gaps**	**Frame**
79.7 bits(195)	2e-16	Compositional matrix adjust.	37/41(90%)	38/41(92%)	0/41(0%)	+3

Query 165 PPQS*QKKGADVWFKELDINTDGAVNFQEFLILVIKMGVAA 287
Sbjct 42 S. YIR..... 8

TABLE: showing Wild Type and Amino acid Variation after DNA molecule have been exposure to SNP mutations at different locations.

Sample No.	**Wild Type (Subject)**	**Amino acid Variation Query)(**
1	S (Serine)	P (Proline)
	Y (Tyrosine)	S (Serine)
	I Isoleucine)	GAB
	R (Arginine)	Q (Glutamine)

3D Protein Structure for CPT



https://www.ncbi.nlm.nih.gov/Structure/pdb/6DS2

FIGURE (2-58): showing the Crystal structure of Ni (II)-bound human calprotectin 3D Protein Structure Triggering receptor expressed on myeloid cells 1 (TREM1).

The Sequence ID: 1Q8M_ALength: 127Number of Matches: 1

Alignment statistics for match #1

Alignment statistics for match #1						
Score	**Expect**	**Method**	**Identities**	**Positives**	**Gaps**	**Frame**
123 bits (308)	3e-33	Compositional matrixes adjust.	71/74(96%)	73/74(98%)	0/74(0%)	-1

Query 230
IELRAAtklteekyelkEGQTLDVKCDYTLEKFASSQKAWQIIRDGEMPKTLACTERPSK 51
Subject 1 M..... 60
Query 50 NSHPVQVGRIGLDD 9
Sbjct 61 .....I.E. 74

FIGURE (2-90): Alignment statistics for TREM protein

TABLE: (2-26) showing Wild Type and Amino Acid Variation after DNA molecule have been exposure to SNP mutations at different locations

Sample No.	Wild Type (Subject)	Amino acid Variation (Query)
1	M (Methionine)	I (Isoleucine)
	I (Isoleucine)	G (Glycine)
	E (Isoleucine)	D (Aspartic acid)

https://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?Dopt=s&uid=25725

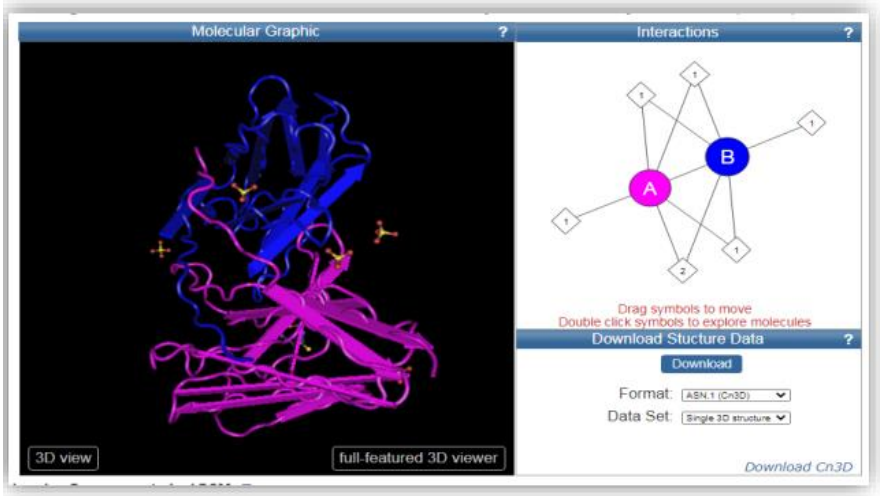


FIGURE (2-91): Crystal structure of the human myeloid cell activating receptor TREM-1 [Homo sapiens]

3D Protein Structure for Neopterin

Sequence ID: L41559.1Length: 626Number of Matches: 1

Score	**Expect**	**Method**	**Identities**	**Positives**	**Gaps**	**Frame**
218 bits (554)	1e-68	Compositional matrixes adjust.	103/104(99%)	104/104(100%)	0/104(0%)	+3

Query 52
MAGKAHRLSAEERDQLLPNLRAVGWSELEGRDAIFKQFHFKDFNRAFGFMTRVALQAE
KL 111
Sbjct 21 .....N..... 200
Query 112 DHHPEWFNVYNKVHITLSTHECAGLSERDINLASFIEQVAVSMT 155
Sbjct 201 332

FIGURE (2-91): Alignment statistics for pterin protein

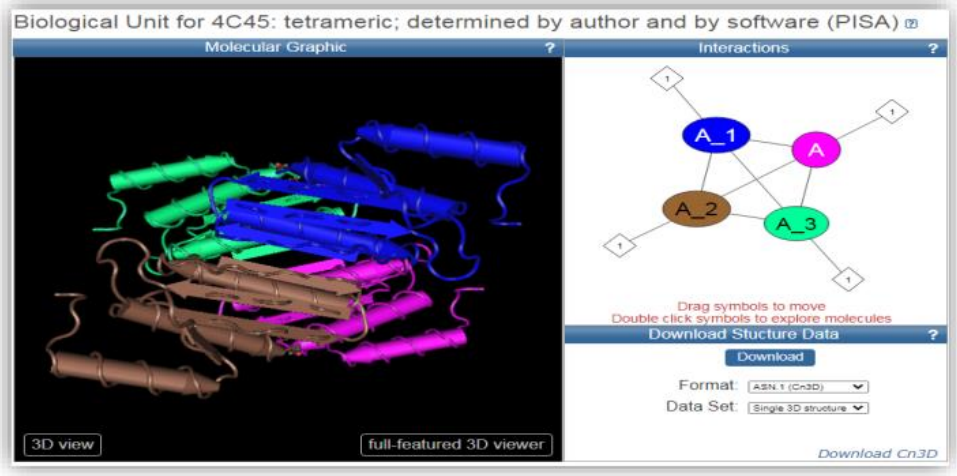


FIGURE (2-94): Crystal structure of the Neopterin

https://www.ncbi.nlm.nih.gov/Structure/pdb/4C45

TABLE: (2-19) showing Wild Type and Amino Acid Variation after DNA molecule have been exposure to SNP mutations at different locations

Sample No.	Wild Type (Subject)	Amino acid Variation (Query)
2	N (Asparagine)	S (Serine)

DISCUSSION

DNA sequencing

The present study sequencing of PCT, CPT, S-TREM1, and Neopetrin were taken from three samples. There was a convergence between study sample one PCT isolated and that of the Gen Bank database (NCBI) with identity 91/96 (95%) in forward PCT. The reverse PCT showed an identity 165/169 (98%) when compared with the NCBI database. The reverse PCT showed an identity 165/169 (98%) when compared with the Gen Bank database (NCBI) that found one Addition mutation was (GC-to GCA), Transition mutations were (TGG to TGA), and two trans version mutations appeared as (AGG to AGC T GG to TGC). There was a convergence between study sample three PCT (control) isolated and that of the GenBank database with identity 164/170 (96%) in the Reverse PCT [9-11]. There was a convergence between study sample one (Cal) isolated and that of the GenBank database (NCBI) with identity 186/190 (98%) in forward Cal that had been recorded four mutations (two trans versions) appeared in (GGG to GGC), (GTA to GTC), (one transition), and (one addition) appeared. On the other side, the reverse Cal has shown an identity 144/145 (99%) when compared with the Gen Bank database - one addition mutation was reported as (CC-to CCC). There was a convergence between study sample

three Cal (Control) isolated and that of the GenBank database (NCBI) with identity 184/191 (96%) in forward Cal that had been recorded. On another side, reverse Cal showed an identity 147/147 (100%) when compared with the Gen Bank database - one transversion mutation appeared in (GTA to GTC). There was a convergence between study sample one s-trem1 isolated and that of the Gen Bank database (NCBI) with identity 344/349 (99%) in forward s-Trem1 that had been recorded two trans version mutations appeared in (TCT to TCG) and (GTA to GTC) and two addition mutations appeared as (GT-to GTG) and (TG-to TGG). The reverse s-trem1 showed an identity 333/335 (99%) when compared with the Gen Bank database (NCBI) one deletion mutation appeared in (CGC to CG-) and one addition mutation appeared as (CC- to CCC), And there was a convergence between study sample. No previous studies about the same this mutation in biomarkers. There was a convergence between the study sample one Neopetrin isolated and that of the Gen Bank database (NCBI) with identity 166/167 (99%) in each case. For example, there was an identity166/169 (98%) when compared with NCBI that found one transition mutations was (GGT to GGG) and two deletion mutations appeared as (TG- to TGT and GA to GA-) respectively. There was a convergence between study sample two Neopetrin isolated and that of the Gene Bank database (NCBI) with identity161/184 (88%) in reverse NeopETrin had six mutations one deletion mutations appeared as (GGG to GG-), one transversion mutation appeared as (AGG to AGC), and four transition mutations. On the other side, there was an identity 165/168 (98%) when compared with Gen Bank database which observed one deletion mutation appearing as (TGA to TG-), and one Transition mutation appeared as (CCC to CCT) [12-14].

Studies explains that due to genetic diversity, PCT concentrations can differ in more than 10% of the population. PCT levels in people with this condition may be two- to threefold higher in those who have the minor genetic variation. It is fair to infer that genetic variants in the examined CALCA SNPs will affect PCT concentrations in these individuals in cases of sepsis and upper and lower respiratory tract infections [15].

Some studies showed that the combined effect of CAL gene polymorphisms and gender may be linked to periodontitis susceptibility in Chinese people [16]. Due to the important role of calprotectin and the lack of knowledge about calprotectin, it is necessary to study further whether any polymorphisms result in differences in protein expression levels [17] showed that it is possible that calprotectin, both at the gene and serum levels, contributes to disease etiology. Also, showed how a genetic variation in TREM-1 affects a person's susceptibility to pneumonia [18].

S-TREM-1 genetic polymorphisms may be significantly correlated only with susceptibility to septic shock in the Chinese Han population [19]. It is noticed that from our results, the gene mutations and polymorphism in all markers are more in sample one and sample two (cases) than in sample three (control). [20] showed that gene mutations play an important role in the susceptibility of patients to pneumonia and sepsis. Also discussed the effect of genetic polymorphisms and their positive impact on calcitonin and CRP levels in patients with infection [21,22].

No previous studies interested with Neopetrin gene polymorphism, so we cannot discuss the present study.

In 3D Protein Structure the present study sequencing of PCT, CPT, S-TREM1, and Neopetrin showed: there is a high convergence between the study PCT isolated and that of the Gen Bank database (NCBI) with identity 47/47(100%) PCT. There is a convergence between the study CPT isolated and that of the Gen Bank database (NCBI) with identity 37/41(90%) in CPT had Four mutations appeared as (S to P), (Y to S), (I to GAB) and (R to Q). There a high convergence between the study S-TREM1 isolated and that of the Gen Bank database (NCBI) with identity71/74 (96%) S-TREM1 had three mutations appeared as (M to I), (I to G) and (E to D) and there a high convergence between study Neopetrin isolated and that of the Gen Bank database (NCBI) with

identity104/104 (100%) Neopetrin had a one mutations appeared as (N to S) No previous studies interested studied in 3D protein with biomarkers so we cannot discuss the present study.

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