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In Silico Analysis and Molecular Modelling of Neutrophil cytosol factor 4/p40^{phox} involved in Chronic Granulomatous Disease

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ABSTRACT

Chronic Granulomatous disease is a primary immunodeficiency disease. This disease first described in the year 1950s.Immune deficiencies are the condition which the body's immune cells are unable to kill any foreign body. In this disease the collection of granulomas/small nodules occurs granulomas (collection of immune cells) i.e. phagocytes when they cluster these phagocytes are unable to kill any pathogen.CGD patients, with a phagocyte NADPH oxidase that is not properly functioning, suffer from recurrent, life-threatening infections with certain bacteria, fungi, and yeasts. Defects occurs in one of the five subunits of NADPH Oxidase. It is caused by mutations in genes encoding protein subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. CGD is characterized by a defective intracellular killing of phagocytosed organisms due to a defective oxidative burst in the neutrophils and macrophages. Includes following proteins gp91^{phox}(CYBB gene),p22^{phox} (CYBA gene), p47^{phox} (NCF1), p67^{phox} (NCF2), p40^{phox}(NCF4). So now in present studies we have used different *Insilico*tools and techniques which includes retrieval of Neutrophil cytosol factor 4 sequence from UniProt database and physiochemical parameters analysed by using Protparam tool. In that leucine had maximum amino acid composition. The structure of protein has very important role in its function.The secondary structure was predicted by using SOPMA tool which indicated that the percentage of Random coils was higher than the percentage of alpha helix and extended strand. Then the 3D structure of Neutrophil cytosol factor 4 was predicted by using SWISS MODEL server and the model was validated by using PROCHECK analysis after validation of the model, the validation score was 93.6%.

Keywords : Chronic granulomatous disease; NADPH Oxidase; gp91^{phox}; p22^{phox}; p47^{phox}'p67^{phox}; p40^{phox}

I. INTRODUCTION

Chronic Granulomatous Disease is generally characterized by infectionsof skin, the airways, lymph nodes, liver, brain and bone.A subgroup of bacteria that produce catalase are the main cause of infections. The most commonly found pathogens includes *Staphylococcus aureus, Aspergillus species, Salmonella species, Klebsiella*.[1]

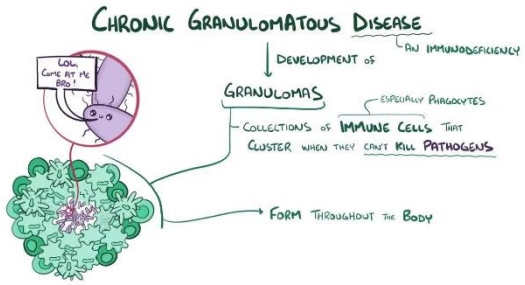


Fig No.1 CGD

Patients with CGD typically first exhibit frequent, severe bacterial andor fungal infections in infancy or childhood. However, delayed diagnosis in maturity as well as female incidence are also possibilities. The CGD only affects around 1/250,000 people worldwide.In almost 90% of individuals with proven CGD, there are significant respiratory burst abnormalities that lead to minimal to no superoxide radical expression. These individuals typically appear as serious or potentially fatal bacterial or fungal illnesses very early in life (typically in infancy).[2]Chronic granulomatous disease arises due to defects in the oxidative bursts. Defects occurs in one of the five subunits of NADPH Oxidase enzyme This enzyme complex plays very important role in catalytic conversion of free radicle superoxide(O2-) and this gives rise to hydrogen peroxide,HOCL and hydroxyl ions this is tough to kill pathogenic bacteria and fungi. So ultimately this gives rise to the Reactive Oxygen Species (ROS).In the normal resting state this enzyme NADPH Oxidase consist of mainly two important components i.e., a membrane bound heterodimer gp91^{phox} and p22^{phox} encoded by CYBB and CYBA genes respectively. This heterodimer (cytochrome b558) is embedded within the lysosomal membrane. And the next group of cytosolic proteins are p47^{phox}, p67^{phox} and p40^{phox} encoded by NCF1, NCF2 and NCF4 respectively. This assembled cytochrome can be stabilized by endoplasmic reticulum membrane by protein 'essential for reactive oxygen species' (EROS), this encoded by a gene.[3]

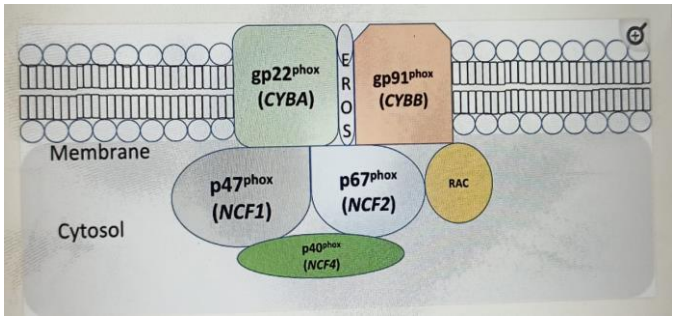


Fig No.2 NADPH Oxidase Complex

CYBC1Patients with CYBC1/Eros lack, a new and uncommon type of CGD, present as loss of respiratory burst and gp91phox articulation in phagocytes. The Neutrophils from patients suffering from CGD are lacking in neutrophil extracellular traps (NETosis), autophagy, and apoptosis. In addition, CGD phagocytes' hyperactivation of NF-B and the inflammasome results in the persistent production of pro-inflammatory cytokines and inflammatory manifestations such as granuloma formation and colitis-like inflammatory bowel disease.[4]

If any defects occur in this gene this may also cause of CGD.Activation of NADPH oxidase in the phagocyte lysosome results in the loss of an electron, which is transferred to molecular oxygen, where it takes on a negative charge and forms superoxide. Hydrogen peroxide, which reacts with superoxide anion to form a highly reactive hydroxyl radical and transforms into hypochlorous acid in the presence of myeloperoxidase and chlorine, can either spontaneously or through superoxide dismutase become superoxide. These reactive oxygen species are produced by phagocytes, allowing potassium and protons to enter the

phagolysosome and activating granule proteases like cathepsin G and elastase. The proteases cause obliteration of phagocytosed microorganisms. Superoxide has both a direct microbicidal effect and the dual function of activating lysosomal molecules to cause death.2.

All these proteinsform an enzyme NADPH Oxidase (NOX) and this generates superoxide ions. This produced superoxide ions as well as its reduced products i.e., Hydrogen peroxide this ultimately gives rise to additional ROS (reactive oxygen species). This occurs in normal healthy person, but in the case of patients suffering from chronic granulomatous disease an enzyme is not properly functioning[5].It was discovered that while neutrophils can ingest bacteria, oxygen deprivation affects their ability to kill them.Memory B cells in patients with CGD have been found to decrease when B cells do not produce enough ROS.When B cells don't make enough ROS, it has been found that patients with CGD have fewer memory B cells.[6]

There are mainly two types of inheritance i.e. X linked and Autosomal recessive CGD.The 50% chance of transmitting a CYBB pathogenic variant in each pregnancy is given to males whose mother is heterozygous for the variant.Heterozygous females are generally not affected by CGD but are significantly at risk for inflammatory conditions. Parents with autosomal recessive CGD-causing pathogenic variants have 25% chance of affected sibling, 50% chance of carrier, and 25% chance of inheriting neither variant.[7]

Patients with X-linked CGD often have a more severe disease course with an earlier presentation age and an earlier death age. Morality rate of patients affected with X Linked CGD is 5% per year and rate of patients suffering from autosomal recessive CGD is 2% per year.Due to lyonization, female X-linked CGD carriers have two populations of phagocytes, and cases of severe skewing of X-chromosome inactivation have been observed in individuals who are susceptible to CGD-like illnesses.[8]

Antifungals and prophylactic antibiotics are typically included in CGD treatment. Immunomodulators, immunosuppressors, or prophylactic recombinant human interferon may be used to prevent infections or inflammatory manifestations.Gene therapy and hematopoietic stem cell transplantation are currently available treatments for CGD.[9].

II. MATERIALS AND METHODS

1.Retreival of Protein sequence

The protein sequence of Neutrophil cytosol factor 4 was retrieved by using UniProt protein database and saved in the FASTA file format. UniProt is the Universal Protein Resource is comprehensive resource for protein sequence and annotation data.The amino acid sequences of proteins can be found in the publicly accessible protein sequence database UniProt[10]

2.Physiochemical Properties Analysis

The physiochemical properties of Neutrophil cytosol factor 4 was analyzed by using Proparam the computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY)[11]

3.Secondary Structure prediction and analyses

The secondary structure was predicted using SOPMA.The self-optimized prediction method (SOPM) has been described as increasing the probability of successful protein secondary structure prediction.In a database of 126 chains of non-homologous (less than 25% identical) proteins, the

SOPM method (SOPMA) accurately predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet, and coil).[12]

4.Homology Modelling and Model Validation

The Neutrophil factor 4 were used for comparative homology modelling by using SWISS MODEL server.An annotated database of fully automated homology-modelling pipeline SWISS-MODEL-generated three-dimensional comparative protein structure models is the SWISS-MODEL Repository. The Storehouse right now contains around 300,000 three-layered models for groupings from the Swiss-Prot and TrEMBL information bases. After modelling, we can check the quality and validation of the model was carried out by PROCHECK Ramchandran plot method using PDBsum server. [13]

III. RESULTS AND DISCUSSION

1.Retrieval of Neutrophil cytosol factor 4 protein

The protein sequence of neutrophil cytosol factor 4 protein was retrieved from UniProt database and stored in the FASTA file in notepad. The protein name, organism name, UniProt KB ID and sequence length is given below in the table no.1

Protein Name	Organism Name	UniProt KB ID	Sequence Length
Neutrophil Cytosol Factor 4	Homo Sapiens	Q15080	339

Table no.1 Retrieval of sequence

2.Physiochemical analysis of protein
The physiochemical properties were analysed by using ProtParam tool and results were enlisted in the given table no.2

Table no.2 Physiochemical parameters of neutrophil cytosol factor 4

Parameters	Values
Total No. of amino acid	339
Molecular Weight	39031.72
Theoretical Pi	6.40
UniProt KB ID	Q15080
The no of positive amino acids (Arg+Lys)	48
The no of negative amino acids (Asp+Glu)	49
Aliphatic index	90.32
GRAVY	-0.364

3.Secondary structure prediction

Table no.2 Physiochemical parameters of neutrophil cytosol factor 4

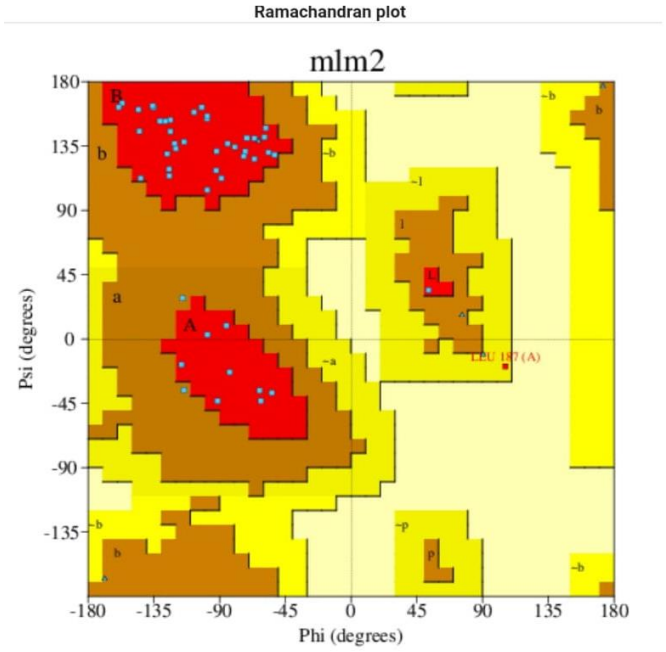
The secondary structure of protein was predicted by using the SOPMA method. The secondary structure elements like alpha helix, beta sheets, extended strands, and random coils were given below in the table no.3.From the given table the percentage of coils of Neutrophil factor 4 is higher than percentage of alpha helix and extended strand.

Secondary Structure elements	Number of Residues	Percentage
Alpha Helix	105	30.97%
Extended Strand	69	20.35%
Beta Turn	23	6.78%
Random Coil	142	41.89%

Table No.3 Secondary Structure Prediction

4. Homology Modelling and Model Validation

The 3D structure of Neutrophil cytosol factor 4was predicted by using SWISS Model server. The sequence template Neutrophil cytosol factor 4 protein were selected as template for prediction of homology modelling. The quality and validation of model was carried out by Ramchandran plot analysis using PDBsum server. Ramchandran plot analysis showed that percentage of favored region is 93.6% which was higher than percentage of additional allowed region it concludes predicted model was reliable and goof quality shown below in the Fig no.3 Further model was visualized in the RasMol visualization software packages given below in fig no 4.



PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	44	93.6%
Additional allowed regions	[a,b,l,p]	2	4.3%
Generously allowed regions	[~a,~b,~l,~p]	1	2.1%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		47	100.0%
End-residues (excl. Gly and Pro)		1	
Glycine residues		5	
Proline residues		2	
Total number of residues		55	

Fig.No.3 Protein Validation by PROCHECK

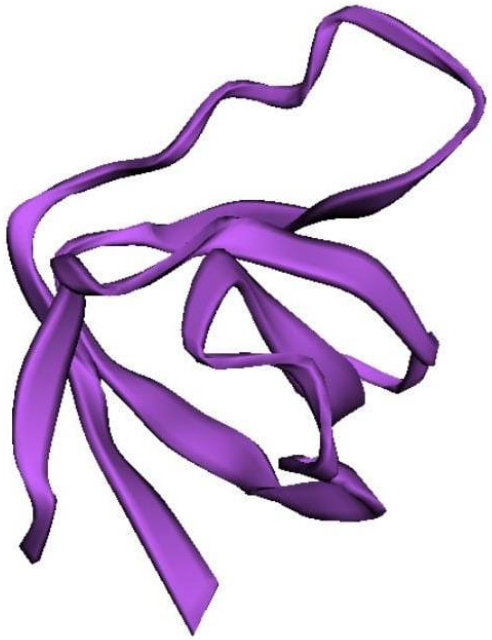


Fig.No.4 Visualization of Predicted 3D structure by RasMol

5.Identification of domain function and analysis

The protein domain and protein family analysis of Neutrophil cytosol factor 4 protein by Pfam database. The domain of neutrophil factor 4 protein and its sequence length was given below in Fig no.4

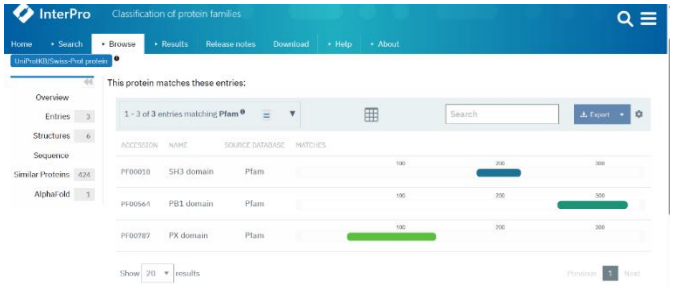


Fig.No.5 Domain Identification with its sequence length

IV. CONCLUSION

The present preliminary investigation mainly leads to understand basic primary, secondary structure and tertiary structure of Neutrophil cytosol factor 4 In-Silico Tools and Technique. The Neutrophil cytosol factor 4 were retrieved from UniProt database and having length of 339.The primary sequence analysis carried out by using Protparam tool and retrieval

having Molecular weight of 39031.72Da,theoretical pI 6.40,the no of positive amino acids (Arg+Lys):48,the no of negative amino acids (Asp+Glu):49.respectiveleyAliphatic index:90.32,and GRAVY:-3.64.Secondary structure of neutrophil cytosol factor 4 was predicted by using SOPMA having alpha helix 69,beta turn 23,extended strand 69,and random coil 142 so protein structure was highly stable. The Modelling of 3D structure of protein neutrophil cytosol factor 4 was build by using SWISS Model 2xf2.Strucutre was validated using PROCHECK having model quality was 93.6%.This present study put molecular insight into the further studies to find structural and functional properties of Neutrophil cytosol factor 4 /P40phox.

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