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FUNCTIONAL AND PHYLOGENETIC ANALYSIS OF NOD2 GENE WHICH IS RESPONSIBLE FOR CROHN'S DISEASE

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ABSTRACT

Crohn's disease is a type of Inflammatory bowel disease (IBD) that may affect any part of the gastrointestinaltract from mouth to anus. The cause of Crohn's disease is believed to be due to a combination of environmental, immune, and bacterial factors in genetically susceptible individuals. It results in a chronic inflammatory disorder, in which the body's immune system attacks the gastrointestinal tract possibly directed at microbial antigens. Nod2 is an intracellular bacterial sensor and its mutations are associated with the development of CD. Nucleotide and Protein sequence for the gene NOD2 is retrieved from National Centre for Biotechnology Information in fasta format. Functional analysis is done by using the AMIGO tool. The gene expression studies were analyzed using insilico tool ACUA. Highly expressed genes is selected on the basis of CAI value. Phylogenetic analysis of target gene is done through using phyML. From the results that the identified conserved motif sequences are the potential protein target sequences for structure based drug designing and molecular drug docking studies. This work would definitely be useful in the field of Clinical Pathology, Computational Entomology and Cheminformatics.

KEYWORDS: Crohn, NOD2. Gene expression, CAI, phylogenetic analysis.

INTRODUCTION

Crohn's disease of the gastrointestinal tract affecting 26–200 per 100,000 in European populations. Along with ulcerative colitis, it is one of the two major forms of inflammatory bowel disease (IBD). The exact causes of Crohn's disease are unknown, though it is likely to involve a disrupted immunological response to gut microbiota in genetically susceptible

individuals. There is currently no known cure and disease is managed by a combination of immune-suppressing medications, dietary changes or surgery.

Bowel obstruction also commonly occurs and those with the disease are at greater risk of bowel cancer. It is less common in Asia and Africa. It has historically been more common in the developed world. Rates have, however, been increasing, particularly in the developing world, since the 1970s. Inflammatory bowel disease resulted in 47,400 deaths in 2015 and those with Crohn's disease have a slightly reduced life expectancy. It tends to start in the teens and twenties, although it can occur at any age. Males and females are equally affected. The disease was named after gastroenterologist Burrill Bernard Crohn, who, in 1932, together with two other colleagues at Mount Sinai Hospital in New York, described a series of patients with inflammation of the terminal ileum of the small intestine, the area most commonly affected by the illness.

About half of the overall risk is related to genetics with more than 70 genes found to be involved. Tobacco smokers are two times more likely to develop Crohn's disease than nonsmokers. It also often begins after gastroenteritis. Diagnosis is based on a number of findings including biopsy and appearance of the bowel wall, medical imaging and description of the disease, Other conditions that can present similarly include irritable bowel syndrome and Behçet's disease.

Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) also known as caspase recruitment domain-containing protein 15(CARD15) or inflammatory bowel disease protein 1 (IBD1) is a protein that in humans is encoded by the*NOD2* gene located on chromosome 16. Crohn's disease with multifactorial traits, that can involve any part of the gastrointestinal tract. In recent years, a dozen genome-wide association scan and meta-analysis were published bringing the number of susceptibility alleles to more than 30 variations. However, the major susceptibility gene for Crohn's disease is NOD2, located on proximal 16q, which is involved in the innate immune response.

Treatment options help with symptoms, maintain remission, and prevent relapse. In those newly diagnosed, a corticosteroid may be used for a brief period of time to quickly improve the disease with another medication such as either methotrexate or a thiopurine used to prevent recurrence. An important part of treatment is the stopping of smoking among those who do. One in five people with the disease are admitted to hospital each year, and half of

those with the disease will require surgery for the disease at some point over a ten-year period. While surgery should be used as little as possible, it is necessary to address some abscesses, certain bowel obstructions, and cancers. Checking for bowel cancer via colonoscopy is recommended every few years, starting eight years after the disease has begun.

MATERIALS AND METHODS

The diseased pathway of target gene identified from the kegg database,it also clearly shows the gene which is responsible for crohn's disease. The protein and nucleotide sequences of NOD2 are retrieve from NCBI in FASTA format. The functional analysis is done through using AMIGO tool. The gene expression of target gene is done using ACUA. Highly expressed sequences are applied in to clustal W, multiple aligned sequence are done through phyML. Finally, the structure analysis of target gene (NOD2) is done through using three tools are disEMBL, PREDYFLEXY, PCOILS.

RESULT AND DISCUSSION

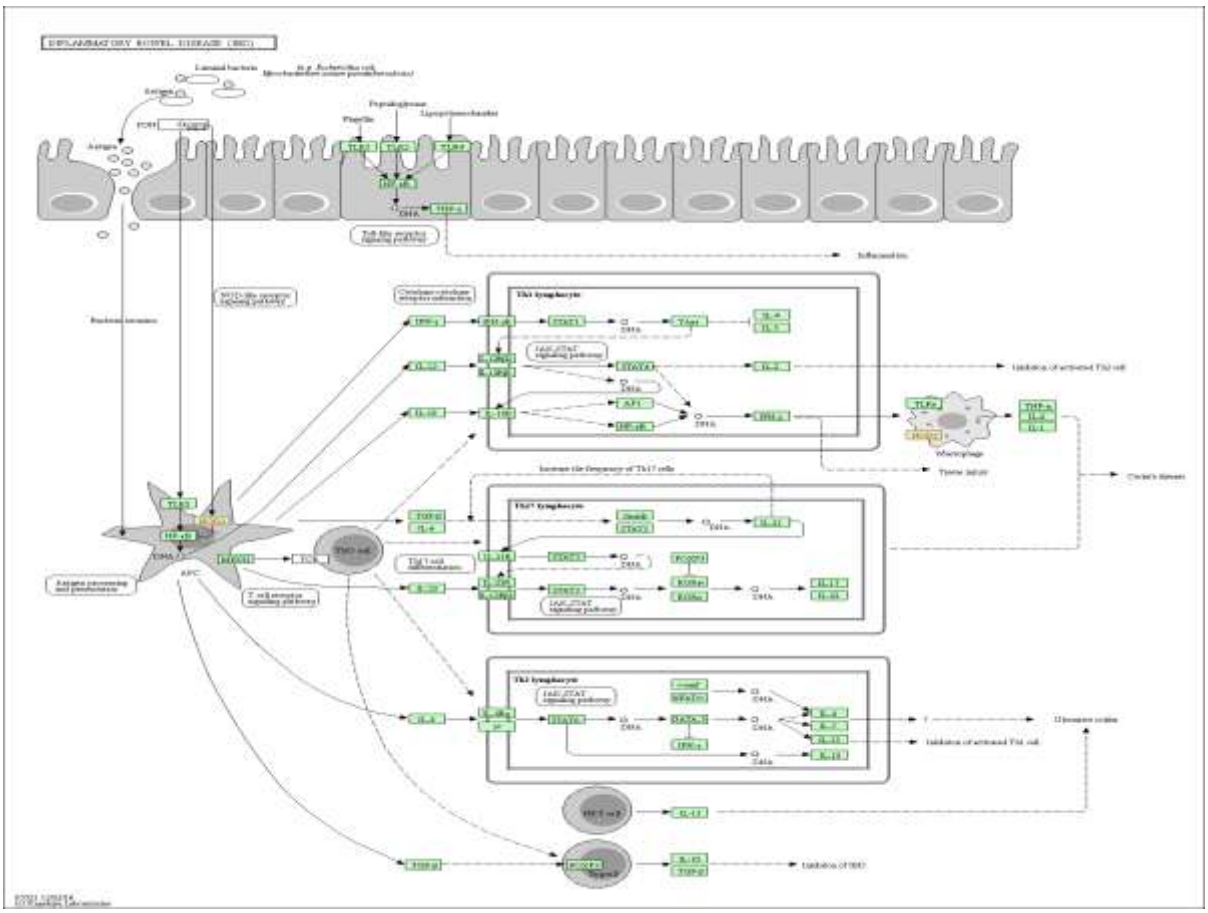

Figure 1: The above result shows the diseased pathway of crohn's disease. The red mark shows the gene (nod2) responsible for crohn's disease.

	Gene/product	Gene/product name	Organism	PANTHER family	Type	Source	Synonyms
☐	*nod2*	nucleotide-binding oligomerization domain containing 2	Danio rerio	family not named pthr24106	protein	ZFIN	NLR-A2 card15
☐	*Nod2*	nucleotide-binding oligomerization domain containing 2	Rattus norvegicus	family not named pthr24106	gene	RGD	
☐	*NOD2*	Nucleotide-binding oligomerization domain-containing protein 2	Hylobates lar		protein	UniProtKB	NOD2_HYLLA CARD15
☐	*NOD2*	Nucleotide-binding oligomerization domain-containing protein 2	Pan troglodytes		protein	UniProtKB	NOD2_PANTR CARD15
☐	*NOD2*	Uncharacterized protein	Sus scrofa		protein	UniProtKB	A0A287BKC9_PIG
☐	*Nod2*	nucleotide-binding oligomerization domain containing 2	Mus musculus	family not named pthr24106	protein	MGI	Card15 F830032C23Rik Nlrc2
☐	*NOD2*	Nucleotide binding oligomerization domain containing 2	Canis lupus familiaris		protein	UniProtKB	F1P9K0_CANLF
☐	*NOD2*	Nucleotide-binding oligomerization domain-containing protein 2	Homo sapiens	family not named pthr24106	protein	UniProtKB	NOD2_HUMAN CARD15 IBD1
☐	*NOD2*	Nucleotide-binding oligomerization domain-containing protein 2	Bos taurus	family not named pthr24106	protein	UniProtKB	NOD2_BOVIN CARD15

Figure 2: The above structure shows the gene ontology of target sequence.

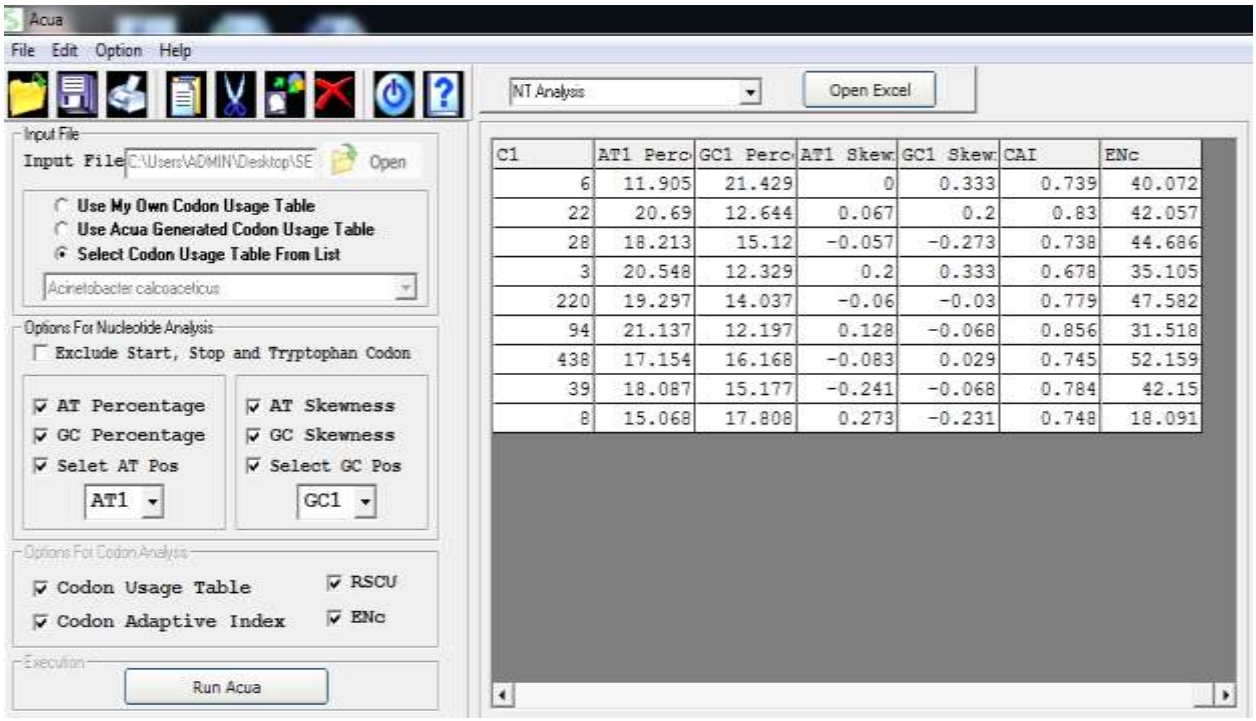

Figure 3: The above table shows the highly expressed sequence of evolutionary related gene (NOD2).

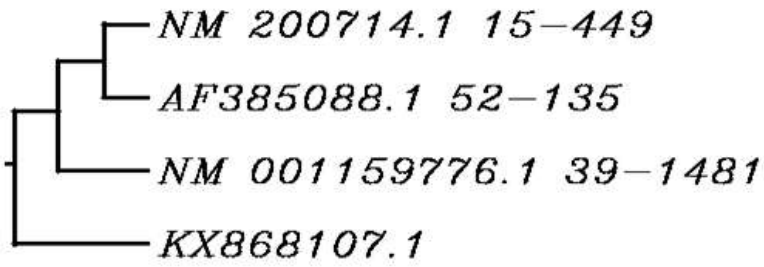

Figure 4: The above tree shows the evolutionary relationship of sequence.

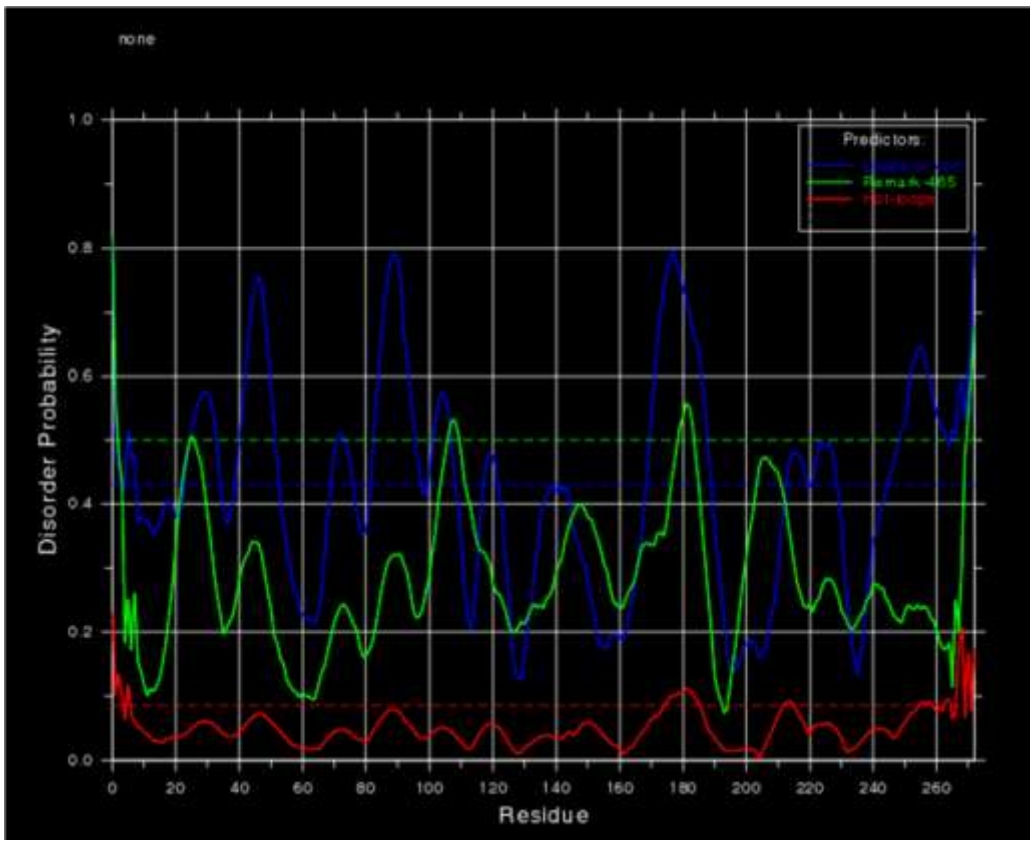

Figure 5: The above graph result shows the structural disordered probability of Nod2 gene.

- The hot loops follows that highly dynamic loops should be considered protein disorder.
- The Remark-465 Non assigned electron densities most often reflect intrinsic disorder, and have been used early on in disorder prediction.
- The Loops/coils are not necessarily disordered, however protein disorder is only found within loops.

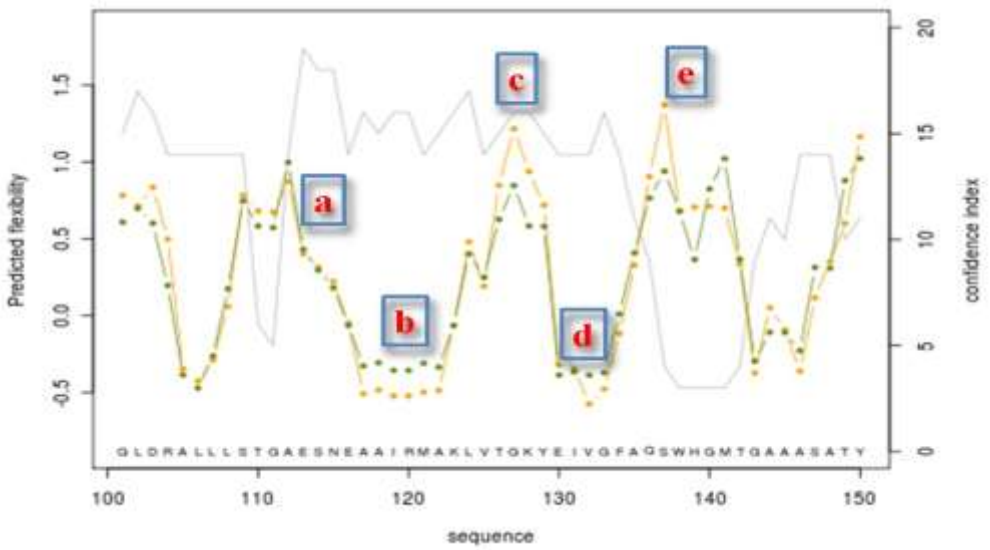

Figure 6: prediction of coil-coiled region and flexibility of NOD2 gene.

CONCLUSION

NOD2 is a cytoplasmic molecule involved in sensing microbial cell wall components and regulating inflammatory processes and apoptosis. Coding region variants in the leucine-rich repeat region of Nod2 may affect host interactions with bacterial lipopolysaccharide. Research suggests that newer corticosteroids, such as budesonide and beclomethasone dipropionate, may be more effective at reducing symptoms. The new study, suggests that wiping out a significant portion of the bacteria in the gut microbiome, and then re-introducing a certain type of "good" bacteria that lacks this enzyme, known as urease, may be an effective approach to better treat these diseases. Finding the genes that contribute to disease represents only one of many steps required to more effectively treat these disorders. Hence, this nod2 gene is taken for studying functional analysis, phylogenetic analysis and the structural analysis. From the result this work a comprehensive study of gene may be further used in research.

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