



Analysis of Variations in Conserved Motifs of Cag A protein in Different Helicobacter Pylori Strains

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Abstract:

Infection with strains of Helicobacter pylori that carry the cytotoxin-associated antigen a (cagA) gene is associated with gastric carcinoma. Recent studies have shed light on the mechanism through which the **cagA gene** product, CagA, elicits pathophysiological actions. Helicobacter pylori infection has an association with histological gastritis, gastric atrophy, gastric cancer, and mucosa-associated lymphoid tissue (MALT) lymphoma in the stomach. There are some strains of **H. pylori** that are capable of triggering a greater inflammatory response in the stomach of its host. This strain carries the cag pathogenicity island (cag PAI). The research work deals with analysis and prediction of structural motif of cagA protein present in different strains of helicobacter pylori. The variations among the motifs in different strains are analyzed. The significant motif showing highest variability is traced. Through this changes in the conserved region of the motif the variability among different strains were studied.

Introduction:

Gastric cancer is commonly referred to as stomach cancer which can develop in any part of the stomach and may spread throughout the stomach and to other organs; particularly the esophagus, lungs, lymph nodes, and the liver. Most stomach cancers start in the mucosa. Stomach cancer causes about 800,000 deaths worldwide per year. There are three main types of stomach cancers: lymphomas, gastric stomal tumors, and carcinoid tumors. Stomach cancer can cause the, Indigestion or burning sensation (heartburn), Loss of appetite, especially for meat, Abdominal discomfort or irritation, Weakness and fatigue. The three main treatments for stomach cancer are surgery, chemotherapy, and radiation therapy.

Helicobacter pylorus (also known as H.pylori) is a spiral-shaped bacterium that lives in the lining of the stomach. Helicobacter pylori CagA protein is associated with severe gastritis and gastric carcinoma.

Keywords: Gastric carcinoma, Gastric atrophy, MALT, pathophysiological.

A major virulence determinant is the H. pylori CagA protein (encoded by the cytotoxin-associated geneA) is injected from the attached Helicobacter pylori into host cells and undergoes tyrosine phosphorylation. In the host cell, CagA is phosphorylated on tyrosine residues and induces rearrangements of the actin cytoskeleton. CagA protein which is translocated from the bacteria into the cytoplasm of host cells by a type IV secretion system. Helicobacter pylorus is one of the most wide-spread bacterial pathogens and infects the human stomach to cause diseases, such as gastritis, gastric ulceration, and gastric cancer.

Methodology:

CagA protein sequence from different strains of Helicobacter pylori were retrieved from GenBank of National Centre for Biotechnology Information (NCBI) and to find the local similarities Basic Local Alignment Search Tool (BLAST) was performed.

Specific motifs which are functionally involved towards infection from CagA protein were retrieved using FINGER PRINT SCAN . From top scoring finger prints the best motifs are sorted out from different strains. Phylogenetic analysis was then performed for different strains using ClustalW which depicts the evolutionary relationship among various strains and evolutionary distance was analyzed between the strains using Clustal Dist.

TEXSHADE was used to screen the conserved residues which were present in motif regions of the protein sequence. The specific motif showing variability among the different strains were studied.

Results and discussion:

Sequence retrieval using NCBI:

CagA protein sequence from different strains (CagA protein [Helicobacter pylori v225d], Cag pathogenicity island protein [Helicobacter pylori F32], Cytotoxin-associated protein A [Helicobacter pylori G27], Cag pathogenicity island protein (cagA, cag26) [Helicobacter pylori Sat464], Cag pathogenicity island protein (cagA, cag26) [Helicobacter pylori Cuz20], Cytotoxin-associated protein A [Helicobacter pylori Gambia94/24], Cytotoxin-associated protein A [Helicobacter pylori

India7], Cytotoxin-associated protein A [Helicobacter pylori Lithuania75], Cag pathogenicity island protein (cag26) [Helicobacter pylori SJM180])of Helicobacter pylori was retrieved using GenBank databases of NCBI.

FINGERPRINT SCAN:

Through FINGERPRINTSCAN the best top scoring motif sequence is selected from different strains. That sequence is shown in the table1.listed below.

Table 1: Table showing the various motifs found in the Cag A Protein

STRAINS	MOTIF SEQUENCE
H.pylori NCTC 11637	NEFKNGKNKDFSKVTQAK
H.pylori v225d	NGGPEARHHDWNTATVGYKNQQ
H.pylori F57	NDFKNGKNKDFSKVTQAK
H.pylori G27	TTTTDIQGLPPEAR
H. pylori Sat464	EHGVKNINTKSGFQK
H. pylori Cuz20	GGINNPFSFYLYKKDQLTG
H.pylori Gambia94/24	EHGIKNANTKGGFQK
H.pylori India7	GFNQKNMKDYSDSFKF
H.pylori Lithuania75	KKNQYFSSFISKSNLDLIDKG
H.pylori SJM180	KEKEKFRNEIKDFQKDSKAY

Phylogenetic analysis:

1. CLUSTAL W DENDOGRAM:

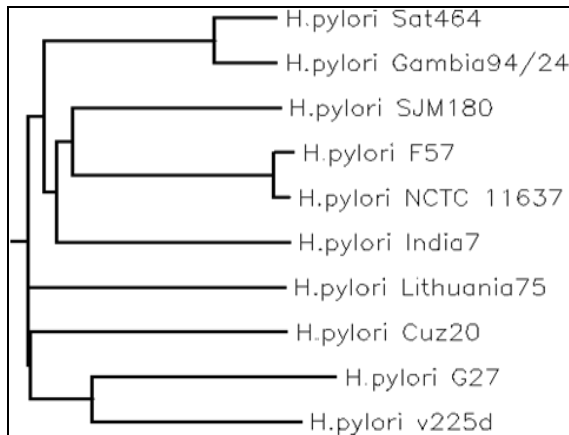


Fig 1(Dendogram)

2. CLUSTAL DISTANCE MATRIX:

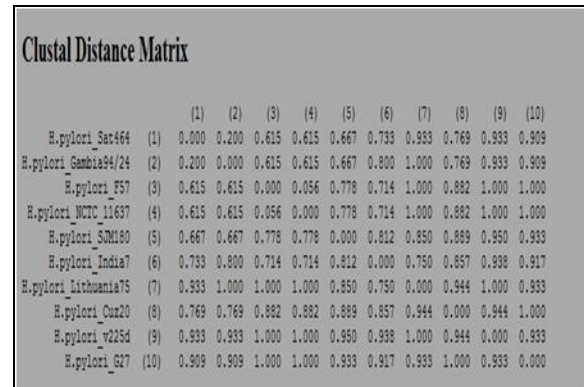


Fig 2(Clustal Distance Matrix)

3. ANALYSIS OF DIFFERENT MOTIFS USING TEXSHADE :

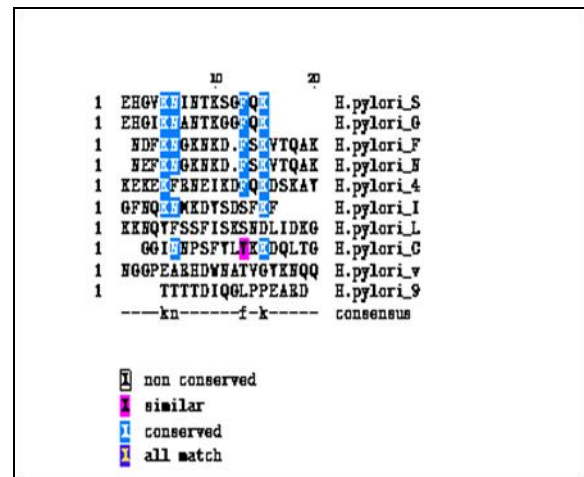


Fig 3(Texshade)

In Phylogenetic analysis the CLUSTAL W shows the evolutionary relationship between different Helicobacter pylori strains. The Helicobacter pylori NCTC 11637(Query sequence) has shown a very close evolutionary relationship with Helicobacter pylori F57 with highest similarity. The Other strains showing nearest similarities with query sequence are Helicobacter pylori SJM 180, Helicobacter pylori India 7. The evolutionary distance of the above species is less compared to the other species. Finally, TEXSHADE is done to determine the conserved regions and similar regions among the motifs. Here the light blue color shows the conserved regions, and pink color shows the similar regions.

Analysis for motif sequence in cag A protein:

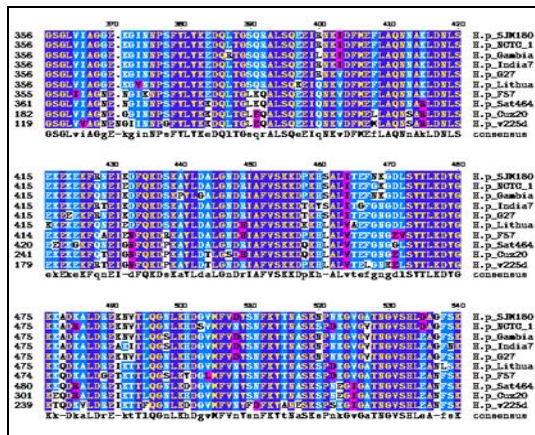


Fig 4(Texshade)

We are using TEXSHADE to screen the conserved residues which are present in motif regions of the protein sequence. Finally the motif sequence “KEKEKFQNEIEDFQKDSKAY” showing the highest variation was selected. This sequence was designed using Argus lab.

Table 2: Specific Motif showing variation in Different strains

STRAINS	MOTIF SEQUENCE
H.pylori SJM180	KEKEKFRNEIKDFQKDSKAY
H.pylori NCTC 1637	KEKEKFQNEIEDFQKDSKAY
H.pylori Gambia94/24	KEKEKFQNEIKDFQKDSKPY
H.pylori India7	KEKEKFRTEIKDFQKDSKAY
H. pylori G27	KEEEKFRNEIKDFQKDSKAY
H.pylori Lithuania75	KEKEKFQNEIEDFQKDSKAY
H.pylori F32	KEKEKFQTEIENFQKDRKAY
H.pylori Sat464	EEKGKFQNEIGNFQKDKPKAY
H.pylori Cuz20	KEKEKFQTEIGNFQKDKPKAY
H.pylori v225d	KEKEKFRTEIGNFQKDKPKAY

Motifs design using Argus lab:

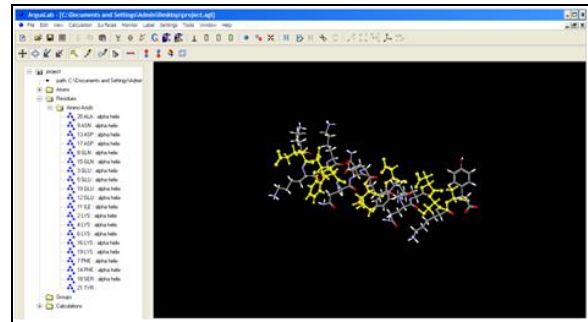


Fig 5 Design of motif in Argus lab

Through ARGUSLAB the structure of motif “KEKEKFQNEIEDFQKDSKAY” is designed and the specific regions which are showing variations due to change in amino acids in the region are displayed in the form ball and stick model. That region is showing in yellow color.

Conclusion:

There are different strains in Helicobacter pylori which are responsible for gastric cancer. Motifs of biological significance like the regions which contribute for the protein function and structure has been studied. Through the analysis of motifs we have analyzed the variations among different strains which might be the possible reason for the evolution of different strains. Future studies may be undertaken on next probable variation and new upcoming strains of H Pylori may be predicted. It may help and fetch interest of pharma community to undertake Research on designing and developing drugs against gastric cancer which may be caused by a new strain in future.

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