W1 – Microbiology, Molecular Genetics and Virulence Factors

Abstract no.: W1.1
PREVALENCE AND CLINICAL SIGNIFICANCE OF HOMA AND HOMB, TWO NOVEL HELICOBACTER PYLORI VIRULENCE MARKERS, IN SLOVENIAN PAEDIATRIC POPULATION
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Background: Although severe gastroduodenal disease mostly appears in adulthood after long-term H. pylori infection, peptic ulcer disease (PUD) may develop in young children, suggesting an involvement of potentially more pathogenic strains. Two novel H. pylori outer membrane proteins, homA and its paralogue homB, were suggested to influence the severity of disease manifestation.

Objective: To determine the prevalence of homA and homB in Slovenian paediatric population and to evaluate their clinical relevance, previously associated with non-ulcer dyspepsia and PUD, respectively.

Material and Methods: A total of 204 H. pylori positive gastric biopsies, obtained from children, were included in the study. The presence of virulence genes was determined by a single polymerase chain reaction (PCR) assay, which generates amplicons of 128-bp and 161-bp for homA and homB, respectively. Each of the genes was compared with density, activity and chronicity of H. pylori infection according to the Updated Sydney Histological Classification.

Results: Strains in which both homA and homB were detected (13/204) and strains with intermediate PCR product lengths (3/204) were excluded from further analysis. Thus, a total of 121/188 (64%) and 64/188 (34%) strains were positive for homA and homB, respectively. There was no statistically significant association between the presence of either homA and activity (p = .73) or chronicity (p = .13), while correlation was found between homA positivity and density (p = .02).

Conclusion: Due to the lack of association between either of genes and severe histological findings, it is unlikely that homA or homB represent important H. pylori virulence markers in children.

Abstract no.: W1.2
IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS IN THE TFS4 TYPE IV SECRETION SYSTEM OF HELICOBACTER PYLORI
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Genome sequence data has determined the duodenal ulcer promoting (dupA) gene to be encoded within a cluster of vir homologous type IV secretion system (T4SS) genes in the plasticity zones of several H. pylori strains. Sequence identity and the presence of characteristic sequence motifs indicates that DupA may be the VirB4 ATPase component of the T4SS, however the function of the T4SS, recently termed Ths4, and the identity of its secretion substrates are unknown.

In this study, we aimed to assess the protein-protein interactions mediated by a dupa-encoded VirD2-like protein using the yeast two-hybrid (Y2H) system. VirD2 proteins are relaxases typically involved in conjugation or interkingdom DNA transfer in association with several other proteins collectively referred to as the relaxosome. A homologue of one other relaxosome protein, VirC1 is also present in dupa.

In a candidate approach, a pairwise Y2H interaction screen determined that VirD2 interacted with itself, VirC1 and an unknown protein encoded adjacent to VirC1. Interactions were generally weak indicating a likely requirement for stabilising factors inherent to a relaxosome complex. An interaction was not observed with a VirD4 coupling protein. In a secondary approach, a high titre genomic library has been constructed from a dupa + dupa + (dupa) H. pylori clinical strain and is in the process of being screened to define the entire repertoire of T4SS proteins that interact with VirD2 and VirD4.

Our preliminary observations are consistent with known interactions in other T4SSs and suggest that Ths4 may function in DNA transfer to a host cell.

Abstract no.: W1.3
SCREENING OF PROPHAGE SEQUENCES AMONG HELICOBACTER PYLORI ISOLATES
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Until recently, Helicobacter pylori was considered a bacterium without prophages. The presence of an incomplete prophage sequence in strain B38 and a complete prophage sequence in strain B45 showed otherwise.

Using a PCR strategy, based on degenerated primers designed after aligning bacteriophage integrate sequences from H. pylori strains B38 and B45, and H. acinomychii prophage II, we found that integrate sequence was present in 21.4% (73/341) of the H. pylori clinical strains tested. The phylogenetic analysis of the sequenced region revealed that strains cluster according to their geographic origin, but not to their pathology. We have applied the same methodology to additional 147 European strains and 77 African strains, determining the presence of integrate sequence in 25.2% (37/147) of the former and in 19.5% (15/77) of the latter. Currently, we have a total of 565 strains screened for the presence of integrate, with 125 positive for this sequence (22.1%). To understand if these integrate sequences belong to remisstent or complete prophages we are also screening for the presence of other prophage coding sequences. Among integrate positive strains, we found 19.2% (5/26) positive strains for the primase sequence and 53.3% (8/15) for the presence of the end of the phage. Presently, we are running the sequencing of the PCR amplified products in order to conduct the phylogenetic analysis. The results reinforce the abundance of prophage sequences in H. pylori and suggest that the majority of them belong to remisstent prophages integrated within the bacterium genome. Work supported by FCT (PTDC/EBB-EBI/119860/2010).

Abstract no.: W1.4
GENE POLYMORPHISMS OF MICRONRNAS IN HELICOBACTER PYLORI-INDUCED HIGH RISK ATROPHIC GASTRITIS AND GASTRIC CANCER
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Background and Aims: Different studies have shown that microRNAs (miRNAs) are deregulated in gastric cancer (GC). Several single nucleotide polymorphisms (SNPs) of genes related to miRNAs were linked with GC and premalignant lesions. The data on the potential association between miRNA SNPs and the risk of GC or Helicobacter pylori-induced atrophic gastritis, however, are scarce and partially conflicting.

The aim of our study was to evaluate potential associations between the presence of GC and high risk atrophic gastritis (HRAG) and SNPs of genes related to mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608.

Methods: Gene polymorphisms were analyzed in 538 subjects (GC: n = 106; HRAG: n = 222, controls: n = 210) of Caucasian origin. Mir-146a C>G (rs2910164), mir-149 T>C (rs2292832), mir-196a-2 C>T (rs1614913), mir-379 A>G (rs15991156), mir-499a A>G (rs3744644) and mir-608 C>G (rs4919510) SNPs were genotyped by RT-PCR.

Results: Frequencies of genotypes in our study are similar to the data reported on subjects of Caucasian ethnicity. There was a tendency for mir-196a-2 C/C genotype to be associated with lower incidence of HRAG (49.0% in controls vs. 47.1% in HRAG, p=.13), while correlation was found between homA positivity and density (p = .02).

Conclusion: Due to the lack of association between either of genes and severe histological findings, it is unlikely that homA or homB represent important H. pylori virulence markers in children.

Abstract no.: W1.5
PHOTODYNAMIC THERAPY USING HELICOBACTER PYLORI-ENCODED LABELLED PAPILLOMA VIRUS IN ELADENOSIS-INDUCED BLADDER CANCER IN A RABBIT MODEL
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