W1 – Microbiology, Molecular Genetics and Virulence Factors

Abstract no.: W1.1

PREVALENCE AND CLINICAL SIGNIFICANCE OF HOMA AND HOMB, TWO NOVEL HELICOBATER 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, *homB* and its paralogue *homA*, 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 Tfs4, and the identity of its secretion substrates are unknown.
In this study, we aimed to assess the protein-protein interactions mediated by a *tfs4*-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 *tfs4*.
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 *tfs3 + /tfs4 + /cag+ 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 Tfs4 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 integrase genes from *H. pylori* strains B38 and B45, and *H. acinonychis* prophage II, we found that integrase 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 integrase 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 integrase gene, with 125 positive for this sequence (22.1%). To understand if these integrase sequences belong to reminiscent or complete prophages we are also screening for the presence of other prophage coding sequences. Among integrase 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 prophages sequences in *H. pylori* and suggest that the majority of them belong to reminiscent prophages integrated within the bacterium genome. Work supported by FCT (PTDC/EBB-EBI/119860/2010).

Abstract no.: W1.4

GENE POLYMORPHISMS OF MICRORNAS 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 (rs11614913), mir-379 A>G (rs61991156), mir-499a A>G (rs3746444) 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. 41.4% in HRAG group, $p = .079$). Allele C of mir-196a-2 SNP was more frequent in controls when compared to HRAG group, 67.8% and 60.1% respectively, however it failed to reach significance level ($p = .087$). We did not find any significant associations for all miRNA polymorphisms in relation to GC or HRAG.
Conclusions: Mir-146a, mir-149, mir-196a-2, mir-379, mir-499a and mir-608 SNPs are not linked with gastric carcinogenesis, and therefore do not appear as potential biomarkers for identifying individuals with higher risk for GC.