Genetic variation in Helicobacter pylori

Pan, Z.

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Chapter 7

Summary and conclusions

This thesis investigates systematically both virulence and non-virulence factors of \( H. pylori \) from a large collection of Chinese patients in comparison with \( H. pylori \) strains isolated from patients in the Netherlands and in other countries as well as \( H. pylori \) strains isolated from different continents. The investigation included the examination of virulence factors, such as (1) and non-virulence factors (non-secretory factors). Chinese \( H. pylori \) strains show a close together with \( H. pylori \) isolated from other Asian countries.

In chapter 4, the prevalence of eight virulence factors in the 20 strains of \( H. pylori \) isolated from patients in China and the Netherlands was compared. The data suggested that the prevalence of the specific virulence factors varies between countries and between different strains of \( H. pylori \). The prevalence of these factors was further analyzed to determine the risk factors for the development of gastric and duodenal ulcers. The results indicated that the prevalence of these factors was higher in patients with ulcer disease compared to those without ulcer disease. This suggests that the variation in the prevalence of these factors may be related to the risk of developing ulcer disease.

In chapter 5, the prevalence of non-virulence factors was studied. The data indicated that the prevalence of these factors varies between countries and between different strains of \( H. pylori \). The results suggested that the prevalence of non-virulence factors is higher in patients with ulcer disease compared to those without ulcer disease. This suggests that the variation in the prevalence of these factors may be related to the risk of developing ulcer disease.
Summary and conclusions

This thesis investigates systematically both virulence and non-virulence factors of *H. pylori* from a large collection of Chinese patients in comparison with *H. pylori* organisms isolated from patients in the Netherlands and in other Western countries as well as *H. pylori* from other East Asian countries. The results indicate that Chinese *H. pylori* is different from *H. pylori* circulating in Western countries in prevalence, distribution and gene sequences of virulence factors (*cagA, vacA*) and non-virulence factors (housekeeping genes). Chinese *H. pylori* form a clone together with *H. pylori* from other Asian countries.

In chapter 2, the prevalence of *cagA* positive *H. pylori* in Chinese patients were analyzed by PCR, Southern Blotting, and colony hybridization and its correlation with both PUD and gastritis was evaluated. The results showed that 98% (47/48) of the *H. pylori* from PUD patients and 100% (35/35) of the *H. pylori* from CG patients were *cagA* positive. Therefore, *cagA* cannot be used as a marker for the presence of PUD in Chinese patients. The result also suggests that there might be some other more important factors other than *cagA* determining the clinical outcome. *CagA* sequence variation was suggested by the lower sensitivity of the PCR with one of the *cagA* primer sets in Chinese *H. pylori* as compared to that in Dutch *H. pylori*. It suggests that allelic variation in *cagA* may exist and that distinct *H. pylori* genotypes may circulate in China and Western Europe.

In chapter 3, the hypothesis that different *cagA* positive *H. pylori* populations may circulate in China and the Netherlands was studied. Twelve Dutch *H. pylori* and ten Chinese *H. pylori* were assessed by sequencing of 243-bp of *cagA* gene and 240-bp of *glmM* gene (phosphoglucomutase; identical to urease C). Based on comparison of the sequence of a 243-nucleotide part of *cagA*, the Dutch (group I) and Chinese (group II) *H. pylori* isolates formed two separate branches with high confidence limits in the phylogenetic tree. These two clusters were not observed when the sequence of a 240-bp part of *glmM* was used in comparison. The number of nonsynonymous substitutions was much higher in *cagA* than in *glmM*, indicating positive selection. The average levels of divergence of *cagA* at the nucleotide and protein levels between group I and II isolates were found to be high, 13.3 and 17.9% respectively. We conclude that in China and The Netherlands, two distinct *cagA*-positive *H. pylori* populations are circulating. The *cagA* gene encodes an immunodominant protein. Whether the immunogenecity of this protein is also different in *H. pylori* from these two countries need to be studied in future.

Studies of *H. pylori* from the West have linked production of vacuolating cytotoxin and a particular signal sequence (s1a) allele of the underlying *vacA* gene to peptic ulcer disease (PUD). In chapter 4, the vacuolating cytotoxin production and the underlying gene among Chinese *H. pylori* were investigated. 76% (35/46) of isolates from PUD patient and 83% (29/35) isolates from CG patients produced vacuolating cytotoxin activity on *Hela* cells (P>0.5). Polymerase chain reaction and DNA sequencing showed that 95 of 96 isolates carried *vacA* s1a alleles. In the mid-region, 78 of 96 isolates carried m2, 14 were m1-like but only 87% identical (DNA-level) to classical m1 and were designated m1b; the other 4
were unusual hybrids (m1b-type proximal, m2-type distal). Isolates with m1b and m1b-m2 alleles produced higher levels of vacuolating activity than did isolates with m2 alleles (P<.01). The results suggest that unlike previous reports, vacuolating cytotoxin production as well as any \textit{vacA} allele do not have specific correlation with PUD in Chinese \textit{H. pylori}. In contrast with the recent data showing that both the m1 and m2 allele of \textit{vacA} of \textit{H. pylori} can induce vacuoles in cultured Eucorico cells but only the m1 allele can induce vacuoles in Hela cells (104), the m2 allele of \textit{vacA} of Chinese isolates also produce cytotoxin activity on Hela cells in \textit{vitro}. Furthermore, the distribution of \textit{vacA} allele is also different from \textit{H. pylori} in West. Recently, data about \textit{vacA} alleles from other Asia countries indicate that the distribution of \textit{vacA} allele and the \textit{vacA} sequence motifs are different from those reported from West (105-109), which are consistent with our founding.

In order to understand whether \textit{vacA} genotype among \textit{H. pylori} isolates from Dutch patients are associated with disease, the cytotoxin activity of the \textit{H. pylori} isolates from 34 PUD patients and 46 patients with functional dyspepsia (FD) was assessed by an \textit{in vitro} assay which is the same as what we used for Chinese \textit{H. pylori}. The \textit{vacA} types and \textit{cagA} status of the isolates were assessed by PCR. Our conclusion from the results presented in chapter 5 is that an association between \textit{vacA} subtypes and disease could not be established in this patient population due to strong linkage between \textit{vacA} s1 type and \textit{cagA}. Similar results was also reported from other group (110).

In order to better understand the population genetic structure and diversity of \textit{H. pylori}, we expanded our study to other East Asia \textit{H. pylori} as well as strains from several regions of the world. In chapter 6, the sequences of fragments of seven housekeeping genes and two virulence-associated genes from 20 strains of \textit{H. pylori} isolated from diverse geographical regions were assessed. Two clonal groupings, the Asian clone and clone 2, were detected in a global collection of \textit{H. pylori}. These clonal grouping are widespread and have probably existed for a long time period. Frequent recombination occurs on a global scale for most genes of \textit{H. pylori} but has not totally disrupted the relationship with the clonal groupings. All six strains isolated from Japanese and coastal Chinese were assigned to the "Asian" clonal grouping, probably reflecting descent from a distinct common ancestor. The clonal groupings were not totally uniform; recombination, as measured by the homoplasy test and compatibility matrices, was extremely common within all genes tested, except \textit{cagA}. The fact that clonal descent could still be discerned despite such frequent recombination possibly reflects founder effects and geographical separation and/or selection for particular alleles of these genes.

This thesis presents a global view of the genetic structure and geographic diversity of \textit{H. pylori} from. The conclusion is that \textit{H. pylori} displays clonal groupings. This clonal grouping and geographic distribution of \textit{H. pylori} should be taken into account when designing strategies for diagnosis and treatment of \textit{H. pylori} infections, and it will be useful in the study of human evolution.
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very unusual hybrid 

C. rufus-type pseudochromosomes were detected in the X:Y ratio of 0.4. The results suggest that under certain environmental conditions, such as the presence of high temperatures and humidity, the hybridization of C. rufus and E. rufus may lead to the formation of novel hybrid pseudochromosomes. This observation highlights the potential for genetic reassortment among related species, which may have implications for evolution and speciation.

In order to better understand the population genetic structure and variability of C. rufus, we expanded our study to other areas in Asia. Our results revealed a complex pattern of genetic diversity within the species. We identified several distinct genetic groups, each characterized by unique patterns of allele frequencies and genetic distances. These findings suggest that C. rufus may be divided into several subpopulations, each adapted to specific environmental conditions. This diversity is likely shaped by a combination of genetic drift, natural selection, and gene flow among populations.