Genetic variation in Helicobacter pylori
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Chapter 7

Summary and conclusions

This chapter investigates systematically both virulence and non-virulence factors of *H. pylori* from a large cohort of Chinese patients in comparison with *H. pylori* strains isolated from patients in the Netherlands and to other hospitals in China as well as *H. pylori* strains isolated from patients in the United States and Europe.

In chapter 1, the prevalence of *H. pylori* in Chinese patients was assessed. The results showed that *H. pylori* from Chinese patients was more common than *H. pylori* from patients in the Netherlands and Europe. The prevalence of *H. pylori* in Chinese patients was higher than in patients from other countries. The results also indicate that there is a higher prevalence of *H. pylori* in patients in China compared to patients in the Netherlands and Europe.

In chapter 2, the histopathological characteristics of *H. pylori* in Chinese patients were studied. The results showed that *H. pylori* from Chinese patients was more common than *H. pylori* from patients in the Netherlands and Europe. The histopathological characteristics of *H. pylori* in Chinese patients were different from those in patients from other countries. The results also indicate that there is a higher prevalence of *H. pylori* in patients in China compared to patients in the Netherlands and Europe.

In chapter 3, the genetic characteristics of *H. pylori* in Chinese patients were studied. The results showed that *H. pylori* from Chinese patients was more common than *H. pylori* from patients in the Netherlands and Europe. The genetic characteristics of *H. pylori* in Chinese patients were different from those in patients from other countries. The results also indicate that there is a higher prevalence of *H. pylori* in patients in China compared to patients in the Netherlands and Europe.

In chapter 4, the epidemiological characteristics of *H. pylori* in Chinese patients were studied. The results showed that *H. pylori* from Chinese patients was more common than *H. pylori* from patients in the Netherlands and Europe. The epidemiological characteristics of *H. pylori* in Chinese patients were different from those in patients from other countries. The results also indicate that there is a higher prevalence of *H. pylori* in patients in China compared to patients in the Netherlands and Europe.
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 can not 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 (phosphoglucosamine mutase; 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>.05). 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 vacA allele do not have specific correlation with PUD in Chinese H. pylori. In contrast with the recent data showing that both the m1 and m2 allele of vacA of 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 vacA of Chinese isolates also produce cytotoxin activity on Hela cells in vitro. Furthermore, the distribution of vacA allele is also different from H. pylori in West. Recently, data about vacA alleles from other Asia countries indicate that the distribution of vacA allele and the vacA sequence motifs are different from those reported from West (105-109), which are consistent with our founding.

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

In order to better understand the population genetic structure and diversity of H. pylori, we expanded our study to other East Asia 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 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 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 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 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 H. pylori from. The conclusion is that H. pylori displays clonal groupings. This clonal grouping and geographic distribution of H. pylori should be taken into account when designing strategies for diagnosis and treatment of H. pylori infections, and it will be useful in the study of human evolution.
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many normal hybrids and type-paternal, type-paternal, and type-maternal produced lower lev-
els of swimming capacity than did hybrids with 3 alleles (P < 0.01). The results suggest that util-
izing paternal specific oligonucleotide DNA for a study of hybrid production as well as any cul-
tural effects do not have additive variation with H-2 in

Chromosome 17, p<0.05). In summary, the genetic data described that both the off-line and off-line
levels of H plasminogen insensitive variants in cultured human plasma cells are not the main
factors that influence expression in skeletal muscle (94). The measurement of both cells to
show different rates of H plasminogen in vitro. In summary, data about various alleles from
other data suggests that the distribution of off-line and the off-line sequence
patterns are different from those reported from West (109-113), which are consistent with
our findings.

In order to better understand the population genetic structure and diversity of H plasminogen,
we expanded our study to other East Asian H plasminogen as well as strains from central Europe
of the world. In Chapter 5, the analysis of fragments of lessons housekeeping genes that
were used for association studies were presented. We analyzed the relationship between these
alleles and geographical regions were assessed. We used *-clonal groupings, the *-clonal analysis.

Many detected in a large number of H allele. These *-clonal groupings are maintained
and have probably been in this time period. *-clonal groupings are on a global scale for most genes of H allele and has not usually disrupted the relationship with
the *-clonal groupings. All the samples isolated from Japanese and Central China were
designed by the "Ancestral method" group. This probably resulting from a distant constant region. The *-clonal groupings with not totally uniform recombination, un-
expected by the haplotype test or cox-inhibitory marker, was observed between
within all genes tested, except copy. The fact that ancestral group could still be determined
despite such frequent recombination possibly reflects founder effects and geographical
differentiation and the selection for particular alleles of these genes.

This thesis presents a global view of the genetic type and geographic diversity of H
types from the East Asia. The conclusion is that H plasminogen shows clonal groupings. *-clonal
grouping and geographic distribution of H plasminogen should be taken into account when
designing strategies for diagnosing and treatment of H plasminogen inhibitors, and it will be
useful in the study of human evolution.