ΔΙΑΛΕΞΗ ΠΡΟΣΚΕΚΛΗΜΕΝΟΥ ΞΕΝΟΥ ΟΜΙΛΗΤΗ

Gastric carcinogenesis: is there a role for genetic susceptibility?

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Introduction

Gastric carcinoma (GC) is one of the most common gastrointestinal malignancies world-wide and it was, for a large part of the 20th century, the leading cause of cancer related death. Although the global incidence and mortality of GC is decreasing in developed countries the incidence of this malignancy in the developing world is increasing. Estimates predict 960.000 new cases in the year 2010 and 1.1 million in the year 2020. The 5-year survival rate of GC is low, and identification and a better control of risk factors, seem to be the most effective means of prevention.

The discovery of *Helicobacter pylori* in the early 1980s has proved a turning point in understanding the pathogenesis of GC. It is one of the most common chronic infections in humans, usually acquired early in childhood and if left untreated, persists for the host's lifetime. The progressive decline in *H. pylori* infection prevalence during the last century in developed countries has been accompanied by a decreasing incidence in GC.[1,2] Presently, *H. pylori* is an established risk factor for GC. Case-control studies have shown that *H. pylori* seropositivity is associated with a significantly increased risk of GC (2.1-16.7-fold greater than in seronegative persons).[3-5] Yet, a striking difference exists between the number of infected individuals and the number that go on to develop malignancy. Hence, progression

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towards disease is likely to depend on the combined effects of bacterial pathogenicity, host susceptibility and environmental factors.

Genetic susceptibility

The key feature by which genetic susceptibility to cancer is recognised clinically is family history. Extensive investigation of families with high risk and high incidence of specific cancer types led to the identification of several genetic cancer syndromes and the underlying cancer related genes. These include hereditary diffuse GC (HDGC) and the CDH1 gene, as will be further discussed. Paradoxically, the largest category of inherited susceptibility to cancer, in terms of expected fraction of cancer incidence, is the one with the weakest genetic effects – susceptibility without evident family clustering. The explanation however is straightforward; despite the high penetrance of cancer related genes, like *CDH1,* the resulting genetic cancer syndromes are fairly rare, hence accounting for a very low fraction of overall cancer incidence. By contrast, cancer susceptibility alleles with a small relative risk but with high frequency could account for a significant fraction of overall cancer incidence. The role of common genetic variation in determining the range of individual susceptibility to GC within the population is increasingly recognised, and will also be discussed.

Hereditary gastric carcinoma

The first evidence for a specific GC susceptibility locus was the identification of a gene predisposing to GC in a large New Zealand Maori family.[6] The predisposing gene mapped to an interval on chromosome 16q22.1 containing the gene for the cell-cell adhesion protein E-cadherin (*CDH1*). Subsequent mutation analysis identified inactivating germline *CDH1* mutations in this family and two other families of Maori ethnicity with familial diffuse GC.[6] The tumours in the described families were poorly differentiated, diffuse adenocarcinomas with signet-ring cells.[6-8]

The *CDH1* gene comprises 16 exons spanning approximately 100 kb of genomic DNA which are transcribed into a 4.5 Kb mRNA encoding a 120 kD glycoprotein. E-cadherin is localised at the adherens junctions on the cell's basolateral surface of epithelial cells, where it mediates homophilic calcium-dependent cell-adhesion and interacts with filaments of actin through catenins. The cadherin family is central to the processes of development, cell differentiation and maintenance of tissue integrity.[9] Disruption of the E-cadherin complex induces loss of cell-adhesion with a concomitant increased cell invasion.[10,11] Germ-line truncating mutations in the *CDH1* gene have now been found in many families with HDGC.[12] Analysis of these families indicates that GC develops in three of every four carriers of a mutant CDH1 gene.[13] The HDGC syndrome was defined by the International GC Linkage Consortium, as any family that fits the following criteria:

(1) two or more documented cases of diffuse GC in first/second degree relatives, with at least one diagnosed before the age of 50, or (2) three or more cases of documented diffuse GC in first/second degree relatives, independently of age.[14] Predictive genetic testing is therefore possible in these families, and carriers of the mutation should undergo clinical surveillance and even prophylactic surgery.

Sporadic gastric carcinoma

There is considerable variation in the individual risk of GC development in *H. pylori*-infected patients. This is evident when we look at the global rate of *H.* pylori infection and the much smaller global incidence of GC. Environmental factors including tobacco smoking, alcohol, and dietary risk factors such as ingestion of nitroso compounds, salt, and smoked foods are, among others recognised factors, permissive factors in the development of GC.

Individual variation in cancer risk has also been associated with specific genetic polymorphisms, that may modify the effect of environmental exposures. These gene-environment interactions, in turn, could help explain the high variation in GC incidence observed around the world. Individual genetic susceptibility may be critical in a variety of processes relevant to gastric carcinogenesis including (*i*) the inflammatory response, which conditions the maintenance, severity and outcome of *H. pylori* infection; (*ii*) mucosal protection in the face of *H. pylori* infection and other carcinogens; (*iii*) protection against oxidative damage and other inductors of DNA damage; (*iv*) the ability of carcinogen detoxification and antioxidant protection; and (*v*) cell proliferation ability (by activation of oncogenes or inactivation of tumour suppressor genes).[15] We will concentrate on the discussion of factors associated with the inflammatory response to *H. pylori* infection.

H. pylori establishes a chronic infection in the gastric mucosa, which leads to a chronic inflammation. The magnitude of this chronic inflammation depends, among other factors, on the host's genetic makeup; some individuals will develop a stronger inflammatory response than others, all this being mediated by an array of anti- and pro-inflammatory cytokines. Genetic polymorphisms directly influence inter-individual variation in the level of cytokine production and this clearly contributes to an individual's ultimate clinical outcome.

The Interleukin (IL)-1 beta cytokine is one of the most powerful physiological inhibitors of gastric acid secretion as well as a key cytokine in the host's inflammatory response to *H. pylori*. These characteristics made it a prime candidate to look for genetic variation associated with susceptibility to GC. It was shown that individuals with the *IL1B-511**T or *IL1B-31**C alleles – which are associated with increased levels of IL1β production[16] – are at increased risk of developing hypochlorhydria and gastric atrophy in response to *H. pylori* infection. Carriers of these alleles have also a 2-3-fold increased risk for development of GC. The same holds true for the allele 2 of the VNTR present in the Interleukin-1 Receptor Antagonist (*IL1RN*) gene, which functions as a regulator of IL-1beta levels.[17-20] It was

also shown that the combined effects of pro-inflammatory *IL1B* genotypes and *H. pylori* bacterial virulence factors (*cagA positive, vacA s1* and *vacA m1*) lead to a substantially increased risk for the development of GC,[21] which consubstantiates the important role of the interaction between the host and *H. pylori*.

The *TNFA*-308*A allele, which is thought to increase the production of TNFα, has also been found to confer an increased risk for the development of GC. This polymorphism has been found to be associated with several other inflammatory diseases such as rheumatoid arthritis and multiple sclerosis.[22] Carriage of this polymorphism increased the odds ratio (OR) for non-cardia GC by approximately 2-fold.[18,20] The combined effect of pro-inflammatory host genetic polymorphisms in the *IL1B*, *IL1RN* and *TNFA* genes in the risk of GC development has also been investigated. For GC the odds of developing disease increased with the number of high-risk genotypes.[20,18]

T helper 1 (Th-1) cells are induced mostly in response to intracellular pathogens. Paradoxically, *H. pylori*–specific gastric mucosal T cells generally present a Th1 phenotype. *IL10* is an anti-inflammatory cytokine, which downregulates the expression of cytokines which are primarily involved in the Th1–driven inflammatory such as *IL1B* and *TNFA*. It was reported that homozygosity for the low *IL10* ATA haplotype (based on three promoter polymorphisms at positions -592, -819 and -1082) increased the risk of noncardia GC with an OR of 2.5.[18]

The *TLR4*+896*G polymorphism was also found to be associated with risk to GC. This polymorphism is thought to result in replacement of a conserved aspartic acid residue with glycine at amino acid 299 (Asp299Gly), and alteration in the extracellular domain of the TLR4 receptor. This renders carriers hyporesponsive to LPS challenge by either disrupting transport of TLR4 to the cell membrane or by impairing ligand binding or protein interactions. Carriers of this polymorphism were found to have an increased risk for the development of GC of 2.4-fold.[23]

A common issue in genetic association studies is its lack of reproducibility among different populations. At least in part, this problem can be ascribed to bad study design. Therefore, it might be useful to recall that genetic association studies should fulfil some criteria: a) there should be no evidence of population admixture; b) the inclusion of a proper population control group which is in Hardy-Weinberg equilibrium is required; c) they should have enough statistical power to detect a certain OR with 80% power, a significance of 0.05 and a given observed proportion of exposed individuals in the population; d) the chosen polymorphisms should be in genes relevant for the development of the disease; e) and these polymorphisms should be functionally relevant. Yet, even with all these criteria being met there are conflicting reports regarding some of the associations.

One of the clearest examples is the *IL8*-251*T/*A polymorphism for which several Asian-based population studies reported a significant association with GC.[24-27] However, in Caucasian-based population studies the association between the *IL8*-251*T/*A polymorphism and risk of GC could not be replicated.[28-30] Conceptually, the discrepant results between

Asian and Caucasian populations may be explained by differences in the genetic background of the populations under study. In Asian populations the IL8-251*A allele may be in linkage disequilibrium with an as yet unidentified sequence variant which is responsible for the association with risk of GC. In this context finding associations with different polymorphisms in different populations would not be unexpected as haplotype structure may vary considerably between distinct populations. Thus, haplotype-based approaches involving genotyping of several genetic markers simultaneously, will constitute a more efficient way of capturing the genetic diversity present in a given genomic region and thus help clarify the association between certain polymorphisms and GC risk in different populations. This hypothesis is supported by a recent meta-analysis study showing that although residual heterogeneity beyond factors addressed in the analysis was observed, the findings provide evidence that there are ethnic-specific associations between IL1B and IL1RN gene polymorphisms and GC risk.[31]

Conclusions

Genetically, it is possible to distinguish two main forms of GC: an hereditary form in which the initiating genetic alteration is inherited and the remaining mutations are acquired somatically; and a sporadic form in which every mutation is of somatic origin and where the environment is thought to play a major role.

Germline mutations of the tumour suppressor gene *CDH1*, that codes for the cell adhesion molecule E-cadherin, are the cause of the HDGC syndrome. Families carrying *CDH1* mutations have a high incidence of diffuse GC, usually in young individuals (less than 45 years of age). The identification of such mutations has proven invaluable in the clinical management of such families and in GC prevention in *CDH1* mutation carriers. The model of HDGC has been also providing valuable insight into the molecular mechanisms underlying the involvement of E-cadherin in onset and progression of cancers of epithelial origin.

In the setting of sporadic-type GC it has been shown that individuals infected with *H. pylori*, a stomach colonizing bacteria, have an increased risk of developing GC. The risk for developing this type of tumour relates to the physiologic and histologic changes that *H. pylori* infection induces in the stomach. Results on record show that the extent of gastric mucosal injury may be related to *H. pylori* strain differences, inflammatory responses governed by host genetics, and interactions between host and bacterial determinants. The combination of these factors, favouring a set of responses with higher magnitude, can eventually result in hypochlorhydria, corpus atrophy, and an increased risk of GC (Figure 1). Abundant evidence has now been collected showing that the risk for sporadic gastric cancer development also depends on host genetic factors. Yet, despite providing important insights into the understanding of the disease pathogenesis, the genetic markers we have at present are not sensitive/specific enough to form the basis of a screening strategy.

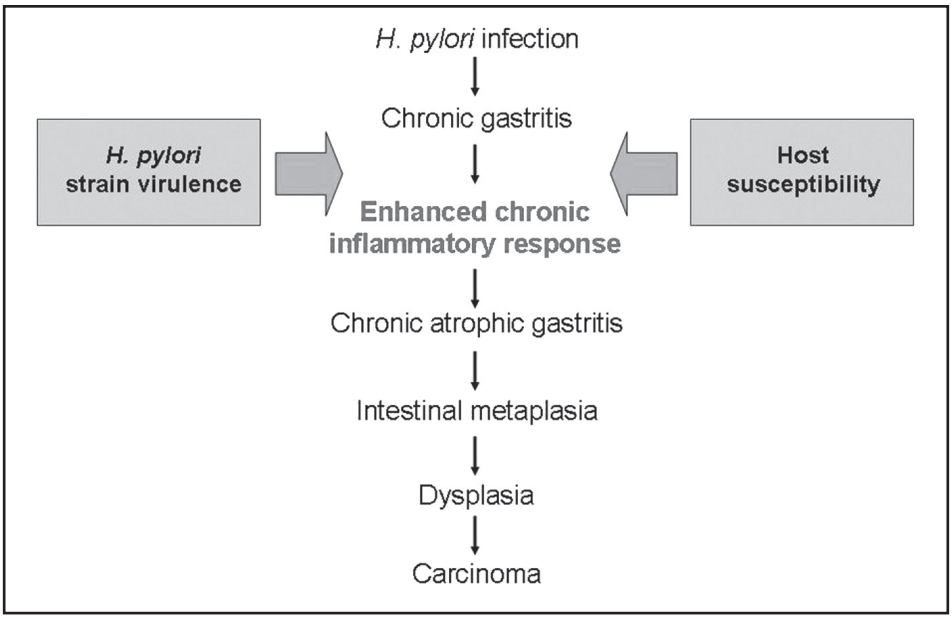

Figure 1. Correa's model of gastric carcinogenesis. The development of an enhanced chronic inflammatory response is influenced by both host genetic susceptibility factors and bacterial virulence factors. This interaction may help explain why some people infected with *H. pylori* develop GC while others do not.

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