HHS Public Access

Author manuscript

Transl Cancer Res. Author manuscript; available in PMC 2017 June 23.

Published in final edited form as:

Transl Cancer Res. 2016 September ; 5(Suppl 3): S557–S560. doi:10.21037/tcr.2016.09.33.

Helicobacter pylori vacuolating cytotoxin and gastric cancer risk: reconsidered

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Although *Helicobacter pylori* cause gastric cancer, cancer develops in a fraction of *H. pylori* infected patients. Based on the notion that polymorphisms in the *H. pylori* vacuolating cytotoxin gene might be a determinant of clinical outcome, Abdi *et al.* used meta-analysis to examine the association between *vacA* gene subtypes and the risk of developing atrophic gastritis, intestinal metaplasia or gastric cancer (1).

H. pylori infection causes gastric mucosal inflammation which underlies the development of peptic ulcer disease and gastric cancer (2). The outcome of any *H. pylori* infection reflects complex interactions between the host, the bacterium and the environment. These interactions are evident clinically as marked geographic variation in the prevalence of *H. pylori*-related diseases (3).

The Abdi *et al.* meta-analysis focuses on one putative *H. pylori* virulence factor, *vacA*, the vacuolating cytotoxin. More than two decade ago, *H. pylori* were noted to differ in terms of ability to produce vacuolating cytotoxicity in vitro in cultured cells (4). The phenomena were subsequently related to the signal (s) and the middle (m) regions of the *vacA* gene (4). Changes in the signal peptide led in a shortened N-terminal portion and an attenuation of vacuolation ability. The most active cytotoxin was termed the s1 allele and the less active form, s2 allele. The m region of the gene encodes a *vacA*-to-host cell binding site; the m1 varient is more effective in binding than the m2 form. Different combinations resulted in

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Provenance: This is a Guest Commentary commissioned by Section Editor Xiaoying Zhou (Institute of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing, China).

Conflicts of Interest: Dr. Graham is a paid consultant and has received research funding from RedHill Biopharma regarding novel *H. pylori* therapies and is a consultant to BioGaia regarding use of probiotics for *H. pylori* infections. And other authors have no conflicts of interest to declare.

Comment on: Abdi E, Latifi-Navid S, Latifi-Navid H, *et al.* Helicobacter pylori vacuolating cytotoxin genotypes and preneoplastic lesions or gastric cancer risk: a meta-analysis. J Gastroenterol Hepatol 2016;31:734-44.

different patterns of cytotoxicity: s1m1 strains produce the greatest level of cytotoxicity, s1m2 strains do not consistently induce vacuolation, and s2m2 strains are not cytotoxic (4).

The presence of marked geographic variation in the prevalence of *vacA* genotypes led to studies to test whether *vacA* genotyping might be usefully clinically. Abdi *et al.* meta-analysis included 33 studies with 1,446 cases and 2,697 controls and included European, Asian and American populations (1). The authors report that the *vacA* s1 genotype was associated with the risk of atrophic gastritis, intestinal metaplasia and gastric cancer [i.e., relative risk (RR) =1.116, 95% CI, 1.019–1.222; RR =1.418, 95% CI, 1.035–1.942; and RR =1.333, 95% CI, 1.115–1.593, respectively]. The *vacA* m1 genotype was associated with intestinal metaplasia and gastric cancer, but failed to statistically influence the risk of atrophic gastritis (RR =1.571, 95% CI, 1.247–1.980 and RR =1.431, 95% CI, 1.180–1.735, respectively). Here, we examine whether the association is a determinant of clinical outcome (i.e., causative) and whether *vacA* genotyping has clinical utility.

In 1965, Bradford Hill formulated nine criteria to distinguish simple association from causation (5) (Table 1). We use those criteria to evaluate the *vacA* associations.

Strength

strong associations with a large increase in RR measured by the incidence (or prevalence) of the condition among the exposed relative to those unexposed are more likely to be causative than weak associations (e.g., the association of death from lung cancer and cigarette smoking is 20 times greater than for nonsmokers) (5,6). Although there are no value that clearly separates “strong” from “moderate” or “weak” associations, RR values below three are considered moderate or weak (7). The Abdi *et al.* meta-analysis (1) reported relatively weak associations (i.e., RR's below 1.5) for both s1 and m1 and for the combination of s1m1.

Consistency

causal association (repeatability) should be consistent in different populations, places and times (5). Fifteen studies in the meta-analysis (1) confirmed the association of the s1m1 genotype with an increased risk for gastric cancer, however studies from Belgian and India only confirmed an association with *vacA* s1 but not with m1 (8,9). In Thailand (10), s, m or s, m, combination showed no statistically significant association with clinical outcome.

Specificity

causation is more certain when the association is limited to specific workers, anatomic sites, and type of disease (5). For example, an exposure that gives rise to a single outcome with no other explanation is likely specific (6). *H. pylori* related diseases are strongly influenced by environmental factor and this criterion is not applicable.

Temporality

temporality relates to exposure and outcome (i.e., the exposure must precede the outcome) (5). The temporality criteria is clear for *H. pylori* as a type I carcinogen (2).

Biologic gradient

biological gradient relates to a dose-response effect (5) (i.e., risk of cancer with an number of cigarettes smoked) (6). With *vacA* one might consider the association between degree of cytotoxicity and outcome. Comparison of area with high prevalence of s1m1 strains and cancer incidence fails to show either a strong or a consistent correlation. For example, s1m1 strains are common in China, Japan and Korea where gastric cancer is common (3). They are also common in India, Thailand, Bangladesh and Pakistan, sites where gastric cancer is rare. For example, in Taiwan *vacA* m2 strains are present in 87.4% (11) and the ASR of gastric cancer is considered high (i.e., 17 cases/100,000 population/year) (12).

Plausibility

causation requires the presence of a biological plausible mechanism that links cause and effect (5). To date no convincing mechanism has been described that can link *vacA* to a particular *H. pylori*-related disease outcome.

Coherence

causality suggest the cause and effect interpretation should not seriously conflict with the generally known of natural history and biology of disease (5,6). The lack of a biologically plausible mechanism does not allow this factor to be tested.

Experiment

in vitro studies suggest that *vacA*-induced vacuolation can disrupt protein trafficking pathways to and from the plasma membrane and thus influence various cell capacities (13). However, the physiological role of vacuolation in vitro remains unclear and animal experiments have failed to confirm a *vacA*-gastric cancer association (14). Experiments using gnotobiotic piglets failed to confirm a role of *vacA* in bacterial colonization, epithelial vacuolation or clinical outcome (15). Although *vacA* mutants colonized less well than their wild type *vacA* counterparts, his loss of *vacA* did not cause any apparent disadvantage (16). A study of the interaction between *H. pylori* and human T84 epithelial cell polarized monolayers using 43,000 element-spotted human cDNA microarrays, failed to detect any gene was specifically related with the presence of the *vacA* gene (17).

Analogy

analogy means the effect of similar factors may be considered. The presence of the s1 *vacA* genotype is strongly associated with the presence other virulence genes particularly the *cag* pathogenicity island, *oipA*, and *babA*. Both CagA and OipA are directly associated with the production of mucosal inflammation which underlies both gastric cancer and peptic ulcer

disease (2). Most likely, the associations ascribed to *vacA* genotypes reflect the presence of these other virulence factors (i.e., CagA, OipA, BabA positive) especially the proinflammatory virulence factors, CagA and OipA.

Summary

Gastric cancer is a multifactorial disease related to longs-tanding gastric mucosal inflammation enhanced by *H. pylori*-specific factors that result in genetic instability (2). Studies attempting to link individual putative *H. pylori* virulence factors to specific disease outcome while ignoring the presence of other factors (e.g., CagA) are likely to produce spurious findings. The outcome of an *H. pylori* infection reflects intimate interactions between the bacteria, the host and the environment. Changes in the environment (e.g., diet) can result in a marked change in disease prevalence (e.g., rapid fall in gastric cancer incidence). Such that any factor can potentially either enhance or reduce the risk of a particular clinical outcome of *H. pylori* related disease. Disease association studies have used an expanding group of *vacA* gene polymorphisms that include the i (intermediate), the d (which refers to a deletion, d, located between the i and m regions), and the c genotypes (a polymorphic site in the three end region of *vacA*) (10). The original predictions made for the s, m, and i regions failed as disease determinants in East Asian and Southeast Asian countries where gastric cancer is a major clinical problem (18). Predictions for d genotype (a deletion, d, located between the i and m regions) base on western countries, failed in East Asia (19). Overall, *vacA* genotyping has generally failed as a consistent and reliable marker of risk and cannot be recommended.

Early in *H. pylori* history, it was suggested that the presence of CagA might serve as a biomarker to identify those at highest risk (3). The Maastricht Consensus III conference (20) reviewed the role of biomarkers for clinical use in *H. pylori* and stated “The detection of *H pylori* pathogenic factors and the study of host genetic polymorphisms is currently not recommended in the management of *H pylori* infection”. This recommendation was based on failure of identification of such facts to “allow a reliable prediction of the outcome at an individual level”. The fact that no *H. pylori* type has been discovered that does not carry a significant risk of development of gastric cancer and peptic ulcer has continued to confirm that recommendation. *vacA* genotype differences most likely reflect genetic drift in populations and thus describe the population and it diseases rather than playing a causative role. In particular *vacA* genotyping studies are often surrogates for the presence of other virulence factors especially, CagA.

Acknowledgments

Funding: Dr. Graham is supported in part by the Office of Research and Development Medical Research Service Department of Veterans Affairs, Public Health Service grant DK56338 which funds the Texas Medical Center Digestive Diseases Center. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the VA or NIH. Professor Yamaoka is supported in part by grants from the National Institutes of Health (DK62813) and the Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan (24406015, 24659200, 25293104, 26640114 and 15H02657). Professor Yamaoka and Dr. Miftahussurur are also supported by the Japan Society for the Promotion of Science (JSPS) Institutional Program for Young Researcher Overseas Visits, the Strategic Funds for the Promotion of Science and Technology from Japan Science and Technology Agency.

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Table 1

Hill's nine criteria for causation

Strength of association
Consistency
Specificity
Temporality
Biologic gradient
Plausibility
Coherence
Experiment
Analogy