

A Review of Polymorphisms in Genes Involved in the Development of Gastric Cancer

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Abstract

Gastric carcinoma and lymphoma are leading causes of cancer mortality throughout the world. This disease is the end result of a long multifactorial process involving a large number of environmental and genetic factors. As a genetic disease, individual variation in cancer risk has been associated with specific alleles of different genes (polymorphisms) in which the modulatory mechanisms of carcinogenesis and the risk of its progression are found. Research at the molecular level has focused on the detection of genetic alterations predisposing to the development and progression of gastric cancer. These studies have been conducted in various populations in which the disease recurs and have been initially based on individual selection of single nucleotide polymorphisms (SNPs) in candidate genes. Important molecular markers have been described and proposed as prognostic markers in this type of patients which has allowed for advances in the understanding of the neoplastic process. This review intends to provide an up to date look at recent studies on gene polymorphisms involved in immunogenic processes, DNA repair mechanisms, responses to detoxification of carcinogenic compounds, mechanisms of tumor suppression and apoptosis which are all processes involved in the development of gastric cancer. Data are also reported from molecular markers associated with this disease from Colombian and foreign genomes already stored in the database of the 1000 Genomes Project.

Keywords

Gastric cancer, susceptibility, genetic polymorphisms.

INTRODUCTION

Gastric cancer (GC), also called stomach cancer, is a type of malignant cell growth with the ability to invade and destroy other tissues and organs, particularly the esophagus and small intestine. Worldwide, it is responsible for about 1 million deaths/year, primarily in Asia and Latin America. (1) In Colombia, the mortality rate in 2012 was 9.1/100,000 inhabitants and average life expectancy after diagnosis was about six months because of late detection in very advanced stages of the disease. (2, 3) Figure 1 shows the countries most affected by gastric cancer in relation to *Helicobacter pylori*. According to Corte, late diagnosis is

largely due to the absence of clinical manifestations and to non-specific symptoms such as dyspepsia. In many cases the tumor is diagnosed late and the survival rate is low. (4)

According to the Lauren classification, the most frequent types of GC are the well differentiated intestinal type which presents as corpus-dominated gastritis with gastric atrophy and intestinal metaplasia, and the diffuse undifferentiated type which presents as gastritis without atrophy. (5, 6) Intestinal tumors predominate in geographical areas with high incidences of GC at a rate of 2 men/1 woman. Worldwide, diffuse type tumors are reported less frequently. (7, 8)

Risk factors play a major role in the development of GC. Some are controversial, but others have been clearly con-

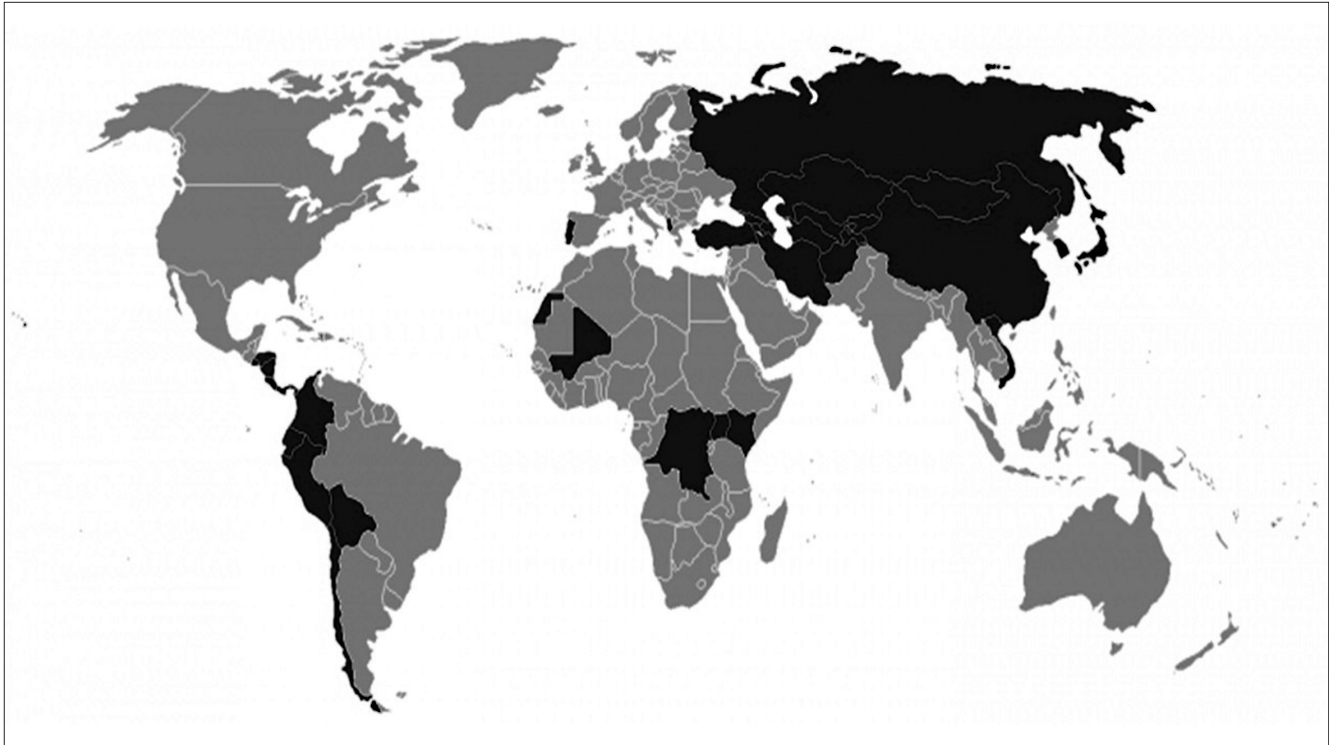


Figure 1. Countries of the world most affected by gastric cancer in relation to *H. pylori*. Map generated by meta-data bibliográfica. International Agency for Research on Cancer (IARC), GLOBOCAN (2002) and PubCan.

firmed. (9) Certain factors such as *H. pylori* infections, tobacco abuse, alcohol consumption, advanced age and poor diets (high-salt and little or no vegetables and fruit) are notorious as the most important risk factors associated with GC. (10, 11).

In addition to environmental risk factors, heredity is extremely relevant to the origin and development of GC even though the majority of diagnosed cases, between 8% and 10%, are sporadic. (4) In this context, genetic factors play an important role in gastric carcinogenesis. (12) Individual genetic susceptibility is associated with specific allele variants (polymorphisms) in a wide variety of genes. They can modify the effect of environmental exposure and gene-environment interaction (1, 11).

Polymorphisms are mutations or changes in DNA whose frequency in a population is above 1%. They constitute one of the major genetic factors involved in susceptibility, risk, and genetic predisposition to disease. Susceptibility is defined as the presence of certain variations in DNA sequences and/or a combination of a number of them (haplotypes) in an individual which may increase the risk of developing a particular disease. (1) Genetic predisposition is defined as increasing susceptibility to a particular disease due to the presence of one or more genetic mutations which are asso-

ciated with increased risk of disease. Genetic risk refers to increased probability of onset of a particular pathology. (12)

The search for genes and genetic polymorphisms involved in susceptibility to disease development involves two strategies of study: genetic linkage analysis and association studies which require case and control sampling to define candidate genes for investigating depending on knowledge of the pathophysiology of the disease in question. (13)

The study of genetic susceptibility to GC includes genes involved in critical processes of gastric carcinogenesis including:

1. The inflammatory and immune response to infection. This includes genes responsible for recognition of *H. pylori* in gastric epithelial cells and genes responsible for activation of signaling pathways that induce proinflammatory and apoptotic responses.
2. Protection of the gastric mucosa, metabolism and detoxification of carcinogenic compounds
3. Repair of oxidative damage, cell proliferation and adhesion, and DNA repair. (14-16)

Bibliographic databases show that investigation of genetic polymorphisms in gastric carcinogenesis has increased in recent years due to advances in DNA analysis technologies

and knowledge of the human genome. (17) As a result of this progress, there are now reports of publications of single nucleotide polymorphisms (SNPs) in candidate genes. (14)

The search for SNPs primarily employs methods such as direct sequencing, restriction fragment length polymorphism (RFLP) analysis, and the chemical cleavage of mismatch (CCM) method using RNase or oligonucleotide-specific alleles for hybridization (ASO). These methods have different sensitivity ranges and require sophisticated reagents and equipment that increase their complexity and operational cost. (18)

The most commonly used technique for the detection of SNP is polymerase chain reaction (PCR) followed by digestion of restriction enzymes (PCR-RFLP). This technique is useful for diagnosis of point mutations, point deletions, and short insertions that generate or eliminate a restriction enzyme recognition site. The method is based on the detection of DNA fragments of different molecular weight or of different lengths, so that, by analyzing the polymorphisms in the size of the restriction fragments, it is possible to determine whether or not a mutation is present and thereby identify an individual's genotype. (19)

This article systematically reviews publications about genetic polymorphisms associated with the development of GC. To do this, a literature search was carried out. It included books and original scientific articles and verified the topic and technical reports. Documents consulted were published between October 2014 and April 2015. The published reports were identified using key words in English and Spanish: gastric cancer, genetic polymorphisms, interleukin genes, tumor necrosis factor, DNA repair, cadherin encoder, epidermal growth factor receptor, glutathione S-transferase encoder, and CYP1A1 and CYP2E1 genes. In addition, bio-information data bases such as 1000 Genomes were consulted.

We also consulted the bibliographies cited in indexed articles. This process took into account the presence of allele variants in genes involved in the following processes:

1. Immunogenetic factors, such as the inflammatory response induced by cytokines
2. Intrinsic variability in proteins involved in DNA repair mechanisms
3. Variability of carried out by metabolic enzymes' responses to detoxification of carcinogenic compounds
4. Tumor suppression mechanisms and mechanisms involved in apoptosis
5. Cellular regulation mechanisms.

These processes are defined below through presentation of the genes involved and the studies of polymorphisms associated with the development of GC.

IMMUNOGENIC FACTORS: INFLAMMATORY RESPONSE INDUCED BY CYTOKINES

Cytokines are signaling proteins that contribute to inflammatory response and are key components in the pathogenesis of diseases such as cancer, metabolic disorders and inflammatory conditions. (20) The cytokines that are the most important actors in the inflammatory response and the main mediators of chronic inflammatory diseases include the families of interleukin 1 (IL-1) and tumor necrosis factor (TNF) family.

The cytokines of the IL-1 family are encoded by three different genes: IL-1A, IL-1B and IL-1RN. These in turn code for IL-1 α , IL-1 β , and the endogenous IL-1 (IL-1ra), respectively. (21) Polymorphisms present in the IL-1 β and IL-1RN genes are related to the development of GC. Those reported in the IL-1 β gene are associated with the inhibitory effect that cytosine has on acid secretion of the stomach. This inhibition facilitates colonization and infection by agents such as *H. pylori* as well as the genesis of pre-neoplastic states that can lead to the development of cancer. (22) Camargo has shown that IL-1 β levels in the gastric mucosa are higher than usual in patients infected with *H. pylori* and in carriers of the IL-1B511TT or IL-1RNA2-A2 genotypes. (23) For the IL-1RN gene, there is a report of a functional polymorphism involving five alleles which is produced by variations in the number of tandem repeats (VNTR) and which generates five possible alleles corresponding to the presence of 2, 3, 4, 5 or 6 replicates of 86 bp. Allele 1 has 4 replicates (412 bp), allele 2 has 2 replicates (240 bp), allele 3 has 3 replicates (326 bp), allele 4 has 5 replicates (498 bp), and allele 5 has 6 replicates (584 bp). The VNTR polymorphism is important for regulation of IL-1Ra levels, human immune response and cancer risk. (21, 24)

In Colombia, polymorphisms of the IL-1 β , IL-1RN and IL-10 genes have evidenced different levels of association with cancer development. A PCR-RFLP study of the IL-1 β gene that used Aval, Alul and TaqI restriction enzymes has analyzed polymorphisms at sites -511 (T/C), -31 (C/T) and +3954 (C/T). This study was performed with two groups of patients. One group had been diagnosed with chronic non-atrophic gastritis and gastric ulcers, both of which are classified as benign diseases and which are associated with normal or high levels of acid secretion in the stomach. The other group of patients had atrophic gastritis, metaplasia, dysplasia and gastric cancer, all of which are classified as premalignant and malignant diseases and which are associated with low acid secretion in the mucosa and which occur infrequently. (22) The analysis for benign diseases indicated that there is a significant difference in the frequency of the CC genotype at position -31 between H.

pylori infected patients (28.57%) and uninfected individuals (8.33%). This suggests that the C genotype may be related to a predisposition to H. pylori infection. However, the authors point out that these results neither support nor contradict the hypothesis that this genotype is related to the development of GC after a previous H. pylori infection has been reported. (22) In this regard, a study in Mexican populations has indicated that carrying the proinflammatory allele IL-1B-31 * C confers increased risk of development of gastric cancer or precancerous lesions. (25)

Patients with the TT genotype for the IL-1B-511 gene in Colombia had a positive association with cancer, as evidenced by a meta-analysis performed in Caucasian populations. (26) This association has not been reported in other Latin American countries such as Venezuela (21)

Contrary to what was observed in Colombian populations, a study in Venezuela found a significant association between the +3954 polymorphism and C allele carriers (CC + CT). (21) In spite of these results, the proinflammatory action and the association with the risk for GC of the IL-1B + 3954 polymorphism has not been clearly demonstrated since some reports show frequencies of the IL-1B + 3954T allele or the heterozygous IL-1B + 3954C/T genotype that are significantly higher in GC cases. Morán points out that the conclusions of a meta-analysis by Wang show that IL-1B + 3954T genotype is not associated with increased risk of development of GC. (21) This shows that results vary depending on the populations in which these polymorphisms are studied and that there is no clear consensus about their role in the pathogenesis of the disease.

Another study has used real time PCR with labeled primers to evaluate the intron 2-VNTR and -819, -1082 (G/A) polymorphisms of the IL-1RN and IL-10 genes in high and low risk GC regions in case groups of patients with histological diagnoses of chronic superficial gastritis, diffuse and normal antral gastritis, gastric cancer, and duodenal ulcers and in a control group. (26). The study showed that IL-10-1082AA is the most common the genotype in all of groups studied (control, GC and duodenal ulcer) but found no association between polymorphisms for this gene and susceptibility to GC. This contrasts with the study of Caucasian populations in which the AA genotype was associated with the development of pathology. Similar results stand out for non-GC associated polymorphisms in IL-10-819, but the distribution of genotypes was similar to that of Caucasian populations in which there is an association of the TT genotype with GC. (26)

Polymorphisms found for the IL-1RN gene were not associated with GC in contrast to what was reported in Brazil where genotype analysis of polymorphisms in a case and control study has demonstrated that the IL-1RN * 2 allele is the most frequent in patients with gastric ulcers and

adenocarcinomas. Carriers of this genotype have higher levels of IL-1B in the gastric mucosa than do those with the IL-1RN1/1 genotype and, therefore, have more severe and prolonged immune responses. (27)

Another group of proinflammatory defensive cytokines is the TNF family. There are two types: tumor necrosis factor alpha (TNF- α) and tumor necrosis factor beta (TNF- β).

TNF- α is an essential cytokine within the normal mechanisms of innate and acquired immunity which has antitumor, antiviral and antimicrobial activity and which also induces tissue growth, tissue differentiation and play a role in immune system regulation. Overexpression of TNF- α consequent to chronic inflammatory and autoimmune diseases can be explained by the presence of certain polymorphisms in its gene. (28) One of the most studied allele variants of this gene is the transition from G to A found at the -308 position of the gene promoter. This polymorphism sometimes generates increased expression which causes greater damage to the gastric tissue. (1)

In Latin America, studies using PCR-RFLP (NcoI) by Torres (1) and Martínez (26) in Colombia and Partida (29) in Mexico have shown an absence of any relationship between the -308 polymorphism of promoter (G/A) and the development of GC. In contrast to these results, in Caucasian populations there is a significant association of the A allele and the A/A genotype with gastric cancer, a result that shows that carriers of the A allele have increased production of TNF- α . This excess can cause severe inflammation in the gastric mucosa that increases the risk of cancer. (30)

Like TNF- α , TNF- β is a cytokine of great importance in immunological and inflammatory processes with cytotoxic activity against tumor cells. Important SNPs have been described for the encoding gene. Among them, a substitution of G for A at position p252 in the first intron is associated with TNF- β overexpression. (31) In the Mexican population, including patients with non-atrophic gastritis, intestinal metaplasia, gastric cancer, duodenal ulcers and asymptomatic patients, the evaluation of the FNT- β p252 (G/A) polymorphism by PCR-RFLP indicates a significant association of the A allele with GC. This fact suggests that the presence of SNP selectively favors the development of severe gastric neoplastic lesions. (29)

This study included an analysis of polymorphisms in genes encoding HSP70 proteins which are considered to be immunogenic factors related to inflammatory and diseases and tumors such as GC. HSP70 proteins act as chaperones of antigenic peptides derived from tumor cells which lead to antitumor immune recognition by cytotoxic T lymphocytes. (32) HSP70-1 (-p190 G/C), HSP70-2 (-p1267 A/G) and HSP70-HOM (-p2437 T/C) polymorphisms showed only the C p190 allele of the HSP70-1 gene, a significant association with GC or with non-atro-

phic gastritis with intestinal metaplasia, a fact that supports the C/C genotype as a possible risk factor. Although polymorphisms in the HSP70-2 and HSP70-HOM genes showed no association with GC, the combined analysis of the frequencies of various genotype combination of SP70 that HSP70-1G/G, HSP70-2A/G and HSP70_HOM T/T were absent in all patients with gastric cancer which suggests that this combination could be a protective factor against the development of this pathology. (29)

INTRINSIC VARIABILITY OF PROTEINS INVOLVED IN DNA REPAIR MECHANISMS

Cells are constantly exposed to a wide variety of genotoxic agents from both endogenous and exogenous sources, hence DNA repair pathways are responsible for maintaining the integrity of the genome. Repair gene XRCC1 (X-ray repair cross-complementing Group 1) is essential in the processes of basic cleavage repair and repair of single-strand breaks. More than 60 validated XRCC1 single nucleotide polymorphisms are listed in the Ensembl database. The best known are changes in exon 6 (Arg194Trp) and in exon 10 (Arg399Gln). Polymorphisms in this gene can produce changes in conserved proteins that alter repair capacity, thus increasing susceptibility to diseases, including cancer. (33)

Another important gene in the DNA repair pathway is that which codes for XRCC3 (X-ray repair cross-complementing Group 3). It acts on breakages of double-stranded DNA and interacts and stabilizes Rad51, a component of the pathway of homologous repair. It repairs lost information at a breakage site and thus prevents chromosomal aberration. The main polymorphism of this gene involves a change of threonine to methionine at codon Thr241Met in exon 7. Although little is known about the functional consequences of this variation, some studies have observed a positive relationship between this polymorphism and increased risk of breast, lung and skin cancer. (34)

The Duarte case and control study of Brazilian patients with chronic gastritis, gastric adenocarcinoma analyzed polymorphisms of the XRCC1 and XRCC3 genes involved in DNA repair mechanisms. (35) The Arg194Trp, Arg399Gln polymorphisms of XRCC1 and the Thr241Met of XRCC3 were identified using PCR-RFLP technique with the restriction enzymes MspI and NlaIII. The results did not show any associations between polymorphisms and increased risks of chronic gastritis and gastric cancer, but there was increased risk of acquiring chronic gastritis when the Arg194Trp, Arg399Gln and Thr241Met alleles were found in one patient. This supports the hypothesis that these three polymorphisms together have an additive effect. The authors say the presence of these polymor-

phisms leads to reduced repair capacity since mutations accumulate in the DNA of the epithelial cells of the stomach because of the inflammatory process caused by *H. pylori* or the influence of environmental factors. (35)

Contrary to what was observed in Brazilian patients, Qiao's study of a Chinese population identified an association of two allele variants in the XRCC1 gene (an A/G substitution at position 280 of exon 9 (Arg/His) and a G/A substitution at position 399 of exon 10 (Arg/Gln)) with susceptibility to gastric cancer. (36)

The results of these studies show that the presence of polymorphisms in the XRCC1 and XRCC3 repair genes is associated with the development of GC, and their combined contribution can increase susceptibility to this disease and other cancers. The authors agree on the importance of including more genetic polymorphisms on the DNA repair pathway to verify gene-gene interactions which may be essential in the etiology of the disease. (35,36)

VARIABILITY OF RESPONSES TO DETOXIFICATION OF CARCINOGENIC COMPOUNDS BY METABOLIC ENZYMES

Cytochrome P450 monooxygenases (CYP1A1, CYP2E1) are known as the most important isoenzymes in the phase I metabolism. They protect against cancer through detoxification of numerous potentially cytotoxic/genotoxic compounds and are involved in the biotransformation of xenobiotics such as polycyclic aromatic hydrocarbons (PAHs), nitrosamines and dioxins such as those in tobacco smoke. (37) Of the phase II enzymes, glutathione S-transferases (GST) are responsible for the detoxification of potentially carcinogenic compounds. (1)

Most of the enzymes involved in the metabolism of xenobiotics are polymorphic and have been associated with differentiated susceptibility to cancer. The genes encoding the three major GST isoenzymes for phase II metabolizing enzymes (GSTP1, GSTT1 and GSTM1) are extensively expressed through the gastrointestinal tract and are highly polymorphic. Two common deletion polymorphisms in the GSTT1 and GSTM1 genes that result in absence of the active protein have been studied most and are associated with a high risk of GC due to poor ability to detoxify carcinogenic compounds. (38, 39)

A case control study of Colombian patients with gastric adenocarcinoma used PCR-RFLP to investigate homozygous deletions of GSTM1 (GSTM1-0) and GSTT1 (GSTT1-0) for the presence or absence of a band of 480bp in GSTT1 and the presence or absence of a 215bp band in GSTM1. (1) In the analysis, the frequency of the GSTM1 deletion polymorphism was higher among patients with GC than among control patients. The GSTM1 deletion

polymorphism results in decreases or suppression of the synthesis of the enzyme involved in detoxification of chemical compounds such as polycyclic aromatic hydrocarbons and nitrosamines, found in cigarette smoke, food, agrochemicals and antineoplastic drugs. (1) The same study found no association of the GSTT1 deletion polymorphism with GC which corresponds with the results of studies in Spanish populations. (1, 39) This suggests an influence of the ethnic group or exposure to different environmental factors on the frequency of the polymorphism.

Other phase I metabolism enzymes for which harmful polymorphisms have been found include those expressed by the CYP1A1 and CYP2E1 genes. The CYP1A1 gene there are two non-synonymous polymorphisms associated with the development of cancer: a transition from T to C resulting from cutting with by the MspI enzyme to produce the CYP1A1 * 2A mutant allele (commonly called m2 allele), and a substitution of A for G at codon 462 in exon 7 (Ile462Val, rs1048943). It is thought that these variations may alter the expression and function of CYP1A1 and potentially influence the balance between metabolic activation and toxic substance detoxification which ultimately leads to susceptibility to cancer. (40) The RsaI/PstI variant of the CYP2E1 gene has been said to lead to a substitution of C for T in which the wild-type allele corresponds to CYP2E1 * 5A and the CYP2E1 * 5B mutant allele (commonly called c2 allele) causing polymorphism of an overexpression of the gene associated with the development of neoplastic processes. (41)

A case and control study of Colombian patients with gastric neoplasia by Castaño reported polymorphisms in CYP1A1 (alleles m1 and m2) and CYP2E1 (alleles c1 and c2). (42) The results for the CYP1A1 gene, indicated that there was no association between the polymorphisms and the development of GC, whereas for the CYP2E1 gene it was reported that the c2 allele in heterozygous or homozygous condition had a significant association with the risk of GC. Susceptibility increased for the three genotypes of this polymorphism when it was associated with smoking. Other studies, such as that by Gao have indicated that homozygous and heterozygous individuals with this polymorphism have increased risks when they smoke and consume large amounts of meat. (43)

In contrast to these results, individuals with a homozygous genotype of the c2 allele in Costa Rica were found to have a lower GC frequency indicating that this allele could act as a protective factor against this neoplasm. (44) It is possible that this genotype protects against GC because it has greater transcriptional activity and, consequently, greater capacity to detoxify substances. The authors of the study considered that other possible risk factors such as age and place of origin should be taken into account since they

could influence association of this polymorphism to the risk of GC.

TUMOR SUPPRESSION MECHANISMS INVOLVED IN APOPTOSIS

Cell division is essential for the normal development of tissue. It requires a series of regulatory proteins that control all phases of the cell cycle. Virtually all types of cancer alter the pathways that govern the cycle and are critical to cell growth. At different stages of the cycle, detection of DNA damage or replication errors can lead to cycle arrest and apoptosis, a mechanism that prevents the accumulation of mutations in DNA with oncogenic potential. Apoptosis is performed by a number of proteins including that encoded by the TP53 gene. (45, 46)

Human nuclear phosphoprotein p53 functions as a transcription factor. It forms a tetrameric complex that recognizes a specific DNA sequence and stimulates the transcription of various genes. It is one of the best known barriers against malignant transformations and constitutes the central component of a system that rapidly destroys cells that have been damaged and have become harmful to the organism. (47, 48) The p53 polymorphism in exon 4, codon 72, has been studied most. It codes for an arginine (Arg) or a proline (Pro). This -342 C/G polymorphism at codon 72 can be identified by PCR-RFLP, with the restriction enzyme BstUI. (49)

Reports indicate that p53 protein encoded by the Pro allele does not efficiently transcript and therefore has diminished repair functions which can trigger neoplastic processes. (47) Studies of the association of this polymorphism with susceptibility to cancer and progression of cancer present results that vary according to ethnicities. There was no association of the Pro/Pro polymorphism with the risk of developing GC in Colombian and Costa Rican populations, (49, 50) but populations in Japan had positive associations of the Pro/Pro genotype with the risk of developing diffuse GC which makes this polymorphism a biomarker for susceptibility to this type of cancer. (51)

Investigations of tumor suppression mechanisms in GC currently also include analysis of genes such as CDH1, an encoder of E-cadherin, which plays a key role in the establishment and maintenance of intercellular adhesion and epithelial architecture. (52)

A study of Mexican patients indicates that a polymorphism in the promoter region of the CDH1 gene (-160 C/A) is associated with a loss of E-cadherin functioning which is related to diffuse familial GC and hereditary diffuse GC. (51) On the other hand, Steinberg (53) highlights the identification of alterations in exon regions 7, 8 and 9 of the E-cadherin gene in patients with gastric tumors from several ethnic groups (African American, Asian, Caucasian

and Hispanic) which is relevant for characterization of potential biomarkers of oncogenicity. The results show three SNP type insertions in exon 8 in Hispanic patients and polynucleotide insertions in the same exon in Asian and Caucasian patients. The authors believe that the insertions originate during carcinogenesis and could be markers of tumor progression. (53)

MOLECULAR MECHANISMS OF CELL REGULATION

Cell growth begins by the binding of a signaling product, a growth factor, to a specific receptor. This is why receptors are important regulators of cellular functions such as cell growth and differentiation. (54)

One family of receptors, the HER receptor family, is characterized by tyrosine kinase activity. These receptors play a crucial role in the control of basic cellular processes such as proliferation, migration, metabolism, cell differentiation and survival. They also play a role in regulation of intercellular communication during processes that play an important role in the development of cancer. (55)

The HER tyrosine kinase family includes four receptors: HER-1, also called epidermal growth factor receptor (EGFR/ERbB1), HER-2 (ERbB2), HER-3 and HER-4. (55) EGFR plays a key role in the control process of many cells, so its abnormal expression is closely related to a wide variety of malignant tumors. (56) Studies of the EGFR gene have identified a polymorphic variant derived from a substitution of G for A which leads to a change from an arginine to a lysine at codon 521 in the extracellular subdomain of the gene. This variant is also known as EGFR-R521K. It is thought that the EGFR-R521K mutant allele may attenuate the ligand binding function, growth stimulation, tyrosine kinase activity and the induction of some proto-oncogenes. (57) On the other hand, studies indicate that the HER-2 membrane receptor is overexpressed in between 13% and 25% of GC patients and that overexpression occurs more frequently in intestinal tumors and in tumors of the esophago-gastric junction. (52) The most investigated variation of this gene is a polymorphism called HER2-I655V which is located in the coding region of the transmembrane domain. It results in a transition from A to G and is associated with the risk of developing cancer since it can destabilize the formation of the receptor's active dimers. (58)

A Mexican case and control study of patients with gastric cancer, premalignant gastric lesions (intestinal metaplasia and atrophic gastritis) and non-atrophic chronic gastritis identified the R21K polymorphism in the EGFR gene and the I655V A/G polymorphism in the ERBB2 gene. The study observed that the frequency distribution of genotypes of both polymorphisms does not differ significantly across the three groups and concluded that they are not

associated with the development of GC in the study population. (59) In addition to the R21K polymorphism, Yang has reported the presence of +2073 A/T SNP in exon 16 in Chinese populations. (60) In a stratified analysis of patients with GC, the T allele was also reported to be significantly associated with an increase of metastasis of lymph nodes.

Given the importance of the EGFR gene in the development of neoplastic processes, there are research reports on kinase domain coding sequences (exons 18-21) and interphase fluorescence in situ hybridization (FISH) analysis of chromosome 7 at position 7p12. The Moutinho study of Portuguese patients diagnosed with GC revealed structural alterations of this receptor, with the presence of missense mutations in exons 20 and 21 of the EGFR gene. (61) One of the mutations in exon 20 (C/T) substitutes alanine for valine. A second mutation in exon 21 (A/G) involves substitution of asparagine for aspartic acid. In addition, silent mutations of exon 20 were identified in a single member of the case group, but were absent in the control group (2301C> T Ala767Ala, 2415 C> T His805His). However, these mutations have not been reported in other studies. Given their location in the kinase domain of EGFR, it is possible to infer that they may affect receptor activity. (61)

FISH analysis of patients with the pathology showed increased numbers of copies in cases of gene amplification and polysomy of chromosome 7. (61) Phenomena of gene amplification suppose a duplication of DNA sequences which creates multiple copies of a gene which may contribute to neoplastic development. Correspondingly, the correlation of polymorphisms and FISH analysis with clinical parameters showed an association between alterations in EGFR (mutation/amplification) and tumor size which was significant in the case of carcinomas that were already invading the basal membrane and spreading to the gastric wall. This suggests that alterations in this gene could lead to invasive behavior of neoplastic cells. Similar results have also been observed in HER-2 where gene amplification also occurs in cases of advanced GC. (62)

These reports of associations demonstrate that the roles of the different polymorphisms that have been identified in the EGFR and HER-2 genes in various geographic areas provide valuable guidance for genetic diagnosis of GC which could become useful for evaluating the prognosis of patients with this disease. (59-62)

Recent advances in sequencing technology have favored the development of global databases of the sequencing of genomes of a large number of people in many populations around the world as a source of knowledge about human genetic variation. Such is the case of the 1000 Genomes project (<http://www.1000genomes.org/>).

Table 1 summarizes the principal polymorphisms of the genes discussed above that are associated with the develop-

ment of GC and includes frequencies of the alleles of each variant in the five study populations of the 1000 Genomes project: American (AMR), African (AFR), East Asians (EAS), European (EUR) and South Asians (SAS).

As for the genes involved in the inflammatory and immune response, there are reports of three types of variants of IL-1 β which are associated with GC: -31C> T (rs1143627), -511T> C (rs16944) and + 3954C> T (rs1143634). According to the allele frequencies reported for the American population, the most frequent alleles are C (55%), T (55%) and C (87%) respectively, with identification of the lowest frequencies of these alleles in the European population. On the other hand, the IL-10 gene reported variants are -1082A> G, (rs1800896), and -819C> T (rs1800871). Allele frequencies for the American population are A (70%) and C (67%), with low frequencies in European and East Asian populations. Another proinflammatory cytokine of importance in apoptosis is TNF, a gene for which there are reports of variants such as -308G> A (rs1800629) and -252A> G (rs900253) with the G allele being more common in America, with frequencies of 93 % and 68%, respectively.

As for genes involved in basal cleavage repair and homologous repair pathways that maintain DNA stability, the XRCC1 gene has -280G> A (rs25489), -194C> T (rs1799782) and - 399G> A (rs25487). The highest frequencies are found in American populations for alleles G (94%), C (88%) and G (88%), respectively while lower frequencies are found in the European population. On the other hand, the XRCC3 gene, with the -241C> T variant (rs861539) shows the highest frequency of the C allele (77%) in the American population.

In the cytochrome P450 xenobiotic metabolism pathway, CYP1A1 presents, among others, the following polymorphic variants: -6235T> C (rs4646903) and -462A> G (rs1048943) with the highest frequencies of alleles T (59%) and A (65%) in America, and lower frequencies in populations of Europe and Africa. The CYP2E1 gene with variant 1053C> T (rs2031920) has the highest frequency for the C allele (88%) in America but also has high frequencies in other populations.

The TP53 gene is important in cell cycle and apoptosis. It has a widely studied variant, -342C> G (rs1042522) whose C allele has the highest frequency (68%) in America, while the African population has the lowest frequency of this allele.

Table 1 shows two variants for the E-cadherin encoding gene (CDHE) which are involved in signaling and cellular communication mechanisms: -160C> A (rs16260) and -1018A> G (rs116093741). The American population shows the highest frequency of the C allele of variant rs16260 while the studies reveal no presence of the G allele

in variant rs116093741 in the populations analyzed where there is 100% fixation of the A allele.

Studies of HER-1 and HER-2 receptor tyrosine kinase coding genes identified -521G> A variants (rs2227983) for the HER-1 gene, with high frequencies of the G allele in American populations and lower frequencies in East Asia. The -655A> G variant (rs1136201) for the HER-2 gene has its highest frequency (86%) in America.

Finally, genes encoding heat shock proteins involved in the cellular response processes include HSP70 with the -190G> C (rs1043618) and + 2437T> C (rs2227956) variants whose G and T alleles have the highest frequencies in the American population. In addition, variants identified in genes encoding HSP90 proteins included polymorphisms such as HSP90AA1, -C> T (rs4947), HSP90AB1, -C> T (rs13296) and HSP90AB1, -C> T (rs13296).

CONCLUSIONS

The study of the factors involved in the development of gastric cancer (GC) is key to understanding the etiology of cancer as well as to identification of populations of individuals who are most susceptible to the disease and who require early monitoring, care and treatment.

In the study of polymorphisms in genes involved as immunogenic factors, it should be mentioned that those reported for interleukin 1 β (IL-1 β), IL-1RN and IL-10 show different levels of association with the development of cancer. The CC genotype at position -31 of the IL-1 β gene is primarily related to a predisposition to *H. pylori* infections in diseases such as chronic non-atrophic gastritis and gastric ulcers. The TT genotype for the IL-1B-511 gene is associated with the development of cancer, and the IL-1RN * 2 allele is common in patients with gastric ulcers and adenocarcinomas. The presence of the A allele of tumor necrosis factor alpha (TNF- α) p308 of the promoter and TNF- β p252 A allele selectively favor the development of severe gastric neoplastic lesions.

For genes involved in DNA repair mechanisms, we can observe the joint association of the Arg194Trp, Arg399Gln and Thr241Met polymorphisms of the XRCC1 and XRCC3 genes with increased risk of acquiring chronic gastritis. Susceptibility markers for GC risk includes the A/G substitution at position 280, exon 9 (Arg/His) and the C/A substitution at position 399, exon 10 (Arg/Gln) in the XRCC1 gene.

The study of genes encoding metabolic enzymes responsible for detoxification of carcinogenic compounds reveals that deletion of GSTM1 has a significant association with the development of GC. This is due to decreases or suppression of synthesis of this enzyme which is responsible

Table 1. Detailed information on 12 genes that are associated with the development of gastric cancer and that signal metabolic pathways, risk factors, variants or polymorphisms and allele frequency according to data provided by the 1000 Genomes project.

Gene	Metabolic Pathway	Risk Factors	Variants	Alleles	AMR %	AFR %	EAS %	EUR %	SAS %
IL-1 β	Inflammatory response	Risk of GC after infection by H. pylori	IL-1 β , -31C>T (rs1143627)	C	55	63	48	35	60
			IL-1 β , -511T>C (rs16944)	T	45	37	72	65	40
			IL-1 β , +3954C>T (rs1143634)	T	55	57	47	35	60
				C	45	43	53	65	40
				C	87	88	98	75	85
				T	13	12	2	25	15
IL-10	JAK-STAT signaling pathway	Smoking	IL-10, -1082A>G, (rs1800896)	A	70	69	95	55	76
	Inflammatory response			G	30	31	5	45	24
	Cytokine-cytokine receptor interaction	Infection by H. pylori	IL-10, -819C>T (rs1800871)	C	67	56	32	76	54
				T	33	44	68	24	46
FNT	Immune and inflammatory response Apoptosis	Infection by H. pylori Alcohol consumption	FNT, -308G>A, (rs1800629)	G	93	88	94	87	95
			FNT, -252A>G, (rs900253)	A	7	12	6	13	5
				A	32	33	32	46	48
				G	68	67	68	54	52
XRCC1	Base cleavage repair	Radiation Ionization	XRCC1, -280G>A, (rs25489)	G	94	97	90	94	90
				A	6	3	10	6	10
		Alkylating agents	XRCC1, -194C>T, (rs1799782)	C	88	93	72	95	89
				T	12	7	28	5	11
		Smoking	XRCC1, -399G>A, (rs25487)	G	88	93	72	95	89
		A	12	7	28	5	11		
XRCC3	Homologous recombination	Radiation Ionization Alkylating agents Smoking	XRCC3, -241C>T, (rs861539)	C	77	81	93	61	79
				T	23	19	7	39	21
Cytochrome P450, family 1, subfamily A	Metabolism of xenobiotics by cytochrome P450 Oxidative stress	Smoking	CYP1A1, -6235T>C, (rs4646903)	T	59	77	57	89	66
			CYP1A1, -462A>G, (rs1048943)	C	41	23	43	11	34
				A	65	99	75	97	87
				G	35	1	25	3	13
Cytochrome P450, family 2, subfamily E	Metabolism of xenobiotics by cytochrome P450 Tryptophan metabolism	Smoking	CYP2E1, -1053C>T, (rs2031920)	C	88	100	80	96	99
				T	12	0	20	4	1
TP53	p53 pathway Cellular cycle	Age Cancer stage	TP53, -342C>G, (rs1042522)	C	68	33	59	71	51
				G	32	67	41	29	49
CDHE	Cellular Communication CAM	Family History of GC	CDHE, -160C>A, (rs16260)	C	75	87	69	72	75
			CDHE, -1018A>G, (rs116093741)	A	25	13	31	28	25
				A	100	100	100	100	100
				G	0	0	0	0	0
EGFR, HER-1	ECM interaction receptor	Smoking Gender	EGFR, -521G>A, (rs2227983)	G	67	94	48	72	65
				A	33	6	52	28	35
ERBB2, HER-2	Mitogen-activated protein kinase Phosphatidylinositol-3 kinase	High BMI/obesity	ERBB2, -655A>G, (rs1136201)	A	86	99	88	75	87
				G	14	1	12	25	13
HSPA1A, HSP70-1	Cellular response to heat stress Endocytosis Ubiquitin Proteasome Pathway	Advanced stage	HSP70-1, -190G>C, (rs1043618)	G	63	20	71	61	59
			HSP70-HOM, +2437T>C, (rs2227956)	C	37	80	29	39	41
				T	92	99	77	84	84
				C	8	1	23	16	16
HSP90AA1	Cellular response to heat stress	Advanced stage	HSP90AA1, -C>T, (rs4947)	C	16	72	20	16	17
				T	84	28	80	84	83
HSP90AB1	Cellular response to heat stress		HSP90AB1, -C>T, (rs13296)	C	64	81	71	68	63
				T	36	19	29	32	37
HSP90B1	Cellular response to heat stress		HSP90B1, -C>G, (rs2070908)	C	56	61	46	65	47
				G	44	39	54	35	53

% Allele frequencies, 1000 Genomes Project Phase 3.

AMR: Americans; AFR: Africans; EAS: East Asians; EUR: European; SAS: South Asians; CDHE: E-cadherin encoding gene; GC: gastric cancer; ECM: extracellular matrix; EGFR: epidermal growth factor receptor; ERBB2: tyrosine kinase 2 receptor; TNF: tumor necrosis factor; HSP90AA1: 90-KD heat shock protein, alpha, class A, member 1; HSP90AB1: heat shock protein 90-KD, beta, class A, member 1; HSP90B1: 90-KD heat shock protein, beta 1; IL-10: interleukin; BMI: body mass index; TP53: p53 tumor protein; XRCC1: Cross-complementing X-ray repair complement group 1; XRCC3: Cross-complement X-ray repair complement group 3.

for detoxification of chemical compounds. On the other hand, the presence of the C2 allele in either heterozygous or homozygous conditions in the CYP2E1 gene is related to susceptibility to the risk of GC associated with smoking. Contradictions regarding this polymorphism indicate that the C2 allele may act in other cases as a protective factor against this neoplasia.

The Pro allele and Pro/Pro genotype in the TP53 gene which encodes the P53 protein are responsible for tumor suppression and apoptotic mechanisms. They have been reported as biomarkers for susceptibility to diffuse-type GC. Similarly, familial and hereditary diffuse GC is associated with a polymorphism in the promoter region of the CDH1 gene (-160 C/A), and there are reports of altered exon regions of this gene in Hispanic patients with several types of GC.

Finally, studies of receptors with tyrosine kinase activity, which are key for control of basic cellular processes, report that mutations in the EGFR gene and gene amplification phenomena have a significant association with tumor size because they confer invasive behavior on neoplastic cells.

In this way, this documentary review of various published reports includes some studies of the numerous polymorphisms located in genes that are involved in the development of GC. Contradictions that exist among these reports indicate the importance of studies of genetic factors in different genes and in various populations, geographic areas and lifestyles in order to conclusively determine the role of the different allele variants in the development of GC. This is paramount in epidemiological research for the identification of genetic markers of susceptibility and/or prognosis.

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