Genotype B of Killer Cell Immunoglobulin-Like Receptor is Related with Gastric Cancer Lesions

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NK cells are important in innate immunity for their capacity to kill infected or cancer cells. The killer cell immunoglobulin-like receptors (KIR) are a family of polymorphic genes with inhibitory and activating functions. The main driving force for gastric cancer (GC) development is a chronic response, which causes an increase of NK cells in the gastric mucosa. The aim of this work was to study polymorphisms in KIR genes in patients with either GC or non-atrophic gastritis (NAG). We studied 242 patients (130 with NAG and 112 with GC) and contrasted with 146 asymptomatic individuals. We analyzed diversity in the content and localization of KIR genes in the different clinical groups studied. Four activating and one inhibitory genes were associated with GC: 2DS1 (OR 3.42), 2DS3 (OR 4.66), 2DS5 (OR 2.25), 3DS1 (OR 3.35) and 2DL5 (OR 3.6). The following were also found as risk factors for GC: Bx genotype (OR 4.2), Bx-Bx centromere-telomere (OR 2.55), cA01|cB03 (OR 36.39) and tB01|tB01 (OR 7.55) gene content and three B motifs (OR 10.9). Polymorphisms in KIR genes were associated with GC and suggest that mutated NK cells may contribute to GC development by increasing gastric mucosa inflammation, leading to constant tissue damage.

NK cells represent a subset of lymphoid cells that are components of innate immunity acting as first line of defense against viral infection and other pathogens, and in the early cellular transformation and tumor surveillance. The functions of NK cells are partly regulated by the family of KIR receptors (killer cell immunoglobulin-like receptor) coded by 11 genes (2DL1, 2DL2/2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS4, 2DS5/2DS3, 2DL3/3DS1, 3DL2 and 3DL3) and two pseudogenes (2DP1 and 3DP1) located on the chromosome 19q13.42–4. Some of these genes may present sequence variations; thus, it has been reported a 22 bp deletion in the second extracellular domain of 2DS4 that affect substantially the sequence of amino acids, whereas the exon 2 can be absent in 3DP1. Also, it has been found that 2DL5 gene is encoded by different loci (A and B). The KIR family is primarily expressed on NK cells, but they can also be expressed on CD4, CD8 and γδ T cells. There are four promoter types based on intermediate promoters (ProI), which are associated with distinct expression in KIR genes, thus ProI correlates with bi-directional promoters, whereas ProI is the first to be expressed by NK cells after Hemopoietic Stem Cell Transplantation. The 3DL3 is not expressed by circulating CD56 dim NK cells, and 2DL4 is expressed by CD56 bright and dim NK cells in a non-variegated manner; and finally, the remaining KIR genes are expressed by CD56 dim NK cells. T cells express 3DL2 more than other KIR genes, probably as a result of ProI activation earlier in the development of T cell. In addition, the KIR gene family has bi-directional promoters, which control variegated expression, whereas ProI correlates with protein expression.

Composition of KIRs may be complex, thus, two haplotypes (A and B) and genotypes (AA and Bx, where x can be A or B) have been reported for KIR based on gene content (Fig. 1). Actually, there are over 500 different Bx genotypes (http://www.allelefrequencies.net). KIR genotype AA is homozygous for the A haplotype, which is an inhibitory haplotype formed by the loci 3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, 2DS4 and 3DL2; whereas Bx genotype has either one (AB heterozygous) or two (BB homozygous) B haplotypes, and is an activator haplotype (formed by 3DL3, 2DS2, 2DL2, 2DL5B, 2DS3/2DS5, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A,

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the main risk factor to develop gastric cancer (GC) and duodenal ulcer. Different immune cells are involved in the innate and adaptive system such as T lymphocytes and natural killer (NK) cells have a critical role in the regulation of the immune response.

In this work we aimed to study polymorphisms in KIR receptors genes and identify any possible association with GC.

2DS3/2DS5, 2DS1, 2DS4 and 3DL2 genes. The A haplotype usually has a fixed number of genes, while B has a variable gene content with additional activating KIR genes. KIR haplotypes consists of two regions, the centromeric region from 3DL3 to 3DP1, and the telomeric region from 2DL4 to 3DL2; and both regions can be cenA or cenB, and telA or telB depending on the haplotype. Based on the gene content 9 centromeric regions (cA01, cA02, cA03, cB01, cB02, CB03, CB04, CB05 and CB06) and 8 telomeric regions (tA01, tB01, tB02, tB03, tB04, tB05, tB06 and tB07) have been described. KIR B haplotype can also be classified according to B content genes, and B content score is calculated by adding the number of cenB and/or telB motifs in each genotype.

Helicobacter pylori (H. pylori) infects the gastric mucosa of over 50% of the world population and represents the main risk factor to develop gastric cancer (GC) and duodenal ulcer. Different immune cells are involved in the development of gastric pathologies by causing a chronic, unregulated mucosal inflammation. Thus, cells of this work may have an activating receptor (KIR A) and/or an inhibitory receptor (KIR B) depending on the haplotype.

The frequency data obtained was analyzed between groups to determine differences in KIR genes between asymptomatic and disease groups. The framework genes of centromeric (3DL3 and 3DP1) and telomeric (2DL4 and 3DL2) regions were present in 100% of the three groups studied. The frequencies of 2DL1 (99.3%, 99.2% and 100%), 2DL3 (97.9%, 96.9% and 92.9%), 2DS4 (93.8%, 100% and 88.4%) and 2DP1 (99.3%, 99.2% and 100%) genes were not statistically different among the groups (Asymptomatic, NAG and GC, respectively). The KIR genes with a significant association with disease are shown in Table 2. When compared with healthy controls, most of the activating and inhibitory genes studied were found significantly associated with either NAG or GC, whereas some showed an increasing tendency of association from NAG to GC, like 2DS1 (OR of 2.56 to OR 5.45), 3DS1 (OR of 3.52 to OR 4.75) and 2DL5 (OR of 3.77 to OR 6.21). In contrast, 2DS3 presented a significantly decreasing tendency of association from NAG to GC, (OR of 187.7 to OR 24.98). 2DS2 and 2DL2 showed significant association with NAG (OR of 3.75 for the two genes), whereas 3DL1 was associated with protection for GC (OR 0.61). The above associations remained significant in a multivariate model of analyses (Table 3), where it can be observed that age was constantly associated with risk for GC, and H. pylori and gender for NAG.

Results
The characteristics of the population studied are described in Table 1. We studied two groups of patients, one with a diagnosis of NAG and the other with GC, formed by 130 and 112 patients respectively, and both were compared with an asymptomatic group (n = 146). It can be observed that the NAG group showed significantly higher seroprevalence to H. pylori and to CagA, with an OR of 3.23 and 2.74, respectively, as compared to the asymptomatic patients.

KIR genes. In order to characterize the KIR genotype frequencies in the study groups, genomic DNA was isolated from peripheral blood leukocytes, and the KIR genes responsible for the activating signals (2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 2DL2, 2DL3, 2DL4, 2DL5, 3DL1, 3DL2, 3DL3), and the two pseudogenes (2DP1 and 3DP1) were genotyped using single specific primer-polymerase chain reaction (SSP-PCR). KIR genotypes were assembled according to the presence or absence of each gene locus.

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![Figure 1. Composition of KIR haplotypes (A and B) based on gene content. KIR genes are tightly organised head-to-tail over approximately 150 kb within the Leukocyte Receptor Complex (LCR). Inhibitory KIR genes are shown in white, activating genes in black and pseudogenes in gray.](image-url)
multivariate analyses showed that 2DS1, 2DS3, 2DS5, 3DS1 and 2DL5 were found as risk for non-atrophic gastritis to gastric cancer (P < 0.00001).

### Table 2. Distribution of KIR genes giving significant differences between clinical groups. a = number of subjects. b Comparisons were made using the asymptomatic group as the reference group. pc < 0.05 and OR (95% CI) were adjusted by age and gender. 'NS = not significant. 2DS2 = 1.13 (0.46–2.72), 2DL2 = 1.13 (0.46–2.72). c Linear trend analysis for 2DS1, 3DS1 and 2DL5 genes from asymptomatic to non-atrophic gastritis to gastric cancer (P < 0.00001).
but was not correlated with disease progression\textsuperscript{30}. The inhibitory genes have also been reported associated with other immune and infectious diseases; 3DL1 in combination with the HLA-B*57 allele showed a protective effect against progression to AIDS in Zambian patients\textsuperscript{31}, and 2DL2 was reported associated with rheumatoid arthritis\textsuperscript{28}.

In our population Bx genotype was frequent and associated with both gastric diseases, although the risk was stronger for NAG than for GC. In our patients Bx genotypes were a combination of A and B haplotypes, with very few B homozygotes, which is in agreement with studies in other human populations in America, including Amerindian groups\textsuperscript{32}. This high frequency of Bx genotype may have resulted from the selection by the infectious and chronic diseases that have been prevalent in our population for many generations; although this selection process may have caused an increased risk for GC in the region.

Our work shows eight KIR genes associated with gastric diseases, five of them were associated with risk for GC (2DS1, 2DS3, 2DS5, 3DS1, and 2DL5) and belong to B haplotype, which is an activator haplotype. This unexpected association might partially be explained by the pathogenesis of GC\textsuperscript{19}, which main risk factor is an infection. \textit{H. pylori} infection is strongly pro-inflammatory and invariably causes a chronic, decades-long inflammation of the gastric mucosa\textsuperscript{20}. In the context of a decades-long mucosal inflammation, NK cells may be constantly and chronically recruited and activated; until in some patients the regulation of this activation might be lost.\textsuperscript{21} Unregulated NK cell may help to increase inflammation leading to mucosal damage and development of precancerous lesions and eventually to GC\textsuperscript{19,22,30}. On the other hand, activating KIR haplotypes would have opposite effects on distinct malignancies depending on whether inflammation is or is not a major component of tumor pathogenesis\textsuperscript{33}. Although, it should be noted that an activator haplotype could also be expected to be associated with increased ability to eliminate tumors\textsuperscript{1,34}. In fact, it was reported that patients with metastatic colorectal cancer had complete response to FOLFIRI (5-fluorouracil, leucovorin and irinotecan) treatment when B haplotype was present\textsuperscript{35}. Interestingly, there was a strong association between the KIR B haplotype and p53 alteration in Basal cell carcinoma tumors, with a higher likelihood that KIR B carriers harbor abnormal p53\textsuperscript{34}.

| Gene | Variable | Non-atrophic gastritis | | Gastric cancer | |
|---|---|---|---|---|---|
| | | p | OR (95% CI)\textsuperscript{a} | p | OR (95% CI)\textsuperscript{a} |
| 2DS1 | H. pylori + male | 0.005 | 2.14 (1.25–3.65) | <0.0001 | 3.41 (1.87–6.22) |
| | ≥ 50 years | 0.013 | 2.01 (1.16–3.47) | <0.0001 | 8.09 (4.48–14.62) |
| 2DS2 | H. pylori + male | 0.016 | 2.33 (1.17–4.64) | NS | NS |
| | ≥ 50 years | 0.014 | 2.01 (1.15–3.51) | NS | NS |
| 2DS3 | H. pylori + male | 0.007 | 0.37 (0.20–0.80) | NS | NS |
| | ≥ 50 years | NS | <0.0001 | 8.57 (4.70–15.66) | NS |
| 2DS5 | H. pylori + male | 0.014 | 2.32 (1.18–4.56) | NS | NS |
| | ≥ 50 years | 0.009 | 2.08 (1.20–3.61) | <0.0001 | 8.90 (4.97–15.94) |
| 3DS1 | H. pylori + male | 0.011 | 2.04 (1.17–3.56) | <0.0001 | 8.37 (4.63–15.13) |
| | ≥ 50 years | 0.013 | 2.02 (1.16–3.53) | NS | NS |
| 2DL2 | H. pylori + male | 0.013 | 2.40 (1.20–4.40) | NS | NS |
| | ≥ 50 years | 0.013 | 2.02 (1.16–3.53) | <0.0001 | 8.75 (4.82–15.91) |
| 2DL5 | H. pylori + male | 0.037 | 2.10 (1.04–4.18) | NS | NS |
| | ≥ 50 years | 0.006 | 2.21 (1.26–3.87) | <0.0001 | 8.75 (4.82–15.91) |
| 3DL1 | H. pylori + male | 0.005 | 0.21 (0.07–0.63) | NS | NS |
| | ≥ 50 years | <0.0001 | 9.93 (5.46–18.05) | NS | NS |

Table 3. Multivariate logistic regression analysis of KIR genes associated with gastric disease. \textsuperscript{a}Comparisons were made with the asymptomatic as the reference group. \textsuperscript{b}Adjusted by the other independent variables. \textsuperscript{c}NS = not significant.
to A. Bx

protection from relapses, and an increased disease-free survival 18; which would suggest that the exacerbated function of the B haplotype contributes to the damage of the gastric disease. A B-score of zero was more frequent in asymptomatics and strongly protective for GC (OR 0.23), and a score of 3 increased the risk for GC almost 11 times (OR of 10.9).

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Concerning the distribution of A and B, we observed that A was more frequent in centromere and telomere of asymptomatic healthy adults, whereas B was more common in both, NAG and GC. The multivariate analysis confirmed a highly significant association of cA01|cB03 with GC, in fact this association (OR 36.39) was over 3 times higher than the association with age (OR 10.65 for >50 years old), which was usually the stronger factor. In addition, whereas tB01|tB01 was also a significant risk factor for GC, tA01|tA01 showed a significant association with protection. To our knowledge, there is no report describing the analysis of gene content in centromere and telomere regions and gastric cancer. Our analysis also confirms that the telomeric part of the KIR B genotype may have a role in the development of gastric diseases, particularly the cluster of genes 2DS1, 2DS3, 2DS5, 3DS1 and 2DL5, which showed a risk association with GC. In contrast, genes 2DS2 and 2DL2 were found as risk for NAG; these genes are located in the centromeric region of B haplotype, and have been reported with high linkage disequilibrium16. Thus, in our population the telomeric region of KIR B was more associated with GC, and the centromeric region with NAG. Within the B haplotype the telomeric region is more diverse, and probably the observed association with GC is due to an unbalanced response by the NK cell and a reduced ability to kill cancer cells. In contrast, the centromeric region is more conserve and the response of the NK cell is probably more balanced and efficient to fight cancer.

In order to determine the participation of B motifs in GC, we evaluated the B score in centromere and telomere, and the multivariate analysis confirmed its importance in GC, showing a trend following the course of the disease. A B-score of zero was more frequent in asymptomatics and strongly protective for GC (OR 0.23), and a score of 2 was associated with NAG, whereas a score of 3 increased the risk for GC almost 11 times (OR of 10.9). These results suggest that the exacerbated function of the B haplotype contributes to the damage of the gastric mucosa, favoring the development of GC. The B-score was previously evaluated in patients with acute myelogenous leukemia, where patients receiving transplant from donors with a B-score of 2 or greater showed a better protection from relapses, and an increased disease-free survival36; which would suggest that the exacerbated function of B results in an efficient response against leukemia.

On the other hand, the A genotype was associated with protection for GC, although only the 3DL1 gene showed a significant protection. This gene is within the telomeric region of A genotype and probably the observed association could be due to the strong linkage disequilibrium that 3DL1 has with the other genes of the A genotype.

It was recently reported that tumor-infiltrating NK cells were decreased in human GC; moreover, the production of IFNγ and TNFα by these cells was impaired by tumor-associated monocytes/macrophages via TGFβ38. In contrast, patients with GC had a better survival when they presented higher concentrations of NK cells, an effect that was more evident in advanced stage cases37. Our work associates the risk to develop GC with the B KIR genotypes and the gene cluster included within the telomeric part. There is a need to better understand the functional role of the diversity in KIR genes content in GC, together with the participation of other factors involved in GC development, such as peptides derived from cancer that are presented by HLA class I molecules to KIR receptors39. Since HLA molecules are ligands of NK cells, they regulate the variation in immune responses to different antigens by selection and suppression/activation of NK cells, and have a relevant role in the combat against GC38. However, for the eight genes that presented association with NAG and GC in our study, only three ligands are known (2DS1-C2, 2DL2-C1 and C2, 3DL1-Bw4)38. In addition, it is known that B allotypes can influence the

| Genotype | Variable | Non-atrophic gastritis | Gastric cancer |
|----------|----------|------------------------|---------------|
|          | P        | OR (95% CI)           | p             | OR (95% CI)           |
| AA       | <0.0001  | 0.073 (0.03–0.18)     | <0.0001       | 0.23 (0.12–0.47)     |
|          | H. pylori | NS                     |               | NS                     |
|          | male     | <0.0001                | 0.22 (0.12–0.40) | NS                     |
|          | ≥50 years | 0.036                  | 1.90 (1.04–3.45) | <0.0001               | 8.18 (4.52–14.91) |
| Bx       | <0.0001  | 13.62 (5.68–32.61)    | <0.0001       | 4.2 (2.11–8.49)      |
|          | H. pylori | NS                     |               | NS                     |
|          | male     | <0.0001                | 0.22 (0.12–0.40) | NS                     |
|          | ≥50 years | 0.036                  | 1.90 (1.04–3.45) | <0.0001               | 8.18 (4.52–14.91) |
| Centromere-Telomere | Variable | P        | OR (95% CI)           | p             | OR (95% CI)           |
|          | cAcA-tAtA | <0.0001              | 0.073 (0.03–0.18) | <0.0001               | 0.23 (0.12–0.47) |
|          | H. pylori | NS                     |               | NS                     |
|          | male     | <0.0001                | 0.22 (0.12–0.40) | NS                     |
|          | ≥50 years | 0.036                  | 1.90 (1.04–3.45) | <0.0001               | 8.18 (4.52–14.91) |
| cBx-tBx  | <0.0001  | 7.08 (3.80–13.17)     | 0.008         | 2.55 (1.27–5.10)     |
|          | H. pylori | 0.027                  | 2.28 (1.10–4.73) | NS                     |
|          | male     | <0.0001                | 0.21 (0.11–0.37) | NS                     |
|          | ≥50 years | NS                     | <0.0001       | 8.73 (4.88–15.61)     |

Table 4. Multivariate logistic regression analysis of distribution of KIR genotype and variables in KIR centromere-telomere and its association with gastric disease. aComparisons were made with the asymptomatic as the reference group. bAdjusted by the other independent variables. cNS = not significant. AA = homozygote to A. Bx = homozygote or heterozygote to B. c = centromere. t = telomere.
binding with 3DL1; thus, in the Bw4 dimorphic position 80, isoluecine (HLA-B*51,*53,*57,*58, HLA-A*24) generally exhibit stronger inhibition than threonine (HLA-B*13, B*27, B*37, B*44)33,39. Besides, 3DL1 and the other KIR genes associated with GC reported in this study are expressed by CD56-dim NK cells, which migrate to acute inflammatory sites and display a higher cytotoxic activity than CD56-bright cells 40, and the B haplotype could also influence the cytotoxic activity on tumor cells. It is necessary to further study the role of HLA-Cw and KIR gene alleles in gastric cancer surveillance since receptor-ligand combinations are important in the regulation of NK cell responses38.

Although one limitation of our study is the sample size, we were still able to identify a strongly significant risk association of a gene cluster located in telomeric region of B genotype with GC. We should consider that GC is a multifactorial disease and consequently a multivariate analysis is necessary to better understand the importance of KIR gene variants in GC. We acknowledge that whereas our work present evidences of a significant association of KIR gene variants with gastric pathology, this association is not probe of causality and further studies are now needed to show that unregulated NK cells in the stomach mucosa may lead to gastric pathology. In conclusion, we found that 2DS1, 2DS3, 2DS5, 3DS1, 2DL5, Bx genotype, cBx-tBx, cA01|cB03, tA01|tB01, tB01|tB01 and B motifs were risk factors for GC. Mutated NK cells may contribute to GC development by increasing gastric mucosa inflammation, leading to constant tissue damage. The impact of the NK cell response on GC control might be determined in part by the genetic variation in KIR genes.

Materials and Methods

Study subjects. A total of 388 unrelated adults were recruited in this study, 146 healthy individuals (asymptomatic), 130 with non-atrophic gastritis (NAG) and 112 with GC. Patients with NAG were adults over 30 years old who were attended for symptoms at the gastroenterology service, whereas GC patients attended the oncology service for GC treatment; both groups attended the Instituto Mexicano del Seguro Social (IMSS) Medical Center in Mexico City. We selected NAG and GC patients without treatment of antibiotics, bismuth compounds, proton pump inhibitors and nonsteroidal anti-inflammatory drugs for at least two weeks prior to the study. GC patients without previous treatment for cancer were selected. Diagnosis was based on endoscopic examination.

Table 5. KIR B content score and its association with gastric disease. *n = number of subjects; *Comparisons were done using the asymptomatic group as the reference group and OR (95% C.I.) adjusted by age and gender. *pc < 0.05. *NS = not significant. AA = homozygote to A. Bx = homozygote or heterozygote to B. c = centromere. t = telomere. B score is the number of cB and/or tB motifs in each genotype 18.

| Genotype | B score | Centromere-Telomere | Asymptomatics n=146 (%) | Non-atrophic gastritis n=130 (%) | OR (95% CI) | Gastric cancer n=112 (%) | OR (95% CI) |
|----------|---------|---------------------|------------------------|-----------------------------|------------|------------------------|------------|
| AA       | 0       | cAcA-tAtA           | 64 (43.8)              | 7 (5.4)                    | 0.04 (0.01–0.12) | 16 (14.3)              | 0.22 (0.08–0.57) |
| Bx       | 1       | cAcA-tAIB           | 54 (37)                | 50 (38.5)                  | NS         | 56 (50)                | NS         |
|          |         | cAcB-tAIA           |                        |                            |            |                        |            |
|          | 2       | cAcA-tBIB           | 25 (17.1)              | 71 (54.6)                  | 9.7 (4.4–21.5) | 26 (23.2)              | NS         |
|          |         | cAcB-tAIA           |                        |                            |            |                        |            |
|          | 3       | cAcB-tBIB           | 3 (2.1)                | 2 (1.5)                    | NS         | 14 (12.5)              | 78.7 (4.9–1246.6) |
|          |         | cAcB-tBIB           |                        |                            |            |                        |            |
|          | 4       | cAcB-tBIB           | 0                     | 0                          | 0          | 0                      | 0          |

Table 6. Multivariate logistic regression analysis of KIR B content score and its association with gastric diseases. *Comparisons were made with the asymptomatic as the reference group. *Adjusted by the other independent variables. *NS = not significant.

| B score | Variable | Non-atrophic gastritis | Gastric cancer |
|---------|----------|------------------------|----------------|
| 0       |          |                        | P          | OR (95% CI) |
|         |          |                        | 0.0001    | 0.073 (0.03–0.18) |
|         | H. pylori| NS                     |          | 0.0001 |
|         | male     | <0.0001                | 0.22 (0.12–0.40) |
|         | ≥50 years| 0.036                  | 1.90 (1.04–3.45) |
| 2       |          |                        | 0.0001    | 6.6 (3.5–12.2) |
|         | H. pylori| 0.013                  | 2.5 (1.2–5.1) |
|         | male     | <0.0001                | 0.21 (0.12–0.38) |
|         | ≥50 years| 0.042                  | 1.8 (1.02–3.3) |
| 3       |          |                        | 0.001     | 10.9 (2.7–43.8) |
|         | H. pylori| NS                     |          |          |
|         | male     | NS                     |          |          |
|         | ≥50 years| <0.0001                | 9.9 (5.4–17.9) |
and histopathology studies\(^4\). The individuals of the asymptomatic group were selected from healthy blood donors who attended the blood bank of the IMSS Medical Center, with an age over 30 years old and without any symptom or medication. To minimize the genomic diversity in different regions of the country of Mexico\(^4\)–\(^4\), all groups of patients included in this study, patients and controls, received medical coverage from the same institute, IMSS at hospitals in the same city, Mexico City. Patients and controls were informed about the nature of the study and those willing to participate were asked to sign an informed consent letter. The study was approved by the ethics committee from the National Council for Research on Health, IMSS, Mexico and all procedures were performed in accordance with relevant guidelines and regulations.

**Collection of samples.** For the NAG patients seven gastric biopsies were taken and processed for histology to study the presence of precancerous lesions and \textit{H. pylori} infection. Biopsies were collected from both the lesser and the greater curvature, four from antrum and three from corpus. Mucosal inflammation was graded according to the Karttunen classification\(^4\), and only patients without precancerous lesions were included. In the case of GC patients, a tissue sample from the tumor lesion and a sample from adjacent non-cancerous tissue was also obtained and the lesion was classified according to the Karttunen classification\(^4\). A sample of 5 ml of peripheral blood was drawn from each patient, and each healthy volunteer, and mononuclear cells were purified by centrifugation through a Ficoll-Hypaque density gradient. DNA was isolated from these cells using the salting-out microtechnique\(^4\) and frozen at \(-70\) °C until genotyping. The serum fraction was frozen at \(-20\) °C until tested.

**Definition of \textit{H. pylori} infection.** Serum samples were tested by ELISA to detect IgG antibodies against \textit{H. pylori} whole-cell extract and against recombinant CagA protein, as previously described\(^4\). Infection was also diagnosed by histology in both antrum and corpus. The patient was considered infected with \textit{H. pylori} when either tests, ELISA and/or histology, were positive, and non-infected when both tests were negative.

**Genotyping of KIR.** The presence of each KIR gene was used to define the KIR gene content of patients. KIR genes were tested using a commercial kit (Invitrogen, Brown Deer, Wisconsin, USA) based on the technique

| Gene content | Variable | Non-atrophic gastritis | Gastric cancer |
|--------------|----------|------------------------|---------------|
|              |          | \(p\) | OR (95% CI)\(^a\) | \(p\) | OR (95% CI)\(^a\) |
| cA01|cA01 | <0.0001 | 0.28 (0.16–0.48) | NS\(^b\) |
| \(H. pylori\) | 0.02 | 2.30 (1.14–4.62) |
| male | <0.0001 | 0.21 (0.12–0.38) |
| \(\geq 50\) years | 0.014 | 2.04 (1.16–3.59) |
| cA01|cB02 | <0.0001 | 2.83 (1.59–5.03) | NS |
| \(H. pylori\) | 0.024 | 2.20 (1.11–4.38) |
| male | <0.0001 | 0.21 (0.13–0.38) |
| \(\geq 50\) years | 0.016 | 1.98 (1.14–3.46) |
| cA01|cB03 | <0.0001 | 0.08 (0.04–0.16) | 0.23 (0.12–0.43) |
| \(H. pylori\) | NS | 36.39 (4.32–306.85) |
| male | NS | NS |
| \(\geq 50\) years | <0.0001 | 10.65 (5.80–19.55) |
| tA01|tA01 | <0.0001 | 0.08 (0.04–0.16) | <0.0001 | 0.23 (0.12–0.43) |
| \(H. pylori\) | NS | NS |
| male | <0.0001 | 0.23 (0.13–0.43) | NS |
| \(\geq 50\) years | NS | <0.0001 | 7.88 (4.32–14.36) |
| tA01|tB01 | 0.001 | 2.51 (1.45–4.35) | 0.016 | 2.06 (1.15–3.70) |
| \(H. pylori\) | NS | NS |
| male | <0.0001 | 0.23 (0.14–0.40) | NS |
| \(\geq 50\) years | 0.013 | 2.02 (1.16–3.50) | <0.0001 | 7.90 (4.44–14.07) |
| tA01|tB0X | <0.0001 | 26.04 (7.45–90.97) | NS |
| \(H. pylori\) | 0.006 | 3.01 (1.38–6.54) |
| male | <0.0001 | 0.30 (0.17–0.54) |
| \(\geq 50\) years | NS | NS |
| tB01|tB01 | NS | 7.55 (2.31–24.70) |
| \(H. pylori\) | NS | NS |
| male | NS | NS |
| \(\geq 50\) years | <0.0001 | 10.45 (5.70–19.16) |

**Table 7.** Multivariate logistic regression analysis of distribution of KIR according to centromeric and telomeric gene content. \(^a\)Comparisons were made with the asymptomatic as the reference group. \(^b\)Adjusted by the other independent variables. \(^c\)NS = not significant. \(c\) = centromere. \(t\) = telomere. The number was according gene content\(^15\)–\(^17\). \(O\(X\) = the number had not been assigned so far.

and histopathology studies\(^4\). The individuals of the asymptomatic group were selected from healthy blood donors who attended the blood bank of the IMSS Medical Center, with an age over 30 years old and without any symptom or medication. To minimize the genomic diversity in different regions of the country of Mexico\(^4\)–\(^4\), all groups of patients included in this study, patients and controls, received medical coverage from the same institute, IMSS at hospitals in the same city, Mexico City. Patients and controls were informed about the nature of the study and those willing to participate were asked to sign an informed consent letter. The study was approved by the ethics committee from the National Council for Research on Health, IMSS, Mexico and all procedures were performed in accordance with relevant guidelines and regulations.
of single specific primer-polymerase chain reaction (SSP-PCR), which can identify 2DL1, 2DL2, 2DL3, 2DL4, 2DL5A, 2DL5B, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1 and 3DP1 genes (including the variants of 2DS4 and 3DP1). PCR reaction and cycling conditions were according the instructions recommended by the manufacturer.

**Statistical analysis.** The gene, genotype, centromere-telomere gene content and B score frequencies in patients with NAG and GC were compared with the asymptomatic group. Chi-squared or Fisher’s exact test were used to test differences among groups, using the Epidat 3.1 Software; p values ≤ 0.05 were considered as significant. The significance of association was assessed using odds ratios (OR) with confidence intervals (CI) of 95%. OR values were corrected for gender and age using a logistic regression model. The analyses were performed using SPSS Statistics 22.0 (IBM SPSS Data Collection). The role of *H. pylori*, gender and age as variables influencing risk factor for GC was estimated in a multivariable logistic regression analysis.

**Data Availability.** The datasets generated and analyzed during the current study are available from the corresponding authors on reasonable request.

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Author Contributions
E.G.H. performed K.I.R. genotyping. O.P.R., M.N.R. and L.R.V. participated in the sample collection. O.P.R. and M.C.P. performed seroprevalence of *H. pylori*. J.T. conceived and participated in design and coordination and provided helpful discussions and helped to edit the manuscript. M.P.R. conceived and participated in statistical analysis, and in design and coordination and edited the manuscript.

Additional Information

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