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SHORT COMMUNICATION

Women with chronic follicular gastritis positive for *Helicobacter pylori* express lower levels of GKN1

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Abstract

In women, serum levels of CTSB, GKN2, LIPF, LIPFG, AZGP1, TOP2A and PGA4 are proposed as predictive markers of gastric cancer. It is unknown whether GKN1 expression varies with the sex of patients with chronic gastritis or gastric cancer. We studied 36 patients with histopathological diagnosis of chronic gastritis from the state of Guerrero, Mexico. PCR was performed for *H. pylori* detection and GKN1 expression was determined by RT-qPCR and western blot. GKN1 mRNA expression was significantly lower in patients with chronic follicular gastritis than in those with chronic chemical gastritis ($p = 0.00071$). The mRNA and protein level of expression of GKN1 were significantly lower in women with chronic follicular gastritis than in men with the same condition ($p = 0.0279$ and $p = 0.0014$, respectively); the lowest levels of GKN1 were detected in women with *H. pylori*-positive follicular gastritis ($p = 0.0175$ and $p = 0.0111$, respectively). Through a bioinformatic analysis, estrogen response elements were identified in the GKN1 promoter.

Keywords Gastrokine 1 · Sex · Chronic gastritis · *Helicobacter pylori*

Introduction

Interactions between etiological factors of gastric cancer (GC) induce alterations in the expression of proto-oncogenes and tumor suppressor genes [1]. In GC and in *Helicobacter pylori* infection, the expression of genes that regulate gastric homeostasis, such as Gastrokine 1 (GKN1) is altered [2]. GKN1 is a tumor suppressor that inhibits inflammation [2]. The transcript and protein of GKN1 decrease in the mucosa of patients with intestinal and diffuse-type GC [3–5] as well as in GC cell lines [6]. It is proposed that GKN1 may be a highly specific diagnostic biomarker for early GC detection [7].

Research on the transcriptome of various organisms indicates that the gene expression profile differs between sexes [8]. Sex is a factor that determines the incidence and mortality of individuals in different types of cancer [9]. In women with GC, serum levels of cathepsin B (CTSB), gastrokine 2 (GKN2), gastric lipase (LIPF), endothelial lipase (LIPFG), zinc alpha glycoprotein 2 (AZGP1), DNA topoisomerase 2-alpha (TOP2A) and pepsin A (PGA4) integrate a profile of promising predictive markers [10]. Currently, it is unknown

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if GKN1 expression varies with the sex of patients with chronic gastritis or GC. In this study, we evaluated GKN1 expression in biopsies of patients with chronic gastritis and with *H. pylori* infection and analyzed the relationship between the level of GKN1 and the sex of the patients.

Material and methods

Patients

Thirty-six biopsies of patients with histopathological diagnosis of chronic gastritis were studied. The patients underwent endoscopy at the Instituto Estatal de Cancerología "Dr. Arturo Beltrán Ortega", Acapulco, Guerrero, Mexico. The patients or their relatives signed a letter of consent. This project was approved by the bioethics committee of the Universidad Autónoma de Guerrero and by the research department of the Instituto Estatal de Cancerología "Dr. Arturo Beltrán Ortega".

Detection of *H. pylori*

Helicobacter pylori DNA was detected using the oligonucleotides HP16SF and HPGR16SR, which amplify a 522 bp fragment of the *16S rRNA* gene, following the methodology described by Román-Román et al. [11].

GKN1 expression

RNA and total proteins were obtained from the biopsies with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. *GKN1* mRNA expression (assay no. Hs00219734) was determined using the TaqMan RNA-to-CT 1 step kit (Thermo Fisher Scientific, Waltham, MA, USA). The relative expression of *GKN1* was calculated by the $2^{-\Delta ct}$ method. *GAPDH* (assay no. Hs99999905_m1) was used as endogenous control. GKN1 protein was detected by western blot with an anti-GKN1 monoclonal antibody at a 1:15,000 dilution (Abnova, Walnut, CA, USA). GAPDH was used as a loading control, and was detected with a specific monoclonal antibody (Santa Cruz, Dallas, TX, USA) at a 1:5000 dilution. Immunocomplexes were revealed by chemiluminescence using Immobilon (Millipore, Burlington, MA, USA) in the ChemiDoc Digital Imaging System (Bio-Rad, Hercules, CA, USA). GKN1 expression was calculated by densitometric analysis of the bands using the Image J program.

Bioinformatic and statistical analyses

Statistical analysis of the data was performed using the GraphPad Prism v5.0 (GraphPad Software, San Diego, CA, USA) and STATA v14.0 (StataCorp, College Station, TX, USA) software. Absolute and relative frequencies of the qualitative variables were obtained, and the differences were calculated by the X^2 test. Means ± standard deviations (SD) or medians were calculated for quantitative variables. The Student's *t* test or the Mann–Whitney U test were used to compare the differences between the groups, respectively. A p value of < 0.05 was considered statistically significant. The bioinformatic analysis tosearch the potential binding sites of the estrogen and androgen receptors within the *GKN1* promoter was done using the ExPASy program [12]. The binding sites ofthe estrogen and androgen receptors to the *GKN1* promoter were selected considering a p value of < 0.001.

Results

Thirty-six patients with chronic gastritis were studied. The average age was 55 years (range 16–84). 47.2% (17/36) of patients were *H. pylori*-positive (Table 1). Based on the Sydney System, 75% (24/32) of the patients had moderate chronic gastritis and of these 84.6% were women and 68.4% were men.

Chronic chemical gastritis was present in 44.4% (16/36) of the patients whereas 55.6% (20/36) had chronic follicular gastritis. *GKN1* mRNA expression was significantly lower in patients with chronic follicular gastritis than in those with chronic chemical gastritis ($p = 0.00071$, Fig. 1a). Likewise, GKN1 protein expression was lower in patients with chronic follicular gastritis (Fig. 2a, b), however this difference was not significant.

The levels of mRNA and protein of GKN1 were different between the types of gastritis and regarding the sex of the patients. GKN1 expression was significantly lower in women with chronic follicular gastritis compared tomales with the same condition ($p = 0.0279$, Fig. 1b; $p = 0.0014$, Fig. 2c, d). Irrespective of the sex, lower levels of GKN1

Table 1 Clinical features of chronic gastritis patients stratified by sex

Feature	Sex		*p* value
	Female $n = 15$	Male $n = 21$	
Age (mean ± SD; years)	54 ± 17.6	55 ± 12.9	0.852^{Ω}
Helicobacter pylori n (%)			
Negative	8 (53.3)	11 (52.4)	0.955^{δ}
Positive	7 (46.7)	10 (47.6)	
Chronic gastritis type *n* (%)			
Chemical	7 (46.7)	9 (42.9)	0.821^{δ}
Follicular	8 (53.3)	12 (57.1)	

$^{\Omega}$Student's *t* test; $^{\delta}X^2$ test.

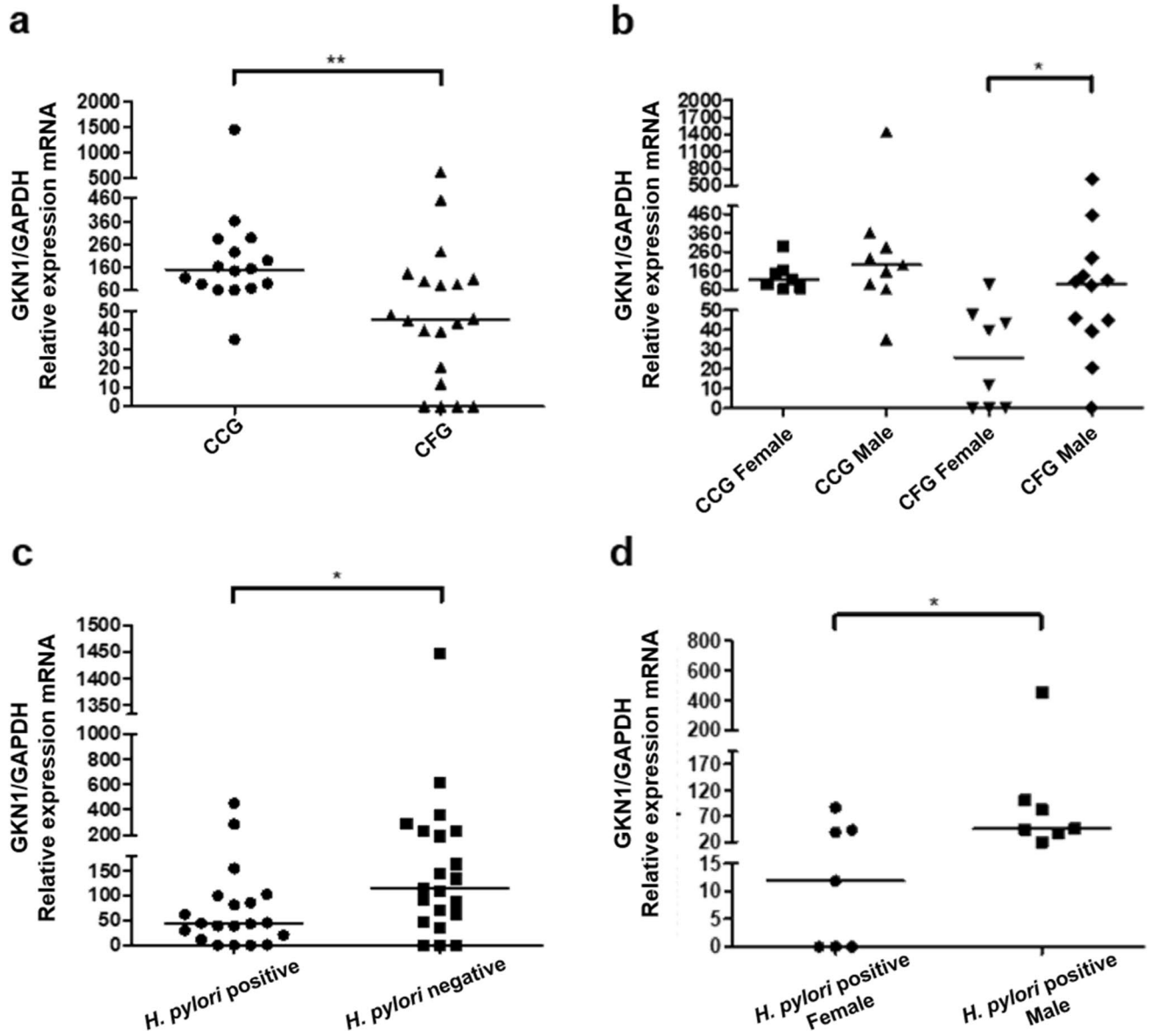

Fig. 1 *GKN1* mRNA expression **a** by the type of gastritis; **b** in chronic chemical gastritis (CCG) and chronic follicular gastritis (CFG) grouped by sex; **c** with and without *H. pylori* in all cases; **d** in *H. pylori*-positive chronic follicular gastritis grouped by sex. The horizontal line represents the median of *GKN1* expression (*$p < 0.05$, **$p < 0.001$)

were detected in *H. pylori*-positive patients ($p = 0.0275$, Fig. 1c; $p = 0.0112$, Fig. 2e, f). Women with *H. pylori*-positive follicular gastritis expressed the lowest levels ($p = 0.0175$, Fig. 1d; $p = 0.0111$, Fig. 2g, h).

By means of a bioinformatic analysis, it was found that the *GKN1* promoter contains four estrogen response elements (ESR1, ESR2, ESRRα and ESRRβ) and an androgen response element (AR), (Fig. 3).

Discussion

GKN1 decreases in mucosa infected with *H. pylori* and is absent in uninfected gastric cancer [5, 13–15]. In this study, the expression of GKN1 was evaluated in men and women with chronic gastritis with and without *H. pylori* infection. In patients with chronic follicular gastritis, the

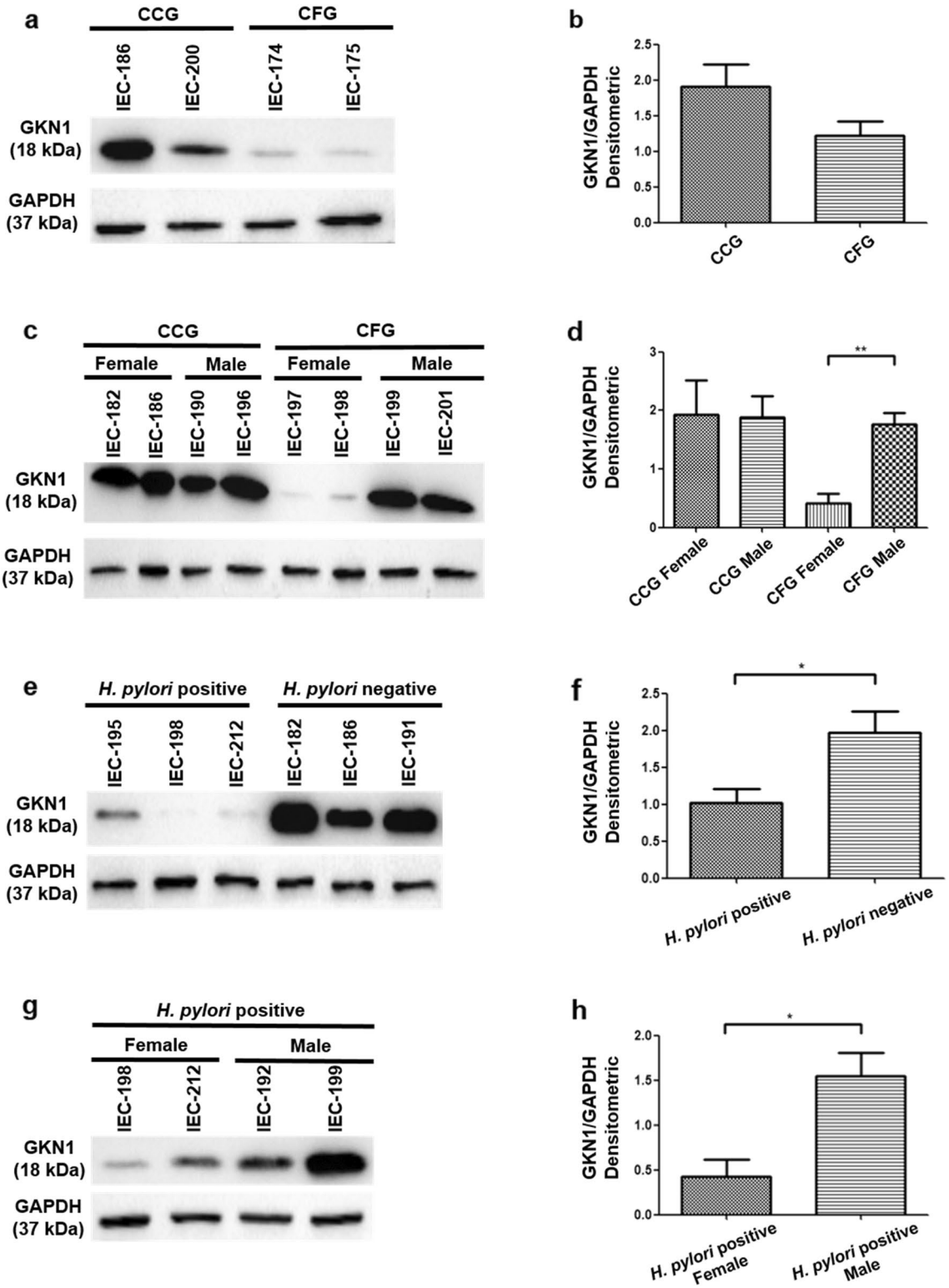

Fig. 2 Western blot of GKN1 **a**, **b** by the type of gastritis; **c**, **d** in chronic chemical gastritis (CCG) and chronic follicular gastritis (CFG) grouped by sex; **e**, **f** with and without *H. pylori* in all cases; **g**, **h** in *H. pylori*-positive follicular gastritis cases grouped by sex. The GKN1 level was normalized with GAPDH

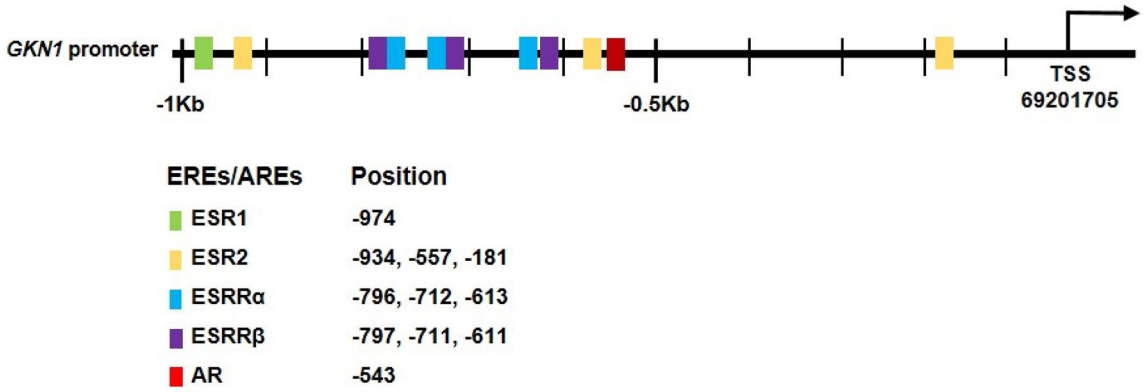

Fig. 3 Estrogen and androgen response elements in the *GKN1* promoter. The colored boxes show the estrogen or androgen binding sites in the *GKN1* promoter from −1 to −1000 bp. *TSS* transcription start site, *EREs* estrogen response elements, *AREs* Androgen Response Elements, *ESR1* estrogen receptor 1, *ESR2* estrogen receptor 2, *ESRRα* estrogen-related receptor alpha, *ESRRβ* estrogen-related receptor beta, *AR* androgen receptor

expression of *GKN1* mRNA was significantly lower than in patients with chronic chemical gastritis ($p < 0.05$). Patients with *H. pylori*-positive chronic gastritis express significantly lower levels of the *GKN1* transcript than the *H. pylori*-negative cases ($p < 0.05$). These data are consistent with those reported by Nardone et al., and Mao et al. [3, 6], who observed that in *H. pylori*-positive gastric mucosa the expression of GKN1 is decreased. The low expression of GKN1 may be due to *H. pylori* promoting the expression of AUF1, a protein that induces degradation of the *GKN1* mRNA [16]. On the other hand, there may be a negative regulation of the *GKN1* transcript translation by non-coding RNAs [17] or degradation of the protein by ubiquitination [18]. These and other mechanisms can act synergistically and cause decrease or silencing of GKN1 expression in the gastric tissue [19].

The mRNA and protein levels of GKN1 were lower in women with chronic follicular gastritis than in men with the same condition ($p = 0.0279$ and $p = 0.0014$, respectively). Women with *H. pylori*-positive chronic follicular gastritis expressed significantly lower levels of transcript and protein than infected men ($p = 0.0175$ and $p = 0.0111$, respectively; Figs. 1d, 2d, h). These findings suggest that, in women, in addition to *H. pylori* status and the type of gastritis, other factors modify the expression of GKN1. Taking the above point into account, it is likely that female hormones are involved in the regulation of GKN1 expression. This hypothesis is strengthened by the results of the bioinformatic analysis that in the GKN1 promoter, four estrogen response elements and an androgen response element are located (Fig. 3). The influence of sex hormones on the expression of GKN1 has not been described, however, it has been proposed that estrogens may have a protective effect on gastric cancer [20–22]. In breast cancer, estrogen was reported to positively regulate the expression of Trefoil 1 protein (TFF1) [23], a protein that contributes to the protection of gastric mucosa [24]. GKN1 expression in women could be influenced by hormonal status. Considering that the age of the women studied varied from 16 to 84 years and that menopause can start at 45, the fluctuation in estrogen production may explain, at least partially, the variation in GKN1 levels. In menopausal women (> 45 years) the minimum estrogen production could be related to the lower levels of GKN1 found. To strengthen these results, it is necessary to increase the sample size and conduct further studies on the differences in the expression of GKN1 in relation to the sex and age of the patients. It is imperative to elucidate the role of estrogens in GKN1 expression regulation.

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Author contributions All authors contributed to the study conception and design. Manuscript writing: JAM, DNMC and GFT. Data collection: JAM, SILN, GECV and EMCM. Data analyses: DNMC, JAM, JOO, MAMC, BIA and HJW. Patient management: SRN, MAJL, and CACS. Final manuscript approval: all authors.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

Ethical approval This study was approved by the Bioethics Committee of the Universidad Autónoma de Guerrero and by the Research Department of the Instituto Estatal de Cancerología.

Informed consent Informed consent was obtained from all the participants.

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