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Short Communication

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Hereditary Gastric Cancer Is Linked With Hereditary Breast and Ovarian Cancer

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Abstract

Background: *Helicobacter pylori* (*H. pylori*), a bacterium which chronically infects the stomach of approximately half the world's population, is a risk factor for the development of gastric cancer (GC). However, the underlying mechanism whereby *H. pylori* infection induces GC development remains unclear. Intermittent injection of the *H. pylori* cytotoxin-associated gene A antigen (CagA) protein into its host cell inhibits nuclear translocation of BRCA1/BRCA2, DNA repair proteins involved in the development of breast cancer/ovarian cancer. Interestingly, hereditary breast and ovarian cancer (HBOC) syndrome is associated with GC development. Here, we aimed to clarify the molecular link between *H. pylori* infection, *BRCA1/2* pathogenic variants (PVs), GC and higher GC incidence in HBOC families.

Methods: We retrospectively reviewed data from Japanese patients undergoing precision treatment using cancer genomic medicine.

Results: We found a higher GC incidence in HBOC families having germline pathogenic variants (GPVs) of *BRCA1/2* (2.95% vs. 0.78% in non-HBOC families). Next, we found that 96.1% of *H. pylori*-infected patients received cancer genomic medicine for advanced GC, and > 16% advanced GC patients had *gBRCA2* PVs. Furthermore, expressing wild-type BRCA1/2 in *Gan* mice (a mouse model of human GC) inhibited GC development. Thus, *gBRAC1/2* PVs and *H. pylori* infection synergistically increase the risk of GC development.

Conclusion: Our study highlights the need to investigate the potential of therapeutic agents against BRCA1/2 PVs to avoid the development of GC in HBOC families. In addition, our results suggest that poly (ADP-ribose) polymerase (PARP) inhibitors could potentially inhibit GC development and progression with gBRCA1/2 PVs.

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Introduction

During gastric cancer (GC) development, external stimuli turn mucosal epithelial cells into cancer precursor cells, which are further transformed into cancerous cells that proliferate uncontrollably [1]. As the tumor grows due to cancer cell proliferation, GC cells gradually infiltrate the submucosa, muscularis propria, and serosa [2, 3]. Then, GC cells crossing outside the serosa get scattered within the abdomen and pelvis, resulting in peritoneal dissemination. In addition, these GC cells directly infiltrate the large intestine, pancreas, diaphragm, and liver, all near the stomach [4] and/or invade lymph vessels and blood vessels, resulting in distant metastasis in multiple organs. Notably, a type of stomach cancer, scirrhous GC, spreads while making the stomach wall hard and thick [5]. Furthermore, scirrhous GC (which is also a refractory malignant tumor) is difficult to diagnose by endoscopic examination.

East Asian countries (Japan, China, and South Korea) have the highest incidence of GC worldwide with > 50,000 GC deaths annually recorded in Japan [6]. *Helicobacter pylori* (*H. pylori*), a pathogenic bacterium that chronically infects the human gastric mucosa, is estimated to infect approximately half of the world's population. *H. pylori* infection causes atrophic gastritis and gastric mucosal lesions such as gastric ulcers. Importantly, *H. pylori* is highly prevalent in these East Asian countries [6]. *H. pylori* infection is associated with the development of GC and some malignant lymphomas [7]. Almost 90% of GC patients are positive for *H. pylori* infection, and *H. pylori* infection is a risk factor for the development of GC [8, 9]. Thus, in East Asia, GC is likely caused by *H. pylori* infection.

H. pylori is broadly classified based on the presence of cytotoxin-associated gene A antigen (CagA). Western countries have a higher predominance of CagA-negative strains but the CagA-positive/CagA-negative ratio in the West is approximately 6:4 [10]. However, *H. pylori* strains isolated in East Asia, including Japan, are predominantly CagA-positive. CagA-positive strains have a much stronger ability to induce gastric mucosal lesions than CagA-negative strains [11-14]. Using a microscopic needle (type IV secretion mechanism), *H. pylori* directly injects CagA protein into gastric epithelial cells.

Thus, there appears to be global variations in growth, spread and prevalence of *H. pylori* strains and GC incidence. For instance, GC incidence in Japan (male 6.07%, female 2.11%) is approximately 5 - 10 times higher than in Western countries (USA: male 0.65%, female 0.33%, UK: male 0.66%, female 0.28%) [15].

Likely, *H. pylori* infects gastric mucosal epithelial cells causing genetic mutation in the infected cells. Accumulation of multiple cancer-related gene mutations can lead to cancer. However, the underlying molecular mechanism whereby *H. pylori* infection induced GC remains unclear. Breast cancer susceptibility genes I and II (*BRCA1/BRCA2*) protect DNA replication by repairing double-strand breaks (DSBs) during nuclear genomic DNA replication, thus maintaining the stability of the cellular genome (genome stability). Therefore, *BRCA1/2* genes are typical tumor suppressor genes. BRCA1 and BRCA2 strongly linked to the onset of hereditary breast and ovarian cancer (HBOC) and the development of some prostate and pancreatic cancers, may also be involved in GC onset [16-19]. Therefore, in this multi-center retrospective observational study, we compared the incidence of GC in 78 HBOC families (i.e., a family having ≥ 1 patients with breast cancer or ovarian cancer and a germline pathogenic variant (GPV) of *BRCA1/2* (g*BRCA1/2*)), with that in 86 non-HBOC families. Furthermore, using a transgenic GC mouse model (*Gan*) modified to express wild-type (wt) *BRCA1/BRCA2*, we tested whether wt *BRCA1/BRCA2* could rescue tumorigenesis in *Gan* mice. Next, we verified if *H. pylori* infections occur in most GC patients belonging to HBOC. Thus, we aimed to clarify the interaction between *H. pylori* infection and *gBRCA1/2* pathogenic variants (PVs) and GC risk. Our results lead us to conjecture that oral administration of PARP inhibitors (which are effective against platinum-sensitive HBOC) will be effective against GC with *gBRCA1/2* PVs.

Materials and Methods

Gan^{tgBrca1} and Gan^{tgBrca2} mice

Gan^{tgBrca1} and *Gan*^{tgBrca2} mice were created by crossing C57BL/6J^{tgBrca1} or C57BL/6J^{tgBrca2} mice with Gan mice (kindly provided by Dr. Oshima M (Kanazawa University School of Medicine, Kanazawa, Ishikawa, Japan)). *Gan* mice are compound transgenic mice created by crossing C2mE and Wnt mice. In *Gan* mice, both the COX-2/PGE2 pathway and Wnt signaling are activated in the gastric mucosa. These mice spontaneously develop ductal GC accompanied by an inflammatory response, with 100% efficiency. Thus, *Gan* mice are considered human GC models, wherein tumors develop through the interaction of Wnt signal activation and inflammatory responses.

The C57BL/6J^{tgBrca1} and C57BL/6J^{tgBrca2} mice used to obtain *Gan*^{tgBrca1} and *Gan*^{tgBrca2} mice were produced as follows.

MMTV-BRCA1 transgenic constructs

A diagram of *BRCA1* cDNAs used for the generation of transgenic animals is shown in Section of Generation and Maintenance of BRCA2 Transgenic Mice (Supplementary Material 1, www.wjon.org). In MMTV-BRCA1 transgenic constructs, the expression of wt *BRCA1* is controlled by the mouse mammary tumor virus-long terminal repeat (MMTV-LTR) promoter. *BRCA1* cDNAs were inserted into the third exon of the rabbit *β-globin* gene (*β-g.*). The bar indicates the Really Interesting New Gene finger motif, the hatched region corresponds to the nuclear localization signals and the negative symbols (-) indicate the negatively charged C-terminal domain with transactivation function.

Generation and maintenance of BRCA1 transgenic (C57BL/6J^{tgBrca1}) mice

Xho I *BRCA1* fragments were microinjected into the male pronucleus of C57BL/6x DBA/2 F1 fertilized mice embryos (CLEA Japan, Inc., Meguro, Tokyo, Japan) and implanted into pseudo-pregnant ICR surrogate mice at the Transgenic/Embryonic Stem Cell Shared Resource Facility (Japan) to obtain founder mice, which were bred with C57BL/6J mice (obtained from CLEA Japan, Inc., Meguro, Tokyo, Japan) to establish the C57BL/6J^{tgBrca1} transgenic mice.

CMV-BRCA2 transgenic constructs

To construct *p236BRCA2*, the *pcDNA3* vector was first modified by inserting a 236-bp fragment of the 5′ untranslated region of *BRCA2* between the KpnI and NotI sites. The assembled full-length *BRCA2* cDNA was then inserted at the XhoI site of this plasmid. The 5′ UTR of *BRCA2* was obtained by RT-PCR using primers 5′-GGTACCGGTG GCGCGAGCTT CTGA-3′ and 5′-GCGGCCGCAACTACGATATTCCTCCAAT-3′. The *pcDNA3* 236HSC WT (*BRCA2*) was a gift from Mien-Chie Hung (Addgene plasmid #16246; RRID:Addgene_16246) [20].

Generation and maintenance of BRCA2 transgenic (C57BL/6J^{tgBrca2}) mice

The *BRCA2* gene was generated using recombination; transgenic mice were generated as described previously.

All animals used in these studies were handled in strict compliance with Shinshu University School of Medicine, Animal Care Committee regulations (approved number: Shinshu University 567-5).

Immunostaining for detection of BrdU-positive cells in tumors

To estimate the BrdU-labeling index, mice were injected IV with 200 µL of BrdU solution (Roche Diagnostics, IN, USA) 1 h before euthanasia. Then, tissue samples were fixed in 70-96% ethanol, embedded and sectioned at 5-µm thickness.

Sections were then stained with anti-BrdU antibody (Roche Diagnostics). The labeling index was calculated by dividing the number of BrdU-positive cells by the total number of nucleated cells.

Clinical study

We carried out a multi-center retrospective observational study of subjects who underwent cancer genomic medicine at cancer medical facilities in Kyoto, Japan. This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) on November 18, 2020, and Kyoto University School of Medicine (Kyoto, Japan) on August 24, 2022, with approval codes NHO R4-04 and M237. All participants provided written informed consent. Our clinical research complied with the Helsinki Statement.

Cancer genomic medicine is being carried out using cancer gene panel testing, which was approved by the Japanese Ministry of Health, Labor and Welfare in June 2019. The following panels are tested for OncoGuide™ NCC oncopanel*; Gene mutation analysis set for cancer genome profiling test (Sysmex Corporation Kobe, Hyogo, Japan), Foundation One CDx**; Foundation One CDx liquid; Foundation One CDx's cancer genome test (Foundation Medicine, Inc., Cambridge, MA, USA); OncoGuide™ NCC oncopanel*; Foundation One CDx**.

Institutional review board approval

This research on human cancer genome information derived from results by cancer genome gene panels was at Kyoto University, affiliated hospitals and National Hospital Organization Kyoto Medical Center in accordance with institutional guidelines (i.e., IRB approval no. M192, H31-cancer-2). Subjects signed an informed consent form after being briefed on the clinical study and agreed with the content of the research.

Ethic Committee Name: IRB of Shinshu University; approval code: M192; approval date: April 5, 2014, and June 16, 2016.

Ethic Committee Name: IRB of National Hospital Organization Headquarter; approval code: H31-cancer-2; approval date: November 9, 2019, and June 17, 2022.

Ethic Committee Name: IRB of Kyoto University; approval code: R34005; approval date: August 1, 2023.

Ethical compliance with human/animal study

This manuscript contains personal and/or medical information about an identifiable individual. This manuscript also contains a case report/case history about identifiable individual. This manuscript is sufficiently anonymized in line with our anonymization policy. Authors obtained directly consent from patient. This study involves human participants and was approved by an Ethics Committee(s) and Institutional Board(s). This study involves the research studies with animals.

The authors attended research ethics education through the Education for Research Ethics and Integrity (APRIN e-learning program (eAPRIN)). The completion numbers for the authors are AP0000151756, AP0000151757, AP0000151769, and AP000351128. Consent to participate was required as this research was considered clinical research.

Statistical analysis

All data are expressed as the mean and standard error of the mean. Normality was verified using the Shapiro-Wilk test. For comparing two groups, the unpaired two-tailed *t*-test or Mann-Whitney U test was used. Multiple comparisons were performed using a one-way analysis of variance with a Tukey *post hoc* test or a Kruskal-Wallis analysis with a *post hoc* Steel-Dwass or Steel test. A P-value < 0.05 was considered statistically significant. All statistical analyses were conducted using the JMP software (SAS Institute, Cary, NC, USA).

Detailed Materials and Methods are contained in the Supplementary Material 1 (www.wjon.org).

Results

GC prevalence in HBOC and non-HBOC families

H. pylori CagA injected into gastric mucosal epithelial cells induces the accumulation of genetic mutations leading to the development of GC [21]. Furthermore, the CagA genotype majorly influences GC development [21, 22]. Thus, CagA may trigger GC. Especially, mutations (i.e., PVs) in *BRCA1/2* appear to indirectly induce transformation of gastric mucosal epithelial cells. Therefore, HBOC families with GPVs of the *BRCA1/2* may have a higher incidence of GC [16-19]. We investigated the number of patients affected by GC in 78 HBOC and 86 non-HBOC families (up to six generations). We found that non-HBOC families had less patients suffering from GC than HBOC families (0.78% vs. 2.95%) (Table 1, Supplementary Materials 2, 3, www.wjon.org). Of 61 people with GC in HBOC families, 58 were infected with *H. pylori* (infection rate 57/61: 93.44%). Of 17 people with GC in non-HBOC families, 16 were infected with *H. pylori* (infection rate 16/17: 94.12%). Compared with the *H. pylori* infection rate (93.44%) in patients with GC in HBOC families, the *H. pylori* infection rate (94.12%) in patients with GC in non-HBOC families was almost the same.

Antitumor effect of wild-type BRCA2 in genetically engineered mice

The *Gan* mice closely mimic human GC, wherein Wnt signal activation and inflammatory responses interact to form tumors. The *Gan* mice are composite transgenic mice created by crossing C2mE-expressing mice and Wnt-expressing mice. Both the COX-2/PGE2 pathway and Wnt signaling are acti-

Table 1. Patients With Hereditary Gastric Cancer in HBOC Families Reflecting the Extent of Genetic Testing[a]

Generation	HBOC family (78 families)		No-HBOC family (86 families)	
	Patients with GC (other tumor[b])	Incidence (%) of GC (total people)	Patients with GC (other tumor[b])	Incidence (%) of GC (total people)
I	12 (24) cases	4.17% (288 cases)	1 (6) case	0.31% (324 cases)
II	36 (174) cases	5.56% (648 cases)	5 (43) cases	0.74% (674 cases)
III	12 (120) cases	2.38% (504 cases)	9 (36) cases	1.73% (521 cases)
IV	0 (6) cases	0.00% (348 cases)	2 (5) cases	0.55% (364 cases)
V	1 (1) case	0.39% (258 cases)	0 (2) cases	0.00% (263 cases)
VI	0 (0) case	0.00% (24 cases)	0 (0) cases	0.00% (41 cases)
Total cases	61 (325) cases	2.95% (2,070 cases)	17 (92) cases	0.78% (2,187 cases)

[a]BRACAnalysis® Diagnostic System (Myriad Genetics G.K., Zurich, Switzerland). [b]HBOC-related cancer (hereditary cancer, i.e., breast cancer, ovarian cancer, prostate cancer, pancreatic cancer). GC: gastric cancer; HBOC: hereditary breast and ovarian cancer.

vated in gastric mucosal epithelial cells of the *Gan* mice [23]. Activation of these two signals in the *Gan* mice causes spontaneous development of ductal-type GC accompanied by an inflammatory response, with 100% efficiency [23] (Figs. 1A, B, and 2). To verify if *BRCA1* or *BRCA2* are involved in the development of ductal-type GC (accompanied by an inflammatory response), we created $Gan^{tgBrca1}$ and $Gan^{tgBrca2}$ mice, which constitutively express wt BRCA1 and BRCA2, respectively (Fig. 1C, D). The proliferation ratio of epithelial cells in the gastric mucosa of wt C57BL/6J mice was 8.54%, as indicated by the percentage of bromodeoxyuridine (BrdU) positive cells (Figs. 1A, E, and 2). Furthermore, the proliferation ratio of ductal-type GC cells in *Gan* mice (34.41%) was higher than that in $Gan^{tgBrca1}$ (14.84%) and $Gan^{tgBrca2}$ (13.69%) mice (Fig. 1B-E). These results suggest that expressing wt *BRCA1* or *BRCA2* in *Gan* mice suppresses the transformation of mucosal epithelial cells into ductal-type GC cells. In particular, the nuclear atypia of epithelial cells in the gastric mucosa of $Gan^{gtBrca2}$ mice was weaker than that in $Gan^{gtBrca1}$ mice (Figs. 1Cc, Dd, and 2). Specifically, the nuclear shapes of epithelial cells in the gastric mucosa of $Gan^{gtBrca2}$ and C57BL/6J wt mice were similar (Figs. 1Aa, Dd, and 2). These results indicate that *BRCA2* GPVs produce more mutations and transformation into ductal-type GC cells than *BRCA1* GPVs.

Relationship between BRCA2 PVs and GC development in human patients

The medical history of a total of 35,311 patients in the cancer genomic medicine precision treatment at Japanese national universities between December 2019 and September 2023 was re-examined using cancer genome panels (8,130 cases using OncoGuide™ NCC oncopanel test (Riken genesis Co., Ltd., Japan) and 27,181 cases using FoundationOne® CDx test (MF Inc., USA)). New therapies for 2,291 cases of advanced GC in Japanese patients were tested using such examinations. We found that 96.1% of advanced GC patients with H. pylori infection (2,200/2,291) underwent cancer genomic panel examinations; recent clinical studies have reported that 5-10% of Japan's total population is infected with H. pylori. GPV and/or somatic pathogenic variant (SPV) in BRCA2 were detected in 382 patients with advanced GC (16.67%: 382/2,291) showing that BRCA2 GPVs and/or SPVs are associated with the onset and aggravation of advanced GC, consistent with our experimental findings showing that constitutive wt BRCA2 expression reduces the aggravation of epithelial cells in the gastric mucosa of GantgBrca2 mice. ERBB2 GPVs and/or SPVs was detected in 521 patients with advanced GC (22.74%: 521/2,291). Our results, here, from analyzing cancer genomic medicine database are similar to those obtained in clinical research conducted by other research institutions [23].

Discussion

GC is the third largest leading cause of cancer-related mortality, accounting for approximately 10% of all cancer-related deaths [24]. Infection of the stomach with *H. pylori* is the greatest risk factor for GC development. The CagA protein produced by *H. pylori* invades the gastric mucosal epithelial cells, binds to intracellular proteins, causes abnormalities in signal transductions that influence cell proliferation and subsequently promotes canceration [25]. However, the physiological mechanism by which gastric mucosal epithelial cells are transformed in a CagA-dependent manner has not been clarified. BRCA1/2 is a tumor suppressor protein in HBOC [16-19]. The CagA protein of *H. pylori* impairs the function of *BRCA1*, which is a homologous recombination repair related gene controlling genome stability, leading to the accumulation of genetic mutations required for canceration of gastric mucosal epithelial cells [19, 26, 27]. *BRCA1/2* are tumor suppressors because inactivating mutations in them cause hereditary breast and ovarian cancers. There exists a common carcinogenic mechanism between the pathogeneses of GC caused by *H. pylori* infection and HBOCs [16]. Here, we found that HBOC families had more GC cases than non-HBOC families (Fig. 3). The cancer genome panel examination revealed that 382 patients with advanced GC (16.67%: 382/2,291) had GPVs and/or SPVs in *BRCA2*. PARP inhibitors are effective against platinum-sensitive HBOC. Therefore, oral administration of PARP inhibitors may be effective against these GCs.

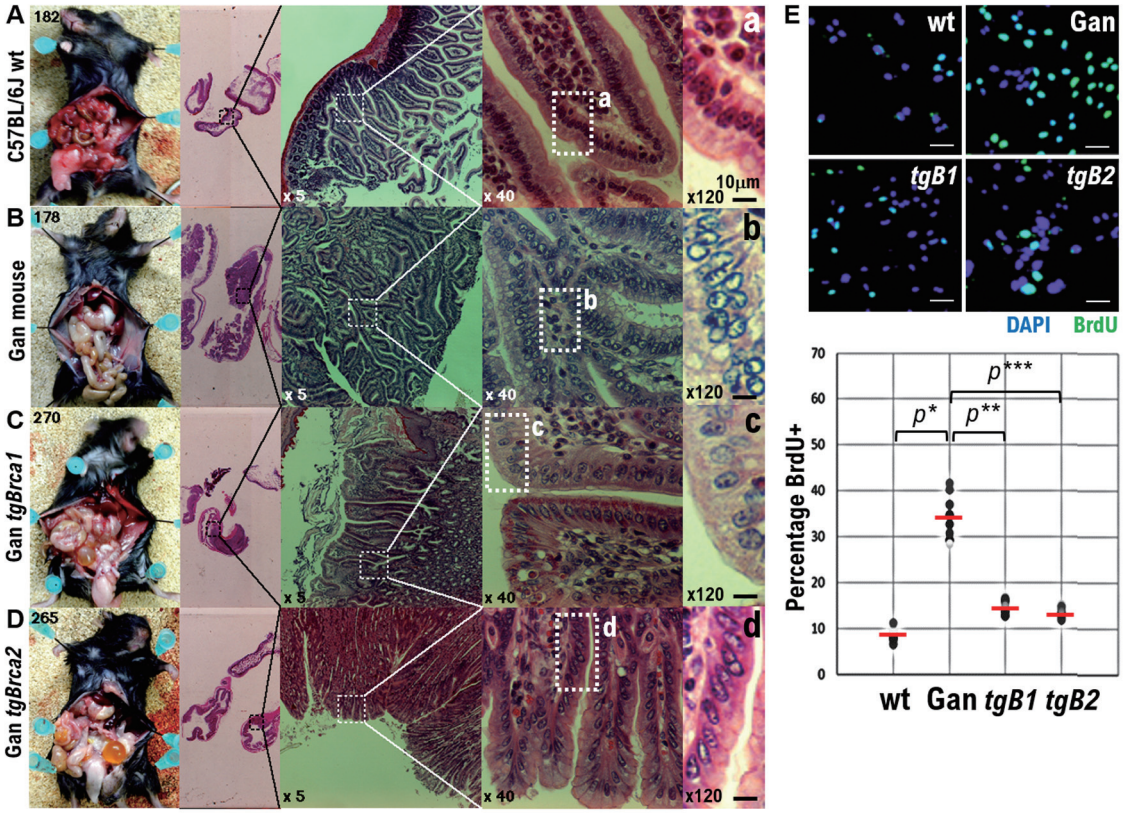

Figure 1. Effects of wild-type *BRCA1* and *BRCA2* expression on gastric cancer (GC) development in *Gan* mice, a mouse model of GC. (A) Photographs showing surgical pathological findings of normal epithelial cells in gastric mucosal tissue of wild-type C57BL6J mice. (B) Photographs showing surgical pathological findings of highly atypical epithelial cells in gastric mucosal tissue developed in *Gan* mice. (C) Photographs showing surgical pathological findings of mildly atypical epithelial cells in the gastric mucosal tissue developed in *Gan*^{tgBrca1} mice constitutively expressing BRCA1. Decreased number of atypical epithelial cells in gastric mucosal tissue. (D) Photographs showing surgical pathological findings of epithelial cells in the gastric mucosal tissue in *Gan*^{tgBrca2} mice constitutively expressing BRCA2. Significantly less atypical epithelial cells can be observed in gastric mucosal tissue. The gastric mucosal tissue of *Gan*^{tgBrca2} mice pathologically resembles normal tissue. (a-d) Enlarged images of tissue areas showing pathological findings of epithelial cells in the gastric mucosal tissue of each mouse at × 40 magnification. In other words, these images have been further enlarged to × 120. Scalebars, 10 μm. (E) Each genetically modified mouse was inoculated with a BrdU solution, and proliferation of epithelial cells in the gastric mucosal tissue of each genetically modified mouse was examined by the number of BrdU-positive cells. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of wild-type C57BL6J mice is extremely small. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of the *Gan* mice was extremely large. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of *Gan*^{tgBrca1} mice was reduced. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of *Gan*^{tgBrca2} mice was significantly reduced. The percentage of number of BrdU-positive cells observed among epithelial cells of the gastric mucosal tissue of each genetically modified mouse is plotted in the graph. *P < 0.001, **P < 0.005, ***P < 0.002.

H. pylori CagA in infected cells (host cells) binds to the oncogenic phosphatase - SRC homology phosphatase t2 (SHP2) encoded by the *PTPN11* gene, enhancing its activity. This in turn activates the Ras-ERK pathway that promotes cell proliferation. Polarity-regulating kinase partitioning-defective 1b (PAR1b), also called microtubule affinity-regulating kinase 2 (MARK2), is a kinase that phosphorylates serine and threonine residues. PAR1b regulates the formation of apical-basal polarity of epithelial cells and microtubules. Furthermore, CagA binds to PAR1b forming a complex near the cell membrane, which suppresses PAR1b kinase activation [16, 28]. A nuclear import signal is an amino acid sequence that serves as a marker for transporting a protein into the nucleus, and generally indicates a region where basic amino acids lysine and arginine are gathered. For nuclear translocation of BRCA1/2, their serine residue (Ser616), located near the nuclear translocation signal, must be phosphorylated by PAR1b [29]. Specifically, the nuclear translocation of BRCA1/2 is inhibited due to PAR1b kinase inactivation by CagA. As a result, BRCA1 decreases in the nucleus, and BRCA1/2 dysfunction causes BRCAness. Subsequently, homologous recombination repair of DSBs in DNA replication forks fails to occur [16, 28]. DNA replication proceeds by opening double-stranded DNA in both directions from the origin of replication. The Y-shaped structure where the DNA has dissociated as replication progresses and the dissociated double-stranded DNA join together is

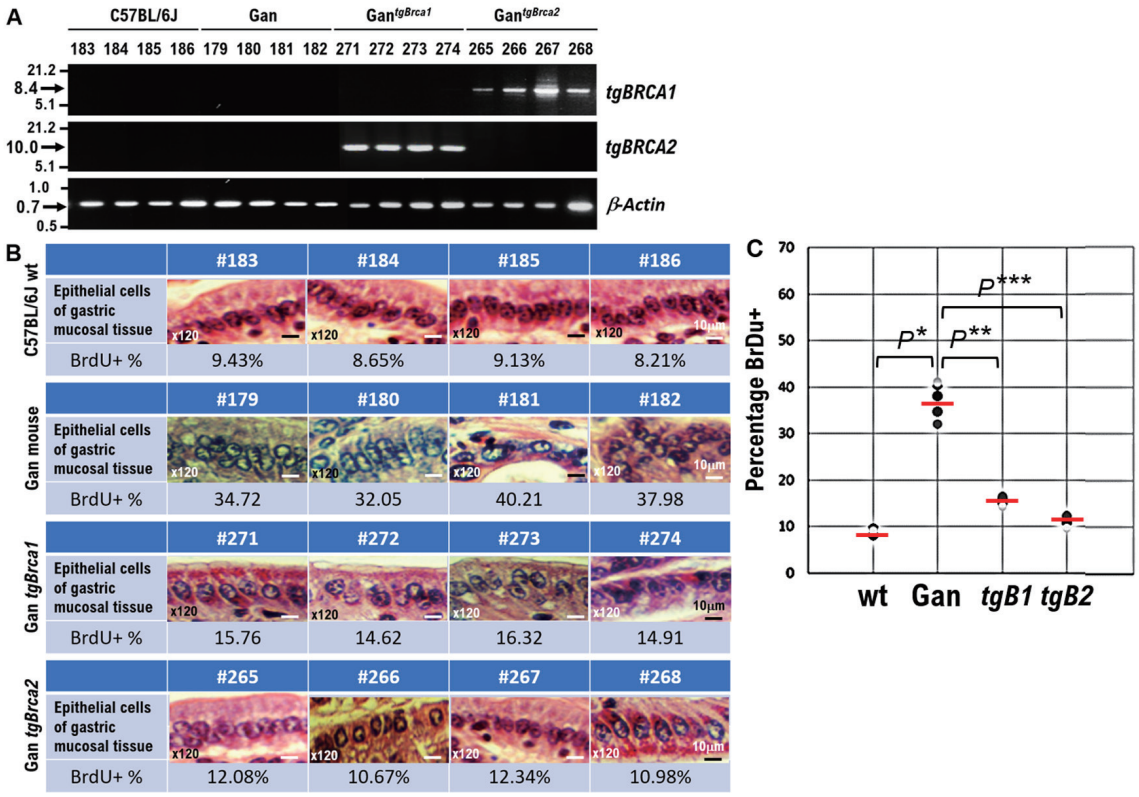

Figure 2. Effects of wild-type *BRCA1* and *BRCA2* expression on gastric cancer (GC) development in *Gan* mice, a mouse model of GC. (A) The results of reverse transcriptase polymerase chain reaction (RT-PCR) showing *Brca 1* wild type or *Brca2* wild type mRNA expression in *Gan*^{tgBrca1} or *Gan*^{tgBrca2} mice. (B) Surgical pathological findings of normal epithelial cells in gastric mucosal tissue of wild type C57BL6J mice (mouse ID number #183, #184, #185, #186). Surgical pathological findings of highly atypical epithelial cells in gastric mucosal tissue in *Gan* mice (mouse ID number #179, #180, #181, #182) and mildly atypical epithelial cells in the gastric mucosal tissue of *Gan*^{tgBrca1} mice (mouse ID number #271, #272, #273, #273) constitutively expressing BRCA1. The atypical epithelial cells in gastric mucosal tissue are reduced in this mouse. The surgical pathological findings of atypical epithelial cells in the gastric mucosal tissue of *Gan*^{tgBrca2} mice (mouse ID number #265, #266, #267, #268) constitutively expressing BRCA2 were significantly reduced. Pathologically, the gastric mucosal tissue of *Gan*^{tgBrca2} mice (mouse ID number #265, #266, #267, #268) resembles normal tissue. These images of the gastric mucosal tissue have been enlarged to × 120. Scalebar, 10 μm. (C) Each genetically modified mouse was inoculated with a BrdU solution, and the proliferation of epithelial cells in the gastric mucosal tissue of each genetically modified mouse was examined using the number of BrdU-positive cells as an indicator. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of wild-type C57BL6J mice is extremely small. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of the *Gan* mice was extremely large. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of *Gan*^{tgBrca1} mice was reduced. The number of BrdU-positive cells in the epithelial cells of the gastric mucosal tissue of *Gan*^{tgBrca2} mice was significantly reduced The percentage of number of BrdU-positive cells observed among epithelial cells of the gastric mucosal tissue of each genetically modified mouse is plotted in the graph. *P < 0.001, **P < 0.005, ***P < 0.001.

called a replication fork. Specifically, *H. pylori*-infected cells exhibit BRCA1-specific repair dysfunction. This may lead to high number of GC cases in HBOC families which also contain GPVs of *BRCA1/2* genes. To ascertain this, we investigated the number of GC cases in 78 HBOC families (one/four/six generations of these families were studied). We found that HBOC families had a higher proportion of GC cases (2.95%) compared to non-HBOC families (0.78%) (Table 1, Supplementary Materials 2, 3, www.wjon.org).

When *H. pylori* inserts CagA into infected cells, BRCA1/2 nuclear translocation is inhibited, resulting in genome instability [16, 28] and making gastric epithelial cells turn into cancer progenitor cells. A recent report demonstrated the expression and intracellular distribution of BRCA1/2 in surgically excised gastric mucosal tumor tissues [16, 28]. In a recent study, BRCA1 was not found in the nucleus of epithelial cells in the gastric mucosa [28]. The fundus gland is an exocrine gland in the stomach composed of parietal cells that secrete gastric acid, mucus cells that secrete mucus to protect the mucous membrane from gastric acid, and principal cells that secrete pepsinogen. Furthermore, in epithelial cells infected with *H. pylori*, nuclear BRCA1 was significantly reduced and there was DSB formation [28]. The lifetime incidence of breast cancer and ovarian cancer, in women with HBOC due to germline mutation and/or inactivating mutations in *BRCA1*/2 genes, is 70-80% and 40%, respectively [30]. Moreover,

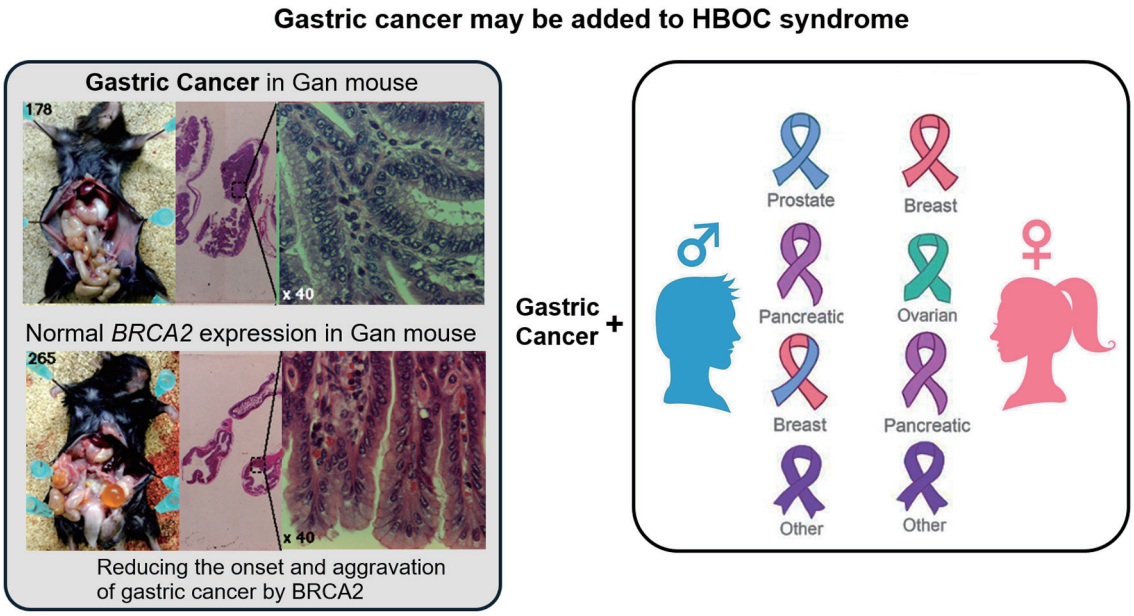

Figure 3. The gastric cancer observed in some patients may be part of the hereditary breast and ovarian cancer (HBOC) syndrome. BRCA1/BRCA2 protect DNA replication forks by repairing damage (DNA double-strand breaks (DSBs)) during nuclear genomic DNA replication. In particular, in maintaining the stability of cellular genome (genome stability). BRCA1/2 genes are typical tumor suppressor genes, and loss-of-function mutations in BRCA1/2 significantly increase the risk of developing breast and ovarian cancers, as well as some prostate and pancreatic cancers. The present results indicate that BRCA1 or BRCA2 pathogenic variants may be involved in the malignant transformation of epithelial cells in the gastric mucosal tissue. Therefore, the gastric cancer observed in some patients could be part of the HBOC syndrome.

approximately 6% of men with HBOC syndrome, who also carry a *BRCA1* mutation, develop breast cancer [31]. On the other hand, *BRCA1/2* is expressed in all cells including mammary gland cells, ovarian epithelial cells, and gastric epithelial cells. In murine gastric stem and progenitor cells, inactivation of *BRCA1* and/or *BRCA2* synergizes with *H. pylori* infection to induce DNA damage [28, 32]. Furthermore, in GC cells, infection-dependent DNA damage is aggravated by mutational inactivation of *BRCA2*, but not by *TRP53*/*Smad4* loss, or *Erbb2* overexpression [28, 32]. However, the reason for the significant increase in the risk of developing specific cancers (such as breast, ovarian, and GCs) with deletion/inactivation of germline *BRCA1/2* is still unknown.

H. pylori is a gram-negative microaerophilic spiral bacillus that sustains infection in the harsh, highly acidic stomach environment [33]. Normally, the *H. pylori* infection established during childhood persists lifelong, unless aggressively eradicated using drugs [34]. *H. pylori* infects approximately half of the world's population and is a resident bacterium [35]. However, some people infected with *H. pylori* develop atrophic gastritis, peptic ulcers, and even GC, forcing *H. pylori* to be categorized as a pathogenic bacterium [36]. Here, we demonstrate the importance of early eradication of *H. pylori* infections in HBOC family members in preventing GC development. Poly ADP-ribose polymerase (PARP) inhibitors inhibit the activity of PARP, which is involved in DNA repair. Normal cells use homologous recombination to repair DSBs, but cancer cells cannot lead to cell death. PARP inhibitors are used as chemotherapy for breast and ovarian cancers. The results of this study indicate that oral administration of PARP inhibitors, which is effective against breast, ovarian, pancreatic, and prostate cancers involving *BRCA1/2* PVs, can be used to treat GCs involving *BRCA1/2* PVs. Therefore, this study will help establish early treatment methods for GC patients with *BRCA1/2* PVs.

Supplementary Material

Suppl 1. Possible hereditary gastric cancer revealed by genetically engineered mice and family history of HBOC.

Suppl 2. HBOC family.

Suppl 3. Non-HBOC family.

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Conflict of Interest

The authors state no competing interest.

Informed Consent

We have obtained informed consent from people participating in clinical studies.

Author Contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. TH, KS and MO: research conduction; TH and KS: writing-original draft; TH, TU and IK: writing-review and editing; IK: visualization; TH and IK: supervision; TH and IK: funding acquisition.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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