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Study of CDH1 germline mutation in hereditary diffuse gastric cancer and lobular breast cancer: a multicenter study in Iran

Marveh Rahmati[1], Elmira Ebrahimi[1], Reza Shirkoohi[1], Habibollah Mahmoodzadeh[2], Ahad Mohammanjad1, Hedyeh Malekisarvar[1], Zahra Valizadeh[1], Marzieh Dehghani[3], Shirin Haghighat[3], Maryam Marzban[4], Kazem Zendehdel[1*]

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1. Cancer Biology Research Center, Cancer Institute, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Surgical Oncology, Cancer Institute, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
3. Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
4. The Persian Gulf Tropical Medicine Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran

ABSTRACT

Introduction: Mutations occurring in the CDH1 gene elevate the susceptibility to the development of hereditary diffuse gastric cancer (HDGC) as well as lobular breast cancer (LBC). LBCs with CDH1 germline mutations are classified as hereditary breast cancers, devoid of any indication of gastric carcinoma. As prophylactic gastrectomy is a recommended course of treatment for individuals with CDH1 mutations, it is advised that genetic testing be conducted for those who meet the criteria established by the National Comprehensive Cancer Network (NCCN). This study aimed to screen patients with HDGC for CDH1 testing and clinical management for the prevention of HDGC. Moreover, patients with LBC are tested for CDH1 mutation.

Materials and Methods: In this multicenter study, thirty-one patients with HDGC were selected among 615 patients and admitted for CDH1 mutation testing. PCR for all 16 exons of CDH1 and Sanger sequencing were performed. Patients with LBC who are negative for BRCA1/2 were also included in this study regardless of gastric cancer history.

Results: The results showed that 5.04% of patients with DGC were identified as HDGC of which 9.6% of individuals had a pathogenic CDH1 mutation and only 15.38% of cases with a family history carried germline mutation in CDH1. Furthermore, 7.6% of patients with LBC and negative for a family history of gastric cancer have CDH1 mutation.

Discussion: All patients who met the NCCN criteria were not CDH1 mutation-positive, indicating other genes should be involved in DGC. Moreover, patients with LBC and negative for BRCA1/2 are recommended for testing the mutation in CDH1.

Keywords: CDH1 mutation, Hereditary Diffuse Gastric Cancer, Lobular breast cancer, Prophylactic gastrectomy

INTRODUCTION:

Gastric cancer (GC) is the most important cancer worldwide with a high incidence in western Asian countries [1]. In Iran, GC is the five most common cancer with an incidence of 7300 cases annually [2], and is the first cause of cancer death [1]. 90% of GC cases are sporadic while 10% of them are familial features [3]. Hereditary GC accounts for a very low percentage of cases (1–3%), and hereditary diffuse gastric cancer (HDGC) is the most important one [4]. HDGC represents an autosomal dominant predisposition to diffuse gastric cancer, which is characterized by a poorly differentiated adenocarcinoma that infiltrates the stomach wall, resulting in the thickening of the wall without the formation of a distinct mass. HDGC is also known as signet ring carcinoma or isolated cell-type carcinoma. The mean age of onset of HDGC is 38 years, with a range of 14-69 years. It is noteworthy that approximately 40% of HDGC families exhibit germline mutations in CDH1, and over 100 different pathogenic germline mutations have been reported across various ethnicities [5].

According to estimates, CDH1 mutation carriers have an approximate cumulative risk of 80% for gastric cancer by the age of 80 years, regardless of gender. Additionally, women with this mutation have a risk of 39%-52% for developing lobular breast cancer (LBC) [6]. The significant risks associated with this condition, combined with the complexities involved in detecting DGC at its early stages, underscore the utmost importance and clinical complexities in the identification and treatment of individuals harboring CDH1 mutations [7].

CDH1 is located on chromosome 16q22.1 [4], autosomal dominant, and encodes the E-cadherin protein, which plays a role in cellular binding. The mutation in this gene leads to loss of cell adhesion, increased growth, invasion, and metastasis [8]. CDH1 mutations encompass small frameshifts, splice-site alterations, nonsense mutations, missense mutations, and large rearrangements. The majority of CDH1 mutations are deemed pathogenic, while certain missense mutations exhibit a detrimental impact on the functionality of E-cadherin. Current clinical recommendations for HDGC prevention are prophylactic gastrectomy in CDH1 carriers [9]. An endoscopy is an option for those who have not had a gastrectomy or are known to have a family with a CDH1-positive mutation [10–12].

In addition to HDGC, CDH1 germline mutations were identified in women diagnosed with LBC who did not have a family history of gastric carcinoma [13]. The most recent International Gastric Cancer Consortium has introduced a new guideline, suggesting genetic testing for bilateral LBC or a family history of two or more cases of LBC [13]. Nevertheless, prophylactic mastectomy is not commonly advised for women affected by CDH1 mutations; instead, they are recommended to undergo annual mammography and breast MRI starting at the age of 35 years [14].

The National Comprehensive Cancer Network (NCCN) guidelines indicate the suitable candidate of patients with HDGC for mutation testing [15] as follows; 1) Patients with DGC before the age of 40 years without a family history. 2) Families with two patients of gastric cancer, one confirmed DGC diagnosed before age 50 years. 3) A person with DGC, regardless of age, but with three confirmed cases of DGC in first or second-degree relative. 4) Personal or familiar history of DGC and lobular breast cancer, one diagnosed before age 50 years. Testing for the CDH1 mutation should also be done in all the individuals in the families of those affected and should be referred to a counseling team for gastrectomy.

In this study, with the aim of investigating the mutation rate of the CDH1 gene in DGC and LBC Iranian samples, the patients and their families, who had fully met the NCCN criteria and carried the CDH1 mutation, were chosen and referred to specialists if necessary.

Patients and Methods:

Patients with Diffuse Gastric Cancer and lobular breast cancer

DGC patients who were referred to Tehran, Shiraz, and

Bushehr hospitals in 2018 and 2022 were selected. The patients who met the NCCN criteria were included in this study and pathologically were double-checked by two specialist pathologists due to difficulties in the determination of diffuse type. "Poorly differentiated adenocarcinoma" or diffuse type was reported as the histopathological type of all the tumors. All the patients filled out the questionnaire and were asked about their cancer history in their families. The questionnaire also has all information about demographic data and clinical features including endoscopy and surgical history. The patients also have informed consent forms. From all suitable candidates, 10 ml of venous blood have been taken in EDTA tubes for further analysis.

16 patients with LBC were also included in this study who were negative for BRCA1/2. 13 out of 16 patients were negative for familiar GC and three with familiar history of GC in their 1st to 3rd -degree relatives.

Ethical code

Ethics Committee of Imam Khomeini Hospital, Tehran University of Medical Sciences, IR.TUMS.IKHC.REC.1397.046, approved this project.

PCR test

DNA was extracted from blood samples using the salting-out method to isolate high yields of DNA. To check the quality of DNA, the optical density (OD) of all samples was read by spectrophotometer NanoDrop™ One/OneC (Thermo Fisher Scientific). To evaluate all 16 exons in the CDH1 gene (NM_004360.4), PCR was performed for each of the 16 exons using their specific primers (Table 1) as follows; 10 pmol of forward and reverse primers with 50-500 ng of extracted DNA were used for PCR. A quality check of PCR products was carried out on 2% gel electrophoresis.

Sequence determination by Sanger sequencing

In order to investigate the mutations in all exons, PCR products were sequenced using Sanger sequencing. The submitted sequences were read using Finch TV software. The suspected mutations were checked by different databases, including http://www.mutationtaster.org/) and their pathogenicity was evaluated using the https:/www.ncbi.nlm.nih.gov/clinvar/ website.

Results:

Clinicopathologic characteristics

31 HDGC patients out of 615 DGC (5.04% %) were selected based on the NCCN criteria. Altogether, the mean age of the cases at diagnosis was 35.03 years. 16 patients with LBC are also included in this study. There are 13 patients without familiar GC and three patients with familiar history of GC in their 1st to 3rd -degree relatives.

Sequencing results and case presenting of DGC

DNA sequencing of the amplified PCR products exhibited five out of 31 patients with DGC-positive CDH1 gene mutation (16.1%). Three of them were predicted to be pathogenic (9.6%). 13 patients out of 31 showed a family history of GC (32.2%) from the mother side or father side in their 1st to 3rd-degree relatives and only 2 patients of 13 displayed positive mutation in CDH1(15.38%). One patient with both lobular breast cancer and DGC (3.2%) was evaluated but it was negative for CDH1 mutation. Table 2. Showed all the patients with familiar GC and genetic testing for CDH1 mutation.

Sequencing results and case presenting of LBC

The results of patients with LBC who are negative for BRCA1/2 showed that one patient had a pathogenic mutation and another had a variant of uncertain significance (VUS). None of them has a family history of gastric cancer.

Pathogenic CDH1 mutation-positive in patients

Patient number 1

Patient number 1 is a 28-year-old male with poorly differentiated adenocarcinoma, diffuse type. He has no family history (Figure 1). The CDH1 genetic test showed the mutation (C:2275G>A) in exon 14, category 4, and was pathogenic (Figure 1 and Table 1).

Table 1. CDH1 specific primers

Number	Primers	Sequence	Product size
Exon 1	Forward	caggtcccataacccaccta	458
	Reverse	gaagaagggaagcggtgac	
Exon 2	Forward	gagtcacccggttccatcta	229
	Reverse	ggtgtgggagtgcaatttct	
Exon 3	Forward	tcttcccacaagttcgctct	675
	Reverse	gattcagtcccagacggtgt	
Exon 4,5	Forward	gctgtctggctaggttggac	617
	Reverse	agctcctcatgtgttcagagc	
Exon 6	Forward	cagagcctaggaaggtgtgg	338
	Reverse	ctttgggcttggacaacact	
Exon 7,8	Forward	attgacccagtcccaaagtg	731
	Reverse	tcaaagacttcgcccatga	
Exon 9	Forward	aatcctttagccccctgaga	382
	Reverse	tctgggaaagtcaccctgtc	
Exon 10	Forward	ccacagttacttttgcaccaa	533
	Reverse	ggaacaggtgaaaggagcac	
Exon 11	Forward	tgttgggattacaggcatga	373
	Reverse	gaggggcaaggaactgaact	
Exon 12	Forward	gaccactgaagagccaggac	445
	Reverse	caatggaaggggtgacatct	
Exon 11	Forward	ccaagcagctctgctctctt	501
	Reverse	ttttatggaggctggcataact	
Exon 14	Forward	accgacttcagggatgtgag	410
	Reverse	tccaaagtgattcagagctgtt	
Exon 15	Forward	cagttggcagtgaaggcatc	401
	Reverse	aaaaggaaaacagggctcca	
Exon 16	Forward	agatgacaggtgtgcccttc	519
	Reverse	tggacatcaccaccatgtaaa	

Patient number 2

Patient number 2 was a 38-year-old woman with DGC. Genetic testing of the CDH1 gene mutation showed that she had a missense mutation (C:2195G>A(p. Arg732Gln) in exon 14, which is in category 5 and pathogenic. Her family history showed her mother also had DGC. The CDH1 genetic testing of her family showed that the brothers were 27, 38, 43, and 46 years old, respectively, and one of her sisters, 44 years old, and her uncle 61 years old genetically mutated in the

Table 2. Genetic reporting of patients with diffuse gastric cancer

Number	Gender	age	Genetic report (CDH1 test)	Location	Variant	Family history of GC
1	Male	28	Positive, category 4 (likely pathogenic	C:2275G>A	Exon 14	None
2	Female	38	positive, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14	Mother : DGC
3	Female	30	positive category 4	c.1711+1A>G	exon 11	Cousin (father side) with GC, 18yrs- Cousin (father side) with GC 33yrs- Grandmother (Mother side) with GC and 85yrs
4	Female	36	positive category 2	c.345G>A (little clinical significance)	Exon 3	None
5	Female	39	positive category 3	c.214G>A (Uncertain clinical significance)	Exon 3	None
6	Female	27	negative	-	-	Mother with GC, 32yrs-Uncle (mother side) with GC and 57yrs
7	Male	27	negative	-	-	Grandfather (mother side) with GC and 60yrs
8	Male	47	negative	-	-	Cousin (mother side) with GC and 22yrs
9	Male	41	negative	-	-	Uncle (father side) with GC over 50yrs
10	Male	37	negative	-	-	And Grandfather with GC over 50 yrs
11	Female	38	negative	-	-	Uncle (mother side) with GC58yrs
12	Male	40	negative	-	-	Father with DGC
13	Female	36	negative	-	-	Aunt (Father side) with GC over 50yrs

exon 14. They were referred to an oncosurgeon for consulting (Figure 2 and Table 3).

Patient number 3

Patient number 3 was 30 years old woman with DGC. Genetic testing of the CDH1 gene mutation showed that she had a missense mutation in exon 11, which is in category 4 and pathogenic (c.1711+1A>G). Her family history showed that her two cousins, from her father's side 18yrs, 33yrs and her grandmother from her mother's side 85yrs diagnosed with GC (Figure 3).

Discussion:

It has been observed that germline mutation in CDH1 accounts for approximately 15-50% of HDGC [16]. Nevertheless, with the implementation of updated

Table 3. Genetic report of patient number 2 and her family members.

number	Gender	Age	Relative	Genetic report (CDH1 test)	Location	Variant
1	Male	62	Brother	Mutation, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14
2	Male	43	Brother	Mutation, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14
3	Male	27	Brother	Mutation, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14
4	Male	38	Brother	Mutation, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14
5	Male	46	Brother	Mutation, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14
6	Female	44	Sister	Mutation, category 5, pathogenic	C:2195G>A(p.Arg732Gln)	Exon 14
7	Female	37	Sister	No mutation	-	Exon 14
8	Male	35	Uncle, mother side	No mutation	-	Exon 14

criteria, the incidence of mutation has decreased to 10-18% in regions with low GC prevalence [17]. The rate of CDH1 mutation in HDGC is unknown in Iran. However, there are some reports sporadically. For example, a study conducted in Isfahan in 1396 showed 11 out of 17 HDGC patients (64.7%) and 4 out of 28 sporadic DGC patients (14.02%) had a mutation in the CDH1 gene. The results showed different mutations in the CDH1 gene which emphasize the importance of investigating all the CDH1 mutation [18]. The other study examined mutations in the CDH1 gene within a solitary family afflicted with HDGC in Iran and reported the presence of a truncating mutation [19].

In our study, 5 mutations in 31 HDGC cases (16.12%) were positive in CDH1 from which 60% were pathogenic and under 40 years old.

A 2015 study from the University of British Columbia in Canada found that 34 out of 183 HDGC patients (19%) had mutations in the CDH1 gene. These individuals were selected exactly according to NCCN criteria. In fact, of these, 84 were families in which at least 2 had GC and at least one had HDGC before age 50. The results of the CDH1 mutation in these families showed that 22 of them (26%) had different CDH1 mutations. There was only one family in which three age-independent DGCs were found, all of which had negative CDH1 mutation results [5].

Another study in Portugal found that of 32 HDGC families and 23 early onset families, 1 had a CDH1 mutation and 1 did not fully meet the criteria for a p53 mutation. It has been suggested that in countries with a high incidence of gastric cancer, the p53 gene be tested as an alternative in families with CDH1-negative [8]. Other experiments also showed the rate of CDH1 positive in HDGC is 40% in England and 30% in Italy) [20,21].

To compare our results to the mentioned reports reveals that the rate of CDH1 mutation is different which showed a reflection of the varied ethnicities or sample size of the study.

Most of the CDH1 positive in HDGC is found in individuals who are less than 40 and have a familiar history [5,8]. Our results confirmed that 3.2% of early onsets less than 40 showed CDH1 positive and 15.38% of families with a historical background of GC carried germline mutation of CDH1. There was no difference in the rates of diffuse gastric cancer between men and

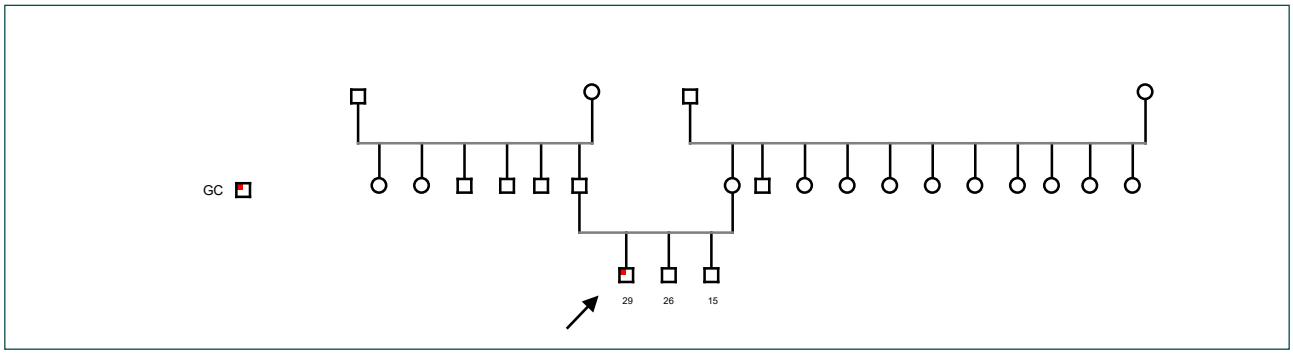

Figure 1. The family tree of patient number 1 who is 28-year-old. He has no family history of GC. The CDH1 genetic test showed the mutation (C:2275G>A) in exon 14, category 4 and was pathogenic. The arrow points to our patient.

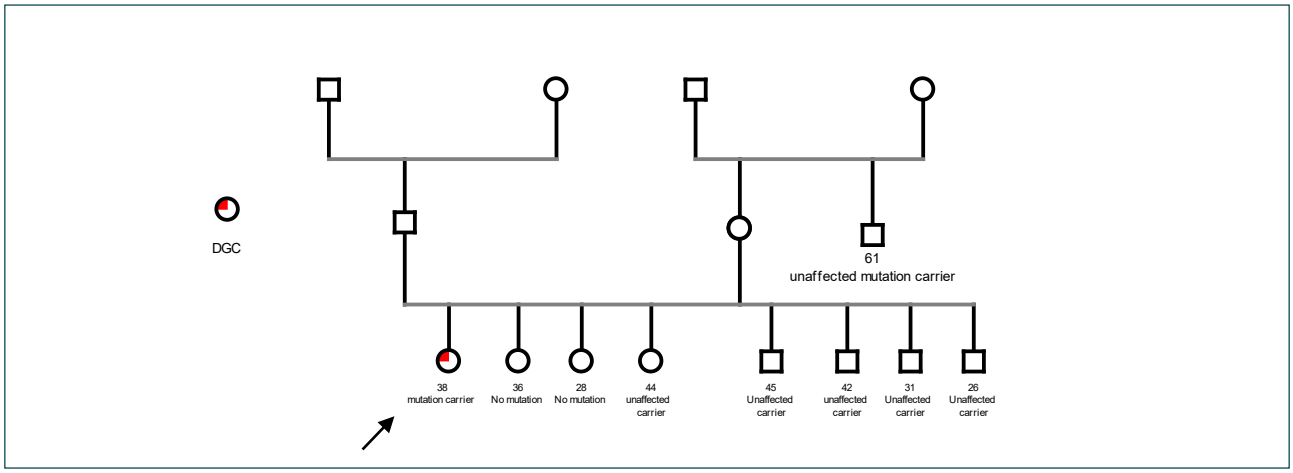

Figure 2. The pedigree belongs to a 38-year-old woman (patient number 2) who has DGC. Genetic testing of the CDH1 gene mutation showed that she had a missense mutation in exon 14, which is in category 5 and is therefore considered pathogenic. His family history was also examined. Their results showed that 4 brothers, one sister and uncle also have similar mutations. The arrow points to our patient.

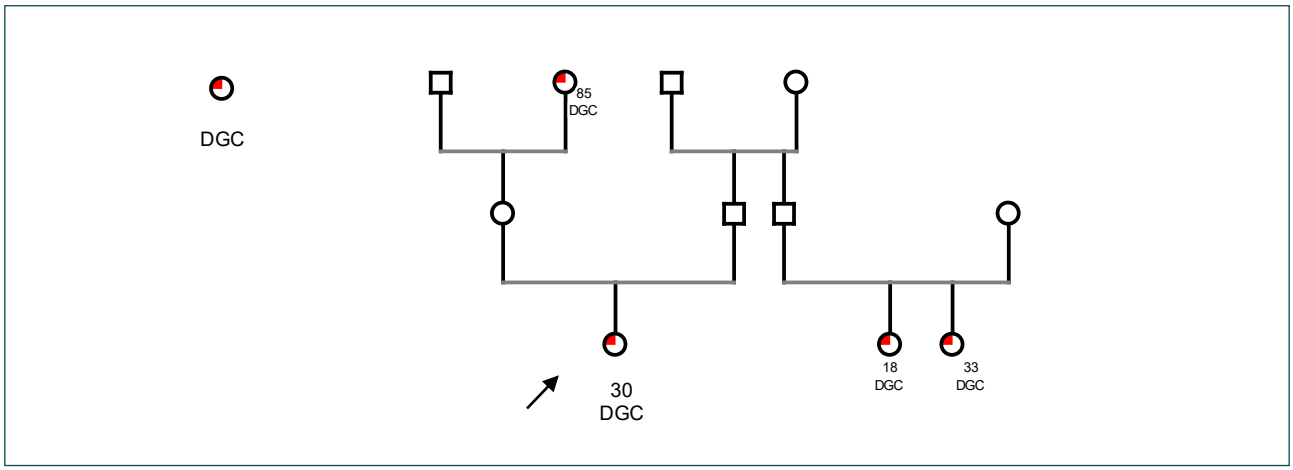

Figure 3. Patient number 3 was 30 years old woman with DGC. Genetic testing of the CDH1 gene mutation showed that she had a missense mutation in exon 11, which is in category 4 and pathogenic (c.1711+1A>G). Her family history showed that her two cousins, from father's side with 18yrs, 33yrs and her grandmother from mother's side with 85yrs diagnosed with GC. The arrow points to our patient.

women (48% men and 52% women) that are the same as previous reports [22]; however, 80.64% of HDGC are women in comparison to men which indicates that the samples were not selected appropriately from all cities in Iran. Within different age groups, diffuse gastric cancer is more predominant in younger patients, with the average age of diagnosis before 40 years, which is considered an early onset for most cancers. Our results are consistent with other reports [23].

The clinical significance of identifying CDH1 mutations in families with HDGC lies in the ability to ascertain the susceptibility of unaffected relatives to developing DGC and BC. Currently, the sole recommended strategies for reducing the risk of GC are gastroscopy with multiple random biopsies or prophylactic total gastrectomy. For individuals who decline or defer gastrectomy, endoscopic surveillance is a viable alternative [4]. In our experiment, the family members of patients with positive CDH1 mutation were also examined. In one of the patient's family members, 6 out of 8 (75%) individuals had the same mutation in the CDH1 gene and they were referred to a surgeon. The best prevention for them is to remove the stomach. However, they refuse total gastrectomy. They were suggested to do an endoscopy every 6 months. Individuals who completely fulfill the criteria but are negative for CDH1 mutation should be analyzed for further mutations in other genes such as CTNNA1 [24].

In this experiment, we have also conducted tests on LBCs to assess the presence of CDH1 mutation. It has been observed that approximately 3% of these LBCs carrying CDH1 germline mutations are classified as "hereditary breast tumors" without any signs of gastric carcinoma [25]. This study marks the first report from Iran regarding LBCs with positive CDH1 mutation. However, at present, there is no indication of prophylactic mastectomy in individuals with CDH1 mutations [26]. The only recommendation is to undergo annual mammography, ultrasound, breast MRI scans, and clinical breast examination starting at the age of 35 [26].

Conclusions:

Our experiment showed the importance of CDH1 mutation in HDGC and the unaffected individuals in a family with a historical background. We also reported for the first time the CDH1 mutation in LBC. This information is expected to aid in the genetic counseling and treatment of individuals at high risk from CDH1-positive HDGC families and LBC.

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