Journal of Gastrointestinal Cancer (2019) 50:420–427
https://doi.org/10.1007/s12029-018-0082-7

ORIGINAL RESEARCH

Novel Variants and Copy Number Variation in *CDH1* Gene in Iranian Patients with Sporadic Diffuse Gastric Cancer

Abbas Moridnia[1] · Mohammad Amin Tabatabaiefar[1] · Mehrdad Zeinalian[1] · Mohammad Minakari[2] · Majid Kheirollahi[1] · Noushin Afshar Moghaddam[3]

Published online: 26 March 2018

Abstract

Introduction The aim of this study was to survey the nucleotide changes and copy number variations (CNV) in the *CDH1* gene in Iranian patients with sporadic diffuse gastric cancer (SDGC).

Materials and Methods In this study, 28 patients were examined who upon gastrectomy had been diagnosed with SDGC according to the familial history and histopathological criteria which was confirmed by the pathologist. DNA extraction was performed from formalin-fixed paraffin-embedded tissues using a phenol-chloroform method following xylene deparaffinization. Determination of DNA sequence by Sanger was performed using PCR amplification of 16 exons and boundaries of intron/exon of CDH1 gene. Multiplex ligation-dependent probe amplification (MLPA) was performed on patients with pathogenic disorders in the sequence.

Results In total, patients included 20 males and 8 females. Of all patients, 12 patients were under 45 years old (early onset gastric cancer, EODC) and 16 patients were older. The tumor was diagnosed in the early TNM stage (I, II) in six patients and in late stages (III, IV) in 19 cases. Altogether, 16 variants (three exonic with one new variant and 13 intronic with nine new variants) were found in DNA sequencing of the *CDH1* gene in five samples. Also, using MLPA, a new duplication in exon 9 and one deletion in exon 2 were detected in two other patients. Altogether, *CDH1* variants were identified in seven out of 28 patients (25%).

Conclusion Our study revealed several novel somatic variants in the *CDH1* gene in Iranian patients with sporadic diffuse GC. Our data supports the hypothesis that mutations in *CDH1* gene, and particularly the mutations we describe, should be considered, even in sporadic cases of gastric cancer. The presence of these mutations in patients raises important issues regarding genetic counseling and diagnostic test in DGC patients.

Keywords Sporadic · Diffuse gastric cancer · *CDH1* · Variant · Iran

Introduction

Gastric cancer (GC) is the fourth common cancer in the world, accounting for 7% of all cancers and 9% of all cancer deaths in 2012 [1]. Diffuse gastric cancer (DGC) accounts for approximately 30% of all GCs, with poor prognosis mainly in young patients [2, 3]. Early onset gastric cancer (EOGC) is defined as gastric cancer at age of 45 or earlier, and it contains

✉ Majid Kheirollahi
mkheirollahi@med.mui.ac.ir

Abbas Moridnia
a_moridnia@med.mui.ac.ir

Mohammad Amin Tabatabaiefar
tabatabaiefar@med.mui.ac.ir

Mehrdad Zeinalian
zeinalianmehrdad@gmail.com

Mohammad Minakari
minakari@med.mui.ac.ir

Noushin Afshar Moghaddam
afsharmoghadam@med.mui.ac.ir

[1] Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-communicable disease and Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, P.O.Box: 81746-73461, Isfahan, Iran

[2] Internal medicine department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

[3] Department of Pathology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

approximately 10% of all gastric cancers. EOGC can occur in two forms of hereditary or sporadic. Epigenetic inactivation and *CDH1* gene mutations are causes of diffused form of EOGC and HDGC [4]. The incidence rate of diffuse-type gastric cancer was increased up to 62% within 1996 and 2000, while it has declined roughly between 2001and 2005 [5]. GCs are morphologically very heterogeneous. Most GCs are sporadic (90%) and a positive family history exists in just about 10% of cases [6], of which 1–3% are hereditary. There are three known hereditary syndromes for GC: hereditary diffuse GC (HDGC), familial intestinal GC (FIGC), and gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) [2, 7]. Despite the known molecular genetic causes in HDGC, most GCs are sporadic, and the susceptibility genes have been poorly investigated. There are two types of GCs, including diffuse and intestinal, but their clinicopathologic profile and epidemiological features are different [8]. The incidence rate of the intestinal GC in most countries is gradually decreasing. In contrary, the incidence of the diffuse type is constant or even increased [9]. Most GCs are multifocal and located beneath a healthy mucosal surface. Histologically, the tumor cells invade the surrounding tissues, and there is no mass formation. In most cases, the intracellular mucin pushes the nucleus to one side that leads to the signet ring cell carcinoma (SRCC) [10, 11].

Although mutations in the *CTNNA1*, *BRCA2*, *STK11*, *SDHB*, *PRSS1*, *ATM*, *MSR1*, and *PALB2* genes are introduced at DGC, but the *CDH1* gene mainly changes in DGC [12]. The deleterious germline mutations in the *CDH1* gene can lead to HDGC [13]. However, there is controversy about the prevalence of the *CDH1* mutations in different studies. While, some studies show the high prevalence of *CDH1* somatic mutations in sporadic diffuse GC cases, others report very rare prevalence of the mutations [14–16]. In addition, epigenetic inactivation of *CDH1* by promoter hypermethylation is the most common occurrence in DGC and plays a vital role as a second-hit mechanism in inactivation of the normal allele in HDGC patients and patients with germline *CDH1* mutations [17].

The *CDH1* gene is located in chromosome 16q22.1 and contains 16 exons with a 4.5-kb mRNA which encodes E-cadherin [18]. Cadherin is a glycoprotein composed of three domains including extracellular, transmembrane, and cytoplasmic domain. E-cadherin is a calcium-dependent cellular adhesion molecule playing a significant role in mechanisms regulating cell adhesions, motion, and proliferation of epithelial cells [19, 20]. Dysregulation of E-cadherin is associated with tumor invasion and progression [18]. The majority of the germline mutations are identified to date as single nucleotide substitutions, which lead to non-synonymous changes or insertion/deletion of a few base pairs, leading to deleterious frameshift mutations [12]. On average, 5% of the familial cases are due to the large deletions including several exons of the gene [21, 22]. Accordingly, some techniques like multiplex ligation-dependent probe amplification (MLPA) method can be used to identify quantitative changes in the *CDH1* gene or other genomic regions in patients with DGC.

To date, according to human gene mutation database (HGMD), 121 variants have been reported for *CDH1* gene. Moreover, large deletions may also be responsible for *CDH1* inactivation [23, 24]. As E-cadherin is a tumor suppressor gene, a second hit is required to disable the wild type allele through diverse mechanisms, including genetic and epigenetic alterations [25]. Based on what has been said, E-cadherin (CDH1) is one of the vital significant tumor suppressor genes in gastric cancer that its inactivation contributes to tumor development via increase in proliferation, metastasis and, invasion. CDH1 structural variations pose as a poor prognostic factor, and its somatic alterations are present in all the clinical settings and histological types of gastric cancer and associated with diverse survival rates. CDH1 is a potential predictive biomarker of response to therapy as its deficiency decreases tumor cell sensitivity to therapy. Since, there is no specific hotspot mutation region in the *CDH1* gene, there is a need to examine the entire gene in GC cells to find mutations. In this study, we report several novel *CDH1* variants and large deletion/duplication in Iranian patients with sporadic diffuse GC.

Materials and Methods

Patients and Sampling

The cases were 28 Iranian patients with SDGC confirmed by pathologist base on histopathological features and absent criteria based on International Gastric Cancer Linkage Consortium (IGCLC) [26]. They were selected among GC patients identified between January 2011 and April 2016 in Al-Zahra hospital, a referral hospital in Isfahan province, central Iran, and Alaa cancer control center, a charity-based foundation for cancer patients in Isfahan. All samples were formalin-fixed paraffin-embedded (FFPE) tumor tissues. The FFPE tumor samples were cut to 5–l0 μm thickness sections for DNA extraction by SDS-proteinase K digestion, phenol chloroform, and ethanol precipitation. Informed consent forms were signed by all the patients or their families. The study was approved by the Review Board of Isfahan University of Medical Sciences, according to the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration.

DNA Sequencing

The obtained DNA was amplified by polymerase chain reaction (PCR). All the coding exons and flanking intronic regions of the *CDH1* gene were amplified using primers designed by authors (Table 1). The PCR product of each reaction was

subjected to direct sequencing on an ABI 3130XL capillary sequencing platform (Applied Biosystems/Life Technologies, Carlsbad, CA, USA). The results of sequencing were obtained as electropherograms and analyzed with the Chromas software version 2.31. Evaluation of intronic variants was performed using Human Splice Finder (version 3.0).

Multiplex Ligation-Dependent Probe Amplification

All cases with no pathogenic variants in the *CDH1* gene were tested for large genomic deletions/duplications using SALSA P083-C2 *CDH1* multiplex ligation-dependent probe amplification (MLPA) kit (MRC-Holland, Amsterdam, the Netherlands). The reactions were performed according to the manufacturer's protocols. The probe ratio (PR) less than 0.7 was considered indicative for a gene dosage reduction, and a PR more than 1.3 could be indicative for increase in the gene dosage.

Table 1 List of primers for PCR of *CDH1* gene

CDH1 F1	GTGAACCCTCAGCCAATCAG
CDH1 R1	GACGACGGGAGAGGAAGG
CDH1 F2	GGTTTCGGTGAGCAGGAG
CDH1 R2	AAGGGGTGTCGTTTGAGC
CDH1 F3	TGGAGAAGGAATGCTCTTGT
CDH1 R3	GCTGAGAAACCTGGATTAGA
CDH1 F4	GTCTGGCTAGGTTGGACTG
CDH1 R4	TCCCTTTCTCTCCTTGGTAC
CDH1 F5	CTGGTTCAGGTAGAGAAAGAAGT
CDH1 R5	AAGCTCCTCATGTGTTCAGAG
CDH1 F6	GCTCAAGTCACCCTCACT
CDH1 R6	GCATATAACACAACAATGGCT
CDH1 F7	TCATCTCCTTGAACTCTTCCA
CDH1 R7	CTTAGACCATCACTGTATTAACTG
CDH1 F8	GGTTCCGTGCCTAGAAGAC
CDH1 R8	ACTTCGCCCATGAGCAGT
CDH1 F9	AATGACACATCTCTTTGCTCTG
CDH1 R9	CACTACAATCTGGGAAAGTCAC
CDH1 F10	TGAGCAGATTTGAGAAGCCA
CDH1 R10	GAACAGGTGAAAGGAGCACAG
CDH1 F11	CATGTTGTTTGCTGGTCCT
CDH1 R11	CCTGACTTTACCACTACACATCT
CDH1 F12	TGGTCTGGTGGAAGGCAAT
CDH1 R12	TTGAAAGGTGGGGATCTGG
CDH1 F13	GCTCTGCTCTCTTCACTCG
CDH1 R13	AGTCTCTTTCCCACATCAGC
CDH1 F14	TCTCAACACTTGCTCTGTCTC
CDH1 R14	CTGTTTCAAATGCCTACCTC
CDH1 F15	AGATCATACAGTTGGCAGTGAA
CDH1 R15	CAGGCAAGCTGAAAACATAGT
CDH1 F16	GTGTGCCCTTCCTTTCACTA
CDH1 R16	CATCACCACCATGTAAAGAGTG

Results

Epidemiological and Clinicopathologic Results

Out of 28 studied cases (20 males and 8 females), 16 patients were older than 45 and 12 were under 45 years old (EOGC). The mean age of the patients at diagnosis was 54.5 years. The mean age of diagnosis for male and female patients were 52.85 and 58.75, respectively. In six patients (21.4%), tumor had been identified in early TNM stage (I, II), and in 19 cases (67.9%) tumor had been identified in late stages (III, IV). In three patients (10.7%), the pathological stage of tumor had not been determined. "Signet ring cell carcinoma" and "poorly differentiated adenocarcinoma" had been reported in 21 (75%) and 7 (25%) of cases as the histopathological type of the tumor, respectively (Table 2).

Table 2 Epidemiologic and clinicopathologic features of sporadic diffuse gastric cancer patients

Gender	Age of diagnosis	Stage	Histopathological type
Male	68	I	Signet ring cell carcinoma
	42	II	Signet ring cell carcinoma
	43	II	Signet ring cell carcinoma
Female	65	IB	Signet ring cell carcinoma
	41	II	Signet ring cell carcinoma
	70	II	Poorly differentiated adenocarcinoma
Male	49	IIIA	Signet ring cell carcinoma
	52	IIIA	Signet ring cell carcinoma
	68	IIIA	Poorly differentiated adenocarcinoma
	57	IIIB	Signet ring cell carcinoma
	42	IIIA	Poorly differentiated adenocarcinoma
	78	IIIC	Signet ring cell carcinoma
	44	IIIA	Poorly differentiated adenocarcinoma
	40	IIIB	Signet ring cell carcinoma
	62	IIIC	Signet ring cell carcinoma
	42	IIIB	Signet ring cell carcinoma
	51	IIIA	Signet ring cell carcinoma
	40	IV	Poorly differentiated adenocarcinoma
	41	IV	Signet ring cell carcinoma
	61	IV	Signet ring cell carcinoma
	44	IV	Signet ring cell carcinoma
Female	75	IIIC	Signet ring cell carcinoma
	43	IIIC	Signet ring cell carcinoma
	70	IIIC	Poorly differentiated adenocarcinoma
	63	IV	Signet ring cell carcinoma
Male	80	–	Poorly differentiated adenocarcinoma
	53	–	Signet ring cell carcinoma
Female	43	–	Signet ring cell carcinoma

Sequence Analysis

Except for three samples which were excluded due to their low quality, all the coding exons and flanking intronic regions of the *CDH1* gene were successfully PCR amplified in all patients, and DNA sequencing of the amplified PCR products showed ten new variants in *CDH1* gene of the tumor samples (Table 3). Exonic alterations were synonymous, and evaluation of intronic variants revealed a potential effect on splicing site in seven different variants.

MLPA Results

We found one duplication in exon 9 (*CDH1* probe 16884-L19717) (Fig. 1a) and one deletion in exon 2 (*CDH1* probe 12657-L14803) (Fig. 1b). In two patients, we found one duplication in exon 9 (*CDH1* probe 16884-L19717) (Fig. 1a), and one deletion in exon 2 (*CDH1* probe 12657-L14803) (Fig. 1b) using the MLPA technique. These deletion and duplication with these probes have not yet been reported (Fig. 2). The normal sample from the same patient was obtained for normalization and as the negative control. The ABI Step One Plus (Applied Biosystems, Foster City, CA, USA) instrument and ΔΔCt method were used for data analysis.

Amino Acid Substitutions

Some synonymous likely benign variants were explored. A single base pair substitution in three samples causes G to A transition, and the result is a single amino acid substitution at codon 116 as p.116L>L (Fig. 3a). In addition, a single base pair substitution in two samples producing T to C transition that leads to a synonymous amino acid substitution at codon 692 as p.692A>A was found (Fig. 3b). Finally, a single base pair substitution in one sample, resulting in a C to T transition leading to a synonymous amino acid substitution at codon 751 as p.751N>N (Fig. 3c) was detected (Table 3).

Intronic Substitutions and Splicing Site Effects

We also identified some likely benign variants in intronic sites. A single base pair substitution consisting a T to G transversion (c.2439+103T>G) in one sample (Fig. 3d), a single base pair substitution including a C to G transversion (c.2296-22C>G) in four samples (Fig. 3e), a base pair deletion in three samples (c.1937-58delA), a single base pair substitution in one sample (c.2164+49G>A), one base pair substitution in one sample (c.2439+52G>A), one single base pair substitution (c.2296-48C>G) in four samples, and finally, one single base pair substitution (c.2296-44T>G) in four samples (NM_004360.3) were found. Bioinformatics analysis using Human Splicing Finder showed that these alterations may be affected by the splicing site, and new potential splice sites could be created (Table 4).

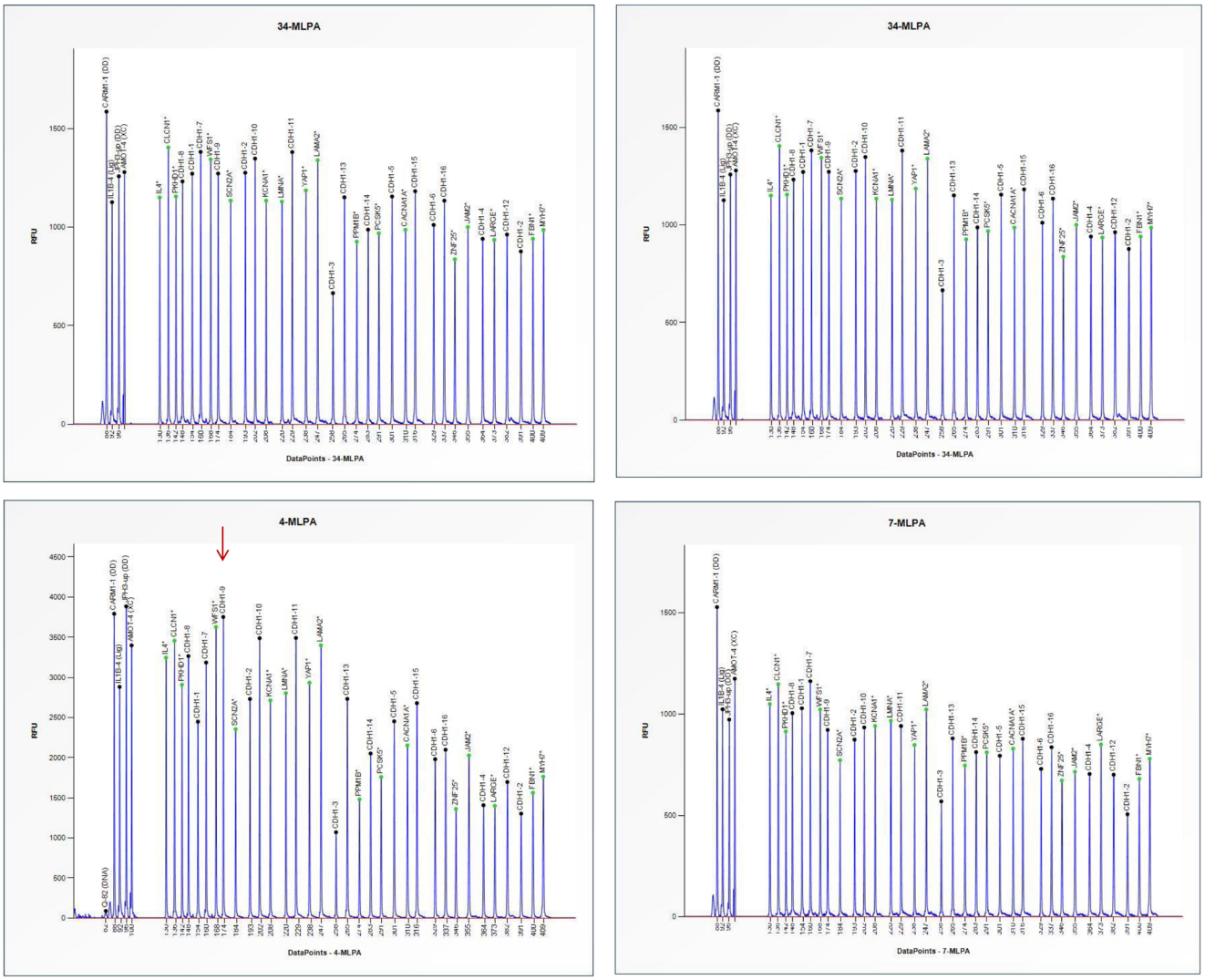
Discussion

GC is the fourth most common cancer worldwide, accounting for 9% of all cancer-related mortality in 2012 [1]. On the other hand, a significant part of all types of GC is diffused type with

Table 3 Exonic and intronic variants in the *CDH1* gene in patients with sporadic diffuse gastric cancer

Sample ID	Substitution	Exon or exon/intron boundary	Deletion	Homozygosity	Amino acid	Chromosome location
4,7, 25	G>A	3	–	Heterozygot*	L116L	NM_004360.3:c.348G>A
4, 5	T>C	13	–	Heterozygot	A692A	NM_004360.3:c.2076T>C rs:1801552
25	C>T	14	–	Heterozygot	N751N	NM_004360.3:c.2253C>T rs:33964119
25	G>T	3	–	Homozygot*	Intronic	NM_004360.3:c.387+47G>T
5	G>A	10	–	Heterozygot*	Intronic	NM_004360.3:c.1321-30G>A
5	T>A	10	–	Heterozygot*	Intronic	NM_004360.3:c.1321-16T>A
4, 5, 7	–	13	A*	–	–	NM_004360.3:c.1937-58delA
5, 7	T>C	13	–	Heterozygot	Intronic	NM_004360.3:c.1937-13T>C rs:2276330
25	G>A	13	–	Heterozygot	Intronic	NM_004360.3:c.2164+49G>A rs:774975562
25	G>A	13	–	Heterozygot*	Intronic	NM_004360.3:c.2164+46G>A
23	G>C	14	–	Heterozygot*	Intronic	NM_004360.3:c.2295+74G>C
4	G>A	15	–	Heterozygot	Intronic	NM_004360.3:c.2439+52G>A rs:33965115
5	T>G	15	–	Heterozygot*	Intronic	NM_004360.3:c.2439+103T>G
4, 7, 23, 25	C>G	15	–	Heterozygot*	Intronic	NM_004360.3:c.2296-48C>G
4, 7, 23, 25	T>G	15	–	Heterozygot*	Intronic	NM_004360.3:c.2296-44T>G
4, 7, 23, 25	C>G	15	–	Heterozygot	Intronic	NM_004360.3:c.2296-22C>G rs:763184960

*New variants



A B

Fig. 1 (**a**) Coffalyser electrophoretogram of the *CDH1* gene. The above electrophoretogram is normal control and below is present patient. Arrow indicates duplication in exon 9. (**b**) Coffalyser electrophoretogram of the *CDH1* gene. Above electrophoretogram is normal control and below is present patient. Arrow indicates deletion in exon 2

very little data in developing countries such as Iran [2, 3]. The global incidence of GC is very different, and the reason for this difference is unknown. GC has different characteristics in regions with low and high risk. In regions with high risk, carcinogenesis factors such as environmental, life style, and specific foods are more possibly associated with the GC, while genetic factors, such as *CDH1* gene mutations are very rare [27]. GC is the most common cause of cancer death in Iran, especially in the North and North West of Iran. Unlike Japan, North America, and Western Europe, the incidence of GC has increased in Iran over the past 30 years [28]. The rate of *CDH1* mutations in diffuse GC prior to 2010 was reported to be between 25 to 50%, using the guidelines established in 1999 [12, 29]. However, using the updated criteria with more specificity, mutation rate in *CDH1* gene was declined to 10 to 18% in countries with low incidence of GC [30–32]. In one study that was conducted on 21 DGC patients in Italy, two of them (9.5%) have shown mutation that one of them was missense by c.670C>T as p.Arg224Cys and one substitution at -63C>A, also stages of disease in this study were 23.8 and 71.5% in early TNM stage (I, II) and in late stages (III, IV), respectively [33]. In another study on 25 patients with sporadic EODGC, 8.0% mutation in the *CDH1* gene was identified by one nonsense as p.Arg335* in exon 7 and one missense as p.Gly239Arg in exon 6 [4].

So far, only one study has been reported in Iran in which *CDH1* gene mutations have been evaluated in a single family with HDGC cases, and a novel truncating mutation has been identified in position 758 (G 758 stop) [34]. In this study, we identified somatic variants in 7 out of 28 (25%) patients with

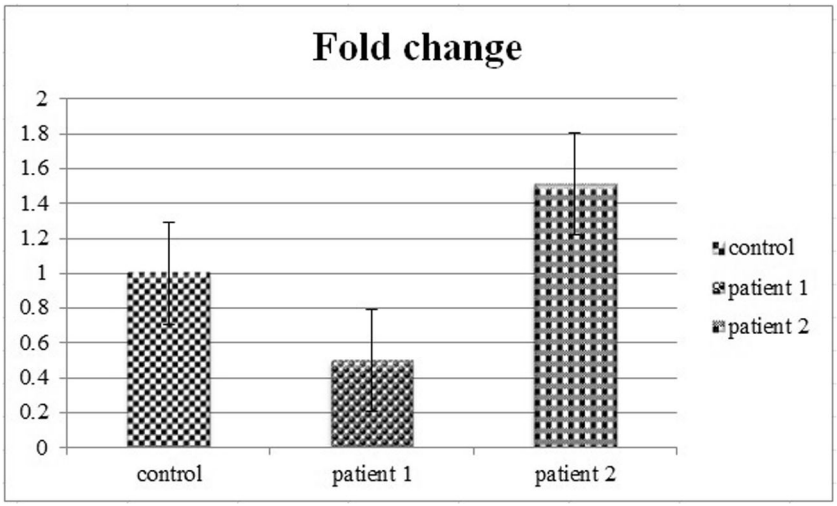


Fig. 2 Fold change graph of exon 2 and 9 *CDH1* gene. Patient 1 indicates heterozygote deletion in exon 2, and patient 2 indicates heterozygote duplication of exon 9

SDGC. Substitution alterations in our samples were located on exons 3, 13, and 14. Substitution c.348G>A is located in exons 3 and c.2076T>C located in exon 13 of *CDH1* gene encoding for extracellular domain of E-cadherin. Since the extracellular domain is necessary for cell-cell adhesion [18], its alterations can rupture the cell adhesion. On the other hand, substitution c.2253C>T located in exon 14 and encoding for the cytoplasmic domain of E-cadherin. This part of E-cadherin plays a central role in tumor-suppressing function. This domain binds to β-catenin and inhibits nuclear signaling pathway of this proto-oncogene [34]. In our study, one novel duplication variant in exon 9 and one large deletion in exon 2 were identified in two cases via MLPA technique. Exon 2 involved in propeptide part of E-cadherin and exon 9 encoding for extracellular domain, and alterations in these regions can lead to impaired production and dysfunction of this protein. In one study, large deletion exon 11 *CDH1* gene by MLPA was reported [35]. Molinaro et al. have demonstrated a large deletion

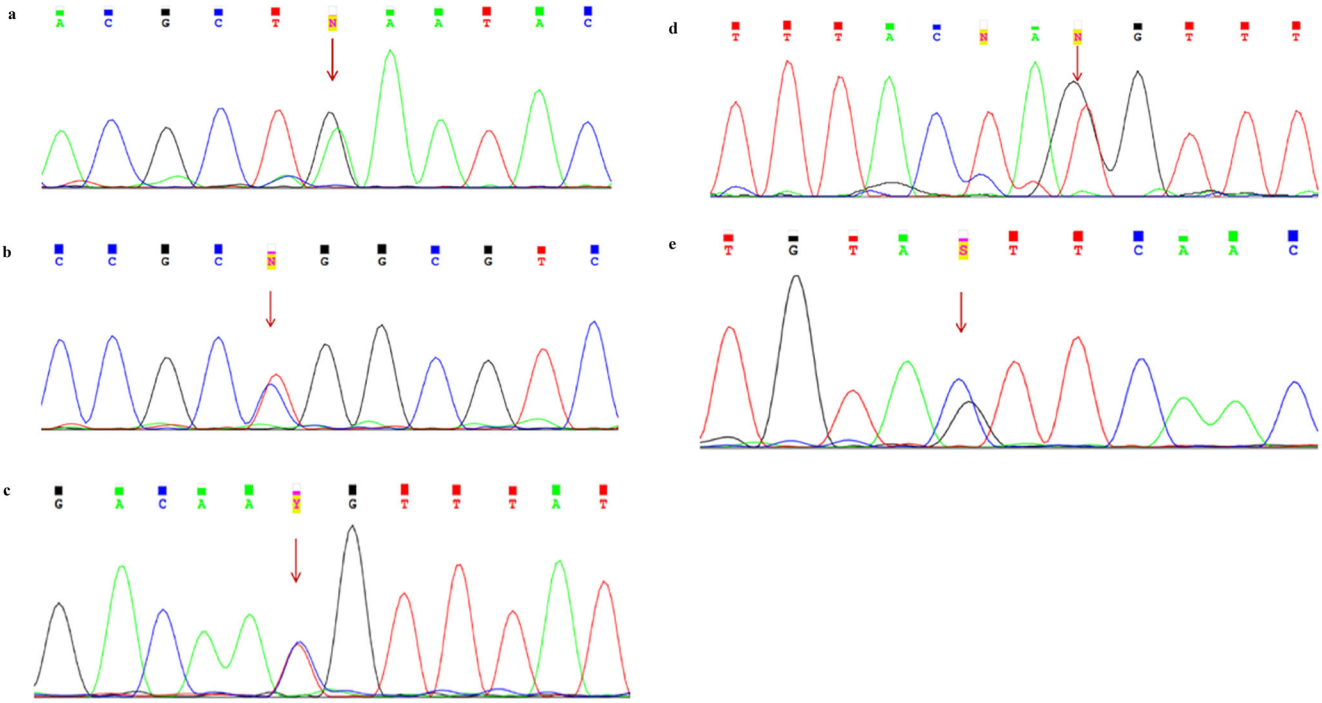


Fig. 3 (**a**) Sequence electropherogram of the exon 3 *CDH1* gene. Arrow indicates location of the base substitution G>A at c.348G>A as p.116L>L. (**b**) Sequence electropherogram of the exon 13 of the *CDH1* gene. Arrow indicates location of the base substitution T>C at c.2076T>C as p.692A>A. (**c**) Sequence electropherogram of the exon 14 of the *CDH1* gene. Arrow indicates location of the base substitution C>T at c.2253 C>T as p.751N>N. (**d**) Sequence electropherogram of the exon/intron boundary 15 of the *CDH1* gene. Arrow indicates location of the base substitution T>G at c.2439+103T>G. (**e**) Sequence electropherogram of the exon/intron boundary 15 of the *CDH1* gene. Arrow indicates location of the base substitution C>G at c.2296-22C>G

Table 4 Intronic variant that can effect splicing sites

Sample	Splice site type	Consensus value (0–100)
NM_004360.3:c.2439+103T>G	Acceptor	85.18
	Acceptor	84.02
	Donor	69.7
NM_004360.3:c.1937-58delA	Acceptor	70.07
NM_004360.3:c.2164+49G>A	Acceptor	66.03
	Donor	73.05
NM_004360.3:c.2439+52G>A	Acceptor	66.39
NM_004360.3:c.2296-48C>G	Acceptor	69.78
NM_004360.3:c.2296-44T>G-c.2296-48C>G	Donor	73.78
NM_004360.3:c.2296-22C>G	Acceptor	82.33

Consensus value: splice site if ≥ 65

in exon 7 and 8 *CDH1* gene in Italian patients with HDGC [36]. Also, Oliveira's study exhibits several large deletions in exons 1, 2, 14, 15, and 16 *CDH1* gene in HDGC families [21]. According to previous studies, about 5% of HDGC probands presents large deletions in the *CDH1* gene in average [21, 22]. However, no duplication variant has been reported in *CDH1* and therefore, this is the first time that a duplication of the gene is reported in our study. Here, we report a novel duplication in exon 9 in the *CDH1* gene, as mentioned above, alterations in this region effect on production and function of E-cadherin.

In conclusion, our study reveals several novel variants in the *CDH1* gene in Iranian patients with sporadic diffuse GC and shows that the prevalence of *CDH1* somatic variants is 25%. Our data support the hypothesis that mutations in *CDH1* gene, and particularly the mutations we describe, should be considered, even in sporadic cases of gastric cancer. The presence of these mutations in patients raises important issues regarding genetic counseling and diagnosis test in DGC patients.

Acknowledgements We sincerely thank the patients and the professional staff of the pathology lab in Al-Zahra hospital (Isfahan, Iran) and also Alaa cancer control center (Isfahan, Iran) for their cooperation in this study.

Funding Information This research was supported by Isfahan University of Medical Sciences, Isfahan, Iran, with grant number 394479.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Ethical Approval In this study, all procedures followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

References

1. Donner I, Kiviluoto T, Ristimäki A, Aaltonen LA, Vahteristo P. Exome sequencing reveals three novel candidate predisposition genes for diffuse gastric cancer. Familial Cancer. 2015;14(2):241–6.
2. Bosman FT, Carneiro F, Hruban RH, Theise ND. WHO classification of tumours of the digestive system, WHO classification of tumours, vol 3. 4th ed. Lyon: IARC Publications; 2010.
3. Oliveira C, Sousa S, Pinheiro H, Karam R, Bordeira–Carriço R, Senz J, et al. Quantification of epigenetic and genetic 2nd hits in CDH1 during hereditary diffuse gastric cancer syndrome progression. Gastroenterology. 2009;136(7):2137–48.
4. Kim S, Chung J-W, Jeong T-D, Park Y-S, Lee JH, Ahn JY, et al. Searching for E-cadherin gene mutations in early onset diffuse gastric cancer and hereditary diffuse gastric cancer in Korean patients. Familial Cancer. 2013;12(3):503–7.
5. Wu H, Rusiecki JA, Zhu K, Potter J, Devesa SS. Stomach carcinoma incidence patterns in the United States by histologic type and anatomic site. Cancer Epidemiol Biomark Prev. 2009;18(7):1945–52.
6. Henson DE, Dittus C, Younes M, Nguyen H, Albores-Saavedra J. Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: increase in the signet ring cell type. Arch Pathol Lab Med. 2004;128(7):765.
7. Worthley D, Phillips K, Wayte N, Schrader K, Healey S, Kaurah P, et al. Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS): a new autosomal dominant syndrome. Gut. 2012;61(5):774–9.
8. Pinheiro H, Oliveira C, Seruca R, Carneiro F. Hereditary diffuse gastric cancer—pathophysiology and clinical management. Best Pract Res Clin Gastroenterol. 2014;28(6):1055–68.
9. Lauren P. The two histological main types of gastric carcinoma, an attempt at a histoclinical classification. Acta Pathol Microbiol Scand. 1965;64:31–49.
10. Hamy A, Letessier E, Bizouarn P, Paineau J, Aillet G, Mirallie E, et al. Study of survival and prognostic factors in patients undergoing resection for gastric linitis plastica: a review of 86 cases. Int Surg. 1998;84(4):337–43.
11. Carneiro F, Huntsman DG, Smyrk TC, Owen DA, Seruca R, Pharoah P, et al. Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening. J Pathol. 2004;203(2):681–7.
12. Kaurah P, MacMillan A, Boyd N, Senz J, De Luca A, Chun N, et al. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. JAMA. 2007;297(21):2360–72.
13. Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, Blair V, et al. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet. 2010;47(7):436–44.
14. Corso G, Carvalho J, Marrelli D, Vindigni C, Carvalho B, Seruca R, et al. Somatic mutations and deletions of the E-cadherin gene predict poor survival of patients with gastric cancer. J Clin Oncol. 2013;2012.2044:4612.
15. Becker K-F, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, et al. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res. 1994;54(14):3845–52.
16. Carvalho J, van Grieken NC, Pereira PM, Sousa S, Tijssen M, Buffart TE, et al. Lack of microRNA-101 causes E-cadherin functional deregulation through EZH2 up-regulation in intestinal gastric cancer. J Pathol. 2012;228(1):31–44.

17. Liu Y-C, Shen C-Y, Wu H-S, Hsieh T-Y, Chan D-C, Chen C-J, et al. Mechanisms inactivating the gene for E-cadherin in sporadic gastric carcinomas. World J Gastroenterol. 2006;12(14):2168–73.
18. Liu X, Chu K-M. E-cadherin and gastric cancer: cause, consequence, and applications. Biomed Res Int 2014. https://doi.org/10.1155/2014/637308.
19. Van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci. 2008;65(23):3756–88.
20. Stemmler MP. Cadherins in development and cancer. Mol BioSyst. 2008;4(8):835–50.
21. Oliveira C, Senz J, Kaurah P, Pinheiro H, Sanges R, Haegert A, et al. Germline CDH1 deletions in hereditary diffuse gastric cancer families. Hum Mol Genet. 2009;18(9):1545–55.
22. Yamada M, Fukagawa T, Nakajima T, Asada K, Sekine S, Yamashita S, et al. Hereditary diffuse gastric cancer in a Japanese family with a large deletion involving CDH1. Gastric Cancer. 2014;17(4):750–6.
23. Pinheiro HB-CR, Seixas S, Carvalho J, Senz J, Oliveira P, Inácio PGL, et al. Allele-specific CDH1 downregulation and hereditary diffuse gastric cancer. Hum Mol Genet. 2010;19:943–52.
24. Moran CJJM, McAnena OJ. CDH1 associated gastric cancer: a report of a family and review of the literature. Eur J Surg Oncol. 2005;31:259–64.
25. Machado J, Oliveira C, Carvalho R, Soares P, Berx G, Caldas C, et al. E-cadherin gene (CDH1) promoter methylation as the second hit in sporadic diffuse gastric carcinoma. Oncogene. 2001;20(12):1525–8.
26. Park J-G, Yang H-K, Kim WH, Caldas C, Yokota J, Guilford PJ. Report on the first meeting of the international collaborative group on hereditary gastric cancer. J Natl Cancer Inst. 2000;92(21):1781–2.
27. Corso G, Marrelli D, Pascale V, Vindigni C, Roviello F. Frequency of CDH1 germline mutations in gastric carcinoma coming from high-and low-risk areas: metanalysis and systematic review of the literature. BMC Cancer. 2012;12(1):1.
28. Almasi Z, Rafiemanesh H, Salehiniya H. Epidemiology characteristics and trends of incidence and morphology of stomach cancer in Iran. Asian Pac J Cancer Prev. 2015;16(7):2757–61.
29. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. Int J Surg Pathol. 2006;14(1):21–33.
30. van der Post RS, Vogelaar IP, Carneiro F, Guilford P, Huntsman D, Hoogerbrugge N, et al. Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet. 2015;52(6):361–74.
31. Hansford S, Kaurah P, Li-Chang H, Woo M, Senz J, Pinheiro H, et al. Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol. 2015;1(1):23–32.
32. Benusiglio PR, Malka D, Rouleau E, De Pauw A, Buecher B, Noguès C, et al. CDH1 germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: a multicentre study. J Med Genet. 2013;50(7):486–9.
33. Corso G, Pedrazzani C, Pinheiro H, Fernandes E, Marrelli D, Rinnovati A, et al. E-cadherin genetic screening and clinicopathologic characteristics of early onset gastric cancer. Eur J Cancer. 2011;47(4):631–9.
34. Ghaffari S, Rafati M, Sabokbar T, Dastan J. A novel truncating mutation in the E-cadherin gene in the first Iranian family with hereditary diffuse gastric cancer. Eur J Surg Oncol. 2010;36(6):559–62.
35. Sugimoto S, Yamada H, Takahashi M, Morohoshi Y, Yamaguchi N, Tsunoda Y, et al. Early-onset diffuse gastric cancer associated with a de novo large genomic deletion of CDH1 gene. Gastric Cancer. 2014;17(4):745–9.
36. Molinaro V, Pensotti V, Marabelli M, Feroce I, Barile M, Pozzi S, et al. Complementary molecular approaches reveal heterogeneous CDH1 germline defects in Italian patients with hereditary diffuse gastric cancer (HDGC) syndrome. Genes Chromosom Cancer. 2014;53(5):432–45.