CDH1 germline mutations in a Chinese cohort with hereditary diffuse gastric cancer

Zhiwen Pan1,2 · Zhixuan Fu3 · Cong Luo4 · Yejiang Bao1 · Mingli Wang1 · Wenming Cao4 · Xiaohong Xu1

Received: 14 April 2021 / Accepted: 17 August 2021 / Published online: 18 September 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Purpose Germline mutations in CDH1 are associated with hereditary diffuse gastric cancer (HDGC) and have been identified in multiple ethnicities. However, CDH1 germline mutations have seldom been documented in Chinese patients with HDGC, and their frequency remains unclear. Here, we aimed to examine the frequency of CDH1 germline mutations in Chinese patients with HDGC. In total, 285 patients who met the International Gastric Cancer Linkage Consortium 2015 testing criteria of HDGC for CDH1 germline mutations were recruited.

Methods All 16 CDH1 exons, including neighboring intronic sequences, were amplified using polymerase chain reaction and screened using Sanger sequencing. Variants were analyzed using Mutation Surveyor V4.0, SIFT, and PolyPhen-2 software.

Results Three nonsense and nine missense CDH1 germline mutations were identified in 21 of 285 index cases (7.4%). Two CDH1 germline mutations, N405Y (Asn405Tyr) and W409X (Trp409Ter), were identified as new variants. In addition, up to 28.6% of CDH1 mutations in the 21 indicated patients were identified as c.1775G>C (E551Q). The frequency of CDH1 mutations was 6.5% (7/108) in HDGC and 7.9% (14/177) in early onset diffuse gastric cancer (EODGC). The mutation detection rates of CDH1 in males and females were 6.7% (4/60) and 8.5% (10/117) in EODGC and 4.6% (3/65) and 9.3% (4/43) in HDGC, respectively.

Conclusion These data reveal, for the first time, the type and frequency of CDH1 germline mutations in Chinese HDGC and demonstrate that germline CDH1 mutations are a noteworthy contributor to the high frequency of HDGC in Chinese.

Keywords CDH1 · Mutation screening · E-cadherin · Hereditary diffuse gastric cancer · Germline mutation

Introduction

Gastric cancer (GC) is considered the fifth-most common malignancy (Torre et al. 2015) and the third leading cause of cancer-related deaths worldwide (Ferlay et al. 2013). However, an obvious geographical difference exists concerning GC incidence, with the highest incidence rates in East Asian countries (particularly Korea, Mongolia, Japan, and China) and the lowest in Northern America and most parts of Africa (Ferlay et al. 2010). In China, GC was estimated as the second-most frequent cancer in men and the third-most frequent in women; it was also the second leading cause of cancer-related death in men and women in 2015 (Chen et al. 2016).

Accordingly, owing to the large population size and high incidence of GC in the country, nearly 42% of all GC cases worldwide occur in China alone (Parkin et al. 2005; Chen et al. 2016).

GC is histologically classified into two major types: intestinal and diffuse. The intestinal type is common in the
general population and is often related to environmental factors and lifestyle. However, diffuse GC (DGC) is more likely associated with genetic variants (Laurén 1965). Hereditary DGC (HDGC), a subset of DGC, is an autosomal dominant cancer syndrome characterized by signet ring cell carcinomas. HDGC can be caused by heterozygous germline mutations in CDH1, which are regarded as the major genetic cause of HDGC (Guilford et al. 1998). CDH1 maps to chromosome 16q22.1, and encodes the cell adhesion protein E-cadherin, a member of the transmembrane glycoprotein family. E-cadherin is involved in calcium-dependent cell-to-cell adhesion and confers cell polarity. Loss of E-cadherin function is expected to affect the epithelia architecture, cell adhesion, and cell polarity, which are correlated with tumor infiltration and metastasis (Suriano et al. 2003a; van Roy and Berx 2008; Humar and Guilford 2009). Male patients carrying germline CDH1 mutations have a 70% risk of developing DGC in their lifetime, whereas 56% of female patients may develop DGC by the age of 80 (Hansford et al. 2015). Clinical criteria for CDH1 mutation screening were established by the International Gastric Cancer Linkage Consortium (IGCLC) in 1999 (Caldas et al. 1999) and updated in 2010 (Fitzgerald et al. 2010) and 2015 (van der Post et al. 2015a). Germline CDH1 mutations have been detected in approximately 30–50% of HDGC families fulfilling the original HDGC criteria (Kaurah et al. 2007). To date, more than 100 different germline CDH1 mutations have been reported in various ethnicities worldwide (Hansford et al. 2015). Notably, these germline mutations are dispersed throughout the CDH1 gene, and no hotspot mutations have been described for HDGC.

Despite the undisputable status of GC as the most common cancer in China, germline CDH1 mutations are seldom documented in patients with HDGC. Here, we present the first report on the type and spectrum of CDH1 germline mutations in the largest index cases of Chinese patients with HDGC to date.

**Methods**

**Patients**

In total, 285 index cases meeting the IGCLC 2015 clinical criteria for HDGC were recruited between February 2013 and July 2018. All diagnoses were confirmed from pathology reports at Zhejiang Cancer Hospital. Clinical information (e.g., age and sex) was obtained from clinical records. This study was conducted in accordance with the recommendations of the Ethics Committee of the Zhejiang Cancer Hospital. The protocol was approved by the Ethics Committee of Zhejiang Cancer Hospital (Approval No. IRB-2019-172). All subjects provided written informed consent in accordance with the Declaration of Helsinki. Patients who did not meet the 2015 clinical criteria for HDGC were excluded from the study.

**CDH1 genetic testing**

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer’s instructions. The entire coding sequence and flanking intronic portions of the CDH1 gene were amplified by polymerase chain reaction (PCR) and sequenced via Sanger sequencing using standard procedures. The primers were designed by the authors and are presented in Table 1. PCR was carried out in a 20 µL volume (detailed conditions available upon request). The amplicons were sequenced on an ABI 3730XL sequencer (Life Technologies, Carlsbad, CA, USA).

**Table 1** Primers used for polymerase chain reaction amplification

| Exon | Forward 5’–3’ | Reverse 5’–3’ | Product size |
|------|---------------|---------------|--------------|
| E1E2 | GCAAGCACCTGTGAGCTT | GCGTAAATTCCAAGGGGTGTC | 1242 |
| E3  | AGACAAAGGAGATTTGCTGTCCTT | TGGTCAAACTGTGGAGAAATGTC | 616 |
| E4E5 | AGCACAGGGCTCTCATTTGTT | GCTCTCTATGGTTAGATACAG | 737 |
| E6  | AGCCTAGAAGGATGTTGCAA | CCAAGAGTTCTGTTCGTCAGG | 380 |
| E7E8 | TATCCCCATAGGAGAATTTGG | GCCATCTCAAGAGATTCGTC | 822 |
| E9  | GGAGATTTGCTTGGACCCCA | ACTGCAAATCCCACATGTC | 522 |
| E10 | GCAGATTTGGAAGGAGCATGTT | AGAGGAGCAGACATCAG | 637 |
| E11 | GACCTAGGCTGATCTGCCCA | ATTTGGGATGCGGACTACCTT | 561 |
| E12 | TGAAAAACCCGAGGACCTT | ACCCTTTGAGCAAGGGTCTC | 585 |
| E13 | AAAAAAAAAAAGACCATCTGCG | GCTGGCATAACCTGGGATG | 496 |
| E14 | ACCGACTTCAAGGGATGTGAG | TGAGCTTCTGTGTCCTGACG | 554 |
| E15 | CTCACAATCTCTTTTGACCCA | GACACACATTCTCTGAGGTCTT | 412 |
| E16 | GTCACTCTCGCCTGAGGTGTC | AAACCACCAGCAACGTGAT | 408 |
Mutation analysis and in silico prediction

Variants were analyzed using the Mutation Surveyor V4.0 software package (Software Genetics, State College, PA, USA). Missense mutations were analyzed using SIFT (Kumar et al. 2009) and PolyPhen-2 (Adzhubei et al. 2010) software to predict the potential functional effects of amino acid substitutions.

Results

Categorization of HDGC cases

The IGCLC clinical criteria for testing germline mutations of CDH1 in HDGC, as revised in 2015, are as follows: (i) two GC cases regardless of age, with at least one confirmed as DGC; (ii) one DGC case before the age of 40 years without a family history of GC; and (iii) a personal or family history of DGC and lobular breast cancer, with one diagnosis occurring before the age of 50 years. Patients meeting the third criterion are relatively rare. Herein, we did not identify any patients meeting the third criterion to include them in the study. According to these criteria for genetic screening, all cases in the present study were classified into two categories: (a) 108 index cases with a family history of HDGC, and (b) 177 patients with early onset diffuse GC (EODGC). Testing for CDH1 germline mutations was performed in 285 unrelated Chinese patients.

Detection of germline CDH1 mutations

Among the 285 patients subjected to CDH1 mutation analysis, 21 index cases were detected as CDH1 germline mutation carriers (7.4%) (Table 2). Among the 21 mutation carriers, 14 were classified as EODGC and seven had a family history of HDGC. Twelve non-synonymous mutations were identified in these patients (Table 3), including three truncating mutations (nonsense mutations) and nine missense mutations. Notably, two of the 12 mutations identified were novel: c.1225G>A (Trp409Ter) and c.1213A>T (Asn405Tyr). Three of the other germline mutations have been reported previously in HDGC cases: c.489C>A (Cys163X), c.1018A>G (Thr340Ala), and c.1118C>T (Pro373Leu). Five additional synonymous mutations were identified in CDH1 exons, which were previously registered in the National Center for Biotechnology Information (NCBI) dbsNP database (https://www.ncbi.nlm.nih.gov/snp/) (Table 4).

We also performed in silico analyses to assess the effect of missense mutations on E-cadherin function. Web-based SIFT (Kumar et al. 2009) and PolyPhen-2 (Adzhubei et al. 2010) software were used to predict whether the amino acid change conferred by variants might alter protein structure and function. The analysis indicated that the mutation E26K is less likely to be a pathogenic variant. However, other mutations were considered pathogenic based on in silico analyses.

Table 2  Summary of CDH1 germline mutation types identified in the study population

| 2015 criteria | Definition | Index Cases | Mutations | Total no. of patients (%) |
|--------------|------------|-------------|-----------|--------------------------|
| 1            | Two or more GC cases, regardless of age, with at least one with confirmed DGC | 108 | 5 | 7 (6.5) |
| 2            | DGC onset at < 40 years | 177 | 7 | 14 (7.9) |
| Total        |             | 285 | 9 | 21 (7.4) |

Table 3  Index cases with CDH1 germline mutations

| ID | Region | Mutation | Pathogenicity |
|----|--------|----------|---------------|
| 20 | E8     | c.1018A>G/p.340T>A | Possible pathogenic |
| 100 | E8     | c.1018A>G/p.340T>A | Possible pathogenic |
| 127 | E4     | c.489C>A/p.163C>X | Pathogenic |
| 140 | E16    | c.2638G>A/p.880E>K | Possible pathogenic |
| 148 | E12    | c.1888C>G/p.630L>V | Possible pathogenic |
| 154 | E8     | c.1118C>T/p.373P>L | Possible pathogenic |
| 171 | E2     | c.76G>A/p.26E>K | Benign |
| 244 | E9     | c.1225G>A/p.409W>X | Pathogenic |
| 260 | E8     | c.1018A>G/p.340T>A | Possible pathogenic |
| 276 | E11    | c.1651G>C/p.551E>Q | Possible pathogenic |
| 302 | E9     | c.1296C>G/p.432N>K | Possible pathogenic |
| 323 | E11    | c.1651G>C/p.551E>Q | Possible pathogenic |
| 340 | E11    | c.1651G>C/p.551E>Q | Possible pathogenic |
| 347 | E3     | c.1877C>T/p.63R>X | Pathogenic |
| 350 | E11    | c.1651G>C/p.551E>Q | Possible pathogenic |
| 371 | E9     | c.1213A>T/p.405N>Y | Possible pathogenic |
| 405 | E8     | c.1018A>G/p.340T>A | Possible pathogenic |
| 410 | E12    | c.1888C>G/p.630L>V | Possible pathogenic |
| 413 | E8     | c.1019C>T/p.340T>M | Possible pathogenic |
| 449 | E11    | c.1651G>C/p.551E>Q | Possible pathogenic |
| 465 | E11    | c.1651G>C/p.551E>Q | Possible pathogenic |
Table 4  CDH1 synonymous variants in exons in HDGC in the study population

| Site | CDH1 variant | Mutation type | Proportion of patients | dbSNP |
|------|--------------|---------------|------------------------|-------|
| E4   | c.393C>T(S131S) | Silent | 1/167 | rs145430811 |
| E13  | c.2076T>C(A692A) | Silent | 137/167 | rs1801552 |
| E13  | c.2079C>T(G693G) | Silent | 1/167 | rs771993728 |
| E13  | c.2103C>T(V701V) | Silent | 1/167 | rs730881656 |
| E14  | c.2253C>T(N751N) | Silent | 33/167 | rs33964119 |

Discussion

To our knowledge, our series of 285 index cases of HDGC with germline CDH1 mutations is the largest reported from China to date. The penetrance of CDH1 germline mutations in DGC is reported to be nearly 80% (Barber et al. 2008; Rogers et al. 2008). Therefore, it is important to identify CDH1 mutation carriers that benefit from gene mutation screening and the implementation of cancer risk reduction strategies. The findings of the present study provide valuable clinical information that is expected to assist clinical and laboratory-based genetic cancer experts in effectively managing patients with germline mutations.

To date, few CDH1 mutations have been reported in HDGCs from Chinese patients (Jiang et al. 2004; Zhang et al. 2006; More et al. 2007). In the current study, we described 12 CDH1 germline mutations in HDGCs. The pathogenic CDH1 germline variant C163X was identified in a patient diagnosed with signet ring cell carcinoma of the stomach at 38 years, whose mother also suffered from GC. This mutation generated a premature stop codon at position 163 of the E-cadherin (C163X) protein and was thus considered pathogenic. The C163X mutation has been previously described in two separate studies (Kluijt et al. 2012; van der Post et al. 2015b) and was detected for the first time in Chinese patients with HDGC in the present study. The R63X mutation was identified in a 40-year-old female patient without a family history of cancer. She also carried an L630V CDH1 mutation. The germline mutation L630V was also detected in two index male patients with EODGC. The germline mutation W409X in the CDH1 gene was reported in a 42-year-old female patient, was first identified in a patient with HDGC, and is a novel CDH1 germline mutation.

The T340A variant was first identified by Kim et al. (2000) and has been reported in more than four distinct patients or families (Oliveira et al. 2002; Suriano et al. 2003b; van der Post et al. 2015b), including a Chinese patient (Zhang et al. 2006). In our study, we described the variant in four unrelated female patients. The nucleotide variant c.1019C>T caused a T340M amino acid variation, which was identified in a female index. Functional analysis suggested a pathogenic role for the T340A mutation in the E-cadherin protein (Suriano et al. 2006; van Roy and Berx 2008). In contrast, the results of van der Post et al. (van der Post et al. 2015b) did not present the variant as a pathogenic mutation but as a variant of uncertain significance; however, the study lacked consistent GC histories and segregation analyses. Thus, based on our findings and previous reports, we classified this variant as a pathogenic mutation.

Roviello et al. (2007) first reported the missense mutation P373L in an Italian family. We detected this mutation in a Chinese patient with HDGC and classified it as pathogenic based on in silico analysis. N405Y was identified in a 37-year-old male patient, was first reported in HDGC cases, and is a novel mutation.

Notably, the E551Q mutation was detected in six unrelated patients, comprising approximately 2.1% of the cohort (6/285) and 28.6% of all mutation carriers identified in this study (6/21). As the germline mutations identified to date were distributed across the CDH1 gene and no hotspot mutation has been reported, this finding was rather unexpected. Therefore, the findings suggest that E551Q may be a hotspot mutation in Chinese patients with HDGC. However, we cannot exclude the possibility that the E551Q mutation may be a benign variant. Further functional analysis is required to confirm this.

The detection rate of CDH1 germline mutations in Chinese patients with HDGC is considered low, as only a few have been reported in several distinct Chinese families with HDGC (Jiang et al. 2004; Zhang et al. 2006; More et al. 2007), although no large cohorts have been examined systematically. The purported rarity of the CDH1 mutation in Chinese patients with HDGC may be related to an insufficient number of patients evaluated or a lack of studies on this disease, and is not associated with the actual prevalence of HDGC in China. The overall CDH1 mutation rate in countries with a high risk of GC, such as Italy (22.2%) (Corso et al. 2012), Japan (15.4%) (Yamada et al. 2011), and Korea (6.3%) (Kim et al. 2013; Choi et al. 2014), is lower than that in low-risk countries, such as Canada, the USA, and the UK (51.6%); however, CDH1 mutations in high-risk GC countries are generally composed of dominant missense mutations (Corso et al. 2012). In our study, the detection rate for the CDH1 mutation was 7.4% (21/285), which was lower than that in low-risk GC countries. In addition, the major type of mutation identified in our study was missense,
CDH1 germline mutations in patients with EODGC without a family history have been documented in the literature (Choi et al. 2014); however, the actual frequency in this subset of DGC has not been described in the Chinese population. The detection rate of the CDH1 mutation in European patients with EODGC was 4.9–10%, which was lower than that reported in HDGC with a family history of GC in each study (Oliveira et al. 2002; Benusiglio et al. 2013, 2015; Hansford et al. 2015). Among areas with a high incidence of GC, the detection rate of CDH1 germline mutations in EODGC was 8% (South Korea) (Kim et al. 2013) and 9.5% (Italy) (Corso et al. 2011). In the present study, among 177 unrelated cases, 14 tested positive for CDH1 mutations, with a 7.9% (14/177) detection rate of CDH1 germline mutations in Chinese patients with EODGC. The CDH1 germline mutation rate in Chinese patients with EODGC was similar to that in patients from Korea and Italy.

It has been reported that patients with EOGC and those with GC at older ages clearly display different clinical and pathological features. For example, patients under the age of 40 are more often female, whereas older patients are mostly male (Brenner et al. 2000; Windham et al. 2002; Lim et al. 2003). Among the 177 patients with EODGC in the present study, female patients outnumbered male patients (177/60), similar to the proportions among patients with EOGC. The detection rate of the CDH1 mutation in EODGC was similar in female patients, and that in male patients was 8.5% (10/117) and 6.7% (4/60). Although there were more female than male patients with EOGC, a similar CDH1 mutation rate was noted between them. This indicates that the CDH1 mutation may not be the main cause of early onset GC in women compared with men. Maeta et al. (1995) speculated that the development of GC in young women may be markedly influenced by natural, biological, and hormonal factors. However, this hypothesis requires further investigation.

It has been estimated that male CDH1 mutation carriers have a 67–70% risk of developing DGC, whereas females exhibit a 56–83% risk by 80 years old (Hansford et al. 2015). Therefore, it is important to identify mutation carriers before they develop lethal symptomatic DGC, which has a survival rate below 20% (Lynch et al. 2005). Based on these findings, despite the low frequency of CDH1 germline mutations in China, we recommend gene screening for people at risk and highlight the importance of early identification of CDH1 mutation carriers, especially for individuals with EODGC, to provide suitable intervention before the emergence of lethal symptoms.

Our study has a limitation in that we did not screen for the CDH1 gene for large-fragment deletions with multiplex ligation-dependent probe amplification. We plan to do this in future studies. To our knowledge, this study is the first to describe the frequency of CDH1 germline mutations in Chinese patients with HDGC. The detection rate of CDH1 germline mutations in Chinese patients with HDGC was 7.4%. These results confirm that patients with HDGC from high-risk areas, such as China, show a lower incidence of CDH1 germline mutations than individuals from low-risk regions. These data should provide useful assistance for genetic counseling and management of patients at risk (Corso et al. 2014).

Acknowledgements We would like to thank Editage (www.editage.com) for English language editing.

Author contributions Conceptualization: ZP, CL, and XX. Data curation: MW, WC, ZP, and ZF. Writing – original draft: ZP and XX. Supervision and writing – review & editing: ZP, ZF, CL, YB, MW, WC, and XX.

Funding This study was supported by the Medical and Health Science Project of Zhejiang Province (2015KYA033, 2016139538, 2017KY021). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability statement All datasets analyzed for this study are included in the manuscript/supplementary files.

Code availability Not applicable.

Declarations

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

Ethical approval The protocol of the study was approved by the Ethics Committee of the Zhejiang Cancer Hospital (Approval No. IRB-2017-47). All subjects provided written informed consent in accordance with the Declaration of Helsinki.

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

Consent to publish All individuals participating in the study have provided the consent to publish the data.

References

Adzhubei IA, Schmidt S, Peshkin L et al (2010) A method and server for predicting damaging missense mutations. Nat Methods 7:248–249. https://doi.org/10.1038/nmeth0410-248
Barber ME, Save V, Carneiro F et al (2008) Histopathological and molecular analysis of gastrectomy specimens from hereditary diffuse gastric cancer patients has implications for endoscopic
patients with a germline mutation in CDH1. Gastroenterology 149:897–906. https://doi.org/10.1053/j.gastro.2015.06.003
van Roy F, Berx G (2008) The cell-cell adhesion molecule E-cadherin. Cell Mol Life Sci 65:3756–3788. https://doi.org/10.1007/s00018-008-8281-1
Windham TC, Termuhlen PM, Ajani JA et al (2002) Adenocarcinoma of the stomach in patients age 35 years and younger: no impact of early diagnosis on survival outcome. J Surg Oncol 81:118–124. https://doi.org/10.1002/jso.10157
Yamada H, Shinmura K, Ito H et al (2011) Germline alterations in the CDH1 gene in familial gastric cancer in the Japanese population. Cancer Sci 102:1782–1788. https://doi.org/10.1111/j.1349-7006.2011.02038.x
Zhang Y, Liu X, Fan Y et al (2006) Germline mutations and polymorphic variants in MMR, E-cadherin and MYH genes associated with familial gastric cancer in Jiangsu of China. Int J Cancer 119:2592–2596. https://doi.org/10.1002/ijc.22206

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.