Short Report

De novo CDH1 mutation in a family presenting with early-onset diffuse gastric cancer


In this report, we describe the first concluded case of a de novo germline mutation in CDH1 in a hereditary diffuse gastric cancer (HDGC) kindred. The incident case was a woman with a personal history of Hodgkin’s lymphoma and diffuse gastric cancer, who was then confirmed to have a CDH1 mutation (c.1792 C>T (R598X)). The patient’s mother was found to have the same CDH1 germline mutation; however, neither maternal grandparent was found to carry the mutation, thus leading to a conclusion that the proband’s mother’s mutation is of de novo origin. This case highlights the importance of recognition of the HDGC syndrome and of testing for CDH1 germline mutations in young individuals with diffuse gastric cancer without a family history of the disease.

Conflict of interest

The authors confirm that there are no potential conflicts of interest with this manuscript.

Hereditary diffuse gastric cancer (HDGC) (OMIM No. 137215) is an autosomal dominant genetic predisposition syndrome caused by germline mutations in the CDH1 gene (1, 2). E-cadherin, the CDH1 gene product, is a calcium-dependent cell membrane protein involved in cell–cell adhesion and confers cell polarity (3). HDGC accounts for 1–3% of all gastric cancers (4, 5). CDH1 mutations are associated with a cumulative risk for diffuse gastric cancer, before the age of 75, of 40–67% for men and 63–83% for women (6, 7), in addition to increased risk of lobular breast cancer and signet ring colon cancer (8). Because of the inability to screen for early diffuse gastric cancer, patients with germline CDH1 mutations are recommended to undergo risk-reducing prophylactic gastrectomy (8–10).

The CDH1 locus is 16q22.1, and is composed of 16 exons spanning 100 kb of genomic DNA. Germline mutations in CDH1 are identified in 30–40% of families clinically defined to have HDGC by the International Gastric Cancer Linkage Consortium (1, 8). Mutations have been found in families of various ethnic backgrounds (7, 11). To date, approximately 100 germline mutations, including large deletions (12), in CDH1 have been published (7, 11, 13). Additionally, a founder mutation (2398delC), confirmed by haplotype...
Shah et al.

analysis, has been identified in four families from Newfoundland (7). At this time, CDH1 is the only known gene associated with HDGC, and no reports of de novo mutations in CDH1 have been published. Herein, we report the first description of a de novo CDH1 mutation in a woman whose daughter was diagnosed with early-onset diffuse gastric cancer.

Methods

Patients

The Memorial Sloan Kettering Cancer Center (MSKCC) Early Onset and Familial Gastric Cancer Registry (NCT00582257, www.clinicaltrials.gov) was created to identify individuals with gastric cancer at high risk for a familial predisposition syndrome for gastric cancer. In this report, the proband’s mother, maternal aunt, and maternal grandfather were enrolled in the MSKCC Gastric Cancer Registry. The proband and her maternal grandfather had died prior to the family contacting our center. This study was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center.

Mutation analysis of CDH1

Full gene sequencing of the CDH1 gene on the proband and single amplicon CDH1 mutation analysis on the probands mother and maternal grandparents was performed at the Molecular Diagnostic Laboratory at City of Hope. Confirmation analysis for the presence or absence of the familial CDH1 mutation (c.1792 C>T (R598X)) in the proband’s mother, maternal grandparents, and maternal aunt was performed in the Diagnostic Molecular Genetics Laboratory at MSKCC.

For confirmation analysis, peripheral blood samples were obtained from the proband’s mother, maternal aunt, and her maternal grandparents. Tumor tissue was also obtained from her maternal grandfather’s gastroesophageal junction (GEJ) adenocarcinoma. Genomic DNA was prepared from 10 ml ethylenediaminetetraacetic acid blood samples or from tumor tissue according to standard procedures. Exon 12 of the CDH1 gene was analyzed for the c.1792 C>T (R598X) mutation. The sequence of the forward primer (in intron 11) is 5’-AGA CTT GGT CTG GTG GAA G-3’. The sequence of the reverse primer (in intron 12) is 5’-ATT GAA AGG TGG GGA TCT GG-3’. Each polymerase chain reaction (PCR) reaction contained 5 μl 10× Qiagen PCR buffer (containing 15 mM MgCl2), 1.5 unit HotStar Taq (Qiagen, Gaithersburg MD), 2 μl 10 mM dNTPs (Invitrogen Co., San Diego, CA, Catalog number: 18427-088), 4 μl 100 ng/μl primers (2 μl for each), 2 μl genomic DNA (approximately 100 ng) and water to make the final volume of 50 μl. Cycling conditions were 95°C for 5 min, 95°C for 30 s (35×), 58°C for 30 s (35×) and 72°C for 30 s (35×) with a final extension at 72°C for 7 min (1×). The PCR products were purified and subjected to direct DNA sequencing analysis (BigDye Terminator v3.1 Cycle Sequencing Kit and 3730 DNA Analyzer, Applied Biosystems, Foster City, CA).

Paternity confirmation

Paternity testing was performed in the Diagnostic Molecular Pathology Laboratory at MSKCC using microsatellite markers routinely employed for bone marrow transplant engraftment studies [GenePrint Fluorescent STR Multiplex – CSF1PO, TPOX, TH01, vWA (Fluorescein), Cat# DC6301; Gene Print Fluorescent STR Multiplex – Gamma STR (Fluorescein) D16S539, D7S820, D13S317, D5S818, Cat# DC6071; both kits were purchased from Promega Corporation, Madison, WI]. PCR amplification and fragment analysis of STR markers were performed according to the manufacturer’s instructions. Markers were analyzed on an ABI 3730 DNA Analyzer and GeneMapper 4.0 software (Applied Biosystems).

Results

Family description

The family is a 24-member, four-generation kindred with two mutation-positive individuals. CDH1 full gene sequencing identified a germline truncating mutation (c.1792 C>T (R598X)) in an affected 25-year-old woman (proband) (Fig. 1). This woman had a personal history of Hodgkin’s lymphoma (syncytial variant nodular sclerosing type) diagnosed at 14 years of age that was successfully treated with combination chemotherapy (adriamycin, cyclophosphamide, bleomycin, vincristine, procarbazine, prednisone, and vinblastine) and radiation to the chest, mediastinum, bilateral supraclavicular, and bilateral neck regions (total 2100 cGy). She was subsequently diagnosed with metastatic diffuse gastric carcinoma with signet ring features at 25 years of age and died of this disease at the age of 26 years.

The proband’s unaffected 50-year-old mother was subsequently found to have the same CDH1 germline mutation as the proband upon single
De novo CDH1 mutation in an HDGC family

amplicon analysis. Prophylactic gastrectomy was performed, and surgical pathology revealed four small foci of intramucosal signet ring adenocarcinoma, 12 benign lymph nodes, chronic gastritis, and gastric hyperplastic polyps; the depth of invasion was to the intramucosal layer and the largest single focus was 1 mm in size.

The proband’s 76-year-old maternal grandfather tested negative for the familial CDH1 germline mutation. He had a personal history of metastatic GEJ adenocarcinoma diagnosed at 77 years of age and a family history of esophageal cancer per verbal report in a brother at age 76 years. Both of these individuals had been cigarette or pipe smokers. The proband’s maternal grandfather died at age 77 years prior to the family joining the MSKCC Gastric Cancer Registry.

The proband’s 73-year-old maternal grandmother and only maternal aunt also both tested negative for the familial CDH1 mutation on single amplicon analysis. The maternal aunt did have a personal history of Wilms’ tumor diagnosed at age 2 years per verbal report (Fig. 1).

De novo CDH1 mutation confirmation

The family history suggests that this familial germline CDH1 mutation had occurred de novo in the proband’s mother. Analysis of eight highly polymorphic short tandem repeat (STR) markers supported the claimed paternity between the proband’s mother and her parents (Table 1). To determine whether the mutation had occurred on the maternal or paternal allele, we investigated several single nucleotide polymorphisms (in Ensembl database) located near the c. 1792 C>T (R598X) mutation in the CDH1 gene. However, we could not take advantage of these single nucleotide polymorphic short tandem repeat (STR) markers supported the claimed paternity between the proband’s mother and her parents (Table 1). To determine whether the mutation had occurred on the maternal or paternal allele, we investigated several single nucleotide polymorphisms (in Ensembl database) located near the c. 1792 C>T (R598X) mutation in the CDH1 gene. However, we could not take advantage of these single nucleotide polymorphic markers.

De novo CDH1 mutation confirmation

The family history suggests that this familial germline CDH1 mutation had occurred de novo in the proband’s mother. Analysis of eight highly polymorphic short tandem repeat (STR) markers supported the claimed paternity between the proband’s mother and her parents (Table 1). To determine whether the mutation had occurred on the maternal or paternal allele, we investigated several single nucleotide polymorphisms (in Ensembl database) located near the c. 1792 C>T (R598X) mutation in the CDH1 gene. However, we could not take advantage of these single nucleotide polymorphic markers.

Table 1. Results of genotype analysis using eight highly polymorphic markers

<table>
<thead>
<tr>
<th>STR marker</th>
<th>Proband’s maternal grandmother</th>
<th>Proband’s mother</th>
<th>Proband’s maternal grandfather</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS818</td>
<td>133, 137</td>
<td>133, 137</td>
<td>133, 137</td>
</tr>
<tr>
<td>D13S317</td>
<td>160, 188</td>
<td>180, 167</td>
<td>188, 167</td>
</tr>
<tr>
<td>D7S820</td>
<td>223, 223</td>
<td>223, 235</td>
<td>289, 235</td>
</tr>
<tr>
<td>D16S539</td>
<td>282, 294</td>
<td>282, 290</td>
<td>290, 290</td>
</tr>
<tr>
<td>vMA</td>
<td>149, 153</td>
<td>149, 153</td>
<td>157, 153</td>
</tr>
<tr>
<td>TH01</td>
<td>189, 181</td>
<td>189, 177</td>
<td>185, 177</td>
</tr>
<tr>
<td>TPOX</td>
<td>232, 232</td>
<td>232, 232</td>
<td>244, 232</td>
</tr>
<tr>
<td>CSF1PO</td>
<td>308, 316</td>
<td>308, 316</td>
<td>308, 316</td>
</tr>
</tbody>
</table>

The sizes (in bp) of both alleles of the corresponding STR marker are shown for each person. The sizes of different marker alleles are analyzed using GeneMapper 4.0. Alleles transmitted to the proband’s mother are shown in bold (the proband’s maternal grandparents have identical allelic pattern for the DSS818 and CSF1PO markers, and are therefore uninformative).
polymorphisms (SNPs) because both parents were homozygous at these locations (data not shown).

On the basis of the proband’s maternal grandfather’s personal and family history of GEJ adenocarcinoma and the concern for somatic mosaicism, we performed CDH1 single amplicon analysis on the proband’s maternal grandfather’s GEJ cancer tissue. The malignancy was negative for the familial CDH1 mutation (Fig. 2). The proband’s only maternal aunt tested negative for the familial CDH1 mutation (confirmed by single amplicon testing at MSKCC). The proband’s only maternal uncle did not undergo CDH1 genetic testing and has since died at age 49 from non-cancer causes.

**Discussion**

In this report, we describe the first case of a concluded de novo mutation in the CDH1 gene in a HDGC kindred. The familial CDH1 mutation (c.1792C>T (R598X)) has been previously described in two families (14, 15). In the current family, this CDH1 mutation was initially identified in a young woman with a personal history of Hodgkin’s lymphoma and diffuse gastric cancer. The mother of this patient was also found to have the CDH1 mutation, but neither of the proband’s maternal grandparents had the familial mutation. Paternity between the proband’s mother and the maternal grandparents was confirmed. The proband’s maternal grandfather’s GEJ adenocarcinoma was also negative for the familial CDH1 mutation and thus the likelihood of somatic mosaicism is reduced. These results lead to the conclusion that the nonsense CDH1 germline mutation identified in this proband and her mother likely represents a de novo event in the proband’s mother. The proband’s unaffected uncle died at the age of 49 years and was not tested for the familial CDH1 mutation prior to his passing, and therefore, germline mosaicism in one of the proband’s maternal grandparents cannot be further excluded.

It is not clear whether the proband’s personal history of Hodgkin’s lymphoma is related to her germline CDH1 mutation. There has been one previous report of a gastric lymphoma in the setting of *Helicobacter pylori* infection reported in a kindred with a germline CDH1 mutation (16); however, there have been no previously reported cases of Hodgkin’s lymphoma. The risk of a second neoplasm following the treatment for Hodgkin’s lymphoma is significant; as high as 5% for all survivors (17), and most frequently include breast cancer, thyroid cancer, and bone or soft tissue sarcoma, although gastric cancer has also been reported (17, 18). The risk of secondary malignancy is associated with increased dose of mediastinal radiation (reviewed by Ng et al. (18)). Radiation-induced genomic instability has been showed to include DNA methylation (19), which is a common mechanism of inactivation of the CDH1 locus in CDH1 mutation carriers (20). It is possible that the treatment the proband received for her Hodgkin’s lymphoma may have resulted in inactivation of CDH1, leading ultimately to the initiation of her early-onset invasive diffuse gastric adenocarcinoma. Tumor tissue from the proband was unavailable to test this hypothesis. It is notable that in Li-Fraumeni syndrome, another rare cancer predisposition syndrome associated with germline mutations within the *p53* tumor suppressor gene, an increased susceptibility to secondary malignancies within prior radiotherapy fields has been observed (21). This case suggests that further assessment of the possible role of radiation-induced carcinogenesis in patients with HDGC is warranted.

To date, over 100 kindreds with CDH1 germline mutations have been identified, and this kindred is the first case of a concluded de novo CDH1 germline mutation to be reported. This suggests that, similar to the *BRCA1* and *BRCA2* cancer susceptibility genes, the rate of de novo mutation in CDH1 is likely very low. In contrast, Li-Fraumeni syndrome has a reported frequency of de novo TP53 mutations of 7–20% (22). There are numerous explanations for this difference, including possibly a lack of recognition of HDGC as
a cancer predisposition syndrome and unfamiliarity with criteria for CDH1 mutation analysis. The most recent CDH1 testing criteria put forth by the International Gastric Cancer Linkage Consortium include: (i) two or more gastric cancer cases in the family, with one being a confirmed diffuse gastric cancer diagnosed before the age of 50 years; (ii) three or more confirmed diffuse gastric cancers in first- or second-degree relatives, independent of age; (iii) diffuse gastric cancer diagnosed before the age of 40 years without additional family history; and (iv) personal or family history of diffuse gastric cancer and lobular breast cancer, with at least one diagnosed prior to the age of 50 years (8). This finding further supports the importance of testing young patients with diffuse gastric cancer for germline CDH1 mutation, in which the mutation detection rate is estimated to be \( \sim 1–2\% \) (23, 24), and highlights the significance of the identification of CDH1 mutations in young diffuse gastric cancer patients without an extended family history of the disease.

In this kindred, the proband's unaffected mother also carried the familial CDH1 mutation, and was confirmed to have evidence of signet ring cell adenocarcinoma within the prophylactic gastrectomy specimen. This finding, when considering the proband's history of early-onset metastatic diffuse gastric cancer, also highlights the intra- and inter-familial variability associated with CDH1 germline mutations.

**Acknowledgements**

This work was supported by the DeGregorio Family Foundation (MAS), FDA Orphan Products grant I1RO1FD003755-01A1 (MAS). We would like to thank Ruben Bacares and Paulo Salazar in the Department of Pathology for their technical assistance.

**References**