



Short Report

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

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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.

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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

analysis, has been identified in four families from Newfoundland (7). At this time, *CDHI* is the only known gene associated with HDGC, and no reports of *de novo* mutations in *CDHI* have been published. Herein, we report the first description of a *de novo CDHI* 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 grandmother 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 *CDHI*

Full gene sequencing of the *CDHI* gene on the proband and single amplicon *CDHI* mutation analysis on the proband's 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 *CDHI* 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 *CDHI* 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. *CDHI* 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 *CDHI* germline mutation as the proband upon single

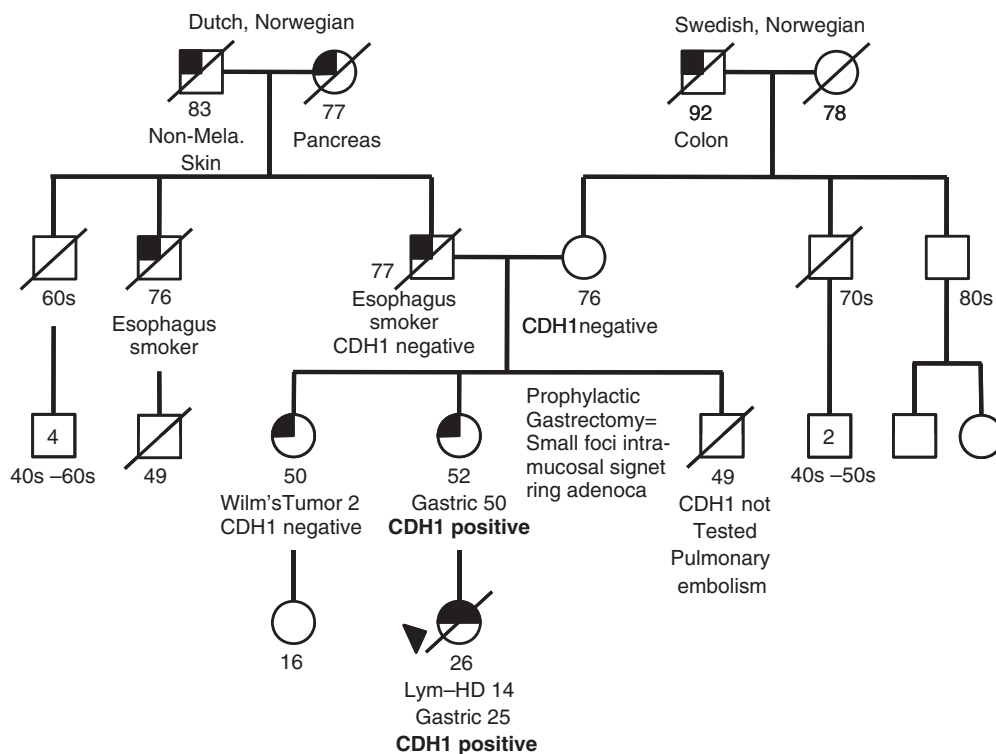


Fig. 1. CDH1 family pedigree.

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

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

STR marker	Proband's maternal grandmother	Proband's mother	Proband's maternal grandfather
D5S818	133, 137	133, 137	133, 137
D13S317	180 , 188	180, 167	188, 167
D7S820	223 , 223	223, 235	289, 235
D16S539	282 , 294	282, 290	290, 290
vMA	149 , 153	149, 153	157, 153
TH01	189 , 181	189, 177	185, 177
TPOX	232 , 232	232, 232	244, 232
CSF1PO	308, 316	308, 316	308, 316

^aThe 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 D5S818 and CSF1PO markers, and are therefore uninformative).

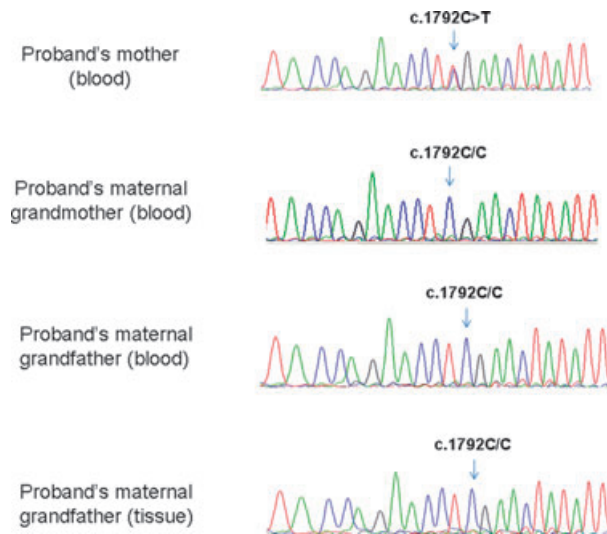


Fig. 2. *CDHI* mutation single amplicon sequencing analysis for the proband's mother, the proband's maternal grandparents, and the proband's maternal grandfather's gastroesophageal junction adenocarcinoma.

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 *CDHI* single amplicon analysis on the proband's maternal grandfather's GEJ cancer tissue. The malignancy was negative for the familial *CDHI* mutation (Fig. 2). The proband's only maternal aunt tested negative for the familial *CDHI* mutation (confirmed by single amplicon testing at MSKCC). The proband's only maternal uncle did not undergo *CDHI* 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 *CDHI* gene in a HDGC kindred. The familial *CDHI* mutation (c.1792C>T (R598X)) has been previously described in two families (14, 15). In the current family, this *CDHI* 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 *CDHI* 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 *CDHI* mutation and thus the likelihood of somatic

mosaicism is reduced. These results lead to the conclusion that the nonsense *CDHI* 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 *CDHI* 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 *CDHI* 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 *CDHI* 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 shown to include DNA methylation (19), which is a common mechanism of inactivation of the *CDHI* locus in *CDHI* mutation carriers (20). It is possible that the treatment the proband received for her Hodgkin's lymphoma may have resulted in inactivation of *CDHI*, 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 *CDHI* germline mutations have been identified, and this kindred is the first case of a concluded *de novo* *CDHI* germline mutation to be reported. This suggests that, similar to the *BRCA1* and *BRCA2* cancer susceptibility genes, the rate of *de novo* mutation in *CDHI* 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 ~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.

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