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CDH1 gene mutations do not contribute in hereditary diffuse gastric cancer in Poland

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Abstract Hereditary diffuse gastric cancer (HDGC) is a cancer susceptibility syndrome characterized by a high risk of diffuse stomach cancer and lobular breast cancer. HDGC is caused by germline mutations in the *CDH1* gene encoding the E-cadherin which is a member of the transmembrane glycoprotein family responsible for calcium-dependent, cell-to-cell adhesion and plays a fundamental role in the maintenance of cell differentiation and the normal architecture of epithelial tissues. Mutations in the *CDH1* gene are detected in 30–46% of families that fulfil strong clinical criteria for HDGC and in about 11% of families fulfilling the modified criteria. In the present study, we investigated germline mutations in the *CDH1* gene in Polish patients with HDGC. The entire coding sequence of *CDH1* gene was analyzed by sequencing in 86 Polish cancer patients from families fulfilling the modified criteria of HDGC. We found several silent mutations including one common variant (c.2076T>C) present in 56 patients, and three rare variants (c.2253C>T, c.1896C>T, c.2634C>T) detected in 2 patients. In addition, we found four rare sequence variants of unknown significance localized in introns. We did not detect any deleterious mutations of the *CDH1* gene. *CDH1* gene mutations are not present in Polish families with HDGC defined by the modified clinical criteria. Further studies of families with HDGC matching the restrictive criteria for HDGC are needed.

Keywords E-cadherin · Hereditary diffuse gastric cancer · Polish population

Abbreviations

CDH1 gene	E-cadherin gene
IGCLC	Gastric Cancer Linkage Consortium
HDGC	Hereditary Diffuse Gastric Cancer
DGC	Diffuse Gastric Cancer
GC	Gastric Cancer
LBC	Lobular Breast Cancer
FDGC	Familial Diffuse Gastric Cancer
FGC	Familial Gastric Cancer
EOGC	Early Onset Gastric Cancer
PCR	Polymerase Chain Reaction
MRI	Magnetic Resonance Imaging
HGVS	Human Genome Variation Society, http://www.hgvs.org/

Introduction

The E-cadherin is a member of the transmembrane glycoprotein family responsible for calcium-dependent, cell-to-cell adhesion and plays a fundamental role in the maintenance of cell differentiation and the normal architecture of epithelial tissues [1–7]. The protein is encoded by the *CDH1* gene which is located on chromosome 16q22.1 and consists of 16 exons. Mutations in *CDH1* gene

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are known to be associated with Hereditary Diffuse Gastric Cancer syndrome (HDGC) [8–16] and also with lobular breast cancer [17–19]. The HDGC has been defined in 1999 by International Gastric Cancer Linkage Consortium (IGCLC) as two or more documented cases of DGC in first or second degree relatives, including at least one case of DGC diagnosed before the age of 50, or three or more documented cases of DGC in first or second degree relatives diagnosed at any age [9, 10]. Among patients that fulfill the above clinical criteria about 30–46% of cases carry a mutation in the *CDH1* gene [11, 15, 20–22]. Afterwards the IGCLC criteria have been modified e.g. including cases diagnosed with lobular breast cancer or signet-ring carcinoma of the colon [12]. The new modified criteria were as follow: (1) two or more documented cases of DGC in first-degree relatives with at least one diagnosed before age 50 years; (1A) two or more cases of GC with at least 1 documented DGC diagnosed before age 50 years; (2) three or more documented cases of DGC in first-degree relatives with diagnoses at any age; (2A) three or more cases of GC, diagnosed at any age, with at least one documented case of DGC; (3) isolated individual diagnosed with DGC at less than 45 years of age; (4) isolated individual diagnosed with both DGC and lobular breast cancer (no other criteria met); (5) one family member diagnosed with DGC and another with lobular breast cancer (no other criteria met); (6) one family member diagnosed with DGC and another with signet-ring carcinoma of the colon (no other criteria met). It has been shown that among patients fulfilling the modified criteria 11% carry *CDH1* mutation [12–14].

Patients with germline mutations in the *CDH1* have a high risk of developing diffuse gastric cancer and female carriers are at high risk of lobular breast cancer [17–19]. The estimated penetrance of *CDH1* mutations is 70–80% for stomach cancer [8, 15, 22, 23] and 39–52% for lobular breast cancer [15, 23]. Because of high penetrance of *CDH1* mutations and almost 100% mortality of patients with symptomatic DGC prophylactic total gastrectomy during the second decade of life is recommended for *CDH1* mutation carriers [22, 24–26]. Referring to updated consensus guidelines for clinical management of HDGC in female carriers surveillance including monthly breast self examination starting at age 35, annual mammogram and breast MRI is recommended [26]. Additionally, in families in which colon cancer was reported enhanced screening with colonoscopy beginning at age 40 or 10 years younger than the youngest diagnosis of colon cancer and repeated at intervals of 3–5 years should be considered [26].

Given the complexity and high mortality of HDGC, not only with regard to the management of the DGC, but also with regard to the risk for other related cancers such as lobular breast cancer or colon cancer, it is very important to identify asymptomatic carriers of *CDH1* mutations in order to apply the appropriate surveillance.

In the current study we sought to determine prevalence of *CDH1* mutations in Polish families with diffuse gastric cancer.

Materials and methods

Patients

For analysis of *CDH1* gene 86 Polish patients from families fulfilling the modified criteria of HDGC were selected. Among these cases 82 were affected by DGC, two cases were diagnosed with lobular breast cancer and two with colon cancer. Only families with GC diagnosed in first and second degree relatives were included to this study. The characteristic of tested families including mean age at stomach cancer diagnosis is presented in the Table 1.

Patients were invited to participate in this study either in person during their hospital stay in participating hospitals throughout Poland or through a mailed invitation. Patients who responded to the personal or mailed invitation were invited to the local study center for an interview. During the interview the goals of the study were explained, informed consent was obtained, genetic counseling was given and a blood sample was taken for DNA analysis. A detailed family history of gastric cancer in first- and second-degree relatives was taken and the medical record and/or pathology report were reviewed and forwarded to the study center in Szczecin. Information was recorded on age at diagnosis and cancer pathology. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland.

Sequencing

Peripheral blood samples from 86 probands were obtained for genomic DNA isolation. DNA was isolated using a standard procedure [27]. The entire coding sequence of *CDH1* gene and intron/exon splice sites, was amplified in PCR reactions using primers and conditions described previously [16]. After purification, PCR products were analysed on an ABI 377 DNA Sequencer according to manufacturer's procedure.

Results and discussion

This is the first study of *CDH1* mutations in the Polish population, in which we screened 86 patients with HDGC. We found four different silent mutations: one common (c.2076T>C) and three rare (c.2253C>T, c.1896C>T,

Table 1 Characteristic of families selected for *CDH1* analysis

Criteria	Number of families	Number of GC in family[a]	Mean age at GC diagnosis in proband (range)	Mean age at GC diagnosis in relatives (range)	Number families with GC in 1st degree relatives only	Number families with with GC in 2nd degree relatives
2 or more cases of GC with at least 1 documented DGC diagnosed before age 50 years	60	2 GC in 32 families 3 GC in 21 families >3 GC in 7 families	45.3 (35–56)	54.1 (22–81)	45	15
3 or more cases of GC, diagnosed at any age, with at least 1 documented case of DGC	16	3 GC in 11 families >3 GC in 5 families	59.2 (52–75)	61.4 (51–75)	15	1
Isolated DGC diagnosed at age 45 or below	6	–	38.8 (16–45)	–	–	–
1 family member diagnosed with DGC and another with lobular breast cancer (no other criteria met)	2	1 DGC in 2 families	44.5 (42–49)	53.5 (49–58)	2	0
1 family member diagnosed with DGC and another with signet-ring carcinoma of the colon (no other criteria met)	2	1 DGC in 2 families	67	54 (53–55)	2	0

[a] Including proband

Table 2 *CDH1* variants detected in 86 Polish patients with HDGC

CDH1 variant[a]	Number of carriers
c.1896C>T (p.=)	1[b]
c.2076T>C (p.=)	56
c.2253C>T (p.=)	1
c.2634C>T (p.=)	1[b]
c.1-44A/G	7
c.172+6T/C	11
c.655+10C/G	2
c.1836-46A/G	1
c.1836-13T/C	14

[a] Description of detected variants according to the HGVS recommendations; (p.=) indicates lack of effect on protein level–silent mutation

[b] Patient with both detected variants

c.2634C>T) variants. In addition, we found five different sequence variants of unknown significance localized in introns (Table 2). We did not detect any pathogenic mutations in the *CDH1* gene.

Germline mutations in *CDH1* gene were originally reported in three Maori families with aggregation of diffuse gastric cancer [8]. Since this report, several studies have investigated the role of *CDH1* mutations in gastric cancer in different ethnic groups [8–22]. It has been reported that *CDH1* mutations underlie approximately 40% of families fulfilling the strong IGCLC criteria for hereditary diffuse gastric cancer (HDGC). However, the criteria for HDGC defined by IGCLC require strong family history with all documented cases of diffuse gastric cancer what refers to small number of all patients affected with gastric cancer. Therefore, *CDH1* gene has been screened in gastric cancer patients fulfilling less restrictive, modified criteria of familial diffuse gastric cancer (FDGC–families with aggregation of gastric cancer and index cases with diffuse gastric cancer but not fulfilling the IGCLC criteria for HDGC), familial gastric cancer (FGC–families with aggregation of gastric cancer, but without histology available on the tumors) and early-onset gastric cancer (EOGC–patients with isolated diffuse gastric cancer diagnosed before age 45 years) [9]. *CDH1* mutations have been found in 11% (21/192) of families with FDGC and almost 7% (17/254) of patients with EOGC [11].

In the present study we screened a relatively large series of 86 cases with HDGC fulfilling the modified criteria (FDGC, EOGC), but we did not find any pathogenic mutations in the *CDH1* gene. We performed a sensitive exon by exon sequencing of the entire gene including surrounding intronic sequences. With this method we are able to detect all small intragenic mutations which are the most common mutations observed in the *CDH1* gene [8–22].

Recently large genomic deletions in *CDH1* gene have been found in patients from HDGC families. In the study of 160 patients from families fulfilling the restrictive criteria HDGC 67 (42%) point or small frameshift mutations and 6 (4%) large genomic deletions in *CDH1* gene were detected [20]. This indicates that large genomic rearrangements constitute only a small proportion of *CDH1* mutations (~8%). It is unlikely that the negative result of our study is due to low sensitivity of methods used for mutation screening.

In summary, results of our study show that *CDH1* mutations do not contribute to diffuse gastric cancer in Poland, however, taking into account limitations of our study which are: less restrictive criteria of gastric cancer patients selection and lack of analysis for large genomic deletions, further studies of HDGC in Polish population are needed.

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