CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling

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CDH1 mutation carriers have a strongly increased risk of developing gastric cancer (GC) and lobular breast cancer (LBC). Clinical data of GC cases and surgical and histological data of prophylactic gastrectomies and mastectomies of all 10 Dutch CDH1 mutation families were collected. In vitro functional assays were performed to analyze the nature of the newly found missense mutation c.1748T>G (p.Leu583Arg). Ten different CDH1 mutations were found. Functional assays gave strong arguments for the pathogenic nature of the p.Leu583Arg mutation. The pedigrees comprised 36 GC cases (mean age 40 years, range 20–72 years) and one LBC case. Twenty-nine/37 carriers alive, aged 18–61 years, underwent prophylactic gastrectomy. Invasive GC-foci and premalignant abnormalities were detected in 2 and 25 patients, respectively. In four patients GC/signetring cell (SRC) foci were diagnosed at preoperative gastroscopy. Long-standing presence of SRCs without progression to invasive carcinoma was shown in two others. Multifocal LBC/LCIS was found in the two prophylactic mastectomy specimens. Clefts of lip and/or palate (CL/P) were reported in seven individuals from three families. The age at onset and aggressiveness of GC is highly variable, which has to be included in counseling on planning prophylactic gastrectomies. The incidence of LBC is expected to increase and prophylactic mastectomy needs to be considered. The relationship between CL/P and CDH1 needs further study to inform future parents from hereditary diffuse gastric cancer (HDGC) families adequately.

While the incidence of gastric cancer (GC) has declined during the last decades, it remains the second cause of cancer related death worldwide.1 In contrast to the intestinal type of GC (IGC), diffuse GC (DGC) has no clear defined risk factors and its incidence has been stable,2 suggesting that genetic factors play a more dominant role in DGC.

The far majority of DGCs is characterized by loss of expression of E-cadherin. E-cadherin is a key cell surface protein involved in intercellular connection and maintenance of epithelial integrity and is encoded by the tumor suppressor gene CDH1.3,4 DGCs have a greater tendency to diffusely invade the gastric wall and often present at an advanced stage.

A minority of DGCs (1–3%) is seen in families with autosomal dominant GC susceptibility.5 Germline E-cadherin inactivating mutations in the CDH1 gene are responsible for the development of DGC in approximately 30% of families with the hereditary diffuse gastric cancer syndrome (HDGC).
Diagnostic criteria for HDGC are formulated by the International Gastric Cancer Linkage Consortium.6,7 Germline CDH1 mutations have first been described in 1998 by Guilford et al. in three Maori kindreds with multiple DGCs at very young age.8 Subsequently, different germline mutations have been identified in families with different ethnic backgrounds worldwide.9–14 The lifetime risk (LTR) for GC in HDGC kindreds is high and is estimated >80%.7 Because of this high risk and the restricted value of current surveillance modalities, prophylactic gastrectomy is recommended as the treatment of choice in CDH1 mutation carriers in preventing advanced GC. In almost all resected stomachs of asymptomatic CDH1 mutation carriers multiple foci of signet ring cells (SRCs) were found.15–17 Female carriers are known to have an additional high risk of developing lobular breast cancer (LBC) with LTR of 60% by the age of 80 years, rising from age 40,6,7,18–21 whereas other tumor sites like colon and prostate might also be associated with HDGC.22,23

In The Netherlands, CDH1 mutation analysis is available in a clinical setting since 1998. We report on the geno-/phenotypical data of all, in total 10, Dutch families with different pathogenic germline mutations in the CDH1 gene and describe the functional studies on the pathogenic nature of a newly found missense mutation. In addition, we summarize the pathological findings after prophylactic gastrectomy and prophylactic mastectomy in mutation carriers in these families. We also discuss the issue of counseling on associated malignancies and prophylactic options and our finding of cleft lip with/without palate (CL/P) in a subset of patients.

Material and Methods
Patients and DNA analyses
Individuals from families with multiple GCs and/or GC at young age were referred to our clinical genetics centers. Family details were obtained and led to the suspicion of HDGC in a subset of families. In index cases from these families, mutation analysis of the CDH1 gene was performed by PCR amplification of all coding exons including intron/exon boundaries, followed by direct sequencing using the bigdye terminator v.1.1 cycle sequencing kit (PE Biosystems, Foster City, CA) and by MLPA analysis using the P08MLPA kit (MRC-Holland, Amsterdam, The Netherlands) in one laboratory.

In vitro functional assays on the p.Leu583Arg mutation
Human E-cadherin cDNA was cloned in pIREs2-EGFP vector according to manufacture instructions (Clontech, Takara Bio) and mutation p.Leu583Arg (c.1748T>G) was induced by site directed mutagenesis as described previously.24 The empty vector (Mock) was used as control.

CHO cells were grown in Alfa-MEM medium (Gibco, Invitrogen) supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen), 1% penicillin-streptomycin (Gibco, Invitrogen), in a humidified incubator with 5% CO2 at 37°C. Transient transfection of the above mentioned vectors was performed with Lipofectamine 2000 (Invitrogen), according to manufacture instructions, and the transfection efficiency was evaluated by flow cytometry before each experiment, using GFP fluorescence.

For aggregation assays, trypsinized cells were resuspended in culture medium, 5 × 104 cells were analyzed by flow cytometry and 2 × 104 cells/well were seeded in 96-well-plate coated with 50 μl of agar solution (100 mg Bacto-Agar in 15 ml of sterile PBS). The plate was incubated at 37°C in a humidified chamber with 5% CO2 for 48 hr and aggregation was evaluated and photographed in an inverted microscope (4× magnification). Experiments always included triplicates and were repeated five times.

For invasion assay, 24-well Matrigel invasion chambers (BD Biocoat) were hydrated, and each chamber was filled with 5 × 104 trypsinized cells and incubated for 24 hr at 37°C and 5% CO2. The cells and Matrigel from the upper side of the filters were removed with a pre-wet “cotton swab” while cells from the bottom of the filter (containing invasive cells) remained untouched. The filters were washed in PBS, fixed in ice-cold methanol for 15 minutes and mounted in glass coverslips with Vectashield/DAPI. The total number of invasive nuclei was counted in a Leica DM2000 microscope. Experiments were repeated five times.

Follow-up in CDH1 positive families, histological evaluation
In families with confirmed HDGC by detection of pathogenic CDH1 mutations, relatives of index patients were counseled on HDGC and were offered presymptomatic DNA-analysis.

Based on current knowledge asymptomatic mutation carriers were advised to undergo a prophylactic gastrectomy. A Swiss roll technique25 was used to study the complete mucosa of the gastrectomy specimens and all abnormalities were mapped (Figs. 1 and 2). Complete clinical data of all proven/supposed mutation carriers in these families were collected and histological specimens of therapeutic and prophylactic gastrectomy specimens were reviewed by expert pathologists. Specifically alterations as described in the context of CDH1 mutation carriers were recorded; the presence of invasive carcinoma defined as infiltration in the gastric wall beyond the level of the muscularis mucosae, of clusters of signet ring cells (SRCs) in the lamina propria <3 mm (also designated as in situ SRC carcinoma),7 of intraepithelial SRCs with pagetoid spread either in the surface epithelial lining or extending deeper in the gastric crypt epithelium12 and any additional mucosal changes were recorded. Furthermore, details were collected about the surgical procedures and complications of the individuals who underwent prophylactic gastrectomy.

Results
Description of the 10 CDH1 mutation families
In family A, two cousins died of GC at ages 23 and 34, while their parents were healthy at that time at an age >60. Several distant relatives had died of GC at a mean young age. After a pathogenic CDH1 mutation was found in this family, 11/32 tested relatives were identified as mutation carriers of whom nine underwent a
prophylactic gastrectomy. Three mutation carriers in this family were born with a cleft lip and palate, an isolated cleft palate and a subtle lip defect (forme fruste of cleft lip), respectively.

In family B, a \textit{CDH1} mutation was found after GC occurred in four family members at ages from 43 to 56. Subsequently, 16/25 relatives tested positive for this mutation, of whom 14 underwent a prophylactic gastrectomy.

A female patient in family C died of GC at age 27, while her father and two of his second degree relatives died of GC at ages between 50 and 60. Two sisters were shown to be carrier of a \textit{CDH1} mutation and underwent prophylactic gastrectomy. One of them was diagnosed with T2N0M0 DGC and has no evidence of disease seven years after surgery. CL/P was reported in three members of this family.

DGC was diagnosed in two sisters in family D from Turkish descent, at ages 37 and 39. Lung cancer and laryngeal cancer were reported in their father and his brother, both at age 60. The \textit{CDH1} mutation detected in DNA of the index patients was also found in two children of one of them. Prophylactic gastrectomy is still under consideration.

In family E, a 42-year-old man was diagnosed with GC after testing positive for a \textit{CDH1} mutation and, despite gastrectomy and chemotherapy, he died 19 months after initial diagnosis. DNA analysis was performed because of his family history with a sister, mother and grandmother dead of GC at ages 26, 43 and 37. His 68-year-old and asymptomatic maternal uncle was tested positive for the \textit{CDH1} mutation.

A mother and daughter and a distant relative in family F died of GC at ages 35, 41 and 72. After her diagnosis LBC at age 44, a second daughter was shown to be carrier of a \textit{CDH1} mutation, which was also found in a third healthy daughter. Both underwent prophylactic gastrectomy, combined with prophylactic mastectomy (contralateral/bilateral). A 20-year-old granddaughter proved to be a mutation carrier and gave birth to a son with a CLP. The boy’s parents recently requested counseling on pre-implantation genetic diagnosis (PGD) of the \textit{CDH1} mutation for future pregnancies.

A 40-year-old female member of the Creole family G finally agreed to the proposal of a prophylactic gastrectomy 10 years after the finding of SRC-foci in her stomach. Gastric surveillance had been performed because of her family history with one brother and two sisters dead from GC at ages 32, 22 and 21. Revised histological examination of gastric biopsies taken seven years before in her niece (daughter of her deceased brother) showed presence of SRC-foci in these biopsies. This niece underwent a prophylactic gastrectomy at age 23. Both gastrectomy specimens showed multiple SRC-foci in the lamina propria, but no invasive carcinoma. Both patients proved to be carrier of a \textit{CDH1} missense mutation that was shown to be a pathogenic mutation by \textit{in vitro}
functional assays. Both parents of our first index patient died at ages >80 without a history of malignancies.

In family H, a 41-year-old female was shown to have a CDH1 mutation after her diagnosis of metastasized GC. Her father died of GC at age 28. Thus far, her 21-year-old daughter and a 39-year-old sister tested positive for the CDH1 mutation.

In family I, a CDH1 mutation was found in a 55-year-old man of Hindustan origin, who was recently diagnosed with DGC. One half-sister was treated for DGC, two years before at age 36. A second half-sister and the son of a half-brother died of DGC at ages 20 and 26, respectively. Their 75-year-old mother, who turned out to be mutation carrier, was never diagnosed with cancer. The CDH1 mutation was also found in the index patient’s affected half-sister alive and in a second healthy, 50-year-old half-sister. One first and one second degree relative of his mother were reported to have had BC and her three brothers died before age 50 of a ‘disease in the belly accompanied by vomiting blood’.

A pathogenic CDH1 mutation was established in DNA of a 27-year-old man with DGC in family J. His both parents were alive without a history of malignancies at ages 72 and 55 and cancer was not reported in their siblings. One distant relative was treated for testicular cancer at age 40 and died of GC at age 82. At this moment, no relatives have been tested for the CDH1 mutation.

Genotypic/phenotypic data of the 10 CDH1 mutation families
Ten different, of which five newly described, CDH1 mutations were detected. GC occurred in 36 patients (M/F: 13/23). Mean age at diagnosis was 40 years (range 20–72 years), with 27 GCs diagnosed before the age of 50 and 10 of them before 30 years of age. Twenty-four of 26 confirmed GCs were classified as DGC, two of them had clear features of an intestinal/mucinous GC. In 19 cases, including the IGCs, SRCs were reported. The IGCs were shown in two patients, in one proven mutation carrier at age 55 and in a second deceased patient at age 52, who was a presumed carrier because of two children being CDH1 mutation carrier, while this mutation was excluded in DNA of his healthy wife. Other malignancies than GC are listed in Table 1. CL/P was reported in seven individuals, of which four mutation carriers and three untested with ≤50% risk (Table 1).

Specifically to investigate the pathogenic effect of the c.1748T>G (p.Leu583Arg) missense mutation in E-cadherin function, we performed functional assays in vitro. We found that CHO cells expressing WT E-cadherin form clear cellular aggregates, while cells expressing p.Leu583Arg exhibit a scattered pattern, resembling cells expressing the empty vector (Mock cells) (Fig. 3b). The graph of Figure 3a represents the percentage of transfection in that experiment. CHO Mock cells were shown to be invasive using the Matrigel invasion assay.26 When WT E-cadherin was expressed, the levels of cell invasiveness decreased, highlighting the role of E-cadherin in invasion suppression. In all experiments, mutant p.Leu583Arg E-cadherin was unable to rescue the invasive behaviour of CHO cells. Based on the findings that mutant p.Leu583Arg abrogates E-cadherin-mediated adhesion and impairs invasion suppression, we consider this germline CDH1 missense mutation pathogenic.

DNA testing and follow-up of mutation carriers
Fifty-eight individuals are known carriers of one of the CDH1 mutations, 50 by direct testing in blood or archival tissue specimens, eight indirectly as derived from the pedigree. Of the 39 asymptomatic carriers alive, 29 opted for prophylactic gastrectomy at a mean age of 36 years (18–61 years), combined with prophylactic mastectomy in two cases.

Prophylactic gastrectomies: Clinical and surgical details
Prophylactic total gastrectomy, with Roux-en-Y-reconstruction, was performed by laparotomy in 23/29 and by laparoscopic procedure in 6/29 cases. Treatment related morbidity was recorded as suture leakage (n=3), in one patient complicated by mediastinitis, and bronchopneumonia (n=2), one resulting in ARDS. Four patients required secondary surgical procedures. Three patients underwent re-intervention, respectively, by endoscopic coagulation and laparotomic resection to complete removal of residual proximal gastric mucosa. Hospitalization admission time varied from 5 to 26 days (mean: 11 days).

Prophylactic gastrectomies: Histological findings
All slides of 28 available gastrectomy specimens were revised for this study. (Pre)malignant changes were seen in 27 of these, consisting of invasive carcinoma in two patients, intramucosal carcinomas in 20 patients and intra-epithelial clusters of SRCs in one patient. In six patients, only very subtle changes were seen with slightly atypical surface epithelial cells with cytoplasmic vacuoles. In one patient, no gastric abnormalities were observed besides gastritis and intestinal metaplasia at age 42.

The presence or absence of SRCs and/or residual gastric mucosa in the resection margins was not systematically documented (Table 2). Gastrointestinal stromal tumours (GISTs) were found in the stomach and duodenum of two prophylactic treated patients, respectively.

Prophylactic mastectomies: Histological findings
Multiple foci of lobular carcinoma in situ (LCIS) and a 0.5-mm sized focus of invasive lobular carcinoma were detected in the contralateral mastectomy specimen of the patient who was treated for LBC before. Her sister’s mastectomy specimens showed bilateral widespread LCIS.

Discussion
Five CDH1 mutations in the Dutch families were not reported previously. The other five mutations were previously found in more than one unrelated patients who were reported not to share common haplotypes and are therefore not recognized as founder mutations.7 Of these, the
Table 1. Genotypic/phenotypic data of the 10 CDH1 mutation families

<table>
<thead>
<tr>
<th>fam</th>
<th>Mutation (prev. reported by)</th>
<th>exon</th>
<th>GC sex: n (age)</th>
<th>Age GC (yr) mean (range)</th>
<th>Other tumours (age)</th>
<th>Individuals &gt;18 yr with no malignancy. mut**</th>
<th>50% mut</th>
<th>CL/P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>c.1404del (p.Ser469fs)</td>
<td>10</td>
<td>M: 3 (25,44,63)</td>
<td>43 (23–65)</td>
<td>BC (&gt;75)</td>
<td>3 (mut)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F: 6 (23,31,34, 39,65,65)</td>
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<td></td>
</tr>
<tr>
<td>B</td>
<td>c.1565 + 2dup; Ref. 27</td>
<td>11</td>
<td>M: 1 (47)</td>
<td>47 (43–55)</td>
<td></td>
<td>16 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F: 3 (43,43,55)</td>
<td></td>
<td>Abd (50); PC (57)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>c.1135_1137 + 5delins5; Ref. 28</td>
<td>8</td>
<td>M: 3 (52*, 58, 58)</td>
<td>40 (27–52)</td>
<td></td>
<td>2 (&lt;50%)</td>
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<td></td>
<td></td>
<td></td>
<td>F: 1 (27)</td>
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<tr>
<td>D</td>
<td>c.2195G&gt;A (p.Arg732Gln)</td>
<td>14</td>
<td>F: 2 (37,39)</td>
<td>38 (37–39)</td>
<td></td>
<td>7 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Missense; Refs. 9,29</td>
<td></td>
<td>M:</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>E</td>
<td>c.1476_1477del (p.Arg492fs)</td>
<td>10</td>
<td>M: 1 (42)</td>
<td>37 (26–43)</td>
<td></td>
<td>1 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Ref. 29)</td>
<td></td>
<td>F: 3 (26,37,43)</td>
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<tr>
<td>F</td>
<td>c.489C&gt;A (p.Cys163X)</td>
<td>4</td>
<td>M:</td>
<td>49 (35–72)</td>
<td></td>
<td>2 4</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F: 3 (35,41,72)</td>
<td></td>
<td>LBC (44)</td>
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<td></td>
</tr>
<tr>
<td>G</td>
<td>c.1748T&gt;G (p.Leu583Arg)</td>
<td>12</td>
<td>M: 1 (32)</td>
<td>29 (21–40)</td>
<td></td>
<td>9 2</td>
<td></td>
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<tr>
<td></td>
<td>Missense</td>
<td></td>
<td>F: 2 (21,22)</td>
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<td></td>
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<tr>
<td>H</td>
<td>c.187C&gt;T (p.Arg63X)</td>
<td>3</td>
<td>M: 1 (28)</td>
<td>35 (28–41)</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ref. 23</td>
<td></td>
<td>F: 1 (41)</td>
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</tr>
<tr>
<td>I</td>
<td>c.811_812delins12 (p.Val271fs)</td>
<td>6</td>
<td>M: 2 (26,55*)</td>
<td>35 (20–55)</td>
<td>BC (50)</td>
<td>13 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F: 2 (20,36)</td>
<td></td>
<td>BC (age?)</td>
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<td></td>
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</tr>
<tr>
<td>J</td>
<td>c.55_74del (p.Ser19fs)</td>
<td>1</td>
<td>M: 1 (27)</td>
<td>27</td>
<td></td>
<td></td>
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<td></td>
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<td>F:</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>36 (M/F: 13/23)</td>
<td>40 (20–72)</td>
<td>12</td>
<td>37** 58 7</td>
<td></td>
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</tr>
</tbody>
</table>

M: male; F: female; GC: gastric cancer (symptomatic/at endoscopy/after prophylactic gastrectomy); BC: breast cancer; LBC: lobular breast cancer; Abd: ‘cancer in the abdomen’ (unspecified); PC: pancreatic cancer; U: uterine cancer; Me: melanoma; Lu: lung cancer; La: laryngeal cancer; CL: cleft lip; CL/P: cleft lip and/or palate; mut: mutation carrier; yrs: years; n: number of GC patients; *intestinal GC, all others diffuse type or non-specified GC. **In 29 of these patients prophylactic gastrectomy was performed, in 2 of them both gastrectomy and mastectomy.

c.2195G>A (p.Arg732Gln) missense mutation was proven to be pathogenic by Brooks-Wilson et al.29

We showed that, in family G, clusters of SRCs were identified in prophylactic gastrectomies of two asymptomatic individuals carrying the c.1748T>G mutation. The pathogenic role of this mutation was clearly reproduced in vitro, emphasizing the role of these studies in predicting the pathogenicity of missense mutations and as a tool for genetic counseling. The findings in all Dutch CDH1 mutation families highlight various known and novel aspects that are important as a guideline for care in families at risk.

Phenotypic variation
Although the number of patients in this series is too small to define a genotype/phenotype correlation, some observations should be noted. In accordance with described CDH1 families worldwide, the majority of GC diagnoses were established before the age of 50.6 However, our data showed a wide variability in age at onset (20–72 years) between, but also within families, with non-penetration in supposed carriers at an age older than 75 years in families A, G, and I. Furthermore, GC was predominantly seen in female patients (M/F: 13/23), consistent with the literature on HDGC but in contrast with IGC cases in the general population with predominance in males.

All confirmed GCs were DGCs with the exception of two intestinal GCs, which, however, also contained typical SRC-components. IGC or intestinal components are reported within the histological spectrum of HDGC.30 A latent, nonproliferative, phase of SRCs has been suggested previously31 and reasoned recently,32 and is now strongly supported by the presence of SRCs 10 and 7 years prior to gastrectomy without progression to invasive cancer in two patients from one family. Remarkable is the variation in aggressiveness of the disease within this family. Another notable point is the finding of only subtle gastric abnormalities in six prophylactic treated patients and absence of typical HDGC-related histological findings in one patient, all from family B. The six patients showed predominantly superficial vacuolized cells without the typical morphology of SRCs, as also described by Fitzgerald et al.7 and Carneiro et al.33 for which the relation to CDH1 mutation carrier status is not fully resolved. Because of the fact that these subtle lesions are the most consequent and striking feature in the gastrectomy specimens in this family and frequently the only putative CDH1-related alteration, it is likely that also these changes fall within the spectrum of characteristic alteration in CDH1 mutation carriers (Fig. 4). Further study is planned to investigate if this phenotype may be characteristic for the type of mutation in this family.
finding of GISTs in two mutation carriers is presumed to be coincidental, since no clear correlation between GISTs and \textit{CDH1} was reported in the literature, while Kawano et al. showed a high incidence of GISTs in stomachs which were resected because of GC (50 microscopic GISTs in 35 patients).34
In contrast to the reported excess of LBCs in some HDGC kindreds, we observed LBC/LCIS in only two patients. This is particularly unexpected because of the high BC incidence in The Netherlands (12.5%) and may be explained by the fact that 18 women died of GC before the age of 50 and only a few women with a mutation or at 50% risk of being mutation carrier have reached the age of ≥50 years without being diagnosed with any type of cancer. The reported prevalence of LCIS in the general population ranges from 0.5% to 3.6%. In women diagnosed with LCIS, approximately 30% will develop an invasive carcinoma, most often of the ducal type. In the general population, LCIS is most likely a risk indicator for BC, but it is not itself a true precursor for invasive disease in most patients. However, the presence of multiple foci of LCIS in two CDH1 mutations carriers suggest a causative relation between the presence of LCIS and the development of invasive LBC in women carrying a CDH1 mutation. A small number of malignancies other than GC and BC were reported. None of these cancer types are known to be related to CDH1 mutations.

Finally, the occurrence of CL/P in seven individuals, all of Caucasian origin, in three of our 10 families supports the suggested association of clefts and CDH1 by Frebourg et al. They described two Caucasian CDH1 mutation families with co-occurrence of CL/P and GC. In human embryos, they showed expression of CDH1 at weeks 4–6 of embryogenesis, i.e., the critical stage of lip and palate development. However, clefts were not reported in 58 other HDGC families with a CDH1 mutation (unpublished data, HDGC consortium Cambridge, 2008), but this may be due to reporting bias. Furthermore, in a study population of 500 individuals with nonsyndromic clefts and 500 controls, Letra et al. only observed a borderline association of CL/P with CDH1. No cancer data were available for this cohort. Given the fact that CL/P in the general population is relatively rare (1–2 in 1,000 births), the CL/Ps in our cohort are considered to be related to the CDH1 mutations, but other unknown factors must have been co-contributing to the disruption of the lip and palate closure.

Variable penetrance for GC and BC as well as variable expression of CL/P might be influenced by the nature of individual mutations, by modifying genes and by environmental risk factors.

Implications for clinical practice and genetic counseling
The restricted value of current surveillance modalities for CDH1 mutation carriers was also emphasized in our study and supports the need for prophylactic gastrectomy, since most of the malignant lesions were not detected at pre-operative gastroscopy. Although prophylactic gastrectomy is expected to be life-saving, this procedure is not without short- and long-term risks. Postsurgical complications that required re-intervention were reported in 4/29 prophylactically operated patients. This number is low compared to postoperative morbidity rates of therapeutic gastrectomy. In most cases, this refers to cancer treatment in mainly older patients with co-morbidity and performed with extensive lymphadenectomy with expected higher risk for complications. Long-term morbidity of prophylactic gastrectomy, such as nutritional deficiencies, food intolerance and diarrhea as well as impact on psychological functioning is seen in daily practice and deserves systematic evaluation. Moreover, long-term study in a larger patient population is needed to evaluate the oncological effectiveness of prophylactic gastrectomy. This will be largely determined by the absence of tumor cells and absence of normal gastric mucosa in the resection margins. A frozen section procedure of proximal and distal margins is warranted during operation and mentioning of this item in pathology reports is important. The Swiss roll technique is the designated pathological procedure to enable localization of lesions precisely. Thus far, no recurrent disease or distant metastases were observed in our patient group.

Regarding to the LBC risk, the IGCLC does not recommend prophylactic mastectomy for all female CDH1 mutation carriers, because of the higher mean age at onset of BC, which could justify a breast surveillance strategy. Since life prognosis is expected to improve after prophylactic gastrectomy, female mutation carriers must be considered to be at high risk for developing BC after gastrectomy. Because the value of surveillance is also limited in early detection of LBC, counseling should include the option of prophylactic mastectomy. For those who choose not to perform a prophylactic mastectomy, LBC surveillance from age 35 years by annual MRI and mammography has been recommended.
In genetic counseling, an important issue is the preferred age to perform prophylactic procedures. Regarding gastrectomy, the youngest and mean age of occurrence of GC in the own family was the main factor in decision making in most of our patients, together with career planning. Especially young female mutation carriers have to take their possible future pregnancies into account in planning of both prophylactic gastrectomy and mastectomy, since postsurgical feeding and nutritional deficiencies could complicate pregnancies and breast feeding will not be possible. Although Kaurah et al. showed no adverse outcomes of seven pregnancies in four women (of whom three CDH1 mutation carriers) after gastrectomy and several studies showed no increase of poor pregnancy outcomes after gastrectomies for reasons other than cancer, a critical attitude toward the sequence of pregnancies and prophylactic surgery is recommended.

In our opinion, there is no reason for informing future parents about the risk of CL/P in offspring as integral part of genetic counseling in all HDGC families. However, taking a family history in HDGC families should include the occurrence of CL/P in relatives. In those families positive for CL/P, current knowledge should be carefully communicated with counsellees, since definite risks of CL/P are not known.

Finally, the lifelong impact of being CDH1 mutation carrier will be, at least for a part of future parents, reason to consider the possibility of PGD and referral of these couples to specialized PGD centers may be indicated.

Conclusions
The observed wide variability of age at onset and aggressiveness of DGC and the long-standing presence of dormant SRCs in some patients has to be balanced against the limited ability of detecting early stages of GC and LBC and the impact of prophylactic surgery, in the optimal timing of genetic counseling, testing, and prophylactic surgery for HDGC family members. In case of prophylactic gastrectomy, surgeons and pathologists need to be focused on complete eradication of both malignant cells and normal gastric mucosa. Despite the observed low incidence of LBC, we recommend to discuss the option of prophylactic mastectomy with female mutation carriers, considering the expected higher incidence of LBC in the future.

The observed high incidence of CL/P supports the hypothesis that CDH1 mutations are involved in the disturbed lip and palate closure. This hypothesis needs to be studied in a larger patient cohort in order to inform future parents from HDGC-families adequately.

The reported complexity of counseling topics as well as surgical and pathological procedures and the expected high physical and psychosocial impact of being a mutation carrier and undergoing prophylactic interventions emphasize the need for centralized care for CDH1 mutation carriers, provided by experts in this field working in multidisciplinary teams. Structured procedures on all aspects of this care are warranted.

References


