LETTER TO JMG

CDH1/E-cadherin germline mutations in early-onset gastric cancer

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Background: Gastric cancer remains a leading cause of cancer deaths worldwide. Genetic factors, including germline mutations in E-cadherin (*CDH1*, MIM#192090) in hereditary diffuse gastric cancer (HDGC, MIM#137215), are implicated in this disease. Family studies have reported *CDH1* germline mutations in HDGC but the role of *CDH1* germline mutations in the general population remains unclear.

Aims: To examine the frequency of *CDH1* germline mutations in a population-based series of early-onset gastric cancer (EOGC <50 years old).

Methods: 211 cases of EOGC were identified in Central-East Ontario region from 1989 to 1993, with archival material and histological confirmation of non-intestinal type gastric cancer available for 81 subjects. Eligible cases were analysed for *CDH1* germline mutations by single-strand conformation polymorphism, variants were sequenced, and tumours from cases with functional mutations were stained for E-cadherin (HECD-1) using immunohistochemistry.

Results: 1155 (89%) of 1296 polymerase chain reactions amplified successfully. One new germline deletion (nt41delT) was identified in a 30-year-old patient with isolated cell gastric cancer. The overall frequency of germline *CDH1* mutations was 1.3% (1/81) for EOGC and 2.8% (1/36) for early-onset isolated cell gastric cancer.

Conclusion: This is the first population-based study, in a low-incidence region, of genetic predisposition to gastric cancer. Combined with our previous report of germline *hMLH1* mutations in two other subjects from this series, it is suggested that 2–3% of EOGC cases in North Americans may be owing to high-risk genetic mutations. These data should inform cancer geneticists on the utility of searching for specific genetic mutations in EOGC.

Keypoints

- This is the first and largest population-based study of genetic predisposition to gastric cancer in a low-incidence region reporting a new germline *CDH1* mutation.
- 2–3% of cases of early-onset gastric cancer (EOGC) in North America may be owing to high-risk genetic mutations.
- Informs geneticists on the use of searching for genetic mutations in EOGC.

International Gastric Cancer Linkage Consortium (IGCLC) provided the first clinical management guidelines for this autosomal dominant hereditary predisposition syndrome, hereditary diffuse gastric cancer (HDGC, MIM#137215), as defined by Guildford *et al* earlier that year.^{8–9} HDGC is caused by germline inactivating mutations in E-cadherin (*CDH1*, MIM#192090). However, other genes are likely to cause HDGC, as only 30% of families with HDGC meeting the primary criteria for HDGC have been found to have *CDH1* germline mutations. To date, 57 distinct functional *CDH1* germline mutations have been reported. Of these, 50 are listed in the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk/ac/gene.php?gene=CDH1>), an additional five functional mutations were reported by Suriano *et al*,¹⁰ and a further two splice mutations associated with cleft lip/palate and HDGC were more recently reported by Frebourg *et al*.¹¹ Most of these mutations result in a truncated, non-functional protein. Although primary criteria are fairly well established for screening of *CDH1* germline mutations in kindreds with gastric cancer, the IGCLC recognise the need for large population-based studies of genetic predisposition to gastric cancer to determine the role of germline mutations in sporadic EOGC. Here, we report the first population-based study of EOGC to determine the frequency of *CDH1* germline mutations in a population at a low risk for gastric cancer.

METHODS

Patients, tissue collection and pathology review

A series of 211 patients with gastric cancer ≤50 years of age diagnosed from 1988 to 1993 in Central-East Ontario region (population approximately 4.8 million) were identified from the Ontario Cancer Registry. The research ethics board of Mount Sinai Hospital, Toronto, Canada, approved all study protocols. Formalin-fixed paraffin-wax-embedded tissue (resections or biopsy specimens) and pathology reports were obtained from 35 hospitals where these patients were treated.

Abbreviations: DGC, diffuse-type gastric cancer; EOGC, early-onset gastric cancer; HDGC, hereditary diffuse gastric cancer; IGCLC, International Gastric Cancer Linkage Consortium; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism

Gastric cancer is the second most common cause of cancer deaths worldwide.¹ Despite an overall decline in the incidence of gastric cancer in older people, the incidence of early-onset (≤50 years old) gastric cancer (EOGC) and familial clustering of gastric cancer remains stable in frequency.² Genetic predisposition to gastric cancer caused by known genes such as *CDH1* and the mismatch repair genes may have an important role in the development of gastric cancer in these cases.³ Autosomal dominant gastric cancer is estimated to account for about 1–3% of all cases.⁴ We have previously reported two new germline mutations (2 of 139 cases) in the *hMLH1* gene in our population-based series of EOGC.⁵

In 1998, Guildford *et al*⁶ first described inactivating germline mutations in E-cadherin (*CDH1* gene) responsible for the development of diffuse-type gastric cancer (DGC) in three families of Maori origin. Additional family studies have shown that E-cadherin is responsible for some families presenting with DGC, according to the Lauren classification.⁷ In 1999, the

Patient identifiers were removed and study numbers assigned. Clinical information (age and sex), gross pathology (Borrmann type,¹² tumour size and tumour location) and details of lymph node involvement were obtained from pathology reports or clinical records, where available. Histopathological features (tumour grade, tumour depth and histological subtype) were assigned.¹³ Two pathologists (JTB and RR) jointly assessed and classified cases according to five classification systems—namely, Carneiro,¹⁴ Goseki,¹⁵ Lauren,¹⁶ Ming¹⁷ and World Health Organization,¹³ and these data have been previously reported.⁵

Germline DNA extraction, polymerase chain reaction and single-strand conformation polymorphism analysis for *CDH1*

Formalin-fixed paraffin-wax embedded tissues from normal gastric mucosa were obtained where possible (138 of 139 patients). Haematoxylin and eosin stained slides were used as reference to microdissect unstained 7–10-µm slides. Normal DNA was extracted using QIAmp DNA minikit (QIAGEN, Mississauga, Ontario, Canada) following the manufacturer's instructions. *CDH1* analysis was not carried out in intestinal or glandular-type gastric cancer (n = 38) as defined by the Carneiro classification. Biopsy specimens were also excluded (n = 22) owing to insufficient material and repeated failure of polymerase chain reaction (PCR) amplification. Formalin-fixed

paraffin-wax embedded germline DNA from 81 samples were screened for *CDH1* mutations using PCR of all exons and intron–exon boundaries, followed by single-strand conformation polymorphism (SSCP) analysis on 8% polyacrylamide, 10% glycerol and 0.5× TBE gels. Primer sequences were based on those reported previously (Berx *et al*,¹⁸ 1995), except for exons 1–5, which were amplified using different primers (EX1F: 5'-TACGGGGGGCGGTGCCT-3'; EX1R: 5'-CTGGGGCGCGGAGC TTG-3'; EX2F: 5'-TACCCCGTTCCATCTACCTTT-3'; EX2R: 5'-GCAATTTCTCGGCCCTTTCC-3'; EX3AF: 5'-GTCTTTAATCTG TCCAATTCC-3'; EX3AR: 5'-GCGTAGACCAAGAAATGGAT-3'; EX3BF: 5'-TACAGTCAAAAGGCCTCTACG-3'; EX3BR: 5'-AAAC AACAGCGAACTTCTCAG-3'; EX4R: 5'-CCAGAGAAACAGAGA AC-3'; EX5F: 5'-CTAATTCTTTTCTTTTCATTTTG-3'; EX5R: 5'-TGGGTGGATGTTACCCCG-3'). Polymerase chain reaction was carried out in 20 µl reaction volume with a buffer of 20 mM Tris-HCl, pH 8.4, 50 mM potassium chloride, 1 mM dNTPs, 1–3.5 mM magnesium chloride, 0.45 µM of each primer, 0.05 U of Taq polymerase (Platinum Taq Invitrogen Life Technologies, Carlsbad, California, USA) and 2.0 µCi α-³²P. Furthermore, 5% dimethyl sulphoxide was added to exon 1 PCR. The following cycling conditions were used: 30 s at 94°C, 30 s at optimised annealing temperatures ranging from 51°C to 68°C and 45 s at 72°C. Reaction products were diluted 1:1 with denaturing buffer (formamide with 0.025% xylene cyanol and 0.025% bromophenol blue) and heated to 94°C for 4 min before loading. Two sets

Table 1 *CDH1* germline sequence variants found in patients with early-onset gastric cancer

Site	nt*change/codon	Effect	Proportion (%) of patients	Proportion (%) of controls	Reference
P	5'UTR(-117)g-a	UNK	1/58 (1.7%)	ND	This study
P	5'UTR(-71)c-g	UNK	1/37 (7.7%)	2/51 (3.9%)	19
			2/58(3.4%)	ND	This study
EX1	nt41delT	Fs term 32	1/59 (1.7%)	ND	This study
IVS1	48(+5)g-c	UNK	1/28 (3.6%)	0/100 (0%)	24
			1/58 (1.7%)	ND	This study
IVS1	48(+6)t-c	Silent	5/13 (38%)	18/51 (35%)	19
			11/28 (39.3%)	27/100 (27%)	24
			1/10 (10%)	75/350 (21.4%)	21
			5/17 (29.4%)	ND	28†
			26/58 (44.8%)	ND	This study
IVS1	48(+15)c-g	UNK	2/58 (3.4%)	ND	This study
EX3	ntA324G/R108R	Silent	2/76 (2.6%)	ND	This study
IVS3	387(+26)c-t	UNK	1/76 (1.3%)	ND	This study
EX6	ntC699T/H233H	Silent	1/65 (1.5%)	ND	This study
EX6	ntG777A/K259K	Silent	1/65 (1.5%)	ND	This study
EX7	ntC933G/L311L	Silent	2/70 (2.9%)	ND	This study
EX8	ntG1062T/G354G	Silent	1/73 (1.4%)	ND	This study
EX12	ntC1896T/H632H	Silent	1/34 (2.9%)	5/100 (5%)	24
			NS	ND	20
			2/71(2.8%)	ND	This study
IVS12	1937(-13)t-c	UNK	2/27 (7.4%)	25/100 (25%)	24
			NS	ND	6
			7/79 (8.9%)	ND	This study
EX13	ntC2076T/A692A	Silent	8/13 (61.5%)	ND	19
			15/27 (55.6%)	20/100(59%)	24
			1/5 (20%)	ND	25
			7/16 (43.8%)	ND	21
			NS	ND	6, 9
			NS	ND	20
			NS	ND	28
			82/87 (94.3%)	48/50 (96%)	27
			57/79 (72.2%)	ND	This study
EX14	ntC2253T/N751N	Silent	NS	NS	28
			4/76 (5.3%)	ND	This study
IVS14	2295(+53)g-a	UNK	1/76 (1.3%)	ND	This study
EX15	ntC2296T/D766D	Silent	1/74 (1.4%)	ND	This study
EX15	ntC2310T/S770S	Silent	1/74 (1.4%)	ND	This study
EX15	ntC2314T/L772L	Silent	1/74 (1.4%)	ND	This study
EX15	ntC2400T/R800R	Silent	1/74 (1.4%)	ND	This study
IVS15	2439(+31)g-a	UNK	1/74 (1.4%)	ND	This study

EX, exon; fs, frameshift; IVS, intervening sequence/intron; ND, not done; NS, not specified; nt, nucleotide; term, termination or premature stop; UNK, unknown; UTR, untranslated region; -, nucleotide position upstream of the specified codon; +, nucleotide position downstream of the specified codon.

*According to Antonarakis and the Nomenclature Working Group *Human Mutation* 1998;11:1–3. Nucleotide positions refer to an E-cadherin sequence deposited in the EMBL/GenBank Data Libraries, accession no. Z13009; the A of the ATG initiator Met codon is denoted nt+1.

† Work by Yabuta showed no changes in splicing as a result of IVS1(+6) variant.

of gel run conditions, 18 h at 4°C and 22 h at room temperature were used except for exon 1, where the experiment was carried out only at 22°C. Products were detected by autoradiography.

DNA sequence analysis

Samples showing aberrant SSCP migration patterns were analysed further by an independent PCR from the original DNA extraction using manual and automated sequencing. PCR was repeated in 30 µl reaction volume with a buffer of 20 mM Tris-HCl, pH 8.4, 50 mM potassium chloride, 1 mM dNTPs, 1–3.5 mM magnesium sulphate, 0.45 µM of each primer and 0.05 U of High Fidelity Taq polymerase (High Fidelity Platinum Taq Invitrogen Life Technologies). Samples were run on a 2% agarose gel and purified using either MinElute or Regular gel extraction kit (Qiagen, Valencia, California, USA) and eluted into a volume of 10–30 µl, according to the manufacturer's instructions. A 2–5 µl sample of purified product was sequenced using Thermosequenase Radiolabeled Terminator Cycle Sequencing kit (Amersham Biosciences, Piscataway, New Jersey, USA) in both directions according to the manufacturer's instructions. Sequencing conditions were as follows: initial denaturation 95°C for 4 min followed by 40 cycles of 95°C for 30 s, 50–60°C for 40 s and 72°C for 40 s, and a final extension of 72°C for 10 min using the Perkin Elmer GeneAmp PCR system 9600 (Perkin-Elmer Life and Analytical Science, Boston, Massachusetts, USA). Sequencing was performed using a Big Dye Terminator V.3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) in a final volume of 20 µl with 5 pm/ml primers and 1 ng/base of purified PCR-DNA sample. Sequencing conditions were initial denaturation at 96°C for 1 min, followed by 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Reaction products were purified using AutoSeq G-50 (Amersham Biosciences) with Sephadex 5–50 columns. The eluent was dried and re-suspended in a formamide:loading dye mixture of ratio 5:1, before loading 1 µl on the ABI Prism 377 DNA sequencer.

E-cadherin immunohistochemistry

E-cadherin immunohistochemistry of mutation positive cases (excluding silent, intronic mutations) was carried out with a monoclonal E-cadherin antibody, HECD-1 (Zymed, San Francisco, California, USA), at a dilution of 1:4 and overnight incubation at 4°C. Next, the ImmPRESS Anti-Mouse immunoglobulin (Ig) kit, a peroxidase micropolymer-labelled anti-mouse IgG secondary antibody, was incubated for 30 min at room temperature (Vector Laboratories, Burlingame, California, USA), followed by peroxidase development using NovaRed (Vector Laboratories) for 7 min at room temperature according to the manufacturer's instructions. Internal positive (normal gastric mucosa) and negative staining controls (lymphocytes) were used. The normal immunohistochemical staining pattern for E-cadherin is membranous. Abnormal E-cadherin staining is characterised by loss of membranous pattern, or non-membranous staining.

RESULTS

A population-based series of 81 cases of gastric cancer was available for CDH1 germline mutational analysis. A total of 1155 of 1296 (89%) PCR reactions amplified successfully, ranging from 73% to 98% per exon or from 25% to 100% per case. Exon 1 contains sequences highly rich guanine and cytosine nucleotides, and was the most difficult to amplify with the greatest number of PCR failures (22/81 cases). Of these, 78% (63/81) were successfully PCR amplified for at least 75% of the 16 CDH1 exons. In all, 319 abnormally migrating SSCP shifts were identified and subsequently sequenced. A high false-positive rate (67%) was observed when using SSCP as a screening technique for the detection of CDH1 variants, as only 105 of 319 (33%) shifts showed

Table 2 CDH1 functional germline mutations in patients with early-onset gastric cancer (EOGC) and comparison of studies on EOGC

Study characteristics			Findings			
Reference	Age (years)n	GC incidence/country	Histology	Methods	#GL	Other
This study	≤50 81	Low/Canada	36 DGC 41 MGC	4 SGC PCR-SSCP	EX1/nt41delT Fs term 32	Loss of E-cad IHC staining
Suriano <i>et al.</i> ¹⁰ 2005	≤35 10	Low/North America (diverse ethnicity)	All DGC	PCR-dHPLC	EX8/nt1063delT Fs term 355 EX9/ntC1285T P429S, in vitro ✓ EX11/nt1619insG Fs term 547	27/nos 27/nos 29/nos* Mom had bilateral brca
Keller <i>et al.</i> ²² 2004	≤45 15	High/Germany	14 DGC 1 MGC	PCR-dHPLC	EX12/mC1901T A634V, in vitro ✓	abn E-cad IHC, diffuse cytoplasmic staining E-cad IHC could not be done
Suriano <i>et al.</i> ²⁵ 2003	≤51 66	Mix/UK 26, USA 6, Portugal 22, Italy 11, Greece 1	37 DGC, 12 IGC, 17 MGC	PCR-SSCP		
Studies on EOGC with negative findings						
Saito <i>et al.</i> ³⁵ 1999	<35 9	High/Japan	All DGC	PCR-SSCP	0/9	
Brooks-Wilson <i>et al.</i> ³⁰ 2004	<50 9	Low/US, UK, Canada	All DGC	Seq and PCR-SSCP	0/9, SNPs only	
Carvalho <i>et al.</i> ³³ 2004	<45 40	Mixed/Netherlands, Finland, USA	21 DGC 10 IGC 9 MGC	PCR-SSCP	0/403 variants	
Oliveira <i>et al.</i> ³⁵ 2004	<45 23	High/Portugal	16 DGC 2 IGC 5 GC	PCR-SSCP	0/23 SNPs only	

abnN, abnormal; brca, breast cancer; del, deletion; CPC, clinicopathological characteristics; DGC, diffuse gastric cancer; dHPLC, denaturing high-performance liquid chromatography; E-cad, E-cadherin; EX, exon; Fs, frameshift; GL, germline mutation found; IGC, intestinal gastric cancer; IHC, immunohistochemistry; ins, insertion; MGC, mixed gastric cancer; nos, not otherwise specified; nt, nucleotide; PCR, polymerase chain reaction; Seq, straight sequencing; SGC, solid gastric cancer; SNPs, single-nucleotide polymorphisms; SSCP, single-strand conformation polymorphism; term, premature stop codon; in vitro ✓, pathogenicity proved by in vitro E-cadherin mutant studies; ✓, male.

variant sequences. Primary data can be found in tables A and B at <http://jmg.bmjournals.com/supplemental>. A novel inactivating mutation was identified in exon 1 (table 1; fig 1). This patient with early-onset diffuse-type gastric cancer (case no 167) was a 30-year-old male, of European descent, who presented with advanced DGC with invasion into the small bowel and colon. On the basis of limited available clinical records, a family history of cancer was not elicited for this case. Immunohistochemical studies confirmed loss of functional membranous E-cadherin staining in the tumour relative to the normal mucosa in this patient (fig 1D). The tumour in this case was found to be microsatellite stable in our previous report.⁵

An additional 19 *CDH1* variants were found, 12 of which have not been previously reported (table 1).^{6-9 19-28} Of the 19 variants, 2 are in the promoter (5'UTR) region, 5 are in introns and 12 are silent variants. The C→G transversion at position IVS1+5 was originally found and reported by Oliveira *et al*²³ in a family of American origin, which did not fulfil the criteria for HDGC. Unfortunately, only one member of the family was available for

analysis for this germline mutation. This transversion was also not found in other families or in 100 controls studied in that report. However, Shapiro and Senapathy²⁸ reported that the G nucleotide occurs at this position in 84% of eukaryotic splice donor sites and is therefore likely to be a single-nucleotide polymorphism of no functional significance.

DISCUSSION

The discovery of genetic factors that have a role in the pathogenesis of gastric cancer is an important step in understanding this disease. In 1998, Guildford *et al*⁶ reported the first germline truncating *CDH1* mutations in Maori families with autosomal dominant DGC. Since then, pathogenic mutations have been described in patients with DGC of various ethnic origins in family studies as reviewed by Oliveira *et al*.⁷ Although somatic *CDH1* mutations cluster in the exon 7–9 hotspot region of the extracellular domain, germline mutations in patients with HDGC and EOGC are dispersed throughout *CDH1*,³⁰ necessitating the analysis of the entire gene. Gastric cancer is a heterogeneous disease with two common histological variants,

Figure 1 Case 167 is a *CDH1* germline mutation-positive case. (A) Polymerase chain reaction–single-strand conformation polymorphism shows an abnormally migrating band “<” in case 167N (N normal DNA), which is not present in the wild-type (WT) control. Sequence confirmation in the sense direction shows delT “*” and the resulting frameshift downstream of this nucleotide. Also note the intronic sequences (in lower case) showing the single-nucleotide polymorphism (SNP) at position IVS1(+6)c-t in both WT control and case 167. (C–F) Photomicrographs showing the tumour histology of case 167. (C, E) Haematoxylin and eosin taken at $\times 4$ and $\times 20$ magnification, respectively, showing isolated cell-type gastric cancer with prominent signet ring cells. (D, F) E-cadherin immunohistochemistry taken at $\times 4$ and $\times 20$ magnification, respectively, showing intact membranous E-cadherin staining in normal glands and loss of staining in tumour cells.

DGC and intestinal gastric cancer. The strong genotype-phenotype correlation between *CDH1* germline mutations and DGC is an example where distinct histopathology can be used as a phenotypic criterion to identify gene carriers in familial epithelial cancer syndromes.⁸ To date, *CDH1* germline mutations have not been found in intestinal gastric cancer. Therefore, patients with intestinal EOGC were not included in this study.

HDGC is a rare cancer susceptibility syndrome. The clinical management guidelines for cases of familial HDGC were first defined by the International Gastric Cancer Linkage Consortium⁸ as any family with (1) two or more documented cases of DGC in first or second-degree relatives, with at least one member diagnosed before the age of 50 years or (2) three or more cases of documented DGC in first/second-degree relatives, independent of age at onset. The major criterion used by our group is the revised clinical criteria originally reported in Brooks-Wilson *et al.*³¹ Our group has found that *CDH1* germline mutations are present in 44% (17/39) of families that meet the primary criteria and belong to a low-incidence region for gastric cancer.¹⁰⁻³¹ They have also been found in 12% (1/8) of families that meet the secondary criteria. In addition, the revised clinical criteria included a third criterion to account for patients with EOGC, which is defined as an isolated individual diagnosed with DGC at <45 years of age.¹⁰⁻³¹ As can be seen in table 2, various cut-off ages published for EOGC have been used in different studies, and in some studies all histology types of gastric cancer were also analysed.

Although there is a diverse ethnic distribution of *CDH1* mutations, it is important to recognise that geographic variation in the incidence rates of gastric cancer might influence the extent to which genetic factors, such as *CDH1* germline mutations, can contribute to the development of gastric cancer outside of the familial setting. Therefore, the IGCLC defined criteria for various gastric cancer syndromes to reflect differences in incidence rates of gastric cancer.⁸ These incidence-based criteria have been supported in subsequent publications showing the paucity of germline *CDH1* mutations in patients with EOGC in high-incidence countries (ie, Japan, Portugal and Germany)²²⁻³¹⁻³² relative to countries with low incidence rates of gastric cancer, such as Canada and the US.²⁵⁻³⁰ This difference is partly explained by environmental factors such as *Helicobacter pylori* infection having an even greater role than genetic predisposition factors such as *CDH1* germline mutations. Therefore, our study is relevant to the development of clinical guidelines for screening of *CDH1* germline mutations in patients with EOGC in low-incidence countries.

To date, four mutations have been reported in sporadic EOGC.¹⁰⁻²²⁻²⁵⁻³⁰ These studies have been limited to smaller single-institutional series or multicentre collaborations. Table 2 summarises *CDH1* germline mutations in EOGC. Among the 81 patients in the current study, we found one case of a germline mutation in a 30-year-old patient, with isolated cell-type gastric cancer or DGC. This inactivating mutation leads to a non-functional protein, which terminates in exon 1 and encodes the signal peptide portion of the mature E-cadherin protein. Our report describes the first and largest population-based study of *CDH1* germline mutations in a series of EOGC. Combining our results with others in table 2, the overall reported frequency of mutations attributable to *CDH1* germline mutations in patients with EOGC, including studies with negative findings,³⁰⁻³¹⁻³³⁻³⁴ is about 2% (5/253). Age data from our population-based series show a skewed age distribution, with 58% (47/81) of people >40 years of age (fig 2). Furthermore, the age summary for patients with non-intestinal-type gastric cancer (54/66 EOGC) provided by Suriano *et al.*²⁶ in their series is similar to our study with 64% (34/54) of their patients >40 years of age. They also found one functional mutation in a 30-year-old patient with DGC. To date, all pathogenic *CDH1* mutations were found in young patients (<35 years). Therefore, a strong

Figure 2 Histogram of age data for early-onset gastric cancer (n=81). *, patients who were mutation positive. Mean (SD) 41.25 (5.991).

argument can be made for *CDH1* germline analysis in young, apparently sporadic cases of DGC.

The frequency of *CDH1* germline mutations in our study is likely to be an underestimate owing to failure of PCR in some exons in some cases, and possibly reduced sensitivity of SSCP for detecting mutations. Overall, formalin-fixed paraffin-wax embedded DNA template is of poor quality compared with fresh blood or frozen tissue DNA, thus leading to more PCR failures. However, we were able to amplify 89% of templates. The PCR failures seemed to be related to the underlying *CDH1* sequence. In particular, exon 1 contains an area highly rich in guanine and cytosine nucleotides and is inherently more difficult to amplify. Finally, the finding of a somatic intragenic deletion of *CDH1* affecting at least exon 8 as the second hit in a Caucasian family with *CDH1* germline splice-site mutation³⁵ highlights another possible shortcoming of all EOGC *CDH1* mutation studies to date that might have missed intragenic deletions because of using only PCR-based mutation detection methods. Therefore, future *CDH1* mutation detection studies may require experimental protocols that can detect intragenic mutations.

Finally, our series of EOGC has also been previously characterised for DNA mismatch repair deficiency, and we reported two cases, aged 35 years and 43 years, with germline mutations in the *hMLH1* gene.⁵ Taken together, about 2% of EOGC cases can be attributed to germline mutations in known genes (1 *CDH1* and 2 *hMLH1*).

SUMMARY

The frequency of inactivating *CDH1* germline mutations in this population-based series of EOGC is 1.2% (1/81). The finding of a mutation in a very young patient, in addition to other publications, supports the recommendation to screen for *CDH1* germline mutations in patients ≤35 years of age presenting with diffuse gastric cancer. Furthermore, our population-based series of EOGC has been useful in supporting estimates for genetic predisposition to gastric cancer of 2.1% (1% is caused by germline MMR mutations and 1% by *CDH1* mutations), which should aid in the development of screening guidelines for patients who are at high risk for EOGC.

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Supplementary tables A and B available at <http://img.bmjournals.com/supplemental>.

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