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Genetic Screening for Familial Gastric Cancer

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Abstract

Approximately 10% of gastric cancer cases show familial clustering but only 1-3% of gastric carcinomas arise as a result of inherited gastric cancer predisposition syndromes. Direct proof that Hereditary Gastric Cancer is a genetic disease with a germline gene defect has come from the demonstration of co-segregation of germline *E-cadherin* (*CDH1*) mutations with early onset diffuse gastric cancer in families with an autosomal dominant pattern of inheritance (HDGC). *E-cadherin* is a transmembrane calcium-dependent cell-adhesion molecule involved in cell-junction formation and the maintenance of epithelial integrity. In this review, we describe the frequency and type of *CDH1* mutations in sporadic and familial gastric cancer. Further we demonstrate the functional significance of some *CDH1* germline missense mutations found in HDGC. We also discuss the *CDH1* polymorphisms that have been associated to gastric cancer. We report other types of malignancies associated to HDGC, besides diffuse gastric cancer. Moreover, we review the data available on putative alternative candidate genes screened in familial gastric cancer. Finally, we briefly discuss the role of low-penetrance genes and *Helicobacter pylori* in gastric cancer. This knowledge is a fundamental step towards accurate genetic counselling, in which a highly specialised pre-symptomatic therapeutic intervention should be offered.

Introduction

Gastric cancer is one of the most common gastrointestinal malignancies world-wide, although in recent decades a decline has been observed in its incidence and associated mortality [1, 2]. Gastric cancer is highly heterogeneous morphologically, but there are two main histotypes of gastric carcinoma: the glandular (intestinal) type and the isolated-cell type (diffuse). A proportion of cases displays a mixed phenotype harbouring in a single tumour of two histological components (glandular and diffuse) [3, 4].

Although the incidence of gastric cancer in older patients is decreasing, the incidence of gastric cancer in younger patients and cases with familial clustering remains quite stable. This suggests that a genetic predisposition may play an important role in the pathogenesis of some forms of gastric cancer [5]. About 10% of cases of gastric cancer show familial clustering [2, 6] but only 1-3% of gastric carcinomas

Table 1. Summary of the germline CDH1 mutation screening studies in families with gastric cancer

Study	Total of families	HDGC families	FDGC families	FIGC families	FGC families	CDH1 truncating mutations	CDH1 missense mutations	% CDH1 mutations in HDGC	% CDH1 mutations in FDGC
Guilford et al, 1998 [9]	3	3	0	0	0	3	0	100%	0%
Gayther et al, 1998 [10]	18	10	0	8	0	3	0	30.0%	0%
Richards et al, 1998 [11]	8	8	0	0	0	2	0	25.0%	0%
Guilford et al, 1999 [12]	6	4	2**	0	0	6**	0	100%	100%
Shinmura et al, 1999 [13]	13	3	0	10	0	0	1	33.3%	0%
Yoon et al, 1999 [85]	5	5	0	0	0	0	2	40%	0%
Iida et al, 1999 [86]	14	0	6	6	2	0	0	0%	0%
Keller et al, 1999 [44]	7	2	5	0	0	1	0	50.0%	0%
Avizienyte et al, 2001 [87]	11	5	4	1	1	0	0	0%	0%
Dussaulx-Garin et al, 2001 [88]	1	1	0	0	0	1	0	100%	0%
Humar et al, 2002 [89]	10	7	3*	0	0	5*	0	57.1%	33.3%
Oliveira et al, 2002 [32]	39	11	24	4	0	3	1	36.4%	0%
Yabuta et al, 2002 [26]	17	2	3	0	12	0	1	50.0%	0%
Wang et al, 2003 [27]	78	0	2**	0	76	0	2**	0%	100%
Oliveira et al, 2004 [28]	1	1	0	0	0	1	0	100%	0%
Jonsson et al, 2002 [25]	3	3	0	0	0	1	0	33.3%	0%
Oliveira et al, in press [30]	32	9	10	3	10	0	1	11.1%	0%
Keller et al, 2004 [29]	28***	2	21	5	0	0	1*	0 %	4.8%
Brooks-Wilson et al, in press [31]	34	26	7*	1	0	10	3*	46.2%	14.3%
TOTAL	**328**	**102**	**87**	**38**	**101**	**36**	**12**	**40.2%**	**8.0%**

*One FDGC families with a germline mutation
**Two FDGC families with missense germline mutations
*** the numbers of families, not included in Ref. (Keller et al 1999) are listed

arise as a result of an inherited gastric cancer predisposition syndrome [7].

In formulating a definition of familial gastric cancer syndromes, a distinction must be made between the histopathological subtypes (diffuse or diffuse with glandular component/mixed versus intestinal) which segregate within families [8]. Gastric cancer was proved to be an inherited disease, primarily in families with aggregations of diffuse gastric cancer.

The syndrome of hereditary diffuse gastric cancer (HDGC) was defined by the International Gastric Cancer Linkage Consortium (IGCLC) [8], as any family that fits the following criteria: (1) two or more documented cases of diffuse gastric cancer in first/second degree relatives, with at least one diagnosed before the age of 50, or (2) three or more cases of documented diffuse gastric cancer in first/second degree relatives, independently of age. The identification of the germline gene defect underlying HDGC came from segregation studies in early onset diffuse gastric cancer families [9-12]. Germline mutations of the *CDH1* gene (EMBL/GenBank Data Libraries# *CDH1* – Z13009) resulting in E-cadherin inactivation have been identified in HDGC (OMIM# Gastric cancer – 137215). Families with aggregation of gastric cancer and index cases with diffuse gastric cancer but not fulfilling the IGCLC criteria for HDGC are termed familial diffuse gastric cancer (FDGC).

The criteria to define hereditary intestinal gastric cancer (HIGC) families were adjusted by the IGCLC depending on the incidence of gastric cancer in the

Table 2. Details from all the CDH1 germline mutations described to date in familial gastric cancer

CDH1 Mutation	Gene location	Mutation type	Predicted premature stop codon	*Reference*
45insT	Exon 1	Frameshift	Codon 32	Oliveira et al, 2002 [32]
49-2A>G	Intron 1	Splice-site	Unknown	Richards et al, 1999 [11]
53delC	Exon 2	Frameshift	Codon 32	Humar et al, 2002 [89]
59G>A	Exon 2	Nonsense (W20X)	Codon 20	Richards et al, 1999 [11]
70G>T	Exon 2	Nonsense (E24X)	Codon 24	Guilford et al, 1999 [12]
185G>T	Exon 3	Missense (G62V)	Ns	Shinmura et al, 1999 [13]
187C>T	Exon 3	Nonsense (R63X)	Codon 63	Gayther et al, 1998 [10]
190C>T	Exon 3	Nonsense (Q64X)	Codon 64	Guilford et al, 1999 [12]
283C>T	Exon 3	Nonsense (Q95X)	Codon 95	Dussaulx-Garin et al, 2001 [88]
372-377delC	Exon 3	Frameshift	Codon 249	Keller et al, 1999 [44]
382delC	Exon 3	Frameshift	Codon 215	Brooks-Wilson et al, in press [31]
531+1G>A	Intron 5	Splice-site	Unknown	Brooks-Wilson et al, in press [31]
586G>T	Exon 5	Nonsense (G196X)	Codon 196	Guilford et al, 1999 [12]
731A>G	Exon 6	Missense (D244G)	Ns	Yoon et al, 1999 [85]
832G>A	Exon 6	Frameshift	Codon 281 Codon 336+18bp int 7	Oliveira et al, 2002 [32]
892G>A	Exon 7	Missense (A298T)	Ns	Brooks-Wilson et al, in press [31]
1003C>T	Exon 7	Nonsense (R335X)	Codon 335	Jonsson et al, 2002 [25]
1008G>T	Exon 7	Splice-site	Codon 349	Guilford et al, 1998 [9]
1018A>G	Exon 8	Missense (T340A)	Ns	Oliveira et al, 2002 [32]
1064insT	Exon 8	Frameshift	Codon 393	Brooks-Wilson et al, in press [31]
1135del8ins5	Exon 8	Splice-site	Codon 386	Oliveira et al, 2004 [28]; Brooks-Wilson et al, in press [31]
1137+1G>A	Intron 8	Donor splice-site	Unknown	Guilford et al, 1999 [12]
1212delC	Exon 9	Frameshift	Codon 417	Brooks-Wilson et al, in press [31]
1226T>C	Exon 9	Missense (W409R)	Ns	Brooks-Wilson et al, in press [31]
1243A>C	Exon 9	Missense (I415L)	Ns	Wang et al, 2003 (two families) [27]
1460T>C	Exon 10	Missense (V487A)	Ns	Yoon et al, 1999 [85]
1472insA	Exon 10	Frameshift	Codon 536	Oliveira et al, 2002 [32]
1476delAG	Exon 10	Frameshift	Codon 547	Brooks-Wilson et al, in press [31]
1487del7	Exon 10	Frameshift	Codon 556	Guilford et al, 1999 [12]
1565+1G>T	Intron 10	Splice-site	Unknown	Humar et al, 2002 [89]
1588insC	Exon 11	Frameshift	Codon 536	Guilford et al, 1999 [12]
1710delT	Exon 11	Frameshift	Unknown	Humar et al, 2002 [89]

Table 2.

1711insG	Exon 11	Frameshift	Codon 587	Gayther et al, 1998 [10]
1711+5G>A	Intron 11	Splice-site	Unknown	Brooks-Wilson et al, in press [31]
1779insC	Exon 12	Frameshift	Codon 604	Brooks-Wilson et al, in press [31]
1792C>T	Exon 12	Nonsense (R598X)	Codon 598	Gayther et al, 1998, Humar et al, 2002 [10,89]
1901C>T	Exon 12	Missense (A634V)	Codon 653	Oliveira et al, in press [30]
2061delTG	Exon 13	Frameshift	Codon 783	Brooks-Wilson et al, in press [31]
2095C>T	Exon 13	Nonsense (Q699X)	Codon 699	Guilford et al, 1998 [9]
2195G>A	Exon 14	Missense (R732Q)	Ns	Brooks-Wilson et al, in press [31]
2295+5G>A	Intron 14	Splice-site	Unknown	Humar et al, 2002 [89]
2310delC	Exon 15	Frameshift	Codon 783	Brooks-Wilson et al, in press [31]
2382-2386insC	Exon 15	Frameshift	Codon 349	Guilford et al, 1998 [9]
2396C>G	Exon 15	Missense (P799R)	Ns	Keller et al, 2004 [29]
2494G>A	Exon 16	Missense (V832M)	Ns	Yabuta et al, 2002 [26]

population. Thus, countries with a high incidence like Japan and Portugal should use the diagnostic criteria analogous to the Amsterdam criteria for HNPCC [13]: (1) at least three relatives should have intestinal gastric cancer and one of them should be a first degree relative of the other two; (2) at least two successive generations should be affected; (3) in one of the relatives, gastric cancer should be diagnosed before the age of 50. In countries with a low incidence (USA, UK) HIGC was defined as (1) at least two first/second degree relatives affected by intestinal gastric cancer, one diagnosed before the age of 50; or (2) three or more relatives with intestinal gastric cancer at any age. No germline genetic defect has been found to date in this type of predisposing disease.

Families with aggregations of gastric cancer and an index case with intestinal gastric cancer are termed familial intestinal gastric cancer (FIGC).

Families with aggregation of gastric cancer, but without histology available on the tumours are termed familial gastric cancer (FGC).

Patients who developed gastric cancer at an early age (< 50 years old) without a familial history of gastric cancer were considered early onset gastric cancer patients.

The CDH1 gene

E-cadherin is a 120 kD glycoprotein localised at adherens junctions of epithelial cells, where it mediates homophilic calcium-dependent cell-adhesion [14, 15]. The *CDH1* gene maps to 16q22.1, comprises 16 exons spanning approximately 100 kb of genomic DNA which are transcribed into a 4.5 Kb mRNA [16]. The E-cadherin modular structure consists of five extracellular domains each ~110 aa in length, with conserved calcium-binding motifs, a transmembrane region and a cytoplasmic domain, which interacts with filaments of actin through catenins [17]. Disruption of the E-cadherin complex is expected to induce loss of cell-adhesion with a concomitant increased cell invasion [18, 19].

E-cadherin and sporadic cancer

Loss of E-cadherin function is one of the crucial steps for tumour progression in several types of human cancer. Despite what has been observed in other types of epithelial cancers, in which E-cadherin expression is down regulated without harbouring gene mutations, namely thyroid, skin, lung, ovary and colon, in sporadic diffuse gastric carcinoma E-cadherin down regulation is often associated with gene mutation [20, 21, 22]. *CDH1* mutations have been described not only in diffuse gastric cancers but also in a specific histological type of breast cancer namely infiltrative lobular breast cancers, another epithelial cancer in which neoplastic cells are dispersed in the stromal tissue [23]. Most of the somatic *CDH1* mutations found in sporadic diffuse gastric carcinomas are missense and in-frame deletions [23]. In contrast to stomach, the mutations found in infiltrating lobular breast cancers were out-of-frame mutations, causing premature stop codons. In both models somatic mutations cluster in exons 7 to 9, in the extracellular domain of the protein.

Table 3. Details from CDH1 germline mutations described to date in early onset gastric cancer patients

CDH1 Mutation	Gene location	Mutation type	Predicted premature stop codon	*Reference*
1901C>T	Exon 12	Missense (A634V)	Codon 653	Suriano and Oliveira et al, 2003 [33]
1619insG	Exon 11	Frameshift	Codon 547	Keller et al, 2004 [29]

Inactivation of *CDH1* in diffuse gastric cancer cell lines and primary lobular breast carcinomas is achieved by 2 genetic hits. Infiltrative lobular breast carcinomas show LOH at the *CDH1 locus* as a second hit. But in the majority of diffuse gastric cancer cases with *CDH1* mutations it was demonstrated that hypermethylation of the *CDH1* promoter region accounts for the inactivation of the second allele [24].

Mutation of *CDH1* in HDGC and Early-onset Gastric Cancer

Germline truncating and missense mutations of the *CDH1* gene resulting in E-cadherin inactivation and/or segregating with the disease have been identified in hereditary diffuse gastric carcinoma. To date, forty eight families harbouring *CDH1* germline mutations have been described, 41 HDGC (40%) and 7 FDGC (8%) (see Table 1 for details) [21, 25-31]. In these families, 45 different *CDH1* germline mutations were found and dispersed along the gene (see Table 2 and Fig. 1 for details). The majority (76.0%) of these *CDH1* germline mutations are frameshift, splice-site and nonsense changes resulting in truncated non active proteins. Guilford et al described for the first time germline *CDH1* mutations in a large percentage of New Zealand Maori HDGC families [9]. Shortly thereafter, *CDH1* germline mutations were described in a broad range of ethnic backgrounds. *CDH1* mutations were also found in a significant percentage of HDGC families of European and American origin [21]. In families of Asian ethnicity no truncating mutations have been identified to date [21]. In 24% of the families *CDH1* germline missense mutations have also been reported [26, 27, 29-33]. These missense mutations are also distributed along the gene, eight germline missense mutations cluster in the extracellular region of the protein, one in the transmembrane domain and two are localised in the intracellular domain of the protein (see Table 2 and Fig. 1 for details).

A total of 104 early-onset apparently sporadic gastric cancer patients were studied for *CDH1* germline mutations. Eighty seven of them had diffuse type or mixed gastric cancer with a diffuse component. Only two of the 104 patients had germline *CDH1* mutations (Table 3). These two mutations were identified in patients with diffuse gastric cancer [29, 33].

Initially it was reported that the mechanism of inactivation of the *CDH1* wild-type allele in tumour cells from HDGC by families was either by promoter methylation or by somatic mutation [34]. The biallelic inactivation leads to diminished or absent E-cadherin immunoreactivity in the neoplastic cells [34]. Recently it was found in a Caucasian family with a *CDH1* germline splice-site mutation in all members affected by gastric cancer, a *CDH1* intragenic deletion of *CDH1*, affecting at least exon 8, as the second hit in one of the tumours [28]. This observation highlights

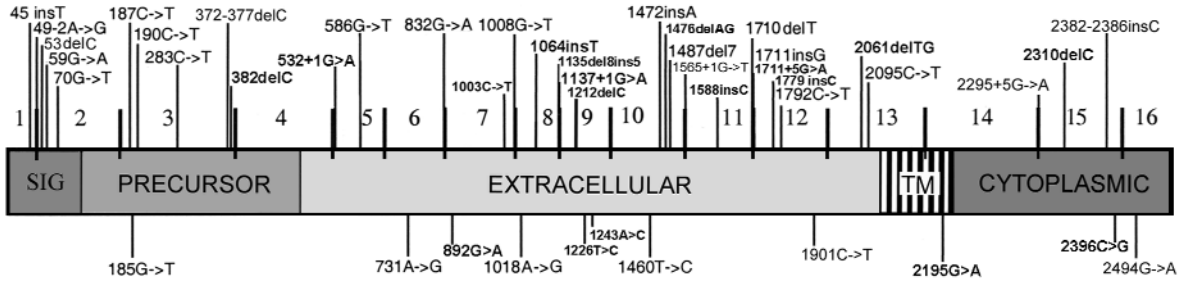

Fig. 1. Scheme of the CDH1 gene with germline mutations described to date in HDGC. Truncating mutations are shown above and missense mutations below the gene. Sig: signal peptide; Precursor: protein precursor domain; TM: transmembrane domain; Cyto. Domain: protein cytoplasmic domain

Table 4. Functional characterization of missense mutations found in gastric cancer probands

CDH1 Construct	Aggregation	Invasion	Pathogenic significance
Wild type	Yes	No	Not applicable
A298T	No	Yes	Yes
T340A	No	Yes	Yes
W409R	No	Yes	Yes
A592T	Yes	No	No
A617T	Yes	No	No
A634V	No	Yes	Yes
R732Q	No	Yes	Yes
P799R	No	Yes	Yes
V832M	No	Yes	Yes

the need of developing experimental protocols to identify, in the setting of HDGC families, the presence of germline or somatic intragenic deletions in *CDH1*, which are easily missed by mutation detection methods based on PCR of genomic DNA.

Functional significance of *CDH1* germline missense mutations

The functional significance associated to *CDH1* germline missense sequence variants is not straightforward. Moreover, due to the lethal nature of the disease there are rarely enough available affected individuals in any family to perform segregation analysis in families carrying germline missense sequence variants. The lack of such knowledge represents a major limitation for the clinical management of these patients and families.

To address this problem, a functional in vitro screen for gastric cancer missense mutations was created [33]. Cell-lines stably expressing the germline E-cadherin sequence variants were established and their effect on the protein ability to mediate cell-cell adhesion and suppress invasion was addressed. To date, we have analysed nine germline missense sequence variants and showed that some of these variants cause impaired or reduced cell-cell adhesion, increased cell motility and invasion, resulting in a scattered cell morphology and invasive phenotype similar to that observed in diffuse gastric carcinoma [29, 31, 33, 35-37] (see Table 4 for details).

In addition, it was shown that the effect of different E-cadherin germline missense mutations in cell morphology and motility was distinct, demonstrating the existence of a genotype-phenotype correlation between different E-cadherin mutations and cell behaviour, likely dependent on the specific E-cadherin domain affected by each mutation [38].

The aforementioned studies indicate that functional assays should be used as an adjunct in deciding on the potential pathogenic role of germline sequence variants, with significant potential to help clinical counselling of the *CDH1* mutation carriers.

CDH1 polymorphisms

There is an increasing number of manuscripts reporting *CDH1* sequence variants in gastric cancer families and also in controls (see Table 5 for details). Two good examples of these sequence variants are single nucleotide polymorphisms located at the promoter region of *CDH1*, the -347G–>GA and the -160C/A. Both sequence variants were described to affect the transcriptional activity of *CDH1*.

The -347G–>GA single nucleotide polymorphism was shown to down regulate the transcriptional activity of the E-cadherin gene by measuring the promoter activity of the -347G–>GA polymorphism. The GA allele decreased the transcriptional efficiency by 10-fold ($p<0.001$) and had a weak transcription factor binding compared to the G allele [39]. In a case-control study performed in a Korean population of 170 individuals (28 probands from gastric cancer families and 142 normal controls) the -347G/GA heterozygous or GA homozygous was associated with FGC patients ($p<0.05$) compared with the G homozygous genotype [39].

The A-allele of the -160C/A polymorphism was shown to decrease the transcriptional efficiency by 68% compared with the C-allele, down regulating E-cadherin expression [40]. Wu and colleagues [41] suggested that individuals who have inherited two copies of the A-allele that reduce transcription of *CDH1* may have a decreased risk of developing gastric cancer in a Taiwanese population. However, no consistent data has been reported about the association between the -160C/A *CDH1* sequence variant and gastric cancer. In a case-control study performed in an Italian population this variant was associated with an increased susceptibility to diffuse gastric cancer. The frequency of the -160A allele was significantly higher ($P<0.005$) in 53 diffuse gastric cancer cases compared to 70 matched controls. The odds ratio associated with the A-allele was 2.27 for CA-heterozygotes (95% CI 1.16-4.44) and 7.84 for AA-homozygotes (95% CI 2.89-21.24) [42]. However, these results were not confirmed in a large series of gastric cancer patients and control populations from Portugal,

Table 5. Polymorphisms identified in CDH1 in gastric cancer probands and normal controls reported to date

Sequence variant	Gene location	Codon	Effect	% patients	% controls	Reference
-71C>G	Promoter		Unknown	1/13 (7.7%)	2/51 (3.9%)	Avizienyte et al, 2000 [87]
-160C>A	Promoter		See text	17/32 (53.1%)	63/114 (55.3%)	Oliveira et al, 2002 [32]
				2/5 (40%)	38/94 (40.4%)	Humar et al, 2002 [89]
				7/28 (25%)	32/142 (22.5%)	Shin et al, 2004 [39]
				31/87 (35.6%)	18/50 (36%)	Wang et al, 2003 [27]
-347G>GA	Promoter		See text	12/28 (42.9%)	39/142 (27.5%)	Shin et al, 2004 [39]
48+6T>C	Intron 1		Unknown	5/13 (38%)	18/51 (35%)	Avizienyte et al, 2000 [87]
				11/28 (39.3%)	27/100 (27%)	Oliveira et al, 2002 [32]
				1/10 (10%)	75/350 (21.4%)	Humar et al, 2002 [89]
				5/17 (29.4%)	nd	Yabuta et al, 2002 [26]
531+10G>C	Intron 4		Unknown	2/34 (5.9%)	nd	Oliveira et al, 2002 [32]
				ns	nd	Guilford et al, 1999 [12]
				ns	nd	Gayther et al, 1998 [10]
532-18C>T	Intron 4		Unknown	2/66 (3.0%)	0/100 (0%)	Suriano and Oliveira et al, 2003 [33]
				2/34 (5.9%)	1/50 (2.0%)	Keller et al, 2004 [29]
918C>T	Exon 7	306	Silent	1/34 (2.9%)	nd	Oliveira et al, 2002 [32]
1029C>G	Exon 8	343	Silent	1/34 (2.9%)	nd	Oliveira et al, 2002 [32]
1774G>A	Exon 12	592	A592T	1/34 (2.9%)	1/50 (2.0%)	Keller et al, 2004 [29]
1849G>A	Exon 12	617	A617T	2/66 (3%)	2/193 (1%)	Suriano and Oliveira et al, 2004 [33]
1896C>T	Exon 12	632	Silent	1/34 (2.9%)	5/100 (5%)	Oliveira et al, 2002 [32]
				ns	nd	Gayther et al, 1998 [10]
1937-13T>C	Intron 12		Unknown	2/27 (7.4%)	25/100 (25%)	Oliveira et al, 2002 [32]
				ns	nd	Guilford et al, 1998, 1999 [9, 12]
1937-27T>G	Intron 12		Unknown	ns	nd	Guilford et al, 1999 [12]
2076C>T	Exon 13	692	Silent	8/13 (61.5%)	nd	Avizienyte et al, 2000 [87]
				15/27 (55.6%)	29/100 (59.0%)	Oliveira et al, 2002 [32]
				1/5 (20%)	nd	Richards et al, 1999 [11]
				7/16 (43.8%)	nd	Iida et al, 1999 [86]
				ns	nd	Guilford et al, 1998, 1999 [9, 12]
				ns	nd	Gayther et al, 1998 [10]
				ns	ns	Yabuta et al, 2002 [26]
				82/87 (94.3%)	48/50 (96%)	Wang et al, 2003 [27]
2253C>T	Exon 14	751	Silent	ns	ns	Yabuta et al, 2002 [26]
2292C>T	Exon 14	764	Silent	1/34 (2.9%)	nd	Oliveira et al, 2002 [32]
2634C>T	Exon 16	878	Silent	1/34 (2.9%)	nd	Oliveira et al, 2002 [32]

nd, Not done; ns, Not specified.

Canada and Germany who have found no significant evidence for an association between stomach cancer and the -160C/A polymorphism in the promoter of *CDH1*. In this report a total of 899 individuals (433 patients and 466 controls) were analysed. The genotype frequencies did not differ significantly between cases and controls, and the genotype-specific risks were not significantly different from unity, with an odds ratio for heterozygotes compared with the common homozygote of 1.3 (95% CI 0.98-1.8) and 1.2 (0.68-2.0) for rare homozygotes compared with common homozygotes [43].

In summary, it is mandatory to clarify the functional

Table 6. Candidate genes analysed in gastric cancer families

Candidate gene	No of families analysed	Germline mutations	Observations	Reference
TP53	66	471C>G (FGC) 847C>T (FDGC)	Family reclassified as Li-Fraumeni Highly conserved residue (Arg 283)	Oliveira et al, in press [30] Keller et al, 2004 [29]
SMAD4	32	0	Probably not relevant for familial gastric cancer	Oliveira et al, in press [30]
Caspase10	32	0	Probably not relevant for familial gastric cancer	Oliveira et al, in press [30]
RUNX3	34	0	Probably not relevant for familial gastric cancer	Keller et al, 2004 [29]
HPP1	34	0	Probably not relevant for familial gastric cancer	Keller et al, 2004 [29]

relevance of the A allele in vivo and to disclose the association of the A/A genotype with GC in larger epidemiologic studies.

Other cancers in HDGC families

In the *CDH1* positive families, family members show other types of malignancy besides diffuse gastric cancer. Breast, colon (namely signet ring cell cancer of the colon), prostate and ovarian carcinomas have been shown to occur in families carrying *CDH1* germline mutations suggesting that non-gastric malignancies can be associated with HDGC [21, 31].

Importantly, breast carcinoma, in particular of the lobular type, has been associated to a positive history of gastric carcinoma [44]. There was reported a gastric cancer patient carrying a germline mutation of *CDH1* who had a mother affected with bilateral breast carcinoma at the age of 49 [29]. An overrepresentation of this tumour type in families with *E-cadherin* germline mutations has been demonstrated [45]. In a recent study, 17 cases of breast cancer were found in families carrying *CDH1* germline mutations, three of which were histologically confirmed as lobular breast carcinomas. This data highlights the need for screening of *CDH1* germline mutations in families with both types of malignancy, diffuse gastric carcinoma and lobular breast carcinoma occurring in the same family.

Familial Gastric Cancer and genes involved in other inherited syndromes

Gastric cancer might also be seen as part of the tumour spectrum in other inherited cancer predisposition syndromes. In particular, gastric cancer has been identified as part of the HNPCC syndrome [46]. As a consequence, the tumours of patients with germline MMR deficiency exhibit a particular phenotype called MSI-H, characterised by a global instability phenomenon affecting microsatellite repetitive sequences [47, 48]. The MSI-H phenotype has been extensively used to pre-screen tumours in cases in which patients should be analysed for h*MLH1* and h*MSH2* [47]. Recently, two tumours from familial gastric cancer probands were detected with MSI-H (one with HDGC and the other with familial gastric cancer). In these two probands germline mutations in h*MLH1* and h*MSH2* were excluded, though other mismatch repair genes may be involved [30].

Gastric cancer has also been recognised as a component of other hereditary cancer syndromes, such as the Li-Fraumeni syndrome [49]. Most of the cases harbouring germline mutations of the *p53* gene have been found in approximately 70% of the families with Li-Fraumeni syndrome. Recently, two gastric cancer families with *p53* germline mutations were identified. One mutation was previously described in a Li-Fraumeni kindred and the other was localised in a highly conserved region of *p53* [29, 30] (Table 6). In these gastric cancer families with *p53* germline mutations, gastric, liver, pancreatic, colon cancers and leukaemia occurred in different members of the families [29, 30]. The presence of *p53* germline mutations in families with a predominance of gastric cancer strengthens the need for *p53* mutation screening in families with aggregations of gastric cancer and no CDH1 mutations.

In addition to HNPCC and Li-Fraumeni syndrome, stomach cancer can also occur in breast and ovarian cancer families. Recently, twenty nine families harbouring gastric and breast malignancies were screened for germline mutations in *BRCA2* and in six of the 29 (20.7%), three frameshift mutations and three missense mutations were identified [50]. Moreover, a *BRCA2* mutation was found in eight of 34 women with ovarian cancer and a family history of stomach cancer [51]. In gastric cancer families with an excess of breast and ovarian tumours, lacking *CDH1*, *p53* or MSI-H tumour phenotype, *BRCA2* is likely to be a candidate gene.

Other candidate genes in Familial Gastric Cancer

In kindreds negative for *CDH1* or *p53* germline mutations, other genes are probably involved. We will address *RUNX3*, *HPP1*, *Caspase-10* and *SMAD4*, which have been shown to be involved in gastric cancer development (mutated in sporadic gastric carcinoma or associated with gastric cancer phenotype in knockout models).

Putative tumour suppressor genes, which are commonly inactivated in sporadic gastric cancers, could also represent good candidate susceptibility genes to familial gastric cancer. *RUNX3*, which belongs to the family of runt domain transcription factors, as well as *HPP1*, encoding a cell surface receptor, which is suggested to play multiple roles in cell growth, maturation and adhesion, have recently been shown to be inactivated by promoter hypermethylation at a high frequency in gastric cancer [52, 53]. Moreover, in the Runx3/Pebp2alphaC null mouse gastric mucosa exhibits hyperplasias due to stimulated proliferation and suppressed apoptosis in epithelial cells. Gastric cancer families that were screened for *RUNX3* and *HPP1*, do not show germline mutations in both genes, suggesting that *RUNX3* and *HPP1* are not important alternative gastric cancer predisposition genes [29]. In 3% of sporadic gastric carcinomas alterations of caspase-10 were described [54]. In vitro expression studies have shown that cells carrying caspase-10 mutations harbour impaired caspase-10-mediated apoptosis, suggesting that somatic alterations of the caspase-10 gene might contribute to the pathogenesis of gastric cancers through the loss of their apoptotic function [54]. Germline mutations in caspase-10 were recently screened in families with gastric cancer and early-onset gastric carcinoma patients, but only a high frequency of sequence variants was found. All variants showed similar frequencies in cases (gastric cancer probands) and in control populations demonstrating its polymorphic nature [30].

In knockout studies *SMAD4* heterozygous mice revealed the presence of *foci* of signet ring carcinoma cells in the stomach [55]. Germline mutations in the *SMAD4* gene were described in a minority of hereditary juvenile polyposis (JPS) [56, 57]. The tumour suppressor gene, *SMAD4*, is a transcription activator that binds specific DNA sequences and whose nuclear localisation is induced after exposure to TGFβ. This gene was searched for germline mutations in gastric cancer families but only sequence variants were found. These sequence variants were either silent or intronic alterations that were present with the same frequency in normal controls pointing to its polymorphic nature [30].

In summary, *RUNX3*, *HPP1*, *Caspase-10* and *SMAD4* can be ruled out as major gastric cancer predisposition genes in families with an excess of gastric carcinoma (see Table 6 for details).

Genetic counselling in HDGC

The IGCLC recommends pre- and post-test genetic counselling for families that either meet or exceed the minimum requirements for HDGC [8].

Testing of asymptomatic at-risk adults for HDGC is available only after an affected family member has been tested and a mutation found. Testing of an asymptomatic at-risk individual is considered predictive testing, not diagnostic testing. Lynch et al [58] describe the genetic counselling process they followed with a large kindred with HDGC. Relevant issues should be discussed with family members seeking predictive testing for HDGC. Discussion should include: (1) the genetics of cancer development and HDGC; (2) the individual's knowledge of HDGC; (3) the individual's reasons for requesting the test; (4) the individual's understanding of the risk for having inherited the mutation based on a family history of HDGC; (5) availability of molecular genetic testing; (6) cancer risk if the individual has inherited the mutation; (7) recommendations for cancer screening and prophylactic surgery; and (8) the possible social impact of positive and negative test results.

Genetic testing in children has always been a controversial issue. Since there have been reports of patients diagnosed with HDGC under the age of 18, it has been suggested that genetic testing in children may be beneficial [8]. Overall, a request from parents for testing of asymptomatic at-risk children requires sensitivity and understanding and thorough rigorous counselling for both the parents and child.

Requests for prenatal testing for conditions such as HDGC that do not affect intellect and have some available treatment are uncommon. Differences in perspective may exist among medical professionals and in families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. Although most centres would consider decisions about prenatal testing to be the choice of the parents, careful discussion of these issues is appropriate.

Helicobacter pylori infection and Familial Gastric Cancer

Among the possible causes of familial aggregation of gastric cancer, exposure to similar environmental factors

such as *H. pylori* infection may contribute to a higher number of affected individuals within the same family.

H. pylori is one of the most common chronic infections in a man, and once acquired early in childhood and if left untreated, persists for the host's lifetime [59]. Risk factors for *H. pylori* acquisition include a low socioeconomic status, household crowding, country of origin and ethnicity, and transmission occurs from person to person [60]. Intrafamilial clustering of the infection also reinforces the importance of person-to-person transmission [61].

A large number of studies provided evidence for the aetiological role of *H. pylori* in gastric carcinoma, and the infection significantly increases the risk of developing both subtypes of gastric carcinoma [62-65]. Despite the well established role of *H. pylori* as a risk factor for gastric cancer, the mechanism of carcinogenesis is still not very clear. The first consequence of *H. pylori* infection is the induction of chronic superficial gastritis. The initiation and promotion of gastric neoplasia may occur via disruption of the epithelial cell proliferation/apoptosis balance and direct damage to host-cell DNA through the synthesis of reactive oxygen species [66, 67].

Nevertheless, only a small fraction of infected individuals develop gastric cancer. This probably depends on a combination of factors, including variation in bacterial pathogenicity. *H. pylori* is genomically diverse and strain differences in virulence factors, including the *cag* pathogenicity island and the vacuolating cytotoxin, have an important role in the development of gastric carcinoma [68-70].

Within the context of familial gastric cancer, it has been shown that first degree relatives of gastric cancer patients have an increased prevalence of *H. pylori* infection [71-73]. Furthermore, *H. pylori*-infected first degree relatives of gastric cancer patients have an increased prevalence of histological and physiological preneoplastic changes, such as atrophic gastritis, intestinal metaplasia and high levels of hypochlorhydria [71, 74].

Although it has not been proven that the eradication of *H. pylori* will result in protection against gastric cancer in first degree relatives of gastric cancer patients, this group is at a significantly higher risk than the general population [72, 74, 75]. Therefore, international consensus guidelines strongly recommended *H. pylori* eradication in first degree relatives of gastric cancer patients [75].

In summary, although familial aggregation of gastric cancer may be mediated by familial clustering of *H. pylori* infection [61], the infection alone cannot explain all of the family aggregation of gastric cancer. Additional genetic and environmental risk factors are likely to contribute to this finding.

Low penetrance genes and genetic susceptibility to gastric cancer

The low frequency of germline mutations in high penetrance genes in familial gastric cancer may be related to an increased susceptibility of these patients to gastric cancer due to low penetrance predisposing genes in association with environmental factors. Patients infected with *H. pylori* are at an increased risk of developing gastric carcinoma [62]. The risk of developing this type of tumour relates to the physiological and histological changes that *H. pylori* infection induces in the stomach [76, 77]. Although there is evidence showing that *H. pylori* infection plays a crucial role in the pathogenesis of gastric carcinoma, a striking difference exists between the number of infected individuals and the number that go on to develop malignancy. Hence, progression towards disease is likely to depend on the combined effects of bacterial pathogenicity, host susceptibility and environmental factors.

The *IL1B-511**T and *IL1RN**2 alleles – which are putatively associated with increased levels of IL1β production [78, 79] – and the *TNFA*-308*A allele – which is thought to increase the production of TNFα [80] – have been found to confer an increased risk of development of gastric carcinoma [69, 81, 82]. The combined effect of pro-inflammatory host genetic polymorphisms in the *IL1B*, *IL1RN* and *TNFA* genes in the risk of gastric carcinoma development has also been investigated. For gastric carcinoma the odds of developing disease increased with the number of high-risk genotypes. Individuals carrying the three high-risk genotypes are at an increased risk of gastric carcinoma with an OR of 9.7 (95% CI 2.6-36.0) [83]. Very similar findings were also reported by El-Omar and colleagues [84].

Results on record support the hypothesis that the extent of gastric mucosal injury may be related to *H. pylori* strain differences, inflammatory responses governed by host genetics, and interactions between host and bacterial determinants. The combination of these factors, favouring a set of responses with higher magnitude, can eventually result in hypochlorhydria, corpus atrophy, and an increased risk of gastric carcinoma. In this context, the *IL1B*, *IL1RN* and *TNFA* genes would play a role in gastric carcinogenesis as low penetrance genes.

In conclusion

Sporadic diffuse gastric cancer cases harbour somatic mutations within the *CDH1* gene of the truncating and missense type, clustered in exons seven to nine. Similarly, approximately 40% of the families that fulfil the criteria

for HDGC show germline mutations of the same gene, ~ 80% of which are of the truncating type and evenly distributed along the gene. In ~20% of cases, germline *CDH1* missense mutations were found and their functional significance was determined by functional assays using an in vitro cell model system. *p53* was found to be mutated in families with an excess of gastric cancer and negative for *CDH1* germline mutations indicating the need of *p53* screen in these types of families. These *p53* germline mutation carriers should have a distinct clinical follow-up. It is of great importance to perform an early and comprehensive screening for *CDH1* mutations in families at an increased risk of developing diffuse gastric cancer, to allow adequate genetic counselling in these families. Efforts must be made to disclose the genetic basis underlying HDGC in families that lack *CDH1* mutations. Familial aggregation of gastric cancer, in the absence of mutations in high penetrance genes, may be in part explained by familial clustering of *H. pylori* infection in combination with an increased host susceptibility.

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