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Advances in Brief

Identification of Germ-Line *E-cadherin* Mutations in Gastric Cancer Families of European Origin[1]

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Abstract

E-cadherin **germ-line mutations have recently been described as a molecular basis for early-onset familial gastric cancer in Maori kindred. We screened 18 gastric cancer families of European origin for germ-line mutations to determine the proportion in which *E-cadherin* mutations occur and the clinical characteristics of the affected families. Truncating mutations were identified in three kindred with familial diffuse gastric cancer. In these families, the age of onset of gastric cancer was variable, the penetrance was incomplete, and one kindred contained individuals with cancers at other sites. Here, we show that a proportion of diffuse gastric cancer families of European origin have germ-line *E-cadherin* mutations; however, these mutations are absent in intestinal gastric cancer families.**

Introduction

Stomach cancer ranks second in the global cancer burden, although a universally declining trend in both sexes has been noted (1). In terms of population-attributable risk, ~8% of gastric cancers in Italy show familial aggregation (2). Earlier studies claimed a familial component only for diffuse gastric carcinoma (3), but more recent reports have found similar proportions of intestinal gastric cancer patients with a positive family history (4, 5). Carriers of *hMSH2* mutations from hereditary nonpolyposis colorectal carcinoma families have a significantly increased risk of stomach cancer (relative risk of 19.3; Ref. 6). However, germ-line mutations of mismatch repair genes are rare in site-specific gastric cancer families (7). A single report of gastric carcinoma in an extended Li-Fraumeni kindred has been published (8). The report of germ-line *E-cadherin* mutations in three Maori kindred with early-onset diffuse gastric cancer represents the first description of a clear molecular basis for familial gastric cancer (9). The importance of this homophilic cell adhesion molecule in tumorigenesis had been established previously with the findings of somatic *E-cadherin* mutations in diffuse gastric cancers (10), lobular breast cancers (11), and gynecological carcinomas (12); the silencing of the *E-cadherin* gene in human carcinomas (13); and the causal role of *E-cadherin* in the transition from adenoma to carcinoma in a mouse model of tumor progression (14). We therefore screened for the presence of germ-line *E-cadherin* mutations in kindred of European origin with familial gastric cancer.

Materials and Methods

Families. Material was collected from 18 gastric cancer families from England, Italy, and Portugal, as well as from families of European origin in the United States and Canada. These families were divided into two groups. The first group was composed of 10 kindred with the following criteria: an index case with diffuse-type gastric carcinoma and at least two other first-degree relatives with confirmed gastric cancer. In seven of these families, there was at least one case diagnosed before the age of 50 years, and in six families for which material was available from multiple cases, pathology confirmed they were diffuse-type gastric carcinoma. The second group included eight kindred with the following criteria: an index case with intestinal-type gastric carcinoma and at least two other first-degree relatives with gastric cancer.

Genomic DNA was isolated using standard methods (15) either from peripheral blood or from frozen normal gastric mucosa collected away from tumor margins. In a few cases in which no fresh material was available, DNA was isolated from paraffin-embedded tissue using a previously described protocol (16).

SSCP[4] and HA methods. The 16 exons of the *E-cadherin* gene were amplified in fragments of 200–400 bp in 34 samples from 18 families. PCR primers were as reported previously (11), except for exons 4 and 5, which were amplified using new primers (EC-EX4F, 5′-CTTGTTCCTCATCTTCTTTC-3′; EC-EX4R, 5′-CTTGGCCAGTGATGCTGTAG-3′; EC-EX5F, 5′-ATTTGGCAGAGAAGTACCAA-3′; and EC-EX5R, 5′-CCCATCACTTCTCCTTAGCA-3′). Genomic DNA (25–100 ng) was amplified under standard PCR conditions (15). Reaction products were diluted 1:1 with a denaturing buffer (formamide with 0.025% xylene cyanol and 0.025% bromophenol blue) and heated to 99°C for 10 min prior to loading onto 0.8× mutation detection enhancement (Flowgen) gels, both with and without 10% glycerol. Gels were run for 12–18 h at 10°C and stained with silver nitrate (15).

Direct DNA Sequencing. The entire *E-cadherin* coding sequence and intron-exon boundaries in 13 affected individuals from 13 different families and any PCR fragments in which a SSCP/HA variant was detected were sequenced. PCR products were purified from 2% low melting point agarose (Life Technologies, Inc.) using Wizard purification columns (Promega). Purified products were sequenced on an ABI373 automated sequencer using TaqFS cycle sequencing (Applied Biosystems/Perkin-Elmer Corp.). All samples in which a SSCP/HA variant or a mutation had been detected were sequenced in both the forward and reverse strands. Mutations were confirmed in an independent PCR product, and all DNA samples available in the respective families were assayed for its presence.

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[4] The abbreviations used are: SSCP, single-strand conformational polymorphism; HA, heteroduplex analysis.

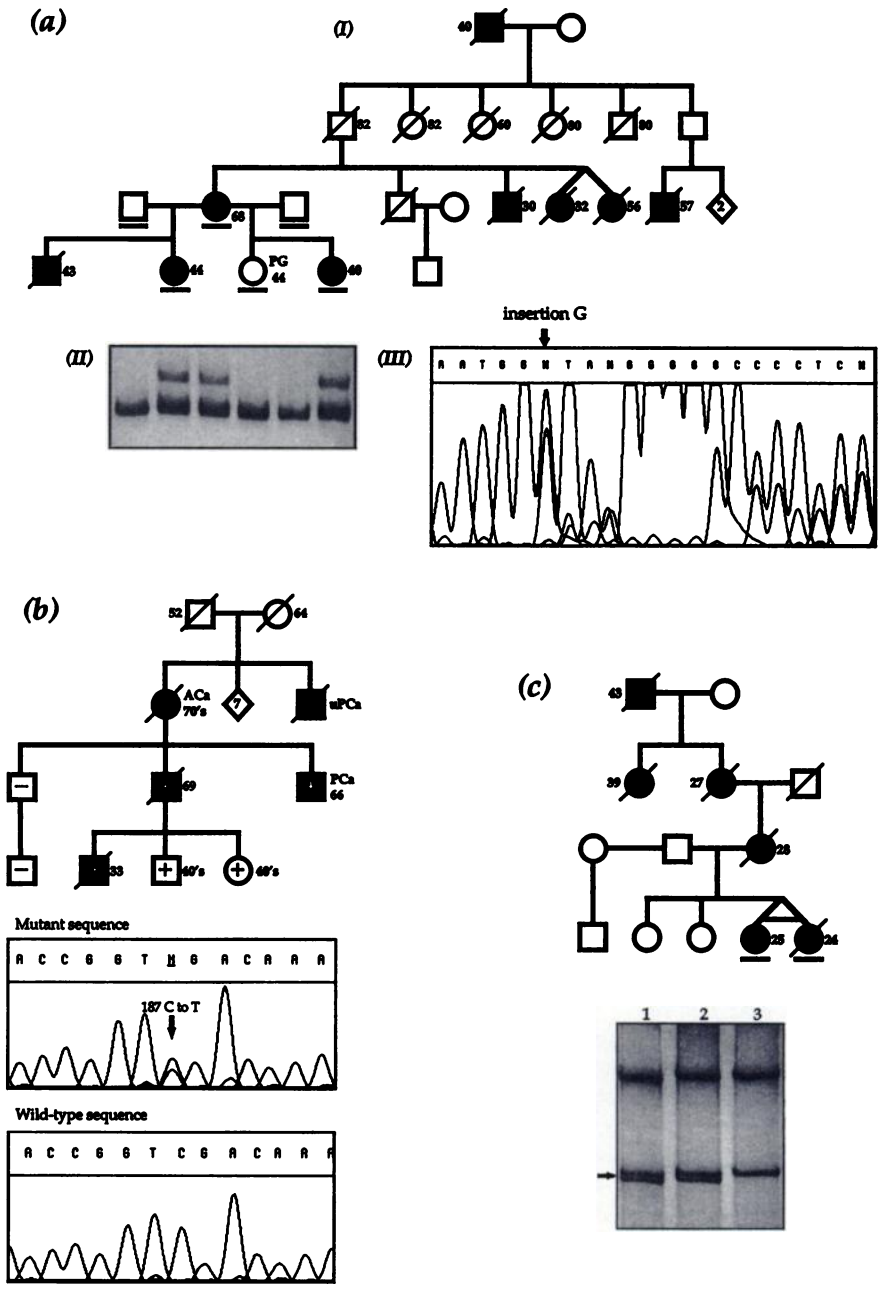

Fig. 1. *a*, (*I*) pedigree of family B contains nine cases of gastric cancer (● and ■) in three different generations. *PG*, individual has undergone prophylactic gastrectomy. *II*, HA of exon 11 in DNA from six different individuals in the family. Lanes are aligned with the corresponding individual in the pedigree. All three affected individuals have a heteroduplex DNA band in addition to the wild-type homoduplex band. *III*, sequence analysis of the three affected individuals demonstrates a frameshift mutation at nucleotide 1711 (1711insG) that is not present in unaffected family members. *b*, pedigree of family C contains two cases of diffuse gastric cancer (■), one case of "abdominal" cancer (*ACa*; gastric *versus* pancreatic), one confirmed case of prostate cancer (*PCa*), and one unconfirmed case of prostate cancer (*uPCa*; *shaded symbols*). The mutation is a substitution of C for a T at nucleotide 187, which creates a nonsense mutation (R63X) at amino acid 63. This mutation is present in all individuals marked + (which includes two gastric cancer cases, one prostate cancer case, and two unaffected individuals) but is not present in two unaffected individuals marked −. An example of a mutant sequence from an affected individual is compared with a wild-type sequence. *c*, pedigree of family D contains six cases of gastric cancer. The mutation in this family (1792 C to T; R598X) creates a SSCP shift (*arrow*) that is present in DNA amplified from both affected identical twins (*Lanes 1* and *2*) but not in DNA amplified from controls (*e.g.*, *Lane 3*). Symbols for *a*, *b*, and *c*: □ and ■, males; ○ and ●, females; ◇, either sex; *symbols with a slash through them*, deceased; *underlined symbols*, individuals from which DNA was available for analysis. *Numbers*, ages at diagnosis. Some details in these pedigrees have been modified to protect the privacy of individual family members; none of these modifications alters the mode of inheritance of the disease.

RT-PCR. RNA was isolated using Trizol reagent (Life Technologies, Inc.) following the manufacture's instructions. Two to 10 μg of total RNA were used to synthesize first-strand cDNA using Superscript II (Life Technologies, Inc.) and following the manufacturer's instructions. The *E-cadherin* transcript was amplified in four overlapping fragments. Primers used to amplify exons 7–10 and 9–11 from cDNA are as described previously (10), whereas new primers were designed to amplify exons 1–7 (EC-EX1F, 5′-ATGGGCCCTTGGAGCCGC-3′; and EC-EX7R, 5′-TGGCACATCATCGTCCGCGT-3′; and EC-EX16R,5′-CTAGTCGTCCTCGCCGCC-3′) and exons 11–16 (EC-EX11F, 5′-GACAGGGAGGATTTTGAGCAC-3′). Amplified products were size-fractionated in 2–3% agarose gels to screen for transcripts of different sizes.

Results and Discussion

We screened the entire coding region of the *E-cadherin* gene, including splice sites, using a combination of nonradioactive SSCP, HA, and sequencing, in at least 1 affected individual from each of 18 kindred with familial gastric cancer.

E-cadherin germ-line mutations were detected in 3 of 10 families with diffuse-type gastric cancer (Table 1). In family B (Fig. 1*a*, *I*), whose pedigree has been reported previously (17), a frameshift insertion leading to truncation of the gene product was detected by HA in three affected cases (Fig. 1*a*, *II*), all with histopathological confirmation of the diagnosis. Sequencing showed a G insertion at nucleotide 1711 in exon 11 (Fig. 1*a*, *III*), which produces a frameshift that creates a downstream stop at codon 587. HA and sequencing confirmed the cosegregation of this mutation with the disease and its absence in three unaffected relatives (one of which had a prophylactic gastrectomy that confirmed absence of gastric carcinoma). In family C, the mutation was a transition at nucleotide 187 (C to T) in exon 3, resulting in a stop codon (R63X; Fig. 1*b*). This mutation was present in the germ line of two individuals affected by diffuse gastric cancer, in the germ line of one individual with prostate cancer diagnosed in his 60s, and in the germ line of two asymptomatic individuals in their 40s. All of the individuals

Table 1 *Germ-line E-cadherin mutations in diffuse gastric cancer families*

Family	No. of gastric cancers	Mean age[a] (yr)	Age range[a] (yr)	Cancers at other sites[b]	*E-cadherin* mutation[c]	Exon	Effect
A	8 (+5 polyps)	48.3 (45)	26–68 (23–58)		WT[e]		
B	9	45.5	30–68		1711 insG	11	fs[f] (587 stop)
C	2 (4)[d]	51	33–69	Pro, Co, En, Br	187 C to T	3	R63X (stop)
D	6	31	23–43		1792 C to T	12	R598X (stop)
E	3	32.3	24–38		WT		
F	5	60.6	45–70	Gy, Lu, Larx, Co	WT		
G	4	68	59–80	Larx, Co, Br, En	WT		
H	4	61.6	46–87		WT		
I	3	58.3	56–62	Lym	WT		
J	3	66.3	64–70		WT		

[a] Mean age and age range of gastric cancer cases (gastric polyp cases in family A).
[b] Pro, prostate cancer; Co, colon cancer; En, endometrial cancer; Br, breast cancer; Gy, unspecified gynecological cancer; Lu, lung cancer; Larx, laryngeal cancer; Lym, non-Hodgkin's lymphoma.
[c] The *E-cadherin* nucleotide positions in reference to the A of the start codon of Genbank accession no. Z13009.
[d] Two cases of confirmed gastric cancer and two more cases of unconfirmed gastric cancer.
[e] WT, wild-type
[f] fs, frameshift.

with the mutation share the same haplotype at 16q22.1 (data not shown). In family D, the mutation was a transition at nucleotide 1792 (C to T) in exon 12 resulting in a stop codon (R598X; Fig. 1*c*). The mutation was present in two identical twins with diffuse gastric cancer. No other samples from this family were available.

In the remaining seven kindred with familial diffuse gastric cancer (Table 1), no *E-cadherin* mutations were detected by sequencing (five families), by SSCP/HA (families H and J), or by RT-PCR analysis of the *E-cadherin* transcript (families G, I, and J). In family A, a large kindred with autosomal dominant familial gastric polyposis/diffuse gastric cancer that we have described previously (18), we were able to exclude the *E-cadherin* locus by multipoint linkage analysis (data not shown). This observation suggests that at least one more susceptibility locus for familial gastric cancer remains to be identified.

No mutations were detected in the eight families with intestinal gastric cancer. Five intestinal gastric cancer families were site specific; three other contained cancers at other sites. This finding, together with the reported absence of somatic *E-cadherin* mutations in sporadic intestinal gastric cancers (10, 19, 20), shows that *E-cadherin* is rarely involved in gastric carcinomas of this type and suggests that distinct tumorigenic pathways are associated with the two different histopathological types of gastric cancer.

Polymorphisms in *E-cadherin* that have been reported previously (12) were also detected in several different individuals: CAC (His) to CAT (His) at codon 632 in exon 12, GCC (Ala) to GCT (Ala) at codon 692 in exon 13, and G to C in nucleotide 10 of intron 4.

In conclusion, this study confirms that germ-line *E-cadherin* mutations occur in a proportion of diffuse gastric cancer families of European origin and establishes a role for *E-cadherin* in susceptibility to gastric cancer in non-Maori populations. Analysis of the families reported here together with the three Maori kindred (9) reveals that the age of onset of gastric cancer is variable (from 14 to 69 years old), the mean age of affected in each involved family ranges from 31 to 51 years, and the penetrance is incomplete (obligatory carriers in their 80s and 90s remain unaffected). The finding of germ-line *E-cadherin* mutations in an individual with prostate cancer (family C) and in two individuals with colon cancer (in one of the Maori kindred; Ref. 9) raises the possibility that the gene is associated with susceptibility to other cancers. These data suggest that the management of families affected with gastric cancer is likely to be complex. A recommendation for genetic screening should be considered in families containing at least one case of diffuse gastric cancer before the age of 50 and at least one other first- or second-degree relative affected by diffuse gastric cancer. The option of prophylactic gastrectomy in mutant gene carriers will need careful consideration and discussion, particularly because the estimates of gene penetrance and lifetime cancer risk are bound to be refined with the description of further families. Further questions that need to be addressed include determining the proportion of germ-line *E-cadherin* mutations among unselected diffuse gastric cancer cases and the identification of genetic and/or environmental modifiers (for example, *Helicobacter pylori* infection) that might account for the variable age of onset and incomplete penetrance.

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