Founder and Recurrent *CDH1* Mutations in Families With Hereditary Diffuse Gastric Cancer

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Context Hereditary diffuse gastric cancer is caused by germline mutations in the epithelial cadherin (*CDH1*) gene and is characterized by an increased risk for diffuse gastric cancer and lobular breast cancer.

Objective To determine whether recurring germline *CDH1* mutations occurred due to independent mutational events or common ancestry.

Design, Setting, and Patients Thirty-eight families diagnosed clinically with hereditary diffuse gastric cancer were accrued between November 2004 and January 2006 and were analyzed for *CDH1* mutations as part of an ongoing study at the British Columbia Cancer Agency. Twenty-six families had at least 2 gastric cancer cases with 1 case of diffuse gastric cancer in a person younger than 50 years; 12 families had either a single case of diffuse gastric cancer diagnosed in a person younger than 35 years or multiple cases of diffuse gastric cancer diagnosed in persons older than 50 years.

Main Outcome Measures Classification of family members as carriers or noncarriers of *CDH1* mutations. Haplotype analysis to assess recurring mutations for common ancestry was performed on 7 families from this study and 7 previously reported families with the same mutations.

Results Thirteen mutations (6 novel) were identified in 15 of the 38 families (40% detection rate). The 1137G>A splicing mutation and the 1901C>T (A634V) missense/splicing mutation occurred on common haplotypes in 2 families but on different haplotypes in a third family. The 2195G>A (R732Q) missense/splicing mutation occurred in 2 families on different haplotypes. The 2064-2065delTG mutation occurred on a common haplotype in 2 families. Two families from this study plus 2 additional families carrying the novel 2398delC mutation shared a common haplotype, suggesting a founder effect. All 4 families originate from the southeast coast of Newfoundland. Due to concentrations of lobular breast cancer cases, 2 branches of this family had been diagnosed as having hereditary breast cancer and were tested for *BRCA* mutations. Within these 4 families, the cumulative risk by age 75 years in mutation carriers for clinically detected gastric cancer was 40% (95% confidence interval [CI], 12%-91%) for males and 63% (95% CI, 19%-99%) for females and the risk for breast cancer in female mutation carriers was 52% (95% CI, 29%-94%).

Conclusions Recurrent *CDH1* mutations in families with hereditary diffuse gastric cancer are due to both independent mutational events and common ancestry. The presence of a founder mutation from Newfoundland is strongly supported.

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GASTRIC CANCER IS THE SECOND most common cause of cancer death worldwide.[1] Two major histopathological variants of this cancer have been described: an intestinal type and a diffuse type.[2] A decline in the overall incidence of gastric cancer can be attributed primarily to a decrease of the intestinal variant of gastric cancer with the diffuse type remaining stable or possibly even increasing.[3]

For editorial comment see p 2410.

The histopathologic appearance of diffuse gastric cancer specimens re-

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veals a pattern of isolated, mucin-filled tumor cells within the wall of the stomach.[4] Decreased expression of the epithelial cadherin (*CDH1*) gene (Online Mendelian Inheritance in Man No. 192090) in cases of diffuse gastric cancer may account for morphological differences between intestinal and diffuse variants. Epithelial cadherin is a transmembrane glycoprotein and plays a major role in epithelial architecture, cell adhesion, and cell invasion. *CDH1* was first associated with gastric cancer when somatic mutations were identified in diffuse gastric cancer specimens.[5] Since then, germline mutations in *CDH1* have been found in families with autosomal dominant susceptibility to hereditary diffuse gastric cancer (HDGC).[6-17]

An autosomal dominant cancer susceptibility syndrome, HDGC has an average age of onset of 38 years for clinically detectable diffuse gastric cancer.[7,18] Germline mutations in *CDH1* are found in 30% to 40% of clinically defined families with HDGC from different ethnic backgrounds, predominantly from low-incident populations.[18,19] The *CDH1* mutation spectrum is heterogeneous and includes point mutations, small deletions, and insertions distributed along the entire coding sequence (TABLE 1).[8,20] The identification of *CDH1* mutations offers the opportunity of cancer risk-reduction strategies for unaffected at-risk individuals.[19,21] Along with a risk of diffuse gastric cancer, there is an excess of lobular breast cancer in families with clinically defined HDGC.[8,9,22-24] This is not unexpected because loss of *CDH1* expression is a cardinal feature of lobular breast cancer and HDGC and both somatic *CDH1* mutations and promoter hypermethylation are found frequently in lobular breast cancer but only rarely in infiltrating ductal carcinoma.[25,26]

In this study, mutation status was assessed in 38 families with diffuse gastric cancer. The analysis of the 38 families revealed 13 different *CDH1* mutations in 15 of these families (40% detection rate). Eight of these mutations were classified as recurring; these included 2 mutations (1 novel, 1 previously reported[10]) that were found in more than 1 family in this study and 6 additional mutations that had been previously reported.[8,11,19,27-29] This was unexpected because earlier mutation screenings typically reported only novel mutations in single families. Of the more than 50 published pathogenic germline *CDH1* mutations, only the 187C>T (R63X) (arginine63stop) truncating mutation,[9,30] the 1018A>G (T340A) (threonine340alanine) mutation,[12,31] the 1792C>T (R598X) (arginine598stop) truncating mutation,[9,13,30] the 1901C>T (A364V) (alanine634valine) mutation,[19,27] and the 1003C>T (R335X) (arginine335stop) truncating mutation[9,32] have been found in more than 1 family. The latter was found in 3 families due to independent mutational events as demonstrated by haplotype analysis.[9] In this study, haplotype analysis was used to determine if 5 of the 8 recurrent mutations within this series occurred due to independent mutational events or founder effects. For 4 of these mutations, at least 2 apparently unrelated families shared the mutation and an associated haplotype at the *CDH1* locus. A novel recurrent *CDH1* mutation was initially found in 2 families from the southeast region of Newfoundland and subsequently in 2 additional families, both originating from this same region, implying a founder effect. The implications of these data on the genetic testing and counseling of families with HDGC, as well as the novel opportunities created by the discovery of founder mutations, appear herein.

METHODS

Patient Ascertainment

As part of an ongoing study at the British Columbia Cancer Agency, 38 families diagnosed clinically with HDGC were accrued between November 2004 and January 2006 and were analyzed to estimate the frequency and penetrance of mutations in the *CDH1* gene. When available, ethnicities for families are included (TABLE 2). In cases in which families are of multiple ethnicities, ethnicity is ascribed based on the branch of the family that carries the *CDH1* mutation. Ethnicities were self-reported by probands. Ethnic background is relevant to ascribing founder effects to mutations.

The majority of families met previously published inclusion criteria.[8,9] Six families had a single case of diffuse gastric cancer diagnosed in a person younger than 35 years; 26 families had at least 2 gastric cancer cases with 1 case diagnosed as diffuse gastric cancer in a person younger than 50 years; 5 families had at least 3 gastric cancer cases at any age, 1 of which was confirmed as diffuse gastric cancer; and 1 family had 2 confirmed diffuse gastric cancer cases, both diagnosed in persons older than 50 years. The majority of families were referred to this study through genetics clinics in North America. Pedigrees and medical records were collected by genetic counselors at the site of origin. When available, pathology and other medical reports were reviewed by study investigators for probands and affected family members.

Mutation screening results were reported to the proband (or next of kin if the proband was deceased) with appropriate counseling and psychological support by the referring genetic counselor. Genetic counseling and carrier testing was then offered to other adult family members. When prophylactic gastrectomies were performed, pathology reports were requested; only abnormalities from gastrectomies analyzed according to a previously published protocol were considered.[33]

Investigations were performed after approval by the clinical research ethics board of the University of British Columbia. Informed consent was obtained from each individual, or next of kin in the case of a deceased individual.

Mutation Analysis

Genomic DNA was purified from peripheral blood leukocytes, using the Puregene DNA Purification Kit (Gentra Systems, Minneapolis, Minn) according to the manufacturer's instruc-

tions. The entire coding sequence and flanking intronic portions of the *CDH1* gene were screened by denaturing high-performance liquid chromatography analysis using a 3500HT WAVE DNA fragment analysis system (Transgenomic Inc, San Jose, Calif). The denaturing high-performance liquid chromatography and polymerase chain reaction (PCR) primers, PCR settings, amplicon length, and resolution temperatures for denaturing high-performance liquid chromatography analysis were as described previously.[9] Bidirectional sequencing of purified PCR products (Qiagen, Mississauga, Ontario) was performed using the ABI BigDye Terminator Sequencing Kit version 3.1 (Applied Biosystems, Foster City, Calif) and an ABI Prism 3100 or 310 Genetic Analyzer (Applied Biosystems).

Missense Mutation Analysis

To assess epithelial cadherin function, aggregation and Matrigel invasion assays (BD Biosciences, Mississauga, Ontario) were performed as previously described.[14,19] Briefly, Chinese hamster ovary (CHO) cells, which do not express endogenous *CDH1*, were transfected with constructs expressing wild-type, R749W, or E781D *CDH1*. Aggregation was assessed using an in-

Table 1. *CDH1* Mutations Described to Date*

Exon No.	Nucleotide Range, Base Pair†	Type of Mutation			
		Deletion or Insertion	Splicing	Nonsense	Missense
1	1-48	41delT 45-46insT		3G>C (start site)	
2	49-163	54delC	49-2A>G	59G>A (W20X) 70G>T (E24X)	
3	164-387	377delC 382delC		187C>T (R63X)‡ 190C>T (Q64X) 283C>T (Q95X)‡	185G>T (G62V)
4	388-531		531 + 2T>A		515C>G (P172R)§
5	532-687		532-18C>T§ 687 + 1G>A	586G>T (G196X)	
6	688-832		715G>A (G239R)‡‖		731A>G (D244G) 808T>G (S270A)
7	833-1008		832G>A 1008G>T	1003C>T (R335X)‡	892G>A (A298T)
8	1009-1137	1062delG 1064-1065insT	1134del8, ins5 1137G>A‡ 1137 + 1G>A		1018A>G (T340A)‡ 1118C>T (P373L)
9	1138-1320	1212delC			126T>C (W409R) 1243A>C (I415L) 1285C>T (P429S)
10	1321-1565	1397-1398delTC‡ 1472-1473insA 1476-1477delAG 1488-1494delCGAGGAC	1565 + 1G>T	1507C>T (Q503X)	1460T>C (V487A)
11	1566-1711	1588-1589insC 1610delC 1619-1620insA 1682-1683insA 1710delT 1711-1712insG	1711 + 5G>A		
12	1712-1936	1779-1780insC	1901C>T (A634V)‡‖	1792C>T (R598X)‡ 1913G>A (W638X)	1774G>A (A592T) 1795A>T (T599S) 1849G>A (A617T) 1876T>A (F626V)¶
13	1937-2164	2064-2065delTG‡	2161C>G 2164 + 5G>A	2095C>T (Q699X)	
14	2165-2295	2276delG	2195G>A (R732Q)‡‖		2245C>T (R749W)
15	2296-2439	2310delC 2386-2387insC 2398delC	2295 + 5G>A		2329G>A (D777N) 2343A>T (E781D) 2396C>G (P799R)
16	2440-4654				2194G>A (V832M)

*The signal domain corresponds to nucleotides 1 to 81; the precursor domain corresponds to nucleotides 82 to 460; the extracellular domain corresponds to nucleotides 461 to 2124; the transmembrane domain corresponds to nucleotides 2125 to 2193; and the cytoplasmic domain corresponds to nucleotides 2194 to 2649.
†Numbering is based on *CDH1* cDNA sequence (GenBank NM_004360) with A in start ATG (methionine) corresponding to position 1.
‡Mutation has been reported in more than 1 family.
§Nucleotide changes that have not been confirmed to be pathogenic mutations and may be polymorphisms.
‖Mutations that have been previously reported to be missense mutations, however, their pathogenic effects likely occur via abnormal splicing based on reports by others and our own experimental results.
¶Mutation described in an individual with lobular breast cancer.

verted microscope following incubation of cells in an agar gel at 37°C for 24 to 48 hours. Prior to assessment of invasion, cells were incubated on top of collagen gels for 22 hours at 37°C. Invasion indices are expressed as ratios between the number of invasive cells inside the gel and the total number of cells. Missense mutations were further analyzed using SIFT (Sorting Intolerant From Tolerant) software version 2 (Fred Hutchinson Cancer Research Center, Seattle, Wash), which uses evolutionary conservation to predict the potential effect of amino acid substitution.[34,35]

Splicing Analysis

Splicing effects were predicted using NNSPLICE software version 0.9 (Berkeley Drosophila Genome Project, Splice Site Prediction by Neural Network, Berkeley, Calif) and NetGene2 software (Center for Biological Sequence Analysis, Lyngby, Denmark).[36,37] These programs assign ideal splice site scores of 1 for donor and acceptor sites.

Reverse transcriptase–PCR (RT-PCR) was performed on putative novel splicing and missense/splicing mutations. The RNA was extracted from white blood cells or gastrectomy specimens using Trizol (Invitrogen, Burlington, Ontario) according to the manufacturer's instructions. Using the SuperScript First-Strand Synthesis System (Invitrogen), 1 µg of RNA was reverse transcribed. The PCR amplification was performed with gene-specific primers (EC5F5′-TGGCCAAGGAGCTGACACAC-3′ and EC7R 5′-GATCTTGGCTGAGGATGGTG-3′ for 715 G>; EC11F5′AACTGGCTGGAGATTAATCCG-3′ and EC15R 5; CAATTTCATCGGGATTGGCAG-3′ for 2195G>A and 2164 + 5G>A) using a PTC-200 DNA Engine Thermal Cycler (MJ Research, Waltham, Mass). The PCR products were either cloned into pCR-TOPO vectors (Invitrogen) or purified from 2% agarose gels followed by sequencing as described above.

Missense mutations with potential splicing effects that had not been previously described were subjected to minigene analysis to validate splicing effects predicted *in silico*. Exons 6 and 14 with flanking intronic sequence were amplified using specific primers (MG6F5′-TATTACTCGAGATGCCTGGCCAGGAAGTCATATATT GAT-3′ and MG6R5-ATATAGATATCCCGGGAGTTTGAAAGTAGCCTGGA-3 for exon 6; MG14F5′-TATTACTCGAGCCCACTCCCCATAGCTGGTTAT G and MG14R 5′-ATATAGATATCAATAAGTAGGCTCTCCAAGACACTG-3′ for exon 14) containing either XhoI (forward primer) or EcoRV (reverse primer) linkers and unidirectionally cloned into pSPL3 vectors. Selected constructs were transfected into COS (*Cercopithecus aethiops*) cells with the Lipofectin Reagent (Invitrogen). The RNA was isolated after 24 hours using Trizol (Invitrogen) according to the manufacturer's instructions. One microgram of RNA was reverse transcribed using MMLV (moloney murine leukemia virus) RT (Invitrogen) followed by PCR amplification using vector-specific primers (SD65′-TCTGAGTCACCTGGACAACC-3′ and SA25′-ATCTCAGTGGTATTTGTGAGC-3′). The PCR products were analyzed on a 2% agarose gel and sequenced as described above.

Table 2. Families Identified With *CDH1* Mutations

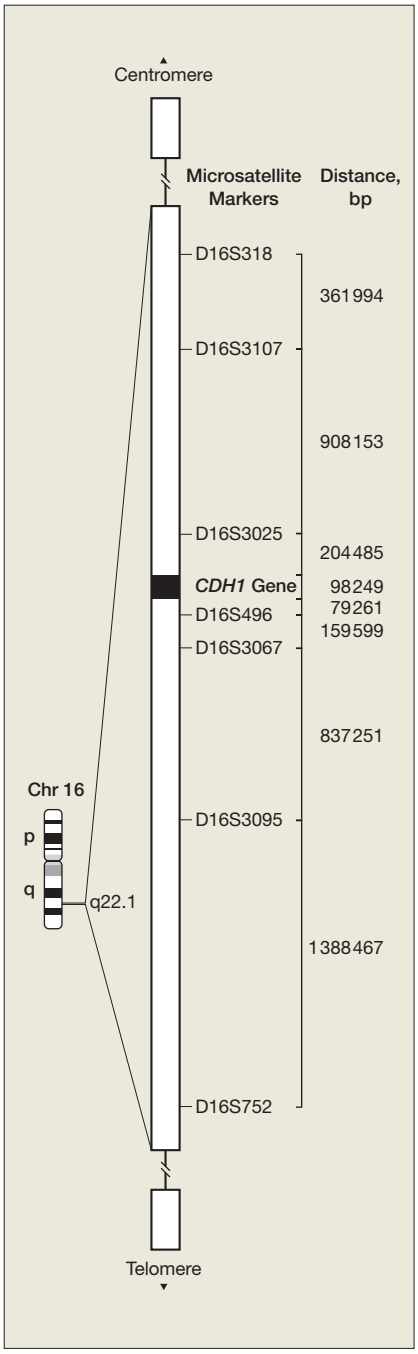
Family	Ethnicity	No. of Gastric Cancer Cases; Diffuse Cancer Cases	Age Range at Gastric Cancer Diagnosis, y	No. of Breast Cancer Cases; Lobular Cancer Cases	Age or Age Range at Breast Cancer Diagnosis, y	Mutation*	Mutation Type	Location	Reference
F1	Unavailable	7; 2	39-76	0		283C>T (Q95X)	Nonsense	Exon 3	11
F2	Filipino	4; 1	29-33	0		715G>A (G239R)	Missense/ splicing	Exon 6	28
F3	Unavailable	7; 1	26-44	1		1137G>A	Splicing	Exon 8	10
F4	Swedish/ Norwegian	3; 2	37-48	0		1137G>A	Splicing	Exon 8	10
F5	English	4; 2	31-82	1	62	1397-1398delTC	Deletion	Exon 10	29
F6	Irish	3; 1	38-52	1	38†	1682-1683insA	Insertion	Exon 11	Novel
F7	English	2; 1	34-45	1	75	1901C>T (A634V)	Missense/ splicing	Exon 12	19, 27
F8	Spanish	10; 1	21-59	0		1913G>A (W638X)	Nonsense	Exon 12	Novel
F9	English/ Scottish	3; 1	37-80	2	65	2064-2065delTG	Deletion	Exon 13	8
F10	Unavailable	3; 1	38-44	0		2164 + 5G>A	Splicing	Intron 13	Novel
F11	English	3; 2†	32-62	4; 1	40-77	2195G>A (R732Q)	Missense/ splicing	Exon 14	8
F12	Columbian	3; 2	36-49	0		2245C>T (R749W)	Missense	Exon 14	Novel
F13	English	2; 2	51-63	1		2343A>T (E781D)	Missense	Exon 15	Novel
F14	Irish/English	17; 2	25-80	13; 3	41-59	2398delC	Deletion	Exon 15	Novel
F15	Irish/English	4; 3	27-48	0		2398delC	Deletion	Exon 15	Novel

*Numbering is based on *CDH1* complementary DNA sequence (GenBank: NM_004360) with the "A" from the start "ATG" (methionine) being equivalent to position 1.
†One individual had both diffuse gastric cancer and lobular breast cancer.

Haplotype Determination

Haplotype analyses were performed using microsatellite markers D16S318, D16S3107, D16S3025, D16S496, D16S3067, D16S3095, and D16S752 surrounding the *CDH1* region (FIGURE 1). Each marker was amplified with PCR using a PTC-200 DNA Engine Thermal Cycler (MJ Research). The PCR products were electrophoresed in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) and analyzed with Genescan version 3.1 software (Applied Biosystems). Primer sequences for microsatellite markers D16S3025, D16S496, D16S3095, and D16S752 have been previously reported.[9] Individual amplification primers for markers D16S318, D16S3107, and D16S3067 are listed in the genome database (http://www.gdb.org).

Figure 1. Positions of Microsatellite Markers on Chromosome 16q22



The 7 microsatellite markers used for haplotype analysis are shown in relation to the *CDH1* gene. Distances in base pairs (bp) between markers are indicated. Chr indicates chromosome.

Penetrance Analysis

Pedigree data from families F14, F15, SF6, and SF7 were used to estimate the penetrance of *CDH1* using a conditional likelihood approach implemented in the Mendel program, as previously described.[38] The conditional likelihood of the pedigree was maximized given the phenotype of the family at ascertainment and the genotype of the index case. The models were parameterized in terms of age-specific log relative risks of gastric and breast cancer compared with background population incidence rates. The cumulative risk of gastric cancer for men and women and the breast cancer risk in women were then estimated from the cumulative incidence estimates. Only cases of clinically detected gastric cancer were considered in this analysis; patients undergoing prophylactic gastrectomy were censored at age of surgery and so occult carcinomas did not contribute to penetrance estimates. To protect the anonymity of the families shown in the pedigrees, unaffected individuals are represented by a sex-neutral symbol (circle within a square). Information about affected individuals is fully displayed. Individuals are not shown in birth order. Complete pedigree data will be made available by the authors upon request for specific research use consistent with protection of participant privacy and prior consent.

RESULTS

Mutation Screening

Mutational analysis of *CDH1* was performed for 38 families. Fifteen of the 38 families were found to have *CDH1* germline mutations (40% detection rate) (Table 2). All mutation carriers were heterozygous for a specific mutation, consistent with autosomal dominant inheritance. Fourteen of the 15 families who tested positive for a mutation had at least 2 gastric cancer cases with 1 case diagnosed as diffuse gastric cancer in a person younger than 50 years (14/26; 54%). One of the 15 families had 2 confirmed diffuse gastric cancer cases, both diagnosed in persons older than 50 years. Thirteen different mutations were found in these 15 families: 6 truncating mutations (2 nonsense, 3 deletions, 1 insertion), 2 splicing mutations, 3 missense/splicing mutations, and 2 missense mutations. Six of the 13 mutations identified herein are novel: 1682-1683insA; 1913G>A (W638X) (tryptophan638stop); 2164+5G>A (splicing); 2245C>T (R749W) (arginine749tryptophan); 2343A>T (E781D) (glutamic acid781aspartic acid); 2398delC. The remaining 7 mutations have been previously reported: 715G>A (G239R) (glycine239arginine, missense/splicing); 283C>T (Q95X) (glutamine95stop); 1137G>A (splicing); 1397-1398delTC; 1901C>T (A634V, missense/splicing); 2064-2065delTG; 2195G>A (R732Q) (arginine732glutamine, missense/splicing).[8,10,11,19,27-29] The 6 truncating mutations were assumed to be deleterious.

To confirm pathogenicity of the 2 novel missense mutations, 2245C>T (R749W) and 2343A>T (E781D), aggregation and invasion were assayed using CHO cells. These are standard methods to assess function of epithelial cadherin missense variants through comparison with wild-type epithelial cadherin, which promotes cell aggregation and inhibits cell invasion.[14,19] Introduction of wild-type *CDH1* into CHO cells resulted in cell aggregation. However, CHO cells

expressing the 2 missense variants failed to produce compact cellular aggregates in soft agar. The CHO cells expressing wild-type *CDH1* had minimal invasion into collagen matrices as expected (mean [SD] invasion index, 0.63% [0.15%]). By contrast, the CHO cells expressing the 2245C>T (R749W) and 2343A>T (E781D) mutations had significantly higher mean (SD) invasion indices of 5.4% (0.15%) and 7.8% (0.36%), respectively ($P<.01$ for both mutations by normal test), demonstrating impaired function of epithelial cadherin. The missense mutation 2245C>T (R749W) is predicted to be pathogenic using SIFT prediction software and this arginine residue is conserved in homologous proteins in cows, rats, mice, dogs, and Chinese soft-shell turtles. The SIFT analysis of the 2343A>T (E781D) mutation suggested that this substitution would be tolerated. However, we believe that this mutation is pathogenic because the functional in vitro results indicate that it affects epithelial cadherin function.

Of the 2 splicing mutations, 1 had been shown previously by RT-PCR to affect splicing (1137G>A).[10] The second (2164 + 5G>A) was predicted by *in silico* analysis to affect splicing at the native donor splice site. This was validated by us through RT-PCR analysis on RNA extracted from white blood cells, which revealed that this mutation caused deletion of exon 13 (228 base pairs).

The 3 mutations classified as missense/splicing have been described previously as pathogenic missense mutations through in vitro functional assays and SIFT analysis.[8,14,19,28] However, further analysis by ourselves or others (in the case of 1901C>T [A634V][39]) indicated that these mutations affect splicing. *In silico* analysis of 715G>A (G239R) and 2195G>A (R732Q) mutations predicted that both of these mutations created new acceptor splice sites. By contrast, similar analysis of the 2 missense mutations (2245C>T [R749W] and 2343A>T [E781D]) did not reveal any splicing effects. Our RT-PCR analysis of the 715G>A (G239R) and 2195G>A (R732Q) missense/splicing mutations using RNA extracted from white blood cells and a gastrectomy specimen, respectively, confirmed the *in silico*–predicted splicing effects. The 715G>A (G239R) mutation caused deletion of the first 29 base pairs from exon 6 and the 2195G>A (R732Q) mutation resulted in complex splicing and deletion of 32 base pairs at the start of exon 14. Previous RT-PCR analysis of the 1901C>T (A634V) missense/splicing mutation demonstrated that this nucleotide substitution causes deletion of the last 37 nucleotides of exon 12 and subsequent frameshift.[39] Novel splicing effects were further validated by minigene analysis, which showed that the 715G>A (G239R) mutation created a preferential splice site in exon 6 and that the 2195G>A (R732Q) mutation activated a cryptic acceptor splice site in exon 14. In comparison, minigene analysis of the 22245C>T (R749W) missense mutation showed normal splicing only.

Haplotype Analysis

Two mutations were found in multiple families in this study. The F3 and F4 families had a single nucleotide substitution (1137G>A) previously reported to result in complex splicing errors.[10] Families F14 and F15 were both found to have a single nucleotide deletion (2398delC) in exon 15. Both families F14 and F15 came from the Canadian province of Newfoundland.

To determine whether recurring mutations represented independent *CDH1* mutational events or were due to common ancestry, haplotype analysis was performed on 5 of the 12 described mutations. These include the mutations that were found in more than 1 family in this series (2398delC and 1137G>A) along with 3 other previously reported mutations (1901C>T [A634V], missense/splicing; 2064-2065delTG; 2195G>A [R732Q], missense/splicing) found in this study for which samples from previously reported families were available (**TABLE 3**).[8,10,19,27]

Seven microsatellite markers surrounding the *CDH1* gene were selected and haplotypes were determined in several family members. These markers span a region of approximately 4 Mb (4 million base pairs of DNA) and are tightly linked to the *CDH1* gene (Figure 1). The disease-associated haplotypes were deduced from allele segregation in known carriers by inspecting genotypes in the families.

The missense/splicing mutation 2195G>A (R732Q) occurred in a previously reported family (SF5)[8] and in family F11 from this study. Three siblings from family F11 and an individual from family SF5 were subjected to haplotype analysis. Families F11 and SF5[9] had haplotypes differing at 2 microsatellite locations (D16S3107 and D16S3095) but were identical at the 3 microsatellite markers most tightly linked to *CDH1* (**TABLE 4**). These data are inconclusive because the mutation could either have arisen independently in these 2 families or it is an ancient mutation with differing haplotypes resulting from 2 recombination events occurring at the more distal markers. This latter possibility is a low-frequency event; however, it may have occurred over many generations.

The missense/splicing mutation 1901C>T (A634V) had been previously reported to occur in a Portuguese individual (family SF2) diagnosed with signet ring cell carcinoma of the stomach at the age of 30 years,[19] a Portuguese family with 2 cases of diffuse gastric cancer (family SF3),[27] and a colorectal carcinoma–derived cell line.[39] This mutation was found in family F7 of English descent. Analysis revealed that families SF2 and SF3 share virtually common haplotypes but differ at microsatellite D16S318 (Table 4), the most centromeric of the markers analyzed (Figure 1). Because D16S318 is relatively distant (approximately 1.5 Mb) from the *CDH1* gene, we believe that a single recombination event has occurred in 1 of the families but that

these families share a common ancestor. The putative haplotype detected in families SF2 and SF3 differed at markers D16S318, D16S3025, D16S496, D16S3067, and D16S3095 from the haplotype cosegregating with the mutation in family F7. We conclude that the mutation 1901C>T (A634V) occurred due to an independent mutational event in family F7.

The 1137G>A splicing mutation had been previously reported in a Brazilian family (SF1) of Italian origin[10] and was reported in 2 families, F3 and F4. Haplotype analysis performed on an individual from each of families F3 and F4 and on 2 affected individuals from family SF1 revealed a common haplotype shared among families F3 and F4 and a different haplotype in family SF1 (Table 4). This result indicates that families F3 and F4 could share a common ancestor. However, the presence of this same mutation in family SF1 is the result of an independent mutational event.

The 2064-2065delTG mutation was identified in family F9 in this study and also had been previously identified in family SF4 of German descent.[8] Haplotypes were determined in an individual from family F9 and in 2 individuals from family SF4. A putative common haplotype was found segregating with the mutation in both families, indicating that families F9 and SF4 share common ancestry. Despite extensive efforts, we have been unable to connect the pedigrees of these 2 families.

The 2398delC mutation (FIGURE 2) was found in families F14 (FIGURE 3) and F15 (FIGURE 4), both of Irish/English ethnicity and originating from Newfoundland. This same mutation was subsequently found in an additional family (SF6) from Newfoundland (Figure 3)[8] and in family SF7

Table 3. Supplementary Families Recruited for Haplotype Analysis

Family	Ethnicity	No. of Gastric Cancer Cases; Diffuse Cancer Cases	Age or Age Range at Gastric Cancer Diagnosis, y	No. of Breast Cancer Cases; Lobular Cancer Cases	Age Range at Breast Cancer Diagnosis, y	Mutation	Mutation Type	Location	Reference
SF1	Italian	3; 2	18-37	0		1137G>A	Splicing	Exon 8	10
SF2	Portuguese	1	30	0		1901C>T	Splicing	Exon 12	19, 27
SF3	Portuguese	2	23-26	0		1901C>T	Splicing	Exon 12	19, 27
SF4	German	3; 3	24-50	0		2064-2065delTG	Deletion	Exon 13	8
SF5	Unavailable	3; 3	36-70	3; 2	44-68	2195G>A	Splicing	Exon 14	8
SF6	Irish/English	5; 2	34-72	3; 1	49-68	2398delC	Deletion	Exon 15	8*
SF7	French	3; 0	35-75	0		2398delC	Deletion	Exon 15	Novel

*Initially classified as negative for *CDH1* mutations.

Table 4. *CDH1*-Associated Haplotypes in Families Carrying Common Mutations

	No. of Affected Individuals Tested	Haplotype No. by Mutation No.						
		D16S318	D16S3107	D16S3025	D16S496	D16S3067	D16S3095	D16S752*
1137G>A								
F3	1	140	295	100	219	145	151	102
F4	1	140	295	100	219	145	151	102
SF1	2	138	283	104	219	139	151	102
1901C>T								
F7	2	140	293	100	219	137	149, 151†	106
SF2	1	138, 148†	293	86	217	131	155	106
SF3	1	132, 136†	293	86	217	131	155	106
2064-2065delTG								
F9	1	140	279	90	219	135	149	114
SF4	2	140	279	90	219	135	149	114
2195G>A								
F11	3	140, 141†	283, 291†	100, 100†	211, 219†	135, 143†	149, 149†	110, 114†
SF5	1	140, 142†	287, 293†	100, 102†	219, 221†	135, 139†	147, 155†	106, 110†
2398delC								
F14	4	140	291	100	207	127	159	114
F15	1	140	291	100	207	127	159	114
SF6	2	140	291	100	207	127	159	114
SF7	2	140	291	100	207	127	159	114

*Indicates tetranucleotide repeat. All other microsatellite markers are dinucleotide repeats (genome database: http://www.gdb.org).
†Microsatellite repeat numbers for both alleles are reported when it was not possible to determine which allele was cosegregating with the mutation.

(Figure 4) in which the mother of the proband originates from Newfoundland. This cluster of 4 families has 29 cases of gastric cancer, 16 cases of breast cancer, and 31 unaffected mutation carriers, of which 12 have had prophylactic gastrectomies. Family SF6 had previously tested negative for *CDH1* mutations[8] but was retested when it was discovered that they originated from Newfoundland. The original false-negative result occurred due to polymorphic sites within primers used for the initial analysis (data not shown); in this study, different primer sets were used for sequencing. Although families SF6 and SF7 showed no apparent relationship to each other or to the original 2 Newfoundland families carrying this mutation, haplotype analysis revealed that all mutation carriers shared a common haplotype. This strongly suggests that the 2398delC mutation is a *CDH1* founder mutation. Furthermore, all 4 families are from communities within a 100-mile radius. Families F14 and SF6 are both from an abandoned island community off the southeast coast of Newfoundland and family SF7 is from the neighboring French islands of St Pierre and Miquelon. We have been able to connect the pedigrees of family F14 and SF6 (Figure 3); however, it is uncertain whether this connection is relevant to the inheritance of the *CDH1* mutation.

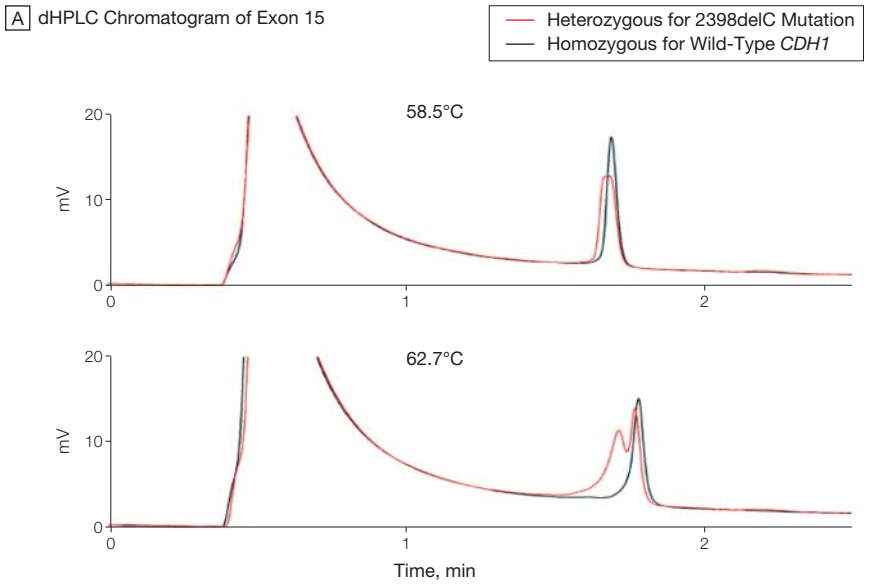
Penetrance Analysis

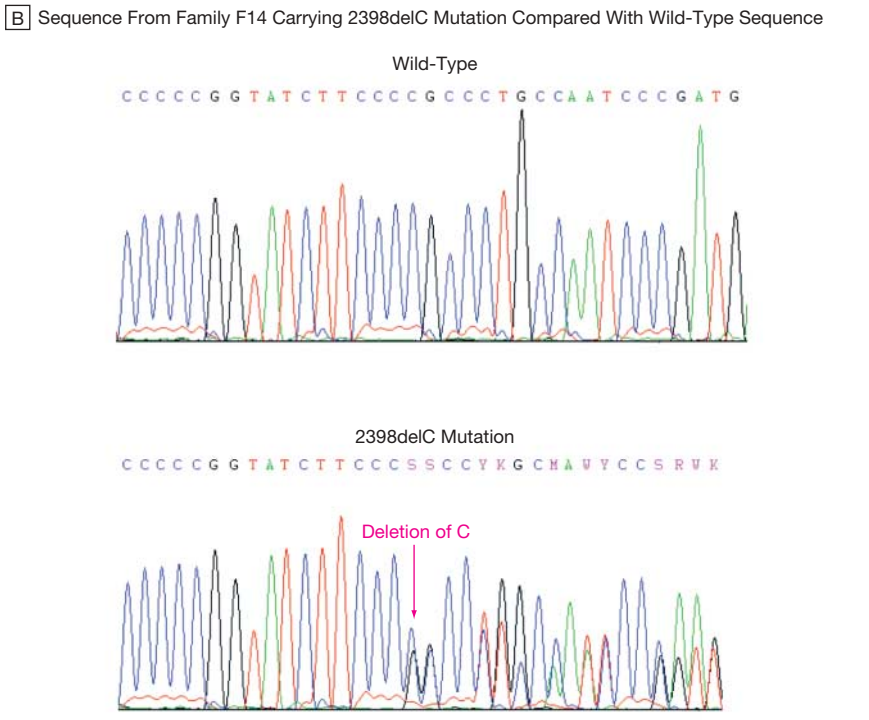
The cumulative risk of gastric cancer by age 75 years in the 4 families with the 2398delC mutation was estimated to be 40% (95% confidence interval [CI], 12%-91%) for males and 63% (95% CI, 19%-99%) for females (TABLE 5). The cumulative risk for breast cancer for females by the age of 75 years was found to be 52% (95% CI, 29%-94%).

Clinical Follow-up

We have performed 129 *CDH1* mutation carrier tests for asymptomatic individuals (age range, 18-77 years) from 11 of the families (9 of the original 15 families plus 2 additional families car-

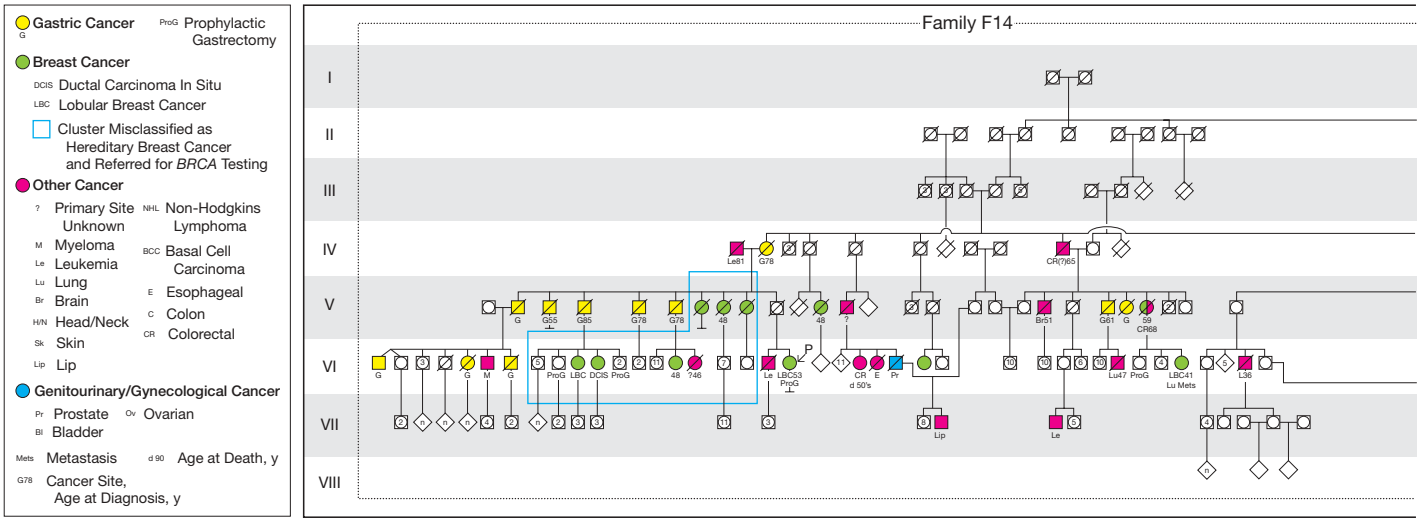
Figure 2. Results of dHPLC and Sequencing From Family F14 Carrying 2398delC Mutation





A, Denaturing high-performance liquid chromatography (dHPLC) chromatograms of exon 15. The red trace is representative of an individual who is heterozygous for the 2398delC mutation and the black trace is representative of an individual who is homozygous for wild-type *CDH1*. Different elution temperatures are used to analyze *CDH1* mutations to maximize the sensitivity of mutation detection and temperatures are unique for each exon. B, Sequencing results from a wild-type sequence (top) and 2398delC mutant sequence (bottom) are shown. These results confirm deletion of C nucleotide at position 2398 (arrow). The presence of S (G or a C nucleotide), Y (C or a T nucleotide), K (G or a T nucleotide), M (A or a C nucleotide), R (A or a G nucleotide), or W (A or a T nucleotide) in the sequence reflects the position of 2 different nucelotides at the positions being analyzed. This occurs because the mutant allele has a deletion at position 2398 (deletion of a C) and so its sequence is shifted with respect to the other wild-type allele.

Figure 3. Pedigrees of Family F14 and SF6



The dashed lines around each family indicate the original pedigree that was received at the time of separate referrals of each proband to the study. Familial links were added subsequent to mutation and haplotype analysis. To protect the anonymity of the families shown in the pedigrees, unaffected individuals are represented by a sex-neutral symbol (circle within a square). Information about affected individuals is fully displayed. Individuals are not shown in birth order. Complete pedigree data will be made available by the authors upon request for specific research use consistent with protection of participant privacy and prior consent.

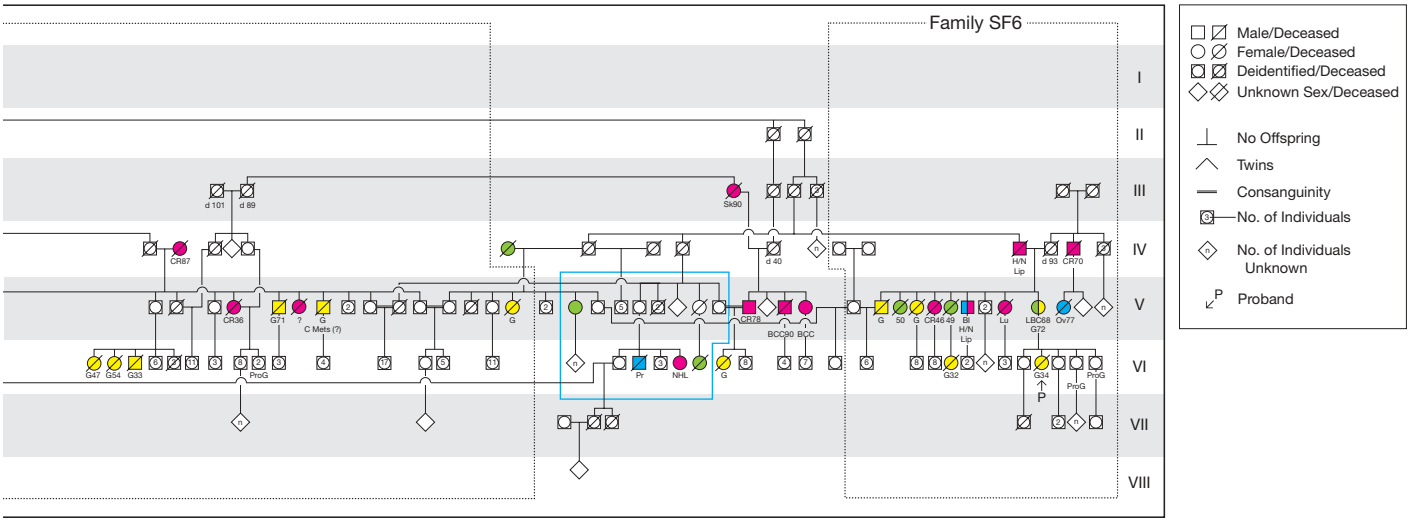
rying the 2398delC mutation) cited in this study. Sixty-one of these tests were from family F14. Fifty-one individuals had positive carrier tests (51/129; 40%) and 23 (45%) of these underwent prophylactic gastrectomies. A pathology report was available for 18 of the specimens and occult cancers were detected in 12 cases (67%). A single individual from family F15 had diffuse gastric cancer detected by endoscopic random biopsy while she was considering a prophylactic gastrectomy.

COMMENT

Gastric cancer is one of the most common gastrointestinal tract malignancies worldwide and increased gastric cancer rates are observed in hereditary nonpolyposis colon cancer, particularly in East Asian families, and in individuals with Li-Fraumeni syndrome or Peutz-Jegher syndrome.[40-42]

The germline defect in *CDH1* underlying HDGC was first identified through a combination of linkage and mutation analysis in 3 Maori families with early onset diffuse gastric cancer[6] and subsequently confirmed in families with different ethnicities.[30] Combining results from this and 2 previous studies, we have found *CDH1* mutations in 53.1% (24/49) of families with 2 or more cases of gastric cancer, with at least 1 diffuse gastric cancer case diagnosed before age 50 years.[8,9] These criteria may be appropriate for HDGC selection in low-incidence populations but is likely too permissive for use in countries such as Japan or Korea where gastric cancer is much more common.

For families with HDGC, the uptake for carrier testing is high and current cancer risk reduction strategies include endoscopic screening and prophylactic gastrectomy. The choice of prophylactic gastrectomy is supported by the finding of early diffuse gastric cancer in the tissue specimens of 21 of 22 *CDH1* mutation carriers who chose to have gastrectomies despite receiving negative endoscopy results.[9,43-45] Furthermore, in this study 23 (45%) of the 51 asymptomatic *CDH1* mutation carriers chose to have prophylactic gastrectomies and additional individuals are either awaiting surgery or are consulting with surgeons to discuss this possibility. A pathology report was available in 18 cases and occult cancer was confirmed in 12 (67%). The age range of individuals undergoing this procedure ranges from 36 to 60 years. The uptake of prophylactic gastrectomies is likely due to poor early detection methods and severity of disease at the time of clinical presentation. One of the difficulties associated with early diagnosis through screening is that early lesions are often multifocal, less than 1 mm in diameter, and underlie normal gastric mucosa.[33] Positron emission tomographic scanning or chromoendoscopy-directed biopsies have recently been recommended as a screening method for at-risk individuals.[46] However, these detection methods appear to have low sensitivity and failed to detect early gastric cancer in 6 patients with HDGC.[47] In this study, a single individual from family F15 was found to have occult cancer from an endoscopic biopsy; this is the only asymptomatic mutation carrier from this study known by us to have cancer detected by this method. Frequent screening by chromoendoscopy as the primary gastric risk reduction strategy in *CDH1* mutation carriers is being used for the previously described Maori family.[6,48] Further study of the risks and benefits of chromoendoscopic screening and prophylactic gastrectomy, including analysis of the long-term quality-of-life implications of gastrectomy will be required for the development of evidence-based management guidelines for this cancer susceptibility syndrome.



Prophylactic gastrectomy is a major surgical procedure associated with predictable morbidity and potential mortality rates (<1%) and thus should only be performed by expert surgeons after counseling by geneticists, dieticians, and gastroenterologists. For HDGC mutation carriers, our recommendations conform to published guidelines,[18] with consideration of genetic testing and commencement of screening in the late teens or early 20s. The timing of gastrectomy is an individual decision. The penetrance of *CDH1* mutations by age 75 years was somewhat lower in men (40%) than in women (63%), a difference that is similar to a previous study in which the cumulative risk of gastric cancer by age 80 years was 67% (95% CI, 39%-99%) for men and 83% (95% CI, 58%-99%) for women.[38] The biologic basis for the increased penetrance among females is not known and is in variance with diffuse gastric cancer incidence rates in males and females, which are approximately equal.[22] The high frequency of occult cancers in prophylactic gastrectomies in comparison with the incomplete penetrance of clinically detected cancer suggests that not all early lesions proceed to lethal carcinomas. The age range for gastric cancer in this series is 18 to 82 years and the youngest fatality from gastric cancer was at age 20 years. The youngest reported death from HDGC was in a 16-year-old adolescent from the Maori family in which the first *CDH1* germline mutations were described.[6] We recommend that gastrectomy should be considered in the early 20s in male mutation carriers whereas female mutation carriers would need to consider the dietary ramifications of prophylactic gastrectomy on pregnancy in their decision-making process and thus may wish to delay the procedure. For mutation carriers awaiting gastrectomy or for those in whom it is not a desirable option for personal or medical reasons, screening every 6 months is recommended. Both more refined penetrance data and an understanding of the effect of gastrectomy on quality of life will be required for the generation of evidence-based management guidelines.

For families with multiple cases of diffuse gastric cancer, mutational *CDH1* screening for diagnostic purposes is difficult due to the large size of the gene and distribution of mutations along the entire gene. Recurring mutations have been described, for example the 1003C>T (R335X) mutation, which was found in 3 families due to separate mutational events in each.[9,32] Other mutations (187C>T [R63X], 1018A>G [T340A], 1792C>T [R598X], and 1901C>T [A634V]) have been independently identified by multiple groups, however further analysis has not been performed to determine whether these are due to common ancestry or represent separate mutational events.[9,12,13,19,27,30,31]

In this study, 38 HDGC families were screened for *CDH1* mutations and 13 different mutations were identified in 15 families. The mutations identified in this study are similar to mutations that previously have been identified in terms of their distribution throughout the *CDH1* gene and mutation type (Table 1).

Five mutations were subjected to haplotype analysis to determine whether they occurred due to independent events or as founder effects. Four of these mutations (1901C>T [A634V], 1137G>A, 2064-2065delTG, and 2398delC) were found to be associated with identical or near identical haplotypes in more than 1 HDGC family. Of these, only the 2398delC mutation was identified multiple times within a genetically homogeneous, ethnically or geographically defined population, and thus is a likely founder mutation.

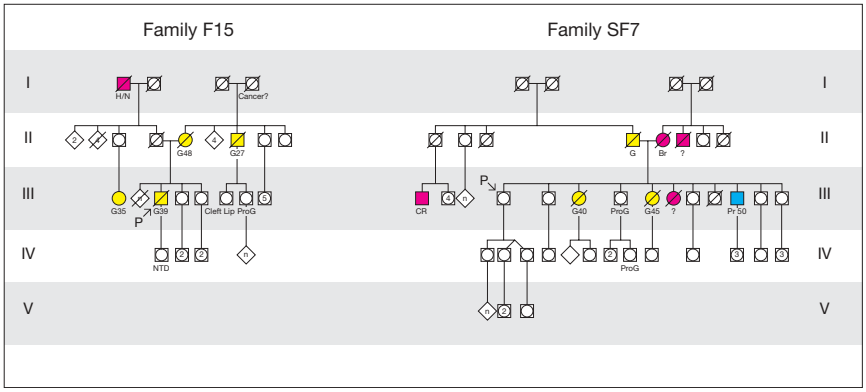
Both the 1901C>T (A634V) and the 1137G>A mutations also occurred as independent mutation events in additional families. Cleft lip and congenital scalp aplasia cutis have been described in siblings with positive test results for the 1137G>A splicing mutation from the previously reported family.[10] In adults, haploid insufficiency of epithelial cadherin results in diffuse gastric cancer susceptibility with no accompanying abnormalities in wound healing or skin integrity. However, at 4 to 6 weeks of embryo development, which coincides with lip and palate development, *CDH1* is expressed in the nasal prominence.[10] There were no known individuals with skin closure abnormalities in either family F3 or F4, which suggests that other genetic or environmental effects (eg, folate insufficiency) may influence this phenotype.

The 2398delC mutation was found on a common haplotype in 4 families (F14, F15, SF6, and SF7; Figure 3 and Figure 4). Aside from the previously reported Maori family with HDGC,[6] a founder mutation in the *CDH1* gene has not been described in the literature for HDGC syndrome. Two of these families originate from or near an abandoned island community off the southeast coast of Newfoundland and the other 2 families are from nearby communities (<100 miles away).

The incidence of mortality from gastric cancer in Newfoundland is the highest in Canada at 1.7 times the Canadian average.[49] Within Newfoundland, the regions this family comes from (Avalon Peninsula and southeast coast) are the highest-risk areas within the province. Consanguinity is known to be increased in the Southern Avalon Peninsula,[50] which increases the possibility of marriage between 2 carriers of the 2398delC mutation. Based on the phenotype of *CDH1* knockout mice, which are lethal during the embryonic stage,[51] we expect that homozygotes for this mutation would not be viable. However, such marriages would increase the heterozygous carrier frequency in sibships. While it is not known whether there is a higher than normal proportion of diffuse gastric cancer cases in this province, an early study describing increased incidence of gastric cancer in Newfoundland[52] shows a single figure of a gastric cancer sample whose pathology matches that of signet ring diffuse gastric cancer identical to that seen in *CDH1* mutation families. There has been speculation that environmental factors such as seabird populations[53] may contribute to this increased risk. However, the finding of a founder population from this region suggests a genetic explanation for these increased gastric cancer rates. If we are correct in attributing the increased rate of gastric cancer in Newfoundland to these founder mutations, then we would expect that more than 40% of the gastric cancer cases in this province could be hereditary. This is in strong contrast to the rest of North America where we estimate that less than 5% of gastric cancer cases are caused by autosomal dominant cancer susceptibility genes.[18,21]

In addition to the consequences of *CDH1* mutations for HDGC patients, female *CDH1* mutation carriers are at increased risk for developing lobular breast cancer.[8,9,22-24] Penetrance studies have shown that female *CDH1* germline mutation carriers have an additional risk of breast cancer, particularly lobular breast cancer, in about 39% (95% CI, 12%-84%) of patients.[38] Within the families with founder mutations from this study, the cumulative risk of breast cancer was 52% (95% CI, 29%-94%), which is slightly higher than the previously reported risk.[38] Family F14 carrying the 2398delC mutation has 13 members with breast cancer; all 3 available pathology reports were confirmed to have invasive lobular breast cancer. Family SF6[8] with the

Figure 4. Pedigrees of Family F15 and SF7



See Figure 3 for symbol key. To protect the anonymity of the families shown in the pedigrees, unaffected individuals are represented by a sex-neutral symbol (circle within a square). Information about affected individuals is fully displayed. Individuals are not shown in birth order. Complete pedigree data will be made available by the authors upon request for specific research use consistent with protection of participant privacy and prior consent.

Table 5. Penetrance Analysis for *CDH1* Mutation for Families F14 and SF6 (2398delC Mutation)

	Cumulative Risk, % (95% Confidence Interval)		
	Gastric Cancer		
	Male	Female	Female Breast Cancer
Age, y			
20	1 (0-9)	3 (1-10)	0
30	3 (0-25)	10 (3-31)	1 (0-7)
40	6 (1-44)	19 (7-48)	6 (1-30)
50	9 (1-55)	26 (9-65)	24 (5-78)
60	14 (93-64)	42 (14-88)	44 (18-93)
75	40 (12-91)	63 (19-99)	52 (29-94)

same mutation also has 2 breast cancer cases with 1 case confirmed as lobular breast cancer (Figure 3). Two branches of this extended family, along with family F11, had been misclassified as breast cancer families due to clustering of lobular breast cancer cases and subsequently tested negative for *BRCA1/2* mutations (Figure 3 and Figure 4). This suggests that families with negative test results for *BRCA1/2* in whom the proband has lobular breast cancer should be reevaluated for HDGC and screened for *CDH1* mutations.

Magnetic resonance imaging with mammography may be more sensitive than mammography alone for detection of breast cancer in high-risk women.[54] Our current recommendations for all female carriers of *CDH1* mutations include referral to high-risk breast cancer screening clinics with regular magnetic resonance imaging and mammography testing starting before age 40 years. Because early lobular breast cancer cases are estrogen-receptor positive and prophylactic tamoxifen trials preferentially reduced risk for estrogen-receptor positive cancer[55] and lobular carcinoma in situ,[56] the recommendation of tamoxifen for women with germline *CDH1* mutation carriers may be prudent if not contraindicated. Prophylactic mastectomy also could be considered and may be particularly appealing for asymptomatic female carriers from families with multiple cases of lobular breast cancer.

This extended family with the 2398delC founder mutation is a useful resource for determining risk-modifying factors in the development of diffuse gastric cancer or lobular breast cancer, such as diet or genetic polymorphisms, and for studying secondary genetic events that lead to cancer formation. The identification of this mutation could permit population-based screening of diffuse gastric cancer within specific regions of Newfoundland. Testing for the founder mutation will be particularly valuable for potential HDGC families from Newfoundland in which there is no known living relative with either diffuse gastric cancer or lobular breast cancer from whom a high-quality peripheral blood DNA sample can be obtained for full *CDH1* genetic screening because testing a single mutation can be readily performed on suboptimal DNA from archival tissue samples. In this study, we have identified other ancestral mutations; however, they cannot be used to streamline *CDH1* testing because they are not associated with a geographically or culturally definable founder population.

Our results confirm that between 30% and 40% of families with a positive family history of gastric cancer and more than 50% of families with 2 diffuse gastric cancer cases diagnosed prior to age 50 years will carry germline mutations in the *CDH1* gene. We also have identified a potential genetic etiology for the increased incidence of gastric cancer in the Canadian province of Newfoundland and have demonstrated a rapid uptake of asymptomatic carrier tests and prophylactic gastrectomies in families testing positive for a *CDH1* gene mutation.

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Author Contributions: Dr Huntsman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Drafting of the manuscript: Kaurah, Boyd, Senz, De Luca, Yim, Pharoah, Huntsman.

Critical revision of the manuscript for important intellectual content: Kaurah, MacMillan, Boyd, De Luca, Chun, Suriano, Zaor, Manen, Gilpin, Nikkel, Connolly-Wilson, Weissman, Rubinstein, Sebold, Greenstein, Stroop, Panzini, McKinnon, Greenblatt, Wirtzfeld, Fontaine, Coit, Yoon, Chung, Lauwers, Pizzuti, Vaccaro, Redal, Oliveira, Tischkawitz, Olschwang, Gallinger, Lynch, Green, Ford, Pharoah, Fernandez.

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