XXV Congreso de la Sociedad Española de Anatomía Patológica y
División Española de la International Academy of Pathology

NEOPLASIAS HEREDITARIAS

Cáncer gástrico familiar y hereditario

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Gastric cancer in familial/hereditary cancer syndromes

Syndromes	Genetic alterations
• Lynch syndrome (HNPCC)	*MMR*
• Li-Fraumeni syndrome	*TP53*
• Peutz-Jeghers syndrome	*STK1*
• Familial adenomatous polyposis	*APC*

GASTRIC CARCINOMA

- Sporadic (90%)
- Familial Aggregation (10%)
- Hereditary (1%)
  Hereditary Diffuse Gastric Cancer (HDGC)

Familial gastric cancer

- Sporadic (90%)
- Familial Aggregation (10%)
  - Familial Gastric Cancer (FGC)
  - Familial Intestinal Gastric Cancer (FIGC)
  - Familial Diffuse Gastric Cancer (FDGC)
- Hereditary (1%)
  - Hereditary Diffuse Gastric Cancer (HDGC)

Risk of gastric cancer development

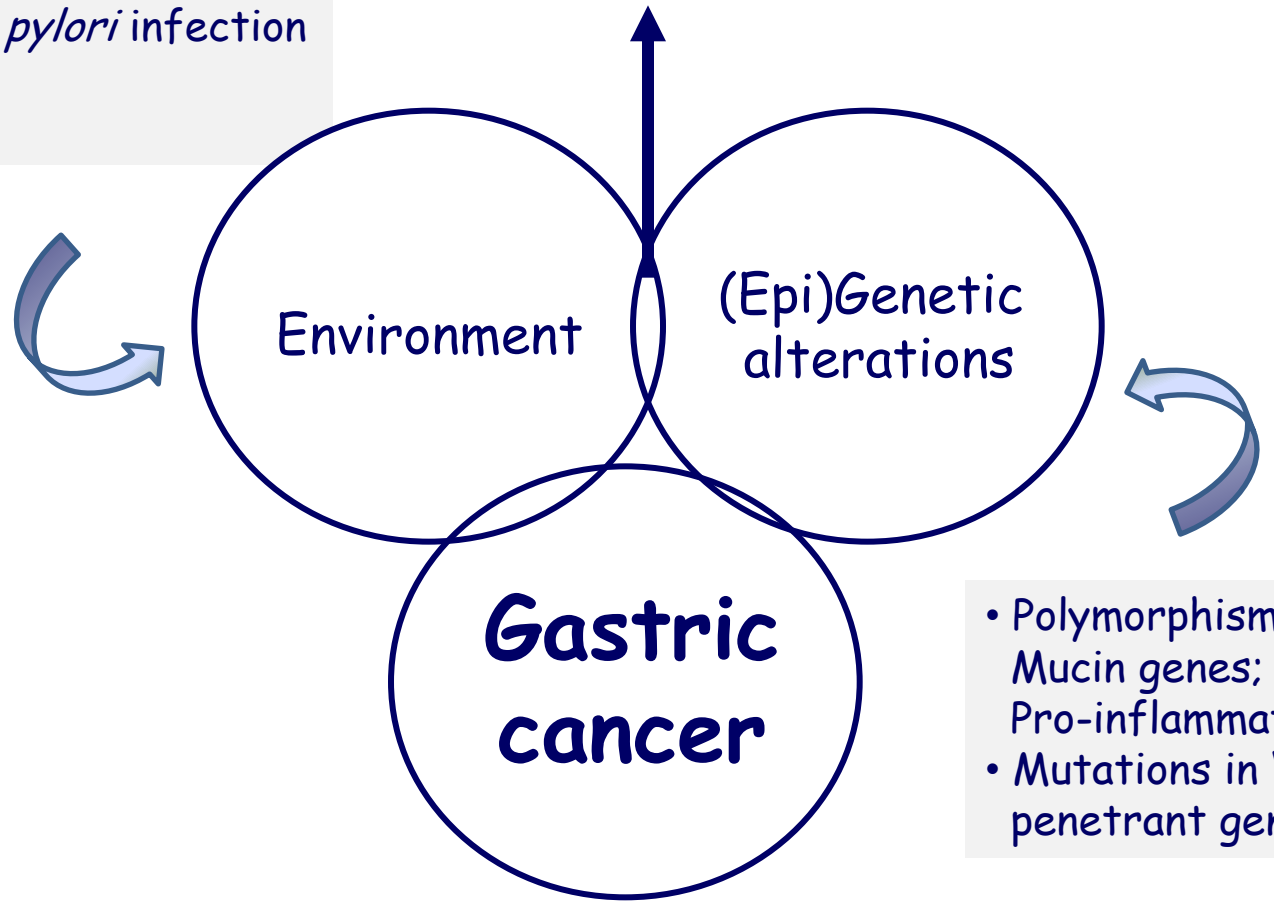
H. pylori virulent genotypes	15 to 17
IL-1 gene polymorphism	3.3
H. pylori virulence & *IL-1B* polymorphism	87

Machado *et al.* Gastroenterology 121: 823, 2001
Figueiredo *et al*, JNCI 94: 1680, 2002

Familial aggregation

Gene-environment interaction

- *Helicobacter pylori* infection
- Diet
- Smoking



- Polymorphisms:
  Mucin genes;
  Pro-inflammatory genes
- Mutations in "low" or "high" penetrant genes

Familial Gastric Cancer

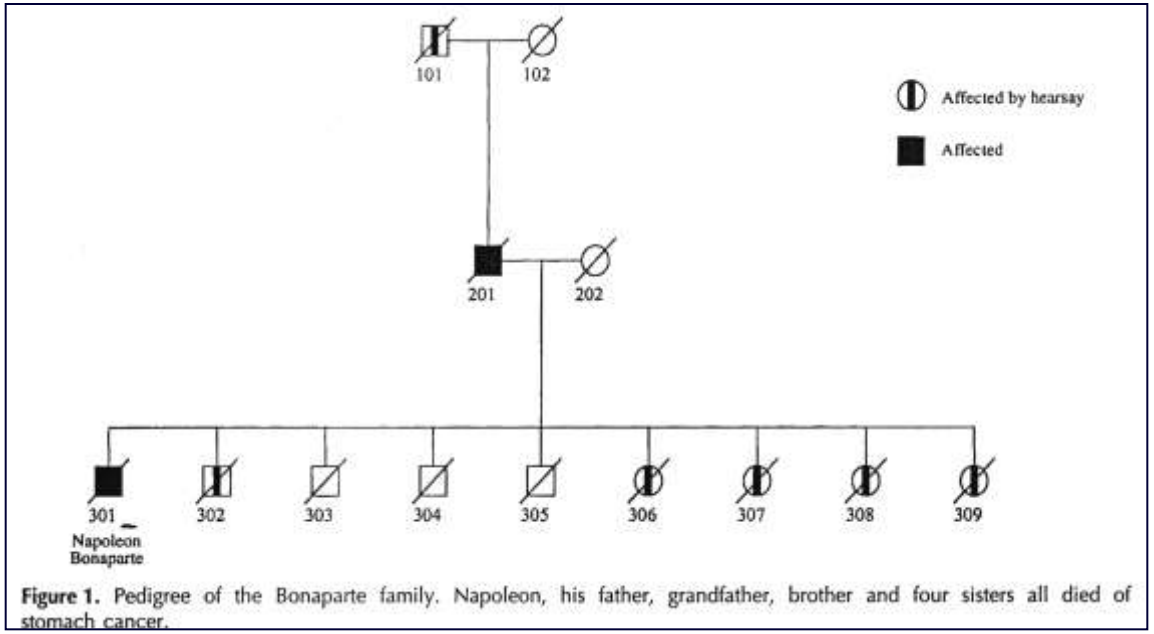
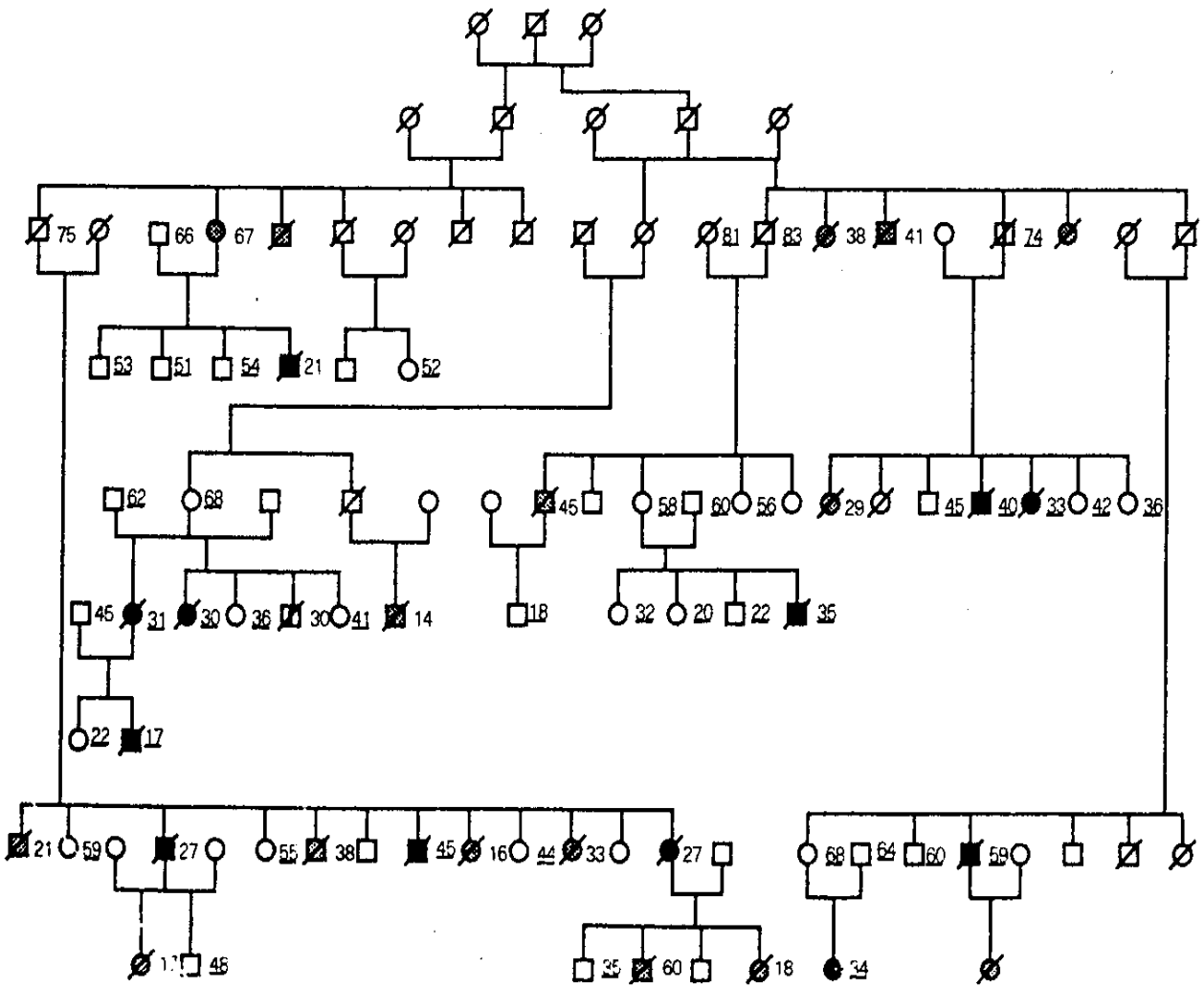
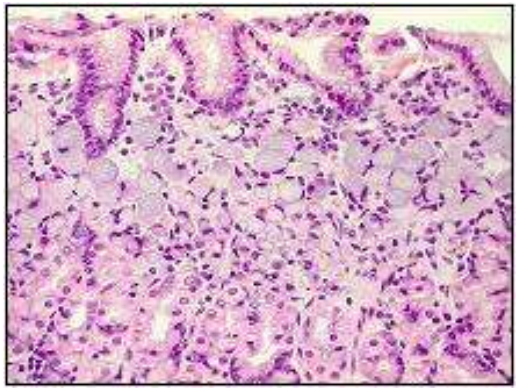


Figure 1. Pedigree of the Bonaparte family. Napoleon, his father, grandfather, brother and four sisters all died of stomach cancer.

Maori kindred



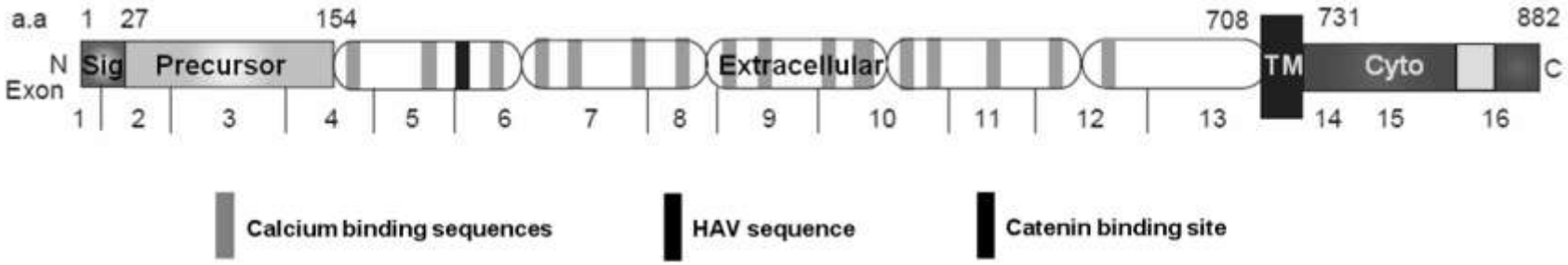


E-cadherin gene *(CDH1)* germline mutations

Hereditary Diffuse Gastric Cancer (HDGC)

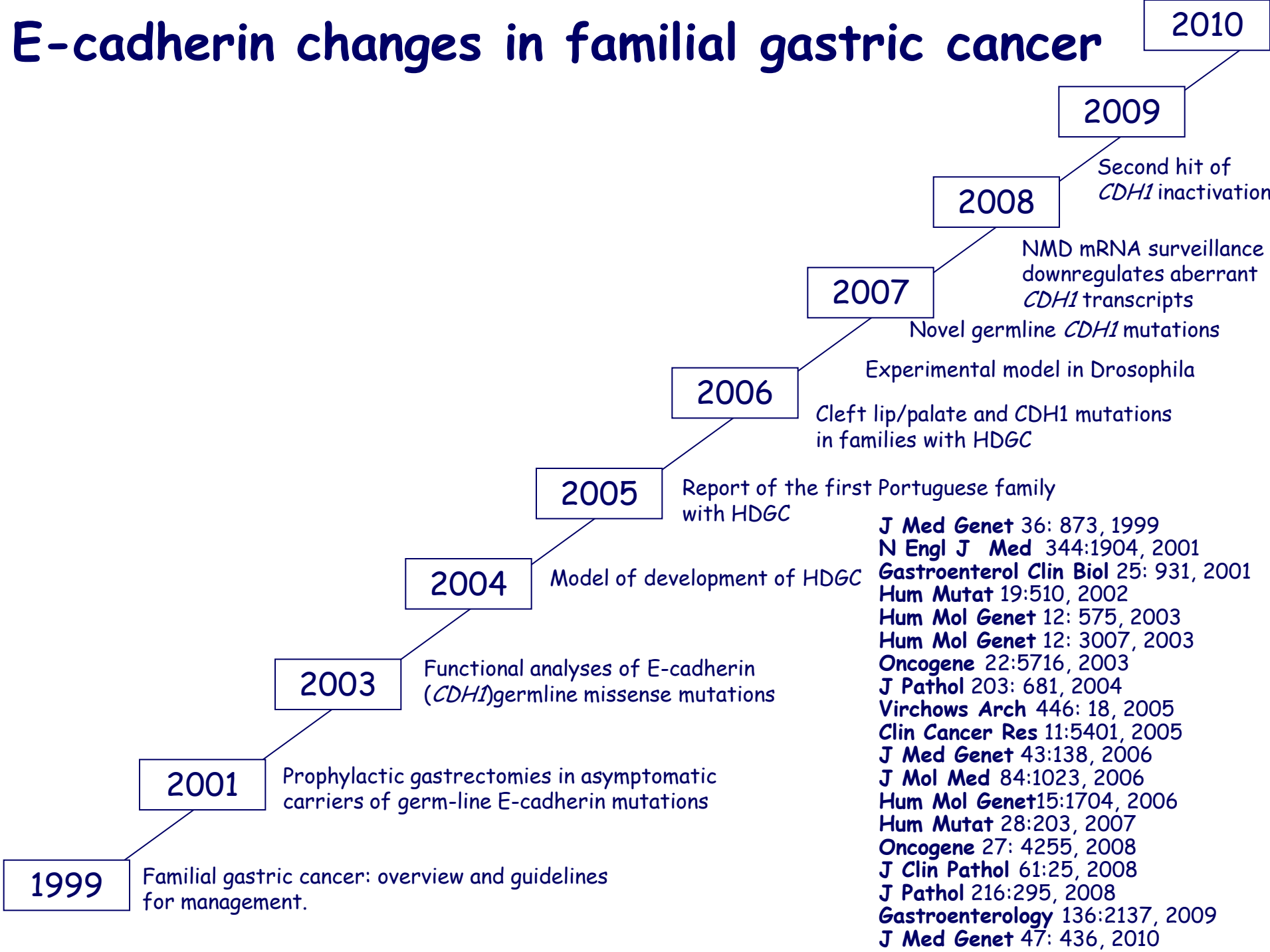
Guilford P *et al*
Nature 392:402,1998

E-cadherin gene (CDH1)
(MIM ID +192090)



Gene map locus: 16q22.1

E-cadherin changes in familial gastric cancer

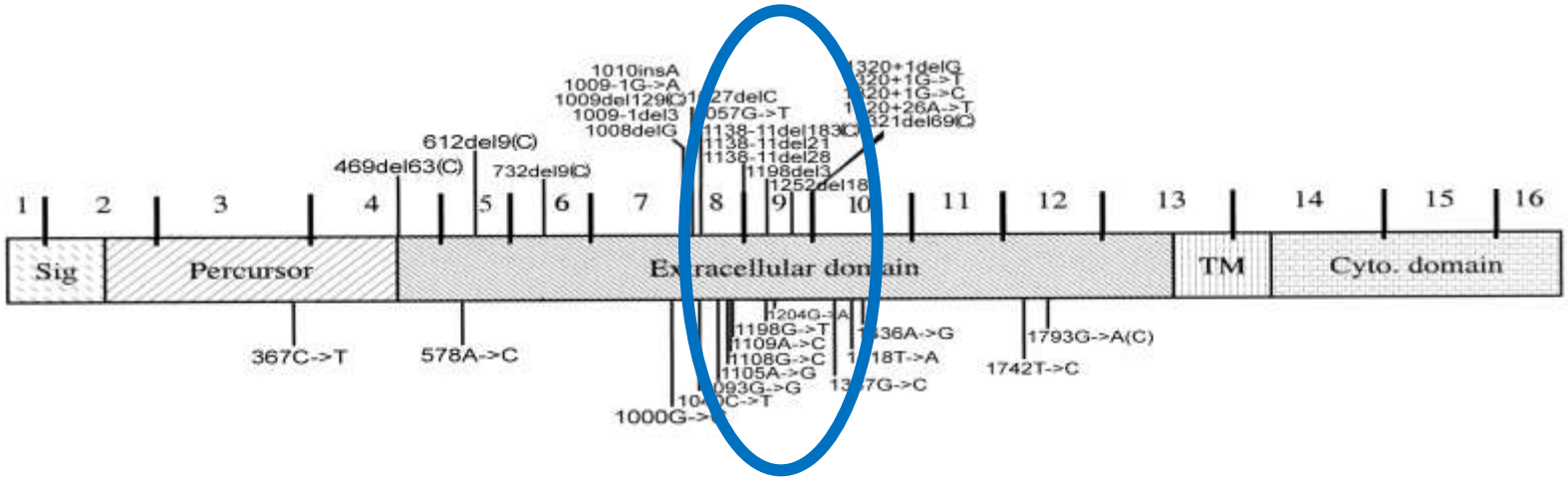


Genetic susceptibility

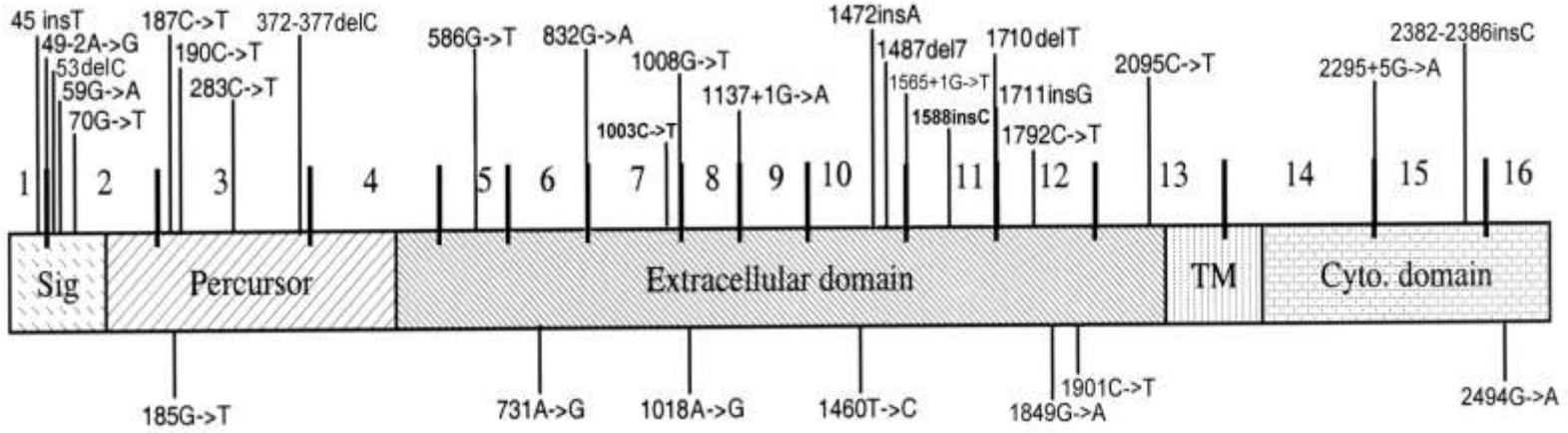
(Germline alterations)

E-cadherin mutations in diffuse gastric cancer

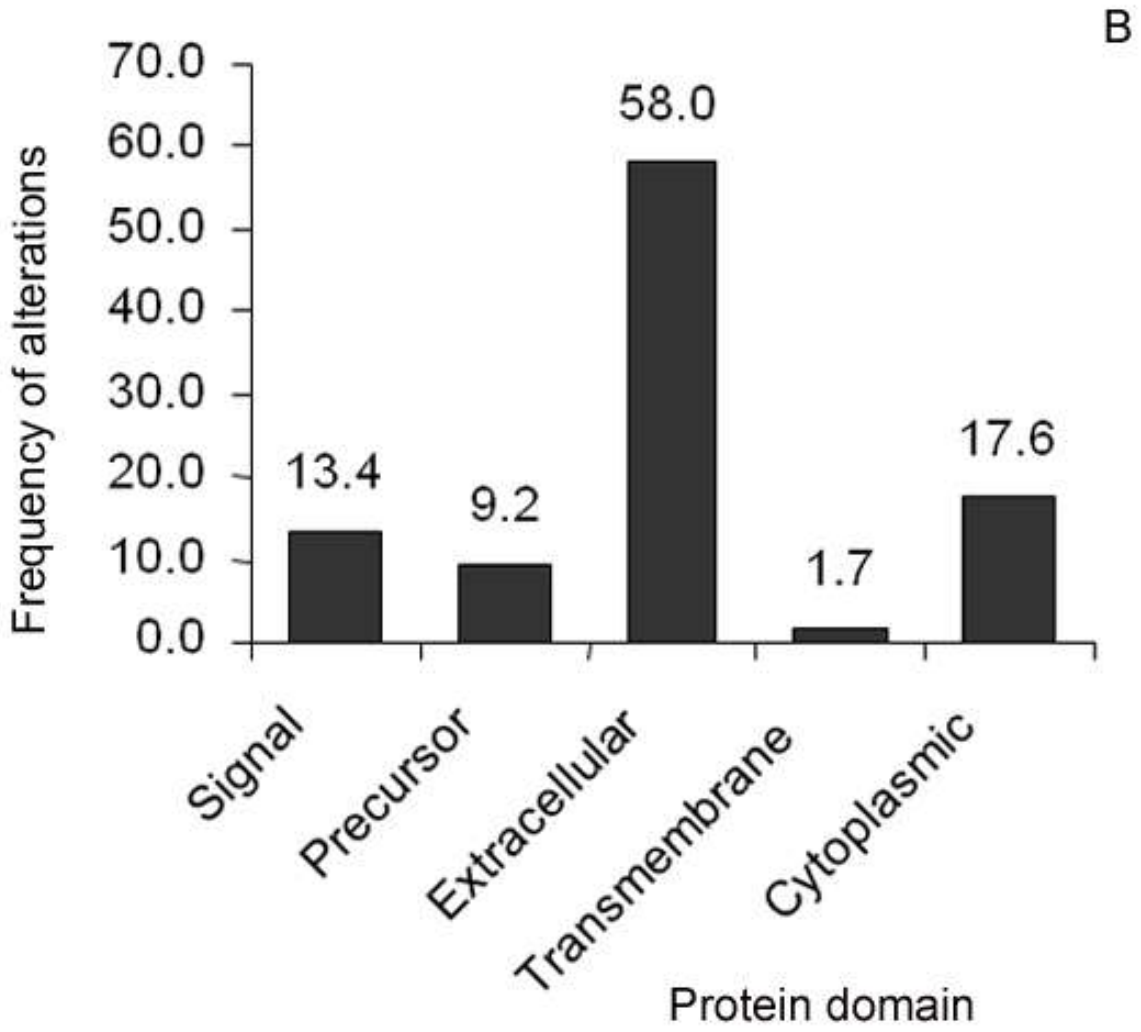
SPORADIC – Somatic mutations



HEREDITARY – Germline mutations

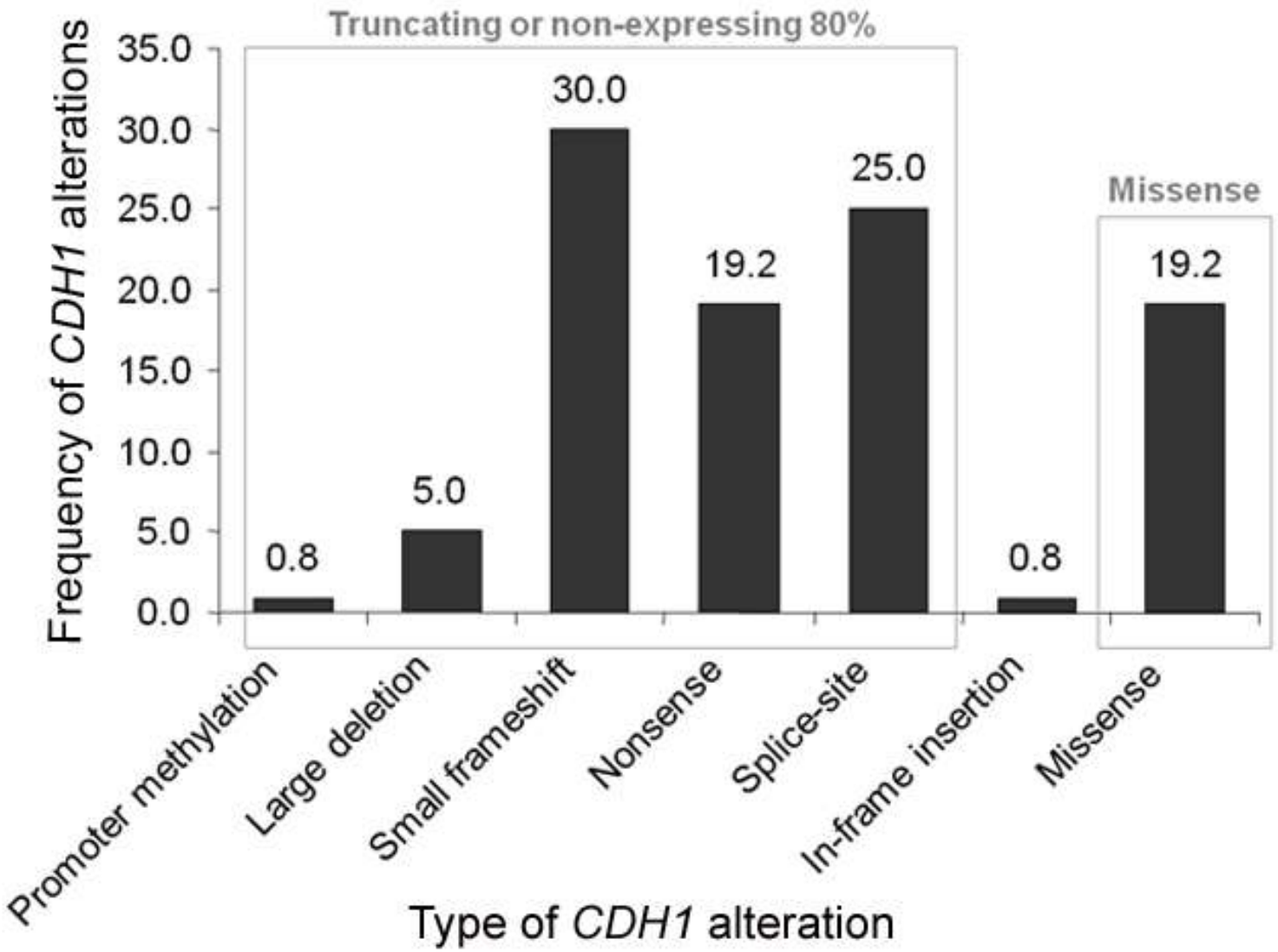


HEREDITARY – Germline mutations



Oliveira C, 2010

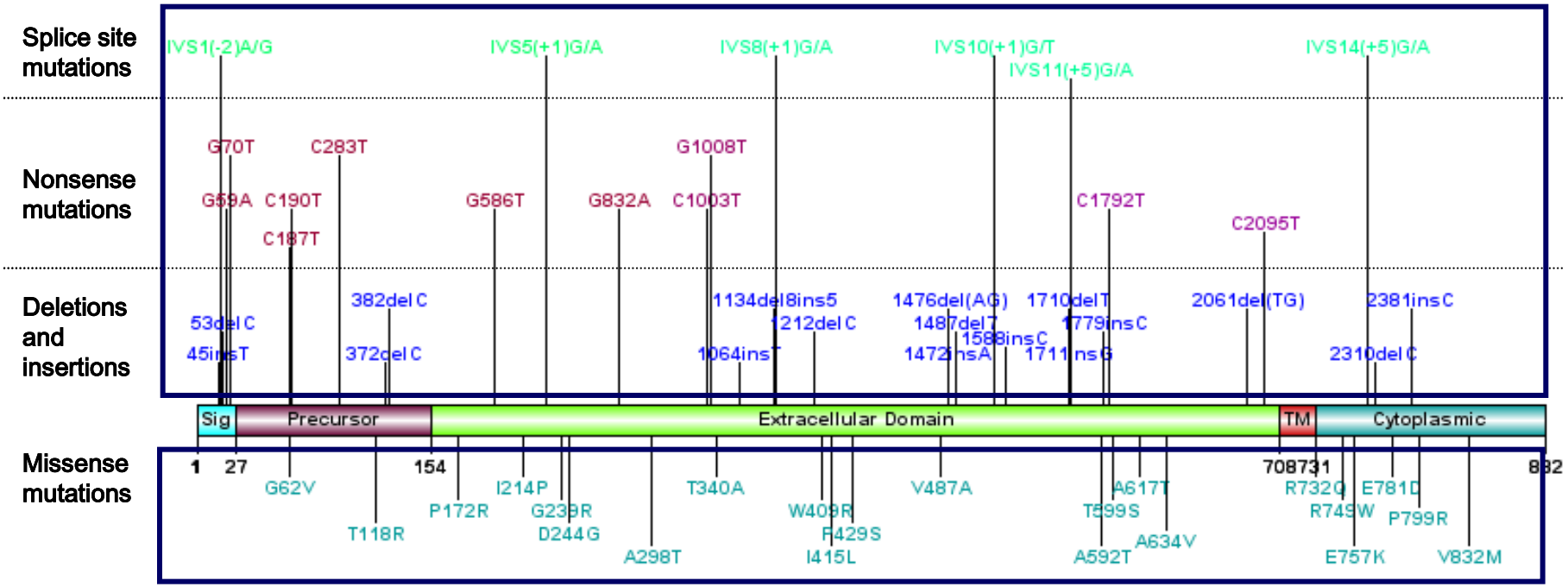
HEREDITARY – Germline mutations



Oliveira C, 2010

What about germline missense *CDH1* mutations in HDGC?

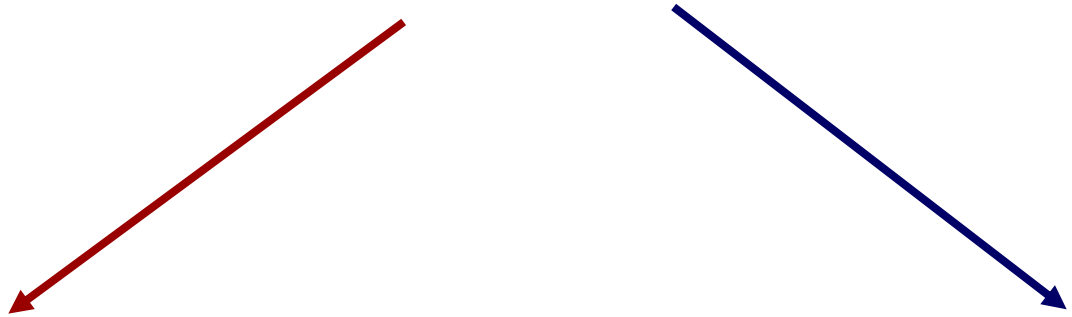
Truncating (~80%)



Missense (~20%)

Functional Assays in CHO cells (aggregation & collagen invasion assays)

Missense mutations affect cell-cell adhesion, motility and invasion



T340A, A634V, W409R, V832M, E757K

Functional Relevant

Adhesion, Motility, Invasion

A617T, ….

Functional Irrelevant

"neutral variants"

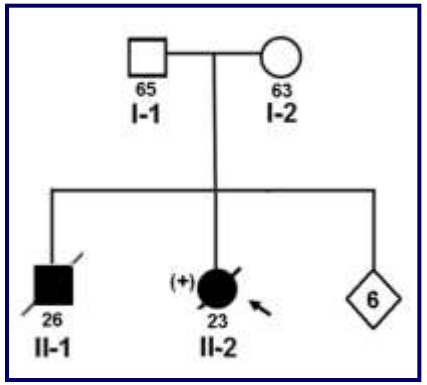
Suriano G *et al.*
Hum Mol Genet 12:3007, 2003

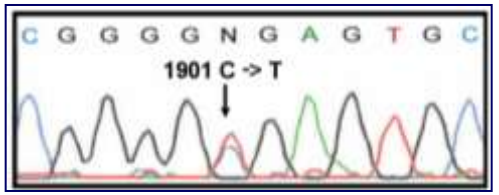
Functional characterization of *CDH1* missense mutations
(IPATIMUP)

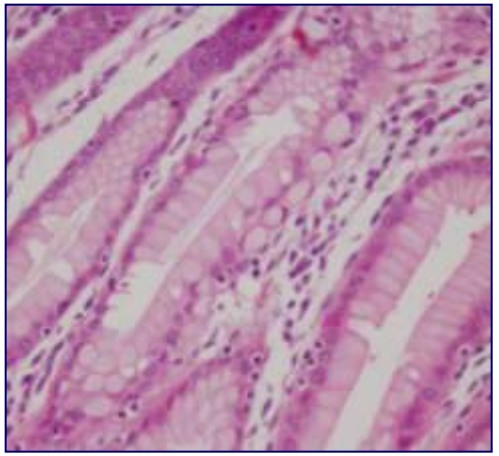
Validation of *CDH1* germline missense mutations

Table 2 E-cadherin germline missense mutations used for the statistical analysis

Variant	<1%	Co-segregation	Recurrence	SIFT[a]	Functional effect
Neutral (*N*)[b]	−	−	−	−	−
T118R	+	−	−	−	+
L214P	+	−	−	+	+
G239R	+	−	−	+	+
A298T	+	−	−	−	+
T340A	+	−	+	−	+
W409R	+	−	−	+	+
P429S	+	−	−	+	+
A592T	−	−	+	−	−
A617T	−	−	+	−	−
A634V	+	−	+	−	+
R732Q	+	−	−	+	+
P799R	+	−	−	+	+
V832M	+	+	−	+	+





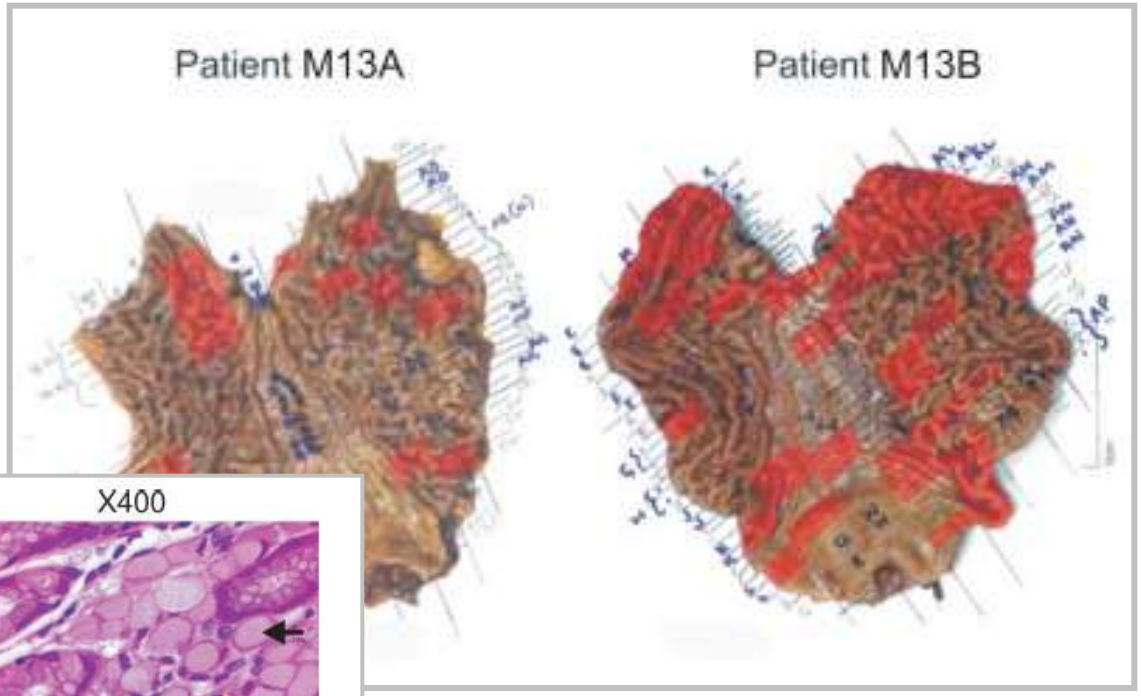


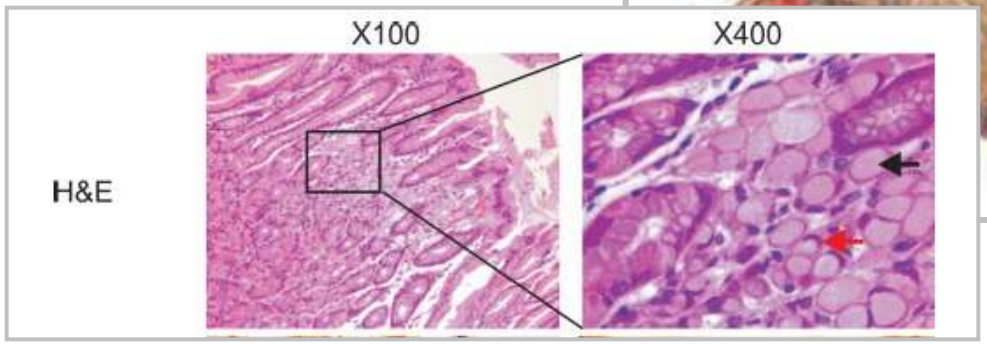
Suriano G *et al*
J Mol Med 84:1023, 2006

In vivo validation of *in vitro* assays of *CDH1* missense mutations

Table 2. Patients' characteristics and foci identified

Patient ID	Germline mutation	Mutation type	Age at surgery (years)	Sex	Length of time in surveillance programme	Number positive endoscopic biopsies/total taken (months prior to surgery)	Number of signet ring cancer foci identified in gastrectomy
M13A	641T>C	Missense	23	F	6 Months	2/24 (2)	16
M13B			20	F	6 Months	6/24 (2)	66

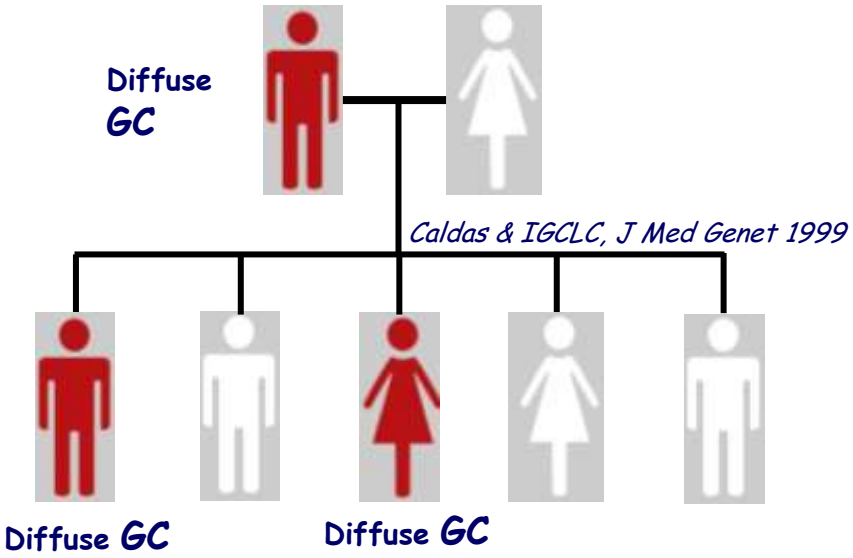


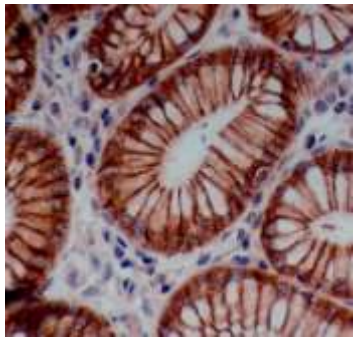


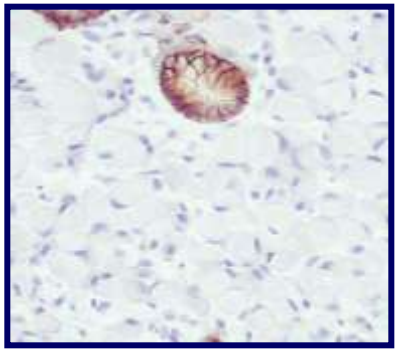
Barber M *et al*
J Pathol 216:295, 2008

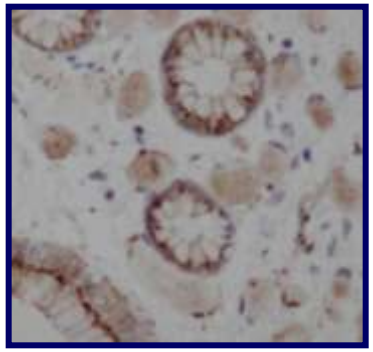
Late 2007

HDGC syndrome









~30% *E-cadherin gene (CDH1)* germline mutations

Guilford et al, Nature, 1998
Gayther et al, Cancer Res, 1999
Oliveira et al, Hum Mutat, 2002
Oliveira C et al, Exp Rev Mol Diagn, 2003
Oliveira et al, Eur J Cancer, 2004
Oliveira et al, Oncogene, 2004
Brooks-Wilson et al, J Med Genet, 2004
Oliveira C et al, Hered Cancer in Clin Pract, 2004
Oliveira C et al, Virchows Archiv, 2005
Suriano G et al, Clin Cancer Res, 2005
Oliveira C et al, Int J Surg Pathol, 2006
Kaurah P et al, JAMA, 2007

Late 2007

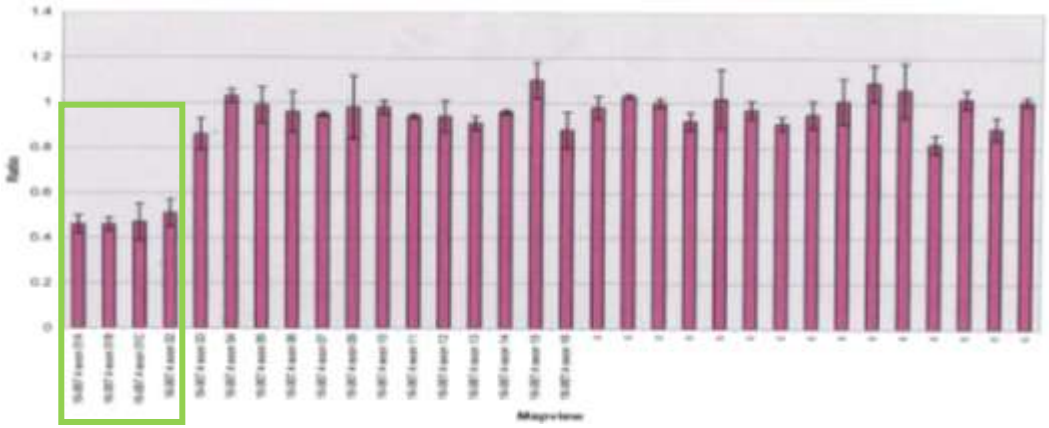
What is the germline defect in ~70% of *CDH1* point mutation negative families?

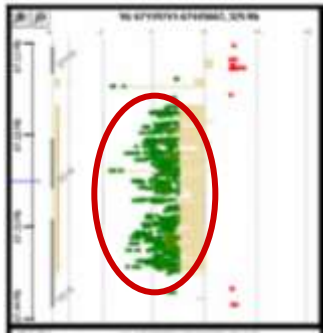
Along 2008 and 2009

- 160 families fulfilling the HDGC criteria, from different geographic backgrounds

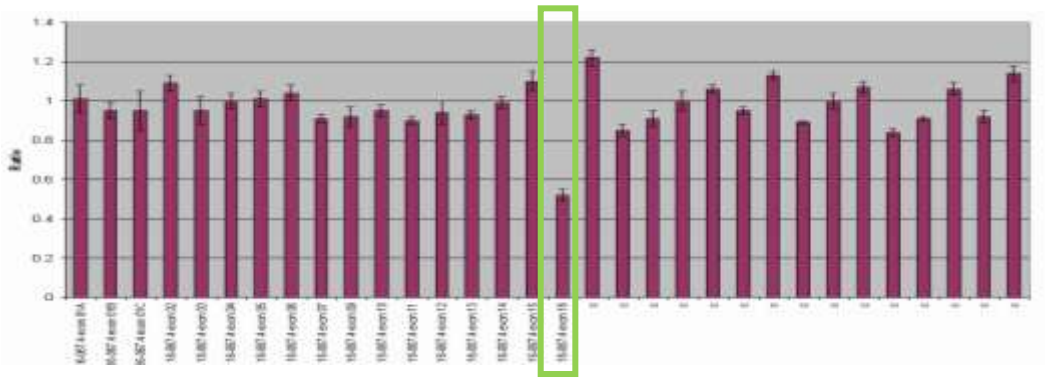
- Search for point mutations and large genomic rearrangements of the *CDH1* gene by MLPA

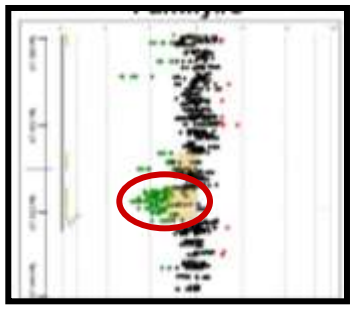
MLPA analysis and Array CGH





Deletions affecting the 5'-end of *CDH1*



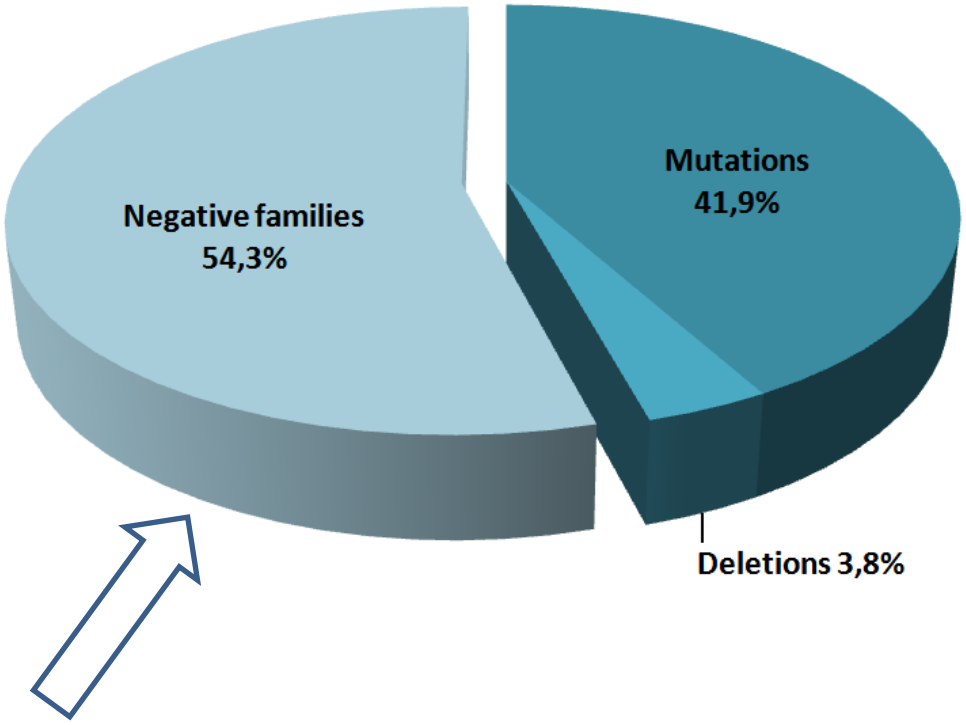


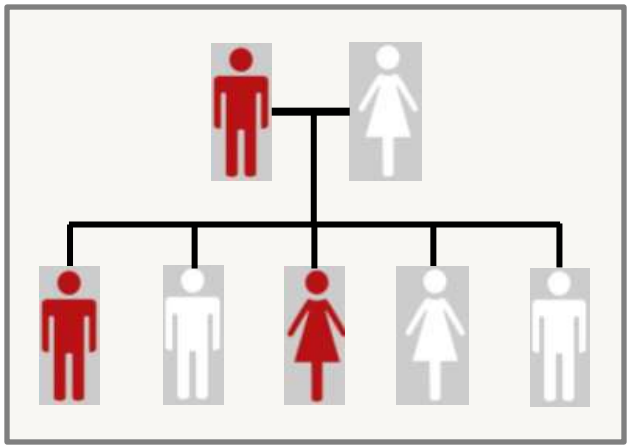
Deletions affecting the 3'-end of *CDH1*

Large Alu associated **germline deletions of *CDH1*** in HDGC families: a new mechanism for disruption of E-Cadherin function

Oliveira O et al, Hum Mol Genet, 2009

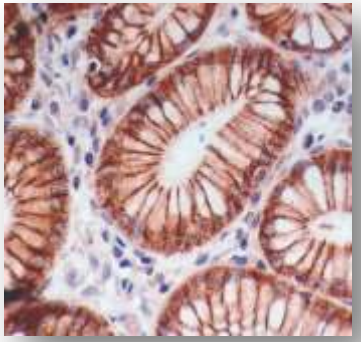
HDGC and E-cadherin gene (*CDH1*)



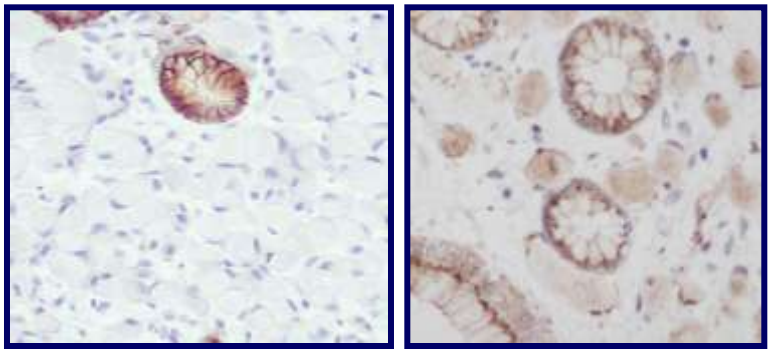


Oliveira O et al, Hum Mol Genet, 2009
Fitzgerald R et al, J Med Genet, 2010

What is the germline defect in >50% of *CDH1* negative families?



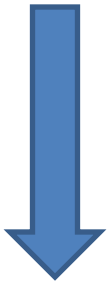
CDH1 point mutation negative



Do other mechanisms inactivating *CDH1* cause cancer in these families?

Into 2010…

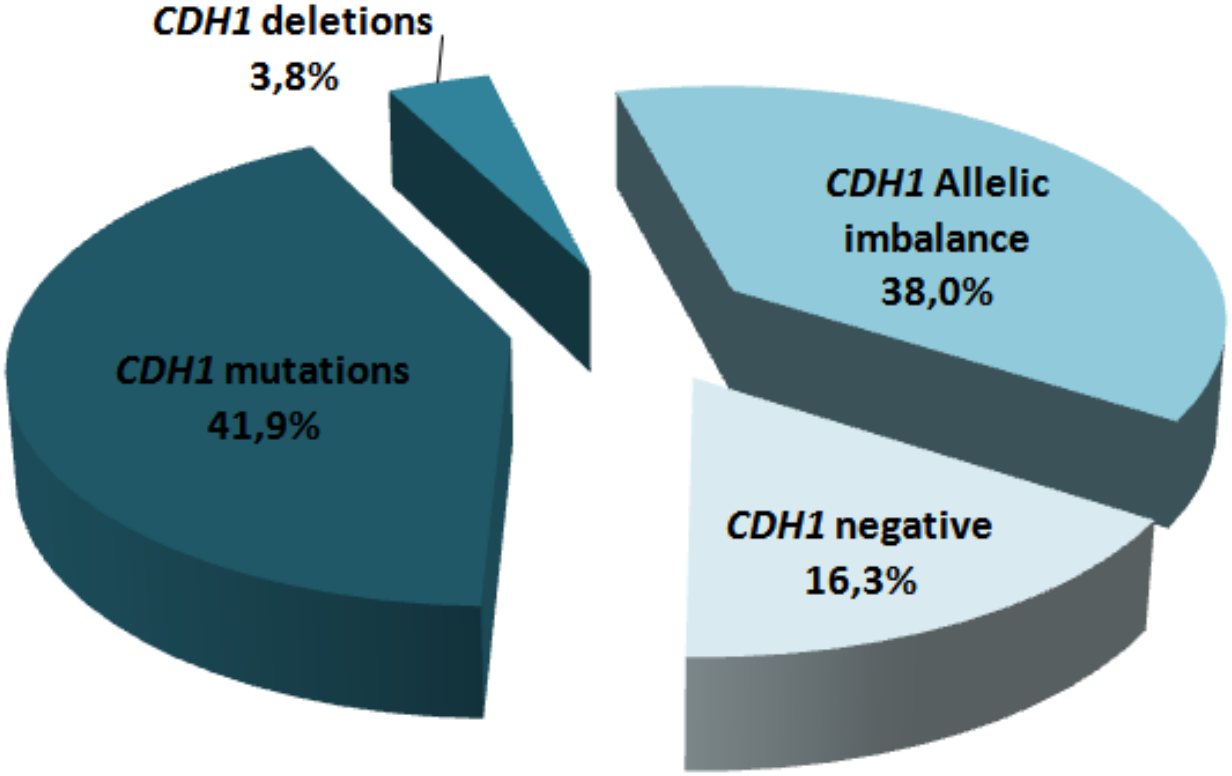
CDH1 allele specific quantification in *CDH1* negative probands



Allelic imbalance – 38% of the cases

Pinheiro H et al. Hum Mol Genet, 2010

2011



E-cadherin gene *(CDH1)* is involved in more than 80% of HDGC cases

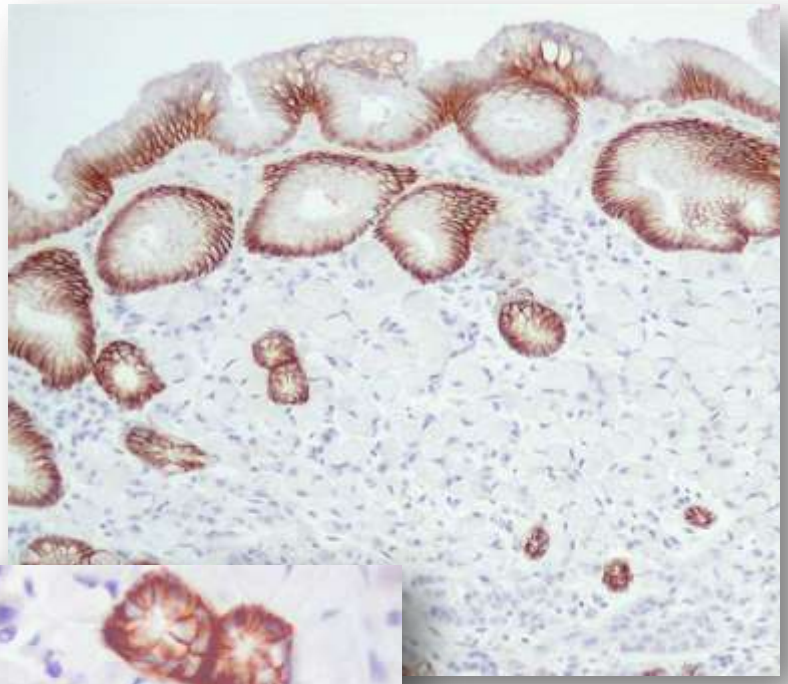
Currently, ongoing sequencing of the full 100kb *CDH1 locus* in 90 HDGC patients

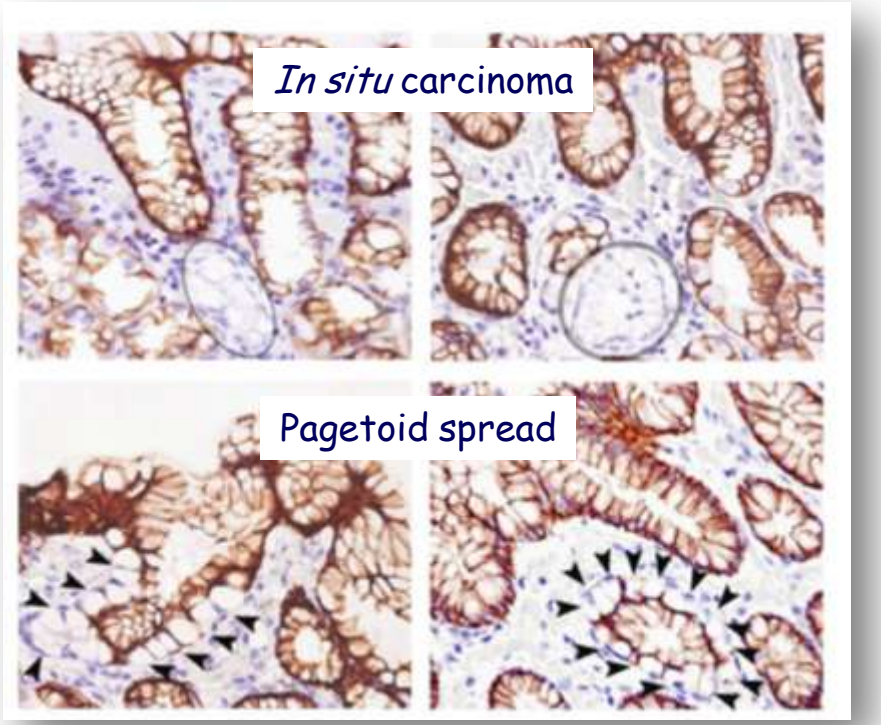
Oliveira O et al, Hum Mol Genet, 2009
Pinheiro H et al. Hum Mol Genet, 2010

Molecular Pathology

(Somatic alterations)

Absent expression of E-cadherin (somatic inactivation of wild allele in the tumour)





CDH1 gene alterations in gastric carcinoma (Sporadic & Hereditary)

"1st HIT"	"2nd HIT"
Mutation	• Promoter methylation • LOH • "Second" mutation • More than one

Grady *et al.* Nat Genet 26:16, 2000
Machado *et al.* Oncogene 20:1525, 2001
Oliveira *et al.* Gastroenterology 136:2137, 2009

Frequency of 2nd hit in HDGC tumours

28 neoplastic lesions (16 primary and 12 LN metastases) from 17 *CDH1* germline mutation carriers (15 HDGC families)

Table 2. Second-Hit Molecular Mechanisms in 28 Neoplastic Lesions

Second-hit mechanism	Total lesions (n = 28)	Primary tumors (n = 16)	Metastases (n = 12)
Methylation alone	9 (32.1%)	8 (50.0%)	1 (8.3%)
LOH alone	7 (25%)	2 (12.5%)	5 (41.7%)
Methylation + LOH	5 (17.9%)	3 (18.8%)	2 (16.7)
No alterations	7 (25%)	3 (18.8%)	4 (33.3%)

The 2nd hit in *CDH1* frequently occurs via epigenetic changes in HDGC primary tumors and LOH in metastases

Oliveira C et al, Gastroenterology, 2009

Clinical features

Familial gastric cancer: overview and guidelines for management

(International Gastric Cancer Linkage Consortium)

Caldas C, Carneiro F, Lynch H *et al*
Eur J Genet 36: 873, 1999

Clinical criteria for the identification of families with HDGC

Criteria for identification of HDGC families were defined by IGCLC in 1999:

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
2) Three or more cases of documented diffuse gastric cancer in first/second degree relatives, independently of age

Caldas C, Carneiro F, Lynch H *et al:* Familial gastric cancer: overview and guidelines for management. **J Med Genet** 36: 873, **1999**

IGCLC criteria for genetic testing were updated in 2010:

1) Idem
2) Idem
3) **Diffuse gastric cancer before the age of 40 years** without a family history
4) Families with diagnoses of **both diffuse gastric cancer and lobular breast cancer**, with one case before the age of 50 years

Fitzgerald R et al: Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. **J Med Genet** 47: 436-444, **2010**

CDH1 associated cancers/syndromes

- Lobular breast cancer
  (estimated cumulative risk of breast cancer for females by the age of 75 is calculated as 52%)
- Cleft lip with or without cleft palate

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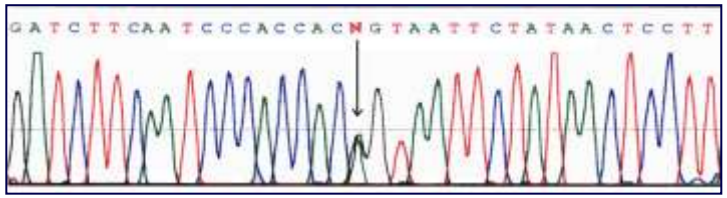
SHORT REPORT

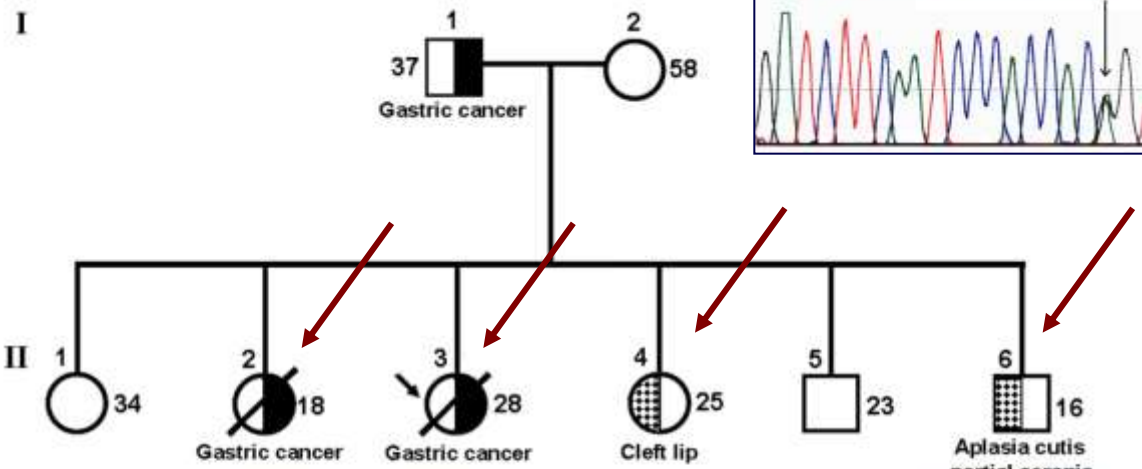
Cleft lip/palate and *CDH1/E-cadherin* mutations in families with hereditary diffuse gastric cancer

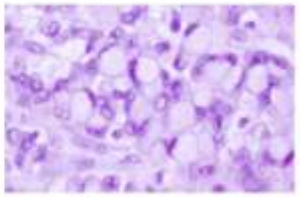
T Frebourg, C Oliveira, P Hochain, R Karam, S Manouvrier, C Graziadio, M Vekemans, A Hartmann, S Baert-Desurmont, C Alexandre, S Lejeune Dumoulin, C Marroni, C Martin, S Castedo, M Lovett, J Winston, J C Machado, T Attié, E W Jabs, J Cai, Ph Pellerin, J P Triboulet, M Scotte, F Le Pessot, A Hedouin, F Carneiro, M Blayau, R Seruca

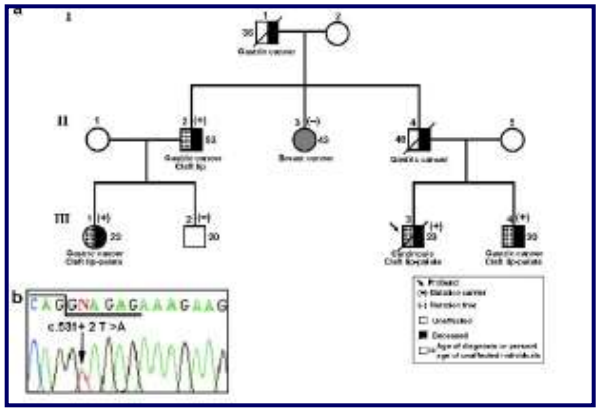
J Med Genet 2006;**43**:138–142. doi: 10.1136/jmg.2005.031385

1137G>A





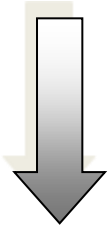




Familial gastric cancer: overview and guidelines for management

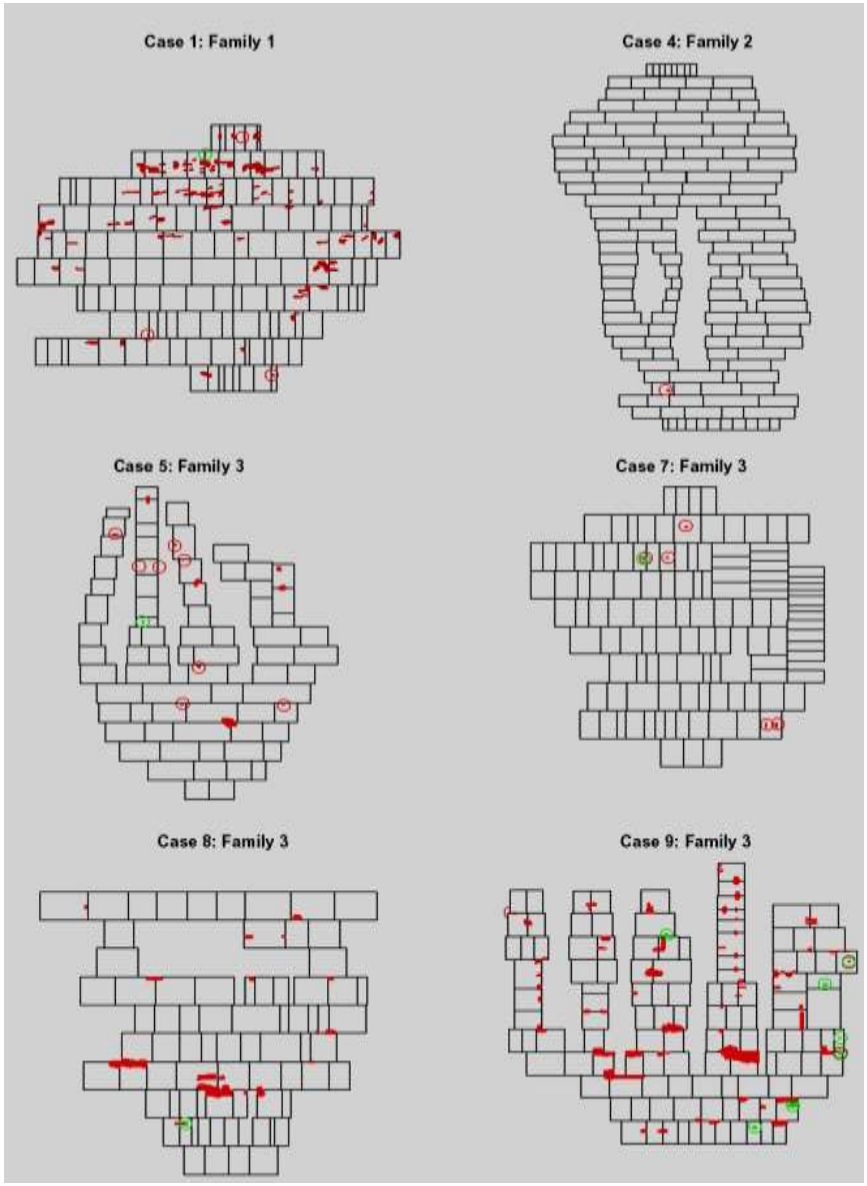
(International Gastric Cancer Linkage Consortium)

Carriers of germline E-cadherin truncating mutations



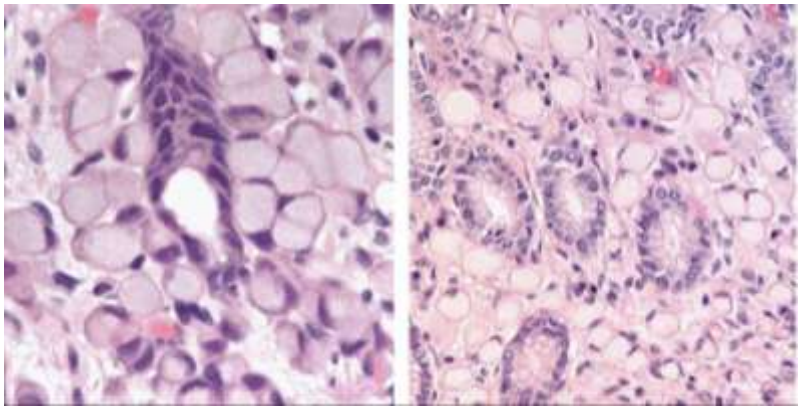
Intensive screening
Prophylactic gastrectomy

Caldas C, Carneiro F, Lynch H *et al*
Eur J Genet 36: 873, 1999

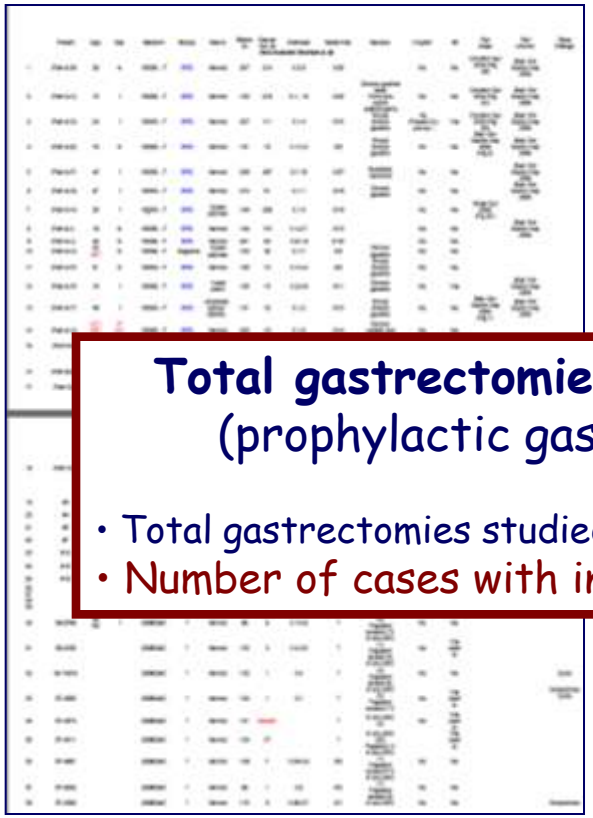


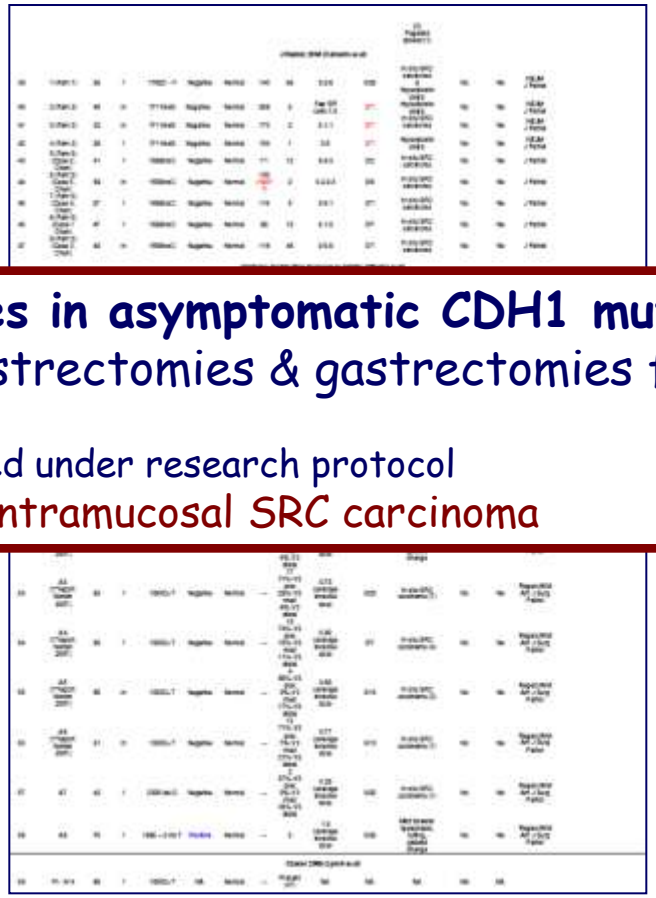
Carneiro F *et al*
J Pathol 203:68, 2004

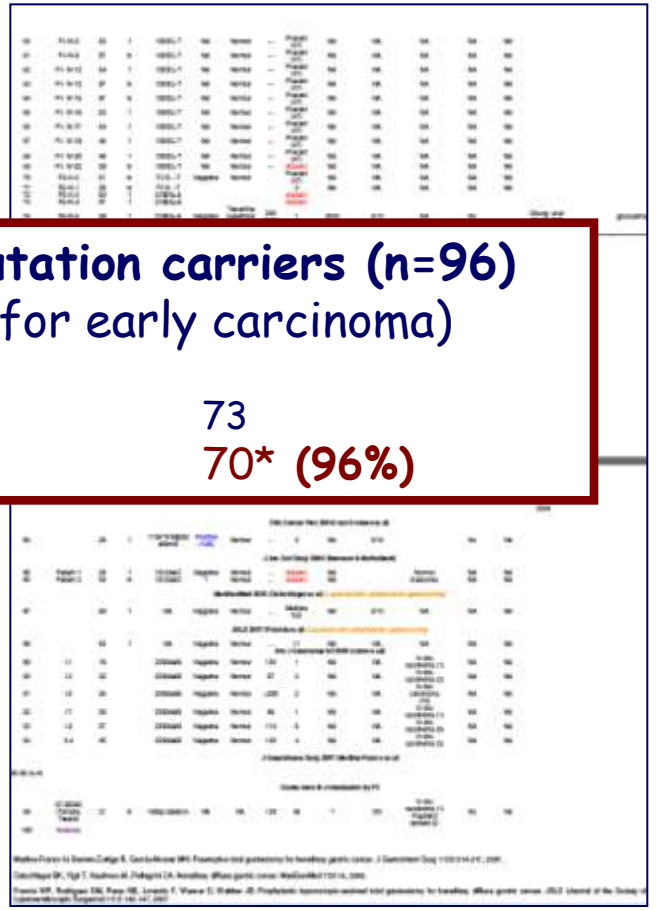
Hereditary diffuse gastric cancer (HDGC)



In 9 prophylactic gastrectomies from North America (Huntsman *et al*, 2001 and Chun et *al*, 2001), all exhibited *foci* of intramucosal diffuse carcinoma





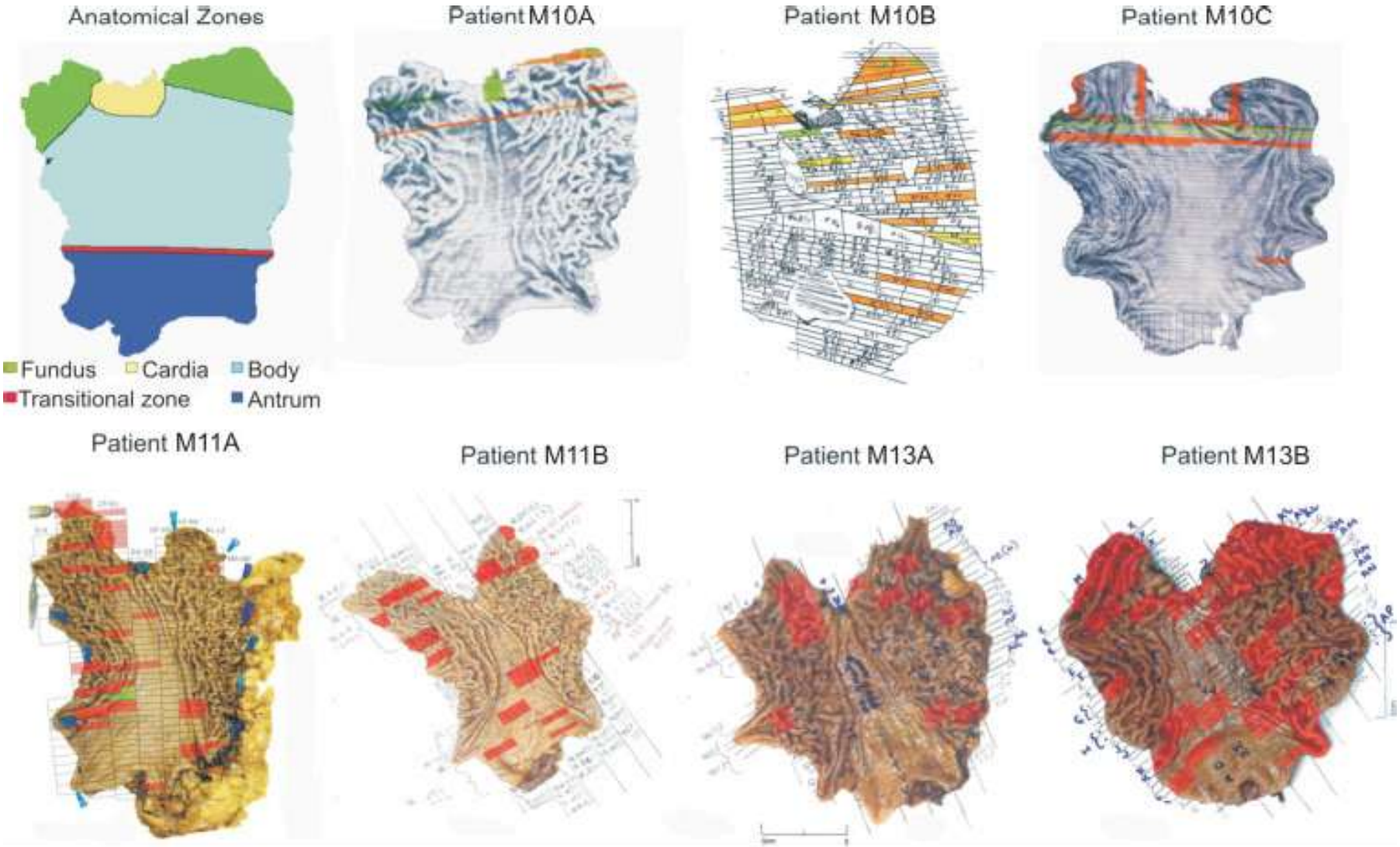


Total gastrectomies in asymptomatic CDH1 mutation carriers (n=96)

(prophylactic gastrectomies & gastrectomies for early carcinoma)

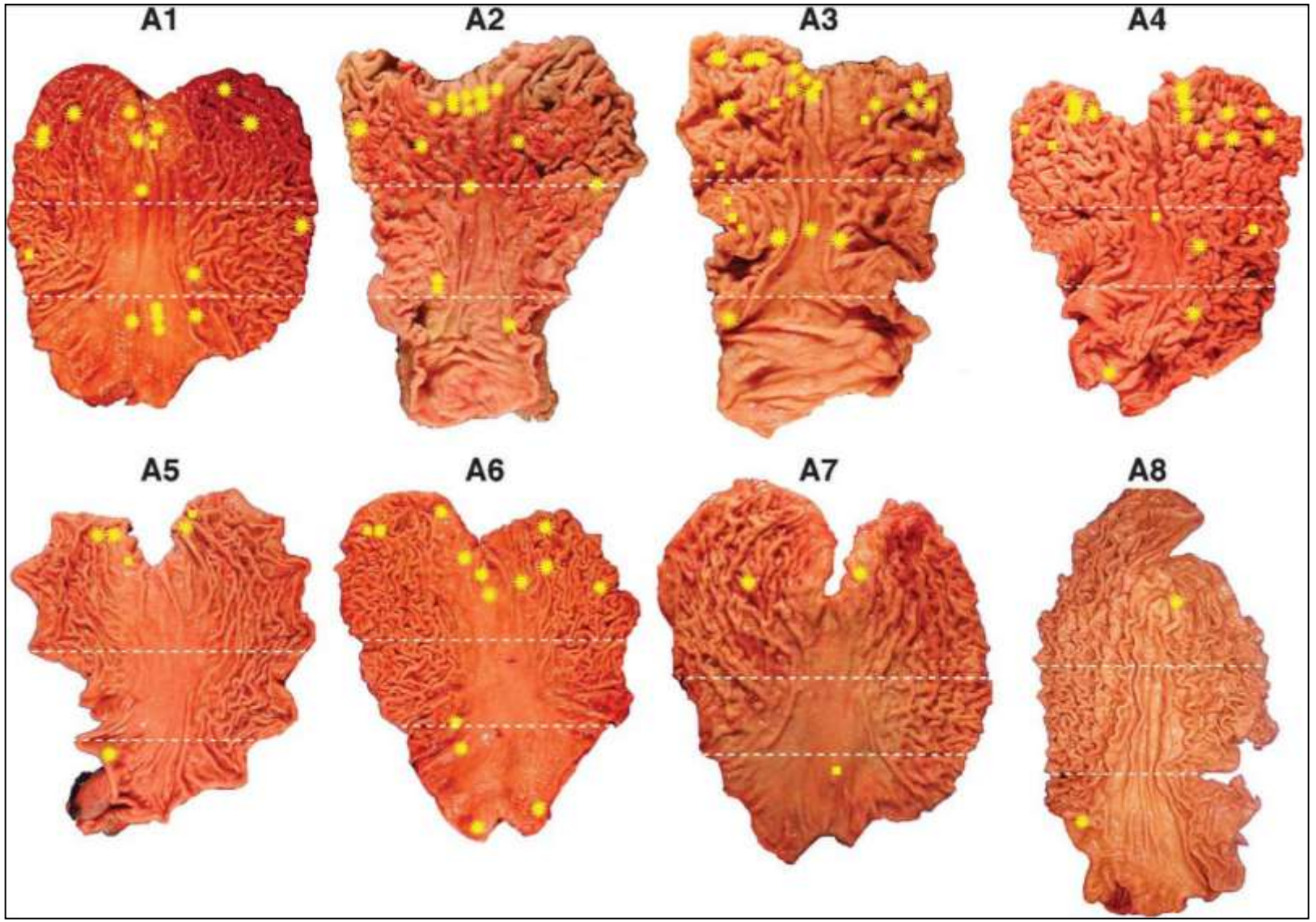
- Total gastrectomies studied under research protocol — 73
- Number of cases with intramucosal SRC carcinoma — 70* **(96%)**

* 2/3 cases negative for SRC intramucosal carcinoma displayed *in situ* carcinoma



The highest number of *foci* was found in the fundus (44.7%) followed by the body (40.2%).

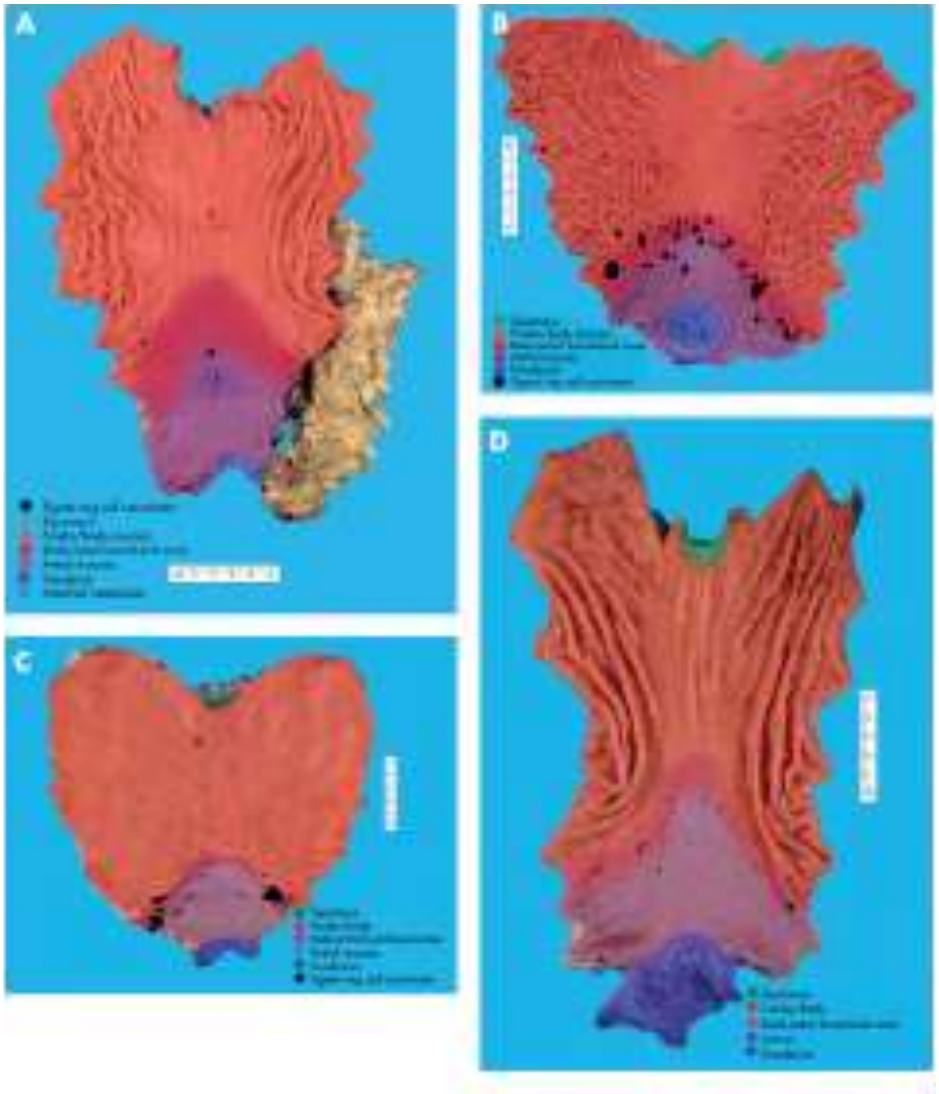
Barber M *et al*
J Pathol 216:295, 2008



70% of the total signet ring cell foci were locted within the proximal 1/3 of the stomach

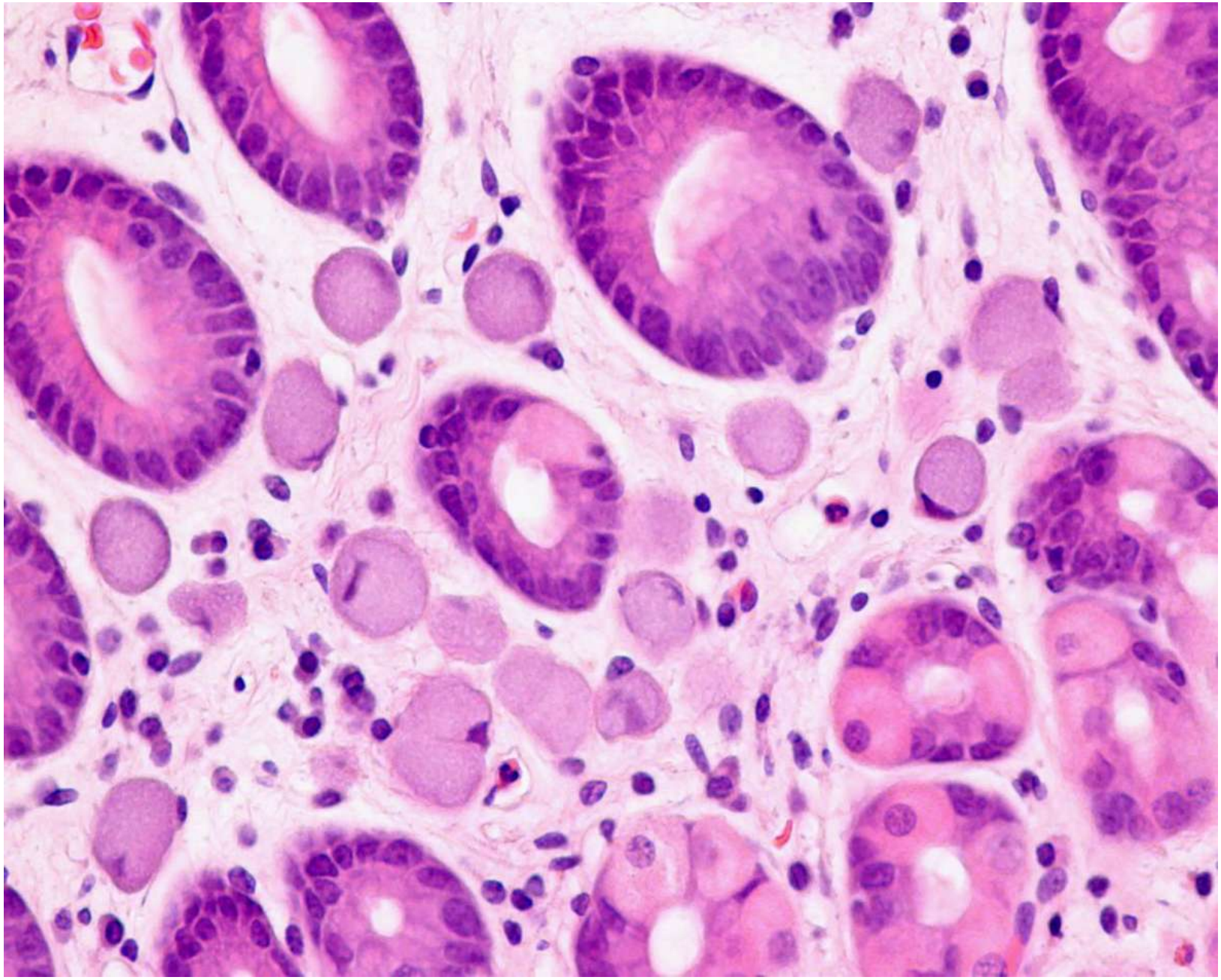
Rogers W *et al*
Am J Surg Pathol 32:799, 2008

Gastrectomies in New Zealand in *CDH1* carriers

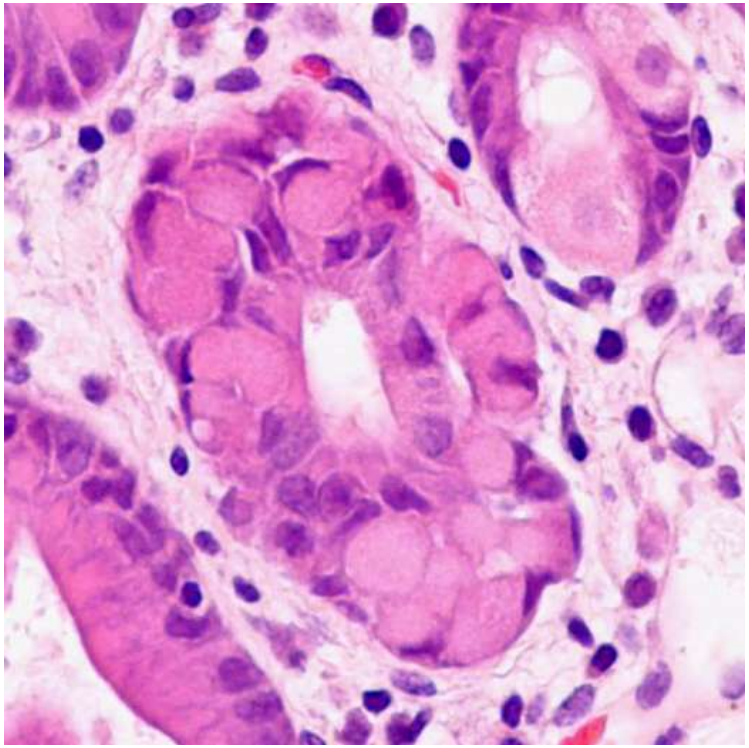


Charlton A *et al*: Hereditary diffuse gastric cancer: predominance of multiple foci of signet ring cell carcinoma in distal stomach and transitional zone. Gut 53;814-820, 2004

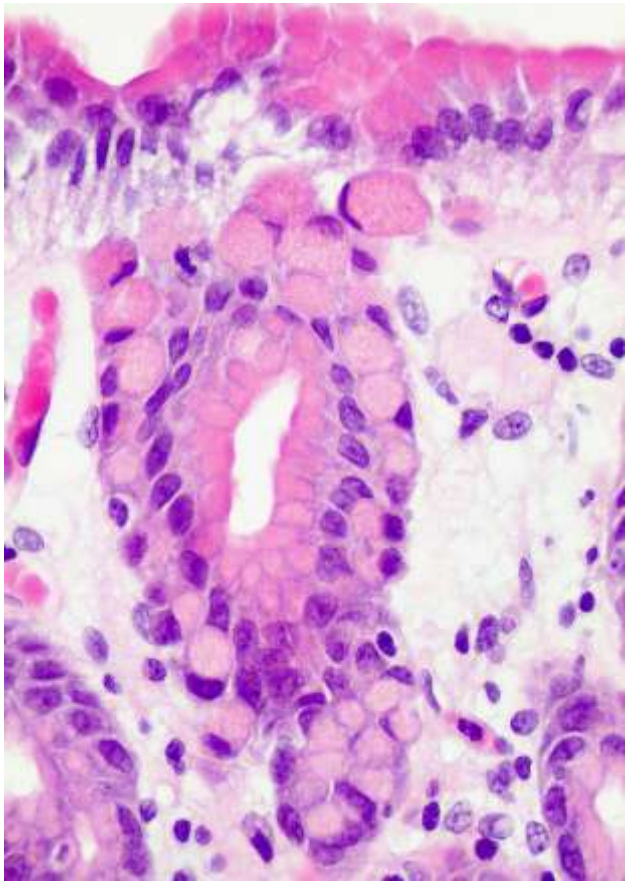
Histopathology



Intramucosal signet-ring cell (diffuse) carcinoma

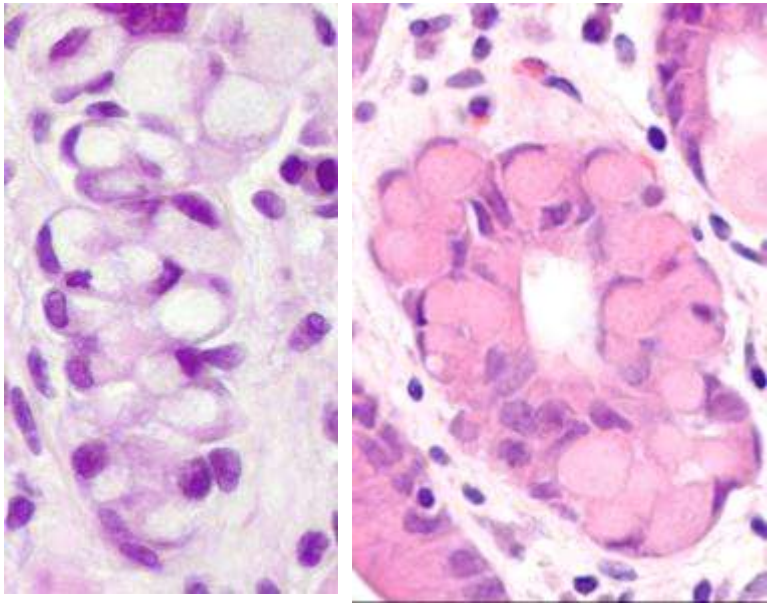


In situ (signet ring cell) carcinoma

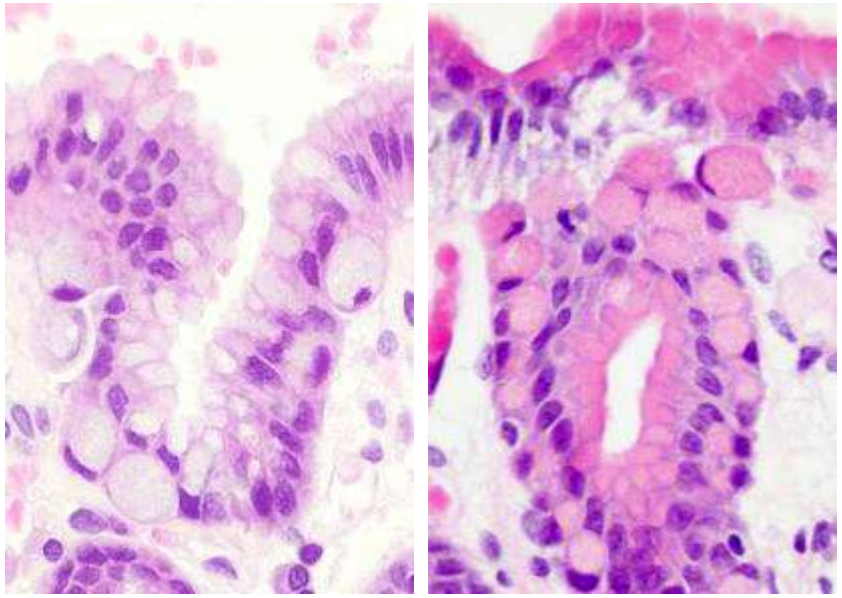


Pagetoid spread of signet ring cells:

Two-layer structure:
an inner layer composed of benign mucous cells
and an outer layer of signet ring cells.



In situ (signet ring cell) carcinoma



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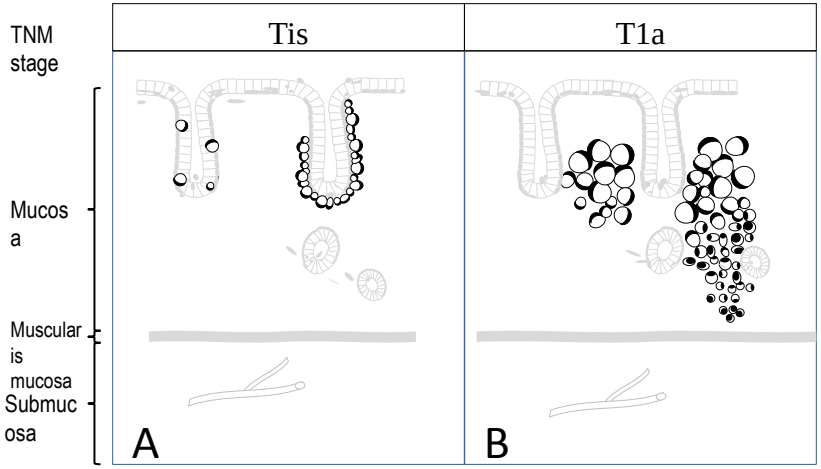


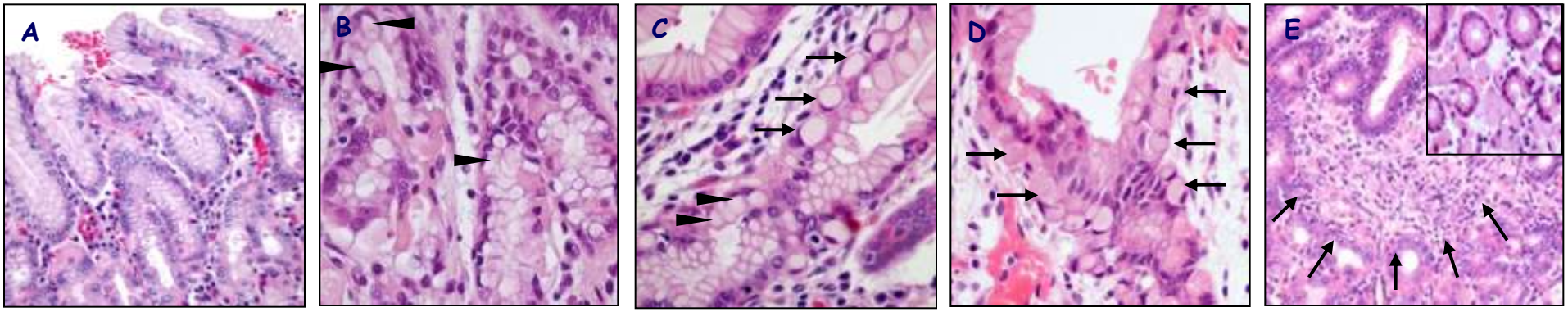
Carneiro F, Charlton A, Huntsman D
4th Edition of WHO book , 2010

Development model of HDGC

?

Inactivation of second allele of *CDH1*

CDH1 germline mutation



Non-neoplastic mucosa with foveolar hyperplasia

In situ signet ring cell carcinoma

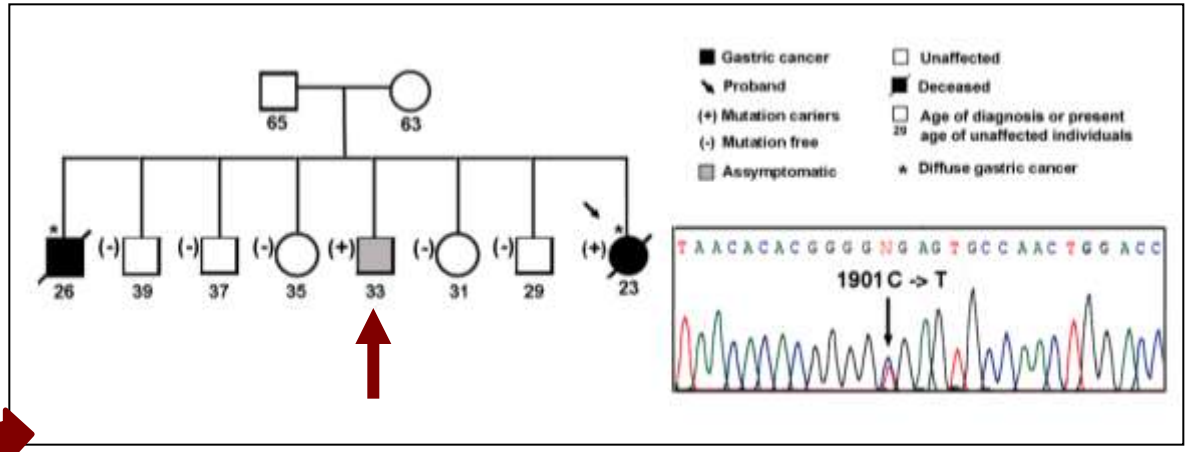
In situ signet ring cell carcinoma and pagetoid spread

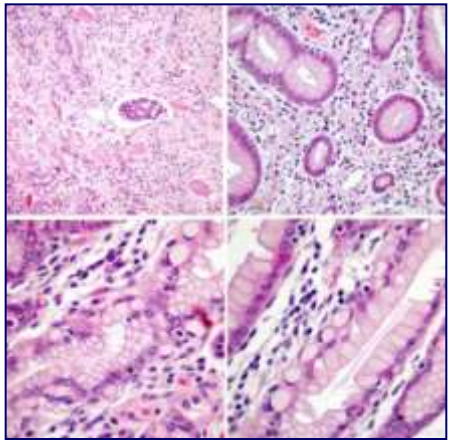
Pagetoid spread of signet ring cell carcinoma

Early invasive (intramucosal) signet ring cell carcinoma

Carneiro F *et al*
J Clin Pathol 61:25, 2008

First Portuguese HDGC Family (Porto)



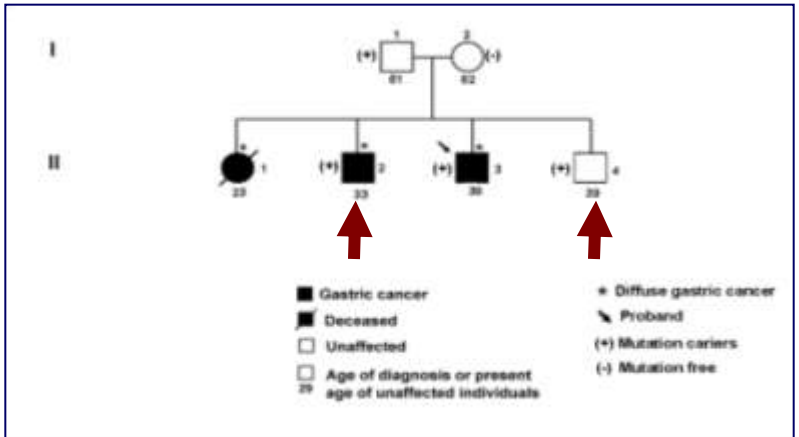


HISTOLOGICAL CLASSIFICATION

CLINICAL HISTORY

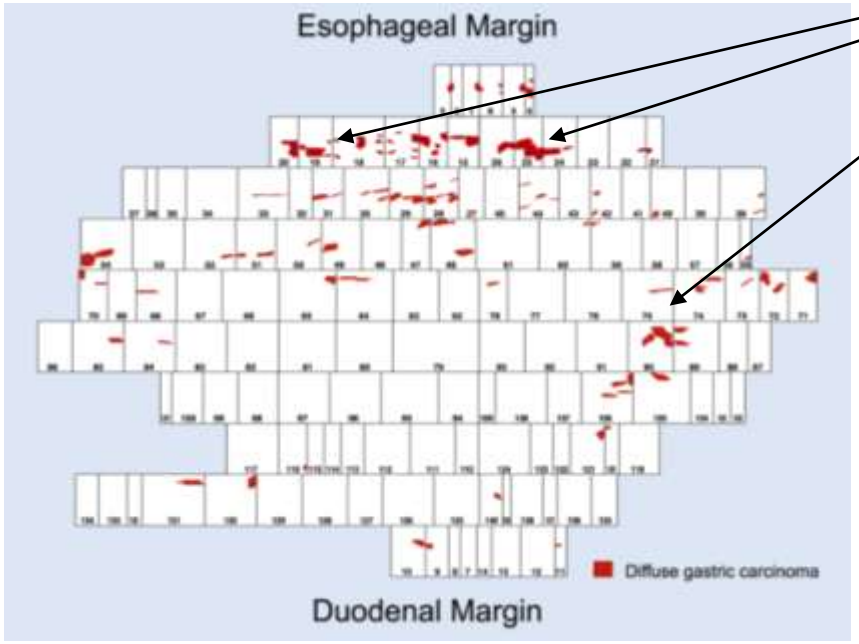
MOLECULAR CLASSIFICATION

Second Portuguese HDGC Family (Coimbra)



Is there room for targeted therapy?

2nd HIT of *CDH1* silencing?



Methylation

DNA demethylating agents

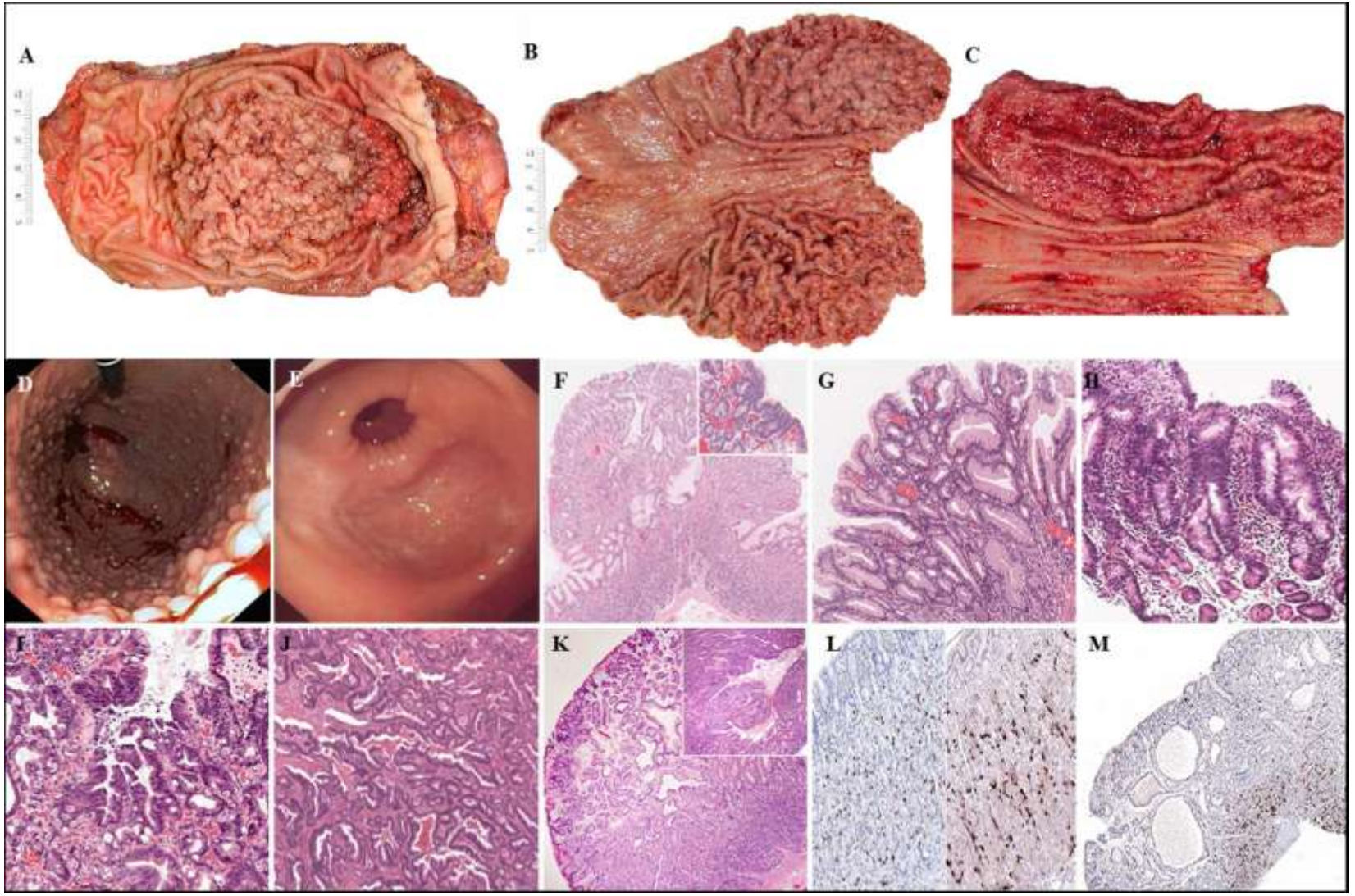
Gene structure alterations

Modulators E-cadherin expression

Partners of E-cadherin signalling

New Hereditary Gastric Cancer Syndrome:

Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS): a new autosomal dominant syndrome.



Submitted (2011)

International Gastric Cancer Linkage Consortium (IGCLC)

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MAYO CLINIC

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25th European Congress of Pathology

2013
Lisbon, Portugal

European Society of Pathology

SPAP

Sociedade Portuguesa de Anatomia Patológica