Familial gastric cancer and germline mutations of E-cadherin

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BACKGROUND: *Most gastric cancer (GC) is sporadic and seem to be mostly related to a cumulative effect of multiple environmental factors. Although the actual importance of genetic factors has not yet been fully documented, GC with familial aggregation has been found to have an incidence of 10% to 30%.*
MATERIALS AND METHODS: *Genetic factors contribute to the well-known autosomal dominant syndrome defined as hereditary diffuse gastric cancer (HDGC) which can be related to germline mutations of the gene encoding E-cadherin gene (CDH1). It has been estimated that 1-3% of cases of GC are due to HDGC.*
DISCUSSION AND CONCLUSION: *The authors review data on CDH1 mutations in HDGC ,CDH1 testing criteria, and treatment. They conclude that cancer pedigrees and screening for CDH1 mutations are essential for improving the management of this disease.*

KEY WORDS: E-Cadherin Gastric cancer, Prophylactic gastrectomy

Introduction

Most cases of gastric cancer (GC) are sporadic and, have a multifactorial etiology. They appear to be related to the cumulative carcinogenic effect of various environmental factors, especially smoking, alchohol, and diet [1]. Other risk factors for GC are H. Pylori infection, and some genetic polymorphisms of certain proinflammatory cytokines [2].
Even though the precise importance of genetic factors has not yet been fully documented, the incidence of familial GC has been reported to range from 10-30% [3]. It is certain that genetic factors are involved in the development of the well known autosomal dominant syndrome called hereditary diffuse gastric cancer (HDGC) which is associated with germline mutations of the gene for E-cadherin (CDH1). Hereditary diffuse gastric cancer constuitutes 1% of all cases of GC [4].

CDH1 and E-cadherin

The CDH1 gene has been mapped to chromosome 16q22.1 and is made up of 16 coding exons (Fig. 1). The gene codes for a 120 kdalton protein called E-cadherin which is a transmembrane glycoprotein expressed on epithelial tissues and responsible for calcium dependent intracellular adhesion. E-cadherin is essential for the stabilization, polarization, and differentiation of the epithelium because it forms intercellur adhesion complexes. Inactivation of this protein contributes to the loss of contact inhibition of growth which results in increased cell motility of tumor cells and distant metastasis for-

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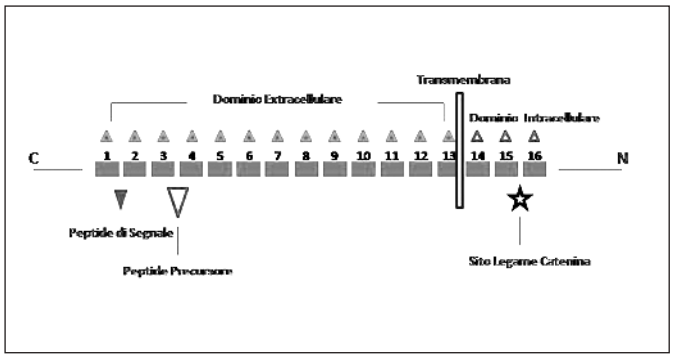

Fig. 1: Schematic structure of the CDH1 gene.

mation. For this reason E-cahedrin is generally considered to act as a tumor suppressor [3]. Deregulation of CDH1 has been frequently observed in patients with inactivating germline mutations and triggers the development of the hereditary diffuse gastric cancer (HDGC) syndrome [4].

The discovery and incidence of hereditary diffuse familial gastric cancer

It is thought that not more than 10% of the cases of GC are familial even though familial aggregation rates of up to 30% have been reported in high incidence areas [5]. However the incidence of hereditary gastric cancer resulting from germline mutations does not exceed 1-3% [6].
In 1964 Jones and colleagues described a Maori family in New Zealand in which numerous individuals developed GC at a young age [7]. In 1998 Parry Guilford was the first to demonstrate germline mutations of the CDH1 gene in three Maori families in which there were numerous cases of GC that appeared to be hereditary. All the individuals affected had diffuse- type GC and were rather young when diagnosed. Pedigree analysis also indicated a risk of lobular breast cancer [4]. Other germline mutations of the CDH1 gene were later identified in different European, American and afro-american families with diffuse-type GC [8].

The International Gastric Cancer Linkage Consortium (IGCLC) and the definition of familial GC

In 1999 the International Gastric Cancer Linkage Consortium (IGCLC) was established to define the clinical criteria for familial GC. The members of this multicenter group came from various countries (Canada, Germany, Portugal, Italy, Japan, Korea, New Zealand, England, and the United States), and proposed mass screening of the CDH1 gene in individuals with familial diffuse-type GC [8].

According to the criteria established by the IGCLC, the probability of identifying a mutation of CDH1 in these individuals ranged from 25 to 30%. Individuals with truncated mutations of the gene encoding E-cadherin were found to have a high risk of developing GC. The estimated cumulative risk of GC by age 80 is 67% for men (95% confidence interval, 39-99) and 83% for women (95% confidence interval, 58-99), with an average age of 40 at diagnosis (range 14-85 years) [9]. The criteria established for defining familial GC distinguished between the two main types the diffuse type and the intestinal type, based on Lauren's classification.

Definition of HDGC and diffuse-type familial GC: 1) Two or more first and/or second generation relatives with documented diffuse GC at least one of whom was $\leq$50 years old when diagnosed, 2) three or more first and/or second degree relatives with documented diffuse GC, independent of age at onset.

Definition of familial intestinal- type GC in high incidence countries (for instance Japan, Portugal) In contrast to diffuse GC, the intestinal type is less clearly defined since no genetic mutations responsible for hereditary transmission have been identified.
The criteria adopted by the Consortium for defining intestinal-type familial (not hereditary) GC are similar to the Amsterdam criteria used for hereditary nonpolyposis colorectal cancer (HNPCC): 1) Three first and/or second degree relatives with documented intestinal GC at least one of whom is a first degree relative, 2) At least two successive generations must be affected, 3) One affected individual who was $\leq$50 years old when diagnosed.

Definition of familial intestinal-type gastric cancer in low incidence countries (for instance United States, Europe) 1) Two or more first and/or second degree relatives with documented intestinal GC at least one of whom was $\leq$50 years old at the time of diagnosis*:* 2) Three or more cases of documented intestinal GC. Independent of age at onset.

Proposal to revise the original criteria

In 2003 Suriano and colleagues studied 66 patients $\leq$ 45 years old with sporadic diffuse-type GC, and identified 5 germline mutations of the CDH1 gene (Table I). These individuals were classified as having early onset diffuse gastric cancer (EOGC) since they had de novo mutations of the gene encoding E-cadherin [10]. To date 281 patients with diffuse-type GC and age when diagnosed $\leq$45 years have been evaluated, and 19 mutations of the gene encoding E-cadherin have been identified (6.8%) (Table I).
The discovery of new mutations in individuals with sporadic diffuse-type GC has made it possible to demon-

TABLE I - *Summary of al families that underwent screening for CDH1*

Reference	N° families	HDGC	CDH1 Mutations (%)	DFGC	CDH1 Mutations (%)	EOGC (Age <51)	CDH1 Mutations (%)	IFGC°	FGC*	Total Mutations (%)	Truncating Mutations	Missense Mutations
Guilford	3	3	3 (100)	–	–	–	–	–	–	3 (100)	3	–
Gayther	18	10	3 (30)	–	–	–	–	8	–	3 (16.7)	3	–
Richards	8	8	2 (25)	–	–	–	–	–	–	2 (25)	2	–
Guilford	6	4	4 (100)	2	2 (100)	–	–	–	–	6 (100)	6	–
Shinmura	13	3	1 (33.3)	–	–	–	–	10	–	1 (7.7)	–	1
Yoon	5	5	2 (40)	–	–	–	–	–	–	2 (40)	–	2
Iida	14	–	–	6	–	–	–	6	2	–	–	–
Keller	7	2	1 (50)	5	–	–	–	–	–	1 (14.3)	1	–
Stone	10	–	–	–	–	–	–	–	10	–	–	–
Saito	9	–	–	–	–	9	–	–	–	–	–	–
Kim	20	–	–	–	–	–	–	–	20	–	–	–
Avizienyte	11	5	1 (20)	4	–	–	–	1	1	1 (9)	–	1
Salahshor	48	–	–	–	–	-	-	-	48	–	–	–
Dussaulux–Garin	1	1	1 (100)	–	–	–	–	–	–	1 (100)	1	–
Humar	10	7	4 (5.7)	3	1 (33.3)	–	–	–	–	5 (50)	5	–
Oliveira	39	11	4 (36.4)	24	–	–	–	4	–	4 (10.3)	3	1
Yabuta	17	2	1 (50)	3	–	–	–	–	12	1 (5.9)	–	1
Wang	78	–	–	2	2 (100)	–	–	–	76	2 (2.6)	–	2
Suriano	66	–	–	–	–	66	5 (7.6)	–	–	5 (7.6)	2	3
Oliveira	1	1	1 (100)	–	–	–	–	–	–	1 (100)	1	–
Graziano	3	3	–	–	–	–	–	–	–	–	–	–
Carvalho	40	–	–	–	–	40	–	–	–	–	–	–
Jonsson	3	3	1 (33.3)	–	–	–	–	–	–	1 (33.3)	1	–
Kusano	3	3	–	–	–	–	–	–	–	–	–	–
Oliveira	32	9	1 (11.1)	10	–	–	–	3	10	1 (3.1)	–	1
Keller	45†	4	–	21	1 (4.8)	15	1 (6.6)	5	–	2 (4.4)	1	1
Brooks–Wilson	38	16	8 (50)	13	4 (30.8)	9	–	–	–	12 (31.6)	10	2
Jiang	5	5	1 (20)	–	–	–	–	–	–	1 (20)	1	–
Concolino	7	7	–	–	–	–	–	–	–	–	–	–
Moran	1	1	1 (100)	–	–	–	–	–	–	1 (100)	1	–
Suriano	30	10	3 (30)	10	3 (30)	10	2 (20)	–	–	8 (26.7)	7	1
Frebourg	2	2	2 (100)	–	–	–	–	–	–	2 (100)	2	–
Rodriguez–Sanjuan	1	1	1 (100)	–	–	–	–	–	–	1 (100)	1	–
Zhang	101	–	2 (1.9)	77	2 (2.6)	24	–	–	–	2 (2.6)	–	2
Bacani	81	–	–	–	–	81	9 (11.1)	–	–	9 (11.1)	7	2
More	36	24	2 (8.3)	12§	6 (60)	–	–	–	–	10 (27.7)	7	3
Roviello	14	14	1 (7.1)	–	–	–	–	–	–	1 (7.1)	–	1
Kaurah	38	26	12 (46.1)	6	1 (16.6)	6	–	–	–	15 (39.5)	8	5
Mayrbaeurln	1	1	1 (100)	–	–	–	–	–	–	1 (100)	8	–
Oliveira	160	160	6 (3.8)	–	–	–	–	–	–	6 (3.8)	6	–
Caron	1	1	1 (100)	–	–	–	–	–	–	1 (100)	1	1
Van Domselaar	1	1	1 (100)	–	–	–	–	–	–	1 (100)	1	–
Corso (in press)	21	–	–	–	–	21	2 (9.5)	–	–	2 (9.5)	–	2
Total	**1048**	**353/1048 (%)**	**72/353 (20.4%)**	**198/1048 (18.9%)**	**22/198 (11.1)**	**281/1048 (26.8%)**	**19/281 (6.8%)**	**37/1048 (3.5%)**	**179/10478 (17.1%)**	**121/1048 (11.5%)**	**89/120 (74.2%)**	**32/120 (26.7%)**

*No information on the criteria adopted, Cases are considered as Familial Gastric Cancer (FGC) . † Five of these 50 families have been previously described.[35] § Two of these families do not meet clinical criteria and are classified as Diffuse Familial Gastric Cancer (DFGC). °IFGC: Intestinal Familial Gastric Cancer.

strate that germline mutations in the CDH1 gene do not only occur in the HDGC syndrome. This has led Brooks-Wilson and colleagues to propose a revision of the clinical criteria. The revised criteria proposed in 2004 consist of the following: 1) two or more first and/or second generation relatives with documented diffuse GC, at least one of whom was ≤50 years old when diagnosed 2) Two or more first and/or second generation relatives with GC, including at least one with diffuse GC, at least one of whom was ≤50 years old when diagnosed

3) Three or more first and/or second degree relatives with documented diffuse GC,independent of age at onset. 4) Three or more first and/or second degree relatives with documented GC including at least one with diffuse GC diffuse GC, independent of age at onset 5) One relative with sporadic diffuse GC who was ≤45 years old when diagnosed 6) An isolated case of diffuse GC associated with lobular breast carcinoma (synchronous or metachronous) 7) Pedigree with at least one relative with diffuse GC and one with lobular breast cancer 8) Pedigree with at least one relative with diffuse GC and one with colon cancer.
However this modification of the criteria has not been universally adopted.The criteria, reviewed during the 7th workshop of the IGCLC held in Cambridge, UK, on November 20-21, 2008, which our group participated in, will be published shortly.

The missense mutations of the CDH1 gene

Missense mutations of the gene encoding E-cadherin make up about 20% of all the identified CDH1 mutations, but their role remains controversial.
In contrast to the high penetrance of truncated mutations (about 70-80%) [4,9] missense mutations result in low-penetrance phenotypes. To date the pathogenicity of these mutations has not been clearly defined and this can lead to difficulties in the management of the patients affected. In order to solve these problems Suriano and colleagues developed an *in vitro* and *in silico* method of demonstrating the true pathogenicity of missense mutations present in germline DNA [10].
The molecular technique analyzes the capacity of cells to form stable cell aggregates and invade the surrounding matrix. The same authors also proposed a statistical model to convalidate the results obtained *in vitro*. The results of these analyses were classified as neutral variants vs mutations.

Other mechanisms of germline inactivation of the CDH1 gene

Oliveira and colleagues recently described a new mechanism of inactivation of the CDH1 gene in a large series of families fulfilling the HDGC criteria [11]. The authors analyzed the extended genomic rearrangement of 160 families that were negative at CDH1 screening using the standard method (multiplex ligation - dependent probe amplification: MLPA) Deletions of E-cadherin were identified in 6 cases (3.8%), and considering the risk of hereditary transmission of these deletions, the authors recommend using this new test for screening high-risk families. It is interesting to note that all the deletions of CDH1 were identified in families from low-risk areas (North America, England) and no such mutations were found in families from high-risk areas (Portugal, central Italy) [11].

Histopathological aspects of HDGC in patients with germline mutations of CDH1

The most common histological form of HDGC is the diffuse with signet-ring cell type. In advanced stages the histology of HDGC resembles that of the sporadic forms,but in the early stages multiple foci of diffuse signet-ring cells can be found confined to the surface of the gastric mucosa 1[2] in the region of the body/fundus. Huntsman and colleagues [13] reported that the majority of the neoplastic foci found in surgical specimens after total gastrectomy were <1cm in diameter and were all located on a macroscopically normal epithelial surface.
Carneiro and colleagues [14] proposed the following histopathological model of the evolution of GC in patients with CDH1 mutations: In the initial phase histology shows a pattern of in situ signet-ring cell carcinoma with some pagetoid spread, then early invasion is followed by pagetoid proliferation of signet-ring cells and finally there is clearly identifiable signet-ring cell carcinoma.
The discrepancy between the large number of foci of infiltrating carcinoma and the small number of foci of carcinoma *in situ* suggests that invasion of the lamina propria by signet-ring cells may take place without *in situ* carcinoma being detected.
The presence of H. Pylori was ruled out in all prophylactic total gastrectomy specimens [14].

Clinical management when clinical criteria for diagnosis of HDGC are fulfilled

Up to 20% of patients with GC can have clinical characteristics of HDGC, and among these about one out of every 3-4 has a germline mutation of the CDH1 gene. In clinical practice patients with the following characteristics should be suspected of having a CDH1 mutation: 1) One or more first and/or second generation relatives with documented diffuse GC, at least one of whom was ≤ 50 years old at the time of diagnosis, 2) Two or more first and/or second degree relatives with documented GC, 3) Age at diagnosis ≤45-50 years. In any of these cases the patient should be offered genetic screening to search for mutations of the gene coding for E-cadherin. If the test is positive genetic counselling should be offered to the patients family members.

Prophylactic gastrectomy

Since the estimated risk of developing GC by the age of 80 is 80%, the IGCLC recommends prophylactic gas-

trectomy to patients with CDH1 mutations. The rationale for this is that microfoci of GC (signet ring cells), have been found in all patients with CDH1 mutations who underwent gastrectomy and were studied following an adequate protocol for pathological examination which included analyis of the entire stomach.
Another reason is that these lesions are difficult to identify on endoscopy even if multiple biopsies are taken.
There is still debate regarding the minimum age at which patients should undergo prophylactic gastrectomy. It seems that initially signet-ring cell carcinoma of the stomach remains in a latent phase with low potential for tumor progression and invasion, as confirmed by a low proliferation index. Moreover, in the families studied so far, the age at diagnosis, and number of foci of carcinoma varied greatly. This makes it seem possible that the syndrome has varying degrees of penetrance which may be linked to provenance, type of mutation, and related environmental factors [15].
Usually prophylactic total gastrectomy is recommended to patients who have reached the age of 20-30, when the risk of developing HDGC outweighs the postoperative mortality risk. The requisite operation is total gastrectomy with Roux-en-Y reconstruction. The resection line at the distal esophagus must be proximal to the Z line, in order to ensure complete removal of the gastric mucosa.

Endoscopic follow-up

Patients with a pathogenic mutation of CDH1 who are postponing surgery (for instance those who are under <20) or who refused prophylactic gastrectomy, should have surveillance endoscopy once a year. Patients with a missense mutation of uncertain pathogenic significance should also be advised to have yearly endoscopies.
Endoscopy should be performed with sedation, by an experienced endoscopist, and should last at least 30 minutes. It is important to emphasize that a result that is macroscopically negative does not exclude the presence of small microscopic foci in the gastric mucosa. For this reason it is recommended to take a large number (approximately 30) of random biopsies (from the fundus, body, body-antral transitional zone, antrum). The role of chromoendoscopy is under debate.

Conclusions

Hereditary diffuse gastric cancer is rare but the disease is of great interest since the molecular and pathogenetic mechanisms involved in gastric carcinogenesis have been clearly identified. For correct clinical management it is essential to obtain the patient's complete personal and family medical history so that sporadic and familial or hereditary forms can be identified in a cancer family tree. The first germline mutation of E-cadherin reported in Italy was identified at the University of Siena, in the Department of General Suergery and Oncology, in a family pedigree fullfilling the clinical criteria of HDGC [16,17]. This discovery made it possible for us to set up laboratories that offer reliable genetic testing for mutations in the CDH1 gene.

Riassunto

Nella maggioranza dei casi, il cancro gastrico (CG) si manifesta in forma sporadica e sembra essere per lo più correlato ad un effetto cumulativo di molteplici fattori ambientali; fumo di tabacco, consumo di alcol e abitudini alimentari sembrano essere i principali fattori di rischio (1). Altri fattori di rischio correlati allo sviluppo del CG sono rappresentati dall'infezione da *H. Pylori* e da diversi polimorfismi genetici di alcune citochine proinfiammatorie (2). Sebbene la reale importanza dei fattori genetici non sia stata ancora completamente documentata, il CG con aggregazione familiare presenta un'incidenza tra il 10% e il 30%. Per certo, i fattori genetici concorrono nella ben nota sindrome autosomica dominante definita come Carcinoma Gastrico Ereditario d'Istotipo Diffuso (o Hereditary Diffuse Gastric Cancer - *HDGC*) correlato a mutazioni costituzionali del gene della caderina-E (*CDH1*). Il *HDGC* rappresenta l'1% di tutti i casi di CG.

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