E-cadherin germline missense mutations in diffuse gastric cancer

G Corso1,2*, D Marrelli1, F Roviello1

Abstract

Introduction
Hereditary diffuse gastric cancer is an autosomal inherited syndrome associated with the E-cadherin germline mutations. Different types of CDH1 germline mutations have been reported; the missense alterations are rarely identified when compared with truncating mutations. The identification of missense mutation represents a clinical burden, since its pathogenicity is still under genetic and clinical studies. The aim of this critical review was to discuss E-cadherin germline missense mutations in diffuse gastric cancer.

Materials and methods
In this paper, we reviewed the literature data about the CDH1 germline missense mutations identified in early-onset and hereditary diffuse gastric tumours. The roles of clinical surveillance and prophylactic gastrectomy were discussed. Mutations reported in breast lobular cancer were excluded.

Results
We identified a total of 31 CDH1 germline missense mutations with different pathogenic impact on E-cadherin function (pathogenic vs. neutral). The majority of these alterations were localized at the extracellular domain of the CDH1 gene. These mutations affected indifferently hereditary and sporadic diffuse gastric carcinomas.

Conclusion
In this critical review, we reported all missense mutations identified to date and discussed about the E-cadherin missense mutation function, clinical management for asymptomatic mutation carriers and the roles of prophylactic total gastrectomy and endoscopic surveillance.

Introduction
The CDH1 gene (OMIM no. 192090) is located on chromosome 16q22.1 and encodes for the E-cadherin protein. This macro-molecule is a transmembrane glycoprotein expressed on epithelial tissue and is responsible for calcium-dependent, cell-to-cell adhesion.

E-cadherin is critical for establishing and maintaining polarized and differentiated epithelia through intercellular adhesion complexes. The human E-cadherin function is to suppress cell invasion; in fact its deregulation is correlated with the infiltrative and metastatic ability of the tumour, with the consequent loss of cell adhesion and concomitant increase in cell motility. In human samples, somatic CDH1 alterations are associated with poor survival and worse prognosis in gastric cancer patients.

E-cadherin germline mutations are responsible for hereditary diffuse gastric cancer (HDGC; OMIM no. 137215) development, an autosomal inherited syndrome. First, it was described in a Maori population with a strong cluster for DGC; to date, various germline CDH1 mutations have been detected from different countries. These E-cadherin constitutional alterations are also described in sporadic early-onset DGC with age at diagnosis of ≤35 years.

The estimated cancer risk in the asymptomatic CDH1 truncating mutation carrier is about 80%; for the missense mutations, the penetrance risk is undefined.

The clinical management of the asymptomatic CDH1 mutations carriers is defined in accordance with the recent guidelines described in the last consensus conference in Cambridge in 2010. However, these guidelines did not include the E-cadherin missense mutations, because the authors are still debating about its clinical aggressiveness.

In this critical review, we reviewed the CDH1 germline missense mutations identified to date and proposed a model of carcinogenesis in mutant carriers. In addition, we discussed about the clinical management of asymptomatic carriers.

Clinical criteria for the definition of hereditary and sporadic diffuse gastric cancer
In 2010, in Cambridge, the International GC Linkage Consortium (IGCLC) revised the clinical criteria for the HDGC definition. In Table 1, we describe these clinical criteria in detail.

Regarding early-onset DGC, recently Corso et al. proved that CDH1 mutation pathogenicity has been assigned only to a few percentage of cases who were recurrently diagnosed before the age of 35 years. At genetic screening, the authors recommended this to the candidate only in cases with confirmed early-onset DGC and those who were ≤35 years of age.

*Corresponding author Email: corso.giov@tiscali.it
1 Department of Medical, Surgical Sciences and Neuroscience, Section of General Surgery and Surgical Oncology, University of Siena, Siena, Italy
2 Molecular Medicine Laboratory, European Institute of Oncology, Milano, Italy

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**E-cadherin missense mutation**

**Description of mutations**

The \(CDH1\) germline mutations identified to date are classified as follows: (a) truncating: point or large deletions, insertion with frameshift; (b) splice-site: frequently identified also in cryptic points; (c) nonsense; (d) silent; (e) intragenic and (f) missense alterations.

E-cadherin germline missense mutations are subclassified into pathogenic and neutral variants. The pathogenic role is demonstrated using specified laboratory tests; Functional *in vitro* assays and *in silico* working models have been developed to characterize HDGC-associated E-cadherin germline missense mutations. Subsequently, additional techniques, such as the proximity ligation assays, are considered for a more accurate and robust evaluation of mutation pathogenic significance for diagnostic purposes in E-cadherin germline missense mutation carriers. The neutral variants are considered as single-nucleotide polymorphisms that are also frequently identified in the control population. For a better understanding about genetic tests, as with *in vitro* and *in silico* analyses, we recommend the exploration of specific bibliographic references.

In this study, we identified a total of 31 germline E-cadherin mutations; in particular, these mutations are located at the signal peptide site (1; 3.3%), precursor domain (2; 6.5%), cytoplasmic domain (6; 19.3%) and extracellular site (22; 70.9%). As shown, we verified that the majority of \(CDH1\) germline missense alterations occurred at the extracellular domain. This gene point probably represents a vulnerable site for those missense mutations. Pathogenicity was assessed in 16 (51.6%) germline mutations, seven (22.6%) were unpathogenic and the functional role of eight cases was unknown.

**Extracellular E-cadherin mutation and epidermal growth factor receptor activity**

E-cadherin constitutes specific complexes with the receptor protein tyrosine kinases, named the epidermal growth factor receptor (EGFR), at basolateral areas of polarized epithelial cells. EGFR has been reported to be involved in a bidirectional cross-talk with E-cadherin. Activated EGFR is implicated in the signalling pathway of cell migration and invasion; the interaction of E-cadherin with EGFR requires an intact extracellular E-cadherin domain. The presence of extracellular E-cadherin missense mutations disturbs its ability to interact with the EGFR, causing an increased receptorial activity. In particular, the presence of E-cadherin missense mutations causes a reduced E-cadherin/EGFR affinity, with an increased autophosphorylation on tyrosine residues leading to enhanced cell motility. These observations suggested that E-cadherin plays an inhibitor function on EGFR activity.

**Clinical observations**

In familial genetic screening, the non-missense mutations (truncating, insertion, etc.) represent the majority. The frequencies of non-missense versus missense \(CDH1\) germline mutations are respectively 72% versus 28%. In particular, only HDGC E-cadherin missense mutations show a frequency of about 20%. Recently, we have verified in a meta-analysis study that the frequency of E-cadherin missense mutations was higher in high-risk areas for gastric carcinoma incidence (Japan, China, North of Portugal, central Italy, etc.). Notably, in this study, we are convinced that geographic variability alone represents an important risk factor for familial GC development. Moreover, we have suspected that missense mutations, with respect to the truncating forms, exercised a different role on gastric carcinogenesis. We could argue that exogenous factors have promoted the pathogenic role of missense mutations in gastric tumour transformation.

In Figure 1, we proposed a model of familial gastric carcinogenesis, and in Figure 2 we illustrated all \(CDH1\) germline mutations described to date. Revising literature data and original reports, we identified a total of 31 germline missense mutations. In detail, 16 (51.6%) missense mutations showed a functional impact and were defined as ‘pathogenic’; seven (22.6%) germline alterations were classified as ‘unpathogenic’ or better as neutral variants without clinical significance; finally, eight (25.8%) mutations were without information, probably never tested. The mean age for pathogenic mutation is 43.7 years (range 23–79) and for unpathogenic alteration is 47.5 years (range 40–62). The majority of pathogenic \(CDH1\) germline missense mutations occurred in familial or hereditary GC cases, whereas these mutations are very rarely identified in a sporadic setting (Table 2).

**Discussion**

The authors have referenced some of their own studies in this review. These referenced studies have been...
Pagetoid elements are characteristics of HDGC development. The mature protein of the CDH1 gene is transcribed into a 4.5-kb mRNA that encodes for the 120-kDa protein E-cadherin. This protein has a signal peptide containing 27 amino acids encoded by exons 1 and 2, a precursor peptide consisting of 154 amino acids encoded by exons 2 to 4 and a mature protein containing 728 amino acids encoded by exons 4 to 16. The mature protein segment has an extracellular domain that includes exons 4–13, a smaller transmembrane domain that includes exons 13 and 14 and the cytoplasmic domain (C-terminal) comprises exons 14–16. 

The missense mutations span throughout the entire CDH1 coding regions. The location of CDH1 germline mutations is identified in familial/hereditary cases (in red), in sporadic gastric cancer (in blue) and in undefined familial setting (black). The choice of prophylactic surgery or endoscopic surveillance in pathogenic missense mutation carriers is still debated. The number of cases with missense mutations treated with prophylactic surgery is rather low to assess a definitive conclusion. 

**Prophylactic total gastrectomy**  
The IGCLC in 2010 recommended the prophylactic total gastrectomy for the treatment of asymptomatic CDH1 mutation carriers. To date, more than 100 gastrectomies have been performed in deleterious E-cadherin mutation carriers. Recently, two CDH1 germline missense mutation carriers underwent prophylactic gastrectomy; microscopic neoplastic foci were detected in both gastric samples. However, previously, it has been described as an asymptomatic carrier of a CDH1 germline missense mutation with a pathogenic role but without evidence of mucosal cancer spreading at the endoscopic surveillance. This individual is still living and cancer free. The authors in this case preferred endoscopic and clinical surveillances.

Clinical management and endoscopic surveillance  
Kluijt and collaborators recently proposed novel guidelines for the surveillance of gastric mucosa in asymptomatic individuals. In particular, these guidelines recommended gastroduodenoscopy at the age of 40 years (or at an age of 5 years younger than the youngest diagnosis in a family) with *Helicobacter pylori* testing and eradication. Particular attention should be given also to diet habits— in GC high incidence areas and in cases with strong familial aggregation.
determined foods, such as high consumption of grilled red meat and meat sauce, are associated with an increased risk of GC development. General recommendations are to modify these dietary habits.

For the asymptomatic CDH1 germline missense mutation carriers, we recommended a multidisciplinary approach with genetic counselling. Taking into consideration the age at onset and gender of affected kindred, we identified, whenever possible, the pathogenic impact, the geographic variability and the age at onset.

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Conclusion
Clinical management of E-cadherin germline missense mutation carriers in asymptomatic cases is complex; a multidisciplinary approach is required. In individuals fulfilling the IGCLC clinical criteria, genetic test is strongly recommended and in missense alterations functional analysis should be considered. The role of prophylactic total gastrectomy in CDH1 pathogenic mutation carriers is still controversial, because the penetrance risk is undefined. Interestingly, two novel cases of prophylactic surgery in two missense mutation carriers were recently described. This new finding opens an innovative approach for a curative treatment of asymptomatic CDH1 germline missense mutation carriers.

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Abbreviations list
EGFR, epidermal growth factor receptor; HDGC, hereditary diffuse gastric cancer; IGCLC, International GC Linkage Consortium

References

Critical review


