Genetics of hereditary diffuse gastric cancer: progress and future challenges

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Hereditary gastric cancer is a rare cancer susceptibility syndrome. One third of hereditary diffuse gastric cancer syndrome families carry germline mutations of the E-cadherin gene. Owing to the limitation of the current endoscopic screening techniques and since no chemoprevention is yet available, total prophylactic gastrectomy is the only option offered to carriers of inactivating mutations in genetic counseling. In this regard, 30% of the E-cadherin germline mutations reported to date are of the missense type, and since their pathogenic significance is not straightforward, the management of carriers of such mutations is suboptimal. In the absence of definitive clinical evidence, functional in vitro studies together with in silico analysis have been used to infer the pathogenic significance of germline missense mutations. Since most of the HDGC families reported to date are negative for E-cadherin germline mutations, the identification of alternative genes underlying the tumorigenesis of diffuse gastric cancer has become an important target for research.

Gastric cancer

Gastric cancer is one of the three leading causes of cancer death worldwide. According to Lauren’s classification, there are two major histopathological variants, namely, an intestinal type and a diffuse type [1]. The intestinal variant shows components of glandular or intestinal architecture together with tubular structures. On the other hand, in the diffuse variant, single cells or poorly cohesive clusters of cells infiltrate the gastric wall. These cells contain a globule of intracellular mucin and an eccentric nucleus, recalling a signet ring.

As much as 90% of gastric cancer cases arise in a sporadic setting, whereas, for the remaining 10%, familial clustering is observed [2–4]. Both familial and sporadic gastric cancers are products of multiple genetic and epigenetic alterations that transform normal gastric epithelial cells into malignant neoplasms. These include, activation of oncogenes through mutation and/or amplification, or biallelic inactivation of tumor suppressor genes through mutation and loss of heterozygosity (LOH) or promoter hypermethylation [5]. It appears that the molecular basis of the differences in morphology and behavior of intestinal versus diffuse gastric cancers could be attributed at least in part to differences in E-cadherin function [6]. The genetic alteration underlying the hereditary forms of intestinal gastric cancer still remains to be identified.

E-cadherin

E-cadherin is a transmembrane glycoprotein critical for establishing and maintaining polarized and differentiated epithelia during development and in adult tissues. Adherens junctions (AJs) cluster, via homophilic interactions, through the extracellular domains of calcium-dependent E-cadherin molecules on the surface of homotypic neighbor cells [7]. β-catenin binds through its Armadillo repeats to the distal region of the E-cadherin tail, thereby stabilizing the E-cadherin molecule and facilitating transport of the newly synthesized protein to the cell surface [8]. β-catenin binds to α-catenin and links components of the AJs to the actin cytoskeleton. p120ctn, another member of the catenin family, interacts with E-cadherin at the level of its juxtamembrane domain. Although static at first, AJs undergo dynamic rearrangement with cadherin molecules entering the endocytosis pathway, being either recycled back to the plasma membrane or ubiquinated for lysosomal degradation [9]. A role for E-cadherin in tumor development is now well established, with many human carcinomas, such as skin, head and neck, lung, breast, thyroid, gastric, colon and ovarian, exhibiting reduced E-cadherin expression relative to their normal cellular counterparts [10]. Experimental evidence supports a role for the E-cadherin complex both in suppressing invasion and metastasis formation [10]. Somatic mutations in cadherin type 1 (CDH1) have been identified in 40–83% of sporadic diffuse-type gastric cancers, but not in sporadic intestinal-type gastric cancers [11]. This observation provided the rationale for considering the CDH1 gene as a candidate susceptibility factor for hereditary diffuse gastric cancer (HDGC) [12].

Keywords: candidate genes, E-cadherin, gastric cancer, germline, hereditary diffuse gastric cancer, mutations, prophylactic gastrectomy
Genetics of hereditary gastric cancer
HDGC is an autosomal dominant inherited gastric cancer susceptibility syndrome. For the majority of families with clustering of gastric cancers, the etiology is likely multifactorial [13]. In 1998, germline truncating mutations of the E-cadherin gene were described in three Maori families with predisposition to diffuse gastric cancer [12]. Similar mutations have since been described in close to 60 other families of different ethnic backgrounds [14]. In 1999, the International GC Linkage Consortium (IGCLC) was formed and the first clinical criteria for HDGC were defined as two or more documented cases of diffuse gastric cancer in first/second degree relatives, with at least one diagnosed before the age of 50; and three or more cases of documented diffuse gastric cancer in first/second degree relatives, independently of age of onset [15]. Recently, on the basis of the results obtained from the ascertainment of 73 new HDGC families, it was demonstrated that the presence in the pedigree of a confirmed diffuse carcinoma diagnosed before the age of 50, together with a heavy history of gastric cancer, represent the optimal criterion for the identification of E-cadherin germline mutations in HDGC families [16,17]. In addition, CDH1 mutations were also identified in early-onset isolated cases of diffuse gastric cancer (age <35 years) and in families with multiple cases of lobular breast cancer (LBC) or any history of gastric cancer [17], confirming the existence of an association between CDH1 mutations and LBC.

Management of hereditary diffuse gastric cancer
The importance of identifying the genetic basis of cancer susceptibility in HDGC families has been underscored by recent reports of endoscopically silent early diffuse gastric cancers in prophylactic gastrectomy samples from germline E-cadherin mutation carriers [17–19]. These findings suggest that prophylactic gastrectomy may be the best treatment for germline mutation carriers and that current endoscopic screening techniques might be inadequate. Nevertheless, the adverse effects on morbidity, mortality, nutritional status, and quality of life associated to this surgery should not be neglected [20]. Recently Show and colleagues discussed the potential of chromoendoscopy for the surveillance of E-cadherin mutation carriers [21]. When this modality is validated and becomes routinely available, it might represent a valid alternative to prophylactic gastrectomy, especially for carriers who choose not to undergo prophylactic gastrectomy for medical or social reasons. To date, total prophylactic gastrectomies have been offered to and performed only on HDGC-carriers of inactivating, highly penetrant germline mutations. Owing to its high morbidity, it was suggested that prophylactic gastrectomy should not be recommended to members of HDGC families in which a causative mutation has not been identified and more in general to individuals with an unconfirmed risk [18]. In this respect, and as observed for other cancer predisposing genes such as BRCA1 or MLH1 [22,23], germline CDH1 mutations of the missense type represent a problem for genetic counseling, since their pathogenic relevance is not straightforward.

CDH1 germline missense mutations
Of the 58 E-cadherin germline mutations reported to date in both hereditary diffuse gastric cancer families and early-onset diffuse gastric cancer cases [14], 19 (33%) are of the missense type (Table 1). These mutations span the entire coding region of the E-cadherin gene, without preferential hot-spot. Contrary to the E-cadherin germline truncating mutations for which 80% disease-penetrance is estimated [6], missense mutations display a low-penetration phenotype, with few mutation carriers affected within pedigrees. This, together with the fact that these HDGC families are usually very small and providing very few individuals that are available for testing, has not allowed segregation analysis within E-cadherin germline missense mutations families. Lacking this clinical information, it appears very difficult to understand the pathogenic significance of missense mutations. In this regard, in silico analysis and in vitro functional assays have been performed to help infer the deleterious nature of E-cadherin germline missense variants [16,17,30,32,35].

Predicting the impact of amino acid substitutions on protein function through evolutionary conservation
This approach assumes that functionally relevant amino acids will be conserved between species. In figure 1 the authors have aligned the E-cadherin protein sequence of five different animal species (human, rat, mouse, dog and chicken) and highlighted in the human sequence the positions at which mutations have been found. If we consider the germline missense mutation W409R, this position only contains the amino acid tryptophan for all the sequences aligned.
Table 1. CDH1 germline missense mutations found to date in the setting of both hereditary diffuse gastric cancer and early onset isolated cases.

<table>
<thead>
<tr>
<th>E-cadherin germline missense mutation</th>
<th>Author/date</th>
<th>Setting</th>
<th>Functional significance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>T118R (exon 3)</td>
<td>Unpublished data</td>
<td>HDGC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>L214P (exon 5)</td>
<td>Unpublished data</td>
<td>HDGC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>G239R (exon 6)</td>
<td>Unpublished data</td>
<td>HDGC</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>D244G (exon 6)</td>
<td>Yoon et al.: (1999)</td>
<td>HDGC</td>
<td>ND</td>
<td>[26]</td>
</tr>
<tr>
<td>T340A (exon 8)</td>
<td>Kim et al.: (2000)</td>
<td>ND</td>
<td>Yes</td>
<td>[27]</td>
</tr>
<tr>
<td>A592T (exon 12)</td>
<td>Salahshor et al.: (2001)</td>
<td>HDGC</td>
<td>No</td>
<td>[31]</td>
</tr>
<tr>
<td>V832M (exon 16)</td>
<td>Yabuta et al.: (2002)</td>
<td>HDGC</td>
<td>Yes</td>
<td>[34]</td>
</tr>
</tbody>
</table>

*Was reported as L599V;
1 Patients were classified according to the Amsterdam criteria for hereditary non-polyposis colorectal cancer (HNPCC).

HDGC: Hereditary diffuse gastric cancer syndrome; CDH1: Cadherin Type 1
This would indicate that substitution to any other amino acid is selected against and that tryptophan is necessary for protein function. Therefore, a change to any other amino acid will be predicted to be deleterious to protein function. On the other hand, considering the germ-line mutation I487L at this position the alignment contains the hydrophobic amino acids isoleucine and valine, therefore it could be predicted that this position can only contain amino acids with hydrophobic character. Since leucine is also a hydrophobic amino acid, the change to I487L could be tolerated, but not changes to other residues, such as charged or polar amino acid. Using this rationale, of the 19 missense mutations reported, 10 (53%) were predicted to affect protein function (highlighted in red, Figure 1), while the remaining 9 (47%) would be tolerated (reported in black, Figure 1).

Other secondary effects, such as reduced mRNA stability or abnormal splicing, are considered below.

In vitro characterization of the functional effect of germ-line missense mutations on the E-cadherin activity is the most informative tool currently available for the classification of mutations.

On the basis of the E-cadherin ability to mediate cell–cell adhesion and suppress cell invasion, the authors have created a functional cell model that determines the impact of missense mutation on the protein function [32]. Using this in vitro model we have characterized 13 of the 19 E-cadherin germ-line missense mutations reported to date (Table 1). With the exception of A617T and A592T, for which only mild effects were observed [30], all the other missense mutations impaired in vitro the E-cadherin ability to mediate cell–cell adhesion and suppress invasion, supporting their pathogenic nature. Interestingly, A592T and A617T were also described to be present in normal population control at polymorphic frequency [30,32] and were also predicted to be tolerated by in silico characterization. Together, these findings suggest that A592T and A617T are neutral variants, which do not influence the risk of hereditary gastric cancer in general, but could have a predisposing role within the specific pedigrees reported.

The above mentioned in vitro system has also provided evidences for an association between the specific location of each mutation in the E-cadherin gene and cell phenotype [36]. It was demonstrated that mutations on the extracellular domain of the protein exhibit enhanced cell motility mediated through ras homolog gene pathway (RhoA) activation; on the contrary, the intracellular V832M mutation adjacent to the β-catenin-binding domain hampers cell motility by destabilizing the E-cadherin/β-catenin functional complex [36]. These differences may account for distinct clinical outcomes depending on the domain affected by the mutations. Using the same subset of mutations, the authors were recently able to demonstrate that in vitro loss of functional E-cadherin renders cells more resistant to apoptotic stimuli [37]. The existence of a possible interplay between E-cadherin and the antiapoptotic B cell CLL/lymphoma 2 (bcl-2) was also demonstrated, bringing new insights into the understanding of the tumorigenic process independent of E-cadherin deregulation. As well worthy of note, the apoptotic agent taxol was used in this study to induce cell death. Taxol is a chemical widely used in the treatment of advanced cancers, including epithelial tumors resulting from E-cadherin loss; these results question the effectiveness of this treatment in these types of tumors and calls for further investigation.

### Figure 1. E-cadherin protein sequences from five different species (human, rat, mouse, chicken and dog) have been aligned.

<table>
<thead>
<tr>
<th></th>
<th>G62V</th>
<th>L214P</th>
<th>D244G</th>
<th>W409R</th>
<th>P429S</th>
<th>T599S</th>
<th>A634V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>G</td>
<td>T</td>
<td>L</td>
<td>G</td>
<td>D</td>
<td>A</td>
<td>T</td>
</tr>
<tr>
<td>Rat</td>
<td>G</td>
<td>S</td>
<td>P</td>
<td>L</td>
<td>G</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>Mouse</td>
<td>G</td>
<td>S</td>
<td>P</td>
<td>L</td>
<td>G</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>Chicken</td>
<td>G</td>
<td>R</td>
<td>P</td>
<td>L</td>
<td>G</td>
<td>D</td>
<td>G</td>
</tr>
<tr>
<td>Dog</td>
<td>G</td>
<td>R</td>
<td>P</td>
<td>L</td>
<td>G</td>
<td>D</td>
<td>G</td>
</tr>
</tbody>
</table>

The positions of germline missense mutations affecting highly conserved residues have been high-lighted in red, in order to imply the degree of conservation.
research on the subject. Another aspect to be considered is that occasionally point mutations could be responsible for the activation of cryptic splice site, as reported for the germline mutation A634V. This missense alteration was identified in a colon cancer cell line and transcript analysis revealed an aberrant splicing associated to the mutation [38]. Interestingly, it was demonstrated that even the full-length mutated protein harbors in vitro dramatic functional consequences on the E-cadherin functions [32], further supporting its pathogenic nature.

**HDGC families negative for CDH1 germline mutations**

Since the first description of E-cadherin germline mutations in families with excess of diffuse gastric cancer and the definition of the HDGC syndrome, mutation analysis in families fulfilling the HDGC clinical criteria have yielded conflicting results. Two thirds of the HDGC families analyzed to date are negative for CDH1 germline mutations [14, 16, 17], suggesting that most of these families might carry mutations in other, yet to be identified susceptibility genes. A candidate gene approach has been applied to identify novel susceptibility genes. Taking into account the biology of E-cadherin, obvious candidate genes for mutations are the E-cadherin binding partners within the adhesion complex, namely β-catenin, γ-catenin, α-catenin and p120-catenin. β-catenin gene (CTNNB1) mutations have been described in intestinal-type gastric cancer and the authors also reported CTNNB1 gene amplification in a mixed-type gastric cancer, but not in diffuse gastric cancer [39]. Oliveira and colleagues screened a series of 32 HDGC families and 23 early-onset gastric cancer patients for β-catenin exon 3 germline mutations, but no mutations were identified [33]. Recently, Huntsman and colleagues assessed the α-catenin, β-catenin, γ-catenin and p120 catenin mutational status in 29 CDH1 germline mutation negative HDGC families from North America and England, but no positive cases were found [6]. From this analysis, it appears that catenins are not major diffuse gastric cancer susceptibility genes.

Other candidate genes for which data on the mutational status in CDH1 germline mutation negative HDGC families are available include: TP53, Caspase-10 and SMAD-4 [30, 33], but none of these genes appear to be a key player in the tumorigenesis of diffuse gastric cancer. Compensating this lack of knowledge is a mandatory step not only towards an improved HDGC families management, but also towards the understanding of the biology of diffuse gastric cancer tumorigenesis.

**Conclusions**

Germline mutations of the E-cadherin gene represent the genetic cause of approximately 30% of HDGC families. E-cadherin germline inactivating mutations have a disease-penetrance in the range of 70 to 80%. Considering that diffuse gastric cancers become symptomatic only when they are incurable and the inadequacy of endoscopy screening, prophylactic total gastrectomy is the only option available for germline mutation carriers. This highlights the importance of genetic screening for the identification of at-risk individuals, also stressing that more effort should be put in the identification of alternative genes responsible for HDGC in families for E-cadherin germline mutations. Of the 58 E-cadherin germline mutations reported to date in both HDGC families and isolated cases, 19 (33%) are missense mutations, representing a clinical problem for the counseling of mutation carriers. In the absence of clinical observations, functional in vitro and in silico analyses of CDH1 missense mutations have been used as an adjunct for the counseling of families.

**Future perspective**

The identification of alternative genes in HDGC families negative for CDH1 mutations as well as the development of evidence-based management of cancer risk for HDGC carriers of E-cadherin germline missense mutations represent the main target of research in HDGC. It is predictable that in the coming years, samples for full genome linkage analysis from HDGC families negative for E-cadherin germline mutations will be available, ultimately leading to the identification of a gene (likely a tumor-suppressor gene) other than E-cadherin responsible for HDGC when mutated.

It is also predictable that on the basis of the in vitro and in silico predictions, as well as clinical observations (i.e., cosegregation within pedigrees), the first prophylactic gastrectomies will also be performed also on carriers of missense mutations. This will enable the creation of more appropriate guidelines for the management of E-cadherin germline mutation carriers of the truncating and missense type. Moreover, by combining in vitro and in vivo studies, signaling pathways disturbed upon E-cadherin deregulation will be identified and their role in cancer progression assessed. These signal molecules will represent the ideal targets for potential therapeutic intervention.
Executive summary

Gastric cancer

- 90% of gastric cancer cases appear in a sporadic setting, whereas familial clustering is observed in the remaining 10%. Of these, only 1–3% are hereditary.
- Intestinal type and diffuse type are the two major histopathological variants of gastric cancer.
- Differences in E-cadherin function could, at least in part, account for differences in morphology and behavior of intestinal versus diffuse gastric cancers.

E-cadherin

- E-cadherin is a transmembrane glycoprotein critical for establishing and maintaining polarized and differentiated epithelia.
- A role for E-cadherin in tumor development is now well established, with many human carcinomas exhibiting reduced E-cadherin expression relative to their normal cellular counterparts.
- Somatic mutations in cadherin Type 1 (CDH1) have been identified in 40–83% of sporadic diffuse-type gastric cancers but not in sporadic intestinal-type gastric cancers, providing a rationale for considering CDH1 as a candidate susceptibility factor for hereditary diffuse gastric cancer (HDGC).

The genetics of hereditary gastric cancer

- HDGC is an autosomal dominant inherited gastric cancer susceptibility syndrome caused by germline mutations of the E-cadherin gene.
- A total of 40% of families with multiple gastric cancers and at least one of the diffuse hystotype diagnosed in an individual under the age of 50 harbor a pathogenic germline CDH1 mutation, representing the optimal screening criterion.

Management of HDGC

- Prophylactic gastrectomy is, at the moment, the only treatment for germline mutation carriers.
- Prophylactic gastrectomies have been offered to and performed only on carriers of E-cadherin germline inactivating mutations.
- CDH1 germline missense mutations appear in 30% of the mutation positive HDGC families. Since the pathogenic significance of missense mutations is not straightforward, the management of these HDGC families is suboptimal.

CDH1 germline missense mutations

- In silico analysis and in vitro functional assays have been used to infer the deleterious nature of E-cadherin germline missense variants.
- Evolutionary conservation uses sequence homology to predict whether an amino acid substitution will affect protein function and hence, potentially alter phenotype.
- In vitro functional studies represent the most powerful tool to address the pathogenic relevance of identified germline missense mutations. This in vitro system has provided important insights into the understanding of the E-cadherin-dependent tumorigenic process.

HDGC families negative for CDH1 germline mutations

- Two thirds of the HDGC families analyzed to date are negative for CDH1 germline mutations.
- A candidate gene approach has been applied to identify novel susceptibility genes.
- α-catenin, β-catenin, γ-catenin and p120-catenin, as well as TP53, Caspase-10 and SMAD-4, have been ruled out as possible alternative genes in HDGC families negative for E-cadherin germline mutations.

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First prolymphocytic gastrectomies in HDGC carriers of E-cadherin germline mutations.


Chromoendoscopic surveillance of E-cadherin germline mutation carriers as opposed to prophylactic gastrectomy.


A functional in vitro system for the functional characterization of E-cadherin germline missense mutations.


Discussion of the possibility that E-cadherin deregulation could render cells more resistant to apoptosis.


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