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Research Article

The Need of CDH1 Germline Mutation Screening in Patients with Gastric Cancer in the West

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1. Abstract

1.1. Background and objectives: Although the international guidelines recommend the CDH1 germline mutations screening in patients at risk of carrying pathogenic mutations, few data exist about the compliance to systematic screening programs carried out in surgical centres.

1.2. Methods: In the present manuscript we report the results of CDH1 germline mutations screening, undertaken in patients with gastric cancer at a high volume Western centre from 2011 to 2016.

1.3. Results: During a 5 years period, we screened 11 patients; among them, we found a pathogenic germline genetic alteration in 6 of them (54.6%). Moreover relative of two cases with germline CDH1 mutation underwent a complete genetic and clinical examination. Of note in both the analysed families, clinically detectable gastric cancers were found in subjects aged 18-19 years.

1.4. Conclusions: Based on our experience, we can conclude that the CDH1 genetic screening should be absolutely offered to high-risk Western patients, in agreement with the most recent international guidelines. Accordingly, the screening should be offered also to families with an index case of diffuse-type gastric cancer and additional cases of gastric cancer with unknown histotype in first- and second-degree relatives. Prophylactic total gastrectomy should be considered in selected cases also under 20 years of age.

2. Key words: Hereditary diffusegastric cancer; CDH1; E-cadherin;Prophylactic gastrectomy

3. Introduction

About 10% of gastric cancers (GC) show a familial aggregation, and 1-3% arises from inherited cancer syndromes[1]. Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer syndrome, characterized by highly penetrant diffuse-type gastric cancer (DGC) with a lifetime cumulative risk of 80%. In addition, women of affected families also have an elevated lifetime risk (about 40%) of lobular breast cancer (LBC) [2].

HDGC is caused in about 40% of cases by germline mutations of CDH1 gene [3] that encodes a transmembrane calcium-dependent protein named E-cadherin, which is predominantly expressed at the basolateral membrane of epithelial cells[3]. Approximately 155 germline mutations in CDH1 gene have been reported so far, including frameshift, splice-site, nonsense and missense mutations, most of which cause an alteration of the protein

function[4]. Large CDH1 deletions are quite rare.

The diagnosis of HDGC is based upon clinical criteria: 1) two or more documented cases of DGC in first- or second-degree relatives, with at least one diagnosed before the age of 50 years or 2) three or more cases of documented DGC in first-/ second-degree relatives, irrespective of age of onset. In all the cases meeting these criteria, the screening of CDH1 germline mutations is mandatory.

Anyway, considering that familial anamnesis is often incomplete and that de novo germline mutations may occur, in the 2010 the International Gastric Cancer Linkage Consortium (IGCLC) established criteria to select cases without diagnosed HDGC syndrome, but nevertheless eligible for screening of CDH1 germline mutations[5]: 1) Two or more GC cases in a family, one DGC <50 2) Three or more DGC cases regardless of age 3) one case of DGC diagnosed under 40 years of age and 3) personal or family history of DGC and LBC, with at least one diagnosed before the age of 50 years[5].

Very recently[6], the IGCLC provided up-dated indications to CDH1 mutations screening in which substantially the previous first two criteria were merged together: "two or more GC cases, with at least one confirmed DGC, in first-/second-degree relatives regardless of age". This new criterion has been introduced in order to include also cases without detailed pathological information.

The youngest age, at which genetic testing should be offered, is still under debate. Rare cases of clinically significant DGC in affected families have been reported even before the age of 16, for example in Maori kindred [3]. According to current international guidelines, genetic testing can be offered after the age of consent (16/18 years) [5].

The consensus reached so far was that individuals with a positive test for a pathogenic CDH1 mutation, should be advised to consider prophylactic gastrectomy regardless of any endoscopic findings. However, as total gastrectomy is associated with weight loss and increased risk of malnutrition, risk-reducing prophylactic gastrectomy (PTG) should be carefully considered during the patient's growth period and is generally recommended only thereafter [7], namely after 21 years of age.

The present study describe the experience of a high volume Western GC centre in screening CDH1 germline mutations, handling kindred with pathogenic CDH1 mutations and making decision on PTG in deleterious mutation carriers. The local experience is discussed according to current scientific literature and some suggestions are provided for the management of patients with GC in Western countries.

4. Results of CDH1 Germline Mutations Screening

From January 2011 to December 2016 a total of 242 patients underwent gastrectomy for GC at General and Upper GI Surgical Division of Verona University. Among them, 11 patients (4.5%), diagnosed with DGC on preoperative biopsies, were selected according to criteria reported in Table 1 to undergo genetic testing for CDH1 mutation screening.

In each of the screened case, the complete analysis of CDH1 gene (OMIM *192090) on DNA from peripheral blood samples was performed. All the CDH1 exons and the flanking introns were analysed by Next-Generation Sequencing (Miseq-Ilumina), confirmation analyses were performed through Sanger sequencing and automated capillary electrophoresis (3130 Genetic Analyzer-Applied Biosystems). The analysis of CDH1 genomic rearrangements, such as gross deletions and duplications, was performed through MLPA (Multiplex Ligation-dependent Probe Amplification). In case of negativity for CDH1 point mutations or genomic rearrangements, mutations in other 93 genes involved in the main hereditary cancer syndromes were searched (TruSight Cancer panel-Illumina). Criteria for CDH1 testing, clinico-pathological and genetic data of the screened cases are reported in **Table 1**.

Of the patients who underwent CDH1 genetic screening, 5 subjects (45.4%) were found to carry pathogenic CDH1 aberrations: 4 subjects were found to carry CDH1 point mutations, while 1 had a CDH1 gross deletion. Of the CDH1-negative patients, 2 presented germline mutations in other cancer predisposition genes (BRCA1 and ATM). The mutation in BRCA1 gene has been shown to have a pathogenic role (ClinVar: pathogenic), while the clinical impact of the mutation in ATM is still unknown but the introduction of a premature stop codon suggests a possible deleterious effect on the protein function. Hence, globally 6 of the 11 screened patients (54.6%) had a definite pathogenic germline genetic alteration.

5. Screening of Relatives of Cases with Pathogenic CDH1 Mutations

For the first two patients in which we found a pathogenic CDH1 mutation (Table 1), as indicated in the genetic counselling, we performed an extensive screening of the relatives. The results are reported in Figure 1 and 2**,** respectively. The screening of the other families is ongoing.

5.1.First family

among 17 screened relatives, 6 (35.3%) were shown to carry the

mutation **(Figure 1)**.

In all the mutations carriers a PTG was proposed. Only 1 of them (a 45 years old sister of the index case) accepted. Her preoperative endoscopy revealed just diffuse atrophy of the gastric mucosa and all biopsies resulted negative for cancer. The breast examination did not evidence any suspected finding.

She underwent total gastrectomy and D2 lymph node dissection, the pathologic report showed 1 focus of Signet Ring Cell adenocarcinoma confined to lamina propria of the mucosa without any nodal metastases (pT1a N0).

In all the mutated cases who refused the PTG, we started a surveillance program including upper GI endoscopy and breast examination. All the endoscopies of mutated subjects resulted macroscopically and pathologically negative except for the 19 years old daughter of the index case. Indeed in this case, the upper-GI endoscopy detected an area of irregular mucosa in the posterior wall of the gastric body. The histological examination revealed an infiltration of poorly cohesive carcinoma (clinical staging $cT1_aN0$). She underwent total gastrectomy and D2 lymph node dissection. Postoperative pathological examination described 2 foci of diffuse-type, both intramucosal lesions (confined to the lamina propria) and typical pagetoid spread of signet ring cells in the surrounding normal mucosa. No nodal metastases were found ($pT1_a$ N0 $_{0/26}$ M0).

The other mutations carriers are still following the endoscopic surveillance, negative to date (median follow-up 31 months, range 18-50).

5.2. Second family

As regards the second family that was completely screened, most (64.3%) of the subjects tested resulted positive for the CDH1 mutation (9 mutation carriers out of 14 tested) **(Figure 2)**.

After adequate genetic counselling, all the patients started the upper GI endoscopic surveillance according to the Cambridge protocol, for females breast imaging examinations including breast MRI was also started.

From these investigations a negativity for GC emerged in all the cases with the exception of two subjects.

Indeed, a second-degree relative of the index case, a 19 years old girl, had a tiny flat lesion whose biopsy was positive for diffuse adenocarcinoma with signet ring cell. She completed the staging performing blood tests (CEA), chest and abdominal CT scan and breast RMI. No suspected lymphadenopathy or secondary lesions were found. The patient underwent total gastrectomy and D2 lymph node dissection. Postoperative pathological examination confirmed that it was an intramucosal tumor (confined to the lamina propria) with signet ring cells, the maximum diameter of the lesion was 2 mm. There were not lymph nodes involved ($pT1_a$ N0 $_{0/41}$).

Very recently, another second-degree relative of the index case, a 35 years old man, was found to have a positive biopsy at upper GI endoscopy. He will undergo total gastrectomy in the next few days.

Among the relatives harbouring the mutation, two subjects decided to undergo prophylactic gastrectomy.

The first was a 35 years old female, who underwent PTG and pathological examination revealed 13 adenocarcinoma foci of diffuse-type, diameter variable from 1 mm to 3 mm, all limited to the lamina propria of the gastric mucosa, distributed in the whole stomach. No lymph nodes were found to be involved ($pT1_a$ N0 $_{0/21}$ M0) **(Figure 3)**. Also her father, a 62 years old man, underwent PTG and the final pathological report documented 5 foci of adenocarcinoma with Laurèn diffuse histology.

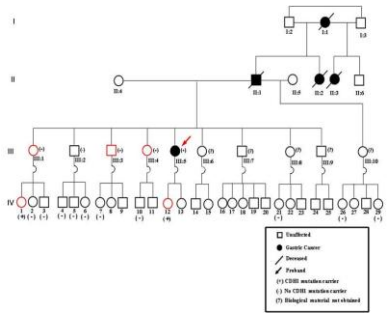

Figure 1. Pedigree chart of case 1.
The index case is indicated by a red arrow. Symbols: squares, males; circles, females; black symbols, gastric cancer affected; slash through symbol, deceased.

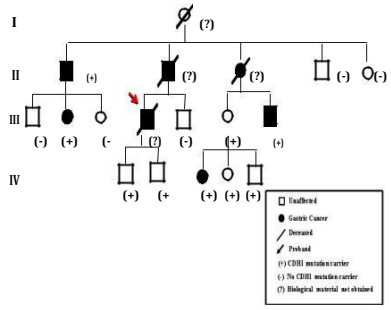

Figure 2. Pedigree chart of case 2.
The index case is indicated by a red arrow. Symbols: squares, males; circles, females; black symbols, gastric cancer affected; slash through symbol, deceased.

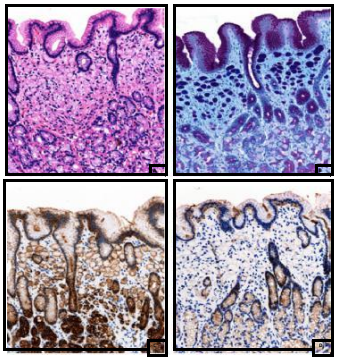

Figure 3. Pathological examination of the 35 years old female of the second family underwent PTG.

Mucosal signet ring cell carcinoma (pT1a) with Hematoxylin and Eosin stain (A), Alcian blue-PAS stain (B), Cytokeratin 8/18 immunostain (C) and E-cadherin immunostain: note the positive membranous stain in non-neoplastic epithelial cells in contrast with neoplastic cells, which have lost E-cadherin expression (D).

The other mutations carriers, endoscopically followed for 18 months each, are negative to date.

6. Discussion

In the present study we report the results of CDH1 germline mutation screening that we started at our high volume Western centre in recent years. We selected patients eligible for the screening according to 2010 IGCLC guidelines[5] in all cases but one case of DGC with other 4 cases of gastric cancer with unknown histotype in first or second degree relatives, all diagnosed after 50 years of age. Of note, this last case would have been included in the screening according to the most recent 2015 IGCLC guidelines [6].

Of the 11 screened patients, 5 subjects (45.4%) were found to carry pathogenic CDH1 aberrations. Hence, the CDH1 mutation detection rate (45.4%) was higher than expected. Indeed the larger studies, using the 2010 IGCLC guidelines, reported a detection rate of 10-18% [6-8].

Of note, the exon 6 c.781G>T p.Glu261* mutation has been reported for the first time in gastric cancer by our group. Moreover, a gross deletion of exons 1 and 2 was found, that is quite uncommon as the frequency of large deletions is about 5%[9]. This suggests the importance to look for CDH1 deletions when CDH1 point mutations are negative.

Another important finding of the present study is the detection of endoscopically-identifiable DGC lesions in patients younger than 20 years in both families, where the relatives of an index case harbouring a deleterious CDH1 mutation were extensively screened for the presence of the same mutation. Of note, in these families the youngest age, at which DGC had been clinically diagnosed, was 39 years before screening implementation. However, according to current guidelines[5,6] prophylactic total gastrectomy in deleterious mutations carriers is currently indicated at an age of at least 21 years, as few cases of CDH1 mutation-mediated gastric cancer have been documented under the age of 20[10,11] and even less cases of clinically significant DGC. On the other hand, there are many concerns about the negative effects of total gastrectomy on metabolism during the growth period.

It should be underlined that in our series both DGC, detected in people aged less than 20 years, although staged as pT1a N0, thus not clinically relevant, were macroscopically detectable. As such, we don't' know whether they were likely to progress in few years, if not resected. Hence, the present findings further support the need to collect more data on the biological behaviour of DGC related to CDH1 mutations, in order to better define the indications to prophylactic total gastrectomy.

In two cases without pathogenic CDH1 mutations, other germline mutations were found in BRCA1 and ATM genes, which have been mainly associated with hereditary breast and ovarian cancers[8]. This observation could have relevant clinical implications, and suggests that germline mutations in cancer predisposition genes should be searched in cases fulfilling the IGCLC criteria for genetic screening, but negative for CDH1 mutations.

We are now completing the genetic screening of relatives in the remaining five families with a mutated index case. We are also studying the molecular mechanism of the second hit in affected subjects.

7. Conclusions

Considering the not negligible rate of deleterious mutations among the screened patients and the possible beneficial impact of mutation detection on mutation carriers, a screening program should be absolutely offered in Western countries to patients at high risk in agreement with the most recent international guidelines.

We can conclude that the CDH1 genetic screening should be absolutely offered to high-risk Western patients in agreement with the more recent international guidelines, including also families with a case of diffuse-type gastric cancer and other cases of gastric cancer with unknown histotype in first- and second-degree relatives regardless of age. In both the families in which the relatives of an index case were extensively screened for CDH1 mutations, we found DGC whit endoscopic detectable lesions in patients younger than 20 years:our findings highlight the need or having more data to better define the biological behaviour of CDH1 mutation related DGC.

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