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SHORT COMMUNICATION

Clinical implications of *CTNNA1* germline mutations in asymptomatic carriers

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Abstract

In 2017, we implemented *CTNNA1* germline analysis in probands suspected of having hereditary diffuse gastric cancer. Here, we report the results from a retrospective series of 41 cases, including the identification of a new family with a *CTNNA1* mutation and the first prophylactic total gastrectomy in an asymptomatic carrier after a normal upper endoscopy. Diffuse gastric cancer foci with loss of catenin alpha-1 expression were seen in the resected tissue, suggesting that *CTNNA1* and *CDH1* germline mutations behave in a similar manner. Life-changing prophylactic total gastrectomy should therefore also be considered in *CTNNA1* mutation carriers.

Keywords Diffuse gastric cancer · *CDH1* · *CTNNA1* · Signet-ring cell · Hereditary cancer

Introduction

Diffuse gastric cancer (DGC) is a gastric cancer subtype characterized by signet-ring cells infiltrating the mucosa and wall as small clusters or scattered individual cells. Germline mutations in cancer susceptibility genes account for a minority of cases, and carriers of such mutations are said to have hereditary DGC (HDGC).

The consensus HDGC testing criteria, which were revised in 2015, are based on family history and age at diagnosis (e.g., two DGC cases in a family, sporadic DGC < age 40), and raise the possibility of genetic susceptibility. When these criteria are fulfilled, genetic testing is recommended [1, 2]. Until recently, *CDH1* was the only known susceptibility gene, with a germline pathogenic variant present in

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14%–20% of probands meeting the HDGC testing criteria [3–5]. *CDH1* codes for the E-cadherin adhesion protein. Testing of probands is beneficial, as the identification of a mutation leads to cascade testing within the family and risk-reducing measures in asymptomatic carriers. The vast majority of *CDH1* mutation carriers hase malignant or premalignant foci in their stomachs that commonly evade detection by upper endoscopy [6, 7]. There is therefore no effective screening approach, and the only consensus recommendation in asymptomatic carriers is prophylactic total gastrectomy (PTG).

In the past five years, truncating germline variants in *CTNNA1* (*catenin alpha-1*), which encode a *CDH1*-binding partner, have been identified in five families with multiples DGC cases [3, 8, 9]. *CTNNA1* is therefore another DGC-susceptibility gene, albeit rarely involved, since 320 probands had to be investigated to identify these families. Given the recent identification of *CTNNA1*, data regarding the benefit of presymptomatic testing and the subsequent management of asymptomatic carriers are lacking. To adequately inform and guide families, this gap in knowledge needs to be filled.

Following earlier reports of the association between *CTNNA1* mutations and HDGC [3, 8], we implemented *CTNNA1* germline analysis in all probands with suspected HDGC. We report herein a retrospective series of 41 probands, including the identification of a new family with a *CTNNA1* mutation and the first prophylactic total gastrectomy in an asymptomatic carrier after a normal upper endoscopy.

Materials and methods

Patient selection

Since 01 January 2017, *CTNNA1* has been included in our "genetic susceptibility to digestive cancer" NGS panel. Sanger sequencing was implemented simultaneously for retrospective patients. This work reports *CTNNA1* results in a retrospective series of *CDH1*-negative probands with suspected HDGC. Cases were selected for analysis because they fulfilled the 2015 testing criteria, or had a personal/family history that was close to these criteria, mainly sporadic DGC between the ages of 40 and 50. They came from two Paris University Hospitals affiliated with the laboratory (La Pitié-Salpêtrière, Saint-Antoine), and from partner cancer genetics clinics located throughout France. All signed an informed consent form.

Gene sequencing

Sequencing was performed at the Pitié-Salpêtrière Cancer Genetics Laboratory. The sequence analysis of the coding exons and exon/intron boundaries of the *CTNNA1* gene (NM_001903.3) was performed on genomic DNA extracted from peripheral lymphocytes for all selected patients. The purified PCR products were sequenced in both directions using the BigDye Terminator v3.1 chemistry (ThermoFisher Scientific). Sequencing reactions were run on an ABI3730 DNA Analyzer and analyzed with the SeqScape software v2.6. All primer sequences and amplification conditions are available on request.

Immunohistochemistry

Catenin alpha-1 protein expression was studied by immunohistochemistry (IHC) in formalin-fixed and paraffin-embedded material. Briefly, IHC staining was performed on one representative tumor block from each case. Sections of 4 µm were incubated with a mouse monoclonal antibody against α-E-catenin for 20 min (clone EP1793Y, Abcam, dilution 1/200). Staining was performed with a Leica immunohistochemistry automate. Catenin alpha 1 was detectable in normal epithelial structures (e.g., the glands of the stomach). Staining was scored according to the percentage of positive tumor cells.

Results

Thirty-two out of 41 probands met the 2015 HDGC testing criteria. A truncating germline variant, *CTNNA1* c.2023C>T, p.Gln675*, was identified in a female with a DGC history at age 58 (III.1, Fig. 1). She died of the disease in 2008. Four first-degree relatives had a history of gastric cancer at the ages of 39, 40 ($n=2$) and 52, 2 of them were confirmed as having DGC (III.3 and III.5). One sister with GC also had ductal breast cancer at age 44. None of the affected relatives were alive. As we had access to tumor tissue for individual III.3, we performed a co-segregation analysis and found the *CTNNA1* variant (Fig. 1). Individual III.2, with no history of cancer, did not carry it. In the proband and individual III.3, catenin alpha-1 expression was lost in 90% and 100% of the DGC tumor cells, respectively.

The *CTNNA1* c.2023C>T, p.Gln675* truncating variant is not reported in the gnomAD database of germline mutations. It is reported once in the TCGA database of somatic mutations, but in a bladder cancer. Given the pathogenic nature of the variant, we then carried out presymptomatic testing in eight adult relatives. Two females aged 32 and 20 were mutation carriers (IV.8 and IV.10, respectively). Screening esophagogastroduodenoscopy (EGD) using white light, narrow band imaging, and coloration with indigo carmine was visually normal in patient IV.10, and no DGC foci were observed in 16 random biopsies. Following multidisciplinary assessment, and considering the *CTNNA1* germline

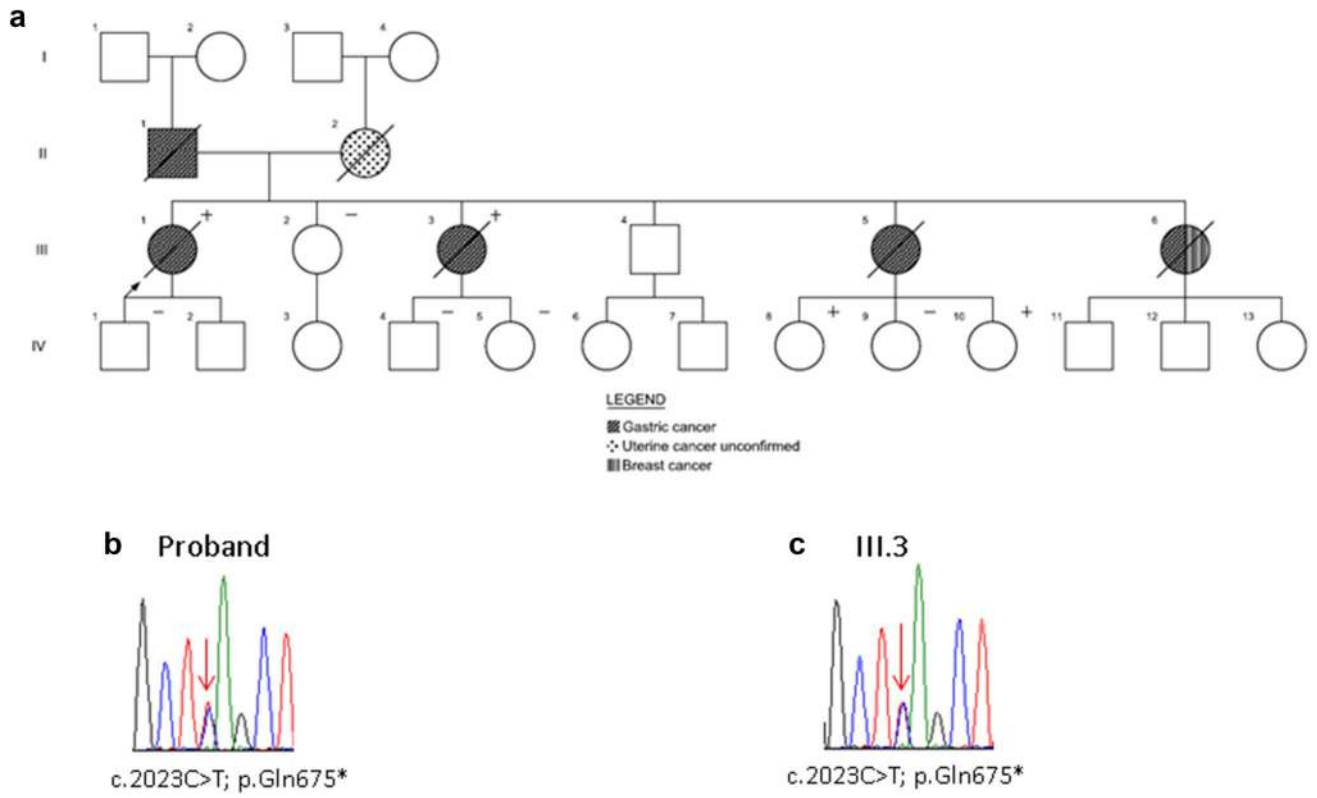

Fig. 1 **a** Pedigree of the family with the c.2023C>T, p.Gln675* *CTNNA1* pathogenic variant. **b** Sanger sequencing, proband's germline DNA. **c** Sanger sequencing of tumor tissue from individual III.3

mutation and the strong family history of DGC, she underwent PTG. The entire stomach was processed into paraffin blocks. All were stained with haematoxylin and eosin and analyzed by two pathologists, who observed three millimeter-scale intramucosal DGC foci out of 148 blocks (Fig. 2). Catenin alpha-1 expression was lost in the tumor foci. In patient IV.8, EGD was also normal, but one of 14 random biopsies contained a 1-millimeter intramucosal DGC focus. She subsequently underwent gastrectomy. The pathologist did not identify any additional foci in the surgical specimen.

Catenin alpha-1 expression was normal (> 50% of tumor cells) in eighteen *CDH1*/*CTNNA1*-negative probands from this retrospective series and for whom DGC biopsies or therapeutic gastrectomy specimens were available (Fig. 3).

Discussion

Data regarding *CTNNA1* presymptomatic testing are scarce, and nonexistent regarding asymptomatic carrier management, making it difficult for cancer geneticists to inform families. While Weren et al. identified the familial mutation in the unaffected mother and daughter of a *CTNNA1* proband, the implications of these findings and subsequent management of the patients are unknown. In this article, we report in detail and for the first time the clinical consequences of a familial *CTNNA1* mutation in two asymptomatic carriers. Despite having a normal EGD and no sign of cancer in multiple random biopsies, the first carrier followed the recommendations of a multidisciplinary team and had a PTG. Three intramucosal DGC foci were observed. The second carrier had a gastrectomy following the identification of an intramucosal focus by EGD.

HDGC is unique in that asymptomatic *CDH1* mutation carriers are advised to undergo PTG [2], and our observations suggest that the same recommendation should be made to *CTNNA1* mutation carriers.

It is now 5 years since the first HDGC family carrying a *CTNNA1* germline mutation was reported [8]. Since then, four additional families have been reported [3, 9], firmly establishing *CTNNA1* as a second DGC susceptibility gene in addition to *CDH1*. In the *CTNNA1*-DGC papers, the authors reported only 5 mutations in a total of 320 *CDH1*-negative probands, using either exome sequencing or a candidate gene approach [3, 8–10]. In our study, only 1/41 probands carried a *CTNNA1* mutation, confirming that the gene only accounts for a small proportion of familial DGC or DGC at a young age. Most of the genetic susceptibility therefore remains unaccounted for.

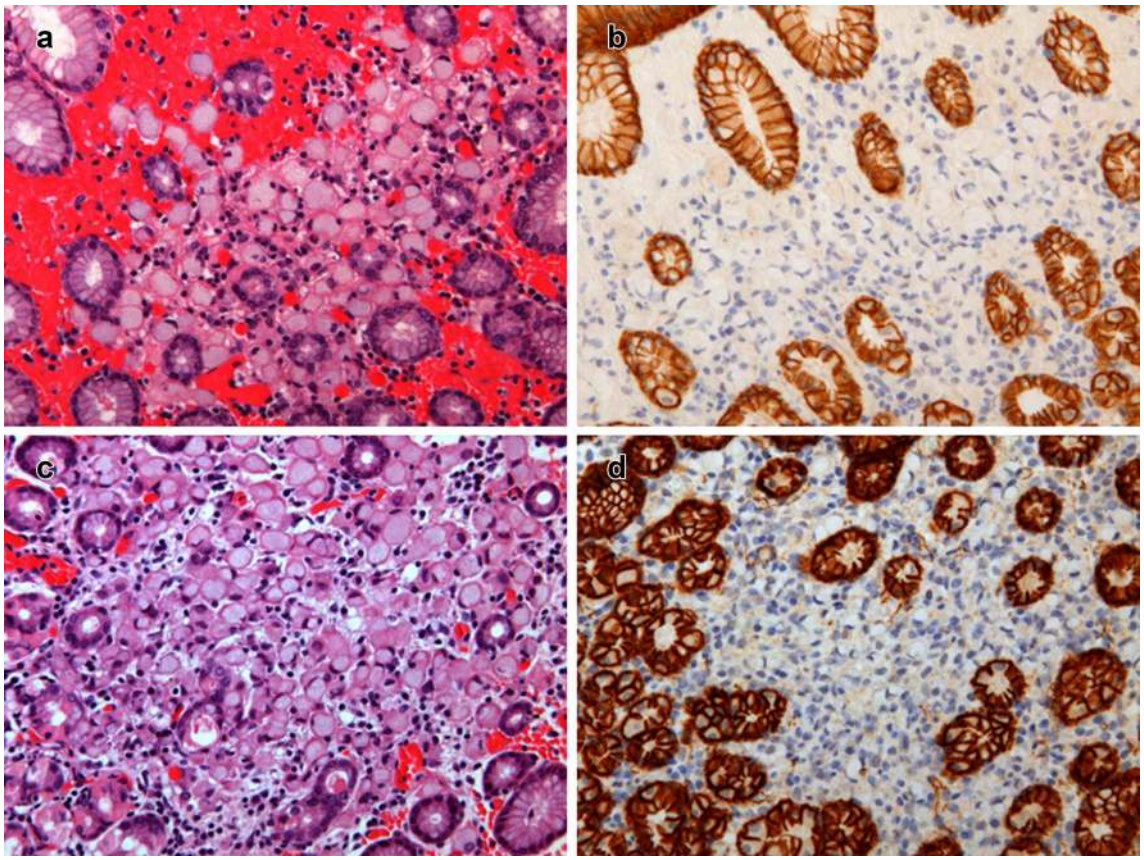

Fig. 2 **a** and **c** Two signet-ring cell foci identified in the lamina propria of the prophylactic total gastrectomy specimen (haematoxylin and eosin staining). **b** and **d** immunohistochemistry. Catenin alpha-1 expression is lost in both tumor foci, while normal tissue shows expression of the protein

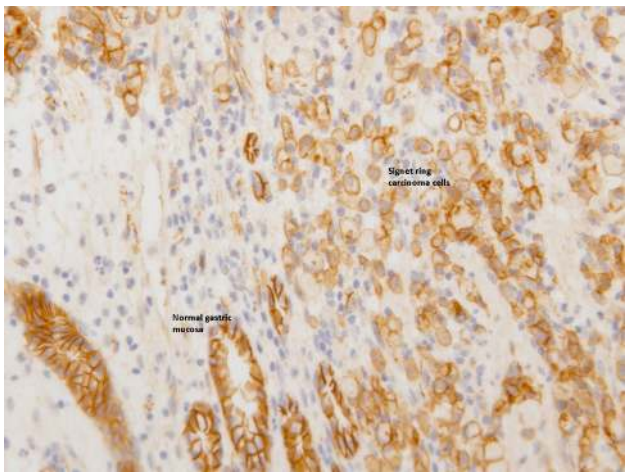

Fig. 3 Diffuse gastric cancer in a patient who does not carry a *CTNNA1* germline mutation. Catenin alpha-1 expression is retained in both normal and tumor cells

In our study, catenin alpha-1 expression loss in DGC was strongly predictive of a *CTNNA1* germline mutation. Indeed, expression was lost or only observed in a minority of cells (10%) in three mutation carriers from the *CTNNA1* family, while it was retained in eighteen wild-type probands. Our observations confirm, in a large number of cases, observations previously made by Majewski [8]. We thus recommend that immunohistochemistry be performed routinely in all patients with suspected HDGC, as absent or weak expression of catenin alpha-1 suggests genetic susceptibility associated with *CTNNA1*. Intriguingly, over 50% of early diffuse gastric cancers in a Korean study had reduced or absent catenin alpha-1 expression [11]. Expression loss was likely due to purely somatic gene inactivation (tumoral mutations, epigenetic changes), as shown in invasive lobular carcinoma of the breast, considering the rarity of *CTNNA1* germline mutations [12]. The high proportion of DGC losing catenin alpha-1 expression in the Korean paper contrasts with our observations, and these discrepancies warrant further exploration in future studies. There are no data to suggest that *CDH1* germline or somatic events affect catenin alpha-1 expression.

The latest guidelines clearly state that PTG is recommended in asymptomatic *CDH1* mutation carriers, regardless of EGD findings [2]. The ultimate objective would be to dispense with PTG in a subset of *CDH1* and *CTNNA1* cases, without putting their lives at risk. That would, however, require a reliable screening tool. In contrast to previous reports, a recent study suggests that EGD, when performed by experts and according to the Cambridge protocol, has good negative predictive value [13]. Indeed it seems to identify large DGC foci at risk of progression to invasive cancer (> 3 mm), thus advising which patients should undergo PTG. Smaller foci, whether there are caught or not by multiple random biopsies may however remain quiescent for long periods. Admittedly, we cannot be sure that the small DGC foci observed in our two *CTNNA1* asymptomatic mutation carriers would have progressed into invasive cancers, but it seemed appropriate to follow the *CDH1* guidelines and offer them risk-reducing surgery. Long-term follow-up of carriers undergoing regular screening EGD, as well as studies in large cohorts, are needed. Should these findings be confirmed [13], the family reported here, like all families carrying *CDH1* and *CTNNA1* mutations, will be relieved to know that at least a subset of their relatives carrying the mutation will be spared the life-changing PTG procedure in the future.

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Author contributions Manuscript writing: PRB, CC, MS and FC. Data collection: PRB, CC, EG, HD, MW, FC. Patient management (clinical): PRB, CC, HD, MW, JB, JN, YP, AM, VC, IC. Genetic analyses: EG, ME, MZ, FS, JD, FC. Pathology: AC, AMG, MS. Final draft approval: all authors.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Ethical standards Retrospective results on a case series are reported in this manuscript. All patients benefited from in-person genetic counseling by a physician with expertise in clinical cancer genetics. They then signed an informed consent form clearly stating that gastric cancer susceptibility genes would be analyzed. Work was done in accordance with French law and National guidelines edited by Health Authorities.

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