Clinical spectrum and pleiotropic nature of CDH1 germline mutations

Joana Figueiredo,1,2 Soraia Melo,1,2,3 Patrícia Carneiro,1,2 Ana Margarida Moreira,1,3,2 Maria Sofia Fernandes,1,2,4 Ana Sofia Ribeiro,1,2 Parry Guilford,5 Joana Paredes,1,3,2 Raquel Seruca1,3,2

ABSTRACT

CDH1 encodes E-cadherin, a key protein in adherens junctions. Given that E-cadherin is involved in major cellular processes such as embryogenesis and maintenance of tissue architecture, it is no surprise that deleterious effects arise from its loss of function. E-cadherin is recognised as a tumour suppressor gene, and it is well established that CDH1 genetic alterations cause diffuse gastric cancer and lobular breast cancer—the foremost manifestations of the hereditary diffuse gastric cancer syndrome. However, in the last decade, evidence has emerged demonstrating that CDH1 mutations can be associated with lobular breast cancer and/or several congenital abnormalities, without any personal or family history of diffuse gastric cancer. To date, no genotype–phenotype correlations have been observed. Remarkably, there are reports of mutations affecting the same nucleotide but inducing distinct clinical outcomes. In this review, we bring together a comprehensive analysis of CDH1-associated disorders and germline alterations found in each trait, providing important insights into the biological mechanisms underlying E-cadherin’s pleiotropic effects. Ultimately, this knowledge will impact genetic counselling and will be relevant to the assessment of risk of cancer development or congenital malformations in CDH1 mutation carriers.

INTRODUCTION

The human CDH1 gene codes for E-cadherin (ENSG0000039068), a cell–cell adhesion glycoprotein that acts as a critical invasion suppressor.12 During embryonic development, E-cadherin is the first adhesion molecule expressed at the 8-cell stage and is essential for the compaction of the morula and subsequent organisation of epithelial tissues.1 Homozygous E-cadherin −/− embryos show severe abnormalities before implantation and do not survive beyond the blastocyst stage.4 In adult epithelia, E-cadherin is crucial for the establishment and maintenance of tissue architecture and homeostasis.1 2 E-cadherin function is primarily affected by homophilic binding of the extracellular domain of cadherins presented on neighbouring cells, forming adherens junctions.5 Furthermore, the cytoplasmic domain of E-cadherin is connected to the actin cytoskeleton through various catenins (α, β, and γ6,7), providing cell structural properties while mediating cellular signalling.8 9 We and others have demonstrated that E-cadherin regulates basic cellular processes such as proliferation, migration, apoptosis and invasion by orchestrating several proteins, namely Hippo and Src family kinase, and signalling pathways involving epidermal growth factor receptor, Notch, Integrin and Laminin.10–12

Given the key role of E-cadherin in tissue homeostasis, the deleterious effects that can arise from E-cadherin loss are not surprising. Loss-of-function germline mutations in the CDH1 tumour-suppressor gene were first described in 1998 as the cause of hereditary diffuse gastric cancer (HDGC) syndrome (OMIM #137215).13 Since the initial report by Guilford et al, many studies have emerged supporting those findings and revealing that HDGC is a rare autosomal dominant disorder that encompasses an increased risk of two cancer types: diffuse gastric cancer (DGC) and lobular breast cancer (LBC).14–17 Although DGC is the dominant tumour type in HDGC, other malignant neoplasms, as well as congenital malformations, have been described in families affected by this syndrome (figure 1).18–21 For that reason, LBC and oral facial clefts (OFCs) have been suggested as early indicators for CDH1 screening and early detection of HDGC.21

Interestingly, with the scientific and technological advances of massive parallel sequencing and the availability of genetic panel testing, a growing body of evidence suggests that CDH1 germline mutations are causative of a disease spectrum independent of the HDGC manifestation.19 22–25 Consequently, we propose that CDH1 is a pleiotropic gene responsible for distinct clinical phenotypes.

In this report, we performed a comprehensive overview of the diseases most frequently associated with CDH1 germline alterations, as well as the type and functional consequences of the mutations found in each setting, which ultimately can provide important insights into the mechanisms underlying this phenomena. For that purpose, data were collected in the PubMed platform, using single or combined search terms such as CDH1, E-cadherin, CDH1/E-cadherin germline mutations, CDH1/E-cadherin variants, gastric cancer, CDH1 susceptibility, familial gastric cancer and HDGC, LBC, colorectal cancer (CRC), cleft lip/palate (CL/P), blepharocheilodontic syndrome (BCDS) and E-cadherin signalling. Available literature from April 1983 to September 2018 was selected and extensively scrutinised.
Cancer genetics

Figure 1  Timeline presenting the key findings related to the clinical phenotypes of CDH1 germline mutation carriers. CRC, colorectal cancer; DGC, diffuse gastric cancer; HDGC, hereditary diffuse gastric cancer; HLBC, hereditary lobular breast cancer; LBC, lobular breast cancer; OFC, oral facial cleft.

Hereditary diffuse gastric cancer

Defects in the Ca\(^{2+}\)-dependent transmembrane protein E-cadherin are frequently associated with several human cancer types, in particular with gastric cancer. The major inherited form of gastric cancer, HDGC, was first identified in a large indigenous New Zealand Māori kindred with a causative germline mutation in CDH1.\(^{13}\) It is well established that CDH1 inactivating germline mutations underlie this highly penetrant cancer syndrome and are found in approximately 40% of families meeting the clinical criteria for HDGC.\(^{14} 15 17 18 21 26–29\) Of relevance, along with a strong aggregation of DGC cases, many HDGC families are also affected by LBC. Accordingly, the cumulative incidence of DGC by 80 years for CDH1 mutation carriers is estimated at 70% for male and 56% for females, and the probability of women developing LBC is 42%.\(^{16} 21\) CRC, appendiceal signet ring cell carcinomas (SRCCs) and CL/P have also been described in HDGC kindreds.\(^{15} 17 20 26–29\) However, of these clinical manifestations, CL/P is the only one considered within the HDGC clinical definition.\(^{21}\)

In the HDGC clinical setting, CDH1 germline abnormalities are evenly distributed throughout the gene with no apparent genotype–phenotype correlation established.\(^{14}\) The great majority of the published germline mutations are expected to lead to truncated proteins or lack of mRNA expression, allowing a straightforward prediction of their pathogenicity.\(^{16} 21 28 35 37\) The most common mutation types are small insertions or deletions (38%).\(^{16}\) Others vary between splice site (21%), nonsense (17%) and large exonic deletions (9%).\(^{16}\) The missense fraction accounts for 16% of the mutations described so far.\(^{16} 33\) The clinical and functional impact of missense mutations still raises controversy among specialists because, in most cases, a full-length protein is preserved, and regular levels of E-cadherin expression are usually produced.\(^{33} 36\) As such, the identification of a CDH1 missense variant requires additional studies to determine E-cadherin functional status and, consequently, its putative pathogenicity.\(^{21} 28 35 37\)

The type and frequency of germline defects in HDGC families are constantly being re-evaluated. The latest review points to a total of 155 germline CDH1 mutations described across multiple ethnicities.\(^{16} 21\) Most are considered pathogenic (126/155), while a small proportion cannot be categorised in terms of their functional relevance (29/155) and are termed variants of uncertain significance (VUSs).\(^{16}\) Newly revised criteria and new methodological approaches to predict their pathogenic nature are thus needed for an improved classification system.

As an autosomal-dominant familial disorder, HDGC CDH1 mutations are inherited in only one allele. For initiation of the neoplastic process, somatic inactivation or downregulation of the second copy of the CDH1 gene must occur.\(^{38–41}\) Epigenetic causes seem to play an essential role in this process. Promoter hypermethylation is the most common established mechanism leading to biallelic CDH1 inactivation, while mutation or deletion of the second CDH1 allele is less frequently described.\(^{38–41}\)

The disease originates with the development of early gastric lesions characterised by spreading of isolated malignant cells.\(^{42}\) Precursor lesions of invasive gastric cancer have been identified as in situ SRCC or pagetoid spread of signet ring cells below the preserved epithelium.\(^{42}\) The pathology of such lesions is unique but not easy to recognise; therefore, their examination and confirmation by experienced pathologists is highly recommended. The presence of in situ lesions, pagetoid spread or multifocal intramucosal signet ring cells in the gastric mucosa provides substantial clues for the diagnosis and management of patients with HDGC, as well as for research.\(^{43}\) It is feasible that many in situ/early invasive lesions are transient, or remain indolent, and do not progress towards an advanced stage.\(^{43} 44\)
Unfortunately, progression to invasive or advanced disease is unpredictable, limiting the utility of scheduled patient follow-up and surveillance. In light of the invasive and lethal nature of diffuse gastric carcinomas and the high degree of penetration of CDH1 mutations, prophylactic total gastrectomy remains the recommended risk-reducing approach for HDGC family mutation carriers.16,30

Recognition of the need for common terminology and management guidelines for this syndrome led to the establishment of the International Gastric Cancer Linkage Consortium, constituted by a multidisciplinary team of experts in 1999.45 Over the last 19 years, this collaborative group, which includes gastroenterologists, oncologists, surgeons, pathologists, geneticists, nutritionists and molecular biologists, has been defining and improving the clinical criteria for early diagnosis of the disease and the identification of patients who should undergo germline CDH1 genetic screening.21,46–47 The latest revised criteria indicate that CDH1 testing should be applied in any case fulfilling one of the following criteria: (1) families with two or more documented cases of gastric cancer at any age, one with confirmed DGC; (2) personal history of DGC before the age of 40 years; and (3) personal or family history of DGC and LBC, with one diagnosed before the age of 50 years.21 Testing could also be considered in: (1) cases of bilateral LBC or family history of two or more cases of LBC, before the age of 50 years; (2) a personal or family history of CLP in a patient with DGC; and (3) in situ signet ring cells and/or pagetoid spread of signet ring cells, as this feature is rarely seen in sporadic cases.21 Whenever possible, testing should start in an affected proband. If the affected proband is deceased, frozen-fixed or formalin-fixed, paraffin-embedded tissue (preferably normal, non-malignant tissue) may still be used as an alternative.21,37

As noted above, carriers of CDH1 pathogenic mutations are advised to undergo a total prophylactic gastrectomy, regardless of the negative endoscopic findings.48–49 Some patients delay this decision due to personal, physical or psychological reasons and, in these cases, annual endoscopic surveillance should be employed.21 Approximately 60% of families that fulfil the current testing criteria for HDGC lack germline CDH1 mutations.19,47–50 These families remain genetically unexplained and may carry pathogenic mutations in other, yet unknown, gastric cancer susceptibility genes. Patients fulfilling the HDGC criteria, who have tested negative for CDH1 germline mutations, are advised to undergo intensive endoscopic screening with gastroenterologists familiar with HDGC.21 The same protocol should be followed by CDH1 VUS carriers.21 Importantly, all individuals undergoing endoscopic examination must be aware that it is possible that lesions at early stage will not be detected by random biopsies due to the multifocal nature of the disease.42,48,51 In addition, bilateral MRI of the breast, starting at age of 30 years, is recommended annually in women with a CDH1 mutation.21

The CTNNA1 gene, which encodes for α-E-catenin, is a strong candidate to explain a minor portion of CDH1-negative HDGC families. Aside from its crucial role in E-cadherin-mediated adhesion, CTNNA1 has been found to be mutated in five HDGC families (five distinct mutations), corroborating the functional significance of CDH1 mutations and the importance of E-cadherin downstream signalling.16,52,53 Despite all the recent efforts in pursuing new candidate genes, CDH1 genetic alterations remain the central cause of HDGC.52,54–56

LBC and hereditary lobular breast cancer (HLBC)

Most hereditary cancer syndromes display specific organ sites preferentially affected by cancer. In this regard, LBC is the second most frequent cancer type associated with HDGC.18,19 In 2013, a study was carried out in 165 unrelated Ile-de-France index cases, selected based on personal and family history of gastric or breast cancers, and it was shown that 18 cases (11%) were CDH1 germline mutation carriers (18 different mutations).19 Three of the carriers were women with a personal history of bilateral LBC below 30 years of age. None of these cases presented family history of DGC in first-degree and second-degree relatives, and as such, they did not meet the HDGC criteria established at that time.19,47 Two of those women were subsequently diagnosed with DGC, while the third woman was subjected to upper gastrointestinal endoscopy with multiple biopsies. Despite no cancer foci being found in the biopsies, the patient underwent prophylactic gastrectomy, and an invasive DGC was identified.19

Remarkably, in the last few years, a number of studies have arisen reporting early-onset LBC cases in CDH1 mutation carriers without any personal or family history of DGC, pinpointing CDH1 as a novel LBC-susceptibility gene.23,57–60 Moreover, HLBC was recently proposed to be an independent clinical entity associated with CDH1 germline alterations.25,58 According to the HDGC consensus guidelines, women diagnosed with bilateral LBC, with or without family history of LBC before the age of 50 years, or women diagnosed with unilateral LBC with family history of LBC with onset age at less of 45 years, are eligible for CDH1 genetic testing.25

Currently, there are four high-penetrance genes identified to be genetically associated with increased breast cancer susceptibility: BRCA1, BRCA2, TP53 and CDH1. Germline mutations in BRCA1 and TP53 are predominantly associated with invasive ductal carcinoma, BRCA2 mutations are associated with both ductal and lobular tumours, while mutations in CDH1 are exclusively associated with LBC, in particular with the invasive subtype.61

Invasive LBC is the second most common histological type of breast cancer, accounting for 5%–15% of invasive breast cancers, and is one of the few histological subtypes harbouring a distinct genetic alteration that is associated with a specific phenotype.62 Indeed, around 90% of invasive LBC display E-cadherin loss, either at the DNA, mRNA or protein level and, in the few tumours preserving expression, E-cadherin integrity is nonetheless impaired.63,64 In contrast, E-cadherin expression is usually unaffected in ductal breast carcinomas.65 Thus, E-cadherin decreased expression can be used as a molecular marker to distinguish ductal from lobular carcinomas.

Loss of E-cadherin expression contributes to the unique histopathological features shared by DGC and LBC. These tumours do not form a well-defined mass and are composed of relatively small, infiltrating and desmoplastic epithelial cells, showing a high predisposition to metastasise to the gastrointestinal tract, gynaecological organs, meninges and peritoneal surface, resembling the DGC cells phenotype.56,67

Common to both cancer types is also the fact that CDH1 germline mutations occur across the entire gene coding sequence.53–54 However, the type and frequency of the genetic alterations differ, with missense mutations constituting the most frequent alteration in HLBC.23,34 Of the 16 novel germline CDH1 variants that have been identified in families with LBC alone, there were six missense mutations (37.5%), three splice site mutations (18.7%), three deletions (18.7%), two insertions (13%), one nonsense mutation (6.2%) and one premature stop codon.
CDH1 variants identified in patients with colorectal cancer (CRC)

| Alteration Location | Type       | Families | CRC cases | Other tumours | References |
|---------------------|------------|----------|-----------|---------------|------------|
| c.45_46insT (p.Gln16Serfs*18) | Exon 1     | Frameshift | 1         | 2             | HDGC and LBC | 33         |
| c.49-2A>G           | Intron 1   | Splice site | 1         | 1             | HDGC and lung. | 31        |
| c.49-2A>C           | Intron 1   | Splice site | 1         | 1             | Breast, skin prostate, bladder, pancreas and throat. | 27        |
| c.1008G>T          | Exon 7     | Splice site | 1         | 2             | HDGC. | 13        |
| c.1018A>G (p.Thr340Ala) | Exon 8 | Missense | 2         | 2             | HDGCf and ovary.† | 71, 33† |
| c.1225T>C (p.Trp409Arg) | Exon 9 | Missense | 1         | 1 (SRCC) DGC. | DGC. | 15        |
| c.1774G>A (p.Ala592Thr)* | Exon 12 | Missense | 2         | 3             | DGC and breast andrenal. | 72, 69† |

For each mutation, the affected site, type of mutation, number of families and carriers affected by CRC and other tumors described in the same genetic background are assigned.

*Classified as non-pathogenic in ref 69.
†Described elsewhere.

DGC, diffuse gastric cancer; HDGC, hereditary diffuse gastric cancer; LBC, lobular breast cancer; SRCC, signet ring cell carcinoma.

The involvement of CDH1 in CRC development has received some support from studies unrelated to HDGC. More specifically, a meta-analysis of genome-wide association data identified the E-cadherin locus as a new susceptibility factor for developing CRC.72 In an attempt to identify additional CRC cases with familial predisposition, comprehensive screening for mutations associated with CRC was performed in 152 patients, resulting in the identification of a single CDH1 mutation.74 In another report, a CDH1 polymorphism was also shown to be a risk factor for CRC development.75 Noteworthy, a large-scale study identified three new susceptibility loci, including the CDH1 locus, in patients with ulcerative colitis (a common form of inflammatory bowel disease).76 These data reinforce that disruption of E-cadherin could also lead to distinct alterations in the colon epithelia, from benign to malignant lesions, though this is a rare event.

Overall, the evidence implicating CDH1 germline mutations in CRC, either dependent or independent of the HDGC cancer spectrum, is still scarce. Accordingly, in current clinical practice, no recommendations are yet approved for CRC screening in CDH1 mutation carriers. Future studies will clarify whether CRC occurs occasionally in HDGC families or if it is a bona fide CDH1-associated disorder.

Cleft lip/palate

OFCs are a heterogeneous group of congenital disorders that result from complex interactions between genetic and environmental risk factors, which affect the lips and oral cavity, causing isolated cleft palate and cleft lip with or without cleft palate.77 Although variable among populations, the incidence of CLP is supposed to be in the range of 1 in 700 to 1 in 1000 newborns, ranking among the most prevalent birth defects.78 Affected individuals display high morbidity, experiencing severe feeding, hearing, speech and psychological problems.77, 78 Around 70% of CL/P cases are estimated to be non-syndromic, occurring as isolated entities in the absence of other congenital anomalies and, as such, intensive research has focused in trying to identify candidate genes and the underlying molecular pathways.76

The biological implication of E-cadherin dysfunction in the aetiology of non-syndromic OFC comes as no surprise, given its overexpression in the critical stages of lip and palate development, namely in the frontonasal prominence at 4 and 5 weeks of embryogenesis and in the lateral and medial nasal prominences at 6 weeks.79 The increased incidence of CL/P cases in CDH1 mutation carriers, when compared with that in the general population, further supports the involvement of E-cadherin in the molecular basis of this congenital defect.

Altogether, at least 12 patients (seven distinct mutations) have already been identified with CDH1-associated CRC from nine families of distinct origins. However, in most cases, the number of patients with CRC identified in these families is not sufficient to include CRC screening in the surveillance of CDH1 mutation carriers.

CDH1-associated CRC from nine families of distinct origins.

Figueiredo J, et al. J Med Genet 2019;56:199–208. doi:10.1136/jmedgenet-2018-105807

J Med Genet: first published as 10.1136/jmedgenet-2018-105807 on 19 January 2019. Downloaded from http://jmg.bmj.com/ on March 16, 2022 by guest. Protected by copyright.
In 2006, it was first reported the association of two different splice site CDH1 mutations, both affecting the E-cadherin extra-cellular domain, with OFC in two HDGC families. These findings were subsequently corroborated by Kluijt and coworkers, who described three variants associated with seven cases of CL/P in Dutch HDGC families carrying CDH1 germline mutations.

Ever since, many studies have shed light on the nature of CDH1 mutations associated with CL/P cases, their penetrance and to what extent the types of mutations or mechanisms lead to either cancer, CL/P or both phenotypes. In a cohort of 81 children with non-syndromic OFC, Vogelaar and coworkers identified three CDH1 missense mutations shown to be functionally relevant in four patients with unknown family history of DGC or LBC. Through exome sequencing, Bureau et al. were also able to identify one common damaging CDH1 mutation in three distant relatives affected with non-syndromic OFC, further highlighting the contribution of E-cadherin dysfunction in the genesis of this congenital defect. However, the definite proof came out when 221 Brazilian probands were sequenced and, when compared with that of non-familial controls, an overall increased CDH1 mutational burden among probands from non-syndromic CL/P families was found. Ultimately, this study was able to establish a consistent role of rare, moderately penetrant, loss of function CDH1 variants in OFC. Indeed, the penetrance of CL/P in CDH1 mutation carriers has been correlated with CDH1 promoter methylation arising as a second hit.

Very recently, a large whole-exome sequencing study in a cohort of 72 multifamilial non-syndromic CL/P revealed 10 pathogenic or likely pathogenic variants in five different genes, CDH1 (2), CTNND1 (5), PLEKHA7 (1), PLEKHA5 (1) and ESRP2 (1), which are functionally linked in the assembly of the epithelial cadherin/catenin complex. These genes were targeted for sequencing in a second non-syndromic CL/P cohort of 497 individuals from 444 families and 10 distinct mutations were identified, providing undisputable evidence for the role of E-cadherin and epithelial adhesion in the molecular mechanism underlying OFC. Notably, the CDH1 variants identified in this study were present in individuals with CL/P alone, with a single exception in a family where one case of gastric cancer was recently diagnosed.

Other association studies have also described CDH1 polymorphisms associated to non-syndromic CL/P. In a Brazilian study, involving 500 individuals with non-syndromic clefts and an equal number of unrelated controls, an association between two genetic variants in CDH1 and susceptibility to non-syndromic CL/P was found. A couple of years later, Song and Zhang revealed that the specific single nucleotide polymorphism rs16260 (C>A), located upstream of the transcriptional start site of the CDH1 promoter, was associated with an increased risk of cleft palate alone in a Chinese Han population.

To date, 23 different CDH1 mutations have been reported in the literature associated with CL/P, seven of which were described in families also segregating gastric cancer (Table 2). Of note is the fact that among the CDH1 mutational repertoire, some mutations increase the risk of CL/P alone, whereas others increase the risk of gastric cancer too. While it is plausible that CDH1 mutations leading to higher invasiveness (in vitro) could increase the risk of gastric cancer, it has been speculated that haploinsufficiency during critical stages of embryonic development could lead to OFC. The current landscape of CDH1 germline mutations associated with gastric cancer, CL/P or both, does not suggest any preferential type or location of CDH1

Table 2 CDH1 mutations found in patients affected by cleft lip/palate (CL/P)

| Alteration | Location | Type | CL/P cases | Cancer history | References |
|------------|----------|------|------------|----------------|------------|
| c.88C>A    | (p.Pro30Thr) | Missense | 2 | 1DGC*, 2LBC* | 22, 24, 28* |
| c.337A>G   | (p.Lys113Glu) | Missense | 1 | 22 |
| c.387+5G>A | Intron 3 | Splice site | 1 | 24 |
| c.531+2T>A | Intron 4 | Splice site | 4 | 3DGC; 1GC | 20 |
| c.531+3A>G | Intron 4 | Splice site | 1 | 22 |
| c.532–18C>T | Intron 4 | Splice site | 3 | 1DGC*; 1 mixed GC* | 22, 28* |
| c.752C>T   | (p.Thr251Met) | Missense | 1 | 22 |
| c.760G>A   | (p.Asp254Asn) | Missense | 8+1* | 24, 81* |
| c.768T>A   | (p.Asn268Lys) | Missense | 6 | 81 |
| c.832+1G>T | Intron 6 | Splice site | 1 | 1DGC | 99 |
| c.1023T>G  | (p.Tyr341*) | Nonsense | 5 | 2DGC* | 24, 100* |
| c.1108G>T  | (p.Asp370Tyr) | Missense | 1 | 22 |
| c.1135_1137+5delins5 | Exon 8 | Splice site | 1 | 2DGC; 1GC | 20 |
| c.1137G>A  | Exon 8 | Splice site | 1 | 2DGC; 1GC | 20 |
| c.1404delC | (p.Thr468Thrfs*13) | Frameshift | 3 | 9GC; 1BC | 17 |
| c.1489G>A  | (p.Glu497Lys) | Missense | 1 | 81 |
| c.1766A>G  | (p.Asn589Lys) | Missense | 1 | 81 |
| c.2143G>T  | (p.Gly715*) | Nonsense | 3 | 79 |
| c.2351G>A  | (p.Arg784His) | Missense | 3 | 24 |
| c.2413G>A  | (p.Asp805Asn) | Missense | 1 | 1DGC* | 22, 16* |
| c.2426_2427delC (p.Asn809delfs*3) | Exon 15 | Frameshift | 4 | 22 |
| c.2439+10C>T | Intron 15 | Splice site | 1 | 22 |
| c.2440–6_2440–4del | Intron 15 | Splice site | 1 | 22 |

The genetic alteration, mutation type, number of CL/P cases and reports of associated cancers are presented. Described elsewhere.
HDGC, hereditary diffuse gastric cancer; LBC, lobular breast cancer; DGC, diffuse gastric cancer; GC, gastric cancer; BC, breast cancer.

Figueiredo J, et al. J Med Genet 2019;56:199–208. doi:10.1136/jmedgenet-2018-105807
germline mutations along the different domains, preventing any sort of differential patient management (figure 2). Moreover, some expert groups—like the Dutch Working Group on Hereditary Gastric Cancer—believe that future parents of CDH1 mutation carriers are not required to be informed regarding the risk of CL/P in their offspring as an integral part of genetic counselling. Nevertheless, occurrence of CL/P abnormalities should be reported in families with HDGC history, so careful counselling can be offered.

Considering all the evidence provided throughout the past decades, the contribution of CDH1 variants to OFC aetiology is unquestionable. However, the incomplete penetrance and variable expressivity of non-syndromic CL/P awards this congenital trait with a complexity that needs to be addressed through a multidisciplinary approach.

Blepharocheilodontic syndrome

Data implicating E-cadherin deregulation in other congenital disorders is growing, further validating the essential role of E-cadherin in human embryological development. BCDS (OMIM #119580), which is a rare birth disorder, was recently added to the set of CDH1-related diseases (figure 3). This syndrome, first described by Elschnig in 1912, usually occurs in sporadic patients but is also reported in large non-consanguineous families, suggesting an autosomal dominant segregation.

BCDS is characterised by bilateral CL/P which is its major feature, dental anomalies (such as conical teeth and tooth agenesis), hair defects and eyelid malformations, namely ectropion of the lower eyelids, euryblepharon and lagophthalmia. In addition, other clinical features, such as imperforate anus, neural tube defect, hypothyroidism due to thyroid gland aplasia or hypoplasia and syndactyly have also been reported in a few patients. Despite the clinical burden and the severe impact on patients’ lives, the causes of such complex condition are yet to be uncovered.

In an attempt to understand the molecular basis of this disease, Freitas et al have screened several genes implicated in syndromes with a similar phenotype, namely TP63 and IRF6 (ectodermal dysplasia syndromes), FOXE1 (orofacial clefting associated with thyroid agenesis) and TBX10 and OR2 (orofacial clefting associated with thyroid agenesis genes). However, they failed to identify a clear causative mutation. Interestingly, in 2016, Nishi et al identified a CDH1 missense mutation in a patient presenting right choanal atresia and several dysmorphic facial features, such as CL/P. Subsequently, two other independent studies, published in 2017 and 2018, used an exome sequencing approach on patients with BCDS and also found mutations in CDH1 and in one other member of the cadherin–catenin complex, CTNND1. Ghoumid et al described three deletions and two heterozygous missense mutations in the CDH1 gene, and three CTNND1 truncating mutations, while Kievit’s team identified seven CDH1 variants (four missense and three deletions), as well as two truncating mutations and one missense variant in CTNND1 (table 3). The first group also detected two asymptomatic parents carrying either a CDH1 or a CTNND1 mutation, suggesting incomplete penetrance of the genotype.

Figure 2  Distribution of CDH1 germline mutations identified in the different disorders. The scheme illustrates E-cadherin signal peptide, precursor sequence, extracellular domains, transmembrane domain (TM) and cytoplasmic domain. The location of CDH1 mutations described in lobular breast cancer (black), colorectal cancer (blue), cleft lip/palate (red) and blepharocheilodontic syndrome (green) is represented.

Figure 3  Illustration of E-cadherin pleiotropic effects. CDH1 germline mutations, inducing loss of E-cadherin expression and function, can result in hereditary cancer or congenital malformations. Diseases such as diffuse gastric cancer and lobular breast cancer, as well as cleft lip/palate and the blepharocheilodontic syndrome, constitute the clinical spectrum of CDH1 mutation carriers.
Of note, all CDH1 mutations described in this context affect the E-cadherin extracellular domain, possibly impairing its ability to homodimerise and, consequently, interfering with its adhesive function. In particular, both studies detected missense mutations at the conserved Asp254-Gln255-Asn-256 ‘linker’ region that comprises a calcium ion-binding site between extracellular domains 1 and 2. As a result, the interaction of these residues with calcium ions may be disrupted, perturbing protein conformation. The CDH1 splice site variant c.1320+1G>C disturbs the consensus donor motif AGgt, which is critical for splicing, and induces exon 9 skipping. This leads to the removal of a major portion of the extracellular domain 3 (EC3), impairing its function. Furthermore, the mutation c.1361_1363del (p.Val454del) removes the hydrophobic cluster located at the c-terminal region of a beta-strand of the EC3 domain.

While Ghoumid et al reported that BCDS CDH1 missense variants behave similarly to HDGC-associated ones, with perinuclear accumulation and accelerated E-cadherin degradation, Kievit et al described a dominant negative effect of BCDS CDH1 variants. The researchers transfected cells with GFP-WT CDH1 and different SNAP-tagged CDH1 variants (including two HDGC-related mutations for comparison) and observed variants (including CDH1 and CTNND1) that dimerise with WT proteins and interfere with the formation of adherens junctions, impairing cell–cell adhesion.

The CTNND1 truncating variants affect p120-catenin isoforms 1–3. These mutations result in premature stop codons, which activate the nonsense-mediated RNA decay mechanism, leading to mRNA degradation and subsequent haploinsufficiency. Kievit et al also identified a missense mutation that affects the highly conserved H3 helix in the fifth armadillo repeat of CTNND1, which is essential for the interaction between p120-catenin and E-cadherin’s cytoplasmic tail. The disruption of p120-catenin/E-cadherin binding destabilises E-cadherin at the membrane and makes E-cadherin available for interaction with proteins from the endocytic machinery, like clathrin adapter proteins and Hakai, that target E-cadherin for degradation. Although no genotype–phenotype correlation was found, a general observation from both studies was that patients with CTNND1 mutations have milder symptoms when compared with patients with CDH1 variants. For example, clefting is less frequent, as is hypothyroidism, neural tube defects and imperforate anus. In the cases where neither CDH1 nor CTNND1 mutations were detected, patients have even less severe features.

Taking these findings into account, it is clear that the function of E-cadherin goes far beyond its invasion and tumour suppressor role. Abnormal E-cadherin expression and signalling can drive the formation of irregular morphological structures, such as defects of palate, lips, eyelids, teeth and neural tube, expanding the clinical spectrum of CDH1-associated diseases to severe congenital malformations.

### CONCLUSION

Compelling evidence suggests that CDH1/E-cadherin is involved in multiple tissue processes, and its dysfunction can result in a plethora of clinical manifestations. It is now widely accepted that DGC and LBC, as well as congenital malformations, such as CL/P and BCDS, are part of the CDH1-disease spectrum. Thus, CDH1 is emerging as a pleiotropic gene.

Pleiotropy is the phenomenon by which the same gene can result in a plethora of effects. In fact, 16.9% of genes and 4.6% of genetic variants have pleiotropic consequences, as shown by the GWAS catalogue. Although the occurrence of pleiotropy is a central phenomena for evolution and has been recognised for over 100 years, the mechanisms by which a single gene can affect multiple traits are still far from being fully understood. Several mechanisms have been proposed to explain the pleiotropic effect of a gene at the molecular level. One interesting explanation is the presence of different protein domains exerting distinct cellular functions. However, in the case of E-cadherin, this is not a valid explanation as mutations affecting the same nucleotide can induce different disorders. Instead, we propose that CDH1 pleiotropy is related to the specific consequences of each genetic alteration, the location, type of mutation, number of BCDS cases, as well as the corresponding reference are displayed.

### Table 3 CDH1 and CTNND1 mutations found in patients with the blepharocLEFToidontic syndrome (BCDS)

| Gene   | Alteration          | Location | Type        | BCDS cases | Reference |
|--------|---------------------|----------|-------------|------------|-----------|
| CDH1   | c.760G>T (p.Asp254Tyr) | Exon 6   | Missense    | 1          | 85        |
| CDH1   | c.760G>A (p.Asp254Asn) | Exon 6   | Missense    | 7          | 86        |
| CDH1   | c.768T>G (p.Asn256Lys) | Exon 6   | Missense    | 2          | 86        |
| CDH1   | c.770A>C (p.Asp257Val) | Exon 6   | Missense    | 1          | 85        |
| CDH1   | c.862G>C (p.Asp288His) | Exon 7   | Missense    | 1          | 86        |
| CDH1   | c.1118C>G (p.Pro373Arg) | Exon 8   | Missense    | 3          | 86        |
| CDH1   | c.1320G>T           | Exon 9   | Splice site? | 2          | 85        |
| CDH1   | c.1320+1G>C         | Intron 9  | Splice site? | 2          | 85        |
| CDH1   | c.1320+1G>A         | Intron 9  | Splice site? | 2          | 86        |
| CDH1   | c.1320+1G>T         | Intron 9  | Splice site? | 1          | 86        |
| CDH1   | c.1320+5G>A         | Intron 9  | Splice site? | 1          | 86        |
| CDH1   | c.1361_1363del (p.Val454del) | Exon 10 | Deletion    | 1          | 85        |
| CDH1   | c.2028C>A (p.Asp676Glu) | Exon 13  | Missense    | 1          | 84        |
| CTNND1 | c.606_627del (p.Pro203Leufs*25) | Exon 6   | Frameshift  | 1          | 85        |
| CTNND1 | c.1093C>T (p.Glu365*) | Exon 7   | Nonsense    | 1          | 85        |
| CTNND1 | c.1372C>T (p.Arg458*) | Exon 7   | Nonsense    | 5          | 86        |
| CTNND1 | c.1595G>A (p.Glu532Asp) | Exon 8   | Missense    | 1          | 86        |
| CTNND1 | c.2098C>T (p.Arg700*) | Exon 14  | Nonsense    | 2          | 85        |
| CTNND1 | c.2489G>A (p.Trp830*) | Exon 16  | Nonsense    | 1          | 86        |

For each genetic alteration, the location, type of mutation, number of BCDS cases, as well as the corresponding reference are displayed.
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each particular mutation. Indeed, we have demonstrated that specific mutations cause E-cadherin to interact differently with its binding partners, activating different signalling pathways and inducing different cell behaviours.939697 Notably, the same mutation can be identified in either DGC families or non-syndromic CL/P cases, suggesting that additional factors such as genetic background may have a role in the development of both diseases.

Overall, this collected knowledge changes the paradigm of E-cadherin impact that, until recently, was only focused on hereditary cancer-predisposing syndromes. From now on, birth defects and congenital anomalies, which tended to be disregarded by clinicians and many researchers, will draw more attention and will be a significant part of genetic (familial) analysis. It is expected that the development of novel tools will improve genetic testing and reveal the biological mechanisms underlying pleiotropic outcomes. These findings will have implications in management of individuals with CDH1 germline alterations and will be crucial to evaluate their risk of cancer or of having a neonate affected by congenital malformations.

Acknowledgements
We would like to thank the patients and families for their participation in CDH1-related studies. We also acknowledge the clinicians who sent us new mutations and information Non Commercial (CC BY-NC 4.0) licence, which most of them of the ICGC consortium. Contributors JF was responsible for study concept and design, JF, SM, PC, AMM, MSF and ASR have drafted the article. PG, JP and RS critically reviewed the manuscript for important intellectual content. All authors approved the final version of the manuscript.

Funding
This work was financed by FEDER funds through the Operational Programme for Competitiveness Factors (COMPETE 2020), Programa Operacional de Competitividade e Internacionalização, Programa Operacional Regional do Norte (Norte 2020) and by National Funds through the Portuguese Foundation for Science and Technology, under the projects PTDIC/MED/GEN-30356/2017, PTDIC/BIM-ONC/1171/2012, PTDIC/BIM-ONC/0281/2014, PTDIC/BBB-IMG/0283/2014, NORT-01-01-04-FEDER-00029; postdoctoral grant SRH/BPD/87705/2012-JF and doctoral grants SRH/BDI/100809/2015-SM and SRH/BDI/114687/2016-AMM. We acknowledge the American Association of Patients with Hereditary Gastric Cancer ‘No Stomach for Cancer’ for funding RS and FJ’s research.

Competing interests
None declared.

Patient consent for publication
Not required.

Provenance and peer review
Not commissioned; externally peer reviewed.

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