

DOUTORAMENTO
CIÊNCIAS BIOMÉDICAS

Inherited predisposition to gastric cancer of different histological subtypes

Joana Guerra

D
2024

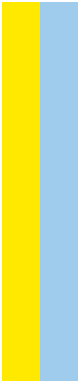


Joana Guerra. Inherited predisposition to gastric cancer of different histological subtypes



Inherited predisposition to gastric cancer of different histological subtypes

Joana Guerra



Joana Sofia Gonçalves Guerra

Inherited predisposition to gastric cancer of different histological subtypes

Dissertation for applying to a Doctoral degree in
Biomedical Sciences submitted to the
ICBAS - School of Medicine and Biomedical
Sciences, University of Porto, Portugal

Supervisor – Professor Manuel R. Teixeira
Category – Guest Full Professor
Affiliation – School of Medicine and Biomedical
Sciences, University of Porto, Portugal

Co-supervisor – Professor Carla Oliveira
Category – Principal Investigator
Affiliation – Institute for Research and Innovation
in Health, University of Porto, Portugal

Co-supervisor – Professor Luis Carvajal-Carmona
Category – Associated Professor
Affiliation – University of California, Davis, USA

Acknowledgments/Agradecimentos

Ao Professor Doutor Manuel Teixeira, Director do Serviço de Genética, por me ter dado a oportunidade de desenvolver este trabalho sob a sua orientação, pelo apoio e disponibilidade durante estes anos.

À Professora Carla Oliveira, co-orientadora deste trabalho, pela ajuda e disponibilidade.

To my co-supervisor Luis Carvajal-Carmona, for the opportunity to develop part of this work in the LCC lab, for the support, and insightful contributions. To Paul for sharing his knowledge with me. To Ana, Carol, Alma, and Graciela for the support, kindness, and friendship during my time in Davis. *Muchas gracias chicas.*

À Manela, Carla, Pedro, Anita e Catarina por me terem ensinado tudo o que sei nesta área. Obrigada pela celebração das conquistas e pelo apoio nos momentos menos bons. Sem dúvida alguma que esta tese não existia sem eles. Esta tese também é um bocadinho vossa. À Ana por todas as gargalhas e momentos bem passados.

A todos os meus colegas do Serviço de Genética do IPO-Porto, que contribuíram para o desenvolvimento de todos os trabalhos apresentados nesta tese.

A todos os co-autores dos artigos incluídos nesta tese pela colaboração.

Aos amigos de uma vida, Catarina, Vera, Wanda, Sara, Nuno, Fábio, Luís e Vítor, obrigada pela amizade, apoio e por todos os bons momentos. Um agradecimento especial ao Vítor pela ajuda nas imagens incluídas nesta tese e nos artigos. Um agradecimento muito especial ao Luís por “estar sempre lá” e por acreditar em mim.

À irmã que não tive, Rak, por toda a amizade e apoio mesmo a 2000 km de distância, por ser o meu “80” e “obrigar-me” a nunca ficar parada.

Às minhas *girls LIR*, Rocha, Oliveira, In-Uba, Tedim e Mendonça, obrigada pela amizade e apoio. Obrigada por serem a minha inspiração de querer sempre fazer mais e melhor.

Aos meus pais, por me apoiarem em todas as escolhas e decisões que tomo, pelo amor incondicional. Nada disto teria sido possível sem vocês.

Apoio financeiro

Fundação para a Ciência e a Tecnologia (bolsa de doutoramento SFRH/BD/138670/2018).

Declaração de honra

Declaro que a presente tese é de minha autoria e não foi utilizada previamente noutro curso ou unidade curricular, desta ou de outra instituição. As referências a outros autores (afirmações, ideias, pensamentos) respeitam escrupulosamente as regras da atribuição, e encontram-se devidamente indicadas no texto e nas referências bibliográficas, de acordo com as normas de referência. Tenho consciência de que a prática de plágio e auto-plágio constitui um ilícito académico.

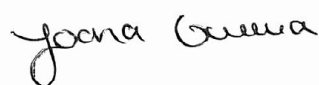
A handwritten signature in black ink, reading "Joana Guerra". The signature is written in a cursive style, with the first name "Joana" and the last name "Guerra" clearly legible.

Table of contents

Acknowledgments/Agradecimentos	3
Declaração de honra.....	5
Table of contents	7
Summary.....	9
Resumo	11
Publications.....	15
List of abbreviations	17
Introduction	21
1. Gastric cancer	21
1.1 Epidemiology and risk factors	21
1.2 Pathology	22
1.2.1 Molecular classification	24
2. Inherited predisposition to gastric cancer	25
2.1 Hereditary diffuse gastric cancer	26
2.1.1 Diagnostic criteria	27
2.1.2 The <i>CDH1</i> gene	28
2.1.3 The <i>CTNNA1</i> gene	29
2.1.4 Clinical surveillance and management	32
2.2 Familial intestinal gastric cancer	33
2.3 Gastric adenocarcinoma and proximal polyposis of the stomach	34
2.4 Gastric cancer in other cancer-associated syndromes	35
2.5 The missing heritability in gastric cancer	37
Aims	41
Paper I	43
Frequency of <i>CDH1</i>, <i>CTNNA1</i> and <i>CTNND1</i> Germline Variants in Families with Diffuse and Mixed Gastric Cancer	43
Paper II	55

Contribution of multi-organ cancer predisposition genes for gastric cancer risk of different histological subtypes	55
General Discussion	83
1. Comparison of the frequencies of <i>CDH1</i> germline variants in families fulfilling the IGCLC criteria for HDGC and families with mixed gastric cancer	83
2. Contribution of <i>CTNNA1</i> and <i>CTNND1</i> germline variants in families with diffuse and mixed gastric cancer	84
3. Contribution of variants in multi-organ cancer susceptibility genes to gastric cancer risk of different histological subtypes	86
4. Validation of the use of NGS for germline variant screening in archival formalin-fixed paraffin-embedded (FFPE) samples.....	89
Conclusions	93
Future Perspectives.....	95
References.....	97

Summary

Familial clustering of gastric cancer (GC) accounts for approximately 10% of the cases, however only 1-3% of GCs result from a known hereditary syndrome. There are three main gastric hereditary syndromes: (1) hereditary diffuse gastric cancer (HDGC); (2) gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS); and (3) familial intestinal gastric cancer (FIGC). The most well-known hereditary form of GC is HDGC caused by germline variants in *CDH1* and, in a minority of cases, by germline variants in *CTNNA1* gene, and it is characterized by an increased risk of diffuse GC and lobular breast cancer. However, while intestinal GC has been completely excluded from the HDGC tumor spectrum, the International Gastric Cancer Linkage Consortium (IGCLC) guidelines do not clarify whether or not mixed GC (with a diffuse and intestinal component) should be considered in the HDGC genetic testing criteria. FIGC has been clinically defined in families with an autosomal dominant inheritance pattern associated with an increased risk of intestinal GC, nevertheless, remains widely understudied and genetically unexplained to date. A lot of efforts have been made to identify the heritable genetic causes of diffuse GC beyond the *CDH1* and *CTNNA1* genes. Despite these efforts, additional genes that predispose to this histological subtype have yet to be identified. Similarly, the heritability of families with non-diffuse GC clustering remains mostly unknown. Moreover, GC has been described in other known hereditary cancer syndromes or as part of the spectrum of moderate-risk cancer predisposition genes. The identification of new GC predisposition genes is extremely important to provide suitable genetic counseling, surveillance, and/or risk reduction measures, and ultimately prevent disease onset.

The major aims of this thesis were: to compare the frequencies of *CDH1* germline variants in families with diffuse and mixed GC to evaluate if *CDH1* testing should or not be considered in patients with mixed GC; To evaluate the contribution of variants in other cancer susceptibility genes to diffuse GC inherited predisposition in families meeting the IGCLC criteria for HDGC without germline variants in *CDH1*; To identify germline variants predisposing to GC, regardless of histology, using a targeted gene panel and/or whole-exome sequencing (WES).

The prevalence of pathogenic *CDH1* variants in our patients with HDGC genetic testing criteria is approximately 3%. In this study, we did not find any pathogenic variant in the *CDH1* gene in a series of 67 patients affected with mixed GC, who would have met the IGCLC 2015 or 2020 testing criteria if this histological type had been considered for genetic testing in the same way as diffuse GC. We also analyzed the *CTNNA1* and *CTNND1* genes

Summary

in 208 cases with diffuse or mixed gastric cancer who had tested negative for *CDH1* pathogenic germline variants. We identified a novel nonsense variant in the *CTNNA1* gene (0.7%) in a woman diagnosed with a poorly differentiated adenocarcinoma with signet ring cells at 27 years old. No deleterious variants were found in *CTNND1*, but six variants of uncertain significance were identified in this gene.

We also analyzed 667 index cases from a multi-center cohort with different histological subtypes using two different sequencing approaches, targeted sequencing and WES to evaluate the contribution of pathogenic/likely pathogenic (P/LP) variants in known multi-tumor high-moderate penetrance cancer predisposition genes for the heritability of GC. We identified 39 patients (6%) with P/LP pathogenic variants in *ATM* (13 cases), *BRCA2* (nine cases), *MLH1* (five cases), *BRCA1*, *PALB2*, *TP53*, *RAD51D* and *CHEK2* (two patients each), and *RAD51C* and *PMS2* (one case each), all mutually exclusive. The P/LP variant prevalence was higher in intestinal (9.3%) than in diffuse (4.2%) or mixed GC (4.9%), with no clear difference per mutated gene by histological subtype. Family history of cancer was also associated with a higher mutation prevalence.

Based on the findings of this thesis, currently there is no evidence that supports including mixed GC in the germline testing of the *CDH1* or *CTNNA1* genes. A deleterious *CTNNA1* germline variant was found in 0.7% of patients meeting the 2020 IGCLC criteria for HDGC testing. Despite this relatively low frequency, screening for the *CTNNA1* gene should be considered in *CDH1*-negative HDGC families. The *CTNND1* gene is an ideal candidate for GC predisposition, but only variants of uncertain significance were identified in this gene. However, larger studies are needed to understand the role of *CTNND1* variants in inherited GC predisposition. Pathogenic or likely pathogenic variants in multi-organ cancer predisposition genes, namely in genes involved in DNA damage repair (Homologous recombination and mismatch repair), explain a significant proportion of GC families, regardless of the histological type. Therefore, these genes should be included in gene panels used for investigating germline variants in GC patients, especially when there is family history of cancer, irrespective of histology subtype, as this would increase the chance of identifying patients that might benefit from prevention and targeted treatment strategies.

Resumo

A agregação familiar de cancro gástrico representa aproximadamente 10% destes casos, no entanto, apenas 1-3% destes resultam de uma síndrome hereditária conhecida. As três principais síndromes hereditárias de cancro gástrico são: (1) cancro gástrico difuso hereditário (*HDGC*); (2) adenocarcinoma gástrico e polipose proximal do estômago (*GAPPS*); e (3) cancro gástrico intestinal familiar (*FIGC*). A forma hereditária mais conhecida é a HDGC, causada por variantes germinativas patogénicas no gene *CDH1* e, numa minoria dos casos, no gene *CTNNA1*. Esta síndrome é caracterizada por um aumento do risco de cancro gástrico do tipo difuso e cancro da mama lobular. No entanto, enquanto o cancro gástrico do tipo intestinal não pertence ao espectro tumoral da HDGC, as *guidelines* do *International Gastric Cancer Linkage Consortium* (IGCLC) não esclarecem se o cancro gástrico do tipo misto (com um componente difuso e intestinal) deve ou não ser considerado nos critérios de teste genético para HDGC. A síndrome FIGC foi definida clinicamente em famílias com um padrão de hereditariedade autossómico dominante associado a um aumento do risco de cancro gástrico do tipo intestinal, no entanto, esta síndrome continua pouco estudada e geneticamente inexplicada até à data. Vários estudos têm surgido nos últimos anos para tentar identificar novos genes que expliquem a predisposição hereditária de cancro gástrico do tipo difuso, para além dos genes já conhecidos *CDH1* e *CTNNA1*. Da mesma forma, as famílias com agregação de cancro gástrico do tipo intestinal ou misto permanecem sem causa genética conhecida. Além disso, o cancro gástrico está descrito noutras síndromes conhecidas de cancro hereditário ou como parte do espectro tumoral de genes associados a um risco moderado para o desenvolvimento de cancro. A identificação de novos genes de predisposição para cancro gástrico é extremamente importante para um aconselhamento genético adequado, vigilância e/ou implementação de medidas de redução de risco.

Este estudo teve como objetivos principais comparar as frequências de variantes patogénicas germinativas no gene *CDH1* em famílias com cancro gástrico do tipo difuso e misto para avaliar se o teste genético deve ou não ser considerado em doentes com cancro gástrico do tipo misto; Avaliar a contribuição de variantes patogénicas noutros genes, já associados a um aumento do risco para o desenvolvimento de cancro, na predisposição hereditária de cancro gástrico do tipo difuso em famílias que preencham os critérios da IGCLC para HDGC e sem variantes patogénicas germinativas no gene *CDH1*; Identificar variantes patogénicas germinativas que possam estar associadas a predisposição

Resumo

hereditária de cancro gástrico, independentemente da histologia, utilizando um painel de genes e/ou *whole exome sequencing* (WES).

No nosso estudo a prevalência de variantes patogénicas no gene *CDH1* em doentes com critérios para teste genético é aproximadamente de 3%. Neste estudo, não encontramos nenhuma variante patogénica no gene *CDH1* numa série de 67 doentes afetados com cancro gástrico do tipo misto, que teriam preenchido os critérios de teste, se este tipo histológico tivesse sido considerado para teste genético da mesma forma que o cancro gástrico do tipo difuso. Também analisamos os genes *CTNNA1* e *CTNND1* em 208 casos de cancro gástrico difuso ou misto sem variantes germinativas patogénicas no gene *CDH1*. Identificamos uma nova variante germinativa no gene *CTNNA1* (0,7%) numa mulher diagnosticada com um adenocarcinoma pouco diferenciado com células em anel de sinete aos 27 anos. Não foram encontradas variantes patogénicas no gene *CTNND1*, mas foram identificadas seis variantes de significado desconhecido neste gene.

Também analisamos 667 casos índice de uma coorte multicêntrica com diferentes subtipos histológicos, usando duas abordagens de sequenciação diferentes, sequenciação com um painel de genes selecionado e WES, para avaliar a contribuição de variantes patogénicas/provavelmente patogénicas em genes de penetrância alta-moderada já associados a risco de desenvolvimento de cancro, para o predisposição hereditária de cancro gástrico. Identificamos 39 doentes (6%) com variantes patogénicas/provavelmente patogénicas nos genes *ATM* (13 casos), *BRCA2* (nove casos), *MLH1* (cinco casos), *BRCA1*, *PALB2*, *TP53*, *RAD51D* e *CHEK2* (dois doentes em cada gene), e *RAD51C* e *PMS2* (um caso em cada gene), todos mutuamente exclusivos. A prevalência de variantes patogénicas/provavelmente patogénicas foi maior no cancro gástrico do tipo intestinal (9,3%) do que no tipo difuso (4,2%) ou misto (4,9%), sem diferenças de gene mutado por subtipos histológicos. A história familiar de cancro também foi associada a uma maior prevalência de mutações.

Baseado nos resultados desta tese, atualmente não há evidência para incluir o cancro gástrico do tipo misto nos critérios de teste genético para pesquisa de variantes germinativas nos genes *CDH1* ou *CTNNA1*. Foi identificada uma variante germinativa patogénica no gene *CTNNA1* em 0,7% dos doentes que preenchiam os critérios da IGCLC para teste genético. Apesar desta frequência relativamente baixa, a pesquisa de variantes germinativas no gene *CTNNA1* deve ser considerada em famílias negativas para variantes patogénicas no gene *CDH1*. O gene *CTNND1* é um candidato ideal de predisposição para cancro gástrico difuso, no entanto apenas variantes de significado desconhecido foram identificadas neste gene. São necessários mais estudos para compreender o papel deste gene na predisposição hereditária para cancro gástrico. Variantes patogénicas ou potencialmente patogénicas em genes associados à predisposição de cancro,

nomeadamente em genes envolvidos na reparação de danos no DNA (recombinação homóloga e reparação de erros de emparelhamento), explicam uma proporção significativa das famílias de cancro gástrico, independentemente do tipo histológico. Desta forma, sugerimos que estes genes sejam incluídos nos painéis de genes usados para identificar variantes germinativas em doentes com cancro gástrico, especialmente quando há história familiar de cancro, independentemente do subtipo histológico, uma vez que a probabilidade de identificar doentes que possam beneficiar de medidas de prevenção e terapias alvo é mais elevada.

Publications

According to “Artigo nº 34, do Decreto-Lei nº 115/2013”, results contained in the following published works were used for this thesis:

PAPER I

Joana Guerra, Carla Pinto, Pedro Pinto, Manuela Pinheiro, Catarina Santos, Ana Peixoto, Carla Escudeiro, Ana Barbosa, Miguel Porto, Inês Francisco, Paula Lopes, Ana Raquel Isidoro, Ana Luísa Cunha, Cristina Albuquerque, Isabel Claro, Carla Oliveira, João Silva, Manuel R Teixeira. **Frequency of *CDH1*, *CTNNA1* and *CTNND1* Germline Variants in Families with Diffuse and Mixed Gastric Cancer.** *Cancers* 2023 Aug 29;15(17):4313.

PAPER II

Joana Guerra*, Ana Estrada-Florez*, Paul Lott*, Carla Pinto, Manuela Pinheiro, Pedro Pinto, Ana Peixoto, Catarina Santos, Ana Barbosa, João Silva, John Suarez-Olaya, Fabian Castro, Graciela Molina, Alejandro Corvalán, Patricio González, Carol Parra, Osvaldo Torres, Cedric Adelsdorfer, Jose Martínez, Adriana Della Valle, Esteban Castelao, Javier Torres, Nora Rios Sarabia, Alfredo Medrano, Magdalena Echeverry de Polanco, Ana Lucia Rivera, Rafael Parra, Alicia Cock-Rada, Mabel Bohorquez, Carolina Sanabria**, Manuel R. Teixeira**, Luis Carvajal-Carmona**. **Contribution of multi-organ cancer predisposition genes for gastric cancer risk of different histological subtypes.** *In preparation.*

* Contributed equally/Joint-first authors.

** Co-Senior authors

List of abbreviations

ACMG	American College of Medical Genetics and Genomics
ACRG	Asian Cancer Research Group
AFAP	Attenuated familial adenomatous polyposis
AMP	Association for Molecular Pathology
APC	<i>APC regulator of WNT signaling pathway</i>
ARID1A	<i>AT-rich interaction domain 1A</i>
ATM	<i>ATM serine/threonine kinase</i>
ATR	<i>ATR serine/threonine kinase</i>
BCD	Blepharocheilodontic syndrome
BMPR1A	<i>Bone morphogenetic protein receptor type 1A</i>
BRCA1	<i>BRCA1 DNA repair associated</i>
BRCA2	<i>BRCA2 DNA repair associated</i>
CDH1	<i>Cadherin-1</i>
CHEK2	<i>Checkpoint kinase 2</i>
CIN	Chromosomal instability
CLDN18	<i>Claudin-18</i>
CTNNA1	<i>Catenin alpha 1</i>
CTNND1	<i>Catenin delta 1</i>
DGC	Diffuse gastric cancer
DOT1L	<i>DOT1-like histone H3K79 methyltransferase</i>
EBV	Epstein-Barr virus
EBV-positive	Epstein-Barr virus-positive
EC	Extracellular cadherin
EGFR	<i>Epidermal growth factor receptor</i>
EMA	European Medicines Agency
EMT	Epithelial-to-mesenchymal transition
EPCAM	<i>Epithelial cell adhesion molecule</i>
ERBB2	<i>Erb-b2 receptor tyrosine kinase 2</i>
FAP	Familial Adenomatous Polyposis
FBXO24	<i>F-box protein 24</i>
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded

List of abbreviations

<i>FGFR2</i>	<i>Fibroblast growth factor receptor 2</i>
FIGC	Familial intestinal gastric cancer
Fmn	Formin proteins
GAPPS	Gastric adenocarcinoma and proximal polyposis of the stomach
GC	Gastric cancer
GS	Genomically stable
<i>H. pylori</i>	<i>Helicobacter pylori</i>
HBOC	Hereditary breast and ovarian cancer syndrome
HDGC	Hereditary diffuse gastric cancer
HER2	Receptor tyrosine-protein kinase erbB-2
IGCLC	International Gastric Cancer Linkage Consortium
<i>INSR</i>	<i>Insulin receptor</i>
JAK2	<i>Janus Kinase 2</i>
JPS	Juvenile polyposis syndrome
KRAS	KRAS proto-oncogene, GTPase
LBC	Lobular breast cancer
LFS	Li-Fraumeni syndrome
LOH	Loss of heterozygosity
LS	Lynch Syndrome
MAP	MUTYH-associated polyposis
<i>MAP3K6</i>	<i>Mitogen-activated protein kinase kinase kinase 6</i>
<i>MET</i>	<i>MET proto-oncogene, receptor tyrosine kinase</i>
<i>MLH1</i>	<i>MutL homolog 1</i>
MMR	Mismatch repair
<i>MSH2</i>	<i>mutS homolog 2</i>
MRE11	MRE11 homolog, double strand break repair nuclease
<i>MSH6</i>	<i>mutS homolog 6</i>
MSI	Microsatellite instability
<i>MSR1</i>	<i>Macrophage Scavenger Receptor 1</i>
MSS	Microsatellite stable
<i>MUTYH</i>	<i>mutY DNA glycosylase</i>
<i>NBN</i>	<i>Nibrin</i>
NGS	Next-generation sequencing
<i>NRAS</i>	<i>NRAS proto-oncogene, GTPase</i>
p120	Catenin delta-1

P/LP	Pathogenic/Likely pathogenic
<i>PALB2</i>	<i>Partner And Localizer of BRCA2</i>
PARP	Poly (ADP-ribose) polymerase
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligands 1
PD-L2	Programmed death ligands 2
PHTS	PTEN hamartoma tumor syndrome
<i>PIK3CA</i>	<i>Phosphatidylinositol-4-5-bisphosphate 3-kinase catalytic subunit alpha</i>
PJS	Peutz-Jeghers syndrome
<i>PMS2</i>	<i>PMS1 homolog 2</i>
<i>PRSS1</i>	<i>Serine Protease 1</i>
PTG	Prophylactic total gastrectomy
<i>RAD51C</i>	<i>RAD51 paralog C</i>
<i>RAD51D</i>	<i>RAD51 paralog D</i>
<i>RHOA</i>	<i>Ras homolog family member A</i>
<i>SMAD4</i>	<i>SMAD family member 4</i>
SRC	Signet ring cell
SRCC	Signet-ring cell carcinoma
<i>STK11</i>	<i>Serine/threonine kinase 11</i>
TCGA	The Cancer Genome Atlas
TMB	Tumor mutation burden
<i>TP53</i>	<i>Tumor protein p53</i>
VAF	Variant allele frequency
VEGF	Vascular endothelial growth factor
VEGFR2	Vascular endothelial growth factor receptor 2
WES	Whole exome sequencing
WHO	World Health Organization
<i>XRCC2</i>	<i>X-ray repair cross complementing 2</i>
YY1	Transcription factor Yin Yang 1

Introduction

1. Gastric cancer

1.1 Epidemiology and risk factors

Overall, the burden of cancer incidence and mortality is quickly increasing worldwide, reflecting both aging and growth of the population, as well as changes in the prevalence and distribution of the main risk factors for cancer. According to GLOBOCAN, in 2020, 19.3 million new cancer cases and almost 10.0 million cancer deaths have been estimated (Sung et al. 2021).

Gastric cancer (GC) remains a global health burden, accounting for a considerable incidence and mortality worldwide. In 2020, GC was the 5th most common cancer, being responsible for over one million new cases, and the 4th leading cause of cancer death worldwide, with an estimated 769,000 deaths (Sung et al. 2021; Morgan et al. 2022). There is a considerable geographic disparity in GC, being incidence rates highest in Eastern Asia, Central and Eastern Europe, and South America, whereas rates in Northern America and Northern Europe are generally low, with the lowest rate in Southern Africa (Ferlay et al. 2021). Portugal has a moderate to high incidence of GC, being the 5th most common cancer and the 3rd leading cause of cancer death (Ferlay et al. 2021).

Several factors have been established to increase the risk of developing GC, such as advanced age, male gender, ethnicity, and genetic factors, but these are neither modifiable nor preventable (Eusebi et al. 2020). However, there are factors that can be modifiable and preventable, with chronic infection with *Helicobacter pylori* (*H. pylori*) being one of the main risk factors. *H. pylori* has been classified as a class I carcinogen by the World Health Organization (WHO), and the prevalence of the infection is extraordinarily high, infecting 50% of the world's population (Eusebi et al. 2020). *H. pylori* can cause chronic gastritis, which may progress to gastric atrophy, metaplasia, and dysplasia, precursor lesions of gastric carcinogenesis (Alipour 2021). Established risk factors beyond *H. pylori* include infection with Epstein-Barr virus (EBV), dietary factors such as high intake of salty and smoked food, red and processed meat, and low consumption of fruits and vegetables, tobacco smoking, alcohol consumption, low physical activity, obesity, and gastroesophageal reflux disease (Ilic and Ilic 2022; Eusebi et al. 2020).

Incidence and mortality of GC have been decreasing for several decades in some countries, which can be attributed to economic development, eradication of *H. pylori*

Introduction

infection, changes in lifestyle, and other environmental factors (Yan et al. 2022; Eusebi et al. 2020). Despite some improvements, GC remains an important cause of cancer mortality. Therefore, screening strategies are important for early detection in high-risk populations and the reduction of the prevalence of *H. pylori* infection, as well as the reduction of other known risk factors, is fundamental to GC prevention (Morgan et al. 2022; Eusebi et al. 2020).

1.2 Pathology

There are several GC histopathological classifications, such as Laurén (Lauren 1965), Nakamura (Nakamura, Sugano, and Takagi 1968), Ming (Ming 1977), Goseki (Goseki, Takizawa, and Koike 1992), Carneiro (Carneiro, Seixas, and Sobrinho-Simões 1995), Solcia (Solcia et al. 2009), the WHO (Nagtegaal et al. 2020), and the Japanese Gastric Cancer Association (Association 2011). The most commonly used are the WHO (Nagtegaal et al. 2020) and the Laurén classification (Lauren 1965).

The WHO classification (Nagtegaal et al. 2020) (Table 1) is based on the recognition of specific histological patterns and classifies five main histopathological subtypes of GC: (1) tubular adenocarcinoma, composed of tubular, glandular, or acinar structures of variable diameter and various degrees of differentiation; (2) papillary carcinoma, presenting finger-like papillary architecture, with some tumors also displaying tubular differentiation (tubulo-papillary phenotype); (3) signet-ring cell carcinoma (SRCC), comprised of tumor cells isolated or in small clusters lacking cellular cohesion; (4) mucinous adenocarcinoma, defined by the presence of mucin pools accounting for > 50% of the tumor; and (5) mixed carcinomas, showing a distinct tubulo-papillary and poorly cohesive component.

According to the Laurén classification (Lauren 1965) (Table 1) gastric carcinomas are separated into two main histological types, intestinal and diffuse. The intestinal type is composed of tumor cells with glandular, tubular, or papillary growth patterns with various degrees of differentiation. The diffuse type displays an infiltrative growth pattern and is characterized by poorly cohesive cells and could have a signet ring cell (SRC) morphology. Tumors presenting both intestinal and diffuse components are named mixed carcinomas. Solid, poorly differentiated, or undifferentiated carcinomas that do not fit into one of these subtypes are placed in the indeterminate category. Although the Laurén classification dates to 1965, it remains widely accepted and used since it differentiates subtypes with different epidemiologic settings, clinicopathologic profiles, and biological behaviors (Gullo et al. 2020). Tubular and papillary carcinomas of the WHO classification roughly correspond to the Laurén intestinal type and poorly cohesive carcinomas correspond to the diffuse type in

Lauren's classification. Intestinal and diffuse GCs are characterized by distinct epidemiological profiles, histogenesis, clinicopathological and molecular features, as well as biological behavior (Gullo, Carneiro, et al. 2018). Intestinal GC usually occurs in elderly patients, predominantly males, and is more common in high-risk regions. In contrast, diffuse GC is more prevalent in young patients, mostly females, and is more common in low-risk areas (Gullo, Carneiro, et al. 2018). While intestinal GC typically metastasizes hematogenously to the liver, diffuse GC commonly has peritoneal dissemination, lymph node metastasis, and a more aggressive phenotype with a higher recurrence rate than intestinal GC (Ooki and Yamaguchi 2022; Chen et al. 2016).

Mixed GC is characterized by the presence of both tubulo-papillary and poorly cohesive-SRC components. These two components can be intermingled, adjacent, or completely separated within the tumor. Although both components are clearly distinguishable, the tumor can be predominantly composed of isolated cells or have a predominant glandular component (Carneiro 1997). Mixed GCs show more aggressive features than "pure" intestinal and diffuse GCs, including larger tumor size, deeper invasion, and a dual metastatic pattern (hematogenous metastases and peritoneal dissemination with lymph node metastases), suggesting a cumulative effect of the adverse behaviors seen in both intestinal and diffuse-type GC (Zheng et al. 2008; Park et al. 2016). Mixed carcinomas appear to have a clonal origin, and the phenotypic divergence has been attributed to somatic mutations in the cadherin-1 (*CDH1*) gene, which seem to be restricted to the poorly cohesive component and have emerged during the progression of some GCs (Gullo, Carneiro, et al. 2018; Machado et al. 1999).

Table 1 – The World Health Organization (WHO) and Laurén histopathological classification of gastric cancer

Laurén classification (Lauren 1965)	WHO classification (Nagtegaal et al. 2020)
Intestinal	Papillary Tubular
	Mucinous
Diffuse	Poorly cohesive, signet ring cell phenotype
	Poorly cohesive, other cell types
Mixed	Mixed
Indeterminate	Undifferentiated
	Rare Variants

Introduction

1.2.1 Molecular classification

Due to the heterogeneous nature of GC, the Lauren and WHO classifications may not fully reflect the diverse characteristics of the disease (Chia and Tan 2016). Over the last few years, the genomic landscape of GC has been extensively studied, providing a better understanding of potential driver alterations involved in GC pathogenesis, which could also lead to the identification of new biomarkers and treatment targets (Chia and Tan 2016). The Cancer Genome Atlas (TCGA) and Asian Cancer Research Group (ACRG) studies have defined distinct molecular GC subtypes based on mutation profile and expression analysis (Cristescu et al. 2015; Cancer Genome Atlas Research Network 2014). The TCGA, based on whole-exome sequencing, DNA methylation arrays, RNA sequencing, and chromosome copy number analysis, proposed a classification divided into four subtypes: Epstein-Barr virus-positive (EBV-positive), microsatellite instability (MSI), genomically stable (GS) and chromosomal instability (CIN) (Cancer Genome Atlas Research Network 2014). These subtypes showed unique molecular features that could potentially guide therapeutic decisions and have been shown to have prognostic significance (Cristescu et al. 2015; Serra et al. 2019). EBV-positive tumors represent approximately 9% of GC, are morphologically associated with prominent immune infiltrate, and frequently display the features of GC with lymphoid stroma (Gullo, Carvalho, et al. 2018; Cancer Genome Atlas Research Network 2014). These tumors are characterized by recurrent *PIK3CA* and *ARID1A* mutations, JAK2 and programmed death ligands 1 and 2 (PD-L1 and PD-L2) amplification, and a high level of DNA hypermethylation (Cancer Genome Atlas Research Network 2014). The MSI phenotype, present in about 22% of the tumors, is characterized by loss of DNA mismatch repair functions, resulting in the accumulation of mutations in short repetitive sequences called microsatellites, mainly due to DNA methylation of the *MLH1* gene promoter (Cancer Genome Atlas Research Network 2014). The GS subtype represents about 20% of cases and is associated with a diffuse histological type. The *CDH1* and *RHOA* genes are recurrently mutated and fusions involving the *CLDN18* gene may be present, with tumors presenting low mutation burden and low somatic copy number alterations (Cancer Genome Atlas Research Network 2014). GC with CIN constitutes the largest group of gastric carcinomas, accounting for 50% of tumors, are associated with intestinal histology, and harbor a higher rate of somatic copy number aberrations. Common alterations in these tumors include *TP53* gene mutations, as well as amplification of tyrosine kinase receptors, such as *ERBB2*, *EGFR*, *MET*, *FGFR2*, and *KRAS/NRAS* (Cancer Genome Atlas Research Network 2014).

The ACRG categorizes GC into four subtypes based on gene expression data, genome-wide copy number microarrays, and targeted gene sequencing. These subtypes

are MSI and microsatellite stable (MSS) with either epithelial-to-mesenchymal transition (MSS/EMT), TP53 activity (MSS/TP53+), or TP53 inactivity (MSS/TP53-) (Cristescu et al. 2015). EMT/MSS tumors are mainly of the diffuse type and occur at an earlier age, and patients have the worst prognosis and the highest recurrence rate (Cristescu et al. 2015). MSI tumors are hypermutated, are mostly of the intestinal histological type, and patients have the best overall prognosis and the lowest recurrence rate, being this subset similar to the TCGA MSI subset (Cristescu et al. 2015; Chia and Tan 2016). Patients with MSS/TP53+ and MSS/TP53- tumors have an intermediate prognosis and recurrence rates, with EBV infection occurring predominantly in the MSS/TP53+ group (Cristescu et al. 2015; Chia and Tan 2016). The MSS/TP53- tumors have the highest frequency of *TP53* mutations, higher aneuploidy, and more amplifications of oncogenes, including *ERBB2*. This subgroup is associated with CIN tumors from the TCGA classification (Cristescu et al. 2015; Chia and Tan 2016).

The classification of GC based on molecular subtypes provides an opportunity for targeted therapy. Biomarkers, particularly MSI, PD-L1, tumor mutation burden (TMB), HER2 and EBV, are increasingly driving new therapy approaches and enabling the identification of patients who might benefit from immunotherapy and targeted therapy (Zeng and Jin 2022). Several targeted therapeutic agents have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for use in advanced GC (Ajani et al. 2022). The CIN subtype, which is characterized by HER2 amplification, can benefit from treatment with trastuzumab, a monoclonal antibody anti-HER2, used as first-line treatment in patients with HER2-positive advanced GC (Alsina et al. 2023; Ajani et al. 2022). Gastric tumors often express high levels of VEGF and secrete pro-angiogenic cytokines. These patients could also benefit from treatment with ramucirumab, a monoclonal antibody against VEGFR2 that inhibits angiogenesis (Lordick et al. 2022; Alsina et al. 2023; Ajani et al. 2022). Furthermore, emerging data suggest that immunotherapies such as programmed cell death protein 1 (PD-1) inhibitors, namely pembrolizumab and nivolumab, demonstrate efficacy in GC, particularly in EBV and MSI-high tumors, due to high levels of neoantigens and PD-L1 positive T cell infiltration (Lordick et al. 2022; Alsina et al. 2023).

2. Inherited predisposition to gastric cancer

Familial clustering of GC accounts for approximately 10% of the cases, however only 1-3% of GCs result from a known hereditary syndrome (van der Post, Vogelaar,

Introduction

Carneiro, et al. 2015; Carneiro 2022). There are three main gastric hereditary syndromes: (1) hereditary diffuse gastric cancer (HDGC); (2) gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS); and (3) familial intestinal gastric cancer (FIGC) (Caldas et al. 1999; Worthley et al. 2012).

The most well-known hereditary form of GC is HDGC caused by germline variants in *CDH1* and, in a minority of cases, by germline variants in catenin alpha-1 gene (*CTNNA1*). It is characterized by an increased risk of diffuse gastric cancer (DGC) and lobular breast cancer (LBC) (Blair et al. 2020).

GAPPS is caused by germline mutations in the promoter 1B of the *APC* gene and is characterized by fundic gland polyposis with focal dysplasia and intestinal adenocarcinoma and with no evidence of colorectal or duodenal polyposis or other heritable gastrointestinal cancer syndromes (Worthley et al. 2012).

FIGC is an autosomal dominant syndrome that is associated with an increased risk of intestinal-type GC (Caldas et al. 1999).

GC can also be observed as part of other hereditary cancer syndromes, namely Familial Adenomatous Polyposis (FAP), Peutz-Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), Lynch Syndrome (LS), Li-Fraumeni syndrome (LFS), *MUTYH*-associated polyposis (MAP), *PTEN* hamartoma tumor syndrome (PHTS), hereditary breast and ovarian cancer syndrome (HBOC) and Cowden syndrome (Carneiro 2022).

The identification of hereditary factors in individuals with a family history of GC is essential for an early diagnosis and effective disease management.

2.1 Hereditary diffuse gastric cancer

HDGC is an autosomal dominant inherited cancer syndrome characterized by an increased risk of DGC and invasive LBC (Blair et al. 2020). In 1998, Guilford *et al.* identified, for the first time, germline variants in the *CDH1* gene as a cause of HDGC in New Zealand Māori families (Guilford et al. 1998). Thereafter, more families have been identified all over the world with the same phenotype and few of these families have also presented cleft lip/palate (Frebourg et al. 2006; Figueiredo et al. 2019; Kluijdt et al. 2012). The International Gastric Cancer Linkage Consortium (IGCLC) established clinical criteria to select patients for screening of *CDH1* germline variants. However, only 10–40% of these families have a pathogenic *CDH1* variant (Hansford et al. 2015; Marwitz et al. 2020; van der Post, Vogelaar, Manders, et al. 2015). This frequency variability can be explained by the different GC incidences between countries. High-incidence countries, such as Portugal, display a lower frequency of *CDH1* germline variants, which contrasts with low-incidence countries (Corso

et al. 2021; Hansford et al. 2015; Corso et al. 2012; Hakkaart et al. 2019). Efforts to uncover potential genes that could explain families without *CDH1* pathogenic germline variants led to the identification of a germline variant in the *CTNNA1* gene by Majewski et al. in 2013 (Majewski et al. 2013). Since then, a few *CTNNA1* pathogenic germline variants have been described in families meeting the IGCLC criteria (Majewski et al. 2013; Hansford et al. 2015; Slavin et al. 2017; Weren et al. 2018; Benusiglio et al. 2019; Marwitz et al. 2020; Clark et al. 2020; Coudert et al. 2022; Lobo et al. 2021). However, the association of *CTNNA1* pathogenic variants with increased LBC risk remains inconclusive.

2.1.1 Diagnostic criteria

In 1999, the IGCLC established for the first-time diagnostic criteria and guidelines for the clinical management of HDGC families (Caldas et al. 1999). Over the years, these criteria have undergone several updates to simplify the screening of *CDH1* germline variants and have been less restrictive regarding age limits and the number of lesions, allowing the identification of more HDGC families (van der Post, Vogelaar, Carneiro, et al. 2015; Fitzgerald et al. 2010). In 2020, the IGCLC published the latest update on testing recommendations. According to the latest guidelines, genetic testing is recommended in individuals who have a confirmed cancer diagnosis and meet one of the following clinical criteria: (1) Two or more GC cases, regardless of age, with at least one histologically confirmed DGC; (2) One or more cases of DGC at any age, and one or more cases of LBC before 70 years; (3) Two or more cases of LBC before 50 years of age. Individual criteria include: (4) Confirmed DGC before 50 years; (5) DGC at any age in individuals of Māori ethnicity; (6) DGC at any age in individuals with a personal or family history (first-degree relative) of cleft lip/cleft palate; history of DGC and LBC both diagnosed before 70 years; (7) Bilateral LBC diagnosed at an age earlier than 70 years; and in situ gastric signet ring cells or pagetoid spread of signet ring cells in individuals before 50 years (Blair et al. 2020).

These guidelines are very clear about not considering the intestinal type and non-lobular breast cancer to fulfill testing criteria, as these histological-type tumors are not part of the syndrome. Additionally, when no *CDH1* pathogenic variant has been identified, genetic testing of *CTNNA1* is recommended. Therefore, according to these guidelines, HDGC is now defined by the “presence of a pathogenic germline *CDH1* or *CTNNA1* variant in either an isolated individual with DGC or in a family with one or more DGC cases in first-degree or second-degree relatives”. Hereditary lobular breast cancer (HLBC) is also defined “by the presence of a pathogenic *CDH1* variant in either an isolated individual with LBC, or a family with one or more LBC cases in first-degree or second-degree relatives, but no

Introduction

known DGC in either situation” (Blair et al. 2020). Families that fulfill the criteria for genetic testing but have no identified pathogenic *CDH1* or *CTNNA1* variants are defined as HDGC-like (Blair et al. 2020).

Moreover, the IGCLC guidelines also aim to increase awareness of the nature of the disease and associated risk factors, improving decision-making and providing better guidance for the management of HDGC families.

2.1.2 The *CDH1* gene

The human *CDH1* tumor suppressor gene is located on chromosome 16q22.1 and encodes cadherin-1, a type I calcium transmembrane glycoprotein expressed in epithelial tissues (Bers et al. 1995). This protein belongs to the family of cell-cell adhesion molecules, and it is essential to maintain cell differentiation, normal tissue architecture, and cell polarity (Kaszak et al. 2020). This protein consists of three domains: an extracellular domain, a transmembrane domain, and a highly conserved cytoplasmic domain. The extracellular domain contains binding sites for Ca^{2+} ions and extends from the cell surface, allowing it to bind to the extracellular domains of the cadherin-1 on adjacent cells through lateral dimerization, allowing a cadherin-cadherin interface and thus cell-cell adhesion. The cytoplasmic domain consists of the juxtamembrane domain and the catenin-binding domain. While the juxtamembrane domain enables the clustering of cadherins and contributes to adhesive stability through catenin delta-1 (also known as p120 catenin), the catenin-binding domain forms a protein complex with catenin alpha-1 via catenin beta-1, which connects cadherin-1 with the actin cytoskeleton. This promotes protein clustering at adherens junction and stabilizes cell adhesion (Figure 1) (Zhao et al. 2021; Kaszak et al. 2020; Humphries and Newham 1998; Gall and Frampton 2013). In addition, the cadherin-catenin complex can trigger signaling pathways and is involved in epithelial-to-mesenchymal transition. The binding of cadherin-1 to β -catenin prevents its translocation to the nucleus and the activation of the Wnt signaling pathway, which promotes cell migration, cell proliferation, cell growth, and induction of apoptosis. Loss of epithelial cell proteins, like cadherin-1, leads to epithelial-to-mesenchymal transition resulting in loss of cell-cell adhesion (Gall and Frampton 2013; Kaszak et al. 2020; Zhao et al. 2021; Collins and Nelson 2015).

In the HDGC, *CDH1* germline variants are distributed across the entire coding sequence, with no apparent genotype–phenotype correlation (Lo et al. 2019). The majority of these variants are truncating, usually caused by frameshift insertions and deletions, splice-site, and nonsense variants, while large rearrangements are less common

(Figueiredo et al. 2019). The clinical and functional impact of missense mutations still raises controversy, but there is no evidence of an association between HDGC phenotype with *CDH1* missense variants of unknown significance (Garcia-Pelaez et al. 2023). According to the two-hit hypothesis, a second-hit somatic event, leading to bi-allelic inactivation, is necessary for cadherin-1 disruption and the development of DGC and LBC. The most common *CDH1* second-hit mechanism is promoter hypermethylation, while loss of heterozygosity (LOH) or a second mutation is less frequent (Barber et al. 2008; Oliveira et al. 2004; Grady et al. 2000).

The cumulative incidence of DGC in *CDH1* carriers by the age of 80 years is approximately 70% for men and 56% for women, using data from families who met the 2010 HDGC clinical criteria (Hansford et al. 2015). However, in recent studies where carriers were not pre-selected using HDGC testing criteria, lower risks were reported, 37%-42% for men, and 24%-33% for women at the age of 80 years (Roberts et al. 2019; Xicola et al. 2019). Estimates of LBC risk in women have been more consistent across studies, which have ranged from 42% to 55% (Roberts et al. 2019; Xicola et al. 2019; Hansford et al. 2015). This variation in GC risk is likely to be highly affected by individual genetic background and lifestyle factors (Blair et al. 2020).

DGC in *CDH1* carriers has been associated with the development of *in situ* SRCC, which is characterized by the presence of SRC with hyperchromatic and depolarized nuclei, located in the basal membrane of a gland, replacing the normal cells. Pagetoid spread of SRC below the preserved epithelium of glands and within the basal membrane has also been observed in *CDH1* variant carriers. These are considered the precursor/preinvasive lesions of HDGC and have not been associated with sporadic DGC (van der Post et al. 2016; Carneiro et al. 2004). However, the precise interval time between the development of cancer *foci* and the risk of symptomatic and potentially incurable disease is unknown (Benesch et al. 2021).

Beyond HDGC predisposition, congenital anomalies such as cleft lip/palate or blepharochelodontic syndrome (BCD) are common in some *CDH1* variant carriers (Figueiredo et al. 2019; Kievit et al. 2018).

2.1.3 The *CTNNA1* gene

The human *CTNNA1* gene is located in chromosome 5q31.2, and encodes the catenin alpha-1 protein, a member of the catenin proteins family (Oda et al. 1993). *CTNNA1* gene is a *CDH1*-binding partner and is involved in intercellular cell adhesion. Catenin alpha-1 consists of three functional domains: an N-terminal β -catenin binding domain, a middle

Introduction

domain that is α -actinin, vinculin and other actin proteins binding domain, and a C-terminal F-actin binding domain (Kobielak and Fuchs 2004). Catenin alpha-1 protein forms a complex with catenin beta-1 to bind the cytoplasmic domain of cadherin-1 to the actin cytoskeleton. While the N-terminal domain of catenin alpha-1 interacts with catenin beta-1, which further binds to cadherin-1, the C-terminal part interacts with the actin filaments (Figure 1) (Gloushankova, Rubtsova, and Zhitnyak 2017; Kobielak and Fuchs 2004; Takeichi 2018). Catenin alpha-1 is essential for establishing the connection between the cadherin-1-catenin beta-1 complex and the actin cytoskeleton. Inactivation of catenin alpha-1 disrupts adherens junctions and disables the connections of the cadherin- catenin beta-1 complex to the actin cytoskeleton, resulting in the inability of cadherin-1 to establish cell-to-cell adhesion (Kobielak and Fuchs 2004).

A germline truncating variant in the *CTNNA1* gene was first identified in a family that fulfilled the HDGC clinical criteria (Majewski et al. 2013). Since then, more than ten pathogenic variants in *CTNNA1* have been described in families meeting the HDGC criteria (Majewski et al. 2013; Hansford et al. 2015; Slavin et al. 2017; Weren et al. 2018; Benusiglio et al. 2019; Marwitz et al. 2020; Clark et al. 2020; Coudert et al. 2022; Lobo et al. 2021). Loss of catenin alpha-1 was observed in gastric tumors from *CTNNA1* germline variants carriers (Majewski et al. 2013; Hansford et al. 2015; Weren et al. 2018; Clark et al. 2020; Benusiglio et al. 2019). Benusiglio and co-workers also reported two asymptomatic *CTNNA1* pathogenic variant carriers, who displayed DGC *foci* in the prophylactic total gastrectomy (PTG) and intramucosal *focus* on esophagogastroduodenoscopy specimens, with loss of catenin alpha-1 expression, suggesting that pathogenic variants in *CDH1* and *CTNNA1* may have similar implications in DGC risk. These observations suggest that a second hit inactivating mechanism occurred somatically at the *CTNNA1* gene (Benusiglio et al. 2019; Carneiro 2022). Additionally, loss of function variants have been described in families without HDGC clinical criteria (Clark et al. 2020; Lobo et al. 2021).

The prevalence of *CTNNA1* pathogenic variants is not well-established, Lobo et al. suggested that these variants seem to account for less than 2% of the families fulfilling HDGC criteria (Lobo et al. 2021). The cumulative lifetime risk of DGC in families with pathogenic *CTNNA1* variants is still uncertain, Coudert and collaborators published the first risk estimates and have estimated penetrance of 49%-57% at 80 years (Coudert et al. 2022). The disease spectrum is also uncertain and the association of *CTNNA1* pathogenic variants with increased LBC risk and cleft lip/palate was not reported thus far. Although some studies have been associating *CTNNA1* and breast cancer (Felicio et al. 2021; Sun et al. 2017; Clark et al. 2020; Shirts et al. 2016), this association remains to be clarified since the subtype of these cancers was not specified.

Beyond HDGC predisposition, germline missense variants in *CTNNA1* have been associated with macular dystrophy patterned-2 (Saksens et al. 2016; Tanner et al. 2021). This autosomal dominant eye disease is characterized by bilateral pigment accumulation in the macular area, similar to butterfly wings patterning (also named butterfly-shaped pigmentary) (van Lith-Verhoeven et al. 2003).

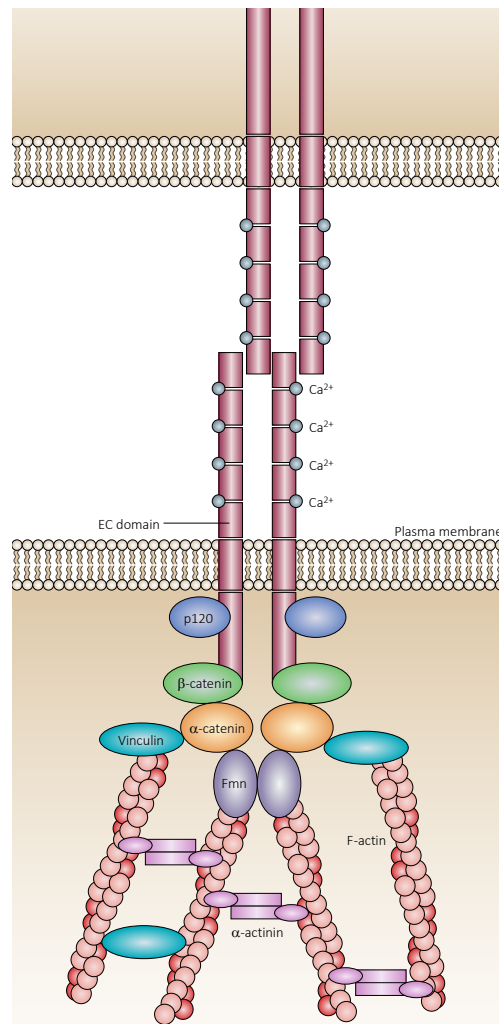


Figure 1 - The extracellular region of cadherin-1, which contains extracellular cadherin (EC) domains and binding sites for Ca^{2+} ions and extends from the cell surface, allowing it to bind to the E-cadherins on a neighboring cell. Stabilization of intercellular adhesion requires the binding of the cytoplasmic domain of cadherin-1 to catenin beta-1. In turn, catenin beta-1 binds to catenin alpha-1, which is central in recruiting cytoskeletal proteins, including the filamentous (F)-actin-nucleating, formin proteins (Fmn), and the actin-binding proteins vinculin, α -actinin. By contrast, catenin delta-1 (p120), binds to E-cadherin through a juxtamembrane domain (Adapted from Kobiela and Fuchs 2004).

2.1.4 Clinical surveillance and management

Prophylactic total gastrectomy is recommended as the standard of care for pathogenic *CDH1* variant carriers, regardless of endoscopic findings, in early adulthood, generally between 20 and 30 years of age (Blair et al. 2020). The diagnosis of early DGC is challenging because SRCC and its precursor lesions are located beneath the epithelium surface and the gastric mucosa is often macroscopically normal, leading to a low endoscopic detection rate (Jacobs et al. 2019; Benesch et al. 2021; Rocha et al. 2018). Therefore, PTG remains the best clinical management option for HDGC patients with a pathogenic *CDH1* variant. The probability of finding at least one *focus* of SRCs in a PTG specimen is estimated to be between 85 to 95%, thus, total gastric mucosa embedding and mapping should be performed to detect DGC in PTG (Rocha et al. 2018; Benesch et al. 2021; Vos et al. 2020). However, PTG also has substantial perioperative risks as well as long-term complications and requires extended follow-up and meticulous postoperative management. The postsurgical complications include early dumping syndrome, resulting from excessive intake of hyperosmotic carbohydrates, diarrhea caused by rapid transit or malnutrition, weight loss, dysphagia, reflux, and maldigestion (Stillman et al. 2022; Muir et al. 2016).

For individuals who refuse or postpone PTG, annual surveillance endoscopies are recommended (Blair et al. 2020). Since most *foci* cannot be reliably visually identified, surveillance endoscopies should include targeted biopsies of visible abnormalities and random biopsies (Lee et al. 2023). The current IGCLC guidelines recommendation for endoscopic surveillance is the Cambridge Protocol, which consists of a systematic examination of the stomach with 30 random biopsies, five each from the prepyloric area, antrum, transition zone, body, fundus, and cardia, in addition to any targeted biopsies of suspicious gastric abnormalities (Blair et al. 2020). In contrast, a recent study suggested that the relatively low occurrence of cancers worse than T1a stage detected endoscopically among these high-risk individuals might indicate the feasibility of endoscopic surveillance and might also be an acceptable alternative to PTG and its associated risks (Asif et al. 2023).

For female variant carriers, breast cancer surveillance with an annual mammogram and/or breast magnetic resonance imaging should begin at age 30, and risk-reducing mastectomy can be considered (Blair et al. 2020; Daly et al. 2021).

Observations of DGC *foci* in PTG and intramucosal *focus* on esophagogastroduodenoscopy specimens from asymptomatic *CTNNA1* pathogenic variant carriers suggest that pathogenic variants in *CDH1* and *CTNNA1* may have similar implications in DGC risk (Benusiglio et al. 2019). Therefore, asymptomatic carriers of

CTNNA1 pathogenic variants should undergo annual endoscopic surveillance and a PTG could be considered, depending on the results of the biopsies and the penetrance of DGC in the family. Regarding breast cancer surveillance, since the association of *CTNNA1* pathogenic variants with increased LBC is still unclear, breast cancer surveillance should be considered based on personal and family history (Blair et al. 2020).

2.2 Familial intestinal gastric cancer

FIGC is characterized by an autosomal dominant inheritance pattern and is associated with an increased risk of intestinal-type GC, but not related to gastric polyposis (Caldas et al. 1999; Carneiro 2022). Diagnostic criteria for FIGC were established in 1999 by the IGCLC and were defined according to GC incidence. In high-incidence countries, the criteria require at least three affected relatives with intestinal GC and one of them should be a first-degree relative of the other two; at least two successive generations should be affected; and in one of the relatives, GC should be diagnosed before the age of 50. In low-incidence countries, the criteria are at least two first-degree or second-degree relatives affected by intestinal GC, one diagnosed before the age of 50; or three or more relatives with intestinal GC at any age (Caldas et al. 1999). FIGC remains widely understudied and almost completely genetically unexplained, with an unknown tumor spectrum and age of onset (Carvalho et al. 2021). Furthermore, given the lack of a genetic cause, clinical criteria have not been validated or revisited since 1999. In a recent study by Carvalho et al, simplified testing criteria for FIGC families were proposed: any family presenting two GC cases, one confirmed of intestinal histology, independently of age, and with or without colorectal cancer or breast cancer in other family members (Carvalho et al. 2021). According to this study, individuals from FIGC families developed intestinal GC at an average age that was higher than those with HDGC but at least 10 years earlier than individuals with sporadic intestinal GC. In addition, the somatic mutational profiles of FIGC tumors were distinctive from those observed in sporadic intestinal GC, exhibiting a significantly higher MSI frequency compared to the sporadic intestinal GC. Given the rarity of germline variants in high penetrance genes in FIGC, Carvalho et al. suggested that FIGC may be a polygenic disease (Carvalho et al. 2021).

FIGC remains largely unknown and genetically unresolved to date, and due to the scarce information about genetic causes and tumor spectrum, the management of FIGC patients is not clear. A few recommendations have been suggested for the management of patients at risk of developing FIGC, including gastroduodenoscopy at the age of 60 years

Introduction

(or at an age 5 years younger than the youngest diagnosis in the family) with *H. pylori* testing and eradication (Carvalho et al. 2021; Corso et al. 2013; Garcia-Pelaez et al. 2022).

2.3 Gastric adenocarcinoma and proximal polyposis of the stomach

GAPPS is an autosomal dominant hereditary syndrome associated with an increased risk of GC, primarily occurring in the context of polyposis of the proximal stomach (Worthley et al. 2012). Germline point variants in the promoter 1B of the *APC* gene have been identified in families with GAPPS, and to date is the only known genetic alteration associated with hereditary predisposition to GAPPS (Li et al. 2016; Repak et al. 2016; Mitsui et al. 2018; Beer et al. 2017). The disease spectrum includes various types of polyps, such as fundic gland polyps, which may exhibit focal foveolar-type dysplasia, hyperplastic polyps, hyperproliferative aberrant pits, adenomas with gastric-type dysplasia, and preinvasive lesions with mixed-type features. Adenocarcinomas occurring in GAPPS are intestinal-type or mixed-type, and 13% of GAPPS patients have been diagnosed with these tumor types (Garcia-Pelaez et al. 2022; Tacheci et al. 2021). GAPPS has been considered a variant of FAP that is restricted to the stomach (Rudloff 2018). GAPPS families display a heterogeneous phenotype, with polyps counts ranging from 30 to hundreds (Rudloff 2018; Worthley et al. 2012). The clinical criteria are (1) gastric polyps restricted to the body and fundus with no evidence of colorectal or duodenal polyposis; (2) >100 polyps in the proximal stomach in the index case or >30 polyps in a first-degree relative; (3) predominantly fundic gland polyps, some having regions of dysplasia (or a family member with either dysplastic fundic gland polyps or gastric adenocarcinoma); and (4) an autosomal dominant pattern of inheritance. Exclusion criteria for GAPPS diagnosis are the diagnosis of other heritable gastric polyposis syndromes and the use of proton pump inhibitors (Worthley et al. 2012). Prolonged use of proton-pump inhibitors might be the cause of fundic gland polyps/polyposis without dysplasia and should be considered in the differential diagnosis (Carneiro 2022).

The *APC* gene encodes the Adenomatous Polyposis Coli protein, a regulator of β -catenin and, consequently, of the WNT signaling pathway. Loss of APC function leads to an increase and cytoplasmic accumulation of β -catenin, which translocate to the nucleus and promotes proliferation (Aoki and Taketo 2007). Germline point variants in *APC* 1B promoter reduce the binding of the transcription factor Yin Yang 1 (YY1) in gastric and colon cancer cells (Li et al. 2016). In the stomach, the 1B promoter has a significantly higher transcription activity compared to the 1A promoter, which is methylated in cells of the gastric mucosa (Hosoya et al. 2009; Li et al. 2016). Therefore, loss of YY1 transcription factor

binding leads to reduced 1B promoter activity and reduced tumor suppressive phenotype in the stomach, which does not occur in the colon because the 1A promoter is preserved and able of compensating the 1B promoter function loss. This explains why *APC* 1B promoter point mutations are not associated with a colonic phenotype typically found in FAP or attenuated familial adenomatous polyposis (AFAP) (Rudloff 2018). LOH or somatic *APC* mutations were found in most fundic gland polyps, affecting a subset of the polyp's epithelial cells (Li et al. 2016; Mitsui et al. 2018).

Given the low number of GAPPS cases reported and patient heterogeneity, the prevalence, and penetrance of GAPPS remain vastly unknown. According to data from very few studies reported in the literature, the penetrance is incomplete, the overall risk for GC ranges from 12% to 25%, and the age of onset is variable, ranging from 23 to 75 years (Worthley et al. 2012; Li et al. 2016; Repak et al. 2016). Due to the scarcity of data and the heterogeneity observed in this syndrome, recommendations for its management should be decided on a case-by-case basis. Currently, endoscopic surveillance with biopsies and/or polypectomies is recommended as a surveillance approach. Furthermore, prophylactic/risk-reduction gastrectomy can also be considered (Worthley et al. 2012; Repak et al. 2016; Tacheci et al. 2021).

2.4 Gastric cancer in other cancer-associated syndromes

GC can also develop in the setting of other inherited cancer disorders, such as hereditary breast and ovarian cancer syndrome (HBOC), Li-Fraumeni syndrome (LFS), Lynch Syndrome (LS), Familial Adenomatous Polyposis (FAP), juvenile polyposis syndrome (JPS), *MUTYH*-associated polyposis (MAP), Peutz-Jeghers syndrome (PJS), and *PTEN* hamartoma tumor syndrome (PHTS) (Table 2). Despite not being the primary cancer, some families exhibit a noticeable clustering of GC, and the lifetime risk of GC in these syndromes is highly variable between populations (Carneiro 2022).

HBOC is an autosomal dominant condition associated with germline variants in *BRCA1* or *BRCA2* genes, which are associated with increased risks of breast and ovarian cancers. However, the risk for additional cancer types, such as GC, has been reported (Jakubowska et al. 2002; Ichikawa et al. 2018; Moran et al. 2012). The cumulative lifetime risk of GC is 3.5% in both male and female carriers of a *BRCA2* pathogenic variant and 1.6% and 0.7% in male and female carriers of a *BRCA1* pathogenic variant, respectively (Li et al. 2022).

LFS is a cancer predisposition autosomal dominant syndrome associated with high risk for a diverse spectrum of childhood- and adult-onset malignancies associated with

Introduction

germline *TP53* deleterious variants. The classic form of LFS is a highly penetrant cancer syndrome, associated with a high lifetime risk for cancer and is characterized by a wide spectrum of neoplasms occurring at a young age. The core tumor spectrum includes soft tissue and bone sarcomas, early-onset breast cancer, brain tumors and adrenal carcinomas, but risk is increased for several other malignancies, including GC (Schneider et al. 1993). Diffuse-type and intestinal-type are both common GC histologies in LFS, and the lifetime risk is estimated to be between 2% to 5% (Masciari et al. 2011; McBride et al. 2014).

LS, the most common gastrointestinal cancer syndrome, is an autosomal dominant inherited condition caused by germline variants in the DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*), as well as deletions in the *EPCAM* gene, and is characterized by an increased risk for colorectal cancer and an increased incidence of extracolonic neoplasms, including ovarian, endometrial, small-bowel, gastric, renal pelvis, and ureter tumors, and skin lesions (Idos and Valle 1993). GC is the second most common extracolonic cancer in these patients, after endometrial cancer, being the most cases of intestinal type, but diffuse or poorly cohesive GCs have also been described (Boland et al. 2022). *MLH1* germline variants have been shown to confer a lifetime risk of 5% to 7%, *MSH2* variants confer a risk of up to 9%, and *MSH6* variants up to 10% (Weiss et al. 2021; Bonadona et al. 2011; Barrow et al. 2009; Møller et al. 2018).

FAP is an autosomal dominant hereditary polyposis syndrome caused by germline variants in the *APC* gene. The classic form of FAP is clinically defined by the presence of 100 or more synchronous colorectal adenomas. Attenuated FAP is a less severe entity, defined as the presence of fewer than 100 adenomatous polyps (Yen et al. 1993). The lifetime risk for intestinal GC in patients with FAP/AFAP is reported to be in the range of 0.1% to 7.1%, but this risk may be increased in patients from geographic areas with a high prevalence of GC, such as Asia, Japan, and Korea (Weiss et al. 2021; Gullo, van der Post, and Carneiro 2021).

MAP is an autosomal recessive disorder due to biallelic variants in the base-excision repair *MUTYH* gene and is characterized by multiple adenomas in the colon and rectum. Patients affected with MAP have a up to 5% lifetime risk of GC (Gullo, van der Post, and Carneiro 2021; Weiss et al. 2021; Win et al. 2016).

PJS is an autosomal dominant syndrome caused by germline variants in the *STK11* gene and is characterized by hamartomatous polyps in the gastrointestinal tract, primarily in the small intestine, and pigmented mucocutaneous lesions (Tomlinson and Houlston 1997). Individuals with PJS have a 29% risk of developing GC (Gullo, van der Post, and Carneiro 2021; Weiss et al. 2021).

JPS is a rare autosomal dominant condition caused by germline variants in the *BMPR1A* or *SMAD4* genes and is characterized by multiple hamartomatous polyps throughout the gastrointestinal tract, predominantly the colorectum, followed by the stomach, jejunum and ileum, and duodenum (Latchford et al. 2012). The GC risk in JPS is thought to arise from the malignant transformation of the adenomatous components present in juvenile polyps. *SMAD4* variants confer an increased risk of gastric polyposis and GC compared to *BMPR1A* variants, with a lifetime risk estimated to be approximately 21% (Weiss et al. 2021; Gullo, van der Post, and Carneiro 2021).

Table 2 – Hereditary cancer syndromes associated with gastric cancer.

Syndrome	Gene	Lifetime gastric cancer risk	Gastric cancer histology
Hereditary breast and ovarian cancer (HBOC)	<i>BRCA1</i>	1.6% in male and 0.7% in female (Li et al. 2022)	Intestinal
	<i>BRCA2</i>	3.5% in female and male (Li et al. 2022)	
Li-Fraumeni (LFS)	<i>TP53</i>	2 - 5% (McBride et al. 2014)	Intestinal or diffuse
Lynch Syndrome (LS)	<i>MLH1</i>	5 - 7% (Weiss et al. 2021)	Intestinal
	<i>MSH2</i>	9% (Weiss et al. 2021)	
	<i>MSH6</i>	10% (Weiss et al. 2021)	
	<i>PMS2</i>	NA	
	<i>EPCAM</i>	NA	
Familial Adenomatous Polyposis (FAP)	<i>APC</i>	0.1 - 7.1% (Weiss et al. 2021)	Intestinal
MUTYH-associated polyposis (MAP)	<i>MUTYH</i>	5% (Weiss et al. 2021)	Intestinal
Peutz-Jeghers syndrome (PJS)	<i>STK11</i>	29% (Weiss et al. 2021)	Intestinal
Juvenile polyposis syndrome (JPS)	<i>SMAD4/</i> <i>BMPR1A</i>	21% (Weiss et al. 2021)	Intestinal or diffuse

NA – not available

2.5 The missing heritability in gastric cancer

A lot of efforts have been made to identify the heritable genetic causes of DGC beyond the *CDH1* and *CTNNA1* genes. Despite these efforts, additional genes that predispose to DGC have yet to be identified. Similarly, the heritability of families with non-DGC clustering remains mostly unknown. FIGC remains widely understudied and genetically unexplained to date, presenting a large disease spectrum and generally occurring at an older age (Carvalho et al. 2021). Likewise, the heritability of families with mixed GC clustering remains to be uncovered. Several studies have suggested a causal role of homologous recombination genes in GC predisposition. Homologous recombination

Introduction

is an essential molecular mechanism for maintaining genomic integrity by repairing DNA double-strand breaks (Stewart et al. 2022). The first evidence of a role for homologous recombination genes in GC was provided by Hansford and colleagues, who analyzed 55 genes known to be associated with hereditary cancer in 183 families with HDGC. Germline pathogenic variants in homologous recombination genes, such as *ATM*, *BRCA2*, and *PALB2* genes, were found in 11% of the families without *CDH1* pathogenic variants and meeting IGCLC 2010 clinical criteria for HDGC (Hansford et al. 2015). Thereafter, more studies came up providing further evidence for a role of homologous recombination genes, such as *BRCA1*, *BRCA2*, *ATM*, *RAD51C*, and *CHEK2*, in GC inherited predisposition either in selected (Sahasrabudhe et al. 2017; Fewings et al. 2018; Zhang et al. 2021; Tedaldi et al. 2019; Liu et al. 2022) and unselected cohorts (Usui et al. 2023; Uson et al. 2022; Slavin et al. 2017; Lu et al. 2015; Zhang et al. 2023). To note that patients with germline pathogenic variants in homologous recombination genes display heterogeneous GC histology, including intestinal, mixed, and diffuse GC (Kupfer 2017; Garcia-Pelaez et al. 2022). The most recent IGCLC guidelines recommend considering *PALB2* testing in unexplained families alongside with other genes associated with an increased risk of GC (Blair et al. 2020). The *PALB2* gene encodes a protein which is recruited to double-strand break sites and mediates DNA repair by interacting with *BRCA1* and *BRCA2*. The *BRCA1/PALB2/BRCA2* complex is necessary for recruitment of *RAD51* and consequently *RAD51*-mediated homologous recombination (Xia et al. 2006; Rahman et al. 2007). Monoallelic pathogenic variants in this gene have been shown to be associated with an increased risk of breast, ovarian, and pancreatic cancers, whereas biallelic pathogenic variants cause a Fanconi anaemia subtype, FA-N (Nepomuceno et al. 2017; Reid et al. 2007). The *ATM*, *CHEK2* and *RAD51C* genes are considered moderate penetrant genes associated with breast and/or ovarian cancer risk (Dorling et al. 2021). The *ATM* gene encodes a cell cycle checkpoint kinase protein, involved in the repair of DNA double-strand breaks through *p53* and *BRCA1* proteins (Jin and Oh 2019). The *CHEK2* gene encodes the Checkpoint kinase 2, a serine threonine kinase involved in cell cycle regulation at the G2 phase and is activated in response to DNA damage and plays an important role in transducing the DNA damage signal to downstream repair proteins (Zannini, Delia, and Buscemi 2014). The *RAD51C* gene, a member of the *RAD51* protein family, is also an essential gene in homologous recombination. *RAD51C* forms a complex with other *RAD51* proteins, including *RAD51B*, *RAD51D*, and *XRCC2*, which is essential for the efficient and accurate repair of DNA damage (Boni et al. 2022). Nonetheless, the clinical implications of deleterious variants in these genes remain unclear, and the evidence is not yet sufficient to recommend surveillance measures for carriers.

The well-established moderate penetrant predisposing genes are still insufficient to explain the heritability in many of these families. Moreover, rare germline variants were identified in other possible and promising candidate genes, including the *MAP3K6*, which encodes a serine/threonine protein kinase that forms a component of protein kinase-mediated signal transduction cascades (Gaston et al. 2014). However, data related to this gene is controversial, as *MAP3K6* variants occur frequently in the general population, being unlikely to be involved in the GC predisposition (Weren et al. 2018; Vogelaar et al. 2017). Germline variants were also identified in other genes such as the *INSR*, which has been shown to affect tumor cell invasion by regulating cadherin-1 glycosylation (de-Freitas-Junior et al. 2013; Donner et al. 2015), *FBXO24*, which may regulate cell proliferation by mediating ubiquitination (Wang et al. 2014; Donner et al. 2015), and *DOT1L* which is involved in DNA damage response (Donner et al. 2015; Kari et al. 2019). However, variants in these genes were not identified in additional GC families, warranting further validation (Donner et al. 2015). Furthermore, germline variants in *MSR1* and *PRSS1* genes were also identified (Liu et al. 2022; Hansford et al. 2015). These genes were previously associated with an increased risk for esophageal and prostate cancer (Xu et al. 2002; Orloff et al. 2011) and for hereditary pancreatitis, respectively (Németh and Sahin-Tóth 2014). Other genes involved in homologous recombination pathway, such as *ATR* and *NBN*, have also been identified in GC families (Fewings et al. 2018; Slavin et al. 2017). The *NBN* gene encodes the NBS1 protein, which associates with MRE11 and RAD50 to form a complex involved in the activation of the ATM and ATR proteins, which have roles in the recruitment of damage repair proteins, cell cycle regulation, and apoptosis (Zhang, Zhou, and Lim 2006).

The role of catenin family proteins in cell adhesion raises the question about their implication in GC predisposition. A good GC predisposition candidate gene is the *CTNND1* gene, another catenin gene, which encodes the p120-catenin, a binding partner of cadherin-1. This protein interacts with the juxtamembrane domain of cadherin-1 and regulates the stability of cell-cell adhesion by controlling the retention of cadherin-1 at the cell surface (Gloushankova, Rubtsova, and Zhitnyak 2017). Moreover, the *CTNND1* gene has been associated with BCD, like the *CDH1* gene. This syndrome is characterized by eyelid malformations, cleft lip/palate, and ectodermal dysplasia (Cox et al. 2018; Kievit et al. 2018; Ghoumid et al. 2017). The involvement of another cadherin–catenin complex gene in cleft palate and BCD makes this gene an ideal candidate for GC predisposition (Benusiglio 2017). Schuetz et al. attempted to identify germline variants in catenin family genes, however, no pathogenic variants were found in 22 HDGC families (Schuetz et al. 2012).

Therefore, the identification of new GC predisposition genes is extremely important to provide suitable genetic counseling, surveillance, and/or risk reduction measures, and ultimately prevent disease onset.

Aims

The overall goal of this study was to identify deleterious germline variants in a wide panel of genes that could explain a significant part of inherited predisposition to GC of different histological subtypes. Specifically, the aims of this thesis were:

1. To compare the frequencies of *CDH1* germline variants in families with diffuse and mixed-type GC to evaluate if *CDH1* testing should or not be considered in patients with mixed-type GC.
2. To evaluate the contribution of variants in other cancer susceptibility genes to diffuse GC inherited predisposition in families meeting the IGCLC criteria for HDGC without germline variants in *CDH1*.
3. To identify germline variants predisposing to GC, regardless of histology, by performing targeted gene panel and/or whole-exome sequencing (WES).
4. To validate the use of next-generation sequencing (NGS) for germline variant screening in archival formalin-fixed paraffin-embedded (FFPE) samples, to allow a more informative molecular study of families with a strong family history of GC in which all affected family members are deceased.

Paper I

Frequency of *CDH1*, *CTNNA1* and *CTNND1* Germline Variants in Families with Diffuse and Mixed Gastric Cancer

Joana Guerra, Carla Pinto, Pedro Pinto, Manuela Pinheiro, Catarina Santos, Ana Peixoto, Carla Escudeiro, Ana Barbosa, Miguel Porto, Inês Francisco, Paula Lopes, Ana Raquel Isidoro, Ana Luísa Cunha, Cristina Albuquerque, Isabel Claro, Carla Oliveira, João Silva, Manuel R Teixeira.

Cancers 2023 Aug 29;15(17):4313.

Article

Frequency of *CDH1*, *CTNNA1* and *CTNND1* Germline Variants in Families with Diffuse and Mixed Gastric Cancer

Joana Guerra ^{1,2}, Carla Pinto ^{1,3,4}, Pedro Pinto ¹, Manuela Pinheiro ¹, Catarina Santos ^{1,3}, Ana Peixoto ^{1,3}, Carla Escudeiro ^{1,3} , Ana Barbosa ^{1,3}, Miguel Porto ¹ , Inês Francisco ⁵, Paula Lopes ⁶, Ana Raquel Isidoro ⁶, Ana Luísa Cunha ⁶, Cristina Albuquerque ⁵, Isabel Claro ^{7,8}, Carla Oliveira ^{9,10,11} , João Silva ^{1,12} and Manuel R. Teixeira ^{1,3,13,*} 

- ¹ Cancer Genetics Group, IPO-Porto Research Center (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center, 4200-072 Porto, Portugal; joana.goncalves.guerra@ipoporto.min-saude.pt (J.G.); carla.a.pinto@ipoporto.min-saude.pt (C.P.); pedropinto18@hotmail.com (P.P.); manuelap@ipoporto.min-saude.pt (M.P.); catarinasantos@ipoporto.min-saude.pt (C.S.); analuisamoura@ipoporto.min-saude.pt (A.P.); carla.escudeiro@ipoporto.min-saude.pt (C.E.); i12756@ipoporto.min-saude.pt (A.B.); masps.porto@gmail.com (M.P.); joao.pinho.silva@ipoporto.min-saude.pt (J.S.)
- ² Doctoral Programme in Biomedical Sciences, School Medicine and Biomedical Sciences, University of Porto (ICBAS-UP), 4050-313 Porto, Portugal
- ³ Department of Laboratory Genetics, Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center, 4200-072 Porto, Portugal
- ⁴ Department of Pathological, Cytological and Thanatological Anatomy, School of Health, Polytechnic Institute of Porto, 4200-072 Porto, Portugal
- ⁵ Molecular Pathobiology Research Unit, Portuguese Oncology Institute of Lisbon, 1099-023 Lisbon, Portugal; mfrancisco@ipolisboa.min-saude.pt (I.F.); calbuque@ipolisboa.min-saude.pt (C.A.)
- ⁶ Department of Pathology, Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center, 4200-072 Porto, Portugal; paulalopes@ipoporto.min-saude.pt (P.L.); anaraquel.isidoro@ulsm.min-saude.pt (A.R.I.); analuisa.cunha@ipoporto.min-saude.pt (A.L.C.)
- ⁷ Gastroenterology Department, Portuguese Oncology Institute of Lisbon, 1099-023 Lisbon, Portugal; iclaro@ipolisboa.min-saude.pt
- ⁸ Familiar Cancer Risk Clinic, Portuguese Oncology Institute of Lisbon, 1099-023 Lisbon, Portugal
- ⁹ i3S-Instituto de Investigação e Inovação em Saúde, 4200-135 Porto, Portugal; carlaol@i3s.up.pt
- ¹⁰ IPATIMUP-Instituto de Patologia e Imunologia Molecular da Universidade do Porto, 4200-135 Porto, Portugal
- ¹¹ FMUP-Faculty of Medicine of the University of Porto, 4100-179 Porto, Portugal
- ¹² Medical Genetics Department, Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center, 4200-072 Porto, Portugal
- ¹³ School of Medicine and Biomedical Sciences (ICBAS), University of Porto, 4050-313 Porto, Portugal

* Correspondence: manuel.teixeira@ipoporto.min-saude.pt; Tel.: +351-225-084-000



Citation: Guerra, J.; Pinto, C.; Pinto, P.; Pinheiro, M.; Santos, C.; Peixoto, A.; Escudeiro, C.; Barbosa, A.; Porto, M.; Francisco, I.; et al. Frequency of *CDH1*, *CTNNA1* and *CTNND1* Germline Variants in Families with Diffuse and Mixed Gastric Cancer. *Cancers* **2023**, *15*, 4313. <https://doi.org/10.3390/cancers15174313>

Academic Editor: Rosalba D'Alessandro

Received: 12 July 2023
Revised: 23 August 2023
Accepted: 25 August 2023
Published: 29 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Simple Summary: Hereditary diffuse gastric cancer (HDGC) is caused by germline pathogenic variants in the *CDH1* and *CTNNA1* genes and is characterized by a high prevalence of diffuse gastric cancer and lobular breast cancer. We aimed to evaluate the contribution of *CTNNA1* and *CTNND1* germline variants to HDGC, as well as to compare the frequencies of *CDH1* and *CTNNA1* (and eventually *CTNND1*) germline variants between patients with diffuse and mixed gastric carcinomas. In this study, we report a deleterious *CTNNA1* germline variant and four *CDH1* pathogenic variants in patients with criteria for genetic testing. None of the cases with mixed gastric cancer carried pathogenic variants in either the *CDH1* or the *CTNNA1* genes, so there is no evidence to use this tumor type in testing criteria.

Abstract: The most well-characterized hereditary form of gastric cancer is hereditary diffuse gastric cancer (HDGC), an autosomal dominant syndrome characterized by an increased risk of diffuse gastric and lobular breast cancer. HDGC is predominantly caused by germline pathogenic variants in the *CDH1* gene, and more rarely in the *CTNNA1* gene. Furthermore, the International Gastric Cancer Linkage Consortium (IGCLC) guidelines do not clarify whether or not mixed gastric cancer

(with a diffuse component) should be considered in the HDGC genetic testing criteria. We aimed to evaluate the contribution of *CTNNA1* and *CTNND1* germline variants to HDGC. Additionally, we also intended to compare the frequencies of *CDH1* and *CTNNA1* (and eventually *CTNND1*) germline variants between patients with diffuse and mixed gastric carcinomas to evaluate if genetic testing for these genes should or should not be considered in patients with the latter. We analyzed the *CDH1* gene in 67 cases affected with early-onset/familial mixed gastric carcinomas and the *CTNNA1* and *CTNND1* genes in 208 cases with diffuse or mixed gastric cancer who had tested negative for *CDH1* pathogenic germline variants. A deleterious *CTNNA1* germline variant was found in 0.7% (1/141) of diffuse gastric cancer patients meeting the 2020 IGCLC criteria, as compared to the rate of 2.8% of *CDH1* deleterious variants found by us in this setting. No deleterious variants were found in *CTNND1*, but six variants of uncertain significance were identified in this gene. We did not find any pathogenic *CDH1*, *CTNNA1* or *CTNND1* variant in index patients with early-onset/familial mixed gastric cancer, so there is no evidence that supports including this tumor type in the testing criteria for germline variants in these genes. The role of the *CTNND1* gene in inherited gastric cancer predisposition is still unclear.

Keywords: hereditary diffuse gastric cancer; mixed gastric cancer; *CDH1* gene; *CTNNA1* gene; *CTNND1* gene

1. Introduction

Familial clustering is observed in approximately 10% of gastric cancer (GC) cases; however, only 1–3% of GC cases result from a known hereditary syndrome [1]. The most well-characterized hereditary form of GC is hereditary diffuse gastric cancer (HDGC), which is an autosomal dominant syndrome characterized by an increased risk of diffuse gastric cancer (DGC) and lobular breast cancer (LBC) [2]. Until recently, *CDH1* was the only known susceptibility gene involved in HDGC, but, in 2013, Majewski and collaborators described a germline variant in the catenin alpha-1 gene (*CTNNA1*) for the first time in a family that fulfilled the HDGC clinical criteria [3]. Since then, to our knowledge, 13 pathogenic variants in *CTNNA1* have been described in 15 families fulfilling the HDGC criteria [3–11]. Additionally, loss-of-function variants have been described in families without HDGC clinical criteria [9,11]. Benusiglio and collaborators reported two asymptomatic *CTNNA1* pathogenic variant carriers, who displayed DGC *foci* in the prophylactic gastrectomy and intramucosal *focus* on esophagogastroduodenoscopy specimens, with loss of catenin alpha-1 expression, suggesting that pathogenic variants in *CDH1* and *CTNNA1* may have similar implications in DGC risk [1,7]. The penetrance of pathogenic *CTNNA1* variants is still unclear, although Coudert and collaborators have recently published the first penetrance estimate for DGC in *CTNNA1* families as 49–57% at 80 years [10]. The association of *CTNNA1* pathogenic variants with increased LBC risk is also still inconclusive; although some studies have been associating *CTNNA1* and breast cancer [9,12–14], this association remains to be clarified since the subtype of these cancers was not specified. Therefore, breast cancer surveillance should be considered based on personal and family history [2].

The *CDH1* and *CTNNA1* genes are members of the cadherin and catenin family, respectively, encoding adherens junction proteins, and they are involved in intercellular cell adhesion [15]. The *CDH1* gene encodes the cadherin-1, a transmembrane protein, and its extracellular domain establishes interactions with cadherin-1 proteins on an adjacent cell, whereas its cytoplasmic domain binds to the members of the catenin protein family, such as catenin delta-1 and catenin alpha-1 via catenin beta-1, promoting protein stabilization [16]. The *CTNNA1* gene encodes the catenin alpha-1 protein, which forms a complex with catenin beta-1 to bind the cytoplasmic domain of cadherin-1 to the actin cytoskeleton by binding directly to the actin filaments [16–18]. This protein is essential for the function of the E-cadherin-catenin complex, and the inactivation of catenin proteins and disruption

of cytoplasmic catenin-cadherin binding results in the inability of cadherin-1 to establish cell-to-cell adhesion [17]. The involvement of the catenin delta-1 protein in cell adhesion raises the question of its implication in GC predisposition. The catenin delta-1 protein is encoded by the *CTNND1* gene, interacts with the juxtamembrane domain of cadherin-1 and regulates the stability of cell–cell adhesion by controlling the retention of cadherin-1 at the cell surface, being crucial to the maintenance and stability of cell adhesion [16]. A single study has been reported that attempted to identify germline variants in the *CTNND1* gene and no pathogenic variants were found in the few families tested [19].

The International Gastric Cancer Linkage Consortium (IGCLC), in 1999, defined clinical criteria to select patients for the screening of *CDH1* germline variants [20]. Since then, these criteria have been updated several times [21,22] and, in 2020, the IGCLC published updated and less restrictive testing recommendations [2]. Moreover, according to these new guidelines, *CTNNA1* testing should be considered when no *CDH1* pathogenic variant has been identified [2]. Despite these updates, the guidelines remain unclear if mixed GC, which harbors the two histological components, intestinal and diffuse, should or should not be considered in the HDGC testing criteria [23,24]. In fact, the IGCLC guidelines are very clear about not considering the intestinal type for *CDH1/CTNNA1* testing, as this cancer type is not part of the syndrome [2], but they provide no guidance as regards to mixed GC.

We aimed to evaluate the contribution of *CTNNA1* and *CTNND1* germline variants to HDGC, as well as to compare the frequencies of *CDH1* and *CTNNA1* (and eventually *CTNND1*) germline variants in patients/families with DGC with those with mixed GC to evaluate the pertinence of genetic testing of these genes in the latter.

2. Materials and Methods

2.1. Patients and Samples

This study included 212 index cases; 193 patients who were referred to the Genetics Department of the Portuguese Oncology Institute of Porto (IPO Porto) between 2012 and 2021 and 19 patients from the Portuguese Oncology Institute of Lisbon referred to genetic counseling between 2014 and 2019. Of the 212 families, 145 met the IGCLC 2015 or 2020 clinical criteria for HDGC testing (5 of the index patients had LBC and all the others had DGC); among the 145 patients that met the IGCLC 2020 criteria, 26 patients did not fulfill the IGCLC 2015 criteria. Germline *CDH1* testing had been performed in these 145 index patients as part of standard clinical genetic testing. The remaining 67 patients were affected with mixed GC and would have met the IGCLC 2015 or 2020 testing criteria if this histological type had been considered for testing in the same way as DGC.

This study was approved by the Ethics Committee of IPO Porto (reference number CES 351/018) and samples for genetic testing were obtained after genetic counseling and informed consent.

Genomic DNA was extracted from peripheral blood samples according to standard protocols.

2.2. Next Generation Sequencing

CDH1 (NM_004360.5) gene analysis was performed in 67 index cases with mixed GC by next-generation sequencing (NGS), using the TruSight Cancer panel (Illumina, Inc., San Diego, CA, USA), with library preparation performed according to the manufacturer's protocol, and the sequencing reaction was carried out using a standard flow cell in the MiSeq platform (Illumina, Inc.) in 2 × 150 bp paired-end runs. Sequencing alignment and variant analysis were performed using a bioinformatics pipeline previously validated by our group [25]. In brief, alignment and variant calling were carried out using NextGENe (v2.4, Softgenetics, State College, PA, USA). NGS as described above had been used as part of clinical care for the 145 index cases tested for *CDH1*.

Sequencing of the *CTNNA1* (NM_001903.5) and the *CTNND1* (NM_001085458.1) genes was performed in 208 cases (with negative *CDH1* testing) using a customized QIASeq Targeted DNA Panel (Qiagen, Antwerp, Belgium). Library preparation was performed

according to the manufacturer's instructions and sequencing was carried out using a micro flow cell in the MiSeq Illumina sequencer (Illumina, Inc.) in a 2×150 bp paired-end run. Sequencing alignment and variant calling were performed using QIAGEN's GeneGlobe data analysis portal (Qiagen). The .vcf files were imported to GeneticistAssistant™ software 1.8 (Softgenetics) for variant annotation. All variants with a variant allele frequency (VAF) less than 10%, minor allele frequency (MAF) greater than 1% and intronic variants at more than 12 bp away from exon–intron boundaries were excluded. For *CDH1* analysis, variants with a frequency higher than 5% in our in-house database were also excluded. For MAF filtering, data were obtained from the 1000 Genomes Project (1000 G; Based on Project Phase III Data), Genome Aggregation Database (gnomAD) and Exome Aggregation Consortium (ExAC) databases.

2.3. Immunohistochemistry

Catenin alpha-1 protein expression was evaluated by immunohistochemistry (IHC) in section from a representative formalin-fixed and paraffin-embedded tumor tissue block. Briefly, a mouse monoclonal antibody against catenin alpha-1 (clone EP1793Y, Abcam, Cambridge, UK) was incubated at 1/200 for 20 min, and staining was performed with the Leica Bond-III instrument according to the manufacturer's instructions. Immunostained slides were evaluated by a pathologist. Diffuse gastric tumors known to be negative for *CTNNA1* and *CDH1* germline variants were used as controls to assess the specificity of the staining.

3. Results

A total of 212 patients were included in this study; 145 of these patients met IGCLC 2020 clinical criteria for HDGC testing and the remaining 67 patients were diagnosed with mixed GC. The *CDH1* gene had been analyzed in the 145 patients that fulfilled the IGCLC criteria as part of standard clinical genetic testing prior to this study and *CTNNA1* gene sequencing was performed in all patients who had tested negative for *CDH1* pathogenic germline variants (Figure 1). Four families have previously been identified with three pathogenic variants in the *CDH1* gene (Table 1). Three of these families were already reported [26,27], two harboring the recurrent splicing variant c.1901C>T, which was identified in two women diagnosed with LBC at 38 and 48 years old, and the third was the c.1565+1G>A variant identified in a man with a cleft lip and diagnosed with DGC at 63 years old. Lastly, the variant c.377del p.(Pro126ArgfsTer89) was identified in a woman diagnosed with DGC at 32 years old (Figure 2a). This variant is classified in ClinVar (ID 406616) as pathogenic and has already been reported in the literature [28].

Table 1. Germline pathogenic variants in the *CDH1* and *CTNNA1* genes identified in families fulfilling genetic testing criteria.

Family	Gene	HGVS Coding	HGVS Protein	Gastric Cancer Histology	Breast Cancer Type	Age of Diagnosis	HDGC Criteria
1 *	<i>CDH1</i>	c.1901C>T	p.(Ala634Val)	-	Lobular breast cancer	39	Yes
2 *	<i>CDH1</i>	c.1901C>T	p.(Ala634Val)	-	Lobular breast cancer	48	Yes
3 *	<i>CDH1</i>	c.1565+1G>A		Diffuse gastric cancer	-	63	Yes
4	<i>CDH1</i>	c.377del	p.(Pro126ArgfsTer89)	Diffuse gastric cancer	-	32	Yes
5	<i>CTNNA1</i>	c.1426C>T	p.(Gln476Ter)	Diffuse gastric cancer	-	27	Yes

* These families have previously been reported in other studies [26,27].

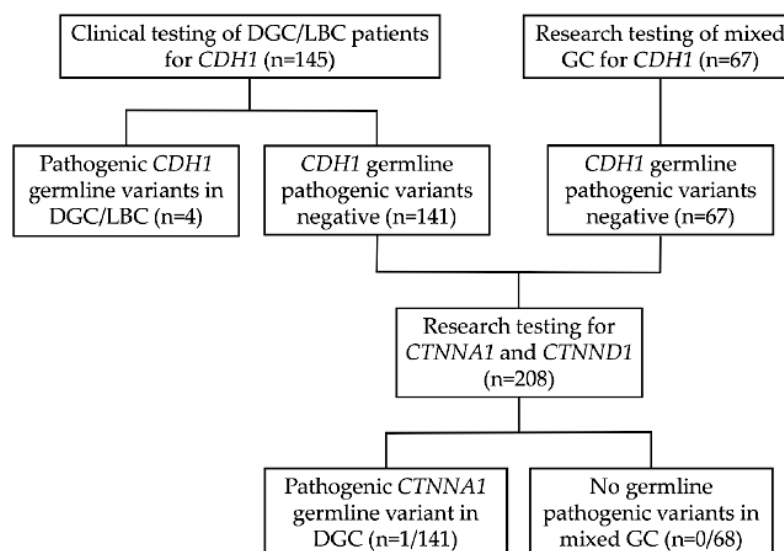


Figure 1. Flow chart representing patient inclusion and subsequent germline analysis for *CDH1*, *CTNNA1* and *CTNND1* genes.

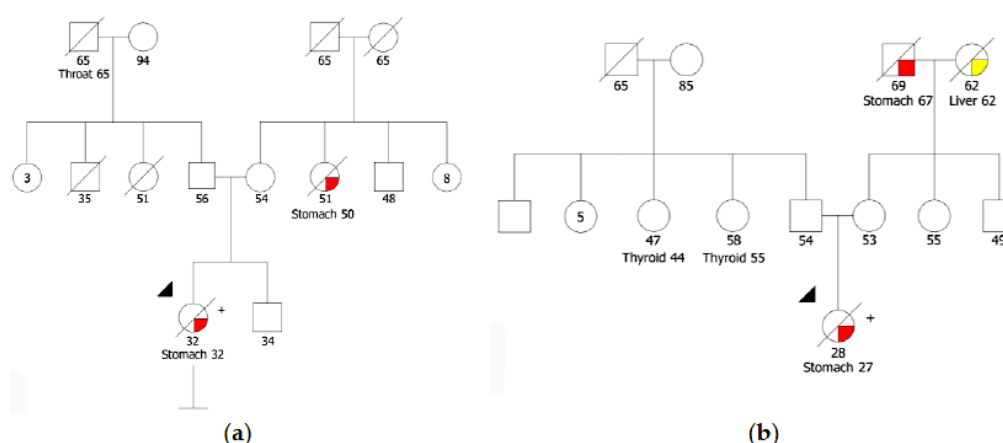


Figure 2. (a) Pedigree of the HDGC family with the *CDH1* variant c.377del p.(Pro126ArgfsTer89); (b) Pedigree of the index patient identified with the *CTNNA1* variant c.1426C>T p.(Gln476Ter).

The *CTNNA1* germline variant c.1426C>T was found in 1 of 141 (0.7%) patients meeting 2020 IGCLC criteria who had previously tested negative for *CDH1* deleterious variants. This is a nonsense variant predicted to lead to premature termination of translation and, consequently, to a truncated protein, p.(Gln476Ter), or to a nonsense-mediated mRNA decay. This variant was identified in a woman diagnosed with a poorly differentiated adenocarcinoma with signet ring cells at age 27 (Figure 2b). There are no *CTNNA1* variant classification guidelines; however, Lobo and collaborators classified all variants described in the literature following the *CDH1* ACMG/AMP guidelines [29,30]. Following these guidelines, the variant identified in our study is classified as pathogenic, using the criteria PVS1 (nonsense variant), PS4 supporting (one family meeting HDGC criteria) and PM2 (variant absent from population databases). We performed immunohistochemistry (IHC) to assess catenin alpha-1 protein expression in the biopsy sample of the *CTNNA1* pathogenic

variant carrier. IHC revealed the loss of catenin alpha-1 expression in the gastric adenocarcinoma and preserved catenin alpha-1 expression in the normal epithelial structures (Figure 3d). We also performed IHC in gastric tumors of ten index cases from this series known to be wild-type for the *CDH1* and *CTNNA1* genes and for whom tumor specimens were available, and catenin alpha-1 expression was retained in both normal and tumor cells (Figure 3b). Regarding family history, the proband's parents are not affected; the maternal grandfather had stomach cancer at age 67 and the maternal grandmother had liver cancer at 62, both deceased. Two paternal cousins were diagnosed with thyroid cancer at 44 and 55 years old, respectively. To date, it was not possible to perform segregation analysis or test the parents of the proband.

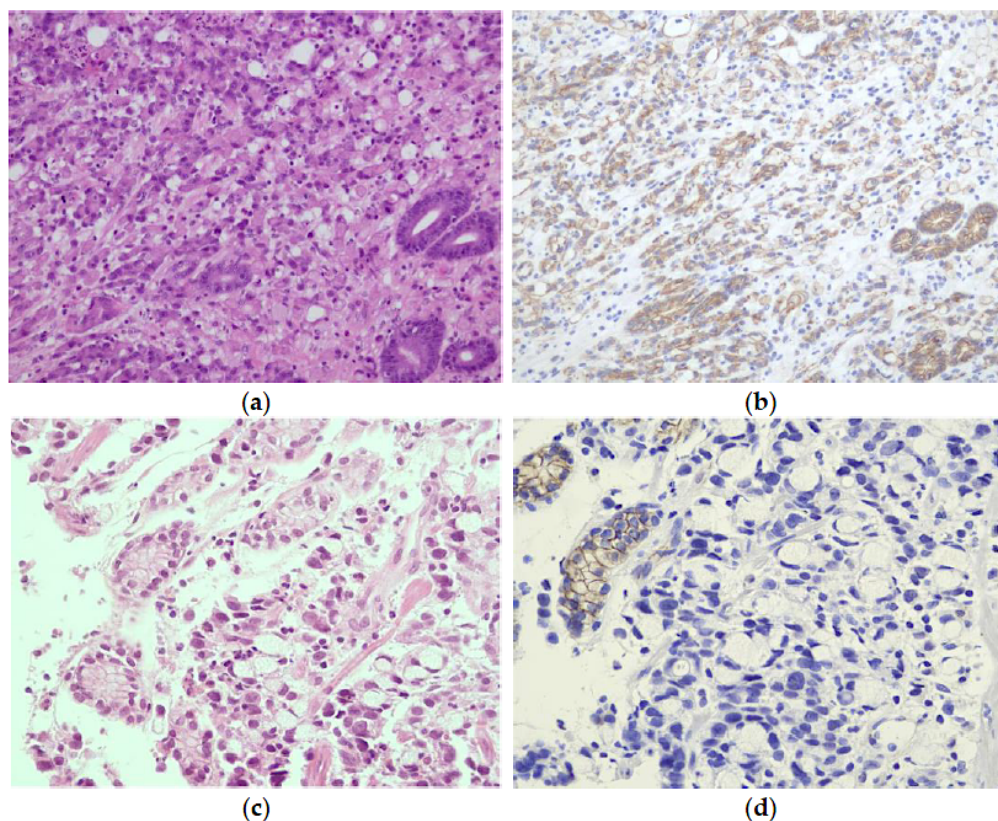


Figure 3. Microscopic findings showing hematoxylin and eosin (H&E) staining in the left column and immunohistochemistry for catenin alpha-1 in the right column. (a,b) Diffuse gastric cancer in a patient who does not carry *CTNNA1* germline variants. Catenin alpha-1 expression is retained in both normal and tumor cells (original magnification, $\times 200$). (c,d) Gastric biopsies of the *CTNNA1* c.1426C>T p.(Gln476Ter) variant carrier show signet ring cells infiltrating the mucosa. Catenin alpha-1 was detectable in normal epithelial structures (e.g., the glands of the stomach), but the expression was completely lost in the tumor cells (original magnification, $\times 400$).

None of the 67 cases with mixed GC carried pathogenic variants in either the *CDH1* or the *CTNNA1* genes. Furthermore, no deleterious *CTNND1* variant was detected in the 208 patients without the *CDH1* pathogenic germline variants. We identified four missense and two synonymous variants in the *CTNND1* gene classified as variants of uncertain significance (VUS) following the ACMG guidelines [29], both in patients with diffuse and mixed gastric cancer subtypes (Table 2).

Table 2. Variants of uncertain significance identified in the *CTNND1* gene.

HGVS Coding	HGVS Protein	Variant Type	GnomAD Frequency	ClinVar Classification	ClinVar ID	ACMG Classification	Number of Samples	Gastric Cancer Histology
c.148G>C	p.Asp50His	missense	N/A	VUS	2391342	VUS	1	Mixed
c.202C>T	p.Arg68Trp	missense	0.0003848	VUS	1311724	VUS	1	Mixed
c.790C>T	p.Arg264Trp	missense	0.0000121	N/A	N/A	VUS	1	Mixed
c.1671C>A	p.Ala557=	synonymous	0.0000040	N/A	N/A	VUS	1	Diffuse
c.2130G>A	p.Lys710=	synonymous	N/A	N/A	N/A	VUS	1	Mixed
c.2474T>C	p.Val825Ala	missense	0.0000392	N/A	N/A	VUS	1	Diffuse

VUS, variant of uncertain significance; N/A, not available.

4. Discussion

Until recently, *CDH1* was the only known susceptibility gene associated with HDGC, but, in 2020, the IGCLC recognized *CTNNA1* as an HDGC predisposition gene and recommended *CTNNA1* testing when no *CDH1* pathogenic variant has been identified [2]. On the other hand, the contribution of *CTNND1* germline variants for GC predisposition is unknown. Furthermore, the IGCLC still does not clarify if mixed GC with a diffuse component should or should not be considered in the clinical criteria for genetic testing.

Prior to this study and as part of clinical care, the 145 index cases that fulfilled the HDGC testing criteria had been analyzed for *CDH1* pathogenic variants, and four families were identified with three pathogenic variants in the *CDH1* gene (Table 1). The prevalence of pathogenic *CDH1* variants in our patients with HDGC genetic testing criteria is approximately 3% (4/145). The prevalence of *CDH1* pathogenic variants in suspected HDGC families varies widely in the literature, which can be mainly explained by the different GC incidences between countries [4]. High-incidence countries, such as Portugal, display a lower frequency of molecularly confirmed HDGC [4,31,32], which can explain the relatively low prevalence of *CDH1* deleterious variants we present here. In this study, we analyzed the two candidate genes, *CTNNA1* and *CTNND1*, in the remaining 141 index cases that were negative for *CDH1* pathogenic variants. We identified a novel nonsense variant in *CTNNA1*, c.1426C>T p.(Gln476Ter) in a woman diagnosed with a poorly differentiated adenocarcinoma with signet ring cells at age 27 (Figure 2b). We used the *CDH1* ACMG/AMP guidelines to classify this variant as pathogenic, as described by Lobo et al. [29,30]. The IHC showed loss of catenin alpha-1 expression in the tumor cells and preserved catenin alpha-1 expression in the normal gastric epithelium of the *CTNNA1* variant carrier. Furthermore, catenin alpha-1 expression was retained in the tumors of ten proband cases, known to be wild-type for *CTNNA1* and *CDH1* genes, suggesting that catenin alpha-1 expression loss in DGC can be predictive of a *CTNNA1* pathogenic variant. Loss or decrease in catenin alpha-1 expression in tumors or biopsy samples of *CTNNA1* carriers has also been demonstrated by several authors [3,7,9]. These observations suggest that a second hit inactivating mechanism occurred somatically at the *CTNNA1* gene; however, the tumor sample was not available to search for this mechanism. Although the prevalence of *CTNNA1* pathogenic variants is not well-established, Lobo et al. suggested that pathogenic variants in *CTNNA1* seem to account for less than 2% of the families fulfilling the HDGC testing criteria [11]. The prevalence of pathogenic *CTNNA1* variants in our families fulfilling 2020 HDGC testing criteria is approximately 1%, which is similar to the frequency described in the literature [11].

According to the latest HDGC guidelines, *CTNNA1* is considered to be a predisposition gene, along with *CDH1*. Annual endoscopy screening following the Cambridge protocol is recommended in asymptomatic carriers and total gastrectomy should be considered depending on the results of the biopsies and the penetrance of DGC in the family [2]. The risk of GC in carriers of pathogenic *CTNNA1* variants is not well defined, but a recent study evaluated DGC risk in *CTNNA1* carriers and estimated a cumulative risk of DGC at 80 years of 49–57% [10]. The association of *CTNNA1* pathogenic variants with high risk of

LBC is also still uncertain; therefore, breast cancer surveillance should be considered based on personal and family history [2].

Beyond HDGC predisposition, the *CTNNA1* and *CDH1* genes have been associated with macular dystrophy patterned [33,34] and cleft lip/palate and blepharochelodontic syndrome (BCD) [35,36], respectively. The *CTNND1* gene has also been associated with BCD [35–37], and the involvement of another cadherin–catenin complex gene in cleft palate and BCD, in addition to its important function in the maintenance and stability of cell adhesion, which makes this gene an ideal candidate for GC predisposition [38]. We therefore analyzed the *CTNND1* gene in 208 patients included in this study, but no pathogenic variants were found in the 141 patients with the 2020 IGCLC criteria negative for *CDH1* variants. Our negative findings support those of Schuetz et al. [19] in 22 families. However, we identified six VUS in the *CTNND1* gene, both in patients with diffuse and mixed gastric cancer subtypes. Here, we report the largest series to date that tested for *CTNND1* variants in suspected HDGC patients, but even larger studies will be needed to clarify if the *CTNND1* gene has any role in GC predisposition.

A proportion of gastric carcinomas display a mixed phenotype presenting the two histological components, intestinal and diffuse [23,24]. Interestingly, while intestinal-type GC has been completely excluded from the HDGC spectrum [20,21] and the IGCLC guidelines are very clear to not test the patients with this histological type, these guidelines do not indicate whether the mixed type should be considered in the criteria for *CDH1* germline variant testing. In this study, we analyzed the *CDH1*, *CTNNA1* and *CTNND1* genes in 67 cases affected with mixed GC and did not find any pathogenic *CDH1*, *CTNNA1* or *CTNND1* variant. To our knowledge, there are no pathogenic *CDH1* variants described in cases with mixed GC in the literature [39,40]. Furthermore, the findings of Machado et al. are in favor of a clonal origin of mixed GC and suggest that an isolated-cell histological component may be originated due to the occurrence of *CDH1* somatic variants [41]. Therefore, there is currently no evidence that supports including this tumor type in germline testing of the *CDH1* or *CTNNA1* genes. However, studies in larger series of mixed GC are necessary to clarify this issue.

5. Conclusions

In this study, a deleterious *CTNNA1* germline variant was found in 0.7% (1/141) of patients meeting the 2020 IGCLC criteria, as compared to the rate of 2.8% of *CDH1* deleterious variants. Despite the relatively low frequency of HDGC families associated with pathogenic germline variants in *CTNNA1*, screening for this gene should be conducted for *CDH1*-negative HDGC families, as recommended in the latest IGCLC guidelines [2]. This will enable the identification of additional *CTNNA1* families, allowing more accurate penetrance estimates and a better-defined disease spectrum. The role of *CTNND1* variants in inherited gastric cancer predisposition is still unclear.

Author Contributions: Conceptualization, M.R.T. and J.G.; methodology, J.G., C.P., P.P., M.P. (Manuela Pinheiro), C.S., A.P., C.E., A.B., M.P. (Miguel Porto), I.F., P.L., A.R.I. and A.L.C.; formal analysis, J.G., C.P., P.P., M.P. (Manuela Pinheiro), C.S. and A.P.; writing—original draft preparation, J.G.; writing—review and editing, J.G., C.P., P.P., M.P. (Manuela Pinheiro), C.S., A.P., C.E., A.B., M.P. (Miguel Porto), I.F., P.L., A.R.I., A.L.C., C.A., I.C., C.O., J.S. and M.R.T.; supervision, M.R.T.; funding acquisition, M.R.T. All authors have read and agreed to the published version of the manuscript.

Funding: Joana Guerra is a research fellow of the Fundação para a Ciência e Tecnologia (SFRH/BD/138670/2018). Manuela Pinheiro and Miguel Porto are funded by the project “P.CCC: Centro Compreensivo de Cancro do Porto”—NORTE-01-0145-FEDER-072678, supported by the Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Portuguese Oncology Institute of Porto (IPO-Porto) (reference number CES 351/018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Carneiro, F. Familial and hereditary gastric cancer, an overview. *Best Pract. Res. Clin. Gastroenterol.* **2022**, *58–59*, 101800. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Blair, V.R.; McLeod, M.; Carneiro, F.; Coit, D.G.; D'Addario, J.L.; van Dieren, J.M.; Harris, K.L.; Hoogerbrugge, N.; Oliveira, C.; van der Post, R.S.; et al. Hereditary diffuse gastric cancer: Updated clinical practice guidelines. *Lancet Oncol.* **2020**, *21*, e386–e397. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Majewski, I.J.; Kluijdt, I.; Cats, A.; Scerri, T.S.; de Jong, D.; Kluin, R.J.; Hansford, S.; Hogervorst, F.B.; Bosma, A.J.; Hofland, I.; et al. An α -E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer. *J. Pathol.* **2013**, *229*, 621–629. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Hansford, S.; Kaurah, P.; Li-Chang, H.; Woo, M.; Senz, J.; Pinheiro, H.; Schrader, K.A.; Schaeffer, D.F.; Shumansky, K.; Zogopoulos, G.; et al. Hereditary Diffuse Gastric Cancer Syndrome: CDH1 Mutations and beyond. *JAMA Oncol.* **2015**, *1*, 23–32. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Slavin, T.; Neuhausen, S.L.; Rybak, C.; Solomon, I.; Nehoray, B.; Blazer, K.; Niell-Swiler, M.; Adamson, A.W.; Yuan, Y.C.; Yang, K.; et al. Genetic Gastric Cancer Susceptibility in the International Clinical Cancer Genomics Community Research Network. *Cancer Genet.* **2017**, *216–217*, 111–119. [\[CrossRef\]](#)
6. Weren, R.D.A.; van der Post, R.S.; Vogelaar, I.P.; van Krieken, J.H.; Spruijt, L.; Lubinski, J.; Jakubowska, A.; Teodorczyk, U.; Aalfs, C.M.; van Hest, L.P.; et al. Role of germline aberrations affecting CTNNA1, MAP3K6 and MYD88 in gastric cancer susceptibility. *J. Med. Genet.* **2018**, *55*, 669–674. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Benusiglio, P.R.; Colas, C.; Guillemin, E.; Canard, A.; Delhomelle, H.; Warcoin, M.; Bellanger, J.; Eyries, M.; Zizi, M.; Netter, J.; et al. Clinical implications of CTNNA1 germline mutations in asymptomatic carriers. *Gastric Cancer* **2019**, *22*, 899–903. [\[CrossRef\]](#)
8. Marwitz, T.; Hüneburg, R.; Spier, I.; Lau, J.F.; Kristiansen, G.; Lingohr, P.; Kalff, J.C.; Aretz, S.; Nattermann, J.; Strassburg, C.P. Hereditary Diffuse Gastric Cancer: A Comparative Cohort Study According to Pathogenic Variant Status. *Cancers* **2020**, *12*, 3726. [\[CrossRef\]](#)
9. Clark, D.F.; Michalski, S.T.; Tondon, R.; Nehoray, B.; Ebrahimzadeh, J.; Hughes, S.K.; Soper, E.R.; Domchek, S.M.; Rustgi, A.K.; Pineda-Alvarez, D.; et al. Loss-of-function variants in CTNNA1 detected on multigene panel testing in individuals with gastric or breast cancer. *Genet. Med.* **2020**, *22*, 840–846. [\[CrossRef\]](#)
10. Coudert, M.; Drouet, Y.; Delhomelle, H.; Svrcek, M.; Benusiglio, P.R.; Coulet, F.; Clark, D.F.; Katona, B.W.; van Hest, L.P.; van der Kolk, L.E.; et al. First estimates of diffuse gastric cancer risks for carriers of CTNNA1 germline pathogenic variants. *J. Med. Genet.* **2022**, *59*, 1189–1195. [\[CrossRef\]](#)
11. Lobo, S.; Benusiglio, P.R.; Coulet, F.; Boussemart, L.; Golmard, L.; Spier, I.; Hüneburg, R.; Aretz, S.; Colas, C.; Oliveira, C. Cancer predisposition and germline CTNNA1 variants. *Eur. J. Med. Genet.* **2021**, *64*, 104316. [\[CrossRef\]](#)
12. Felicio, P.S.; Grasel, R.S.; Campacci, N.; de Paula, A.E.; Galvão, H.C.R.; Torrezan, G.T.; Sabato, C.S.; Fernandes, G.C.; Souza, C.P.; Michelli, R.D.; et al. Whole-exome sequencing of non-BRCA1/BRCA2 mutation carrier cases at high-risk for hereditary breast/ovarian cancer. *Hum. Mutat.* **2021**, *42*, 290–299. [\[CrossRef\]](#)
13. Sun, J.; Meng, H.; Yao, L.; Lv, M.; Bai, J.; Zhang, J.; Wang, L.; Ouyang, T.; Li, J.; Wang, T.; et al. Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients. *Clin. Cancer Res.* **2017**, *23*, 6113–6119. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Shirts, B.H.; Casadei, S.; Jacobson, A.L.; Lee, M.K.; Gulsuner, S.; Bennett, R.L.; Miller, M.; Hall, S.A.; Hampel, H.; Hisama, F.M.; et al. Improving performance of multigene panels for genomic analysis of cancer predisposition. *Genet. Med.* **2016**, *18*, 974–981. [\[CrossRef\]](#)
15. van Roy, F. Beyond E-cadherin: Roles of other cadherin superfamily members in cancer. *Nat. Rev. Cancer* **2014**, *14*, 121–134. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Gloushankova, N.A.; Rubtsova, S.N.; Zhitnyak, I.Y. Cadherin-mediated cell-cell interactions in normal and cancer cells. *Tissue Barriers* **2017**, *5*, e1356900. [\[CrossRef\]](#)
17. Kobiela, A.; Fuchs, E. Alpha-catenin: At the junction of intercellular adhesion and actin dynamics. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 614–625. [\[CrossRef\]](#)
18. Takeichi, M. Multiple functions of α -catenin beyond cell adhesion regulation. *Curr. Opin. Cell Biol.* **2018**, *54*, 24–29. [\[CrossRef\]](#)
19. Schuetz, J.M.; Leach, S.; Kaurah, P.; Jeyes, J.; Butterfield, Y.; Huntsman, D.; Brooks-Wilson, A.R. Catenin family genes are not commonly mutated in hereditary diffuse gastric cancer. *Cancer Epidemiol. Biomark. Prev.* **2012**, *21*, 2272–2274. [\[CrossRef\]](#)
20. Caldas, C.; Carneiro, F.; Lynch, H.T.; Yokota, J.; Wiesner, G.L.; Powell, S.M.; Lewis, F.R.; Huntsman, D.G.; Pharoah, P.D.; Jankowski, J.A.; et al. Familial gastric cancer: Overview and guidelines for management. *J. Med. Genet.* **1999**, *36*, 873–880. [\[PubMed\]](#)
21. van der Post, R.S.; Vogelaar, I.P.; Carneiro, F.; Guilford, P.; Huntsman, D.; Hoogerbrugge, N.; Caldas, C.; Schreiber, K.E.; Hardwick, R.H.; Ausems, M.G.; et al. Hereditary diffuse gastric cancer: Updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. *J. Med. Genet.* **2015**, *52*, 361–374. [\[CrossRef\]](#)

22. Fitzgerald, R.C.; Hardwick, R.; Huntsman, D.; Carneiro, F.; Guilford, P.; Blair, V.; Chung, D.C.; Norton, J.; Ragunath, K.; Van Krieken, J.H.; et al. Hereditary diffuse gastric cancer: Updated consensus guidelines for clinical management and directions for future research. *J. Med. Genet.* **2010**, *47*, 436–444. [\[CrossRef\]](#) [\[PubMed\]](#)
23. WHO Classification Tumours Editorial Board. (Ed.) *WHO Classification of Tumours: Digestive System Tumours*, 5th ed.; IARC Press: Lyon, France, 2019.
24. Lauren, P. The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol. Microbiol. Scand.* **1965**, *64*, 31–49. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Pinto, P.; Paulo, P.; Santos, C.; Rocha, P.; Pinto, C.; Veiga, I.; Pinheiro, M.; Peixoto, A.; Teixeira, M.R. Implementation of next-generation sequencing for molecular diagnosis of hereditary breast and ovarian cancer highlights its genetic heterogeneity. *Breast Cancer Res. Treat.* **2016**, *159*, 245–256. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Garcia-Pelaez, J.; Barbosa-Matos, R.; Lobo, S.; Dias, A.; Garrido, L.; Castedo, S.; Sousa, S.; Pinheiro, H.; Sousa, L.; Monteiro, R.; et al. Genotype-first approach to identify associations between CDH1 germline variants and cancer phenotypes: A multicentre study by the European Reference Network on Genetic Tumour Risk Syndromes. *Lancet Oncol.* **2023**, *24*, 91–106. [\[CrossRef\]](#)
27. Paulo, P.; Pinto, P.; Peixoto, A.; Santos, C.; Pinto, C.; Rocha, P.; Veiga, I.; Soares, G.; Machado, C.; Ramos, F.; et al. Validation of a Next-Generation Sequencing Pipeline for the Molecular Diagnosis of Multiple Inherited Cancer Predisposing Syndromes. *J. Mol. Diagn.* **2017**, *19*, 502–513. [\[CrossRef\]](#)
28. Keller, G.; Vogelsang, H.; Becker, I.; Hutter, J.; Ott, K.; Candidus, S.; Grundei, T.; Becker, K.F.; Mueller, J.; Siewert, J.R.; et al. Diffuse type gastric and lobular breast carcinoma in a familial gastric cancer patient with an E-cadherin germline mutation. *Am. J. Pathol.* **1999**, *155*, 337–342. [\[CrossRef\]](#)
29. Richards, S.; Aziz, N.; Bale, S.; Bick, D.; Das, S.; Gastier-Foster, J.; Grody, W.W.; Hegde, M.; Lyon, E.; Spector, E.; et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **2015**, *17*, 405–424. [\[CrossRef\]](#)
30. Lee, K.; Krempely, K.; Roberts, M.E.; Anderson, M.J.; Carneiro, F.; Chao, E.; Dixon, K.; Figueiredo, J.; Ghosh, R.; Huntsman, D.; et al. Specifications of the ACMG/AMP variant curation guidelines for the analysis of germline CDH1 sequence variants. *Hum. Mutat.* **2018**, *39*, 1553–1568. [\[CrossRef\]](#)
31. Corso, G.; Corso, F.; Bellerba, F.; Carneiro, P.; Seixas, S.; Cioffi, A.; La Vecchia, C.; Magnoni, F.; Bonanni, B.; Veronesi, P.; et al. Geographical Distribution of E-cadherin Germline Mutations in the Context of Diffuse Gastric Cancer: A Systematic Review. *Cancers* **2021**, *13*, 1269. [\[CrossRef\]](#)
32. Corso, G.; Marrelli, D.; Pascale, V.; Vindigni, C.; Roviello, F. Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: Metanalysis and systematic review of the literature. *BMC Cancer* **2012**, *12*, 8. [\[CrossRef\]](#)
33. Saksens, N.T.; Krebs, M.P.; Schoenmaker-Koller, F.E.; Hicks, W.; Yu, M.; Shi, L.; Rowe, L.; Collin, G.B.; Charette, J.R.; Letteboer, S.J.; et al. Mutations in CTNNA1 cause butterfly-shaped pigment dystrophy and perturbed retinal pigment epithelium integrity. *Nat. Genet.* **2016**, *48*, 144–151. [\[CrossRef\]](#)
34. Tanner, A.; Chan, H.W.; Pulido, J.S.; Arno, G.; Ba-Abbad, R.; Jurkute, N.; Robson, A.G.; Egan, C.A.; Knight, H.; Calcagni, A.; et al. Clinical and Genetic Findings in CTNNA1-Associated Macular Pattern Dystrophy. *Ophthalmology* **2021**, *128*, 952–955. [\[CrossRef\]](#)
35. Kievit, A.; Tessadori, F.; Douben, H.; Jordens, I.; Maurice, M.; Hoogeboom, J.; Hennekam, R.; Nampoothiri, S.; Kayserili, H.; Castori, M.; et al. Variants in members of the cadherin-catenin complex, CDH1 and CTNND1, cause blepharocheilodontic syndrome. *Eur. J. Hum. Genet.* **2018**, *26*, 210–219. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Ghoumid, J.; Stichelboud, M.; Jourdain, A.S.; Frenois, F.; Lejeune-Dumoulin, S.; Alex-Cordier, M.P.; Lebrun, M.; Guerreschi, P.; Duquenois-Martinot, V.; Vinchon, M.; et al. Blepharocheilodontic syndrome is a CDH1 pathway-related disorder due to mutations in CDH1 and CTNND1. *Genet. Med.* **2017**, *19*, 1013–1021. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Cox, L.L.; Cox, T.C.; Moreno Uribe, L.M.; Zhu, Y.; Richter, C.T.; Nidey, N.; Standley, J.M.; Deng, M.; Blue, E.; Chong, J.X.; et al. Mutations in the Epithelial Cadherin-p120-Catenin Complex Cause Mendelian Non-Syndromic Cleft Lip with or without Cleft Palate. *Am. J. Hum. Genet.* **2018**, *102*, 1143–1157. [\[CrossRef\]](#)
38. Benusiglio, P.R. CDH1 germline mutations: Different syndromes, same management? *Genet. Med.* **2017**, *19*, 965–966. [\[CrossRef\]](#)
39. Keller, G.; Vogelsang, H.; Becker, I.; Plaschke, S.; Ott, K.; Suriano, G.; Mateus, A.R.; Seruca, R.; Biedermann, K.; Huntsman, D.; et al. Germline mutations of the E-cadherin(CDH1) and TP53 genes, rather than of RUNX3 and HPP1, contribute to genetic predisposition in German gastric cancer patients. *J. Med. Genet.* **2004**, *41*, e89. [\[CrossRef\]](#) [\[PubMed\]](#)
40. van der Post, R.S.; Vogelaar, I.P.; Manders, P.; van der Kolk, L.E.; Cats, A.; van Hest, L.P.; Sijmons, R.; Aalfs, C.M.; Ausems, M.G.; Gómez García, E.B.; et al. Accuracy of Hereditary Diffuse Gastric Cancer Testing Criteria and Outcomes in Patients With a Germline Mutation in CDH1. *Gastroenterology* **2015**, *149*, 897–906.e19. [\[CrossRef\]](#)
41. Machado, J.C.; Soares, P.; Carneiro, F.; Rocha, A.; Beck, S.; Blin, N.; Berx, G.; Sobrinho-Simões, M. E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas. *Lab. Invest.* **1999**, *79*, 459–465. [\[CrossRef\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Paper II

Contribution of multi-organ cancer predisposition genes for gastric cancer risk of different histological subtypes

Joana Guerra^{1,2,3*}, Ana Estrada-Florez^{4,5*}, Paul Lott^{4*}, Carla Pinto^{3,6}, Manuela Pinheiro¹, Pedro Pinto¹, Ana Peixoto^{1,3}, Catarina Santos^{1,3}, Ana Barbosa^{1,3}, João Silva^{1,3}, John Suarez-Olaya⁵, Fabian Castro⁵, Graciela Molina⁷, Alejandro Corvalán⁸, Patricio González⁹, Carol Parra¹⁰, Osvaldo Torres¹¹, Cedric Adelsdorfer¹², Jose Martínez¹³, Adriana Della Valle¹⁴, Esteban Castelao¹⁵, Javier Torres¹⁶, Nora Rios Sarabia¹⁶, Alfredo Medrano¹⁷, Magdalena Echeverry de Polanco⁵, Ana Lucia Rivera¹⁸, Rafeal Parra¹⁹, Alicia Cock-Rada^{20,21}, Mabel Bohorquez⁵, Carolina Sanabria^{18**}, Manuel R. Teixeira^{1,3,22,23**}, Luis Carvajal-Carmona^{4,24**}

1. Cancer Genetics Group, IPO-Porto Research Center (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center, 4200-072 Porto, Portugal.
2. Doctoral Programme in Biomedical Sciences, School Medicine and Biomedical Sciences, University of Porto (ICBAS-UP), 4050-313 Porto, Portugal.
3. Department of Laboratory Genetics, Portuguese Oncology Institute of Porto (IPO-Porto)/Porto Comprehensive Cancer Center, 4200-072 Porto, Portugal.
4. Genome Center and Department of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, USA.
5. Grupo de Citogenética, Filogenia y Evolución de Poblaciones, Facultades de Ciencias y Facultad de Ciencias de la Salud, Universidad del Tolima, Ibagué, Colombia.
6. Department of Pathological, Cytological and Thanatological Anatomy, School of Health, Polytechnic Institute of Porto, 4200-072 Porto, Portugal.
7. Escuela de Medicina, Facultad de Ciencias de la Salud, Universidad Autónoma de Chile, Providencia, Chile.
8. Departamento de Hematología y Oncología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile.
9. Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Paper II

10. Laboratorio de Investigación en Nutrición y Alimentos, Departamento de Salud, Comunidad y Gestión, Facultad de Ciencias de la Salud, Universidad de Playa Ancha, Valparaíso, Chile.
11. Servicio de Cirugía Digestiva, Hospital Clínico Regional de Concepción Dr. Guillermo Grant Benavente, Concepción, Chile.
12. Servicio de Cirugía, Hospital Dr. Gustavo Fricke de Viña del Mar, Viña del Mar, Chile.
13. Servicio de Cirugía, Hospital Dr. Eduardo Pereira Ramírez, Valparaíso, Chile.
14. Grupo Colaborativo Uruguayo: Investigación de Afecciones Oncológicas Hereditarias, Montevideo, Uruguay.
15. Complejo Hospitalario Universitario de Vigo, Vigo, Spain.
16. Unidad de Investigación en Enfermedades Infecciosas y Parasitarias, Unidad Médica de Alta Especialidad en Pediatría, Instituto Mexicano del Seguro Social, México City, México.
17. Dirección general, Unidad Médica de Alta Especialidad en Oncología Instituto Mexicano del Seguro Social (IMSS), México City, México.
18. Grupo de Investigación en Biología del Cáncer, Instituto Nacional de Cancerología, Bogotá, Colombia.
19. Departamento de Patología, Instituto Nacional de Cancerología, Bogotá, Colombia.
20. Instituto de Cancerología Las Américas, Medellín, Colombia.
21. Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia.
22. School of Medicine and Biomedical Sciences (ICBAS), University of Porto, Porto, Portugal.
23. European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS), Porto, Portugal.
24. Department of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, California.

* Contributed equally/Joint-first authors.

** Co-Senior authors

Abstract

Approximately 10% of gastric cancer (GC) cases exhibit familial clustering, yet only 1-3% can be explained by a known hereditary syndrome, being Hereditary Diffuse Gastric Cancer (HDGC) the most well-established predisposing disease for diffuse gastric cancer. Familial intestinal gastric cancer (FIGC) has been defined clinically, but it remains mostly genetically unexplained. Likewise, the heritability of mixed GC remains to be uncovered. Here, we evaluated the contribution of pathogenic and likely pathogenic (P/LP) variants in well-established and multi-organ cancer predisposition genes for GC risk in a large multi-center study involving 667 GC patients. We identified 39 patients (6%) with P/LP pathogenic variants in *ATM* (13 cases), *BRCA2* (nine cases), *MLH1* (five cases), *BRCA1*, *PALB2*, *TP53*, *RAD51D* and *CHEK2* (two patients each), and *RAD51C* and *PMS2* (one case each), all mutually exclusive. The P/LP variant prevalence was higher in intestinal (9.3%) than in diffuse (4.2%) or mixed GC (4.9%) (p -value=0.048), with no clear difference per mutated gene by histological subtypes. Family history of cancer was associated with higher mutation prevalence (p -value=0.041). Only sixteen of the 39 mutation carriers fulfilled the criteria for genetic testing of at least one cancer predisposition syndrome. Our findings indicate that multi-organ cancer predisposition genes should be included in gene panels used for investigating germline variants in GC patients, especially when there is family history of cancer but irrespective of histology subtype, as this would increase the chance of identifying patients that might benefit from prevention and targeted treatment strategies.

Introduction

Gastric cancer (GC) is the fifth most commonly diagnosed cancer and the fourth cause of cancer-related deaths worldwide (Sung et al. 2021). GC familial clustering occurs in approximately 10% of the cases; however, only 1-3% of GCs result from a known hereditary syndrome (Carneiro 2022). The most well-known inherited form of GC is Hereditary Diffuse Gastric Cancer (HDGC), an autosomal dominant syndrome associated with the presence of diffuse-type gastric carcinomas caused by germline variants in the *CDH1* or *CTNNA1* genes (Blair et al. 2020). Pathogenic variants in these two genes, however, only account for ~42% of HDGC families (Hansford et al. 2015; Lobo et al. 2021). On the other hand, familial intestinal gastric cancer (FIGC) has been clinically defined in families with an autosomal dominant inheritance pattern of the disease (Caldas et al. 1999), but the genetic cause predisposing to intestinal-type GC development has not yet been identified. Similarly, as mixed GC is typically not considered in the tumor spectrum of HDGC syndrome (Guerra et al. 2023), the heritability of families with mixed GC clustering remains to be uncovered.

In the past few years, we and others have identified GC families and patients with germline mutations in multi-tumor cancer genes such as *ATM*, *BRCA1*, *BRCA2*, and *PALB2*, suggesting that certain multi-organ cancer predisposition genes may be involved in GC predisposition (Sahasrabudhe et al. 2017; Fewings et al. 2018; Tedaldi et al. 2019). Further dissecting the genetics of GC is extremely important to provide suitable genetic counseling, surveillance, and/or risk reduction measures and ultimately prevent disease onset.

Here, we investigated the prevalence of pathogenic and likely pathogenic (P/LP) germline variants in the multi-organ cancer predisposition genes *ATM*, *BRCA2*, *BRCA1*, *PALB2*, *TP53*, *RAD51D*, *RAD51C*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, and *PMS2* in 667 early-onset and/or familial GC patients recruited in a multi-center study of several high-risk countries in Latin American and Europe.

Materials and methods

Study population

In our study, we included 667 index GC patients from three cohorts, which included cases from a multi-center Hispanic Gastric Cancer Genetics Collaborative Group

(HGCGCG), the Portuguese Oncology Institute of Porto (IPO-Porto) and the Hereditary Cancer Program of the Instituto Nacional de Cancerología (INC) in Colombia. HGCGCG includes patients, recruited from Chile, Colombia, Mexico, Spain, and Uruguay. The IPO-Porto and INC cohorts included patients referred for cancer genetic risk assessments at the largest cancer hospitals in Portugal and Colombia, respectively. The inclusion criteria for our study were: i) patients diagnosed with GC by age 50 years or ii) GC diagnosed after 50 years old and family history of cancer in first- and/or second-degree relatives. All diffuse GC patients were negative for *CDH1* and *CTNNA1* germline P/LP variants. This study included 334 cases with diffuse GC, 193 with intestinal GC, 103 with mixed GC, and 37 with GC of unknown histology. Patient clinical information is shown in Table 1.

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics committees from each collaborating institution in Colombia (University of Tolima, Hospital Federico Lleras Acosta, Hospital Hernando Moncaleano, and Instituto Nacional de Cancerología), Mexico (Instituto Mexicano del Seguro Social), Spain (Complejo Hospitalario Universitario de Vigo), Portugal (IPO-Porto), Chile (Universidad de Playa Ancha) and Uruguay (Grupo Colaborativo Uruguayo: Investigación de Afecciones Oncológicas Hereditarias).

Genomic DNA was extracted from peripheral blood samples according to standard protocols.

Table 1. Clinicopathological characteristics of the patients enrolled in the study, which included GC cases from Instituto Nacional de Cancerología in Colombia (INC), the Portuguese Oncology Institute of Porto (IPO-Porto), and the Hispanic Gastric Cancer Genetics Collaborative Group (HGCGCG).

	Cohort			All cohorts (%)
	INC (%)	IPO-Porto (%)	HGCGCG (%)	
Number of patients	70 (10.5)	258 (38.7)	339 (50.8)	667 (100)
Inclusion criteria				
Age at diagnosis $\leq$ 50 years	10 (14.3)	33 (12.8)	95 (28)	138 (20.7)
Age at diagnosis $\leq$ 50 years and with FH of cancer in first and/or second degree relatives	36 (51.4)	96 (37.2)	67 (19.8)	199 (29.8)
Age at diagnosis $>$ 50 years, and with FH of cancer in first and/or second degree relatives	24 (34.3)	129 (50)	177 (52.2)	330 (49.5)

Table 1 (continued)

	Cohort			All cohorts (%)
	INC (%)	IPO-Porto (%)	HGCGCG (%)	
Gender				
Female	46 (65.7)	133 (51.6)	151 (44.5)	330 (49.5)
Male	24 (34.3)	125 (48.4)	188 (55.5)	337 (50.5)
Age				
Average (SD)	46.5 (13.9)	50.5 (12.8)	53.6 (13.9)	51.7 (13.7)
Country				
Chile	-	-	33 (9.7)	33 (4.9)
Colombia	70 (100)	-	188 (55.5)	258 (38.7)
Mexico	-	-	82 (24.2)	82 (12.3)
Portugal	-	258 (100)	-	258 (38.7)
Spain	-	-	29 (8.6)	29 (4.3)
Uruguay	-	-	7 (2.1)	7 (1)
Family History (Any Cancer)				
No	10 (14.3)	32 (12.5)	78 (24.2)	120 (18.5)
Yes	60 (85.7)	225 (87.5)	244 (75.8)	529 (81.5)
No data	-	1	17	18
Family History Gastric cancer				
No	33 (47.1)	109 (42.4)	213 (66.1)	355 (54.7)
Yes	37 (52.9)	148 (57.6)	109 (33.9)	294 (45.3)
No data	-	1	17	18
Histology				
Diffuse	39 (60.9)	133 (51.6)	162 (52.6)	334 (53)
Intestinal	21 (32.8)	63 (24.4)	109 (35.4)	193 (30.6)
Mixed	4 (6.3)	62 (24)	37 (12)	103 (16.3)
Unknown	6	-	31	37
Stage				
I	7 (10.8)	67 (28)	47 (24.5)	121 (24.4)
II	15 (23.1)	35 (14.6)	50 (26)	100 (20.2)
III	20 (30.8)	74 (31)	72 (37.5)	166 (33.5)
IV	23 (35.4)	63 (26.4)	23 (12)	109 (22)
Unknown	5	19	147	171

FH: Family history

Targeted sequencing

Targeted sequencing was performed in 328 cases, 258 from IPO-Porto in Portugal and 70 from the Instituto Nacional de Cancerología in Colombia.

The Portuguese cohort was analyzed using the TruSight Cancer Panel (Illumina, Inc., USA), with library preparation performed according to the manufacturer's protocol, and 2x150 bp paired-end sequencing carried out in the MiSeq platform (Illumina, Inc.). Bioinformatic analyses were performed using a previously validated pipeline (Pinto et al. 2016).

The Instituto Nacional de Cancerología cohort was analyzed using the Canadian Consortia Inherited Cancer panel (Illumina, Inc.) with library preparation performed according to the manufacturer's protocol and 2x150 bp paired-end sequencing in the MiSeq platform (Illumina, Inc.). Variant calling and annotation were carried out with the SOPHiA DDM® platform using the ILL1IC1G3_TSC algorithm (Sophia Genetics, Saint-Sulpice, Switzerland).

In both cohorts, the following genes were analyzed: *ATM* (NM_000051.3), *BRCA2* (NM_000059.3), *BRCA1* (NM_007294.3), *PALB2* (NM_024675.3), *TP53* (NM_000546.5), *RAD51D* (NM_002878.3), *RAD51C* (NM_058216.2), *CHEK2* (NM_007194.3), *MLH1* (NM_000249.3), *MSH2* (NM_000251.2), *MSH6* (NM_000179.2), and *PMS2* (NM_000535.5). All variants with a variant allele frequency (VAF) $\leq 10\%$, minor allele frequency (MAF) $> 1\%$, with a frequency in our in-house database higher than 5%, and/or intronic variants at more than 12bp away from exon-intron boundaries, were excluded. For MAF filtering, data was obtained from the 1000 Genomes Project (1KGP), Genome Aggregation Database (gnomAD), and Exome Aggregation Consortium (ExAC) databases.

Whole exome sequencing

Whole exome sequencing (WES) was performed in the remaining 339 cases from Chile (n=33), Colombia (n=188), Mexico (n=82), Spain (n=29), and Uruguay (n=7) using the kit Agilent SureSelect Human All Exome V7 (Agilent Technologies, Santa Clara, CA, USA) and sequenced in a NovaSeq6000 (Illumina, Inc.) S4 150PE aiming 100x raw depth (~6Gb per sample). Alignment and variant calling were performed using Illumina Dragen v3.8. Variant calls were re-calibrated using GATK v4 variant recalibration. Variants were annotated with Illumina Nirvana and Annovar. Variants with a population MAF $> 5\%$ were excluded. Data from the same genes listed above were analyzed in these 339 samples.

Paper II

After variant filtering, all the variants that had been described as pathogenic/likely pathogenic in ClinVar or supported by literature evidence were retained as deleterious. Additionally, nonsense, frameshift, as well as canonical splice site variants, were also retained.

Tumor analysis

DNA extraction from formalin-fixed paraffin-embedded (FFPE) tumor samples belonging to P/LP germline variant carriers identified in this study was performed using the Cobas® DNA Sample Preparation Kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocol. DNA libraries were prepared using the Illumina DNA Prep with Enrichment protocol (Illumina, Inc.), with the TruSight Hereditary Cancer panel (Illumina, Inc.), according to the manufacturer's protocol, followed by 2x150 bp paired-end sequencing in the NextSeq 550 platform (Illumina, Inc.). Bioinformatic analyses were performed using a previously validated pipeline with a cut off VAF $\leq 5\%$ (Pinto et al. 2016). This analysis was performed with the objective of finding a second (somatic) hit in the genes mutated in the germline.

Statistical analysis

Statistical analysis was carried out with R (RStudio 2023.12.0). The statistical significance of association between different variables was performed using the Fisher's exact test or Pearson's Chi-squared test. Analysis of variance (ANOVA) was used for mean comparison between groups and the F-test statistic was used to assess statistical significance. P-values < 0.05 were considered statistically significant.

Results

Patients with pathogenic/likely pathogenic germline variants

We identified 39 patients (5.85%) with a P/LP germline variant in the *ATM*, *BRCA2*, *BRCA1*, *PALB2*, *TP53*, *RAD51D*, *RAD51C*, *CHEK2*, *MLH1*, or *PMS2* genes (Table 2), all mutually exclusive. *ATM* was the most frequently mutated gene, with 13 patients (1.95%) carrying P/LP variants. Six of these patients had diffuse GC, three presented mixed GC, and the remaining four had intestinal GC. These patients were diagnosed between the ages

of 32 and 61 years old and most of the patients (9 out of 13) had family history of GC. Two of the four patients without a family history of GC, had family history of other cancers, including breast and colon cancer. Nine patients (1.35%) were identified with a *BRCA2* P/LP variant; four were diagnosed with diffuse GC, one with mixed GC, two with intestinal GC, and two had GC of unknown histology. The age of diagnosis ranged between 44 and 81 years, four of them had family history of GC and five did not have GC family history, but had a family history of other cancer types, including breast and ovary cancer. Five patients (0.75%) presented *MLH1* pathogenic variants, two having diffuse GC, and three having intestinal GC. The age of diagnosis ranged between 38 and 55 years, and three of them had family history of GC. Two of the *MLH1* variant carriers did not have GC family history but presented history of colorectal cancer in a first and/or second-degree relatives. Two patients (0.30%) harbored pathogenic variants in the *PALB2* gene and were diagnosed with intestinal GC at 62 and 64 years respectively and both had GC family history (one of them also had family history of breast, colon, and pancreatic cancer). Two *BRCA1* pathogenic variants were identified in two patients (0.30%), one of them with a GC of diffuse histology at age 43 years and without cancer family history, and the other with a GC of unknown histology at 66 years old with GC family history. Two patients (0.30%) were carriers of *TP53* P/LP variants, both with intestinal GC (at 62 and 74 years old) and with GC family history, and one of them also presented family history of breast and brain cancer. We identified two LP variants in the *CHEK2* gene in two patients (0.30%) with intestinal GC at 55 and 64 years old, without GC family history but with family history of other cancers, including breast, prostate, and pancreatic cancer. One pathogenic variant in the *RAD51D* gene was identified in two patients (0.30%), one with diffuse GC at 57 years old without family history of GC but with family history of breast cancer, and the other with mixed GC at 45 years old with GC family history. One patient diagnosed with intestinal GC at 45 years old without family history of GC, but with family history of thyroid cancer, harbored a pathogenic *RAD51C* variant (0.15%). Lastly, one patient with intestinal GC diagnosed at 51 years old, without GC family history but with family history of pancreatic cancer, harbored one pathogenic variant in the *PMS2* gene (0.15%). Detailed clinical characteristics of the germline P/LP variant carriers are shown in supplementary table 1, and supplementary table 2 shows the details of P/LP variant carriers, stratified by gene. Figure 1 shows the landscape of the germline P/LP variants identified in this study, distributed by gender, age, family history of GC and any cancer, histology, dataset, and country of origin.

Genetic testing criteria of the pathogenic/likely pathogenic variant carriers

Out of these 39 patients with a P/LP variant, 22 presented GC family history and 17 had no GC family history. However, 14 of these latter 17 carriers without GC family history reported first and/or second-degree relatives with other cancers and only three of them had no cancer family history. Among the 39 patients with a P/LP variant, 11 out of the 14 carriers with diffuse GC fulfilled the HDGC criteria for genetic testing of the *CDH1/CTNNA1* genes and two of these 11 also fulfilled criteria for Lynch syndrome evaluation (both with a pathogenic variant in the *MLH1* gene). Out of the remaining three carriers with diffuse GC who did not meet HDGC criteria, one had criteria for Hereditary Breast and Ovarian Cancer (HBOC) syndrome genetic testing and presented a LP variant in the *BRCA2* gene. Of the remaining 25 carriers diagnosed with intestinal, mixed, or unknown GC, 21 did not fulfill the criteria for genetic testing of known predisposition cancer syndromes. Of the other four carriers, one *BRCA2* pathogenic variant carrier and one *CHEK2* LP variant carrier met the criteria for HBOC syndrome genetic testing, one *TP53* pathogenic variant carrier fulfilled the Chompret criteria for *TP53* genetic testing if applied to the family, and one *MLH1* pathogenic variant carrier met the criteria for Lynch syndrome evaluation. Overall, 23 carriers did not meet criteria for genetic testing for any known cancer predisposition syndrome.

Distribution of pathogenic/likely pathogenic variant carriers by inclusion criteria

Our study included patients with early onset (diagnosed up to the age of 50, with or without a family history of cancer) and patients with late-onset (diagnosed after the age of 50) and with a family history of any cancer. We found that the prevalence of P/LP variants in patients with early-onset GC with and without family history of cancer was 7% (14/199) and 2% (3/138), respectively (p -value=0.073). In patients with GC diagnosed after the age of 50 with family history of cancer, the prevalence of P/LP variants was 7% (22/330), which was not statistically different compared with the other two groups (p -value=0.117). The difference in P/LP variant frequency in carriers diagnosed before and after 50 years of age was also not significant (17/337 vs 22/330 carriers; p -value=0.412), but the difference between the group with and without family history of cancer was statistically significant (36/529 vs 3/138 carriers; p -value=0.041).

Tumor histology, age, and gender distribution in pathogenic/likely pathogenic variant carriers

When stratified by tumor histology, we found a P/LP variant prevalence of 9.3% (18/193), 4.9% (5/103), and 4.2% (14/334) among patients with intestinal, mixed, and diffuse tumors, respectively (p -value=0.048). The average age of diagnosis in these 39 P/LP variant carriers was 54 years (range: 32 to 81y), with no significant difference observed between patients with diffuse GC (52 years), mixed tumors (54 years), and intestinal tumors (56 years) (p -value=0.586). The average age of diagnosis among non-carriers was 52 years old, with no significant differences noted when compared with carriers overall (p -value=0.222) and across tumor types, namely, 47 years old for diffuse GC (p -value=0.171), 55 years old for mixed GC (p -value=0.7772), and 57 years old for intestinal GC (p -value=0.810). We found a P/LP variant in more women than men (21/330 female vs 18/337 male carriers) and the age of onset was younger in female P/LP variant carriers (53 years in women vs. 56 years in men), but the differences were not statistically significant. The comparison of female carriers and female non-carriers revealed average ages of diagnosis of 53 and 50 years old, respectively (p -value=0.281). Similarly, male carriers and male non-carriers had average ages of diagnosis of 56 and 53 years old, respectively (p -value=0.465).

Country of origin and cohort distribution in pathogenic/likely pathogenic variant carriers

When stratified by country of origin, the P/LP variant prevalence was 7.3% in Mexico, 6.1% in Chile, 5.8% in Portugal, 5.8% in Colombia, and 3.4% in Spain. We did not detect P/LP variants in Uruguay, likely due to the small sample size ($n=7$). Furthermore, when stratifying by cohort/sequencing method, the P/LP variant prevalence in the INC Colombia cohort (targeted sequencing approach) was 10% (7/70), the IPO-Porto cohort (targeted sequencing approach) was 5.8% (15/258), and HGCGCG cohort (WES approach) was 5.0% (17/339). Supplementary table 2 shows details of P/LP variant carriers, stratified by cohort.

Tumor analysis of gastric carcinomas of P/LP variant carriers

We performed targeted sequencing of eight available gastric carcinomas from carriers of pathogenic germline variants in *ATM* (five cases), *PALB2* (one case), and *RAD51D* (two cases) genes in order to search for the second hit mechanism. All germline

Paper II

variants were detected by the NGS-based tumor test and the variant allele frequency (VAF) ranged between 27% and 79% (Supplementary table 4). The tumor with the *PALB2* germline variant presented a VAF of 79%, which is suggestive of a biallelic inactivation. The VAF of the other germline variants were not suggestive of loss of heterozygosity, nor did we identify a somatic pathogenic variant indicative of a second hit.

Table 2. Clinical characteristics of the germline pathogenic variant carriers

Gene	HGVS coding	HGVS protein	Effect	Dataset/Country	Sample ID	Gender	Age of DX	Histology	FH of GC
ATM	NM_000051.4:c.640del	NP_000042.3:p.(Ser214ProfsTer16)	Frameshift Deletion	IPO-Porto/Portugal	EI_1166	F	57	Diffuse	N
	NM_000051.4:c.1235+1G>A	NP_000042.3:p.?	Splice site	INC/Colombia	EI_1005	F	44	Diffuse	Y
	NM_000051.4:c.2250G>A	NP_000042.3:p.?	Splice site	INC/Colombia	EI_1048	F	35	Diffuse	N
	NM_000051.4:c.3171dup	NP_000042.3:p.(Trp1058MetfsTer5)	Frameshift Insertion	HGCGCG/Colombia	I_27122	F	49	Diffuse	Y
	NM_000051.4:c.3381_3384del	NP_000042.3:p.(Gln1128MetfsTer5)	Frameshift Deletion	HGCGCG/Chile	I_27254	M	40	Diffuse	Y
	NM_000051.4: c.3802del	NP_000042.3:p.(Val1268Ter)	Nonsense	IPO-Porto/Portugal	EI_1110	F	32	Intestinal	N
	NM_000051.4:c.4110-1G>C	NP_000042.3:p.?	Splice site	HGCGCG/Colombia	I_27102	M	56	Intestinal	Y
	NM_000051.4:c.4852C>T	NP_000042.3:p.(Arg1618Ter)	Nonsense	IPO-Porto/Portugal	EI_1104	F	62	Intestinal	Y
	NM_000051.4:c.5497-2A>C	NP_000042.3:p.?	Splice site	HGCGCG/Mexico	I_26254	M	42	Intestinal	Y
	NM_000051.4:c.7681_7682del	NP_000042.3:p.(Leu2561GlyfsTer9)	Frameshift Deletion	HGCGCG/Colombia	I_9149	F	50	Mixed	N
	NM_000051.4: c.8851-1G>C	NP_000042.3:p.?	Splice site	IPO-Porto/Portugal	EI_1083	F	61	Mixed	Y
	NM_000051.4:c.9079dup	NP_000042.3:p.(Ser3027LysfsTer36)	Frameshift Insertion	IPO-Porto/Portugal	EI_1077	M	54	Mixed	Y
	NM_000051.4:c.9079dup	NP_000042.3:p.(Ser3027LysfsTer36)	Frameshift Insertion	IPO-Porto/Portugal	EI_1143	M	60	Diffuse	Y
BRCA2	NM_000059.4: c.156_157insAlu	NP_000050.3:p.?	Frameshift Insertion	IPO-Porto/Portugal	EI_1080	M	60	Mixed	Y
	NM_000059.4:c.2979G>A	NP_000050.3:p.(Trp993Ter)	Nonsense	INC/Colombia	EI_1061	M	80	Intestinal	Y

Paper II

Table 2 (continued)

Gene	HGVS coding	HGVS protein	Effect	Dataset/Country	Sample ID	Gender	Age of DX	Histology	FH of GC
BRCA2	NM_000059.4:c.3264dup	NP_000050.3:p.(Gln1089SerfsTer10)	Frameshift Insertion	HGCGCG/Mexico	I_2714	F	81	Intestinal	N
	NM_000059.4:c.3860del	NP_000050.3:p.(Asn1287IlefsTer6)	Frameshift Deletion	HGCGCG/Spain	I_26895	F	55	Intestinal	Y
	NM_000059.4:c.4329dup	NP_000050.3:p.(Asn1444Ter)	Frameshift Insertion	HGCGCG/Colombia	I_27118	F	75	Diffuse	Y
	NM_000059.3:c.4740_4741dup	NP_000050.3:p.(Glu1581ValfsTer37)	Frameshift Insertion	HGCGCG/Chile	I_26873	M	44	Unknown	N
	NM_000059.4: c.7258del	NP_000050.3:p.(Glu2420AsnfsTer49)	Frameshift Deletion	IPO-Porto/Portugal	EI_1139	F	76	Diffuse	Y
	NM_000059.4:c.8168A>G	NP_000050.3:p.(Asp2723Gly)	Missense	HGCGCG/Mexico	I_2757	F	45	Diffuse	N
	NM_000059.4:c.9382C>T	NP_000050.3:p.(Arg3128Ter)	Nonsense	IPO-Porto/Portugal	EI_1137	M	40	Diffuse	N
MLH1	NM_000249.4:c.213_215del	NP_000240.1:p.(Glu71del)	Frameshift Deletion	INC/Colombia	EI_1055	F	38	Intestinal	Y
	NM_000249.4:c.676C>T	NP_000240.1:p.(Arg226Ter)	Nonsense	HGCGCG/Mexico	I_26265	M	48	Diffuse	N
	NM_000249.4:c.790+1G>A	NP_000240.1:p.?	Splice site	INC/Colombia	EI_1058	M	46	Intestinal	N
	NM_000249.4:c.790+1G>A	NP_000240.1:p.?	Splice site	INC/Colombia	EI_1047	M	55	Diffuse	Y
	NM_000249.4:c.1731G>A	NP_000240.1:p.?	Splice site	HGCGCG/Mexico	I_26197	F	46	Intestinal	Y
PALB2	NM_024675.4:c.1240C>T	NP_078951.2:p.(Arg414Ter)	Nonsense	IPO-Porto/Portugal	EI_1109	F	62	Intestinal	Y
	NM_024675.4:c.2288_2291del	NP_078951.2:p.(Leu763Ter)	Nonsense	HGCGCG/Colombia	I_12495	M	64	Intestinal	Y
TP53	NM_000546.6:c.809T>C	NP_000537.3:p.(Phe270Ser)	Missense	HGCGCG/Mexico	I_26633	M	74	Intestinal	Y
	NM_000546.5: c.1010G>A	NP_000537.3:p.(Arg337His)	Missense	IPO-Porto/Portugal	EI_1106	M	62	Intestinal	Y

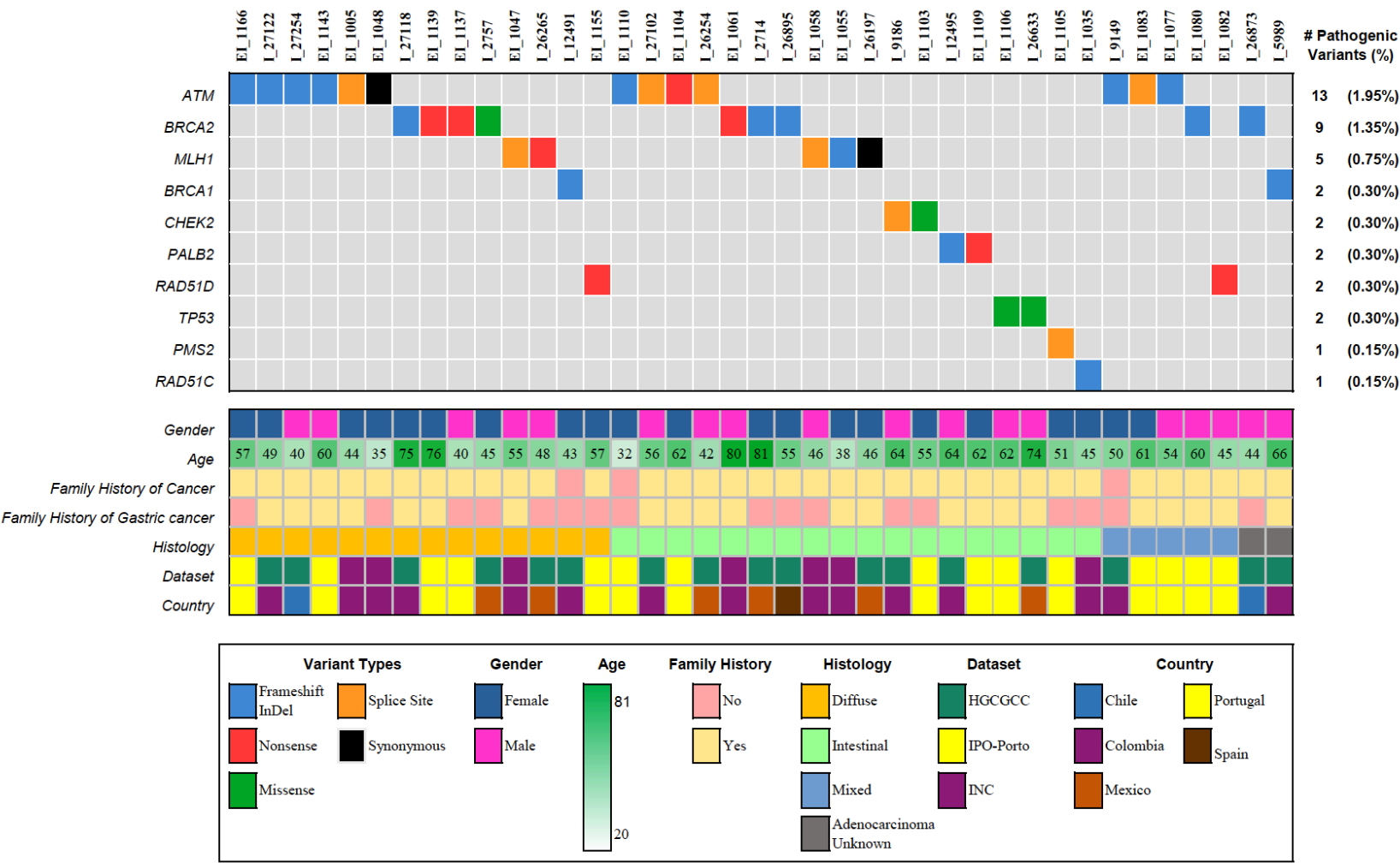
Table 2 (continued)

Gene	HGVS coding	HGVS protein	Effect	Dataset/Country	Sample ID	Gender	Age of DX	Histology	FH of GC
RAD51D	NM_002878.4:c.694C>T	NP_002869.3:p.(Arg232Ter)	Nonsense	IPO-Porto/Portugal	EI_1082	M	45	Mixed	Y
	NM_002878.4:c.694C>T	NP_002869.3:p.(Arg232Ter)	Nonsense	IPO-Porto/Portugal	EI_1155	F	57	Diffuse	N
BRCA1	NM_007294.4:c.1674del	NP_009225.1:p.(Gly559ValfsTer13)	Frameshift Deletion	HGCGCG/Colombia	I_5989	M	66	Unknown	Y
	NM_007294.4:c.3331_3334del	NP_009225.1:p.(Gln1111AsnfsTer5)	Frameshift Deletion	HGCGCG/Colombia	I_12491	F	43	Diffuse	N
CHEK2	NM_007194.4:c.349A>G	NP_009125.1:p.(Arg117Gly)	Missense	IPO-Porto/Portugal	EI_1103	F	55	Intestinal	N
	NM_007194.4:c.846+1G>C	NP_009125.1:p.?	Splice site	HGCGCG/Colombia	I_9186	M	64	Intestinal	N
RAD51C	NM_058216.3:c.561_562del	NP_478123.1:p.(His187GlnfsTer15)	Frameshift Deletion	INC/Colombia	EI_1035	F	45	Intestinal	N
PMS2	NM_000535.7:c.803+2T>G	NP_000526.2:p.?	Splice site	IPO-Porto/Portugal	EI_1105	F	51	Intestinal	N

HGVS: Human Genome Variation Society; DX: Diagnosis; FH: Family history; GC: Gastric cancer; F: Female; M: Male; Y: Yes; N: No

Paper II

Figure 1 – Germline pathogenic/likely pathogenic variants landscape identified in gastric cancer index patients.



Discussion

This study aimed to evaluate the contribution of P/LP variants in known multi-tumor, moderate-high penetrance, cancer predisposition genes for the risk of GC, regardless of the histological subtype. Overall, 39 index patients out of 667 (6%) carried P/LP variants in known cancer predisposition genes (*ATM*, *BRCA1*, *BRCA2*, *PALB2*, *TP53*, *RAD51D*, *RAD51C*, *CHEK2*, *MLH1*, and *PMS2* genes) (Table 2, Figure 1). All these genes play a crucial role in DNA damage repair. Namely, homologous recombination genes have been associated with GC predisposition either in selected (Sahasrabudhe et al. 2017; Fewings et al. 2018; Zhang et al. 2021; Tedaldi et al. 2019; Liu et al. 2022; Calvello et al. 2023) and unselected GC cohorts (Usui et al. 2023; Uson et al. 2022; Slavin et al. 2017; Lu et al. 2015; Zhang et al. 2023). The prevalence of P/LP variants in these cancer predisposition genes in selected studies reported in the literature, in which all histological types were analyzed, ranges between 3% to 11% (Sahasrabudhe et al. 2017; Calvello et al. 2023; Tedaldi et al. 2019; Zhang et al. 2021). Here we report a prevalence of 6%, which is in accordance with the prevalence previously reported. Note that in our cohort, patients with germline P/LP variants display heterogeneous GC histology, including intestinal, mixed, and diffuse GC, which also agrees with previous studies (Sahasrabudhe et al. 2017; Calvello et al. 2023; Tedaldi et al. 2019). When stratified by tumor histology, the prevalence of P/LP variant was higher in carriers diagnosed with intestinal GC (9.3%), compared to carriers diagnosed with diffuse and mixed GC (4.2% and 4.9%, respectively). These differences could be attributed to the lack of studies on intestinal GC, which remains largely genetically unexplained to date. Additionally, the testing criteria for this histological type are not very specific or well-defined (Carvalho et al. 2021). Therefore, when we studied these cases using a panel of known cancer predisposition genes, the probability of identifying a P/LP variant that could explain this type of tumor in affected families is significantly increased. The P/LP variant prevalence was higher in the group of patients with family history regardless of diagnosis age (7% in both) than in the group of patients without family history (2%). Among the 39 patients with a P/LP variant, 23 did not fulfill genetic testing criteria for any known cancer predisposition syndrome and 16 met criteria for genetic testing for specific hereditary cancer syndromes, such as HDGC, Lynch, HBOC or Li-Fraumeni syndromes. However, of the patients who fulfilled criteria for genetic testing for known cancer predisposition syndromes, only five would have been correctly identified if a single gene analysis had been performed. Therefore, regardless of GC histology type, we recommend testing index GC

Paper II

patients with family history of cancer, irrespective of the age of diagnosis, using a panel of known cancer predisposition genes.

The average age of diagnosis of the pathogenic variant carriers was 54 years old, which is very similar to the average age of diagnosis of non-carriers (52 years old). These similarities can be explained by the moderate penetrance of the genes studied, which lead to a later onset of the disease compared to established syndromes like HDGC, where the average age of diagnosis is 40 years old (Garcia-Pelaez et al. 2023; Roberts et al. 2019). This difference regarding the age at diagnosis has been reported before in the literature. Those with *CDH1* pathogenic variants tended to be diagnosed at a younger age compared to non-carriers, whereas the age at diagnosis for individuals with a pathogenic variant in the other known cancer predisposition genes was similar to that of non-carriers in the literature (Usui et al. 2023; Calvello et al. 2023). Notably, our observations are in accordance with a previous study indicating that individuals from FIGC families develop intestinal GC at a higher average age compared to those with HDGC (Carvalho et al. 2021). Moreover, our cohort was selected based on early-onset GC and/or family history, so the families negative for pathogenic variants in genes studied can have a predisposition genetic cause not yet identified. We noticed a slight difference in the age of onset between female and male P/LP variant carriers (53 years in women vs. 56 years in men), along with variations in carrier numbers (21 female vs 18 male). Curiously, these differences are not observed in other known cancer predisposition syndromes, such as HDGC and Lynch syndrome, where GC risk is typically higher in men (Hansford et al. 2015; Kim et al. 2020). Although the difference was not statistically different, our finding of a potentially increased risk of GC for female carriers of P/LP variants needs further study.

We analyzed eight tumors from carriers of pathogenic germline variants in *ATM*, *PALB2*, and *RAD51D* genes in order to search for the second hit mechanism. Only the tumor with the *PALB2* variant presented a VAF of 79%, which is suggestive loss of heterozygosity and biallelic inactivation. These results suggest that a haploinsufficiency mechanism may be operative in gastric tumorigenesis in several of the patients with germline P/LP variants in these multi-organ cancer predisposition genes. In fact, several studies showed no evidence of a second hit mechanism in *ATM* and *RAD51D*-mutated tumors, suggesting that the effect of the pathogenic variants in these genes is possibly mediated by haploinsufficiency (Yap et al. 2022; Renault et al. 2018; Borja et al. 2023; Lim et al. 2023).

A limitation of this study is the lack of data regarding *Helicobacter pylori* infection, so we cannot assess the association between germline pathogenic variants in homologous

recombination genes and *Helicobacter pylori* infection on GC risk that emerged recently (Usui et al. 2023).

Nevertheless, this study is a multi-center cohort with a large case series, which shows that known multi-tumor, high-moderate penetrance cancer predisposition genes explain a significant proportion of GC families, regardless of the histological type. The potential association of homologous recombination genes with GC predisposition may be clinically relevant, not only for cancer prevention but also because these patients may benefit of targeted therapies, such as PARP inhibitors (Bang et al. 2015; Wang et al. 2021; Lin et al. 2018). Likewise, patients with pathogenic germline variants in DNA mismatch repair (MMR) genes can also benefit from PD-1/PD-L1 inhibitors since these tumors are microsatellite instability (MSI)-high (Lordick et al. 2022). In conclusion, our results show that, in addition to *CDH1* and *CTNNA1* testing in families fulfilling the HDGC criteria, multi-organ cancer predisposition genes should be included in gene panels used for investigating germline variants in GC patients, especially when there is family history of cancer and irrespective of histology subtype, as there is often significant phenotypic overlap between various cancer predisposition syndromes.

Supplementary material

Supplementary table 1 – Detailed clinical characteristics of the germline pathogenic/likely pathogenic variant carriers.

Supplementary table 2 - Details of pathogenic/likely pathogenic variant carriers, stratified by gene.

Supplementary table 3 - Details of pathogenic/likely pathogenic variant carriers, stratified by cohort.

Supplementary table 4 - Pathogenic germline variants detected in the tumor samples.

Acknowledgments

We are indebted to all patients who participated in the study. We are grateful for the financial support from Universidad del Tolima, Colombia (Projects 160120516, 470115, 30113, 350113, 160114, 450110, 40218, 250120; contract 398-2017; grant 001-2019, M.E. Bohorquez-Lozano and M. Polanco-Echeverry); MINCIENCIAS, Colombia (grant

Paper II

110565843382; contract 204-2015, M.E. Bohorquez-Lozano; Graduate Studentships 647/2014, A.P. Estrada-Florez); L'OREAL-UNESCO-ICETEX-COLCIENCIAS, Colombia (project 3900917/2017, A.P. Estrada-Florez); Instituto Mexicano del Seguro Social and Consejo Nacional de Ciencia y Tecnología, México (FIS/IMSS/PROT/PRI0/13/027 and Fronteras de la Ciencia 2015-01-773, J. Torres); AACR (Fellowship 21-40-69-ESTR, A.P. Estrada-Florez); The Auburn Community Cancer Endowed Chair in Basic Research, U.S.(L.G. Carvajal-Carmona); the Heart, BrEast, and BrAin HeaLth Equity Research (HEAL-HER) Program, U.S. (L.G. Carvajal-Carmona) and the U.S. NCI of the NIH (grants R01CA223978, R21CA199631, U54CA233306, and P30CA093373, L.G. Carvajal-Carmona). Joana Guerra is a research fellow of the Fundação para a Ciência e Tecnologia (SFRH/BD/138670/2018). Manuela Pinheiro is a research fellow of the Liga Portuguesa Contra o Cancro. We acknowledge the support of the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS).

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Author Contributions

JG, AEF, PL, MRT, and LCC conceptualized and designed the study. MRT, LCC, CS, JT, and MB acquired funding. PL, DM, and KC curated data and developed the pipeline for WES. JG, PP, CP, and CS performed targeted sequencing. JG and AEF performed validation of the variants. JG, PP, AEF, and PL completed the data analysis. All authors reviewed and interpreted the data. JG and AEF drafted the article and all authors reviewed and edited the article. MB, JT, CS, MRT, and LCC supervised the study. JSO, FC, and ALR processed samples. MRT, JS, CS, MB, JT, RPM, EC, ADV, RD, ACR, GM, AC, NRS, AM, collected samples and data. All authors had final responsibility for the decision to submit for publication.

References

Bang, Y. J., S. A. Im, K. W. Lee, J. Y. Cho, E. K. Song, K. H. Lee, Y. H. Kim, J. O. Park, H. G. Chun, D. Y. Zang, A. Fielding, J. Rowbottom, D. Hodgson, M. J. O'Connor, X. Yin, and W. H. Kim. 2015. 'Randomized, Double-Blind Phase II Trial With Prospective Classification by ATM Protein Level to Evaluate the Efficacy and Tolerability of

- Olaparib Plus Paclitaxel in Patients With Recurrent or Metastatic Gastric Cancer', *J Clin Oncol*, 33: 3858-65.
- Blair, V. R., M. McLeod, F. Carneiro, D. G. Coit, J. L. D'Addario, J. M. van Dieren, K. L. Harris, N. Hoogerbrugge, C. Oliveira, R. S. van der Post, J. Arnold, P. R. Benusiglio, T. M. Bisseling, A. Boussioutas, A. Cats, A. Charlton, K. E. C. Schreiber, J. L. Davis, M. D. Pietro, R. C. Fitzgerald, J. M. Ford, K. Gamet, I. Gullo, R. H. Hardwick, D. G. Huntsman, P. Kaurah, S. S. Kupfer, A. Latchford, P. F. Mansfield, T. Nakajima, S. Parry, J. Rossaak, H. Sugimura, M. Svrcek, M. Tischkowitz, T. Ushijima, H. Yamada, H. K. Yang, A. Claydon, J. Figueiredo, K. Paringatai, R. Seruca, N. Bougen-Zhukov, T. Brew, S. Busija, P. Carneiro, L. DeGregorio, H. Fisher, E. Gardner, T. D. Godwin, K. N. Holm, B. Humar, C. J. Lintott, E. C. Monroe, M. D. Muller, E. Norero, Y. Nouri, J. Paredes, J. M. Sanches, E. Schulpen, A. S. Ribeiro, A. Sporle, J. Whitworth, L. Zhang, A. E. Reeve, and P. Guilford. 2020. 'Hereditary diffuse gastric cancer: updated clinical practice guidelines', *Lancet Oncol*, 21: e386-e97.
- Borja, Nicholas A., Rachel Silva-Smith, Marilyn Huang, Dipen J. Parekh, Daniel Sussman, and Mustafa Tekin. 2023. 'Atypical ATMs: Broadening the phenotypic spectrum of ATM-associated hereditary cancer', *Frontiers in Oncology*, 13.
- Caldas, C., F. Carneiro, H. T. Lynch, J. Yokota, G. L. Wiesner, S. M. Powell, F. R. Lewis, D. G. Huntsman, P. D. Pharoah, J. A. Jankowski, P. MacLeod, H. Vogelsang, G. Keller, K. G. Park, F. M. Richards, E. R. Maher, S. A. Gayther, C. Oliveira, N. Grehan, D. Wight, R. Seruca, F. Roviello, B. A. Ponder, and C. E. Jackson. 1999. 'Familial gastric cancer: overview and guidelines for management', *J Med Genet*, 36: 873-80.
- Calvello, M., M. Marabelli, S. Gandini, E. Marino, L. Bernard, M. Dal Molin, G. Di Cola, C. Zanzottera, G. Corso, N. Fazio, L. Gervaso, U. Fumagalli Romario, M. Barberis, A. Guerrieri-Gonzaga, L. Bertario, D. Serrano, and B. Bonanni. 2023. 'Hereditary Gastric Cancer: Single-Gene or Multigene Panel Testing? A Mono-Institutional Experience', *Genes (Basel)*, 14.
- Carneiro, F. 2022. 'Familial and hereditary gastric cancer, an overview', *Best Pract Res Clin Gastroenterol*, 58-59: 101800.
- Carvalho, J., P. Oliveira, J. Senz, C. São José, S. Hansford, S. P. Teles, M. Ferreira, G. Corso, H. Pinheiro, D. Lemos, V. Pascale, F. Roviello, D. Huntsman, and C. Oliveira. 2021. 'Redefinition of familial intestinal gastric cancer: clinical and genetic perspectives', *J Med Genet*, 58: 1-11.
- Fewings, E., A. Larionov, J. Redman, M. A. Goldgraben, J. Scarth, S. Richardson, C. Brewer, R. Davidson, I. Ellis, D. G. Evans, D. Halliday, L. Izatt, P. Marks, V. McConnell, L. Verbist, R. Mayes, G. R. Clark, J. Hadfield, S. F. Chin, M. R. Teixeira, O. T. Giger, R. Hardwick, M. di Pietro, M. O'Donovan, P. Pharoah, C. Caldas, R. C. Fitzgerald, and M. Tischkowitz. 2018. 'Germline pathogenic variants in PALB2 and other cancer-predisposing genes in families with hereditary diffuse gastric cancer without CDH1 mutation: a whole-exome sequencing study', *Lancet Gastroenterol Hepatol*, 3: 489-98.
- Garcia-Pelaez, J., R. Barbosa-Matos, S. Lobo, A. Dias, L. Garrido, S. Castedo, S. Sousa, H. Pinheiro, L. Sousa, R. Monteiro, J. J. Maqueda, S. Fernandes, F. Carneiro, N. Pinto, C. Lemos, C. Pinto, M. R. Teixeira, S. Aretz, S. Bajalica-Lagercrantz, J. Balmaña, A. Blatnik, P. R. Benusiglio, M. Blanluet, V. Bours, H. Brems, J. Brunet, D. Calistri, G.

- Capellá, S. Carrera, C. Colas, K. Dahan, R. de Putter, C. Desseignés, E. Domínguez-Garrido, C. Egas, D. G. Evans, D. Feret, E. Fewings, R. C. Fitzgerald, F. Coulet, M. Garcia-Barcina, M. Genuardi, L. Golmard, K. Hackmann, H. Hanson, E. Holinski-Feder, R. Hüneburg, M. Krajc, K. Lagerstedt-Robinson, C. Lázaro, M. J. L. Ligtenberg, C. Martínez-Bouzas, S. Merino, G. Michils, S. Novaković, A. Patiño-García, G. N. Ranzani, E. Schröck, I. Silva, C. Silveira, J. L. Soto, I. Spier, V. Steinke-Lange, G. Tedaldi, M. I. Tejada, E. R. Woodward, M. Tischkowitz, N. Hoogerbrugge, and C. Oliveira. 2023. 'Genotype-first approach to identify associations between CDH1 germline variants and cancer phenotypes: a multicentre study by the European Reference Network on Genetic Tumour Risk Syndromes', *Lancet Oncol*, 24: 91-106.
- Guerra, J., C. Pinto, P. Pinto, M. Pinheiro, C. Santos, A. Peixoto, C. Escudeiro, A. Barbosa, M. Porto, I. Francisco, P. Lopes, A. R. Isidoro, A. L. Cunha, C. Albuquerque, I. Claro, C. Oliveira, J. Silva, and M. R. Teixeira. 2023. 'Frequency of CDH1, CTNNA1 and CTNND1 Germline Variants in Families with Diffuse and Mixed Gastric Cancer', *Cancers (Basel)*, 15.
- Hansford, S., P. Kaurah, H. Li-Chang, M. Woo, J. Senz, H. Pinheiro, K. A. Schrader, D. F. Schaeffer, K. Shumansky, G. Zogopoulos, T. A. Santos, I. Claro, J. Carvalho, C. Nielsen, S. Padilla, A. Lum, A. Talhouk, K. Baker-Lange, S. Richardson, I. Lewis, N. M. Lindor, E. Pennell, A. MacMillan, B. Fernandez, G. Keller, H. Lynch, S. P. Shah, P. Guilford, S. Gallinger, G. Corso, F. Roviello, C. Caldas, C. Oliveira, P. D. Pharoah, and D. G. Huntsman. 2015. 'Hereditary Diffuse Gastric Cancer Syndrome: CDH1 Mutations and Beyond', *JAMA Oncol*, 1: 23-32.
- Kim, J., D. Braun, C. Ukaegbu, T. G. Dhingra, F. Kastrinos, G. Parmigiani, S. Syngal, and M. B. Yurgelun. 2020. 'Clinical Factors Associated With Gastric Cancer in Individuals With Lynch Syndrome', *Clin Gastroenterol Hepatol*, 18: 830-37.e1.
- Lim, B. W. X., N. Li, S. Mahale, S. McInerney, M. Zethoven, S. M. Rowley, J. Huynh, T. Wang, J. E. A. Lee, M. Friedman, L. Devereux, R. J. Scott, E. K. Sloan, P. A. James, and I. G. Campbell. 2023. 'Somatic inactivation of breast cancer predisposition genes in tumors associated with pathogenic germline variants', *J Natl Cancer Inst*, 115: 181-89.
- Lin, Xiaoting, Dongshao Chen, Cheng Zhang, Xiaotian Zhang, Zhongwu Li, Bin Dong, Jing Gao, and Lin Shen. 2018. 'Augmented antitumor activity by olaparib plus AZD1775 in gastric cancer through disrupting DNA damage repair pathways and DNA damage checkpoint', *Journal of Experimental & Clinical Cancer Research*, 37: 129.
- Liu, Z. X., X. L. Zhang, Q. Zhao, Y. Chen, H. Sheng, C. Y. He, Y. T. Sun, M. Y. Lai, M. Q. Wu, Z. X. Zuo, W. Wang, Z. W. Zhou, F. H. Wang, Y. H. Li, R. H. Xu, and M. Z. Qiu. 2022. 'Whole-Exome Sequencing Among Chinese Patients With Hereditary Diffuse Gastric Cancer', *JAMA Netw Open*, 5: e2245836.
- Lobo, S., P. R. Benusiglio, F. Coulet, L. Boussemart, L. Golmard, I. Spier, R. Hüneburg, S. Aretz, C. Colas, and C. Oliveira. 2021. 'Cancer predisposition and germline CTNNA1 variants', *Eur J Med Genet*, 64: 104316.
- Lordick, F., F. Carneiro, S. Cascinu, T. Fleitas, K. Haustermans, G. Piessen, A. Vogel, and E. C. Smyth. 2022. 'Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up', *Ann Oncol*, 33: 1005-20.

- Lu, Charles, Mingchao Xie, Michael C. Wendl, Jiayin Wang, Michael D. McLellan, Mark D. M. Leiserson, Kuan-lin Huang, Matthew A. Wyczalkowski, Reyka Jayasinghe, Tapahsama Banerjee, Jie Ning, Piyush Tripathi, Qunyu Zhang, Beifang Niu, Kai Ye, Heather K. Schmidt, Robert S. Fulton, Joshua F. McMichael, Prag Batra, Cyriac Kandoth, Maheetha Bharadwaj, Daniel C. Koboldt, Christopher A. Miller, Krishna L. Kanchi, James M. Eldred, David E. Larson, John S. Welch, Ming You, Bradley A. Ozenberger, Ramaswamy Govindan, Matthew J. Walter, Matthew J. Ellis, Elaine R. Mardis, Timothy A. Graubert, John F. Dpersio, Timothy J. Ley, Richard K. Wilson, Paul J. Goodfellow, Benjamin J. Raphael, Feng Chen, Kimberly J. Johnson, Jeffrey D. Parvin, and Li Ding. 2015. 'Patterns and functional implications of rare germline variants across 12 cancer types', *Nature Communications*, 6: 10086.
- Pinto, P., P. Paulo, C. Santos, P. Rocha, C. Pinto, I. Veiga, M. Pinheiro, A. Peixoto, and M. R. Teixeira. 2016. 'Implementation of next-generation sequencing for molecular diagnosis of hereditary breast and ovarian cancer highlights its genetic heterogeneity', *Breast Cancer Res Treat*, 159: 245-56.
- Renault, A. L., N. Mebirouk, L. Fuhrmann, G. Bataillon, E. Cavaciuti, D. Le Gal, E. Girard, T. Popova, P. La Rosa, J. Beauvallet, S. Eon-Marchais, M. G. Dondon, C. D. d'Enghien, A. Laugé, W. Chemlali, V. Raynal, M. Labbé, I. Bièche, S. Baulande, J. O. Bay, P. Berthet, O. Caron, B. Buecher, L. Faivre, M. Fresnay, M. Gauthier-Villars, P. Gesta, N. Janin, S. Lejeune, C. Maugard, S. Moutton, L. Venat-Bouvet, H. Zattara, J. P. Fricker, L. Gladiéff, I. Coupier, G. Chenevix-Trench, J. Hall, A. Vincent-Salomon, D. Stoppa-Lyonnet, N. Andrieu, and F. Lesueur. 2018. 'Morphology and genomic hallmarks of breast tumours developed by ATM deleterious variant carriers', *Breast Cancer Res*, 20: 28.
- Roberts, M. E., J. M. O. Ranola, M. L. Marshall, L. R. Susswein, S. Graceffo, K. Bohnert, G. Tsai, R. T. Klein, K. S. Hruska, and B. H. Shirts. 2019. 'Comparison of CDH1 Penetrance Estimates in Clinically Ascertained Families vs Families Ascertained for Multiple Gastric Cancers', *JAMA Oncol*, 5: 1325-31.
- Sahasrabudhe, R., P. Lott, M. Bohorquez, T. Toal, A. P. Estrada, J. J. Suarez, A. Brea-Fernández, J. Cameselle-Teijeiro, C. Pinto, I. Ramos, A. Mantilla, R. Prieto, A. Corvalan, E. Norero, C. Alvarez, T. Tapia, P. Carvallo, L. M. Gonzalez, A. Cock-Rada, A. Solano, F. Neffa, A. Della Valle, C. Yau, G. Soares, A. Borowsky, N. Hu, L. J. He, X. Y. Han, P. R. Taylor, A. M. Goldstein, J. Torres, M. Echeverry, C. Ruiz-Ponte, M. R. Teixeira, and L. G. Carvajal-Carmona. 2017. 'Germline Mutations in PALB2, BRCA1, and RAD51C, Which Regulate DNA Recombination Repair, in Patients With Gastric Cancer', *Gastroenterology*, 152: 983-86.e6.
- Slavin, T., S. L. Neuhausen, C. Rybak, I. Solomon, B. Nehoray, K. Blazer, M. Niell-Swiler, A. W. Adamson, Y. C. Yuan, K. Yang, S. Sand, D. Castillo, J. Herzog, X. Wu, S. Tao, T. Chavez, Y. Woo, J. Chao, P. Mora, D. Horcasitas, and J. Weitzel. 2017. 'Genetic Gastric Cancer Susceptibility in the International Clinical Cancer Genomics Community Research Network', *Cancer Genet*, 216-217: 111-19.
- Sung, H., J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, and F. Bray. 2021. 'Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', *CA Cancer J Clin*, 71: 209-49.

Paper II

- Tedaldi, G., F. Pirini, M. Tebaldi, V. Zampiga, I. Cangini, R. Danesi, V. Arcangeli, M. Ravegnani, R. Abou Khouzam, C. Molinari, C. Oliveira, P. Morgagni, L. Saragoni, M. Bencivenga, P. Ulivi, D. Amadori, G. Martinelli, F. Falcini, G. N. Ranzani, and D. Calistri. 2019. 'Multigene Panel Testing Increases the Number of Loci Associated with Gastric Cancer Predisposition', *Cancers (Basel)*, 11.
- Uson, P. L. S., Jr., K. L. Kunze, M. A. Golafshar, G. Botrus, D. Riegert-Johnson, L. Boardman, M. J. Borad, D. Ahn, M. B. Sonbol, A. Kahn, M. Klint, E. D. Esplin, R. L. Nussbaum, A. K. Stewart, T. Bekaii-Saab, and N. J. Samadder. 2022. 'Germline Cancer Testing in Unselected Patients with Gastric and Esophageal Cancers: A Multi-center Prospective Study', *Dig Dis Sci*, 67: 5107-15.
- Usui, Y., Y. Taniyama, M. Endo, Y. N. Koyanagi, Y. Kasugai, I. Oze, H. Ito, I. Imoto, T. Tanaka, M. Tajika, Y. Niwa, Y. Iwasaki, T. Aoi, N. Hakozaiki, S. Takata, K. Suzuki, C. Terao, M. Hatakeyama, M. Hirata, K. Sugano, T. Yoshida, Y. Kamatani, H. Nakagawa, K. Matsuda, Y. Murakami, A. B. Spurdle, K. Matsuo, and Y. Momozawa. 2023. 'Helicobacter pylori, Homologous-Recombination Genes, and Gastric Cancer', *N Engl J Med*, 388: 1181-90.
- Wang, Y., K. Zheng, Y. Huang, H. Xiong, J. Su, R. Chen, and Y. Zou. 2021. 'PARP inhibitors in gastric cancer: beacon of hope', *J Exp Clin Cancer Res*, 40: 211.
- Yap, T. A., A. Ashok, J. Stoll, E. Mauer, V. M. Nepomuceno, K. L. Blackwell, J. E. Garber, and F. Meric-Bernstam. 2022. 'Prevalence of Germline Findings Among Tumors From Cancer Types Lacking Hereditary Testing Guidelines', *JAMA Netw Open*, 5: e2213070.
- Zhang, C., D. Zhu, Y. Qu, M. Shi, J. Ma, Y. Peng, B. Zhu, H. Tao, T. Ma, and T. Hou. 2023. 'Profiling of the genetic features of Chinese patients with gastric cancer with HRD germline mutations in a large-scale retrospective study', *J Med Genet*.
- Zhang, Y. J., Y. Yang, Q. Wei, T. Xu, X. T. Zhang, J. Gao, S. Y. Tan, B. R. Liu, J. D. Zhang, X. B. Chen, Z. J. Wang, M. Qiu, X. Wang, L. Shen, and X. C. Wang. 2021. 'A multicenter study assessing the prevalence of germline genetic alterations in Chinese gastric-cancer patients', *Gastroenterol Rep (Oxf)*, 9: 339-49.

Supplementary table 1 - Detailed clinical characteristics of the germline pathogenic/likely pathogenic variant carriers.

Gene	Chr position	HGVS coding	HGVS protein	Effect	ACMG Classification	ClinVar Variation ID	ClinVar Significance	RSID	Dataset/Country	Sample ID	Gender	Age of DX	Histology	FH of GC	FH of any cancer
ATM	11:108244090	NM_000051.4:c.640del	NP_000042.3:p.(Ser214ProfsTer16)	Frameshift Deletion	Pathogenic	188895	Pathogenic/Likely Pathogenic	rs786204543	IPO-Porto/Portugal	EI_1166	F	57	Diffuse	N	Y
	11:108249103	NM_000051.4:c.1235+1G>A	NP_000042.3:p.?	Splice site	Pathogenic	553402	Likely Pathogenic	rs1555069881	INC/Colombia	EI_1005	F	44	Diffuse	Y	Y
	11:108256340	NM_000051.4:c.2250G>A	NP_000042.3:p.?	Splice site	Pathogenic	3044	Pathogenic/Likely Pathogenic	rs1137887	INC/Colombia	EI_1048	F	35	Diffuse	N	Y
	11:108272735	NM_000051.4:c.3171dup	NP_000042.3:p.(Trp1058MetfsTer5)	Frameshift Insertion	Pathogenic	1454465	Pathogenic	-	HGCGCC/Colombia	I_27122	F	49	Diffuse	Y	Y
	11:108279586	NM_000051.4:c.3381_3384del	NP_000042.3:p.(Gln1128MetfsTer5)	Frameshift Deletion	Pathogenic	141734	Pathogenic/Likely Pathogenic	rs587781971	HGCGCC/Chile	I_27254	M	40	Diffuse	Y	Y
	11:108284280	NM_000051.4:c.3802del	NP_000042.3:p.(Val1268Ter)	Nonsense	Pathogenic	127374	Pathogenic	rs587779834	IPO-Porto/Portugal	EI_1110	F	32	Intestinal	N	N
	11:108288976	NM_000051.4:c.4110-1G>C	NP_000042.3:p.?	Splice site	Pathogenic	834436	Likely Pathogenic	rs1060501692	HGCGCC/Colombia	I_27102	M	56	Intestinal	Y	Y
	11:108295002	NM_000051.4:c.4852C>T	NP_000042.3:p.(Arg1618Ter)	Nonsense	Pathogenic	187207	Pathogenic	rs762083530	IPO-Porto/Portugal	EI_1104	F	62	Intestinal	Y	Y
	11:108304673	NM_000051.4:c.5497-2A>C	NP_000042.3:p.?	Splice site	Pathogenic	449345	Pathogenic/Likely Pathogenic	rs786203796	HGCGCC/Mexico	I_26254	M	42	Intestinal	Y	Y
	11:10831929	NM_000051.4:c.7681_7682del	NP_000042.3:p.(Leu2561GlyfsTer9)	Frameshift Deletion	Likely Pathogenic	-	-	-	HGCGCC/Colombia	I_9149	F	50	Mixed	N	N
	11:108365081	NM_000051.4:c.8851-1G>C	NP_000042.3:p.?	Splice site	Likely Pathogenic	1361736	Uncertain Significance	-	IPO-Porto/Portugal	EI_1083	F	61	Mixed	Y	Y
	11:108365416	NM_000051.4:c.9079dup	NP_000042.3:p.(Ser3027LysfsTer36)	Frameshift Insertion	Pathogenic	135788	Pathogenic/Likely Pathogenic	rs587780645	IPO-Porto/Portugal	EI_1077	M	54	Mixed	Y	Y
	11:108365416	NM_000051.4:c.9079dup	NP_000042.3:p.(Ser3027LysfsTer36)	Frameshift Insertion	Pathogenic	135788	Pathogenic/Likely Pathogenic	rs587780645	IPO-Porto/Portugal	EI_1143	M	60	Diffuse	Y	Y
BRCA2	13:32319165	NM_000059.4:c.156_157insAlu	NP_000050.3:p.?	Frameshift Insertion	Pathogenic	126018	Pathogenic	-	IPO-Porto/Portugal	EI_1080	M	60	Mixed	Y	Y
	13:32337334	NM_000059.4:c.2979G>A	NP_000050.3:p.(Trp993Ter)	Nonsense	Pathogenic	37812	Pathogenic	rs80358544	INC/Colombia	EI_1061	M	80	Intestinal	Y	Y
	13:32337618	NM_000059.4:c.3264dup	NP_000050.3:p.(Gln1089SerfsTer10)	Frameshift Insertion	Pathogenic	37830	Pathogenic	rs80359380	HGCGCC/Mexico	I_2714	F	81	Intestinal	N	Y
	13:32338208	NM_000059.4:c.3860del	NP_000050.3:p.(Asn1287IlefsTer6)	Frameshift Deletion	Pathogenic	51545	Pathogenic	rs80359406	HGCGCC/Spain	I_26895	F	55	Intestinal	N	Y
	13:32338681	NM_000059.4:c.4329dup	NP_000050.3:p.(Asn1444Ter)	Frameshift Insertion	Likely Pathogenic	-	-	-	HGCGCC/Colombia	I_27118	F	75	Diffuse	Y	Y
	13:32339092	NM_000059.3:c.4740_4741dup	NP_000050.3:p.(Glu1581ValfsTer37)	Frameshift Insertion	Pathogenic	220160	Pathogenic	rs864622401	HGCGCC/Chile	I_26873	M	44	Unknown	N	Y
	13:32355110	NM_000059.4:c.7258del	NP_000050.3:p.(Glu2420AsnfsTer49)	Nonsense	Likely Pathogenic	-	-	-	IPO-Porto/Portugal	EI_1139	F	76	Diffuse	Y	Y
	13:32363370	NM_000059.4:c.8168A>G	NP_000050.3:p.(Asp2723Gly)	Missense	Pathogenic	38141	Pathogenic	rs41293513	HGCGCC/Mexico	I_2757	F	45	Diffuse	N	Y
MLH1	13:32394814	NM_000059.4:c.9382C>T	NP_000050.3:p.(Arg3128Ter)	Nonsense	Pathogenic	52826	Pathogenic	rs80359212	IPO-Porto/Portugal	EI_1137	M	40	Diffuse	N	Y
	3:37000956	NM_000249.4:c.213_215del	NP_000240.1:p.(Glu71del)	Frameshift Deletion	Pathogenic	90067	Pathogenic	rs63751642	INC/Colombia	EI_1055	F	38	Intestinal	Y	Y
	3:37012098	NM_000249.4:c.676C>T	NP_000240.1:p.(Arg226Ter)	Nonsense	Pathogenic	17087	Pathogenic	rs63751615	HGCGCC/Mexico	I_26265	M	48	Diffuse	N	Y
	3:37014545	NM_000249.4:c.790+1G>A	NP_000240.1:p.?	Splice site	Pathogenic	90356	Pathogenic	rs267607789	INC/Colombia	EI_1047	M	55	Diffuse	Y	Y
	3:37014545	NM_000249.4:c.790+1G>A	NP_000240.1:p.?	Splice site	Pathogenic	90356	Pathogenic	rs267607789	INC/Colombia	EI_1058	M	46	Intestinal	N	Y
PALB2	3:37042331	NM_000249.4:c.1731G>A	NP_000240.1:p.?	Splice site	Pathogenic	89857	Pathogenic	rs63751657	HGCGCC/Mexico	I_26197	F	46	Intestinal	Y	Y
	16:23635306	NM_024675.4:c.1240C>T	NP_078951.2:p.(Arg414Ter)	Nonsense	Pathogenic	128117	Pathogenic	rs180177100	IPO-Porto/Portugal	EI_1109	F	62	Intestinal	Y	Y
TP53	16:23629862	NM_024675.4:c.2288_2291del	NP_078951.2:p.(Leu763Ter)	Nonsense	Pathogenic	232125	Pathogenic/Likely Pathogenic	rs786659571	HGCGCC/Colombia	I_12495	M	64	Intestinal	Y	Y
	17:7673811	NM_000546.6:c.809T>C	NP_000537.3:p.(Phe270Ser)	Missense	Pathogenic	376594	Pathogenic/Likely Pathogenic	rs1057519986	HGCGCC/Mexico	I_26633	M	74	Intestinal	Y	Y
RAD51D	17:7670699	NM_000546.6:c.1010G>A	NP_000537.3:p.(Arg337His)	Missense	Pathogenic	12379	Pathogenic/Likely Pathogenic	rs121912664	IPO-Porto/Portugal	EI_1106	M	62	Intestinal	Y	Y
	17:35103298	NM_002878.4:c.694C>T	NP_002869.3:p.(Arg232Ter)	Nonsense	Pathogenic	127893	Pathogenic	rs587780104	IPO-Porto/Portugal	EI_1082	M	45	Mixed	Y	Y
BRCA1	17:35103298	NM_002878.4:c.694C>T	NP_002869.3:p.(Arg232Ter)	Nonsense	Pathogenic	127893	Pathogenic	rs587780104	IPO-Porto/Portugal	EI_1155	F	57	Diffuse	N	Y
	17:43093856	NM_007294.4:c.1674del	NP_009225.1:p.(Gly559ValfsTer13)	Frameshift Deletion	Pathogenic	37425	Pathogenic	rs80357600	HGCGCC/Colombia	I_5989	M	66	Unknown	Y	Y
CHEK2	17:43092196	NM_007294.4:c.3331_3334del	NP_009225.1:p.(Gln1111AsnfsTer5)	Frameshift Deletion	Pathogenic	37523	Pathogenic	rs80357701	HGCGCC/Colombia	I_12491	F	43	Diffuse	N	N
	22:28725338	NM_007194.4:c.349A>G	NP_009125.1:p.(Arg117Gly)	Missense	Likely Pathogenic	128071	Pathogenic/Likely Pathogenic	rs28909982	IPO-Porto/Portugal	EI_1103	F	55	Intestinal	N	Y
RAD51C	22:28710005	NM_007194.4:c.846+1G>C	NP_009125.1:p.?	Splice site	Likely Pathogenic	219543	Pathogenic/Likely Pathogenic	rs864622149	HGCGCC/Colombia	I_9186	M	64	Intestinal	N	Y
	17:58696846	NM_058216.3:c.561_562del	NP_478123.1:p.(His187GlnfsTer15)	Frameshift Deletion	Pathogenic	372486	Pathogenic/Likely Pathogenic	rs1057517812	INC/Colombia	EI_1035	F	45	Intestinal	N	Y
PMS2	7:5997324	NM_000535.7:c.803+2T>G	NP_000526.2:p.?	Splice site	Pathogenic	1761741	Pathogenic	-	IPO-Porto/Portugal	EI_1105	F	51	Intestinal	N	Y

Chr: Chromosome; HGVS: Human Genome Variation Society; ACMG: American College of Medical Genetics and Genomics;

DX: Diagnosis FH: Family history; GC: Gastric cancer; F: Female; M: Male; Y: Yes; N: No

Paper II

Supplementary table 2 - Details of pathogenic/likely pathogenic variant carriers, stratified by gene.

Gene	Inclusion criteria			Histology				Country						Gender		FH of Gastric cancer		FH of Any cancer	
	<50	<50_FH	>50_FH	Diffuse	Intestinal	Mixed	Unknown	Chile	Colombia	Mexico	Portugal	Spain	Uruguay	F	M	Yes	No	Yes	No
<i>ATM</i>	2	5	6	6	4	3		1	5	1	6			8	5	9	4	11	2
<i>BRCA2</i>		3	6	4	3	1	1	1	2	2	3	1		5	4	4	5	9	
<i>MLH1</i>		4	1	2	3				3	2				2	3	3	2	5	
<i>BRCA1</i>	1		1	1			1		2					1	1	1	1	1	1
<i>CHEK2</i>			2		2				1		1			1	1		2	2	
<i>PALB2</i>			2		2				1		1			1	1	2		2	
<i>RAD51D</i>		1	1	1		1					2			1	1	1	1	2	
<i>RAD51C</i>		1			1				1					1			1	1	
<i>TP53</i>			2		2					1	1				2	2		2	
<i>PMS2</i>			1		1						1			1			1	1	

FH: Family history; F: Female; M: Male

Supplementary table 3 - Details of pathogenic/likely pathogenic variant carriers, stratified by cohort.

	INC	IPO- PORTO	HGCGCG	Total
Total Number of patients	70	258	339	667
Number of pathogenic/Likely pathogenic carriers	7	15	17	39
<i>ATM</i>	2	6	5	13
<i>BRCA2</i>	1	3	5	9
<i>MLH1</i>	3		2	5
<i>BRCA1</i>			2	2
<i>CHEK2</i>		1	1	2
<i>PALB2</i>		1	1	2
<i>RAD51D</i>		2		2
<i>TP53</i>		1	1	2
<i>PMS2</i>		1		1
<i>RAD51C</i>	1			1
Histology				
Intestinal	4	6	8	18
Diffuse	3	5	6	14
Mixed		4	1	5
Unknown			2	2
Inclusion criteria				
Age at diagnosis $\leq$ 50 years		1	2	3
Age at diagnosis $\leq$ 50 years and with family history of cancer in first and/or second degree relatives	5	2	7	14
Age at diagnosis $>$ 50 years, and with family history of cancer in first and/or second degree relatives	2	12	8	22
Family history of gastric cancer				
Yes	4	9	9	22
No	3	6	8	17

Paper II

Supplementary table 4 - Pathogenic germline variants detected in the tumor samples.

Patient	Histology type	Gene	HGVS coding	HGVS protein	VAF
EI_1077	Mixed	<i>ATM</i>	NM_000051.3:c.9079dup	NP_000042.3:p.(Ser3027LysfsTer36)	0.37
EI_1104	Intestinal	<i>ATM</i>	NM_000051.3:c.4852C>T	NP_000042.3:p.(Arg1618Ter)	0.54
EI_1110	Intestinal	<i>ATM</i>	NM_000051.3:c.3802del	NP_000042.3:p.(Val1268Ter)	0.52
EI_1143	Diffuse	<i>ATM</i>	NM_000051.3:c.9079dup	NP_000042.3:p.(Ser3027LysfsTer36)	0.59
EI_1166	Diffuse	<i>ATM</i>	NM_000051.3:c.640del	NP_000042.3:p.(Ser214ProfsTer16)	0.56
EI_1082	Mixed	<i>RAD51D</i>	NM_002878.3:c.694C>T	NP_002869.3:p.(Arg232Ter)	0.27
EI_1155	Diffuse	<i>RAD51D</i>	NM_002878.3:c.694C>T	NP_002869.3:p.(Arg232Ter)	0.51
EI_1109	Intestinal	<i>PALB2</i>	NM_024675.3:c.1240C>T	NP_078951.2:p.(Arg414Ter)	0.79

HGVS: Human Genome Variation Society; VAF: Variant allele frequency

General Discussion

1. Comparison of the frequencies of *CDH1* germline variants in families fulfilling the IGCLC criteria for HDGC and families with mixed gastric cancer

In 1999, the IGCLC established for the first time diagnostic criteria to select patients for the screening of *CDH1* germline variants (Caldas et al. 1999). Since then, these criteria have been updated several times, to simplify the screening of *CDH1* germline variants, and have been less restrictive regarding age limits and the number of lesions, allowing the identification of more HDGC families (van der Post, Vogelaar, Carneiro, et al. 2015; Fitzgerald et al. 2010; Blair et al. 2020). The mixed GC harbors the two histological components, intestinal and diffuse (Lauren 1965; 'WHO Classification Tumours Editorial Board (ed). 2019 WHO classification of tumours: digestive system tumours'). Interestingly, while intestinal-type GC has been completely excluded from the HDGC tumor spectrum (Caldas et al. 1999; van der Post, Vogelaar, Carneiro, et al. 2015) and the IGCLC guidelines are very clear to not test the patients with this histological type, they provide no guidance regarding mixed GC.

Prior to this study and as part of clinical care, 145 index cases that fulfilled the HDGC testing criteria had been analyzed for *CDH1* deleterious variants, and four families were identified with three pathogenic variants in the *CDH1* gene. The prevalence of pathogenic *CDH1* variants in our patients with HDGC genetic testing criteria is approximately 3% (4/145). The prevalence of *CDH1* pathogenic variants in suspected HDGC families varies widely in the literature, between 7 to 40%, which can be mainly explained by the different GC incidences between countries and the genetic testing criteria modifications over time (Hansford et al. 2015; Adib et al. 2022). High-incidence countries, such as Portugal, display a lower frequency of molecularly confirmed HDGC (Corso et al. 2021; Hansford et al. 2015; Corso et al. 2012), which can explain the relatively low prevalence of *CDH1* deleterious variants we here present.

In this study, we analyzed the *CDH1* gene in a series of 67 patients affected with mixed GC, who would have met the IGCLC 2015 or 2020 testing criteria if this histological type had been considered for genetic testing in the same way as DGC (Paper I). We did not find any pathogenic variant in the *CDH1* gene in this cohort, nor in the additional 41 patients with mixed GC from other countries included in Paper II. Concordant with our results in these 108 patients,

General Discussion

to our knowledge, there are no pathogenic *CDH1* variants described in cases with mixed GC in the literature (Keller et al. 2004; van der Post, Vogelaar, Manders, et al. 2015). Furthermore, the findings of Machado et al. are in favor of a clonal origin of mixed GC and suggest that an isolated-cell histological component may be originated due to the occurrence of *CDH1* somatic variants (Machado et al. 1999).

Therefore, there is currently no evidence that supports including this tumor type in germline testing of the *CDH1* or *CTNNA1* genes. However, studies in larger series of mixed GC are necessary to clarify this issue.

2. Contribution of *CTNNA1* and *CTNND1* germline variants in families with diffuse and mixed gastric cancer

Until recently, *CDH1* was the only known susceptibility gene involved in HDGC, however only a few of these families are explained by pathogenic variants in this gene (Hansford et al. 2015; Marwitz et al. 2020; van der Post, Vogelaar, Manders, et al. 2015). In 2013, efforts to uncover potential genes that could explain these families without *CDH1* pathogenic germline variants led to the identification of a germline variant in the *CTNNA1* gene by Majewski et al. (Majewski et al. 2013). Since then, to our knowledge, 13 pathogenic variants in the *CTNNA1* gene have been described in 15 families fulfilling the HDGC criteria (Majewski et al. 2013; Hansford et al. 2015; Slavin et al. 2017; Weren et al. 2018; Benusiglio et al. 2019; Marwitz et al. 2020; Clark et al. 2020; Coudert et al. 2022; Lobo et al. 2021). In the latest guidelines, the IGCLC has already recognized *CTNNA1* as an HDGC predisposition gene and has recommended *CTNNA1* testing when no *CDH1* pathogenic variant has been identified (Blair et al. 2020).

In this study, we analyzed the *CTNNA1* gene in the remaining 141 index cases fulfilling the IGCLC HDGC testing criteria that were negative for *CDH1* pathogenic variants (Paper I), as well as the 67 patients affected with mixed GC. We identified a novel nonsense variant in *CTNNA1*, NM_001903.5:c.1426C>T p.(Gln476Ter), in a woman diagnosed with a poorly differentiated adenocarcinoma with SRC at age 27. We used the *CDH1* ACMG/AMP guidelines to classify this variant as pathogenic, as described by Lobo et al. (Richards et al. 2015; Lee, Krempely, et al. 2018; Lobo et al. 2021). Immunohistochemistry analysis showed loss of catenin alpha-1 expression in the tumor cells and preserved catenin alpha-1 expression in the normal

gastric epithelium of the *CTNNA1* variant carrier. Furthermore, catenin alpha-1 expression was retained in the tumors of ten other proband cases known to be wild-type for *CTNNA1* and *CDH1* genes, suggesting that catenin alpha-1 expression loss in DGC is predictive of a *CTNNA1* pathogenic variant. Loss or decrease of catenin alpha-1 expression in lesions in gastrectomy specimens or biopsy samples of *CTNNA1* carriers has also been demonstrated by several authors (Clark et al. 2020; Majewski et al. 2013; Benusiglio et al. 2019). These observations suggest that a second hit inactivating mechanism occurred somatically at the *CTNNA1* gene, however, the tumor sample was not enough to search for a second hit inactivating mechanism. The incidence of *CTNNA1* pathogenic variants is still not well-established, but Lobo et al. suggested that pathogenic variants in *CTNNA1* seem to account for less than 2% of the families fulfilling HDGC testing criteria (Lobo et al. 2021). The prevalence of pathogenic *CTNNA1* variants in our families fulfilling 2020 HDGC testing criteria is approximately 1%, which is similar to the frequency described in the literature (Lobo et al. 2021).

Benusiglio and collaborators reported two asymptomatic *CTNNA1* pathogenic variant carriers, who displayed DGC *foci* in the PTG and intramucosal *focus* on esophagogastroduodenoscopy specimens, with loss of catenin alpha-1 expression, suggesting that pathogenic variants in *CDH1* and *CTNNA1* may have similar implications in DGC risk. In fact, annual endoscopy screening following the Cambridge protocol is recommended by the latest IGCLC guidelines in asymptomatic carriers and total gastrectomy should be considered depending on the results of the biopsies and the penetrance of DGC in the family (Blair et al. 2020). The risk of GC in carriers of pathogenic *CTNNA1* variants is not well defined, but a recent study evaluated DGC risk in *CTNNA1* carriers and estimated a cumulative risk of DGC at 80 years of 49-57% (Coudert et al. 2022). The disease spectrum is also uncertain and the association of *CTNNA1* pathogenic variants with increased LBC risk and cleft lip/palate, seen in *CDH1* carriers, was not reported thus far. Although some studies have been associating *CTNNA1* and breast cancer (Felicio et al. 2021; Sun et al. 2017; Clark et al. 2020; Shirts et al. 2016), this association remains to be clarified since the subtype of the breast cancers was not specified. Therefore, breast cancer surveillance should currently be considered based on personal and family history (Blair et al. 2020).

Beyond HDGC predisposition, the *CTNNA1* and *CDH1* genes have been associated with macular dystrophy patterned (Saksens et al. 2016; Tanner et al. 2021) and cleft lip/palate and BCD (Kievit et al. 2018; Ghomid et al. 2017), respectively. Another gene involved in BCD and cleft palate is the *CTNND1* gene (Cox et al. 2018; Kievit et al. 2018; Ghomid et al. 2017), which is another cadherin–catenin complex gene. The role of catenin family proteins in cell

General Discussion

adhesion raises the question about their implication in GC predisposition. The involvement of another cadherin–catenin complex gene in cleft palate and BCD, in addition to its important function in the maintenance and stability of cell adhesion, makes this gene an ideal candidate for GC predisposition (Benusiglio 2017). The *CTNND1* gene encodes the p120-catenin, a binding partner of cadherin-1, and its protein interacts with the juxtamembrane domain of cadherin-1 and regulates the stability of cell-cell adhesion by controlling the retention of cadherin-1 at the cell surface (Gloushankova, Rubtsova, and Zhitnyak 2017).

We, therefore, analyzed the *CTNND1* gene in the 208 patients (141 patients fulfilling the 2020 IGCLC criteria for HDGC testing and negative for *CDH1* variants, and 67 patients affected with mixed GC) included in this study, but no pathogenic variants were found (Paper I). However, we identified six VUS in the *CTNND1* gene, both in patients with diffuse and mixed GC subtypes. Our negative findings support those of Schuetz et al. in 22 families (Schuetz et al. 2012). We reported the largest series to date tested for *CTNND1* variants in suspected HDGC patients, but even larger studies will be needed to clarify if the *CTNND1* gene has any role in GC predisposition.

3. Contribution of variants in multi-organ cancer susceptibility genes to gastric cancer risk of different histological subtypes

Histologically, GC can be classified into two main types according to the Laurén classification, intestinal and diffuse (Lauren 1965). Tumors presenting both intestinal and diffuse components are classified as mixed carcinomas. The two main known GC syndromes can also be divided according to the histological GC type. The most well-known hereditary form of GC is HDGC and is characterized by an increased risk of DGC (Blair et al. 2020). The GC hereditary syndrome associated with intestinal GC is FIGC. However, while a small proportion of suspected HDGC cases is explained by germline variants in *CDH1* and *CTNNA1* genes, FIGC remains widely understudied and genetically unexplained to date (Carvalho et al. 2021). Similarly, as mixed GC is normally not considered in the tumor spectrum of HDGC syndrome (Paper I), the heritability of families with mixed GC clustering remains to be uncovered. Moreover, GC has been described in other known hereditary cancer syndromes or as part of the spectrum of moderate-risk cancer predisposition genes.

In this study, we aimed to evaluate the contribution of pathogenic/likely pathogenic variants in known multi-tumor high-moderate penetrance cancer predisposition genes for the heritability of GC, regardless of the histological subtype, studying 667 index cases from a multi-center cohort. We analyzed 334 index cases diagnosed with DGC, negative for *CDH1* and *CTNNA1* pathogenic variants, 103 diagnosed with mixed GC, 193 diagnosed with intestinal GC, and 37 with unknown histology. A combination of two different sequencing approaches was used. Targeted sequencing was performed in 258 index cases from IPO-Porto in Portugal and in 70 index cases from the Instituto Nacional de Cancerologia in Colombia. Whole exome sequencing (WES) was performed in the remaining 339 cases from Chile (n=33), Colombia (n=188), Mexico (n=82), Spain (n=29) and Uruguay (n=7) (Paper II).

Overall, 39 out of 667 (6%) index patients presented a pathogenic or likely pathogenic variant in known cancer predisposition genes (*ATM*, *BRCA2*, *BRCA1*, *PALB2*, *TP53*, *RAD51D*, *RAD51C*, *CHEK2*, *MLH1*, and *PMS2*). Among the 39 patients with a pathogenic/likely pathogenic variant, 23 did not fulfill criteria genetic testing of any known cancer predisposition syndrome and 16 met criteria for genetic testing for specific hereditary cancer syndromes, such as HDGC, Lynch, HBOC or Li-Fraumeni syndromes. However, of these patients who fulfilled criteria for genetic testing for a known cancer predisposition syndrome, only five would have been correctly identified if a single gene test had been performed. The average age of diagnosis of the pathogenic variant carriers was similar to the average age of diagnosis of non-carriers. This similarity can be explained by the moderate penetrance associated with these genes, which leads to a later onset of the disease compared to established syndromes like HDGC, in which the average age of diagnosis is 40 years old (Garcia-Pelaez et al. 2023; Roberts et al. 2019). The variation in the age at diagnosis was also noted in other studies, which showed that *CDH1* pathogenic variants were diagnosed at a younger age compared to non-carriers, whereas the age at diagnosis for individuals with a pathogenic variant in the other known cancer predisposition genes was similar to that of non-carriers (Usui et al. 2023; Calvello et al. 2023). Moreover, our cohort was selected based on early-onset GC and/or family history of cancer, so the families negative for pathogenic variants in genes studied can have a genetic predisposition not yet identified. Our study included patients with early onset (diagnosed before the age of 50, with or without a family history of cancer) and patients with late-onset (diagnosed after the age of 50) and with a family history of any cancer. We found that the prevalence of P/LP variants was higher in the group of patients with family history of cancer, irrespective of the age of diagnosis of the index case. These pathogenic or likely pathogenic variants were identified in patients with different histological subtypes, with 4.2% (14/335), 4.9% (5/103), and

General Discussion

9.1% (1/196) of the diffuse, mixed, and intestinal GC, respectively. The higher prevalence in carriers diagnosed with intestinal GC compared to carriers diagnosed with diffuse and mixed GC could be attributed to the lack of studies on intestinal GC, which remains largely genetically unexplained (Carvalho et al. 2021). Sequencing a panel of known cancer predisposition genes is therefore likely to increase the probability of identifying a pathogenic/likely pathogenic variant in patients with this tumor type.

The prevalence of pathogenic/likely pathogenic variants in the known cancer predisposition genes reported in the literature in selected studies, in which all histological types were analyzed, ranges between 3% to 11% (Sahasrabudhe et al. 2017; Calvello et al. 2023; Tedaldi et al. 2019; Zhang et al. 2021). Here we report a prevalence of 6%, which is in accordance with the prevalence reported previously. Of note, the patients with germline pathogenic/likely pathogenic variants display heterogeneous GC histology, including intestinal, mixed, and diffuse GC, which is also in accordance with the published studies (Sahasrabudhe et al. 2017; Calvello et al. 2023; Tedaldi et al. 2019).

This study is a multi-center cohort with a large case series, in which these known multi-tumor high-moderate penetrance predisposition cancer genes explain a portion of these GC families, regardless of the histological type. In the era of multigene panel testing, NGS provides a distinctive method for efficiently and affordably studying several genes in a huge number of patients. Therefore, multi-organ cancer predisposition genes should be included in gene panels used for investigating germline variants in GC patients, especially when there is family history of cancer but irrespective of histology subtype, as there is often significant phenotypic overlap between various cancer predisposition syndromes. However, further studies are still necessary, since only a small part of GC heritability could be explained by variants in these known cancer genes. The identification of germline variants in these known multi-tumor high-moderate penetrance predisposition cancer genes would allow suitable genetic counseling, surveillance, and/or risk reduction measures, as already occurs in several hereditary cancer syndromes.

The potential association of homologous recombination genes with GC predisposition may have not only significance for screening and/or prophylaxis, but as well as for targeted treatment strategies. Recently, Usui et al. showed that carriers of pathogenic germline variants in homologous recombination genes and *H. pylori* infection had an increased risk of GC, suggesting that *H. pylori* infection modifies the risk of GC in carriers of deleterious variants in these genes. A possible mechanism is that DNA damage caused by *H. pylori* infection is enhanced when there is a reduced DNA damage repair capacity due to deleterious variants in the homologous recombination genes. The eradication of *H. pylori* infection in these carriers

would mitigate the risk of GC (Usui et al. 2023). However, despite the increased risk in pathogenic variant carriers in homologous recombination genes, there are currently no recommended GC surveillance guidelines besides guidance offered by family history. Endoscopic surveillance and *H. pylori* test could be offered to these patients with family history and other risk factors, but further studies are warranted to define a management strategy for GC risk among these carriers (Buckley et al. 2022). Deficiency in homologous recombination leads to inefficient repair of double-strand DNA breaks (Prakash et al. 2015), which can be therapeutically exploited. Tumors with these defects are sensitive to platinum-based chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors (Isaac, Karapetyan, and Tamkus 2018; D'Andrea 2018). These individuals could potentially benefit from targeted therapies, such as PARP inhibitors, which have been used with success in several tumor types, including GC (Bang et al. 2015; Wang et al. 2021; Lin et al. 2018). For patients with pathogenic germline variants in MMR genes it is currently recommended to start GC screening at 30 to 40 years with esophagogastroduodenoscopy and surveillance should be performed every 2 to 4 years according to individual risk and family history of GC (Weiss et al. 2021). Loss of function of MMR genes leads to MSI-high/mismatch repair deficient phenotype and these tumors are sensitive to PD-1/PD-L1 inhibitors, an FDA-approved therapy for deficient mismatch repair and MSI-high solid tumors (Le et al. 2015; Le et al. 2017).

4. Validation of the use of NGS for germline variant screening in archival formalin-fixed paraffin-embedded (FFPE) samples.

Germline genetic testing is generally performed on genomic DNA extracted from peripheral blood leucocytes, however, there are families with a strong family history of GC without an affected member alive or available for genetic testing. Genetic testing in unaffected individuals in these families is not ideal and a negative test result is uninformative. Therefore, the possibility of identifying germline pathogenic variants in archival tissue of affected relatives is advantageous. This will allow a more informative molecular study of families with a strong family history of GC in which all affected family members are deceased or not available. We have previously been able to identify germline variants in archival tumor samples in ovarian cancer patients (subsequently confirmed in a constitutional DNA sample), namely *BRCA1* or *BRCA2* germline pathogenic variants in 13.3% of the patients (Peixoto et al. 2020) and germline

General Discussion

deleterious variants in a panel of ovarian cancer predisposition genes in 21.1% of the patients (Barbosa et al. 2020).

In this study, we aimed to validate the use of NGS for germline variant testing in archival FFPE samples of the gastric adenocarcinomas of some of the patients with pathogenic germline variants identified in our study. Moreover, this analysis can also identify a genomic second-hit mechanism. The two-hit hypothesis for most tumor suppressor genes associated with inherited cancer predisposition postulates that the wild-type allele is somatically inactivated due to a second alteration in the same gene. These include copy number alterations, pathogenic point variants, small insertions/deletions, or epigenetic modifications (Knudson 2001). The detection of a somatic second-hit mechanism is an important tool to determine the functional impact of a variant in cancer predisposition genes, especially when evaluating a tumor not belonging to the typical spectrum of neoplasias in a particular syndrome (Yurgelun et al. 2019; Van Marcke et al. 2020; Mandelker et al. 2017). We studied eight available tumors from carriers of pathogenic germline variants in *ATM*, *PALB2*, and *RAD51D* genes (Paper II). All germline variants were detected by the NGS-based tumor test and the variant allele frequency (VAF) ranged between 27% and 79%. The deletion of the wild-type allele in tumors with germline pathogenic variants can be inferred from the high VAF of the mutant allele (Peixoto et al. 2020). In the tumors analyzed, only the *PALB2* variant found in an intestinal gastric tumor had a high VAF (79%), which is suggestive of a LOH due to the loss of the wild-type allele. The VAF of the other germline variants was not suggestive of a biallelic inactivation by deletion, neither did we identify a somatic pathogenic variant that could explain the second hit mechanism. Failure to observe LOH may result from technical limitations of NGS or low tumor purity, especially in tumors that have high levels of infiltrating lymphocytes, which may also introduce further complexity to the interpretation of allele frequency (Lim et al. 2023). The inactivation of the second allele can also result from an epigenetic mechanism, which we did not study. It is also possible that a haploinsufficiency mechanism may be operative in gastric tumorigenesis in several of the patients with germline pathogenic/likely pathogenic variants in these multi-organ cancer predisposition genes (Santarosa and Ashworth 2004). LOH or a pathogenic somatic variant affecting the *PALB2* gene has been described in *PALB2*-related cancers, but bi-allelic inactivation was not detected in other studies (Li et al. 2019; Casadei et al. 2011; Lee, Li, et al. 2018). Two-hit inactivation has also been observed in cancers associated with *ATM* and *RAD51D* pathogenic variants, though in a low percentage of the tumors tested. In fact, haploinsufficiency is considered a potential mechanism underlying the

tumorigenesis of *ATM* and *RAD51D*-mutated cancers (Borja et al. 2023; Yap et al. 2022; Renault et al. 2018; Lim et al. 2023; Weigelt et al. 2018).

Although the detection of germline variants based on tumor testing appears interesting and advantageous, there are several factors that can influence its accuracy. FFPE samples present several challenges when used in genetic testing, as DNA extracted from this type of sample is usually of poor quality and highly fragmented. A factor that also must be considered is the age of the FFPE block, which has a significant impact on the quality and the number of variants detected (Steiert et al. 2023; Terraf et al. 2022). Furthermore, a small subset of germline variants is currently not detected by this approach, since the accurate detection of large rearrangements in tumor samples using NGS is not yet optimized (Peixoto et al. 2020; Terraf et al. 2022; Wallace 2016). Although large rearrangements are relatively rare in these cancer predisposition genes (Póczy et al. 2021), in our population we have a founder rearrangement that is also not detected using conventional NGS bioinformatic algorithms, the *BRCA2* c.156_157insAlu variant, which was found in a patient with a mixed GC in our study (Paper II). Moreover, the VAF of SNVs and indels variants can be lower than the recommended thresholds to consider germline testing for variants detected in tumor-only sequencing (>20% for indels and >30% for SNVs), which may also limit the ability to detect these variants (Terraf et al. 2022; Mandelker et al. 2019). Other limitations in studying eventual germline variants in tumor tissue include the requirement to confirm the germline origin in a matched normal sample or in a family member, which may not always provide informative results.

Having these potential limitations in mind, the possibility of exploring the underlying genetic cause of cancer in a deceased individual is a powerful additional tool to provide a more precise risk assessment to the families without an available proband for diagnostic genetic testing, which subsequently may allow better surveillance management (Bennett et al. 2021).

Conclusions

The main conclusions of this thesis are:

1. None of the cases with mixed GC carried pathogenic variants in either the *CDH1* or the *CTNNA1* genes, so currently no evidence supports including this tumor type in the germline testing criteria of the *CDH1* or *CTNNA1* genes.
2. A deleterious *CTNNA1* germline variant was found in 0.7% (1/141) of patients meeting the 2020 IGCLC criteria for HDGC testing, as compared to the rate of 2.8% of *CDH1* deleterious variants. Despite this relatively low frequency, screening for the *CTNNA1* gene should be considered in *CDH1*-negative HDGC families.
3. The *CTNND1* gene is an ideal candidate for GC predisposition, but only variants of uncertain significance were identified in this gene. However, larger studies are needed to understand the role of *CTNND1* variants in inherited GC predisposition.
4. Thirty-nine out of 667 (6%) index patients with different GC histologies presented a pathogenic or likely pathogenic variant in multi-organ cancer predisposition genes, namely in genes involved in DNA damage repair (Homologous recombination and MMR), which are more common when there is family history of cancer independently of the age of diagnosis of the index case.
5. It is feasible to perform germline variant screening in archival FFPE samples, to allow a more informative molecular study of families with a strong family history of GC in which all affected family members are deceased.

Future Perspectives

The following points will be addressed in future studies:

1. We aim to perform segregation studies in families identified with a pathogenic or likely pathogenic variant in cancer predisposition genes.
2. We aim to analyze the gastric tumors of patients with pathogenic germline variants in cancer predisposition genes identified in this study to search for other second hit inactivating mechanisms, as well as to search for mutational signatures associated with homologous recombination and MMR deficiency, to confirm the involvement of these genes in the pathogenesis of these gastric tumors.
3. As only part of the heritability of GC is explained by known cancer susceptibility genes, we aim to identify new GC predisposing genes in the families analyzed by WES.

References

- Adib, E., T. El Zarif, A. H. Nassar, E. W. Akl, S. Abou Alaiwi, T. H. Mouhieddine, E. D. Esplin, K. Hatchell, S. M. Nielsen, H. Q. Rana, T. K. Choueiri, D. J. Kwiatkowski, and G. Sonpavde. 2022. 'CDH1 germline variants are enriched in patients with colorectal cancer, gastric cancer, and breast cancer', *Br J Cancer*, 126: 797-803.
- Ajani, J. A., T. A. D'Amico, D. J. Bentrem, J. Chao, D. Cooke, C. Corvera, P. Das, P. C. Enzinger, T. Enzler, P. Fanta, F. Farjah, H. Gerdes, M. K. Gibson, S. Hochwald, W. L. Hofstetter, D. H. Ilson, R. N. Keswani, S. Kim, L. R. Kleinberg, S. J. Klempner, J. Lacy, Q. P. Ly, K. A. Matkowskyj, M. McNamara, M. F. Mulcahy, D. Outlaw, H. Park, K. A. Perry, J. Pimiento, G. A. Poultsides, S. Reznik, R. E. Roses, V. E. Strong, S. Su, H. L. Wang, G. Wiesner, C. G. Willett, D. Yakoub, H. Yoon, N. McMillian, and L. A. Pluchino. 2022. 'Gastric Cancer, Version 2.2022, NCCN Clinical Practice Guidelines in Oncology', *J Natl Compr Canc Netw*, 20: 167-92.
- Alipour, M. 2021. 'Molecular Mechanism of Helicobacter pylori-Induced Gastric Cancer', *Gastrointest Cancer*, 52: 23-30.
- Alsina, M., V. Arrazubi, M. Diez, and J. Tabernero. 2023. 'Current developments in gastric cancer: from molecular profiling to treatment strategy', *Nat Rev Gastroenterol Hepatol*, 20: 155-70.
- Aoki, Koji, and Makoto M. Taketo. 2007. 'Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene', *Journal of Cell Science*, 120: 3327-35.
- Asif, B., A. L. Sarvestani, L. A. Gamble, S. G. Samaranayake, A. L. Famiglietti, G. A. Fasaye, M. Quezado, M. Miettinen, L. Korman, C. Koh, T. Heller, and J. L. Davis. 2023. 'Cancer surveillance as an alternative to prophylactic total gastrectomy in hereditary diffuse gastric cancer: a prospective cohort study', *Lancet Oncol*, 24: 383-91.
- Association, Japanese Gastric Cancer. 2011. 'Japanese classification of gastric carcinoma: 3rd English edition', *Gastric Cancer*, 14: 101-12.
- Bang, Y. J., S. A. Im, K. W. Lee, J. Y. Cho, E. K. Song, K. H. Lee, Y. H. Kim, J. O. Park, H. G. Chun, D. Y. Zang, A. Fielding, J. Rowbottom, D. Hodgson, M. J. O'Connor, X. Yin, and W. H. Kim. 2015. 'Randomized, Double-Blind Phase II Trial With Prospective Classification by ATM Protein Level to Evaluate the Efficacy and Tolerability of Olaparib Plus Paclitaxel in Patients With Recurrent or Metastatic Gastric Cancer', *J Clin Oncol*, 33: 3858-65.

References

- Barber, M., A. Murrell, Y. Ito, A. T. Maia, S. Hyland, C. Oliveira, V. Save, F. Carneiro, A. L. Paterson, N. Grehan, S. Dwerryhouse, P. Lao-Sirieix, C. Caldas, and R. C. Fitzgerald. 2008. 'Mechanisms and sequelae of E-cadherin silencing in hereditary diffuse gastric cancer', *J Pathol*, 216: 295-306.
- Barbosa, A., P. Pinto, A. Peixoto, J. Guerra, C. Pinto, C. Santos, M. Pinheiro, C. Escudeiro, C. Bartosch, J. Silva, and M. R. Teixeira. 2020. 'Gene Panel Tumor Testing in Ovarian Cancer Patients Significantly Increases the Yield of Clinically Actionable Germline Variants beyond BRCA1/BRCA2', *Cancers (Basel)*, 12.
- Barrow, E., L. Robinson, W. Alduaij, A. Shenton, T. Clancy, F. Laloo, J. Hill, and D. G. Evans. 2009. 'Cumulative lifetime incidence of extracolonic cancers in Lynch syndrome: a report of 121 families with proven mutations', *Clin Genet*, 75: 141-9.
- Beer, A., B. Streubel, R. Asari, C. Dejaco, and G. Oberhuber. 2017. 'Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) - a rare recently described gastric polyposis syndrome - report of a case', *Z Gastroenterol*, 55: 1131-34.
- Benesch, M. G. K., S. R. Bursey, A. C. O'Connell, M. G. Ryan, C. L. Howard, C. C. Stockley, and A. Mathieson. 2021. 'CDH1 Gene Mutation Hereditary Diffuse Gastric Cancer Outcomes: Analysis of a Large Cohort, Systematic Review of Endoscopic Surveillance, and Secondary Cancer Risk Postulation', *Cancers (Basel)*, 13.
- Bennett, S., E. Alexander, H. Fraser, N. Bowers, A. Wallace, E. R. Woodward, F. Laloo, A. M. Quinn, S. Huang, H. Schlecht, and D. G. Evans. 2021. 'Germline FFPE inherited cancer panel testing in deceased family members: implications for clinical management of unaffected relatives', *Eur J Hum Genet*, 29: 861-71.
- Benusiglio, P. R. 2017. 'CDH1 germline mutations: different syndromes, same management?', *Genet Med*, 19: 965-66.
- Benusiglio, P. R., C. Colas, E. Guillermin, A. Canard, H. Delhomelle, M. Warcoin, J. Bellanger, M. Eyries, M. Zizi, J. Netter, F. Soubrier, Y. Parc, A. Mourregot, A. Maran Gonzalez, V. Cusin, J. A. Denis, I. Coupier, M. Svrcek, and F. Coulet. 2019. 'Clinical implications of CTNNA1 germline mutations in asymptomatic carriers', *Gastric Cancer*, 22: 899-903.
- Berx, G., K. Staes, J. van Hengel, F. Molemans, M. J. Bussemakers, A. van Bokhoven, and F. van Roy. 1995. 'Cloning and characterization of the human invasion suppressor gene E-cadherin (CDH1)', *Genomics*, 26: 281-9.
- Blair, V. R., M. McLeod, F. Carneiro, D. G. Coit, J. L. D'Addario, J. M. van Dieren, K. L. Harris, N. Hoogerbrugge, C. Oliveira, R. S. van der Post, J. Arnold, P. R. Benusiglio, T. M. Bisseling, A. Boussioutas, A. Cats, A. Charlton, K. E. C. Schreiber, J. L. Davis, M. D.

- Pietro, R. C. Fitzgerald, J. M. Ford, K. Gamet, I. Gullo, R. H. Hardwick, D. G. Huntsman, P. Kaurah, S. S. Kupfer, A. Latchford, P. F. Mansfield, T. Nakajima, S. Parry, J. Rossaak, H. Sugimura, M. Svrcek, M. Tischkowitz, T. Ushijima, H. Yamada, H. K. Yang, A. Claydon, J. Figueiredo, K. Paringatai, R. Seruca, N. Bougen-Zhukov, T. Brew, S. Busija, P. Carneiro, L. DeGregorio, H. Fisher, E. Gardner, T. D. Godwin, K. N. Holm, B. Humar, C. J. Lintott, E. C. Monroe, M. D. Muller, E. Norero, Y. Nouri, J. Paredes, J. M. Sanches, E. Schulpen, A. S. Ribeiro, A. Sporle, J. Whitworth, L. Zhang, A. E. Reeve, and P. Guilford. 2020. 'Hereditary diffuse gastric cancer: updated clinical practice guidelines', *Lancet Oncol*, 21: e386-e97.
- Boland, C. R., M. B. Yurgelun, K. A. Mraz, and P. M. Boland. 2022. 'Managing gastric cancer risk in lynch syndrome: controversies and recommendations', *Fam Cancer*, 21: 75-78.
- Bonadona, V., B. Bonaïti, S. Olschwang, S. Grandjouan, L. Huiart, M. Longy, R. Guimbaud, B. Buecher, Y. J. Bignon, O. Caron, C. Colas, C. Noguès, S. Lejeune-Dumoulin, L. Olivier-Faivre, F. Polycarpe-Osaer, T. D. Nguyen, F. Desseigne, J. C. Saurin, P. Berthet, D. Leroux, J. Duffour, S. Manouvrier, T. Frébourg, H. Sobol, C. Lasset, and C. Bonaïti-Pellié. 2011. 'Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome', *Jama*, 305: 2304-10.
- Boni, J., A. Idani, C. Roca, L. Feliubadaló, E. Tomiak, E. Weber, W. D. Foulkes, A. Orthwein, Z. El Haffaf, C. Lazaro, and B. Rivera. 2022. 'A decade of RAD51C and RAD51D germline variants in cancer', *Hum Mutat*, 43: 285-98.
- Borja, Nicholas A., Rachel Silva-Smith, Marilyn Huang, Dipen J. Parekh, Daniel Sussman, and Mustafa Tekin. 2023. 'Atypical ATMs: Broadening the phenotypic spectrum of ATM-associated hereditary cancer', *Frontiers in Oncology*, 13.
- Buckley, K. H., B. A. Niccum, K. N. Maxwell, and B. W. Katona. 2022. 'Gastric Cancer Risk and Pathogenesis in BRCA1 and BRCA2 Carriers', *Cancers (Basel)*, 14.
- Caldas, C., F. Carneiro, H. T. Lynch, J. Yokota, G. L. Wiesner, S. M. Powell, F. R. Lewis, D. G. Huntsman, P. D. Pharoah, J. A. Jankowski, P. MacLeod, H. Vogelsang, G. Keller, K. G. Park, F. M. Richards, E. R. Maher, S. A. Gayther, C. Oliveira, N. Grehan, D. Wight, R. Seruca, F. Roviello, B. A. Ponder, and C. E. Jackson. 1999. 'Familial gastric cancer: overview and guidelines for management', *J Med Genet*, 36: 873-80.
- Calvello, M., M. Marabelli, S. Gandini, E. Marino, L. Bernard, M. Dal Molin, G. Di Cola, C. Zanzottera, G. Corso, N. Fazio, L. Gervaso, U. Fumagalli Romario, M. Barberis, A. Guerrieri-Gonzaga, L. Bertario, D. Serrano, and B. Bonanni. 2023. 'Hereditary Gastric

References

- Cancer: Single-Gene or Multigene Panel Testing? A Mono-Institutional Experience', *Genes (Basel)*, 14.
- Carneiro, F. 2022. 'Familial and hereditary gastric cancer, an overview', *Best Pract Res Clin Gastroenterol*, 58-59: 101800.
- Carneiro, F., D. G. Huntsman, T. C. Smyrk, D. A. Owen, R. Seruca, P. Pharoah, C. Caldas, and M. Sobrinho-Simões. 2004. 'Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening', *J Pathol*, 203: 681-7.
- Carneiro, F., M. Seixas, and M. Sobrinho-Simões. 1995. 'New elements for an updated classification of the carcinomas of the stomach', *Pathol Res Pract*, 191: 571-84.
- Carneiro, Fátima. 1997. 'Classification of gastric carcinomas', *Current Diagnostic Pathology*, 4: 51-59.
- Carvalho, J., P. Oliveira, J. Senz, C. São José, S. Hansford, S. P. Teles, M. Ferreira, G. Corso, H. Pinheiro, D. Lemos, V. Pascale, F. Roviello, D. Huntsman, and C. Oliveira. 2021. 'Redefinition of familial intestinal gastric cancer: clinical and genetic perspectives', *J Med Genet*, 58: 1-11.
- Casadei, S., B. M. Norquist, T. Walsh, S. Stray, J. B. Mandell, M. K. Lee, J. A. Stamatoyannopoulos, and M. C. King. 2011. 'Contribution of inherited mutations in the BRCA2-interacting protein PALB2 to familial breast cancer', *Cancer Res*, 71: 2222-9.
- Chen, Y. C., W. L. Fang, R. F. Wang, C. A. Liu, M. H. Yang, S. S. Lo, C. W. Wu, A. F. Li, Y. M. Shyr, and K. H. Huang. 2016. 'Clinicopathological Variation of Lauren Classification in Gastric Cancer', *Pathol Oncol Res*, 22: 197-202.
- Chia, N. Y., and P. Tan. 2016. 'Molecular classification of gastric cancer', *Ann Oncol*, 27: 763-9.
- Clark, D. F., S. T. Michalski, R. Tondon, B. Nehoray, J. Ebrahimzadeh, S. K. Hughes, E. R. Soper, S. M. Domchek, A. K. Rustgi, D. Pineda-Alvarez, M. J. Anderson, and B. W. Katona. 2020. 'Loss-of-function variants in CTNNA1 detected on multigene panel testing in individuals with gastric or breast cancer', *Genet Med*, 22: 840-46.
- Collins, C., and W. J. Nelson. 2015. 'Running with neighbors: coordinating cell migration and cell-cell adhesion', *Curr Opin Cell Biol*, 36: 62-70.
- Corso, G., F. Corso, F. Bellerba, P. Carneiro, S. Seixas, A. Cioffi, C. La Vecchia, F. Magnoni, B. Bonanni, P. Veronesi, S. Gandini, and J. Figueiredo. 2021. 'Geographical Distribution of E-cadherin Germline Mutations in the Context of Diffuse Gastric Cancer: A Systematic Review', *Cancers (Basel)*, 13.

- Corso, G., D. Marrelli, V. Pascale, C. Vindigni, and F. Roviello. 2012. 'Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature', *BMC Cancer*, 12: 8.
- Corso, G., F. Roncalli, D. Marrelli, F. Carneiro, and F. Roviello. 2013. 'History, pathogenesis, and management of familial gastric cancer: original study of John XXIII's family', *Biomed Res Int*, 2013: 385132.
- Coudert, M., Y. Drouet, H. Delhomelle, M. Svrcek, P. R. Benusiglio, F. Coulet, D. F. Clark, B. W. Katona, L. P. van Hest, L. E. van der Kolk, A. Cats, J. M. van Dieren, B. Nehoray, T. Slavin, I. Spier, R. Hüneburg, S. Lobo, C. Oliveira, L. Boussemart, L. Masson, J. Chiesa, M. Schwartz, B. Buecher, L. Golmard, A. M. Bouvier, V. Bonadona, D. Stoppa-Lyonnet, C. Lasset, and C. Colas. 2022. 'First estimates of diffuse gastric cancer risks for carriers of CTNNA1 germline pathogenic variants', *J Med Genet*, 59: 1189-95.
- Cox, L. L., T. C. Cox, L. M. Moreno Uribe, Y. Zhu, C. T. Richter, N. Nidey, J. M. Standley, M. Deng, E. Blue, J. X. Chong, Y. Yang, R. P. Carstens, D. Anand, S. A. Lachke, J. D. Smith, M. O. Dorschner, B. Bedell, E. Kirk, A. V. Hing, H. Venselaar, L. C. Valencia-Ramirez, M. J. Bamshad, I. A. Glass, J. A. Cooper, E. Haan, D. A. Nickerson, H. van Bokhoven, H. Zhou, K. N. Krahn, M. F. Buckley, J. C. Murray, A. C. Lidral, and T. Roscioli. 2018. 'Mutations in the Epithelial Cadherin-p120-Catenin Complex Cause Mendelian Non-Syndromic Cleft Lip with or without Cleft Palate', *Am J Hum Genet*, 102: 1143-57.
- Cristescu, R., J. Lee, M. Nebozhyn, K. M. Kim, J. C. Ting, S. S. Wong, J. Liu, Y. G. Yue, J. Wang, K. Yu, X. S. Ye, I. G. Do, S. Liu, L. Gong, J. Fu, J. G. Jin, M. G. Choi, T. S. Sohn, J. H. Lee, J. M. Bae, S. T. Kim, S. H. Park, I. Sohn, S. H. Jung, P. Tan, R. Chen, J. Hardwick, W. K. Kang, M. Ayers, D. Hongyue, C. Reinhard, A. Loboda, S. Kim, and A. Aggarwal. 2015. 'Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes', *Nat Med*, 21: 449-56.
- D'Andrea, Alan D. 2018. 'Mechanisms of PARP inhibitor sensitivity and resistance', *DNA Repair*, 71: 172-76.
- Daly, M. B., T. Pal, M. P. Berry, S. S. Buys, P. Dickson, S. M. Domchek, A. Elkhanany, S. Friedman, M. Goggins, M. L. Hutton, B. Y. Karlan, S. Khan, C. Klein, W. Kohlmann, A. W. Kurian, C. Laronga, J. K. Litton, J. S. Mak, C. S. Menendez, S. D. Merajver, B. S. Norquist, K. Offit, H. J. Pederson, G. Reiser, L. Senter-Jamieson, K. M. Shannon, R. Shatsky, K. Visvanathan, J. N. Weitzel, M. J. Wick, K. B. Wisinski, M. B. Yurgelun, S. D. Darlow, and M. A. Dwyer. 2021. 'Genetic/Familial High-Risk Assessment: Breast,

References

- Ovarian, and Pancreatic, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology', *J Natl Compr Canc Netw*, 19: 77-102.
- de-Freitas-Junior, J. C., S. Carvalho, A. M. Dias, P. Oliveira, J. Cabral, R. Seruca, C. Oliveira, J. A. Morgado-Díaz, C. A. Reis, and S. S. Pinho. 2013. 'Insulin/IGF-I signaling pathways enhances tumor cell invasion through bisecting GlcNAc N-glycans modulation. an interplay with E-cadherin', *PLoS One*, 8: e81579.
- Donner, Ilikki, Tuula Kiviluoto, Ari Ristimäki, Lauri A. Aaltonen, and Pia Vahteristo. 2015. 'Exome sequencing reveals three novel candidate predisposition genes for diffuse gastric cancer', *Familial Cancer*, 14: 241-46.
- Dorling, L., S. Carvalho, J. Allen, A. González-Neira, C. Luccarini, C. Wahlström, K. A. Pooley, M. T. Parsons, C. Fortuno, Q. Wang, M. K. Bolla, J. Dennis, R. Keeman, M. R. Alonso, N. Álvarez, B. Herraiz, V. Fernandez, R. Núñez-Torres, A. Osorio, J. Valcich, M. Li, T. Törnngren, P. A. Harrington, C. Baynes, D. M. Conroy, B. Decker, L. Fachal, N. Mavaddat, T. Ahearn, K. Aittomäki, N. N. Antonenkova, N. Arnold, P. Arveux, M. G. Ausems, P. Auvinen, H. Becher, M. W. Beckmann, S. Behrens, M. Bermisheva, K. Białkowska, C. Blomqvist, N. V. Bogdanova, N. Bogdanova-Markov, S. E. Bojesen, B. Bonanni, A. L. Børresen-Dale, H. Brauch, M. Bremer, I. Briceno, T. Brüning, B. Burwinkel, D. A. Cameron, N. J. Camp, A. Campbell, A. Carracedo, J. E. Castela, M. H. Cessna, S. J. Chanock, H. Christiansen, J. M. Collée, E. Cordina-Duverger, S. Cornelissen, K. Czene, T. Dörk, A. B. Ekici, C. Engel, M. Eriksson, P. A. Fasching, J. Figueroa, H. Flyger, A. Försti, M. Gabrielson, M. Gago-Dominguez, V. Georgoulas, F. Gil, G. G. Giles, G. Glendon, E. B. G. Garcia, G. I. G. Alnæs, P. Guénel, A. Hadjisavvas, L. Haeberle, E. Hahnen, P. Hall, U. Hamann, E. F. Harkness, J. M. Hartikainen, M. Hartman, W. He, B. A. M. Heemskerk-Gerritsen, P. Hillemanns, F. B. L. Hogervorst, A. Hollestelle, W. K. Ho, M. J. Hoening, A. Howell, K. Humphreys, F. Idris, A. Jakubowska, A. Jung, P. M. Kapoor, M. J. Kerin, E. Khusnutdinova, S. W. Kim, Y. D. Ko, V. M. Kosma, V. N. Kristensen, K. Kyriacou, I. M. M. Lakeman, J. W. Lee, M. H. Lee, J. Li, A. Lindblom, W. Y. Lo, M. A. Loizidou, A. Lophatananon, J. Lubiński, R. J. MacInnis, M. J. Madsen, A. Mannermaa, M. Manoochehri, S. Manoukian, S. Margolin, M. E. Martinez, T. Maurer, D. Mavroudis, C. McLean, A. Meindl, A. R. Mensenkamp, K. Michailidou, N. Miller, N. A. Mohd Taib, K. Muir, A. M. Mulligan, H. Nevanlinna, W. G. Newman, B. G. Nordestgaard, P. S. Ng, J. C. Oosterwijk, S. K. Park, T. W. Park-Simon, J. I. A. Perez, P. Peterlongo, D. J. Porteous, K. Prajzandanc, D. Prokofyeva, P. Radice, M. U. Rashid, V. Rhenius, M. A. Rookus, T. Rüdiger, E. Saloustros, E. J.

- Sawyer, R. K. Schmutzler, A. Schneeweiss, P. Schürmann, M. Shah, C. Sohn, M. C. Southey, H. Surowy, M. Suvanto, S. Thanasiythichai, I. Tomlinson, D. Torres, T. Truong, M. Tzardi, Y. Valova, C. J. van Asperen, R. M. Van Dam, A. M. W. van den Ouweland, L. E. van der Kolk, E. M. van Veen, C. Wendt, J. A. Williams, X. R. Yang, S. Y. Yoon, M. P. Zamora, D. G. Evans, M. de la Hoya, J. Simard, A. C. Antoniou, Å Borg, I. L. Andrulis, J. Chang-Claude, M. García-Closas, G. Chenevix-Trench, R. L. Milne, P. D. P. Pharoah, M. K. Schmidt, A. B. Spurdle, M. P. G. Vreeswijk, J. Benitez, A. M. Dunning, A. Kvist, S. H. Teo, P. Devilee, and D. F. Easton. 2021. 'Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women', *N Engl J Med*, 384: 428-39.
- Eusebi, Leonardo Henry, Andrea Telese, Giovanni Marasco, Franco Bazzoli, and Rocco Maurizio Zagari. 2020. 'Gastric cancer prevention strategies: A global perspective', *Journal of Gastroenterology and Hepatology*, 35: 1495-502.
- Felicio, Paula S., Rebeca S. Grasel, Natalia Campacci, Andre E. de Paula, Henrique C. R. Galvão, Giovana T. Torrezan, Cristina S. Sabato, Gabriela C. Fernandes, Cristiano P. Souza, Rodrigo D. Michelli, Carlos E. Andrade, Bruna Durães De Figueiredo Barros, Marcus M. Matsushita, Timothée Revil, Jiannis Ragoussis, Fergus J. Couch, Steven N. Hart, Rui M. Reis, Matias E. Melendez, Patricia N. Tonin, Dirce M. Carraro, and Edenir I. Palmero. 2021. 'Whole-exome sequencing of non-BRCA1/BRCA2 mutation carrier cases at high-risk for hereditary breast/ovarian cancer', *Human Mutation*, 42: 290-99.
- Ferlay, J., M. Colombet, I. Soerjomataram, D. M. Parkin, M. Piñeros, A. Znaor, and F. Bray. 2021. 'Cancer statistics for the year 2020: An overview', *Int J Cancer*.
- Fewings, E., A. Larionov, J. Redman, M. A. Goldgraben, J. Scarth, S. Richardson, C. Brewer, R. Davidson, I. Ellis, D. G. Evans, D. Halliday, L. Izatt, P. Marks, V. McConnell, L. Verbist, R. Mayes, G. R. Clark, J. Hadfield, S. F. Chin, M. R. Teixeira, O. T. Giger, R. Hardwick, M. di Pietro, M. O'Donovan, P. Pharoah, C. Caldas, R. C. Fitzgerald, and M. Tischkowitz. 2018. 'Germline pathogenic variants in PALB2 and other cancer-predisposing genes in families with hereditary diffuse gastric cancer without CDH1 mutation: a whole-exome sequencing study', *Lancet Gastroenterol Hepatol*, 3: 489-98.
- Figueiredo, J., S. Melo, P. Carneiro, A. M. Moreira, M. S. Fernandes, A. S. Ribeiro, P. Guilford, J. Paredes, and R. Seruca. 2019. 'Clinical spectrum and pleiotropic nature of CDH1 germline mutations', *J Med Genet*, 56: 199-208.

References

- Fitzgerald, R. C., R. Hardwick, D. Huntsman, F. Carneiro, P. Guilford, V. Blair, D. C. Chung, J. Norton, K. Ragunath, J. H. Van Krieken, S. Dwerryhouse, and C. Caldas. 2010. 'Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research', *J Med Genet*, 47: 436-44.
- Frebourg, T., C. Oliveira, P. Hochain, R. Karam, S. Manouvrier, C. Graziadio, M. Vekemans, A. Hartmann, S. Baert-Desurmont, C. Alexandre, S. Lejeune Dumoulin, C. Marroni, C. Martin, S. Castedo, M. Lovett, J. Winston, J. C. Machado, T. Attié, E. W. Jabs, J. Cai, P. Pellerin, J. P. Triboulet, M. Scotte, F. Le Pessot, A. Hedouin, F. Carneiro, M. Blayau, and R. Seruca. 2006. 'Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer', *J Med Genet*, 43: 138-42.
- Gall, T. M., and A. E. Frampton. 2013. 'Gene of the month: E-cadherin (CDH1)', *J Clin Pathol*, 66: 928-32.
- Garcia-Pelaez, J., R. Barbosa-Matos, S. Lobo, A. Dias, L. Garrido, S. Castedo, S. Sousa, H. Pinheiro, L. Sousa, R. Monteiro, J. J. Maqueda, S. Fernandes, F. Carneiro, N. Pinto, C. Lemos, C. Pinto, M. R. Teixeira, S. Aretz, S. Bajalica-Lagercrantz, J. Balmaña, A. Blatnik, P. R. Benusiglio, M. Blanluet, V. Bours, H. Brems, J. Brunet, D. Calistri, G. Capellá, S. Carrera, C. Colas, K. Dahan, R. de Putter, C. Desseignés, E. Domínguez-Garrido, C. Egas, D. G. Evans, D. Feret, E. Fewings, R. C. Fitzgerald, F. Coulet, M. Garcia-Barcina, M. Genuardi, L. Golmard, K. Hackmann, H. Hanson, E. Holinski-Feder, R. Hüneburg, M. Krajc, K. Lagerstedt-Robinson, C. Lázaro, M. J. L. Ligtenberg, C. Martínez-Bouzas, S. Merino, G. Michils, S. Novaković, A. Patiño-García, G. N. Ranzani, E. Schröck, I. Silva, C. Silveira, J. L. Soto, I. Spier, V. Steinke-Lange, G. Tedaldi, M. I. Tejada, E. R. Woodward, M. Tischkowitz, N. Hoogerbrugge, and C. Oliveira. 2023. 'Genotype-first approach to identify associations between CDH1 germline variants and cancer phenotypes: a multicentre study by the European Reference Network on Genetic Tumour Risk Syndromes', *Lancet Oncol*, 24: 91-106.
- Garcia-Pelaez, J., R. Barbosa-Matos, C. São José, S. Sousa, I. Gullo, N. Hoogerbrugge, F. Carneiro, and C. Oliveira. 2022. 'Gastric cancer genetic predisposition and clinical presentations: Established heritable causes and potential candidate genes', *Eur J Med Genet*, 65: 104401.
- Gaston, D., S. Hansford, C. Oliveira, M. Nightingale, H. Pinheiro, C. Macgillivray, P. Kaurah, A. L. Rideout, P. Steele, G. Soares, W. Y. Huang, S. Whitehouse, S. Blowers, M. A. LeBlanc, H. Jiang, W. Greer, M. E. Samuels, A. Orr, C. V. Fernandez, J. Majewski, M. Ludman, S. Dyack, L. S. Penney, C. R. McMaster, D. Huntsman, and K. Bedard.

2014. 'Germline mutations in MAP3K6 are associated with familial gastric cancer', *PLoS Genet*, 10: e1004669.
- Ghoumid, J., M. Stichelbout, A. S. Jourdain, F. Frenois, S. Lejeune-Dumoulin, M. P. Alex-Cordier, M. Lebrun, P. Guerreschi, V. Duquennoy-Martinot, M. Vinchon, J. Ferri, M. Jung, S. Vicaire, C. Vanlerberghe, F. Escande, F. Petit, and S. Manouvrier-Hanu. 2017. 'Blepharocheilodontic syndrome is a CDH1 pathway-related disorder due to mutations in CDH1 and CTNND1', *Genet Med*, 19: 1013-21.
- Gloushankova, N. A., S. N. Rubtsova, and I. Y. Zhitnyak. 2017. 'Cadherin-mediated cell-cell interactions in normal and cancer cells', *Tissue Barriers*, 5: e1356900.
- Goseki, N., T. Takizawa, and M. Koike. 1992. 'Differences in the mode of the extension of gastric cancer classified by histological type: new histological classification of gastric carcinoma', *Gut*, 33: 606-12.
- Grady, W. M., J. Willis, P. J. Guilford, A. K. Dunbier, T. T. Toro, H. Lynch, G. Wiesner, K. Ferguson, C. Eng, J. G. Park, S. J. Kim, and S. Markowitz. 2000. 'Methylation of the CDH1 promoter as the second genetic hit in hereditary diffuse gastric cancer', *Nat Genet*, 26: 16-7.
- Guerra, J., C. Pinto, P. Pinto, M. Pinheiro, C. Santos, A. Peixoto, C. Escudeiro, A. Barbosa, M. Porto, I. Francisco, P. Lopes, A. R. Isidoro, A. L. Cunha, C. Albuquerque, I. Claro, C. Oliveira, J. Silva, and M. R. Teixeira. 2023. 'Frequency of CDH1, CTNNA1 and CTNND1 Germline Variants in Families with Diffuse and Mixed Gastric Cancer', *Cancers (Basel)*, 15.
- Guilford, P., J. Hopkins, J. Harraway, M. McLeod, N. McLeod, P. Harawira, H. Taite, R. Scoular, A. Miller, and A. E. Reeve. 1998. 'E-cadherin germline mutations in familial gastric cancer', *Nature*, 392: 402-5.
- Gullo, I., F. Carneiro, C. Oliveira, and G. M. Almeida. 2018. 'Heterogeneity in Gastric Cancer: From Pure Morphology to Molecular Classifications', *Pathobiology*, 85: 50-63.
- Gullo, I., J. Carvalho, D. Martins, D. Lemos, A. R. Monteiro, M. Ferreira, K. Das, P. Tan, C. Oliveira, F. Carneiro, and P. Oliveira. 2018. 'The Transcriptomic Landscape of Gastric Cancer: Insights into Epstein-Barr Virus Infected and Microsatellite Unstable Tumors', *Int J Mol Sci*, 19.
- Gullo, I., F. Grillo, L. Mastracci, A. Vanoli, F. Carneiro, L. Saragoni, F. Limarzi, J. Ferro, P. Parente, and M. Fassan. 2020. 'Precancerous lesions of the stomach, gastric cancer and hereditary gastric cancer syndromes', *Pathologica*, 112: 166-85.

References

- Gullo, I., R. S. van der Post, and F. Carneiro. 2021. 'Recent advances in the pathology of heritable gastric cancer syndromes', *Histopathology*, 78: 125-47.
- Hakkaart, C., L. Ellison-Loschmann, R. Day, A. Sporle, J. Koea, P. Harawira, S. Cheng, M. Gray, T. Whaanga, N. Pearce, and P. Guilford. 2019. 'Germline CDH1 mutations are a significant contributor to the high frequency of early-onset diffuse gastric cancer cases in New Zealand Māori', *Fam Cancer*, 18: 83-90.
- Hansford, S., P. Kaurah, H. Li-Chang, M. Woo, J. Senz, H. Pinheiro, K. A. Schrader, D. F. Schaeffer, K. Shumansky, G. Zogopoulos, T. A. Santos, I. Claro, J. Carvalho, C. Nielsen, S. Padilla, A. Lum, A. Talhouk, K. Baker-Lange, S. Richardson, I. Lewis, N. M. Lindor, E. Pennell, A. MacMillan, B. Fernandez, G. Keller, H. Lynch, S. P. Shah, P. Guilford, S. Gallinger, G. Corso, F. Roviello, C. Caldas, C. Oliveira, P. D. Pharoah, and D. G. Huntsman. 2015. 'Hereditary Diffuse Gastric Cancer Syndrome: CDH1 Mutations and Beyond', *JAMA Oncol*, 1: 23-32.
- Hosoya, K., S. Yamashita, T. Ando, T. Nakajima, F. Itoh, and T. Ushijima. 2009. 'Adenomatous polyposis coli 1A is likely to be methylated as a passenger in human gastric carcinogenesis', *Cancer Lett*, 285: 182-9.
- Humphries, M. J., and P. Newham. 1998. 'The structure of cell-adhesion molecules', *Trends Cell Biol*, 8: 78-83.
- Ichikawa, H., T. Wakai, M. Nagahashi, Y. Shimada, T. Hanyu, Y. Kano, Y. Muneoka, T. Ishikawa, K. Takizawa, Y. Tajima, J. Sakata, T. Kobayashi, H. Kemeyama, H. Yabusaki, S. Nakagawa, N. Sato, T. Kawasaki, K. Homma, S. Okuda, S. Lyle, and K. Takabe. 2018. 'Pathogenic germline BRCA1/2 mutations and familial predisposition to gastric cancer', *JCO Precis Oncol*, 2.
- Idos, G., and L. Valle. 1993. 'Lynch Syndrome.' in M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp and A. Amemiya (eds.), *GeneReviews*(®) (University of Washington, Seattle
- Copyright © 1993-2023, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.: Seattle (WA)).
- Ilic, M., and I. Ilic. 2022. 'Epidemiology of stomach cancer', *World J Gastroenterol*, 28: 1187-203.
- Isaac, D., L. Karapetyan, and D. Tamkus. 2018. 'Association of Germline PALB2 Mutation and Response to Platinum-Based Chemotherapy in Metastatic Breast Cancer: A Case Series', *JCO Precis Oncol*, 2: 1-5.

- Jacobs, M. F., H. Dust, E. Koeppe, S. Wong, M. Mulholland, E. Y. Choi, H. Appelman, and E. M. Stoffel. 2019. 'Outcomes of Endoscopic Surveillance in Individuals With Genetic Predisposition to Hereditary Diffuse Gastric Cancer', *Gastroenterology*, 157: 87-96.
- Jakubowska, A., K. Nej, T. Huzarski, R. J. Scott, and J. Lubiński. 2002. 'BRCA2 gene mutations in families with aggregations of breast and stomach cancers', *British Journal of Cancer*, 87: 888-91.
- Jin, Mei Hua, and Do-Youn Oh. 2019. 'ATM in DNA repair in cancer', *Pharmacology & Therapeutics*, 203: 107391.
- Kari, Vijayalakshmi, Sanjay Kumar Raul, Jana Maria Henck, Julia Kitz, Frank Kramer, Robyn Laura Kosinsky, Nadine Übelmesser, Wael Yassin Mansour, Jessica Eggert, Melanie Spitzner, Zeynab Najafova, Holger Bastians, Marian Grade, Jochen Gaedcke, Florian Wegwitz, and Steven A. Johnsen. 2019. 'The histone methyltransferase DOT1L is required for proper DNA damage response, DNA repair, and modulates chemotherapy responsiveness', *Clinical Epigenetics*, 11: 4.
- Kaszak, I., O. Witkowska-Piłaszewicz, Z. Niewiadomska, B. Dworecka-Kaszak, F. Ngosa Toka, and P. Jurka. 2020. 'Role of Cadherins in Cancer-A Review', *Int J Mol Sci*, 21.
- Keller, G., H. Vogelsang, I. Becker, S. Plaschke, K. Ott, G. Suriano, A. R. Mateus, R. Seruca, K. Biedermann, D. Huntsman, C. Döring, E. Holinski-Feder, A. Neutzling, J. R. Siewert, and H. Höfler. 2004. 'Germline mutations of the E-cadherin(CDH1) and TP53 genes, rather than of RUNX3 and HPP1, contribute to genetic predisposition in German gastric cancer patients', *J Med Genet*, 41: e89.
- Kievit, A., F. Tessadori, H. Douben, I. Jordens, M. Maurice, J. Hoogeboom, R. Hennekam, S. Nampoothiri, H. Kayserili, M. Castori, M. Whiteford, C. Motter, C. Melder, M. Cunningham, A. Hing, N. M. Kokitsu-Nakata, S. Vendramini-Pittoli, A. Richieri-Costa, A. F. Baas, C. C. Breugem, K. Duran, M. Massink, P. W. B. Derksen, IJcken W. F. J. van, L. van Unen, F. Santos-Simarro, P. Lapunzina, V. L. Gil-da Silva Lopes, E. Lustosa-Mendes, M. Krall, A. Slavotinek, V. Martinez-Glez, J. Bakkers, K. L. I. van Gassen, A. de Klein, M. H. van den Boogaard, and G. van Haaften. 2018. 'Variants in members of the cadherin-catenin complex, CDH1 and CTNND1, cause blepharocheilodontic syndrome', *Eur J Hum Genet*, 26: 210-19.
- Kim, J., D. Braun, C. Ukaegbu, T. G. Dhingra, F. Kastrinos, G. Parmigiani, S. Syngal, and M. B. Yurgelun. 2020. 'Clinical Factors Associated With Gastric Cancer in Individuals With Lynch Syndrome', *Clin Gastroenterol Hepatol*, 18: 830-37.e1.

References

- Kluijft, I., E. J. Siemerink, M. G. Ausems, T. A. van Os, D. de Jong, J. Simões-Correia, J. H. van Krieken, M. J. Ligtenberg, J. Figueiredo, E. van Riel, R. H. Sijmons, J. T. Plukker, R. van Hillegersberg, E. Dekker, C. Oliveira, A. Cats, and N. Hoogerbrugge. 2012. 'CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling', *Int J Cancer*, 131: 367-76.
- Knudson, A. G. 2001. 'Two genetic hits (more or less) to cancer', *Nat Rev Cancer*, 1: 157-62.
- Kobielak, A., and E. Fuchs. 2004. 'Alpha-catenin: at the junction of intercellular adhesion and actin dynamics', *Nat Rev Mol Cell Biol*, 5: 614-25.
- Kupfer, S. S. 2017. 'Gaining Ground in the Genetics of Gastric Cancer', *Gastroenterology*, 152: 926-28.
- Latchford, A. R., K. Neale, R. K. Phillips, and S. K. Clark. 2012. 'Juvenile polyposis syndrome: a study of genotype, phenotype, and long-term outcome', *Dis Colon Rectum*, 55: 1038-43.
- Lauren, P. 1965. 'The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification', *Acta Pathol Microbiol Scand*, 64: 31-49.
- Le, D. T., J. N. Durham, K. N. Smith, H. Wang, B. R. Bartlett, L. K. Aulakh, S. Lu, H. Kemberling, C. Wilt, B. S. Lubner, F. Wong, N. S. Azad, A. A. Rucki, D. Laheru, R. Donehower, A. Zaheer, G. A. Fisher, T. S. Crocenzi, J. J. Lee, T. F. Greten, A. G. Duffy, K. K. Ciombor, A. D. Eyring, B. H. Lam, A. Joe, S. P. Kang, M. Holdhoff, L. Danilova, L. Cope, C. Meyer, S. Zhou, R. M. Goldberg, D. K. Armstrong, K. M. Bever, A. N. Fader, J. Taube, F. Housseau, D. Spetzler, N. Xiao, D. M. Pardoll, N. Papadopoulos, K. W. Kinzler, J. R. Eshleman, B. Vogelstein, R. A. Anders, and L. A. Diaz, Jr. 2017. 'Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade', *Science*, 357: 409-13.
- Le, D. T., J. N. Uram, H. Wang, B. R. Bartlett, H. Kemberling, A. D. Eyring, A. D. Skora, B. S. Lubner, N. S. Azad, D. Laheru, B. Biedrzycki, R. C. Donehower, A. Zaheer, G. A. Fisher, T. S. Crocenzi, J. J. Lee, S. M. Duffy, R. M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R. H. Hruban, L. D. Wood, N. Cuka, D. M. Pardoll, N. Papadopoulos, K. W. Kinzler, S. Zhou, T. C. Cornish, J. M. Taube, R. A. Anders, J. R. Eshleman, B. Vogelstein, and L. A. Diaz, Jr. 2015. 'PD-1 Blockade in Tumors with Mismatch-Repair Deficiency', *N Engl J Med*, 372: 2509-20.
- Lee, C. Y. C., A. Olivier, J. Honing, A. M. Lydon, S. Richardson, M. O'Donovan, M. Tischkowitz, R. C. Fitzgerald, and M. di Pietro. 2023. 'Endoscopic surveillance with

- systematic random biopsy for the early diagnosis of hereditary diffuse gastric cancer: a prospective 16-year longitudinal cohort study', *Lancet Oncol*, 24: 107-16.
- Lee, J. E. A., N. Li, S. M. Rowley, D. Cheasley, M. Zethoven, S. McInerny, K. L. Goringe, P. A. James, and I. G. Campbell. 2018. 'Molecular analysis of PALB2-associated breast cancers', *J Pathol*, 245: 53-60.
- Lee, K., K. Krempely, M. E. Roberts, M. J. Anderson, F. Carneiro, E. Chao, K. Dixon, J. Figueiredo, R. Ghosh, D. Huntsman, P. Kaurah, C. Kesserwan, T. Landrith, S. Li, A. R. Mensenkamp, C. Oliveira, C. Pardo, T. Pesaran, M. Richardson, T. P. Slavin, A. B. Spurdle, M. Trapp, L. Witkowski, C. S. Yi, L. Zhang, S. E. Plon, K. A. Schrader, and R. Karam. 2018. 'Specifications of the ACMG/AMP variant curation guidelines for the analysis of germline CDH1 sequence variants', *Hum Mutat*, 39: 1553-68.
- Li, Anqi, Felipe C. Geyer, Pedro Blecua, Ju Youn Lee, Pier Selenica, David N. Brown, Fresia Pareja, Simon S. K. Lee, Rahul Kumar, Barbara Rivera, Rui Bi, Salvatore Piscuoglio, Hannah Y. Wen, John R. Lozada, Rodrigo Gularte-Mérida, Luca Cavallone, Zoulikha Rezoug, Tu Nguyen-Dumont, Paolo Peterlongo, Carlo Tondini, Thorkild Terkelsen, Karina Rønlund, Susanne E. Boonen, Arto Mannerma, Robert Winqvist, Marketa Janatova, Pathmanathan Rajadurai, Bing Xia, Larry Norton, Mark E. Robson, Pei-Sze Ng, Lai-Meng Looi, Melissa C. Southey, Britta Weigelt, Teo Soo-Hwang, Marc Tischkowitz, William D. Foulkes, Jorge S. Reis-Filho, Morteza Aghmesheh, David Amor, Leslie Andrews, Yoland Antill, Rosemary Balleine, Jonathan Beesley, Anneke Blackburn, Michael Bogwitz, Melissa Brown, Matthew Burgess, Jo Burke, Phyllis Butow, Liz Caldon, Ian Campbell, Alice Christian, Christine Clarke, Paul Cohen, Ashley Crook, James Cui, Margaret Cummings, Sarah-Jane Dawson, Anna De Fazio, Martin Delatycki, Alex Dobrovic, Tracy Dudding, Pascal Duijf, Edward Edkins, Stacey Edwards, Gelareh Farshid, Andrew Fellows, Michael Field, James Flanagan, Peter Fong, John Forbes, Laura Forrest, Stephen Fox, Juliet French, Michael Friedlander, David Gallego Ortega, Michael Gattas, Graham Giles, Grantley Gill, Margaret Gleeson, Sian Greening, Eric Haan, Marion Harris, Nick Hayward, Ian Hickie, John Hopper, Clare Hunt, Paul James, Mark Jenkins, Rick Kefford, Maira Kentwell, Judy Kirk, James Kollias, Sunil Lakhani, Geoff Lindeman, Lara Lipton, Lizz Lobb, Sheau Lok, Finlay Macrea, Graham Mann, Deb Marsh, Sue-Anne McLachlan, Bettina Meiser, Roger Milne, Sophie Nightingale, Shona O'Connell, Nick Pachter, Briony Patterson, Kelly Phillips, Mona Saleh, Elizabeth Salisbury, Christobel Saunders, Jodi Saunus, Clare Scott, Rodney Scott, Adrienne Sexton, Andrew Shelling, Peter Simpson, Allan

References

- Spigelman, Mandy Spurdle, Jennifer Stone, Jessica Taylor, Heather Thorne, Alison Trainer, Georgia Trench, Kathy Tucker, Jane Visvader, Logan Walker, Mathew Wallis, Rachael Williams, Ingrid Winship, Kathy Wu, Mary Anne Young, and Investigators kConFab. 2019. 'Homologous recombination DNA repair defects in PALB2-associated breast cancers', *npj Breast Cancer*, 5: 23.
- Li, J., S. L. Woods, S. Healey, J. Beesley, X. Chen, J. S. Lee, H. Sivakumaran, N. Wayte, K. Nones, J. J. Waterfall, J. Pearson, A. M. Patch, J. Senz, M. A. Ferreira, P. Kaurah, R. Mackenzie, A. Heravi-Moussavi, S. Hansford, T. R. M. Lannagan, A. B. Spurdle, P. T. Simpson, L. da Silva, S. R. Lakhani, A. D. Clouston, M. Bettington, F. Grimpen, R. A. Busuttil, N. Di Costanzo, A. Boussioutas, M. Jeanjean, G. Chong, A. Fabre, S. Olschwang, G. J. Faulkner, E. Bellos, L. Coin, K. Rioux, O. F. Bathe, X. Wen, H. C. Martin, D. W. Neklason, S. R. Davis, R. L. Walker, K. A. Calzone, I. Avital, T. Heller, C. Koh, M. Pineda, U. Rudloff, M. Quezado, P. N. Pichurin, P. J. Hulick, S. M. Weissman, A. Newlin, W. S. Rubinstein, J. E. Sampson, K. Hamman, D. Goldgar, N. Poplawski, K. Phillips, L. Schofield, J. Armstrong, C. Kiraly-Borri, G. K. Suthers, D. G. Huntsman, W. D. Foulkes, F. Carneiro, N. M. Lindor, S. L. Edwards, J. D. French, N. Waddell, P. S. Meltzer, D. L. Worthley, K. A. Schrader, and G. Chenevix-Trench. 2016. 'Point Mutations in Exon 1B of APC Reveal Gastric Adenocarcinoma and Proximal Polyposis of the Stomach as a Familial Adenomatous Polyposis Variant', *Am J Hum Genet*, 98: 830-42.
- Li, S., V. Silvestri, G. Leslie, T. R. Rebbeck, S. L. Neuhausen, J. L. Hopper, H. R. Nielsen, A. Lee, X. Yang, L. McGuffog, M. T. Parsons, I. L. Andrulis, N. Arnold, M. Belotti, Å Borg, B. Buecher, S. S. Buys, S. M. Caputo, W. K. Chung, C. Colas, S. V. Colonna, J. Cook, M. B. Daly, M. de la Hoya, A. de Pauw, H. Delhomelle, J. Eason, C. Engel, D. G. Evans, U. Faust, T. N. Fehm, F. Fostira, G. Fountzilas, M. Frone, V. Garcia-Barberan, P. Garre, M. Gauthier-Villars, A. Gehrig, G. Glendon, D. E. Goldgar, L. Golmard, M. H. Greene, E. Hahnen, U. Hamann, H. Hanson, T. Hassan, J. Hentschel, J. Horvath, L. Izatt, R. Janavicius, Y. Jiao, E. M. John, B. Y. Karlan, S. W. Kim, I. Konstantopoulou, A. Kwong, A. Laugé, J. W. Lee, F. Lesueur, N. Mebirouk, A. Meindl, E. Mouret-Fourme, H. Musgrave, J. Ngeow Yuen Yie, D. Niederacher, S. K. Park, I. S. Pedersen, J. Ramser, S. J. Ramus, J. Rantala, M. U. Rashid, F. Reichl, J. Ritter, A. Rump, M. Santamariña, C. Saule, G. Schmidt, R. K. Schmutzler, L. Senter, S. Shariff, C. F. Singer, M. C. Southey, D. Stoppa-Lyonnet, C. Sutter, Y. Tan, S. H. Teo, M. B. Terry, M. Thomassen, M. Tischkowitz, A. E. Toland, D. Torres, A. Vega, S. A. Wagner, S. Wang-

- Gohrke, B. Wappenschmidt, B. H. F. Weber, D. Yannoukakos, A. B. Spurdle, D. F. Easton, G. Chenevix-Trench, L. Ottini, and A. C. Antoniou. 2022. 'Cancer Risks Associated With BRCA1 and BRCA2 Pathogenic Variants', *J Clin Oncol*, 40: 1529-41.
- Lim, B. W. X., N. Li, S. Mahale, S. McInerney, M. Zethoven, S. M. Rowley, J. Huynh, T. Wang, J. E. A. Lee, M. Friedman, L. Devereux, R. J. Scott, E. K. Sloan, P. A. James, and I. G. Campbell. 2023. 'Somatic inactivation of breast cancer predisposition genes in tumors associated with pathogenic germline variants', *J Natl Cancer Inst*, 115: 181-89.
- Lin, Xiaoting, Dongshao Chen, Cheng Zhang, Xiaotian Zhang, Zhongwu Li, Bin Dong, Jing Gao, and Lin Shen. 2018. 'Augmented antitumor activity by olaparib plus AZD1775 in gastric cancer through disrupting DNA damage repair pathways and DNA damage checkpoint', *Journal of Experimental & Clinical Cancer Research*, 37: 129.
- Liu, Z. X., X. L. Zhang, Q. Zhao, Y. Chen, H. Sheng, C. Y. He, Y. T. Sun, M. Y. Lai, M. Q. Wu, Z. X. Zuo, W. Wang, Z. W. Zhou, F. H. Wang, Y. H. Li, R. H. Xu, and M. Z. Qiu. 2022. 'Whole-Exome Sequencing Among Chinese Patients With Hereditary Diffuse Gastric Cancer', *JAMA Netw Open*, 5: e2245836.
- Lo, W., B. Zhu, A. Sabesan, H. H. Wu, A. Powers, R. A. Sorber, S. Ravichandran, I. Chen, L. A. McDuffie, H. S. Quadri, J. D. Beane, K. Calzone, M. M. Miettinen, S. M. Hewitt, C. Koh, T. Heller, S. Wacholder, and U. Rudloff. 2019. 'Associations of CDH1 germline variant location and cancer phenotype in families with hereditary diffuse gastric cancer (HDGC)', *J Med Genet*, 56: 370-79.
- Lobo, S., P. R. Benusiglio, F. Coulet, L. Boussemart, L. Golmard, I. Spier, R. Hüneburg, S. Aretz, C. Colas, and C. Oliveira. 2021. 'Cancer predisposition and germline CTNNA1 variants', *Eur J Med Genet*, 64: 104316.
- Lordick, F., F. Carneiro, S. Cascinu, T. Fleitas, K. Haustermans, G. Piessen, A. Vogel, and E. C. Smyth. 2022. 'Gastric cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up', *Ann Oncol*, 33: 1005-20.
- Lu, Charles, Mingchao Xie, Michael C. Wendl, Jiayin Wang, Michael D. McLellan, Mark D. M. Leiserson, Kuan-lin Huang, Matthew A. Wyczalkowski, Reyka Jayasinghe, Tapahsama Banerjee, Jie Ning, Piyush Tripathi, Qunyuan Zhang, Beifang Niu, Kai Ye, Heather K. Schmidt, Robert S. Fulton, Joshua F. McMichael, Prag Batra, Cyriac Kandoth, Maheetha Bharadwaj, Daniel C. Koboldt, Christopher A. Miller, Krishna L. Kanchi, James M. Eldred, David E. Larson, John S. Welch, Ming You, Bradley A. Ozenberger, Ramaswamy Govindan, Matthew J. Walter, Matthew J. Ellis, Elaine R. Mardis, Timothy A. Graubert, John F. Diersio, Timothy J. Ley, Richard K. Wilson, Paul

References

- J. Goodfellow, Benjamin J. Raphael, Feng Chen, Kimberly J. Johnson, Jeffrey D. Parvin, and Li Ding. 2015. 'Patterns and functional implications of rare germline variants across 12 cancer types', *Nature Communications*, 6: 10086.
- Machado, J. C., P. Soares, F. Carneiro, A. Rocha, S. Beck, N. Blin, G. Berx, and M. Sobrinho-Simões. 1999. 'E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas', *Lab Invest*, 79: 459-65.
- Majewski, I. J., I. Kluijft, A. Cats, T. S. Scerri, D. de Jong, R. J. Kluin, S. Hansford, F. B. Hogervorst, A. J. Bosma, I. Hofland, M. Winter, D. Huntsman, J. Jonkers, M. Bahlo, and R. Bernards. 2013. 'An α -E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer', *J Pathol*, 229: 621-9.
- Mandelker, D., M. Donoghue, S. Talukdar, C. Bandlamudi, P. Srinivasan, M. Vivek, S. Jezdic, H. Hanson, K. Snape, A. Kulkarni, L. Hawkes, J. Y. Douillard, S. E. Wallace, E. Rial-Sebbag, F. Meric-Bersntam, A. George, D. Chubb, C. Loveday, M. Ladanyi, M. F. Berger, B. S. Taylor, and C. Turnbull. 2019. 'Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group', *Ann Oncol*, 30: 1221-31.
- Mandelker, D., L. Zhang, Y. Kemel, Z. K. Stadler, V. Joseph, A. Zehir, N. Pradhan, A. Arnold, M. F. Walsh, Y. Li, A. R. Balakrishnan, A. Syed, M. Prasad, K. Nafa, M. I. Carlo, K. A. Cadoo, M. Sheehan, M. H. Fleischut, E. Salo-Mullen, M. Trottier, S. M. Lipkin, A. Lincoln, S. Mukherjee, V. Ravichandran, R. Cambria, J. Galle, W. Abida, M. E. Arcila, R. Benayed, R. Shah, K. Yu, D. F. Bajorin, J. A. Coleman, S. D. Leach, M. A. Lowery, J. Garcia-Aguilar, P. W. Kantoff, C. L. Sawyers, M. N. Dickler, L. Saltz, R. J. Motzer, E. M. O'Reilly, H. I. Scher, J. Baselga, D. S. Klimstra, D. B. Solit, D. M. Hyman, M. F. Berger, M. Ladanyi, M. E. Robson, and K. Offit. 2017. 'Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing', *Jama*, 318: 825-35.
- Marwitz, T., R. Hüneburg, I. Spier, J. F. Lau, G. Kristiansen, P. Lingohr, J. C. Kalff, S. Aretz, J. Nattermann, and C. P. Strassburg. 2020. 'Hereditary Diffuse Gastric Cancer: A Comparative Cohort Study According to Pathogenic Variant Status', *Cancers (Basel)*, 12.
- Masciari, Serena, Akriti Dewanwala, Elena M. Stoffel, Gregory Y. Lauwers, Hui Zheng, Maria Isabel Achatz, Douglas Riegert-Johnson, Lenka Foretova, Edaise M. Silva, Lisa Digianni, Sigitas J. Verselis, Katherine Schneider, Frederick P. Li, Joseph Fraumeni,

- Judy E. Garber, and Sapna Syngal. 2011. 'Gastric cancer in individuals with Li-Fraumeni syndrome', *Genetics in Medicine*, 13: 651-57.
- McBride, Kate A., Mandy L. Ballinger, Emma Killick, Judy Kirk, Martin H. N. Tattersall, Rosalind A. Eeles, David M. Thomas, and Gillian Mitchell. 2014. 'Li-Fraumeni syndrome: cancer risk assessment and clinical management', *Nature Reviews Clinical Oncology*, 11: 260-71.
- Ming, S. C. 1977. 'Gastric carcinoma. A pathobiological classification', *Cancer*, 39: 2475-85.
- Mitsui, Yasuhiro, Reiko Yokoyama, Shota Fujimoto, Kaizo Kagemoto, Shinji Kitamura, Koichi Okamoto, Naoki Muguruma, Yoshimi Bando, Hidetaka Eguchi, Yasushi Okazaki, Hideyuki Ishida, and Tetsuji Takayama. 2018. 'First report of an Asian family with gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) revealed with the germline mutation of the APC exon 1B promoter region', *Gastric Cancer*, 21: 1058-63.
- Møller, P., T. T. Seppälä, I. Bernstein, E. Holinski-Feder, P. Sala, D. Gareth Evans, A. Lindblom, F. Macrae, I. Blanco, R. H. Sijmons, J. Jeffries, H. F. A. Vasen, J. Burn, S. Nakken, E. Hovig, E. A. Rødland, K. Tharmaratnam, W. H. de Vos Tot Nederveen Cappel, J. Hill, J. T. Wijnen, M. A. Jenkins, K. Green, F. Laloo, L. Sunde, M. Mints, L. Bertario, M. Pineda, M. Navarro, M. Morak, L. Renkonen-Sinisalo, M. D. Valentin, I. M. Frayling, J. P. Plazzer, K. Pylvanainen, M. Genuardi, J. P. Mecklin, G. Moeslein, J. R. Sampson, and G. Capella. 2018. 'Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database', *Gut*, 67: 1306-16.
- Moran, A., C. O'Hara, S. Khan, L. Shack, E. Woodward, E. R. Maher, F. Laloo, and D. G. Evans. 2012. 'Risk of cancer other than breast or ovarian in individuals with BRCA1 and BRCA2 mutations', *Fam Cancer*, 11: 235-42.
- Morgan, E., M. Arnold, M. C. Camargo, A. Gini, A. T. Kunzmann, T. Matsuda, F. Meheus, R. H. A. Verhoeven, J. Vignat, M. Laversanne, J. Ferlay, and I. Soerjomataram. 2022. 'The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: A population-based modelling study', *EClinicalMedicine*, 47: 101404.
- Muir, Jennifer, Melyssa Aronson, Mary-Jane Esplen, Aaron Pollett, and Carol J. Swallow. 2016. 'Prophylactic Total Gastrectomy: a Prospective Cohort Study of Long-Term Impact on Quality of Life', *Journal of Gastrointestinal Surgery*, 20: 1950-58.

References

- Nagtegaal, I. D., R. D. Odze, D. Klimstra, V. Paradis, M. Rugge, P. Schirmacher, K. M. Washington, F. Carneiro, and I. A. Cree. 2020. 'The 2019 WHO classification of tumours of the digestive system', *Histopathology*, 76: 182-88.
- Nakamura, K., H. Sugano, and K. Takagi. 1968. 'Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances', *Gan*, 59: 251-8.
- Németh, B. C., and M. Sahin-Tóth. 2014. 'Human cationic trypsinogen (PRSS1) variants and chronic pancreatitis', *Am J Physiol Gastrointest Liver Physiol*, 306: G466-73.
- Nepomuceno, T. C., G. De Gregoriis, F. M. B. de Oliveira, G. Suarez-Kurtz, A. N. Monteiro, and M. A. Carvalho. 2017. 'The Role of PALB2 in the DNA Damage Response and Cancer Predisposition', *Int J Mol Sci*, 18.
- Network, Cancer Genome Atlas Research. 2014. 'Comprehensive molecular characterization of gastric adenocarcinoma', *Nature*, 513: 202-9.
- Oda, T., Y. Kanai, Y. Shimoyama, A. Nagafuchi, S. Tsukita, and S. Hirohashi. 1993. 'Cloning of the human alpha-catenin cDNA and its aberrant mRNA in a human cancer cell line', *Biochem Biophys Res Commun*, 193: 897-904.
- Oliveira, C., J. de Bruin, S. Nabais, M. Ligtenberg, C. Moutinho, F. M. Nagengast, R. Seruca, H. van Krieken, and F. Carneiro. 2004. 'Intragenic deletion of CDH1 as the inactivating mechanism of the wild-type allele in an HDGC tumour', *Oncogene*, 23: 2236-40.
- Ooki, A., and K. Yamaguchi. 2022. 'The dawn of precision medicine in diffuse-type gastric cancer', *Ther Adv Med Oncol*, 14: 17588359221083049.
- Orloff, M., C. Peterson, X. He, S. Ganapathi, B. Heald, Y. R. Yang, G. Bebek, T. Romigh, J. H. Song, W. Wu, S. David, Y. Cheng, S. J. Meltzer, and C. Eng. 2011. 'Germline mutations in MSR1, ASCC1, and CTHRC1 in patients with Barrett esophagus and esophageal adenocarcinoma', *Jama*, 306: 410-9.
- Park, H. K., K. Y. Lee, M. W. Yoo, T. S. Hwang, and H. S. Han. 2016. 'Mixed Carcinoma as an Independent Prognostic Factor in Submucosal Invasive Gastric Carcinoma', *J Korean Med Sci*, 31: 866-72.
- Peixoto, A., P. Pinto, J. Guerra, M. Pinheiro, C. Santos, C. Pinto, R. Santos, C. Escudeiro, C. Bartosch, R. Canário, A. Barbosa, A. Gouveia, A. Petiz, M. H. Abreu, S. Sousa, D. Pereira, J. Silva, and M. R. Teixeira. 2020. 'Tumor Testing for Somatic and Germline BRCA1/BRCA2 Variants in Ovarian Cancer Patients in the Context of Strong Founder Effects', *Front Oncol*, 10: 1318.
- Pinto, P., P. Paulo, C. Santos, P. Rocha, C. Pinto, I. Veiga, M. Pinheiro, A. Peixoto, and M. R. Teixeira. 2016. 'Implementation of next-generation sequencing for molecular diagnosis

- of hereditary breast and ovarian cancer highlights its genetic heterogeneity', *Breast Cancer Res Treat*, 159: 245-56.
- Pócza, T., V. K. Grolmusz, J. Papp, H. Butz, A. Patócs, and A. Bozsik. 2021. 'Germline Structural Variations in Cancer Predisposition Genes', *Front Genet*, 12: 634217.
- Prakash, R., Y. Zhang, W. Feng, and M. Jasin. 2015. 'Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins', *Cold Spring Harb Perspect Biol*, 7: a016600.
- Rahman, N., S. Seal, D. Thompson, P. Kelly, A. Renwick, A. Elliott, S. Reid, K. Spanova, R. Barfoot, T. Chagtai, H. Jayatilake, L. McGuffog, S. Hanks, D. G. Evans, D. Eccles, D. F. Easton, and M. R. Stratton. 2007. 'PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene', *Nat Genet*, 39: 165-7.
- Reid, Sarah, Detlev Schindler, Helmut Hanenberg, Karen Barker, Sandra Hanks, Reinhard Kalb, Kornelia Neveling, Patrick Kelly, Sheila Seal, Marcel Freund, Melanie Wurm, Sat Dev Batish, Francis P. Lach, Sevgi Yetgin, Heidemarie Neitzel, Hany Ariffin, Marc Tischkowitz, Christopher G. Mathew, Arleen D. Auerbach, and Nazneen Rahman. 2007. 'Biallelic mutations in PALB2 cause Fanconi anemia subtype FA-N and predispose to childhood cancer', *Nature Genetics*, 39: 162-64.
- Renault, A. L., N. Mebirouk, L. Fuhrmann, G. Bataillon, E. Cavaciuti, D. Le Gal, E. Girard, T. Popova, P. La Rosa, J. Beauvallet, S. Eon-Marchais, M. G. Dondon, C. D. d'Enghien, A. Laugé, W. Chemlali, V. Raynal, M. Labbé, I. Bièche, S. Baulande, J. O. Bay, P. Berthet, O. Caron, B. Buecher, L. Faivre, M. Fresnay, M. Gauthier-Villars, P. Gesta, N. Janin, S. Lejeune, C. Maugard, S. Moutton, L. Venat-Bouvet, H. Zattara, J. P. Fricker, L. Gladieff, I. Coupier, G. Chenevix-Trench, J. Hall, A. Vincent-Salomon, D. Stoppa-Lyonnet, N. Andrieu, and F. Lesueur. 2018. 'Morphology and genomic hallmarks of breast tumours developed by ATM deleterious variant carriers', *Breast Cancer Res*, 20: 28.
- Repak, R., D. Kohoutova, M. Podhola, S. Rejchrt, M. Minarik, L. Benesova, M. Lesko, and J. Bures. 2016. 'The first European family with gastric adenocarcinoma and proximal polyposis of the stomach: case report and review of the literature', *Gastrointest Endosc*, 84: 718-25.
- Richards, S., N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W. W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, and H. L. Rehm. 2015. 'Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the

References

- American College of Medical Genetics and Genomics and the Association for Molecular Pathology', *Genet Med*, 17: 405-24.
- Roberts, M. E., J. M. O. Ranola, M. L. Marshall, L. R. Susswein, S. Graceffo, K. Bohnert, G. Tsai, R. T. Klein, K. S. Hruska, and B. H. Shirts. 2019. 'Comparison of CDH1 Penetrance Estimates in Clinically Ascertained Families vs Families Ascertained for Multiple Gastric Cancers', *JAMA Oncol*, 5: 1325-31.
- Rocha, J. P., I. Gullo, X. Wen, V. Devezas, M. Baptista, C. Oliveira, and F. Carneiro. 2018. 'Pathological features of total gastrectomy specimens from asymptomatic hereditary diffuse gastric cancer patients and implications for clinical management', *Histopathology*, 73: 878-86.
- Rudloff, U. 2018. 'Gastric adenocarcinoma and proximal polyposis of the stomach: diagnosis and clinical perspectives', *Clin Exp Gastroenterol*, 11: 447-59.
- Sahasrabudhe, R., P. Lott, M. Bohorquez, T. Toal, A. P. Estrada, J. J. Suarez, A. Brea-Fernández, J. Cameselle-Teijeiro, C. Pinto, I. Ramos, A. Mantilla, R. Prieto, A. Corvalan, E. Norero, C. Alvarez, T. Tapia, P. Carvallo, L. M. Gonzalez, A. Cock-Rada, A. Solano, F. Neffa, A. Della Valle, C. Yau, G. Soares, A. Borowsky, N. Hu, L. J. He, X. Y. Han, P. R. Taylor, A. M. Goldstein, J. Torres, M. Echeverry, C. Ruiz-Ponte, M. R. Teixeira, and L. G. Carvajal-Carmona. 2017. 'Germline Mutations in PALB2, BRCA1, and RAD51C, Which Regulate DNA Recombination Repair, in Patients With Gastric Cancer', *Gastroenterology*, 152: 983-86.e6.
- Saksens, N. T., M. P. Krebs, F. E. Schoenmaker-Koller, W. Hicks, M. Yu, L. Shi, L. Rowe, G. B. Collin, J. R. Charette, S. J. Letteboer, K. Neveling, T. W. van Moorsel, S. Abu-Ltaif, E. De Baere, S. Walraedt, S. Banfi, F. Simonelli, F. P. Cremers, C. J. Boon, R. Roepman, B. P. Leroy, N. S. Peachey, C. B. Hoyng, P. M. Nishina, and A. I. den Hollander. 2016. 'Mutations in CTNNA1 cause butterfly-shaped pigment dystrophy and perturbed retinal pigment epithelium integrity', *Nat Genet*, 48: 144-51.
- Santarosa, Manuela, and Alan Ashworth. 2004. 'Haploinsufficiency for tumour suppressor genes: when you don't need to go all the way', *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 1654: 105-22.
- Schneider, K., K. Zelle, K. E. Nichols, and J. Garber. 1993. 'Li-Fraumeni Syndrome.' in M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp and A. Amemiya (eds.), *GeneReviews*(®) (University of Washington, Seattle
- Copyright © 1993-2023, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.: Seattle (WA)).

- Schuetz, J. M., S. Leach, P. Kaurah, J. Jeyes, Y. Butterfield, D. Huntsman, and A. R. Brooks-Wilson. 2012. 'Catenin family genes are not commonly mutated in hereditary diffuse gastric cancer', *Cancer Epidemiol Biomarkers Prev*, 21: 2272-4.
- Serra, O., M. Galán, M. M. Ginesta, M. Calvo, N. Sala, and R. Salazar. 2019. 'Comparison and applicability of molecular classifications for gastric cancer', *Cancer Treat Rev*, 77: 29-34.
- Shirts, B. H., S. Casadei, A. L. Jacobson, M. K. Lee, S. Gulsuner, R. L. Bennett, M. Miller, S. A. Hall, H. Hampel, F. M. Hisama, L. V. Naylor, C. Goetsch, K. Leppig, J. F. Tait, S. M. Scroggins, E. H. Turner, R. Livingston, S. J. Salipante, M. C. King, T. Walsh, and C. C. Pritchard. 2016. 'Improving performance of multigene panels for genomic analysis of cancer predisposition', *Genet Med*, 18: 974-81.
- Slavin, T., S. L. Neuhausen, C. Rybak, I. Solomon, B. Nehoray, K. Blazer, M. Niell-Swiler, A. W. Adamson, Y. C. Yuan, K. Yang, S. Sand, D. Castillo, J. Herzog, X. Wu, S. Tao, T. Chavez, Y. Woo, J. Chao, P. Mora, D. Horcasitas, and J. Weitzel. 2017. 'Genetic Gastric Cancer Susceptibility in the International Clinical Cancer Genomics Community Research Network', *Cancer Genet*, 216-217: 111-19.
- Solcia, E., C. Klersy, L. Mastracci, P. Alberizzi, M. E. Candusso, M. Diegoli, F. Tava, R. Riboni, R. Manca, and O. Luinetti. 2009. 'A combined histologic and molecular approach identifies three groups of gastric cancer with different prognosis', *Virchows Arch*, 455: 197-211.
- Steiert, Tim A, Genís Parra, Marta Gut, Norbert Arnold, Jean-Rémi Trotta, Raúl Tonda, Alice Moussy, Zuzana Gerber, Peter M Abuja, Kurt Zatloukal, Christoph Röcken, Trine Folseraas, Marit M Grimsrud, Arndt Vogel, Benjamin Goeppert, Stephanie Roessler, Sebastian Hinz, Clemens Schafmayer, Philip Rosenstiel, Jean-François Deleuze, Ivo G Gut, Andre Franke, and Michael Forster. 2023. 'A critical spotlight on the paradigms of FFPE-DNA sequencing', *Nucleic Acids Research*, 51: 7143-62.
- Stewart, Mark D, Diana Merino Vega, Rebecca C Arend, Jonathan F Baden, Olena Barbash, Nike Beaubier, Grace Collins, Tim French, Negar Ghahramani, Patsy Hinson, Petar Jelinic, Matthew J Marton, Kimberly McGregor, Jerod Parsons, Lakshman Ramamurthy, Mark Sausen, Ethan S Sokol, Albrecht Stenzinger, Hillary Stires, Kirsten M Timms, Diana Turco, Iris Wang, J Andrew Williams, Elaine Wong-Ho, and Jeff Allen. 2022. 'Homologous Recombination Deficiency: Concepts, Definitions, and Assays', *The Oncologist*, 27: 167-74.

References

- Stillman, Mason D., Nicole Kusche, Sabrina Toledano, Kimberly J. Hilfrank, Changhwan Yoon, Joel T. Gabre, Sheila D. Rustgi, Chin Hur, Fay Kastrinos, Sandra W. Ryeom, and Sam S. Yoon. 2022. 'Short and long-term outcomes of prophylactic total gastrectomy in 54 consecutive individuals with germline pathogenic mutations in the CDH1 gene', *Journal of Surgical Oncology*, 126: 1413-22.
- Sun, J., H. Meng, L. Yao, M. Lv, J. Bai, J. Zhang, L. Wang, T. Ouyang, J. Li, T. Wang, Z. Fan, T. Fan, B. Lin, and Y. Xie. 2017. 'Germline Mutations in Cancer Susceptibility Genes in a Large Series of Unselected Breast Cancer Patients', *Clin Cancer Res*, 23: 6113-19.
- Sung, H., J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, and F. Bray. 2021. 'Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', *CA Cancer J Clin*, 71: 209-49.
- Tacheci, Ilja, Rudolf Repak, Miroslav Podhola, Lucie Benesova, Jiri Cyrany, Jan Bures, and Darina Kohoutova. 2021. 'Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS) – A Helicobacter-opposite point', *Best Practice & Research Clinical Gastroenterology*, 50-51: 101728.
- Takeichi, M. 2018. 'Multiple functions of α -catenin beyond cell adhesion regulation', *Curr Opin Cell Biol*, 54: 24-29.
- Tanner, A., H. W. Chan, J. S. Pulido, G. Arno, R. Ba-Abbad, N. Jurkute, A. G. Robson, C. A. Egan, H. Knight, A. Calcagni, R. L. Taylor, E. Lenassi, G. C. Black, A. T. Moore, M. Michaelides, A. R. Webster, and O. A. Mahroo. 2021. 'Clinical and Genetic Findings in CTNNA1-Associated Macular Pattern Dystrophy', *Ophthalmology*, 128: 952-55.
- Tedaldi, G., F. Pirini, M. Tebaldi, V. Zampiga, I. Cangini, R. Danesi, V. Arcangeli, M. Ravegnani, R. Abou Khouzam, C. Molinari, C. Oliveira, P. Morgagni, L. Saragoni, M. Bencivenga, P. Ulivi, D. Amadori, G. Martinelli, F. Falcini, G. N. Ranzani, and D. Calistri. 2019. 'Multigene Panel Testing Increases the Number of Loci Associated with Gastric Cancer Predisposition', *Cancers (Basel)*, 11.
- Terraf, P., F. Pareja, D. N. Brown, O. Ceyhan-Birsoy, M. Misyura, S. Rana, E. O'Reilly, M. I. Carlo, C. Aghajanian, Y. Liu, F. Derakhshan, G. Jayakumaran, B. Weigelt, M. Walsh, Z. Stadler, K. Offit, M. Ladanyi, M. Robson, A. Zehir, J. S. Reis-Filho, and D. Mandelker. 2022. 'Comprehensive assessment of germline pathogenic variant detection in tumor-only sequencing', *Ann Oncol*, 33: 426-33.
- Tomlinson, I. P., and R. S. Houlston. 1997. 'Peutz-Jeghers syndrome', *J Med Genet*, 34: 1007-11.

- Uson, P. L. S., Jr., K. L. Kunze, M. A. Golafshar, G. Botrus, D. Riegert-Johnson, L. Boardman, M. J. Borad, D. Ahn, M. B. Sonbol, A. Kahn, M. Klint, E. D. Esplin, R. L. Nussbaum, A. K. Stewart, T. Bekaii-Saab, and N. J. Samadder. 2022. 'Germline Cancer Testing in Unselected Patients with Gastric and Esophageal Cancers: A Multi-center Prospective Study', *Dig Dis Sci*, 67: 5107-15.
- Usui, Y., Y. Taniyama, M. Endo, Y. N. Koyanagi, Y. Kasugai, I. Oze, H. Ito, I. Imoto, T. Tanaka, M. Tajika, Y. Niwa, Y. Iwasaki, T. Aoi, N. Hakozaiki, S. Takata, K. Suzuki, C. Terao, M. Hatakeyama, M. Hirata, K. Sugano, T. Yoshida, Y. Kamatani, H. Nakagawa, K. Matsuda, Y. Murakami, A. B. Spurdle, K. Matsuo, and Y. Momozawa. 2023. 'Helicobacter pylori, Homologous-Recombination Genes, and Gastric Cancer', *N Engl J Med*, 388: 1181-90.
- van der Post, R. S., I. Gullo, C. Oliveira, L. H. Tang, H. I. Grabsch, M. O'Donovan, R. C. Fitzgerald, H. van Krieken, and F. Carneiro. 2016. 'Histopathological, Molecular, and Genetic Profile of Hereditary Diffuse Gastric Cancer: Current Knowledge and Challenges for the Future', *Adv Exp Med Biol*, 908: 371-91.
- van der Post, R. S., I. P. Vogelaar, F. Carneiro, P. Guilford, D. Huntsman, N. Hoogerbrugge, C. Caldas, K. E. Schreiber, R. H. Hardwick, M. G. Ausems, L. Bardram, P. R. Benusiglio, T. M. Bisseling, V. Blair, E. Bleiker, A. Boussioutas, A. Cats, D. Coit, L. DeGregorio, J. Figueiredo, J. M. Ford, E. Heijkoop, R. Hermens, B. Humar, P. Kaurah, G. Keller, J. Lai, M. J. Ligtenberg, M. O'Donovan, C. Oliveira, H. Pinheiro, K. Ragunath, E. Rasenberg, S. Richardson, F. Roviello, H. Schackert, R. Seruca, A. Taylor, A. Ter Huurne, M. Tischkowitz, S. T. Joe, B. van Dijck, N. C. van Grieken, R. van Hillegersberg, J. W. van Sandick, R. Vehof, J. H. van Krieken, and R. C. Fitzgerald. 2015. 'Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers', *J Med Genet*, 52: 361-74.
- van der Post, R. S., I. P. Vogelaar, P. Manders, L. E. van der Kolk, A. Cats, L. P. van Hest, R. Sijmons, C. M. Aalfs, M. G. Ausems, E. B. Gómez García, A. Wagner, F. J. Hes, N. Arts, A. R. Mensenkamp, J. H. van Krieken, N. Hoogerbrugge, and M. J. Ligtenberg. 2015. 'Accuracy of Hereditary Diffuse Gastric Cancer Testing Criteria and Outcomes in Patients With a Germline Mutation in CDH1', *Gastroenterology*, 149: 897-906.e19.
- van Lith-Verhoeven, Janneke J. C., Bellinda van den Helm, August F. Deutman, Arthur A. B. Bergen, Frans P. M. Cremers, Carel B. Hoyng, and Paulus T. V. M. de Jong. 2003. 'A Peculiar Autosomal Dominant Macular Dystrophy Caused by an Asparagine Deletion at Codon 169 in the Peripherin/RDS Gene', *Archives of Ophthalmology*, 121: 1452-57.

References

- Van Marcke, C., R. Helaers, A. De Leener, A. Merhi, C. A. Schoonjans, J. Ambroise, C. Galant, P. Delrée, F. Rothé, I. Bar, E. Khoury, P. Brouillard, J. L. Canon, P. Vuylsteke, J. P. Machiels, M. Berlière, N. Limaye, M. Vikkula, and F. P. Duhoux. 2020. 'Tumor sequencing is useful to refine the analysis of germline variants in unexplained high-risk breast cancer families', *Breast Cancer Res*, 22: 36.
- Vogelaar, I. P., R. S. van der Post, J. H. J. van Krieken, L. Spruijt, W. A. van Zelst-Stams, C. M. Kets, J. Lubinski, A. Jakubowska, U. Teodorczyk, C. M. Aalfs, L. P. van Hest, H. Pinheiro, C. Oliveira, S. N. Jhangiani, D. M. Muzny, R. A. Gibbs, J. R. Lupski, J. de Lig, Lelm Vissers, A. Hoischen, C. Gilissen, M. van de Vorst, J. J. Goeman, H. K. Schackert, G. N. Ranzani, V. Molinaro, E. B. Gómez García, F. J. Hes, E. Holinski-Feder, M. Genuardi, Mgem Ausems, R. H. Sijmons, A. Wagner, L. E. van der Kolk, I. Bjørnevoll, H. Høberg-Vetti, A. G. van Kessel, R. P. Kuiper, M. J. L. Ligtenberg, and N. Hoogerbrugge. 2017. 'Unraveling genetic predisposition to familial or early onset gastric cancer using germline whole-exome sequencing', *Eur J Hum Genet*, 25: 1246-52.
- Vos, E. L., E. E. Salo-Mullen, L. H. Tang, M. Schattner, S. S. Yoon, H. Gerdes, A. J. Markowitz, D. Mandelker, Y. Janjigian, K. Offitt, D. G. Coit, Z. K. Stadler, and V. E. Strong. 2020. 'Indications for Total Gastrectomy in CDH1 Mutation Carriers and Outcomes of Risk-Reducing Minimally Invasive and Open Gastrectomies', *JAMA Surg*, 155: 1050-57.
- Wallace, A. J. 2016. 'New challenges for BRCA testing: a view from the diagnostic laboratory', *Eur J Hum Genet*, 24 Suppl 1: S10-8.
- Wang, Y., K. Zheng, Y. Huang, H. Xiong, J. Su, R. Chen, and Y. Zou. 2021. 'PARP inhibitors in gastric cancer: beacon of hope', *J Exp Clin Cancer Res*, 40: 211.
- Wang, Z., P. Liu, H. Inuzuka, and W. Wei. 2014. 'Roles of F-box proteins in cancer', *Nat Rev Cancer*, 14: 233-47.
- Weigelt, B., R. Bi, R. Kumar, P. Blecua, D. L. Mandelker, F. C. Geyer, F. Pareja, P. A. James, F. J. Couch, D. M. Eccles, F. Blows, P. Pharoah, A. Li, P. Selenica, R. S. Lim, G. Jayakumaran, N. Waddell, R. Shen, L. Norton, H. Y. Wen, S. N. Powell, N. Riaz, M. E. Robson, J. S. Reis-Filho, and G. Chenevix-Trench. 2018. 'The Landscape of Somatic Genetic Alterations in Breast Cancers From ATM Germline Mutation Carriers', *J Natl Cancer Inst*, 110: 1030-34.
- Weiss, J. M., S. Gupta, C. A. Burke, L. Axell, L. M. Chen, D. C. Chung, K. M. Clayback, S. Dallas, S. Felder, O. Gbolahan, F. M. Giardiello, W. Grady, M. J. Hall, H. Hampel, R.

- Hodan, G. Idos, P. Kanth, B. Katona, L. Lamps, X. Llor, P. M. Lynch, A. J. Markowitz, S. Pirzadeh-Miller, N. J. Samadder, D. Shibata, B. J. Swanson, B. M. Szymaniak, G. L. Wiesner, A. Wolf, M. B. Yurgelun, M. Zakhour, S. D. Darlow, M. A. Dwyer, and M. Campbell. 2021. 'NCCN Guidelines® Insights: Genetic/Familial High-Risk Assessment: Colorectal, Version 1.2021', *J Natl Compr Canc Netw*, 19: 1122-32.
- Weren, R. D. A., R. S. van der Post, I. P. Vogelaar, J. H. van Krieken, L. Spruijt, J. Lubinski, A. Jakubowska, U. Teodorczyk, C. M. Aalfs, L. P. van Hest, C. Oliveira, E. J. Kamping, H. K. Schackert, G. N. Ranzani, E. B. Gómez García, F. J. Hes, E. Holinski-Feder, M. Genuardi, Mgem Ausems, R. H. Sijmons, A. Wagner, L. E. van der Kolk, A. Cats, I. Bjørnevoll, N. Hoogerbrugge, and M. J. L. Ligtenberg. 2018. 'Role of germline aberrations affecting CTNNA1, MAP3K6 and MYD88 in gastric cancer susceptibility', *J Med Genet*, 55: 669-74.
- 'WHO Classification Tumours Editorial Board (ed). 2019 WHO classification of tumours: digestive system tumours'. *IARC Press, Lyon*, 5th edn.
- Win, A. K., J. C. Reece, J. G. Dowty, D. D. Buchanan, M. Clendenning, C. Rosty, M. C. Southey, J. P. Young, S. P. Cleary, H. Kim, M. Cotterchio, F. A. Macrae, K. M. Tucker, J. A. Baron, T. Burnett, L. Le Marchand, G. Casey, R. W. Haile, P. A. Newcomb, S. N. Thibodeau, J. L. Hopper, S. Gallinger, I. M. Winship, N. M. Lindor, and M. A. Jenkins. 2016. 'Risk of extracolonic cancers for people with biallelic and monoallelic mutations in MUTYH', *Int J Cancer*, 139: 1557-63.
- Worthley, D. L., K. D. Phillips, N. Wayte, K. A. Schrader, S. Healey, P. Kaurah, A. Shulkes, F. Grimpen, A. Clouston, D. Moore, D. Cullen, D. Ormonde, D. Mounkley, X. Wen, N. Lindor, F. Carneiro, D. G. Huntsman, G. Chenevix-Trench, and G. K. Suthers. 2012. 'Gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS): a new autosomal dominant syndrome', *Gut*, 61: 774-9.
- Xia, B., Q. Sheng, K. Nakanishi, A. Ohashi, J. Wu, N. Christ, X. Liu, M. Jasin, F. J. Couch, and D. M. Livingston. 2006. 'Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2', *Mol Cell*, 22: 719-29.
- Xicola, R. M., S. Li, N. Rodriguez, P. Reinecke, R. Karam, V. Speare, M. H. Black, H. LaDuca, and X. Llor. 2019. 'Clinical features and cancer risk in families with pathogenic CDH1 variants irrespective of clinical criteria', *J Med Genet*, 56: 838-43.
- Xu, J., S. L. Zheng, A. Komiya, J. C. Mychaleckyj, S. D. Isaacs, J. J. Hu, D. Sterling, E. M. Lange, G. A. Hawkins, A. Turner, C. M. Ewing, D. A. Faith, J. R. Johnson, H. Suzuki, P. Bujnovszky, K. E. Wiley, A. M. DeMarzo, G. S. Bova, B. Chang, M. C. Hall, D. L.

References

- McCullough, A. W. Partin, V. S. Kassabian, J. D. Carpten, J. E. Bailey-Wilson, J. M. Trent, J. Ohar, E. R. Bleecker, P. C. Walsh, W. B. Isaacs, and D. A. Meyers. 2002. 'Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk', *Nat Genet*, 32: 321-5.
- Yan, L., Y. Chen, F. Chen, T. Tao, Z. Hu, J. Wang, J. You, B. C. Y. Wong, J. Chen, and W. Ye. 2022. 'Effect of Helicobacter pylori Eradication on Gastric Cancer Prevention: Updated Report From a Randomized Controlled Trial With 26.5 Years of Follow-up', *Gastroenterology*, 163: 154-62.e3.
- Yap, T. A., A. Ashok, J. Stoll, E. Mauer, V. M. Nepomuceno, K. L. Blackwell, J. E. Garber, and F. Meric-Bernstam. 2022. 'Prevalence of Germline Findings Among Tumors From Cancer Types Lacking Hereditary Testing Guidelines', *JAMA Netw Open*, 5: e2213070.
- Yen, T., P. P. Stanich, L. Axell, and S. G. Patel. 1993. 'APC-Associated Polyposis Conditions.' in M. P. Adam, G. M. Mirzaa, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. W. Gripp and A. Amemiya (eds.), *GeneReviews*(®) (University of Washington, Seattle Copyright © 1993-2023, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.: Seattle (WA)).
- Yurgelun, M. B., A. B. Chittenden, V. Morales-Oyarvide, D. A. Robinson, R. F. Dunne, M. M. Kozak, Z. R. Qian, M. W. Welch, L. K. Brais, A. Da Silva, J. L. Bui, C. Yuan, T. Li, W. Li, A. Masuda, M. Gu, A. J. Bullock, D. T. Chang, T. E. Clancy, D. C. Linehan, J. J. Findeis-Hosey, L. A. Doyle, A. R. Thorner, M. D. Ducar, B. M. Wollison, N. Khalaf, K. Perez, S. Syngal, A. J. Aguirre, W. C. Hahn, M. L. Meyerson, C. S. Fuchs, S. Ogino, J. L. Hornick, A. F. Hezel, A. C. Koong, J. A. Nowak, and B. M. Wolpin. 2019. 'Germline cancer susceptibility gene variants, somatic second hits, and survival outcomes in patients with resected pancreatic cancer', *Genet Med*, 21: 213-23.
- Zannini, Laura, Domenico Delia, and Giacomo Buscemi. 2014. 'CHK2 kinase in the DNA damage response and beyond', *Journal of Molecular Cell Biology*, 6: 442-57.
- Zeng, Yongji, and Ramon U. Jin. 2022. 'Molecular pathogenesis, targeted therapies, and future perspectives for gastric cancer', *Seminars in Cancer Biology*, 86: 566-82.
- Zhang, C., D. Zhu, Y. Qu, M. Shi, J. Ma, Y. Peng, B. Zhu, H. Tao, T. Ma, and T. Hou. 2023. 'Profiling of the genetic features of Chinese patients with gastric cancer with HRD germline mutations in a large-scale retrospective study', *J Med Genet*.
- Zhang, Y. J., Y. Yang, Q. Wei, T. Xu, X. T. Zhang, J. Gao, S. Y. Tan, B. R. Liu, J. D. Zhang, X. B. Chen, Z. J. Wang, M. Qiu, X. Wang, L. Shen, and X. C. Wang. 2021. 'A multicenter

- study assessing the prevalence of germline genetic alterations in Chinese gastric-cancer patients', *Gastroenterol Rep (Oxf)*, 9: 339-49.
- Zhang, Ying, Junqing Zhou, and Chang U. K. Lim. 2006. 'The role of NBS1 in DNA double strand break repair, telomere stability, and cell cycle checkpoint control', *Cell Research*, 16: 45-54.
- Zhao, H., H. Hu, B. Chen, W. Xu, J. Zhao, C. Huang, Y. Xing, H. Lv, C. Nie, J. Wang, Y. He, S. Q. Wang, and X. B. Chen. 2021. 'Overview on the Role of E-Cadherin in Gastric Cancer: Dysregulation and Clinical Implications', *Front Mol Biosci*, 8: 689139.
- Zheng, H. C., X. H. Li, T. Hara, S. Masuda, X. H. Yang, Y. F. Guan, and Y. Takano. 2008. 'Mixed-type gastric carcinomas exhibit more aggressive features and indicate the histogenesis of carcinomas', *Virchows Arch*, 452: 525-34.