Investigation on the Role of *PALB2* Gene in *CDH1*-Negative Patients With Hereditary Diffuse Gastric Cancer

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INTRODUCTION: **Not all patients with hereditary diffuse gastric cancer (HDGC) are found to carry germline pathogenic variants in the associated gene *CDH1*, which translates into a challenging clinical management and poor cancer prevention. Thus, several studies have searched for other candidate genes, among which stands *PALB2*. Our work explores the implication of this known cancer gene in HDGC.**

METHODS: **We searched for germline *PALB2* variants by Sanger sequencing in a series of 58 patients with HDGC who tested negative for *CDH1* alterations.**

RESULTS: **No clearly pathogenic variants in *PALB2* were found in these patients. Only 5 rare genetic variants were identified, 3 of which were classified as variants of uncertain significance.**

DISCUSSION: **Despite the promising association between *PALB2* and HDGC suggested by certain works in the literature, our findings do not support *PALB2* as a high predisposition gene for HDGC. Larger studies are needed to define its role in this disease and therefore improve cancer prevention.**

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INTRODUCTION

Gastric cancer is the third deadliest type of cancer worldwide (1). The best characterized inherited one is the hereditary diffuse gastric cancer (HDGC), which follows a pattern of autosomal dominant inheritance. This condition confers an increased risk to develop diffuse gastric cancer, together with lobular breast cancer in women. This entity is associated with germline pathogenic variants in the *CDH1* gene that result in E-cadherin cell adhesion protein disfunction. The identification of these variants allows for a proper management of the disease, the establishment of the prognosis, and the correct genetic counseling, as total gastrectomy is currently the best prophylactic measure. However, up to 70% of the patients with HDGC lack germline alterations in the mentioned gene (2). Therefore, several efforts have been focused in the search for other genetic causes of HDGC to improve cancer prevention.

In this scenario, recent studies suggest that pathogenic variants in *PALB2* could be the cause of HDGC in several families (3–6). *PALB2* protein is involved in DNA repair through the Fanconi anemia and the homologous recombination repair pathways. Germline pathogenic variants in this gene are associated with hereditary breast and pancreatic cancers (7), but the role of *PALB2* as susceptibility gene of HDGC is still unclear. Based on this, we decided to explore the possible implication of *PALB2* in a new series of *CDH1*-negative HDGC patients.

METHODS

Patients

The patients visited the familial cancer consultancy of the University Hospital of Fuenlabrada or other centers for genetic counseling and their samples were referred to the Spanish National Cancer Research Center for the study of the *CDH1* gene. All patients signed an informed consent allowing further research and this project was approved by the University Hospital of Fuenlabrada ethics committee. The 58 patients selected for this study were unrelated individuals who met the HDGC clinical criteria (8) and in whom no pathogenic variants (point variants, deletions, or duplications) were found in *CDH1* gene. Approximately 60% of the individuals were women (age range at diagnosis: 20–68 years, median age at diagnosis: 42 years) and 40% men (age range: 24–76, median: 52). Ninety-three percent of the patients were European Iberian individuals coming from Spain, together with 5% African (Moroccan) and 2% South American (Colombian) individuals. Signed informed consent was obtained from all patients. All data were anonymized.

Genetic studies

The genetic analyses were performed on DNA extracted from peripheral blood leukocytes samples. All patients had been previously screened for alterations in *CDH1*, by Sanger sequencing and by multiplex ligation-dependent probe amplification with

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Table 1. Rare germline *PALB2* variants found in *CDH1*-negative HDGC patients

PALB2 variant	MAF (gnomAD)	Interpretation	Patient ID	Sex	Cancer (age at diagnosis)	Family history
c.48+10dup	ND	VUS	P1	F	DGC (48)	1 SDR with GC (42)
c.833_834delinsAT; p.(L278H)	ND	VUS	P2	F	DGC (37)	2 FDR with GC (52; 63)[a] and 1 SDR with GC
c.1194G>A; p.(V398V)	0.075%	LB	P3	M	DGC (54)	2 FDR with DGC (54; 64)[a]
c.2748G>A; p.(E916E)	ND	VUS	P4	M	DGC (56)	2 FDR with DGC (44; 47),[a] one of them also developed PC (cause of death)
c.2816T>G; p.(L939W)	0.094%	LB	P3	M	DGC (54)	2 FDR with DGC (54; 64)[a]

Age at cancer diagnosis is indicated in brackets.
Decision criteria for variant interpretation are mentioned in Methods. The nomenclature of the variants refers to the canonical transcript NM_024675.4.
DGC, diffuse gastric cancer; F, female; FDR, first-degree relative; GC, gastric cancer; ID, identification; LB, likely benign; M, male; ND, not described; PC, pancreatic cancer; SDR, second-degree relative; VUS, variant of uncertain significance.
[a]Indicates individual(s) deceased due to cancer.

SALSA P083-D1 (MRC Holland). Analysis of *PALB2* was performed by Sanger sequencing. The primers were designed with Primer3Plus tool and are available on request. Presence of the selected variants of interest was confirmed in a second sample.

Variant interpretation

The selected variants in *PALB2* were those with a minor allele frequency <1% (according to gnomAD). Decision on the variant interpretation was made considering the American College of Medical Genetics and Genomics guidelines (9), information from public databases (ClinVar, Leiden Open Variation Database, dbSNP, Ensembl, gnomAD, and Human Gene Mutation Database) and *in silico* pathogenicity predictors (PolyPhen, SIFT, and Condel).

RESULTS

No clearly pathogenic variants in *PALB2* were identified in any of the 58 *CDH1*-negative HDGC patients in this study. We found 5 rare genetic variants in *PALB2* (minor allele frequency <1%), 3 of them were classified as variants of uncertain significance (VUS) and the other 2 as likely benign variants. These variants were found in HDGC families without breast cancer cases (Table 1). DNA samples from the family relatives were not available; therefore, cosegregation studies could not be performed. Several aspects of the mentioned VUS such as their unreported frequency in general population or location near a splice site could suggest a pathogenic effect.

DISCUSSION

The link between *PALB2* pathogenic variants and HDGC was first found through whole exome sequencing and multiplexed targeted sequencing by other authors (3–6). This preliminary association was based in a reduced number of cases (10 families with HDGC) (3–6) with up to 1 identified case for every 100 *CDH1*-negative HDGC individuals tested. Similarly, our targeted study in 58 patients revealed a low number of *PALB2* variants, and we were unable to demonstrate the pathogenicity of any of them. Therefore, this detection frequency seems minimal, and it is probably unsafe to consider *PALB2* as an HDGC predisposition gene until larger series are studied. However, the possibility of testing a treatment with poly-ADP ribose polymerase inhibitors in the derived tumors in those patients carrying *PALB2* pathogenic variants could be a promising alternative to gastrectomy (6), and thus, it is a good incentive to continue researching in this topic.

CONFLICTS OF INTEREST

Guarantor of the article: Miguel Urioste, MD, PhD.
Specific author contributions: Marta Carreño and Laura Pena-Couso, share co-first authorship. Marta Carreño, MSc, and Laura Pena-Couso, PhD, contributed equally to this work. M.C., L.P.-C., and M.U. were involved in the design of the study. J.P. and M.U. were involved in patient recruitment. M.C., L.P.-C., and F.M. performed experiments and analyzed data. M.C., L.P.-C., F.M., and M.U. interpreted the results. M.C., L.P.-C., and M.U. elaborated the manuscript. All authors approved the final draft submitted.
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Study Highlights

WHAT IS KNOWN

- ✓ Genetic testing is a crucial part in HDGC diagnosis to allow for optimal clinical management.
- ✓ Germline pathogenic variants in *CDH1* do not account for all patients with HDGC.
- ✓ The implication of *PALB2* in HDGC predisposition is poorly documented.

WHAT IS NEW HERE

- ✓ Targeted analysis of *PALB2* in 58 *CDH1*-negative HDGC patients revealed only 3 VUS.
- ✓ No strong evidence for *PALB2* as a HDGC predisposition gene is found.

TRANSLATIONAL IMPACT

- ✓ The diagnosis of HDGC patients might not benefit from including *PALB2* genetic testing.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359–86.

2. Oliveira C, Seruca R, Carneiro F. Genetics, pathology, and clinics of familial gastric cancer. Int J Surg Pathol 2006;14:21–33.
3. Sahasrabudhe R, Lott P, Bohorquez M, et al. Germline mutations in PALB2, BRCA1, and RAD51C, which regulate DNA recombination repair, in patients with gastric cancer. Gastroenterology 2017;152:983–6.
4. Fewings E, Larionov A, Redman J, et al. 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 2018;3:489–98.
5. Hansford S, Kaurah P, Li-Chang H, et al. Hereditary diffuse gastric cancer syndrome: CDH1 mutations and beyond. JAMA Oncol 2015;1:23–32.
6. Carvajal-Carmona LG. PALB2 as a familial gastric cancer gene: Is the wait over? Lancet Gastroenterol Hepatol 2018;3:451–2.
7. Nepomuceno TC, De Gregoriis G, Bastos de Oliveira FM, et al. The role of PALB2 in the DNA damage response and cancer predisposition. Int J Mol Sci 2017;18:1886.
8. van der Post RS, Vogelaar IP, Carneiro F, et al. Hereditary diffuse gastric cancer: Updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. J Med Genet 2015;52:361–74.
9. Richards S, Aziz N, Bale S, 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;17:405–23.