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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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.

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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>Tdup    | ND           | VUS            | P1         | F   | DGC (48)                 | 1 SDR with GC (42) |
| c.834delinsAT | 0.075%       | LB             | P2         | F   | DGC (37)                 | 2 FDR with GC (52; 63) | 1 SDR with GC |
| c.1194G>A     | 0.094%       | VUS            | P3         | M   | DGC (54)                 | 2 FDR with DGC (54; 64) |
| c.2748>C     | ND           | VUS            | P4         | M   | DGC (56)                 | 2 FDR with DGC (44; 47) | 1 of them also developed PC (cause of death) |

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.

*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.
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