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Germline Mutations in *PALB2*, *BRCA1*, and *RAD51C*, Which Regulate DNA Recombination Repair, in Patients with Gastric Cancer

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[a]In memoriam

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Abstract

Up to 10% of cases of gastric cancer are familial, but so far, only mutations in *CDH1* have been associated with gastric cancer risk. To identify genetic variants that affect risk for gastric cancer, we collected blood samples from 28 patients with hereditary diffuse gastric cancer (HDGC) not associated with mutations in *CDH1* and performed whole-exome sequence analysis. We then analyzed sequences of candidate genes in 333 independent HDGC and non-HDGC cases. We identified 11 cases with mutations in *PALB2*, *BRCA1*, or *RAD51C* genes, which regulate homologous DNA recombination. We found these mutations in 2 of 31 patients with HDGC (6.5%) and 9 of 331 patients with sporadic gastric cancer (2.8%). Most of these mutations had been previously associated with other types of tumors and partially co-segregated with gastric cancer in our study. Tumors that developed in patients with these mutations had a mutation signature associated with somatic homologous recombination deficiency. Our findings indicate that defects in homologous recombination increase risk for gastric cancer.

Keywords

stomach; tumor; WES; interaction

Worldwide, gastric cancer (GC) is the fifth most commonly diagnosed malignancy and the third cause of cancer-related deaths [1]. Up to 10% of cases show familial clustering, suggesting a genetic basis [2]. *CDH1* mutations are a known cause of hereditary diffuse gastric

cancer (HDGC), explaining ~ 40% of cases [3,4], but the genetics of non-HDGC remain largely unknown. To identify novel GC genes, we analyzed *CDH1* mutation-negative HDGC cases using whole exome sequencing (WES) followed by candidate gene targeted analyses in independent HDGC and non-HDGC cases.

WES of 28 *CDH1*-negative European HDGC cases identified three with candidate causal variants (Table 1): nonsense (p.Arg414Ter) and splice site (c.3201+1G>T) *PALB2* mutations, and a nonsense *RAD51C* (p.Arg237Ter) mutation. No deleterious mutations were seen in other known cancer genes (Supplementary methods). *PALB2* and *RAD51C* are both critical in homologous recombination (HR), a major DNA repair pathway [5]. Both of the above *PALB2* mutations have been previously reported as pathogenic in breast cancer (BC) families [6] and *RAD51C* p.Arg237Ter is reported as pathogenic in ClinVar[7].

We then performed targeted sequencing of *PALB2* and *RAD51C,* their interaction partners *BRCA1/2* and *CDH1* in 173 additional Latin American GC cases. Based upon enrichment of HR mutations in our discovery cohort and a recent report showing multiple intestinal, diffuse and mixed histology gastric tumors with a somatic HR deficiency signature [8] , our validation cohort included both HDGC and non-HDGC cases of diffuse and non-diffuse histology (Supplementary methods). Targeted sequencing identified four additional mutation carriers: two sharing a known Hispanic *BRCA1* founder mutation (p.Gln1111Asnfs) [9] and two with novel *PALB2* mutations (p.Pro918Gln and p.Lys628_Cys630del) with predicted deleterious effects. Residue Pro918 falls in the PALB2 WD40 domain, which mediates interactions with BRCA2, RAD51 and RAD51C, whereas Lys628-Cys630 resides in the binding domain of MRG15, a transcription regulator and whose PALB2 interaction is required for homology directed DNA double-strand break repair indicating potential pathogenicity of these two novel mutations [10, 11].

In a third phase of the study, we genotyped all six *PALB2, RAD51C* and *BRCA1* mutations described above plus four known Hispanic *BRCA1/2* founder mutations (Supplementary methods) in 160 independent Latin American non-HDGC cases and found three additional mutation carriers, one with a *BRCA1* mutation (p.Gly559Valfs) and two with *PALB2* mutations (p.Lys628_Cys630del and p.Arg414Ter, Table 1). Interestingly, during the preparation of this manuscript, our clinic-based Portuguese collaborator (MT), identified one additional GC case (GM037589) with *PALB2* p.Arg414Ter. None of the seven *PALB2*, *RAD51C* and *BRCA1* mutations, detected in 11 unrelated Caucasian and Latin American cases, was detected in 1,170 population-matched controls (see mutation details in Supplementary Table 1).

Clinical details of our mutation carriers are given in Table 1. Most of them had diffuse histology, two had HDGC syndrome (CG-05 and GM022584) and one reported history of hereditary breast and ovarian cancer (HBOC, case CG-36, not shown). These mutation carriers were predominantly non-smokers and/or negative for *Helicobacter pylori* infection (Table 1), which suggest that GC risk in most of these cases was not driven by these two known environmental risk factors[12].

To obtain additional evidence of the causality of our HR gene mutations, we carried out loss of heterozygosity (LOH), mutational signature and co-segregation analyses in available samples from tumors and relatives. For LOH and mutational signatures, we performed WES in four available tumor samples from three *PALB2* (CG-12/p.Arg414Ter, CG-028/ p.Lys628_Cys630del and 3CG-103/p.Pro918Gln) and *RAD51C* mutation carriers (Table 1). We found no LOH or compound heterozygosity in these tumor samples (not shown). Interestingly, when we analyzed the somatic WES data for mutational signatures, we found that all four tumors were enriched for a signature indicative of HR defects [13, 14], providing evidence for the causality of these mutations (Supplementary methods, Supplementary Figures 1 and 2).

Figure 1 shows available pedigrees from mutation carriers. Case 3CG-103 and her daughter were both diagnosed with GC and carried the *PALB2* p.Pro918Gln mutation (Figure 1A). GM037589, a *PALB2* p.Arg414Ter carrier, developed GC and BC and had a sister diagnosed with ovarian and endometrial cancer who also carried *PALB2* p.Arg414Ter (Figure 1B). The *RAD51C* p.Arg237Ter carrier's son died of colon cancer but did not carry the mutation (Figure 1C). We found that GC was the predominantly diagnosed malignancy among unavailable relatives of these carriers (Figures 1A–1D). Although we did not have access to samples from relatives of the *PALB2* p.Lys628_Cys630del carriers, our local collaborators found this mutation co-segregating in an unrelated breast cancer family (unpublished). Albeit limited, our co-segregation data partially support GC causality of *PALB2* mutations. The *RAD51C* co-segregation data is however inconclusive but the presence of a strong HR signature in the gastric tumor (see above) of this mutation carrier warrants further studies on *RAD51C* as a candidate GC gene.

In summary, our study identified eleven cases with mutations in *PALB2*, *BRCA1* and *RAD51C*, three closely-related HR genes. Some of these mutations are known to be pathogenic in other cancer types. Out of 362 cases analyzed, 6.45% of the HDGC cases (2 out of 31) and 2.7% (9 out of 331) of non-HDGC cases had *PALB2, BRCA1* or *RAD51C* mutations, suggesting that HR genes play a role in GC risk. Our data also provide evidence of a germline basis for the recently reported HR mutational signature in gastric tumors and strengthens the evidence for a causal role of these genes, specifically *PALB2,* in GC, as previously observed [4, 15]. Future larger studies are needed to definitively assign causality and understand the penetrance and prevalence of HR gene mutations in GC and to further understand if and why some individuals from HBOC families with HR gene mutations develop GC. Further characterizations of the GC histology in HR gene mutation carriers are also needed as we found instances where the same mutation was found in cases with different histologies (CG-12 and CG-008 with *PALB2* p.Arg414ter and CG-039 and CG-028 with PALB2 p.Lys628_Cys630del, Table 1). *CDH1* mutation negative families might benefit from HR gene testing and increased endoscopic surveillance and targeted therapies, such as PARP inhibitors [8].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GC	gastric cancer
HDGC	hereditary diffuse gastric cancer
WES	whole exome sequencing
LOH	loss of heterozygosity
BC	breast cancer
R	homologous recombination

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Author names in bold designate shared co-first authorship

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Table 1

Details of clinical information of the mutation carriers

Mutation details	ID	Age of onset	Sex	Histology	Satisfied HDGC criteria ?	*Helicobacter Pylori* infection	History of smoking
PALB2 c.1240C>T, p.Arg414Ter	CG-12[a*]	69	M	Intestinal	No	NA	NA
	CG-008[c]	48	F	Diffuse	NA	NA	Yes
	GM037589	46	F	NA	No	Negative	No
PALB2 c.3201+1G>T	CG-05[a]	50	M	Diffuse	Yes	Negative	No
PALB2 c. 1882_1890delGCAGGACTT, p.Lys628_Cys630del	CG-039[b]	47	F	Diffuse	NA	Negative	No
	CG-028[c*]	81	M	Intestinal	NA	Negative	Yes
PALB2 c.2753C>A, p.Pro918Gln	3CG-103[b*]	79	F	Mixed	No	Negative	Yes
BRCA1 c.3331_3334delCAAG, p.Gln1111Asnfs	CG-036[b]	67	F	Diffuse	No	NA	No
	CG-059[b]	54	M	Diffuse	No	NA	No
BRCA1 c.1674delA, p.Gly559Valfs	CG-001[c]	65	M	NA	No	Positive	Yes
RAD51C c.709 C>T, p.Arg237Ter	GM022584[a*]	73	M	Diffuse	Yes	Negative	No

Identified by:

[a] WES,

[b] targeted sequencing or

[c] genotyping.

[*] LOH and mutational signature analyzed.

NA: Not available