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BRIEF COMMUNICATION

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# A comprehensive study evaluating germline *FANCG* variants in predisposition to breast and ovarian cancer

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## Abstract

**Background:** Monoallelic germline pathogenic variants (GPVs) in five Fanconi anemia (FA) genes (*BRCA1/FANCS*, *BRCA2/FANCD1*, *PALB2/FANCN*, *BRIP1/FANCJ*, and *RAD51C/FANCO*) confer an increased risk of breast (BC) and/or ovarian (OC) cancer, but the role of GPVs in 17 other FA genes remains unclear.

**Methods:** Here, we investigated the association of germline variants in *FANCG/XRCC9* with BC and OC risk.

**Results:** The frequency of truncating GPVs in *FANCG* did not differ between BC (20/10,204; 0.20%) and OC (8/2966; 0.27%) patients compared to controls (6/3250; 0.18%). In addition, only one out of five tumor samples showed loss-of-heterozygosity of the wild-type *FANCG* allele. Finally, none of the nine functionally tested rare recurrent missense *FANCG* variants impaired DNA repair

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activities (FANCD2 monoubiquitination and FANCD2 foci formation) upon DNA damage, in contrast to all tested *FANCG* truncations.

**Conclusion:** Our study suggests that heterozygous germline *FANCG* variants are unlikely to contribute to the development of BC or OC.

**KEYWORDS**
breast cancer, Fanconi anemia complementation group G, functional analysis, germline genetic testing, hereditary tumors, ovarian cancer

## 1 | BACKGROUND

The Fanconi anemia (FA) genes encode at least 22 proteins that form multiprotein complexes involved in the resolution of interstrand DNA interstrand cross-links and the precise repair of DNA double-strand breaks via homologous recombination.[1] While biallelic germline pathogenic variants (GPVs) in these genes cause FA (a syndrome characterized by morphologic abnormalities, bone marrow failure, and increased risk of malignancy development), monoallelic GPVs in five FA genes (including *BRCA1/FANCS*, *BRCA2/FANCD1*, *PALB2/FANCN*, *BRIP1/FANCJ*, *RAD51C/FANCO*) confer an increased risk of breast (BC) and/or ovarian (OC) cancer. The role of monoallelic GPVs in other FA genes in BC/OC predisposition remains unclear.[2,3]

*FANCG/XRCC9* encodes a protein of the FA core complex. The primary role of the FA core complex is to monoubiquitinate FANCD2, leading to FANCD2-FANCI heterodimerization (formation of ID2 complex) and the subsequent activation of downstream DNA repair effectors.[4] Notably, within the core complex, FANCG interacts with BRCA1 and BRCA2, the proteins encoded by two major hereditary BC/OC predisposition genes.[4]

Biallelic GPVs in *FANCG* cause FA complementation group G (FA-G; OMIM#614082). *FANCG*, with *FANCA* and *FANCC*, belongs to the most frequently mutated genes responsible for approximately 80% of FA patients worldwide.[5] Heterozygous *FANCG* GPVs have been identified episodically in cancer patients and their association with cancer risk remains uncertain.[6] Carriers have been described in patients with BC,[7,8] OC,[9] and pancreatic[10–12] cancers, a tumor spectrum characteristic for carriers of GPVs in established FA cancer predisposition genes (including *BRCA1*, *BRCA2*). Moreover, our previous study identified 5/1333 (0.38%) *FANCG* GPVs in OC patients (included in this dataset), suggesting a possible association of *FANCG* GPVs with OC.[13] To clarify the role of *FANCG* GPVs in BC/OC predisposition, we performed a case–control analysis and the functional in vitro testing of selected germline *FANCG* variants.

## 2 | MATERIALS AND METHODS

The frequencies of GPVs (truncating/spliceogenic) and rare missense *FANCG* variants (Table 1) were retrieved from the CZECANCA (CZEch CANcer panel for Clinical Application) database version 6 (May 20, 2023), a collection of anonymized phenotype/genotype data from the Czech national consortium (www.czecanca.cz) for germline genetic testing, as we described previously.[14] The datasets included 10,204 female BC patients (including 6753 patients who met national germline genetic testing criteria based on NCCN guidelines[15] and 3451 patients who did not meet testing criteria but were analyzed identically), 2966 unselected OC patients (1333 from previous study and 1633 newly added; all indicated for germline genetic testing in the Czech Republic), and 3250 female population-matched controls (adult volunteers who did not meet germline genetic testing criteria). All individuals provided written informed consent with genetic testing approved by the Ethics Committee of the First Faculty of Medicine and General University Hospital in Prague and were Czechs of Central European origin. A burden case–control analysis was performed to determine the risk in *FANCG* GPVs carriers.

The selected germline *FANCG* variants were functionally evaluated in vitro. Briefly, endogenous *FANCG* was knocked out in U2OS cells (FANCG-KO) by CRISPR/Cas9 technology using the pX458 plasmid (Addgene #48138) expressing sgRNAs targeting exons 1 and 4. pEGFP-C1-FANCG-T2A-Puro plasmids for the expression of wild-type FANCG or its individual variants were generated by Gibson assembly and were stably transfected into FANCG-KO cells. FANCG variants were tested by treating the reconstituted cells with mitomycin (MMC) followed by evaluation of FANCD2 monoubiquitination by immunoblotting, localization of FANCD2 in nuclear DNA repair foci by high content ScanR microscopy, and by colony formation and survival assay (Figure 1C–J, details available upon request). Four recurrent missense variants (Table 1; $MAF_{gnomAD}$ >0.002) and wild-type FANCG were considered positive, fully functional controls, while truncating

**TABLE 1** Identified germline *FANCG* variants.

| Exon | TPR | Variant description (NM_004629.2) | | ClinVar | | InterVar | Breast ca. (*N* = 10,204) | Ovarian ca. (*N* = 2966) | CTRLs (*N* = 3250) |
|---|---|---|---|---|---|---|---|---|---|
| (i) | # | c. | p. | Variant ID | Review status | Class | *N* (%) | *N* (%) | *N* (%) |
| *Truncating variants* | | | | | | | | | |
| i1 | | 85-1G>C | ?[b] | n.a. | n.a. | n.a. | – | – | 1 (0.03) |
| 4 | | 313G>T | Glu105Ter[a,b] | 6712 | 5**-- | P | 9 (0.09) | 2 (0.07) | 3 (0.09) |
| 4 | | 373_374del | Val125ProfsTer29 | n.a. | n.a. | n.a. | 1 (0.01) | – | – |
| 5 | | 520del | Ser174LeufsTer8 | n.a. | n.a. | n.a. | – | 1 (0.03) | – |
| 5 | | 522_523del | Lys175GlyfsTer14 | n.a. | n.a. | n.a. | – | 1 (0.03) | – |
| 5 | | 560delC | Pro187GlnfsTer5[b] | 1452560 | 5*--- | n.a. | 1 (0.01) | – | – |
| 10 | | 1158delC | Ser387ProfsTer16[a] | 619961 | 5/4**-- | n.a. | – | 1 (0.03) | – |
| 10 | | 1158dupC | Ser387LeufsTer9[a] | 623182 | 5/4**-- | n.a. | 5 (0.05) | 1 (0.03) | – |
| 10 | | 1183_1192del | Glu395TrpfsTer5[a,b] | 41224 | ---- | n.a. | 2 (0.02) | – | 1 (0.03) |
| 10 | | 1309_1310dup | Asp437GlufsTer82 | 1073453 | 5**-- | n.a. | 2 (0.02) | – | – |
| i11 | | 1480+1G>T | ? | n.a. | n.a. | n.a. | – | – | 1 (0.03) |
| 13 | | 1642C>T | Arg548Ter[a,b] | 574728 | 5**-- | P | – | 1 (0.03) | – |
| 14 | | 1772del | Leu591ArgfsTer3[a,b] | 2136764 | 4/3*--- | n.a. | – | 1 (0.03) | – |
| All truncating variants | | | | | | | 20 (0.20) | 8 (0.27) | 6 (0.18) |
| odds ratio (95% confidence interval); *p*-value | | | | | | | 1.1 (0.4–2.7); 0.9 | 1.5 (0.5–4.2); 0.5 | Ref. |
| *Rare missense variants* | | | | | | | | | |
| 2 | | 109C>G | Leu37Val[a] | 1319087 | 3*--- | LB | 4 (0.04) | – | |
| 2 | | 122A>G | Gln41Arg | n.a. | n.a. | LB | 2 (0.02) | – | 1 (0.03) |
| 4 | | 338G>A | Arg113Lys | 526405 | 3*--- | LB | | – | 3 (0.09) |
| 4 | | 401A>C | Glu134Ala | n.a. | n.a. | VUS | 1 (0.01) | – | – |
| 4 | | 418C>T | His140Tyr | n.a. | n.a. | VUS | 1 (0.01) | – | – |
| 4 | | 421C>T | Arg141Cys | 239967 | 3**-- | VUS | | 1 (0.03) | – |
| 4 | | 422G>A | Arg141His | 999134 | 3**-- | LB | 2 (0.02) | – | – |
| 4 | | 464G>A | Arg155His[a] | 456236 | 3**-- | LB | 2 (0.02) | 2 (0.07) | – |
| 4 | | 486A>T | Leu162Phe | 1416142 | 3**-- | LB | 1 (0.01) | – | 1 (0.03) |
| 5 | | 517G>A | Ala173Thr[a] | 2085419 | 3*--- | LB | 1 (0.01) | – | 2 (0.06) |
| 5 | | 518C>T | Ala173Val[a] | n.a. | n.a. | VUS | – | – | 3 (0.09) |

(Continues)

TABLE 1 (Continued)

| Exon | TPR | Variant description (NM_004629.2) | | ClinVar | | InterVar | Breast ca. ($N=10,204$) | Ovarian ca. ($N=2966$) | CTRLs ($N=3250$) |
|---|---|---|---|---|---|---|---|---|---|
| (i) | # | c. | p. | Variant ID | Review status | Class | *N* (%) | *N* (%) | *N* (%) |
| 5 | | 580C>G | Pro194Ala | n.a. | n.a. | VUS | 1 (0.01) | – | – |
| 6 | | 724C>T | Arg242Trp | 912780 | 3**-- | VUS | – | – | 1 (0.03) |
| 6 | 1 | 761C>T | Ser254Phe | 1319083 | 3*--- | VUS | 2 (0.02) | – | 1 (0.03) |
| 7 | 1 | 794C>T | Ala265Val | 912779 | 3**-- | VUS | – | – | 1 (0.03) |
| 7 | 1 | 835T>G | Trp279Gly | 1336044 | 3*--- | VUS | 1 (0.01) | 1 (0.03) | – |
| 7 | | 910G>C | Glu304Gln | 2577889 | 3*--- | VUS | 1 (0.01) | – | – |
| 7 | | 917T>C | Leu306Pro | 2202538 | 3*--- | VUS | 1 (0.01) | – | – |
| 7 | | 919G>A | Val307Ile[a] | n.a. | n.a. | VUS | 3 (0.03) | – | – |
| 8 | | 956C>T | Pro319Leu | n.a. | n.a. | LB | 1 (0.01) | – | 2 (0.06) |
| 8 | | 992C>T | Pro331Leu | n.a. | n.a. | LB | 1 (0.01) | – | – |
| 8 | | 992C>G | Pro331Arg[a] | 802483 | 3**-- | LB | 6 (0.06) | – | 3 (0.09) |
| 8 | | 1027C>G | Gln343Glu | 914731 | 3*--- | VUS | 1 (0.01) | – | – |
| 8 | 2 | 1070C>T | Thr357Met | 1806248 | 3**-- | LB | 2 (0.02) | – | – |
| 8 | 2 | 1076G>A | Arg359Lys | 1199306 | 3*--- | VUS | 2 (0.02) | – | – |
| 9 | | 1143G>C | Arg381Ser | n.a. | n.a. | VUS | 1 (0.01) | – | 1 (0.03) |
| 10 | | 1157C>A | Pro386His | 914728 | 3**-- | LB | – | 2 (0.07) | – |
| 10 | | 1268G>A | Arg423His | 456228 | 3**-- | VUS | 2 (0.02) | 2 (0.07) | – |
| 10 | | 1298G>A | Arg433Gln | 644471 | 3*--- | LB | – | 1 (0.03) | – |
| 10 | | 1328A>G | Lys443Arg | n.a. | n.a. | LB | 1 (0.01) | – | – |
| 10 | 3 | 1367A>T | His456Leu | 579904 | 3**-- | VUS | 2 (0.02) | 1 (0.03) | – |
| 10 | 3 | 1402G>A | Ala468Thr[a] | n.a. | n.a. | VUS | 1 (0.01) | 1 (0.03) | 1 (0.03) |
| 12 | | 1492A>C | Asn498His | 836334 | 3**-- | LB | – | 1 (0.03) | – |
| 12 | | 1498G>A | Glu500Lys[a] | 2056153 | 3*--- | VUS | 5 (0.05) | – | 2 (0.06) |
| 12 | | 1505G>T | Gly502Val | 1721737 | 3*--- | VUS | 2 (0.02) | – | – |
| 12 | 4 | 1546G>A | Ala516Thr | 2043453 | 3*--- | VUS | 2 (0.02) | – | – |
| 12 | 4 | 1558C>T | Arg520Cys | n.a. | n.a. | VUS | – | 1 (0.03) | – |
| 12 | 4 | 1586A>G | Gln529Arg | 2038189 | 3*--- | VUS | 1 (0.01) | – | – |
| 13 | | 1643G>A | Arg548Gln | 1692835 | 3*--- | LB | 1 (0.01) | 1 (0.03) | – |

TABLE 1 (Continued)

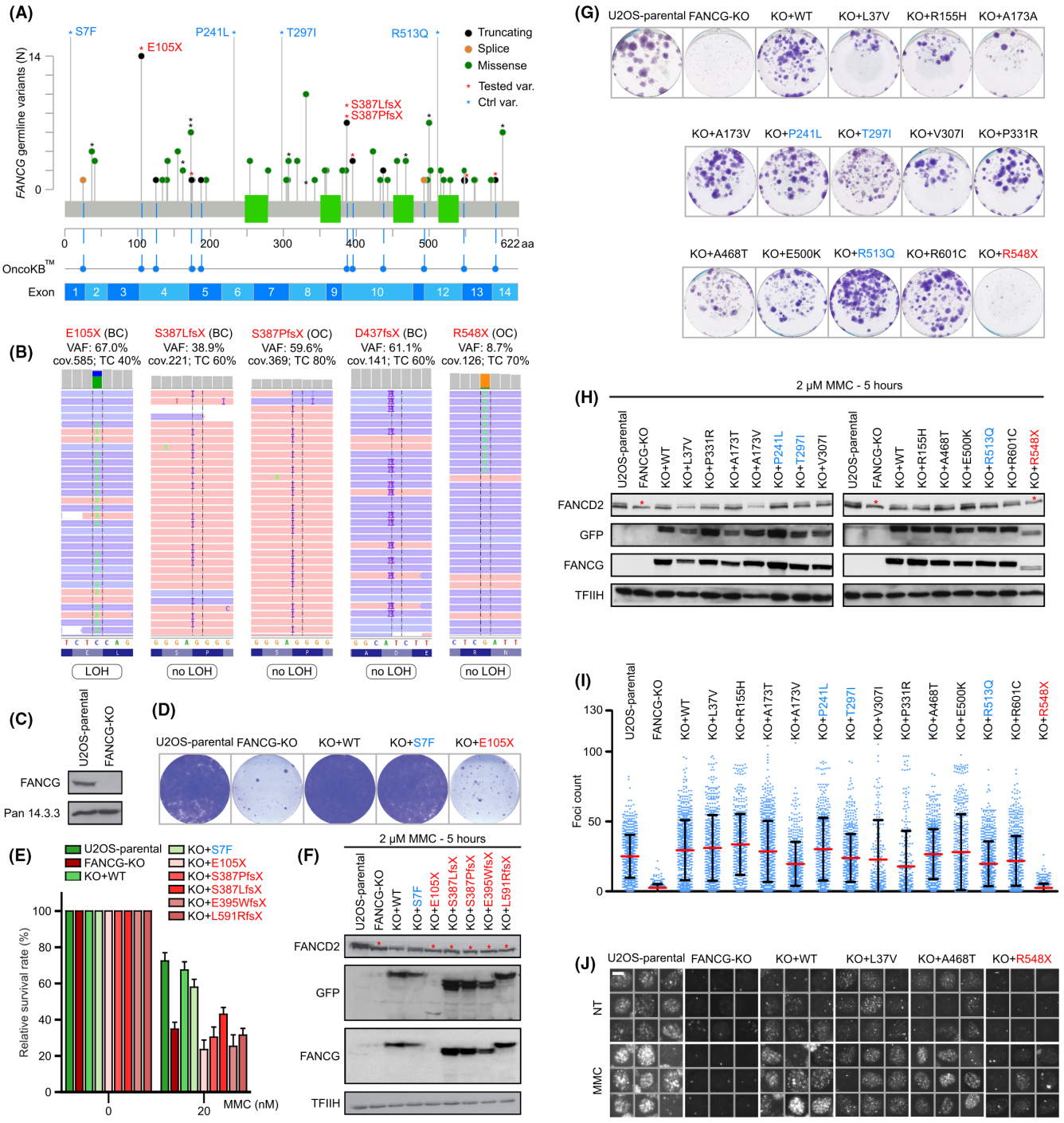
| Exon | TPR | Variant description (NM_004629.2) | | ClinVar | | InterVar | Breast ca. ($N = 10,204$) | Ovarian ca. ($N = 2966$) | CTRLs ($N = 3250$) |
|---|---|---|---|---|---|---|---|---|---|
| (i) | # | c. | p. | Variant ID | Review status | Class | *N* (%) | *N* (%) | *N* (%) |
| 13 | | 1685A>G | Asp562Gly | n.a. | n.a. | VUS | – | 1 (0.03) | – |
| 13 | | 1688G>A | Arg563Gln | 846051 | 3**-- | VUS | 1 (0.01) | – | – |
| 13 | | 1754C>G | Ala585Gly | n.a. | n.a. | VUS | 1 (0.01) | – | – |
| 14 | | 1801C>T | Arg601Cys[a] | 666016 | 3*--- | VUS | 3 (0.03) | 1 (0.03) | – |
| 14 | | 1802G>A | Arg601His | n.a. | n.a. | LB | – | – | 2 (0.06) |
| All rare missense variants | | | | | | | 59 (0.58) | 16 (0.54) | 24 (0.74) |
| *Frequent missense variants* | | | | | | | | | |
| 1 | | 20C>T | Ser7Phe[a] | 134358 | 1/2/3*--- | LB | 30 (0.30) | 9 (0.30) | 12 (0.37) |
| 6 | | 722C>T | Pro241Leu[a] | 221037 | 2/3*--- | VUS | 10 (0.10) | – | 1 (0.03) |
| 7 | | 890C>T | Thr297Ile[a] | 134367 | 1/2**-- | LB | 27 (0.26) | 8 (0.27) | 9 (0.27) |
| 12 | | 1538G>A | Arg513Gln[a] | 134361 | 1/2/3*--- | LB | 112 (1.10) | 41 (1.38) | 42 (1.29) |
| All frequent missense variants | | | | | | | 179 (1.75) | 58 (1.96) | 64 (1.97) |

*Note*: InterVar (https://wintervar.wglab.org/). ClinVar review status was 2023/12/01 and express ClinVar numerical classification (class 1–5 when available) and aggregate ClinVar review status (zero to four stars: ---- to ****).

Abbreviations: c., cDNA; (i), intron; LB, likely benign; n.a., not available; P, pathogenic; p., protein; TPR, tetratricopeptide repeat (numbers 1–4); VUS, variant of unknown significance.

[a]Variant analyzed by functional assays in this study.

[b]Variant described in FA patient.




GPVs and FANCG-KO cells served as negative, functionally dead controls.

Loss-of-heterozygosity (LOH) at the *FANCG* locus was evaluated in five *FANCG* GPV carriers using in-house 359 genes NGS panel, analyzing DNA from formalin-fixed paraffin-embedded (FFPE) tumor samples. LOH was detected using the Copy Number Variant Detection module (CLC Genomics Workbench v23.0.5) along with the frequency of detected mutations of germline origin, considering the percentage of tumor fraction in the analyzed FFPE sample.

## 3 | RESULTS

We identified 57 different, rare heterozygous, germline *FANCG* variants (Figure 1A; Table 1) including 13 frameshift, stop-gain, or spliceogenic variants considered as GPVs (localized before the most N-terminal GPV identified in FA-G patients, c.1795_1804del—ClinVar ID: 6718). However, the frequency of GPVs in patients with BC (20/10,204; 0.20%) or OC (8/2966; 0.27%) did not differ from that in controls (6/3250; 0.18%; Table 1). The frequency of *FANCG* GPV carriers in BC was insignificantly higher among patients who

**FIGURE 1** (A) Distribution of germline *FANCG* variants identified in patients and controls (created using https://www.cbioportal.org/). Asterisks indicate variants included in functional testing (blue—missense variants with minor allele frequency (MAF) >0.002 in gnomAD database that were selected as fully-functional controls; red—truncations). All truncations and splicing alterations were considered pathogenic by OncoKB (www.oncokb.org). Exon structure corresponds to NM_004629.2 reference. (B) DNA sequencing from breast (BC) and ovarian (OC) cancer FFPE samples available from five patients carrying truncating *FANCG* variants. (C–J) Functional characterization of DNA damage response in FANCG variants expressed in U2OS-FANCG-KO cells lacking endogenous FANCG. (C) Immunoblot showing the level of endogenous FANCG in U2OS-parental and U2OS-FANCG-KO cells (Santa Cruz, sc-393,382). As loading control was used protein Pan 14-3-3 (Santa Cruz, sc-133,233). (D) Colony formation assay of parental U2OS, U2OS-FANCG-KO (FANCG-KO), and FANCG-KO stably transfected with truncated FANCG (red text) or fully-functional FANCG-S7F missense variant (blue text) after treatment with 1 nM MMC for 14 days. Colonies were fixed with ethanol (70% v/v) and stained with crystal violet. Note that FANCG-KO cells and FANCG-KO cells expressing the most frequent truncating variant p.E105X fail to grow in MMC. (E) Survival assay of parental U2OS cells, FANCG-KO cells, and FANCG-KO stably transfected with FANCG variants demonstrates that all analyzed truncating variants fail to rescue survival following MMC treatment. Relative cell proliferation was determined by resazurin assay ($n=3$; mean with SD displayed). (F) Parental U2OS, FANCG-KO cells, and FANCG-KO cells stably transfected with FANCG variants were treated with MMC (2 μM, 5 h) and analyzed by immunoblotting with FANCD2 antibody (Abcam, ab108928) to visualize FANCD2 monoubiquitination. A red asterisk indicates the lack of FANCD2 monoubiquitination in FANCG-KO cells and in all cells expressing analyzed truncating variants. Immunoblotting for GFP (Roche, 11,814,460,001), FANCG (Santa Cruz, sc-393,382), and transcription factor TFIIH (sc-293; Santa Cruz) were used as loading controls. (G) A colony formation assay indicates that all tested missense variants rescued cell growth following MMC treatment (1 nM, 7 days). (H) Immunoblotting performed as in 1F demonstrated rescue of FANCD2 monoubiquitination in FANCD-KO cells expressing all analyzed missense variants in contrast to its loss in FANCG-KO controls and FANCG-KO cells expressing the C-terminal truncating variant p.R548X. (I) Quantitative analysis of FANCD2 nuclear foci formation. U2OS, FANCG-KO, and reconstituted FANCG-KO stables cell lines were treated with 2 μM MMC for 5 h, pre-extracted, fixed and stained with DAPI and FANCD2 antibody (Abcam, ab108928) and imaged using Olympus ScanR microscope equipped with 60×/1.42 OIL objective. The number of nuclear FANCD2 foci was determined using spot detection module in ScanR analysis software. Each dot represents one cell, red bar indicates mean, and bars are SDs. Representative out of two independent experiments. Note that FANCD2 foci do not form in FANCG-KO cells and all tested missense variants rescued FANCD2 foci formation. FANCG-KO expressing p.R548X truncation (red text) served as negative control (at least 270 cells were analyzed per condition). (J) Representative microscopy images from (I) showing FANCD2 foci formation in nuclei stained with DAPI after 2 μM MMC treatment (5 h) in U2OS cells, reduced foci formation in FANCG-KO cells and FANCG-KO cells expressing p.R548X and rescued FANCD2 foci formation in FANCG-KO cells expressing wild-type FANCG and all missense variants (scale bars 10 μm). VAF, variant allele frequency; cov., coverage; TC, percentage of tumor cells in sequenced sample; LOH/no LOH, presence/absence of loss of heterozygosity.

were not indicated for germline genetic testing than among those who were indicated [10/3451 (0.29%) vs. 10/6753 (0.15%); $p=0.19$]. We found no evidence for the association of *FANCG* GPVs and ER-negative BC identified in 4/17 (23.5%) carriers and 2321/8383 (27.7%) all BC patients with known ER status, suggested by Nierenberg et al. recently.[8] In addition, the analysis of five available tumor samples from patients with *FANCG* GPVs revealed only one case of LOH at the *FANCG* locus (Figure 1B).

To test the pathogenicity of *FANCG* missense variants, we expressed selected variants in U2OS FANCG-KO cells and evaluated their overall DNA repair capacity following genotoxic treatment. Specifically, we assessed FANCD2 monoubiquitination and its localization to nuclear foci as a readout of the FANCG-dependent FA core complex functionality. Our assays confirmed a clear defect in the overall DNA repair and FA core complex activity in FANCG truncations (Figure 1D–F) but we found no evidence of functional impairment in any of the FANCG missense variants tested (Figure 1G–J).

## 4 | DISCUSSION

Although heterozygous GPVs in five FA genes (*BRCA1*, *BRCA2*, *PALB2*, *BRIP1*, or *RAD51C*) confer high/moderate BC/OC risk, we found no association between *FANCG* GPVs and BC/OC risk. Regarding the case–control evidence for OC predisposition, our results are in agreement with a previous study by Song et al. who also found no association between GPVs in other FA genes (including *FANCG*) and OC risk. Specifically, Song's et al. identified 11/6184 (0.17%) *FANCG* GPVs carriers in OC patients compared to 8/6089 (0.13%) such carriers in controls (OR = 1.4; 95% CI 0.5–3.4).[9] In addition, we detected LOH, an important marker of allelic imbalance indicating the presence of a driver mutation in a tumor suppressor gene, in only one of the five tumors analyzed. Finally, our in vitro functional assays showed that all rare missense variants analyzed did not affect the role of FANCG in DNA repair. Taken together, our study strongly suggests that heterozygous germline *FANCG* variants (including GPVs) do not confer an increased risk of BC or OC.

## AUTHOR CONTRIBUTIONS

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Research data supporting this publication are provided in this article. Details of the methods are available from the corresponding author upon reasonable request.

## ETHICS STATEMENT

All individuals provided written informed consent with genetic testing approved by the Ethics Committee of the First Faculty of Medicine and General University Hospital in Prague and the study was conducted in accordance with the Declaration of Helsinki.

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