Integration of Tumour Sequencing and Case-Control Data to Assess Pathogenicity of *RAD51C* Missense Variants in Familial Breast Cancer

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Abstract

Background: While protein truncating variants in *RAD51C* have been shown to predispose to triple negative breast cancer (TNBC) and ovarian cancer, little is known about the pathogenicity of missense (MS) variants.

Methods: The frequency of rare *RAD51C* MS variants were assessed in the BEACCON study of 5,734 familial breast cancer cases and 14,382 population controls, and integrated with tumour sequencing data from 21 cases carrying a candidate variant to assess bi-allelic inactivation.

Results: Collectively, a significant enrichment of rare missense variants was detected in cases (MAF < 0.001, OR 1.57, 95%CI 1.00 - 2.44, p = 0.05), particularly for variants with a REVEL score > 0.5 (OR 3.95, 95%CI 1.40 - 12.01, p = 0.006). Despite the large sample size, the majority of variants detected were very rare, precluding definitive conclusions about pathogenicity based solely on the case-control data. Sequencing of 21 tumours from carriers of one of eight candidate MS variants, identified four cases with bi-allelic inactivation through loss of the wild-type allele, while six lost the variant allele and ten remained heterozygous. Loss of the wild-type alleles corresponded strongly with ER- and triple-negative breast tumours and a high homologous recombination deficiency score.

Conclusions: Using this approach, the p.Gly264Ser variant, which was previously suspected to be pathogenic based on small case-control analyses and loss of activity in *in vitro* functional assays, was shown to be benign with similar prevalence in cases and controls, and eight out of nine tumours showing loss of the variant allele or retention of heterozygosity. Conversely, the combined case-control and tumour sequencing data identified p.Ile144Thr, p.Arg212His, p.Gln143Arg and p.Gly114Arg as variants warranting further investigation.

Background

Protein truncating variants in *RAD51C* predispose to high grade serous ovarian cancer and triple negative breast cancer (TNBC), and when these cancers occur in carriers of truncating variants they exhibit bi-allelic inactivation (1–3). Few studies have investigated whether missense (MS) variants of *RAD51C* exert similar penetrance as protein truncating variants. Breast cancer case-control studies to date have identified potentially predisposing *RAD51C* MS variants, such as p.Gly264Ser (4–6), p.Gln143Arg (7, 8) and pArg258His (9, 10), while target protein and cellular assays have suggested functional impact and pathogenicity of variants including p.Cys135Tyr and p.Gly264Ser (5, 8, 9). However, the sample sizes in these studies were small, with conflicting evidence for many variants. We analysed data from the BEACCON study of 5,734 familial breast cancer cases and 14,382 population controls for rare *RAD51C* MS variants (MAF < 0.005). To further investigate the potential pathogenicity of candidate variants, we exploited the fact that *RAD51C* appears to conform to the Knudsen's "two-hit" hypothesis, and performed tumour sequencing from variant carriers to assess for bi-allelic inactivation and associated homologous recombination deficiency (HRD). We have previously demonstrated the utility of this reproach for *RAD51C*

loss of function (LoF) variants which revealed the presence of bi-allelic inactivation in the form of loss of heterozygosity (LOH) in TNBCs that was also associated with high HRD scores and mutational signature 3 (1). In this study, case-control analysis data was combined with tumour sequencing, *in silico* prediction tools and pedigree segregation to assess the pathogenicity of *RAD51C* missense variants.

Methods

Cohorts

The case cohort comprised of female index patients diagnosed with breast cancer from 5,734 hereditary breast and ovarian cancer (HBOC) families identified from the Variants in Practice (ViP) Study (combined Victorian and Tasmanian Familial Cancer Centres, Australia) and Pathology North (NSW Health Pathology, Newcastle, Australia). The cases were determined eligible for clinical genetic testing for hereditary breast cancer predisposition genes based on personal and/or family history by a specialist Familial Cancer Clinic. All case subjects have been tested negative for *BRCA1*/*BRCA2* pathogenic variants prior to recruitment. The controls were 14,382 cancer-free female subjects from the Lifepool Study (http://www.lifepool.org/) in Victoria, Australia (BreastScreen Victoria). The average age of first diagnosis in cases was 45.8 years (range, 17-85), while the average age of controls in this study was 64.4 years (range, 40-97), indicating a design that enrich for lifetime cancer-free controls. Family history of cancer was recorded for all cohort subjects by questionnaire or in-person interview. Cases ascertained through ViP study were provided with detailed pedigrees with breast and ovarian cancer family history verified against state cancer registries, and tumour pathology reports.

Targeted sequencing of germline DNA of cases and controls

The coding region and exon-intron boundaries (at least 10 bp of each intron) of *RAD51C* from germline DNA were amplified using a custom designed HaloPlex Targeted Enrichment Assay panel (Agilent Technologies, Santa Clara, CA) and the libraries were sequenced on a HiSeq2500 Genome Analyzer (Illumina, San Diego, CA) as previously described (11-14). Loss-of-function (LoF) variants were defined as stop-gained, frame-shift or essential splice-site variants. Missense (MS) variants were defined as non-synonymous single nucleotide variants.

Sequencing of tumour DNA of RAD51C MS carriers

Tumour DNA was collected from cancer cells in formalin fixed, paraffin embedded (FFPE) slides by needle microdissection under the microscope. For targeted sequencing, all exons of *RAD51C* and 487 additional genes (including 27 breast cancer driver genes, total targeted region of 1.337 Mb), and for whole-exome sequencing, all exons were amplified using an Agilent SureSelect XT Custom Panel (15).

For Sanger sequencing, the relevant RAD51C region was amplified using exon-specific primers through polymerase chain reaction and sequenced using ThermoFisher Scientific BigDye Terminator v3.1 kit. The libraries were sequenced on an Illumina Next Seq 500 (75 bp paired end reads). Promoter hyper-methylation was determined by Sanger sequencing of bisulfite-converted tumour DNA using Qiagen EpiTect Bisulfite Kit.

Identification of MS variants

Sequencing results were aligned to the g1 k x27 h19 reference genome using the Burrows-Wheeler Alignment tool (16), SNP variant calling was carried out using GATK UnifiedGenotyper v2.4 (Broad Institute, Cambridge, MA), Platypus (17) and Varscan (18), and variants were annotated using the Ensembl Variant Effect Predictor (19) as previously described (1). Rare MS variants were identified in canonical transcript by at least two variant callers, with sequencing quality ≥30, allele frequency ≥20% and MAF present at ≤0.005 for MS variants in non-Finnish European in gnomAD (Version 2.1, released 17 October 2018) (20). Manual examination of BAM files and Sanger sequencing was carried out for ambiguous variants to remove sequencing artefacts.

Homologous recombination deficiency (HRD) score calculation

A copy number plot was generated for each tumour using copywriteR package in R studio (21). From the plot, an HRD score was calculated for each tumour sample as a sum of the occurrence of telomeric allelic imbalances, large-scale state transitions and homologous recombination deficiency–loss of heterozygosity (HRD–LOH) as described previously (22).

Sliding window analysis

MS variants were separated into each unique window of N amino acids, then Fisher's Exact Test was performed using the counts of variants in the case and control samples. P-values were then adjusted based on the null distribution estimated by randomising the sample labels of each variant and recalculating the optimal p-value for each iteration.

Mutational Signature analysis

Rare somatic mutations were identified after filtering against germline variants, removing intron variants, sequencing read depth ≥20, allele frequency ≥10% and MAF present at ≤0.0001 for in non-Finnish European in gnomAD. As the number of somatic mutations was low in individual targeted panel

sequenced samples, mutations were pooled into groups according to variant type and/or tumour pathology. Mutational signatures were generated using the DeconstructSig package in R (23).

Statistical analysis

Odds ratios and Fisher's exact test (2-sided) were examined for the case-control analysis, with a two-tailed p-value of ≤0.05 designated as statistically significant, and confidence intervals were calculated using conditional Maximum Likelihood Estimate. Benjamini-Hochberg adjustment was used for multiple test corrections (24). All calculations were carried out using R-in built function in R 3.3.2 (25).

Results

Likely Pathogenic Variants were Enriched in the Case Cohort

A total of 51 unique rare MS variants (MAF < 0.005) were detected in 65 cases (1.13%) and 134 controls (0.91%) (OR 1.22, 95% CI 0.89–1.65, p = 0.21) (Table 1). Several parameters were used to enrich for potentially pathogenic variants including population frequency, location in known function domains, *in silico* pathogenicity prediction and tumour phenotype. Consistent with the hypothesis that rare variants are more likely to be deleterious (26), a reduction of the population frequency threshold resulted in increasing odds ratios that reached statistical significance at MAF < 0.0001 (OR 1.87, 95%CI 1.14–3.03, p = 0.01). Similarly, higher CADD and REVEL score thresholds that should enrich for pathogenic variants were associated with higher odds ratios, especially for a REVEL score of > 0.5 (OR 3.95, 95%CI 1.40–12.01, p = 0.006). Two overlapping functional domains are present in the N-terminal third of RAD51C protein (Holliday junction activity: amino acids 1-126; Interaction with RAD51B, RAD51D and XRCC3: amino acids 79–136) and a significant enrichment of MS variants in cases was observed in the interaction domain (OR 10.04, 95%CI 0.99–494.1, p = 0.03), although the number of variants was low (n = 5), resulting in a wide confidence interval.

Table 1

Frequencies of *RAD51C* missense variants in case and control cohorts according to different filtering criteria to enrich for likely pathogenic variants.

	Groups	Carrier Frequency		Sample Size		p-value	OR (95% CI)
		Case (%)	Control (%)	Case	Control		
Rarity	MAF < 0.005	65 (1.13%)	134 (0.91%)	5734	14382	0.21	1.22 (0.89–1.65)
	MAF < 0.001	35 (0.61%)	56 (0.38%)			0.05	1.57 (1.00-2.44)
	MAF < 0.0001	32 (0.56%)	43 (0.29%)			0.01	1.87 (1.14–3.03)
In-silico	CADD > 20	59 (1.03%)	125 (0.85%)			0.29	1.19 (0.85–1.63)
	CADD > 25	17 (0.30%)	22 (0.15%)			0.05	1.94 (0.97–3.83)
	REVEL > 0.3	17 (0.30%)	19 (0.13%)			0.02	2.25 (1.10–4.57)
	REVEL > 0.5	11 (0.19%)	7 (0.05%)			0.006	3.95 (1.40-12.01)
Functional domain	Interaction domain	4 (0.070%)	1 (0.01%)			0.03	10.04 (0.99–493.1)
	Holliday domain	6 (0.10%)	7 (0.05%)			0.21	2.15 (0.60–7.48)
Hormone receptor subtype	ER-positive	23 (1.04%)	137 (0.93%)	2209		0.64	1.09 (0.67–1.71)
	ER-negative	20 (1.58%)		1262		0.04	1.67 (0.99–2.70)

MAF: Minor Allele Frequency; CADD: Combined Annotation-Dependent Depletion score (34); REVEL: rare exome variant ensemble learner score (35); ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; TN: triple-negative.

HER2-positive	7 (1.21%)	579	0.51	1.27 (0.50–2.7)1
HER2-negative	29 (1.20%)	2426	0.27	1.26 (0.81–1.89)
TN	13 (1.49%)	871	0.11	1.58 (0.81–2.80)
Non-TN	23 (1.08%)	2125	0.55	1.14 (0.70–1.78)

MAF: Minor Allele Frequency; CADD: Combined Annotation-Dependent Depletion score (34); REVEL: rare exome variant ensemble learner score (35); ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; TN: triple-negative.

Subgroup analysis based on hormone receptor status was carried out on case subjects where detailed pathology data was available from the Variant in Practice (ViP) study (n = 3,645). Consistent with previous findings for *RAD51C* LoF carriers, rare MS variants were significantly enriched in the ER-negative breast cancer subgroup (OR 1.67, 95%CI 0.99–2.70, p = 0.04), with a similar but non-significant trend in TNBC cases (OR 1.58, 95%CI 0.81–2.80, p = 0.11).

The distribution and frequency of rare MS variants across RAD51C in the 5,734 cases and 14,382 controls is summarised in Fig. 1. While rare MS variants were distributed across the entire gene, cases showed higher frequencies in the first half of the gene. The position-based odds ratio analysis showed a higher case-control odds ratio for variants located between amino acid positions 82 and 136, coinciding with the interaction domain.

Variants Of Interest Detected In Cases And Controls

Details of the 51 rare *RAD51C* MS variants identified in this study including case-control numbers, *in silico* pathogenicity prediction and literature evidence are summarised in Supplementary Table 1. Also included is the reference variant p.Ala126Thr (MAF = 0.0054), a generally-accepted benign variant. All of the variants were very rare (MAF ≤ 0.0001), with the exception of p.Gly264Ser (MAF = 0.0034). Despite the large sample size, most variants were detected in less than three subjects, therefore the frequencies alone were not adequately powered to confirm or refute pathogenicity. The data did, however, suggest that two previously-identified variants, p.Ala126Thr and p.Gly264Ser, do not represent high penetrance alleles. p.Ala126Thr was detected in 68 (1.19%) cases and 133 (0.9%) controls (OR 1.29, p = 0.10), similar to the allele frequency reported in gnomAD database. Similarly, p.Gly264Ser was detected with equal frequencies in cases (n = 30, 0.52%) and controls (n = 80, 0.54%) (OR 0.96, p = 0.92).

Sequencing Of Tumours From Ms Variant Carriers

Twenty invasive breast tumours and one high grade serous ovarian tumour from 21 cases were sequenced using a targeted gene panel that included all exons and intron boundaries of *RAD51C* (Table 2). These tumours were from cases that carried one of eight heterozygous candidate variants (p.Gly264Ser, p.Gln143Arg, p.Ile144Thr, p.Arg212His, p.Asp242Asn, p.Ile244Val, p.Arg258His and p.Leu262Val) as well as one homozygous p.Gly264Ser carrier. Of the 20 germline heterozygous carrier tumours, four were found to harbour a second hit through loss of the wild-type allele (loss of heterozygosity, LOH). However, another five had lost the mutant allele while eleven others remained heterozygous. On further investigation, none of the heterozygous cases showed evidence of promoter hyper-methylation or somatic point mutations in *RAD51C*. Of the eight tumours from heterozygous carriers of the p.Gly264Ser allele, only four showed copy number loss with three of these involving loss of the variant allele. Importantly, both the p.Gly264Ser homozygous carrier and the case with loss of the wild-type allele had HRD scores below those indicative of loss of homologous recombination function (27).

Table 2
Molecular analysis of 21 tumours from *RAD51C* missense variant carriers.

Sample	Variant	Hormone Receptor/ HER2 Status	Allele status	HRD Score	Promoter Hyper-Methylation	Tp53 Somatic Mutation
1	p.Gly264Ser	TN	Germline homozygous	37	-	Mutated
2		TN	Wild-type loss	15	No	Mutated
3		TN	Variant loss	122	No	Mutated
4†		TN	Variant loss	N/A	No	N/A
5		TN	Heterozygous	39	NA	Mutated
6†		ER-/ HER2-	Variant loss	43	No	Mutated
7		ER+/HER2-	Heterozygous	10	NA	Wild-type
8		ER+/HER2-	Heterozygous	29	No	Wild-type
9		ER+/HER2+	Heterozygous	28	No	Mutated
10	p.Glu143Arg	TN	Variant loss	67	No	Mutated
11		ER-/HER2+	Heterozygous	12	NA	Wild-type
12		ER+/HER2-	Heterozygous	6	No	Wild-type
13		ER+/HER2-	Heterozygous	N/A	No	N/A
14	p.Ile144Thr	TN	Wild-type loss	78	No	Mutated
15		ER+/HER2-	Heterozygous	18	No	Wild-type
16	p.Arg212His	ER-/HER2+	Wild-type loss	47	No	Mutated
17		ER+/HER2-	Heterozygous	10	No	Wild-type
18	p.Asp242Asn	ER+/HER2+	Variant loss	49	No	Wild-type
19	p.Ile244Val	TN	Variant loss	78	No	Mutated
20	p.Arg258His	OvCa	Wild-type loss	70	No	Mutated

All samples were sequenced using targeted panel, with the exception of samples 11 and 20 with whole-exome and samples 4 and 13 with exon-specific Sanger sequencing. HRD: homologous recombination deficiency.

†carriers are 1st degree related

Sample	Variant	Hormone Receptor/ HER2 Status	Allele status	HRD Score	Promoter Hyper-Methylation	Tp53 Somatic Mutation
21	p.Leu262Val	ER+/HER2-	Heterozygous	28	No	Wild-type
All samples were sequenced using targeted panel, with the exception of samples 11 and 20 with whole-exome and samples 4 and 13 with exon-specific Sanger sequencing. HRD: homologous recombination deficiency.						
†carriers are 1st degree related						

Loss of the wild-type allele was identified in two triple-negative tumours carrying p.Ile144Thr and p.Arg212His variants respectively, with both showing high HRD scores, while ER-positive tumours carrying these variants remained heterozygous. An ovarian tumour carrying p.Arg258His also showed LOH and had a high HRD score of 70. Among four tumours sequenced that carried the p.Glu143Arg variant, the one triple-negative case was found to have lost the variant allele, while the one ER-negative and two ER-positive tumours remained heterozygous. All three tumours carrying a germline p.Leu262Val, p.Ile244Val or p.Asp242Asn variant were also showed to remained heterozygous.

Previous studies have shown that breast tumours from individuals carrying a LoF mutation in *RAD51C* accompanied with loss of the wild-type allele were associated with single base substitution mutational signature 3 (1, 28), and this was assessed for tumours from carriers of candidate MS variants. To achieve the minimum recommended number of somatic mutations ($n \geq 40$) for mutational signature estimation, tumours were grouped into those carrying variants of unknown significance (VUS) and those carrying the benign variant p.Gly264Ser (Supplementary Fig. 1). The contribution of signature 3 in tumours carrying a VUS was similar to the tumours carrying the p.Gly264Ser variant. When stratified based on tumour pathology, triple-negative tumours had a higher proportion of signature 3 and higher HRD scores but these were similar in both VUS and benign variant carriers. Whole exome sequencing of a high grade serous ovarian cancer carrying the p.Arg258His variant (case 20) was shown to have lost the wild-type and accompanied by a large proportion of signature 3 and other smaller signatures related to nucleotide excision repair.

Pedigree Segregation Of Ms Variant Carriers

Nine additional family members from seven families, (representing three different variants), were available to examine the segregation of the germline variant detected in the index case (Supplementary Fig. 2). Four of the families carried the p.Gly264Ser variant which was found to be present in two affected first degree relatives (FDR) (ER + BC 43, BC 50), but absent in two affected second degree relatives (ER + BC 38, lobular ER + BC 56) of the respective index cases. The variant p.Gln143Arg was present in a first degree relative diagnosed with TNBC (age 55), ER-positive breast cancer (age 72) and high grade serous ovarian cancer (age 74), while none of the three unaffected FDR, tested from p.Gln143Arg families carried

the variant. Finally, the daughter of an index case carrying the p.Gln137Arg variant remained unaffected but is currently only 35 years old.

Discussion

Germline protein truncating variants in *RAD51C* are known to be associated with predisposition to developing high grade serous ovarian cancer and TNBC (1, 5, 29) but whether there are missense variants of equivalent penetrance is unclear. Data from the BEACCON study has demonstrated that collectively, rare *RAD51C* MS variants are enriched in familial breast cancer, and consistent with protein truncating variants, are more strongly associated with ER-negative and TNBC. Based on an excess in cases and *in silico* predictions, this study has identified a number of potentially pathogenic variants, however definitive designation is challenging due to the low frequency among the population. Nevertheless, our data does exclude some variants as being moderate- to high-penetrance variants. For example, p.Gly264Ser has previously been reported in several small studies to be associated with ovarian and/or breast cancer (4, 5) (6), which was consistent with functional assays showing this variant caused partial reduction of RAD51C cellular function including cell survival, mitomycin C sensitivity and homologous recombination activity (5, 9). However, in the more highly powered BEACCON study, the p.Gly264Ser allele was detected at similar frequencies in cases and controls and was not associated with loss of the wild-type allele in breast cancers from carriers. In addition, the tumour from the homozygous p.Gly264Ser carrier did not show a high HRD score, indicating that its HR pathway remained intact. The data strongly suggests that despite *in vitro* functional assays showing p.Gly264Ser reduces the activity of RAD51C, it is not associated with increased risk of breast cancer. Taken together, our data conflict with the suggestion that this variant may be pathogenic and highlight the need for caution when extrapolating from the results of functional assays to clinical classification of variants.

A number of rare variants previously have been reported as likely pathogenic, including p.Gln143Arg (7, 8), p.Arg258His (7, 9, 10), p.Cys135Tyr (7, 8, 30), p.Ile144Thr (7, 31) and p.Val169Ala (4, 5). In this study, p.Gln143Arg was detected in 0.7% of cases (n = 4), including one TNBC and two with family history of ovarian cancer, and 0.2% of controls (n = 3), consistent with the observed *RAD51C* phenotypes. Pedigree segregation of family 22 also supported that the variant p.Gln143Arg segregated with two subjects affected with ductal breast cancer. Previously described as unlikely to be pathogenic (32), p.Arg212His was detected in this study in two cases (0.03%) and no controls, while also predicted as deleterious by all five *in silico* tools. p.Val169Ala on the other hand was identified in 12 control subjects, three-fold higher than the case frequency, making it unlikely to be a pathogenic variant. Among 12 tumours sequenced across seven germline variants, bi-allelic inactivation and high HRD scores were observed in ER-negative breast cancers and an ovarian cancer of p.Ile144Thr, p.Arg212His and p.Arg258His carriers but not in tumours of p.Glu143Arg, p.Asp242Asn, p.Ile244Val and p.Leu262Val carriers. Promoter hyper-methylation, which has been observed in *BRCA1/2* tumours, appears unlikely to be an important mechanism for *RAD51C* (33), with no instances observed in the tumours examined. Although the number of tumours and family members sequenced for each variant was still low, when combined with the case-

control results, the data provides support for further investigation of those variants identified in this study as candidates by expansion or pooling of databases.

While this study generated evidence against the pathogenicity of p.Gly264Ser, there are several limitations to interpreting results for other variants. Despite a large sample size of ~ 20,000 subjects, the power of the study was limited in its capacity to identify and assess individual rare variants. For the variants examined here, most of which have a MAF of ~ 10^{-5}, to securely identifying an odds ratio of > 2 would require a sample size of several million (~ 4.7 million total cases and controls by standard power calculation). Such numbers seem unachievable even with extensive international collaboration. The statistical power is further eroded by the fact that recent findings indicate that only the rarer TN subset of breast cancer is attributable to *RAD51C* (1–3). Given these limitations of case control analyses, insights from tumour sequencing, that includes identifying a "second hit" and characteristic genome alterations may offer the best avenue for validating or refuting a role for RAD51C MS variants in breast cancer predisposition.

Conclusions

Evidence from this study supports an association of *RAD51C* MS variants with familial breast cancer but due to their rarity this study was not sufficiently powered on its own to identify individual pathogenic variants. Tumour sequencing provided an additional tool to interrogate the *in vivo* consequences of candidate variants and was able to robustly classify some variants a benign. Case-control and tumour sequencing show that the p.Gly264Ser variant is unlikely to be a moderate- to high- penetrance variant, despite *in vitro* assays showing partial functional impairment. These findings raise questions about the validity of functional assays as accurate predictors of variant pathogenicity. Overall, integrating case-control data with tumour sequencing provides a powerful strategy to clarify the role of *RAD51C* MS variants in breast cancer predisposition.

Abbreviations

TNBC
Triple negative breast cancer
MS
Missense
MAF
Minor allele frequency
HRD
Homologous recombination deficiency
LoF
Loss-of-function
LOH
Loss of heterozygosity

HBOC
Hereditary breast and ovarian cancer
FFPE
Formalin fixed, paraffin embedded
ViP
Variant in Practice Study
VUS
Variant of unknown significance
FDR
First degree relative(s)

Declarations

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committees at each participating ViP study recruitment centre and the Peter MacCallum Cancer Centre (Approval # 09/29). All participants provided informed consent for genetic analysis of their germline DNA (cases and controls) and tumour DNA (cases only).

Consent for publication

Not applicable

Availability of data and materials

All sequencing data are deposited to European Genome-phenome Archive which are available upon request to corresponding author. Standard R codes were used. Code requests should be addressed to Prof. Ian Campbell.

Competing interests

The authors declare no competing financial interests.

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Authors' contributions

B.W.X.L. contributed to tumour processing and sequencing, data analysis, and manuscript writing; N.L. contributed to germline data collection and data analysis; S.M.R. contributed to generating sequencing libraries and sample management; E.R.T. contributed to study design and data analysis; M.Z. contributed to bioinformatics analysis and plotting; S.M. and L.D., contributed to collection of study materials or patients; R.J.S. contributed to provision of patients' material and data interpretation; E.K.S. contributed to data interpretation and manuscript revision; P.A.J. contributed to study design, clinical interpretation, and manuscript revision; I.G.C. contributed to study design, data analysis and manuscript revision. All authors contributed to drafting, revising and final approval of the manuscript.

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Figures

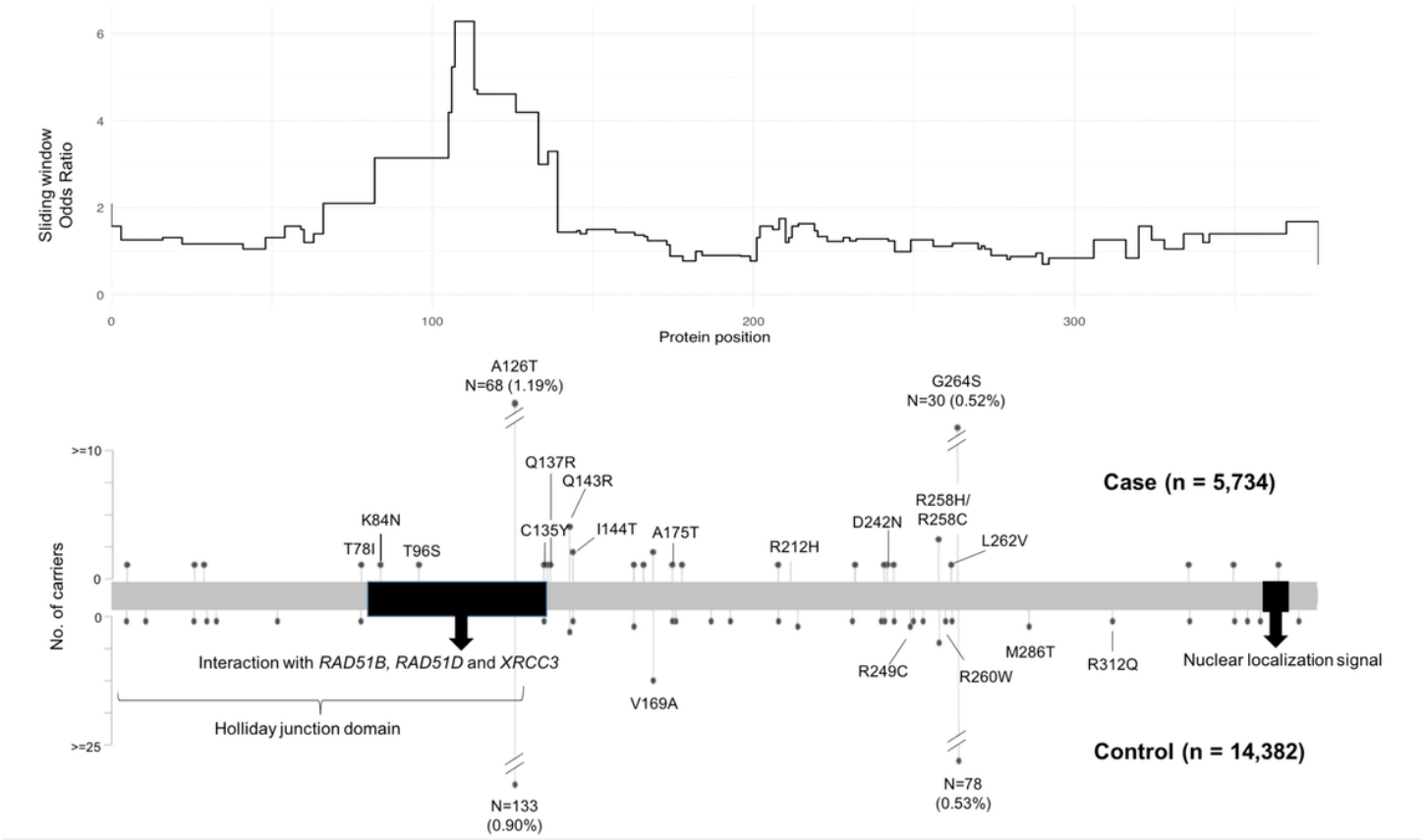

Figure 1

The location and frequency of RAD51C missense variants detected in cases (n = 5,734) and controls (n = 14,382) generated using cBioPortal. Sliding window plot shows the case-control odds ratio in position-based analysis. Key variants are marked with protein change and variants of interest are pointed with arrows. Holliday junction domain includes protein position 1 to 126, domain interacting with RAD51B, RAD51D and XRCC3 include protein position 79 to 136. Note the y-axis scale is different for cases and controls, accounting for the control cohort being more than twice as larger than the case cohort.

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