Germline *RAD51C* mutations confer susceptibility to ovarian cancer

To the Editor:

In 2010, Meindl and colleagues proposed that germline *RAD51C* mutations confer high risk for breast and ovarian cancer, comparable to *BRCA1* and *BRCA2* mutations[1,2]. However, multiple follow-up studies have provided no supportive evidence that *RAD51C* mutations predispose to breast cancer[3–12].

Following the original report, we began investigating the role of other RAD51 paralogs in breast and ovarian cancer susceptibility. This led to our recent discovery that germline *RAD51D* mutations predispose to ovarian cancer[13]. We identified truncating *RAD51D* mutations in 8 of 911 familial breast-ovarian cancer pedigrees and 1 of 1,060 population controls. Our analysis of simultaneous association with both breast and ovarian cancer risk showed that *RAD51D* mutations confer a sixfold increased risk of ovarian cancer (relative risk (RR) = 6.30, 95% confidence interval (CI) = 2.86–13.85; $P = 4.8 \text{ °— } 10^{-6}$) but do not affect or cause only a small increase in breast cancer risk (RR = 1.32, 95% CI = 0.59–2.96; $P = 0.50$). This result was supported by our analysis of 737 familial breast cancer pedigrees with no ovarian cancer, in which we detected no *RAD51D* mutations.

These findings prompted us to reevaluate the role of *RAD51C* in cancer susceptibility. We sequenced the full coding region and intron-exon boundaries of *RAD51C* in 1,132 probands from families with a history of ovarian cancer occurring with or without breast cancer, 272 individuals with ovarian cancer from a hospital-based unselected case series and 1,156 population-based controls (**Supplementary Tables 1** and **2** and **Supplementary Methods**). We identified 12 mutations that result in premature protein truncation in cases compared to 1 such mutation in controls ($P = 0.009$) (**Table 1** and **Supplementary Fig. 1**). Nine mutations were identified among the 1,132 familial cases, and there was a higher prevalence of mutations in families with multiple ovarian cancer cases: 4 mutations were detected in 311 families with 2 or more cases of ovarian cancer, and 2 mutations were detected in the 67 families with 3 or more cases of ovarian cancer. Three mutations were identified among the 272 individuals with ovarian cancer unselected for family history, suggesting that ~1% of ovarian cancer cases harbor germline *RAD51C* mutations.

We also identified a total of 12 nonsynonymous *RAD51C* variants (**Supplementary Table 3**). Four variants were identified in cases and controls; only one, c.790G>A, encoding a p.Gly264Ser amino-acid change, showed any evidence of association with cancer ($P = 0.02$), consistent with other studies[2,9,12]. Of note, this variant is predicted to be benign by *in silico* analyses and has limited impact on RAD51C function[2]. The remaining eight nonsynonymous variants were each identified in a single individual; there was no significant difference in the overall frequency ($P = 0.36$), position or predicted functional effects of these variants between cases and controls (**Supplementary Table 3**). These data exemplify the inherent complexities of evaluating the clinical consequences of missense variants (outside simple Mendelian disorders) and underscore why non-truncating and truncating variants should be considered separately. Analyzing controls for specific rare variants detected in cases and concluding that their absence in controls is evidence of pathogenicity can result in over-interpretation of the data. Such findings confirm that the specific variant is rare but can seldom provide conclusive evidence of disease association. Full sequencing of the gene in both cases and controls is a more appropriate analysis, as it allows the spectrum of variants in cases and controls to be directly compared. Functional and conservation data can be useful in the evaluation of variants, but *in vitro* functional effects do not necessarily imply that the variant has clinical sequelae. Moreover, as we and others have shown (for example, in studies of the breast cancer susceptibility genes *BRIP1* and *ATM*), such an assumption can result in incorrect attribution of pathogenicity[14,15]. Better information is provided when mutational and functional analyses are equally ascertained in both cases and controls.

To estimate the risk associated with *RAD51C* mutations, we undertook modified segregation analysis, in which we simultaneously modeled the risks of ovarian and breast cancer and incorporated control data and information from the full pedigrees of mutation-positive and mutation-negative families (**Supplementary Methods**). The relative risk of ovarian cancer for *RAD51C* mutation carriers was estimated to be 5.88 (95% CI = 2.91–11.88; $P = 7.65 \times 10^{-7}$), which constitutes a >9% cumulative risk by age 80. In contrast, there was no evidence of an association with breast cancer (RR = 0.91, 95% CI = 0.45–1.86; $P = 0.8$). Thus, the cancer risk estimates for *RAD51C* mutations were similar to those estimated for *RAD51D* mutations[13].

These data are fully consistent with the results presented by Meindl *et al.* and provide a likely explanation for why Meindl *et al.* identified *RAD51C* mutations only in breast cancer cases that had relatives with ovarian cancer and not in 620 familial breast cancer pedigrees without ovarian cancer. As *RAD51C*

Table 1 Cancer history in *RAD51C* mutation carriers

ID[a]	Mutation[b]	Protein alteration	Cancer (age at diagnosis)
Breast and/or ovarian cancer familial series			
Fam1	c.837+5G>T		Ovarian (46 years)
			Breast (45 years)
Fam2	c.1026+5_1026+7delGTA[c]		Breast (35 years)
Fam3	c.706-2A>G		Ovarian (42 years)
			Breast (65 years)
Fam4	c.577C>T	p.Arg193*	Ovarian (69 years)
Fam5	c.397C>T	p.Gln133*	Ovarian (61 years)
Fam6	c.664C>T	p.Gln222*	Breast (35 years)
Fam7	c.955C>T	p.Arg319*	Ovarian (59 years)
Fam8	c.397C>T	p.Gln133*	Breast (36 years)
Fam9	c.706-2A>G		Breast (36 years)
Unselected ovarian cancer series			
	c.704dupA		Ovarian (63 years)
	c.706–2A>G		Ovarian (56 years)
	c.904+5G>T		Ovarian (54 years)
Control series			
	c.1026+5_1026+7delGTA[c]		

[a]Family IDs correspond to those in **Supplementary Figure 1**. [b]Mutation nomenclature corresponds to Ensembl Transcript ID ENST00000337432. [c]This mutation is predicted to abolish the exon 8 splice-donor site.

mutations predispose to ovarian cancer, an apparent association may be inferred with any additional phenotype studied in relatives of mutation-positive ovarian cancer cases if the exact relationships between family members (both affected and unaffected) and mutation segregation with each of the phenotypes is not taken into account. These factors need to be considered in risk calculations; failure to do so will inevitably lead to overestimation of the risk of the second phenotype. Our results are also consistent with the data presented in other follow-up studies[3–12]. Analysis of large series of ovarian cancer cases from the general population would now be of value to better estimate the frequency of and risk conferred by *RAD51C* and *RAD51D* mutations and to inform clinical implementation of these genes.

The identification of *RAD51C* as a cancer predisposition gene was an important discovery[1,2]. However, we note that Meindl *et al.* did not present any risk analyses to quantify the extent of associations between *RAD51C* mutations and risk of breast and/or ovarian cancer, and we believe that their data warranted a more cautious interpretation. As we enter an era in which mutational data will become readily obtainable, appropriate genetic and epidemiological experiments are required for the clinical promise of genetic research to be realized.

Note: Supplementary information is available on the Nature Genetics website.

AUTHOR CONTRIBUTIONS
N.R., C.L. and C.T. designed the experiment. M.W.-P., C.T., K.S., D.E., D.G.E., BCSC (UK), M.G. and N.R. coordinated recruitment of cases and samples. C.L., R.M.M.X., E. Ramsay, D.H., A.R. and S.S. performed sequencing of *RAD51C*. C.T., E. Ruark and A.C.A. performed statistical analyses. N.R. oversaw all aspects of the study.

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COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Meindl *et al.* reply:

Loveday *et al.*[1] claim, as do Pelttari *et al.*[2], that *RAD51C* is a predisposing gene for ovarian cancer. However, their screening results do not falsify or disprove our assertion that *RAD51C* is a predisposing gene for breast cancer and ovarian cancer[3]. Indeed, we found that *RAD51C* mutations segregated with breast cancer in two out of the seven families with breast cancer and ovarian cancer we analyzed[3]. Furthermore, Vuorela *et al.*[4] found an in-frame deletion in one individual with breast cancer from a family with four cases of breast cancer and four cases of ovarian cancer. However, they were unable to establish segregation in this pedigree.

The skepticism of Loveday *et al.* toward a pathogenic role for missense mutations is unwarranted. In general, these authors refuse to accept the causality of missense mutations in *RAD51C* in breast cancer. In fact, most of the variants discussed here are predicted to affect amino-acid residues conserved in at least three of the five RAD51 paralogs, and the effects of the variants have been characterized by functional approaches. It is, of course, easier to classify a truncating mutation as pathogenic. We note that Clague *et al.*[5] recently reported a missense variant in *RAD51C*, which seems to compromise the interaction between the RAD51C protein and its interacting partners RAD51B and XRCC3.

The statistical arguments presented[1] might be valid only for a subgroup of families or populations. Here we agree with Rahman and colleagues that *RAD51C*, as well as *RAD51D*, have to be validated in larger cohorts to generate reasonable clinical proposals or conclusions. Rahman *et al.*, as in our study[3], found the p.Gly264Ser alteration in RAD51C (encoded by a c.790G>A mutation) overrepresented in families with breast cancer and ovarian cancer compared to controls. However, there was also a statistically significant overrepresentation of this variant in individuals with ovarian cancer from Australia[6]. Although screening of samples of larger size is required, these observations are consistent with population-specific effects.

AUTHOR CONTRIBUTIONS
A.M. wrote the paper and designed the concept. K.E., S.E., A.B., D.E. and N.D. provided experimental or clinical data. R.K.S. designed the concept and collected clinical data. D.S. supervised the experiments.

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