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A Finnish founder mutation in *RAD51D*: analysis in breast, ovarian, prostate, and colorectal cancer

Liisa M Pelttari¹, Johanna Kiiski¹, Riikka Nurminen², Anne Kallioniemi², Johanna Schleutker^{2,3}, Alexandra Gylfe⁴, Lauri A Aaltonen⁴, Arto Leminen¹, Päivi Heikkilä⁵, Carl Blomqvist⁶, Ralf Bützow^{1,5}, Kristiina Aittomäki^{4,7}, and Heli Nevanlinna¹

¹Department of Obstetrics and Gynecology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland ²Institute of Biomedical Technology/BioMediTech, University of Tampere and Fimlab Laboratories, Tampere, Finland ³Department of Medical Biochemistry and Genetics, University of Turku, Turku, Finland ⁴Department of Medical Genetics, Genome-Scale Biology Research Program, University of Helsinki, Helsinki, Finland ⁵Department of Pathology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland ⁶Department of Oncology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland ⁷Department of Clinical Genetics, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

Abstract

Background—*RAD51D* and *RAD54L* are involved in homologous recombination, and rare mutations in *RAD51D* were recently found in breast-ovarian cancer families. This study investigated *RAD51D* and *RAD54L* for mutations in breast and ovarian cancer patients in the Finnish population.

Methods—The study sequenced the *RAD51D* and *RAD54L* genes in 95 breast and/or ovarian cancer families and genotyped the identified mutation in an additional 2200 breast and 553 ovarian cancer patients and 2102 population controls. To investigate the role of the mutation in other common cancers, 1094 prostate and 980 colorectal cancer patients were genotyped.

Results—In the screening of *RAD51D*, one deleterious founder mutation c.576+1G>A was identified in two breast-ovarian cancer families. No mutations were found in *RAD54L*. Altogether, the c.576+1G>A mutation was detected in 5/707 patients with a personal or family history of ovarian cancer (OR 9.16, 95% CI 1.07 to 78.56; p=0.024), with the highest frequency among breast-ovarian cancer families (3/105 vs 1/1287 controls, OR 37.82, 95% CI 3.90 to 366.91;

Correspondence to Dr Heli Nevanlinna, Helsinki University Central Hospital, Department of Obstetrics and Gynecology, Biomedicum Helsinki, PO Box 700, FIN-00029 HUS, Finland; heli.nevanlinna@hus.fi.

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$p=0.0016$), but no elevated frequency among breast cancer patients/families (2/2105, $p=1$). The mutation was not found among prostate or colorectal cancer patients.

Conclusions—The results of this study on familial and unselected breast, ovarian, colorectal, and prostate cancer patients suggest that *RAD51D* is primarily a moderate penetrance susceptibility gene for ovarian cancer, with clinical significance for the carriers.

Most of the high risk familial breast and ovarian cancer is caused by germline mutations in *BRCA1* and *BRCA2* genes that are important for DNA repair.^{1 2} Mutations in several other DNA repair genes have also been found to increase the risk of breast cancer.³ Recently, pathogenic mutations in *RAD51* paralogs *RAD51C4* and *RAD51D5* involved in homologous recombinational repair, were identified in breast and ovarian cancer families. A homozygous *RAD51C* mutation has also been found in Fanconi anaemia-like disorder.⁶ All mutations in *RAD51C* and *RAD51D* in the studies by Meindl *et al*⁴ and Loveday *et al*⁵ were found in families with both breast and ovarian cancer, and all the mutations were unique for each family. We identified two deleterious *RAD51C* founder mutations in Finnish patients with a personal or family history of ovarian cancer, with a highly increased risk for familial ovarian cancer but not for breast cancer without a family history of ovarian cancer.⁷ In the Finnish population, strong founder mutations typical for isolated populations have been found previously in *BRCA1* and *BRCA2*.⁸ Recurrent mutations have also been found in moderate penetrance genes *PALB2* and *CHEK2*.^{9 10} *RAD54L* encodes a protein that associates with *RAD51* in homologous recombination.¹¹ To our knowledge, the association of *RAD54L* with familial breast and ovarian cancer has not been previously studied.

In this study we have sequenced the *RAD51D* and *RAD54L* genes in 95 familial breast or ovarian cancer patients to investigate the role of these genes for cancer predisposition in the Finnish population. We identified one recurrent deleterious mutation c.576+1G>A in *RAD51D* in two patients. To study further the cancer risks associated with the *RAD51D* mutation we genotyped the identified c.576+1G>A mutation in an extensive series of breast and ovarian cancer patients as well as in prostate and colorectal cancer patients and population controls. To confirm the pathogenic nature of the mutation, we analysed tumour samples from mutation carriers to see whether there is loss of heterozygosity. We also performed haplotype analysis to find out whether the identified mutation is a Finnish founder mutation.

Patients and Methods

First, we screened the coding region and exon–intron boundaries of the *RAD51D* and *RAD54L* genes for germline variation in 95 familial *BRCA1/2* and *RAD51C* negative breast or ovarian cancer patients ascertained at the Helsinki University Central Hospital. Fifty-one of the patients are from families with multiple breast cancer cases, 40 from families with both breast and ovarian cancer, and four from ovarian cancer families. Primers and PCR conditions are listed in supplementary table 1. We identified the c.576+1G>A mutation in two breast cancer patients who both had strong familial background of breast and ovarian cancer. We also detected four previously known polymorphisms rs28363258, rs9901455, rs4796033, and rs45496096 among the patients. No mutations were detected in the *RAD54L* gene; however, analysis in larger patient series will be needed to assess the role of *RAD54L*.

in breast and/or ovarian cancer susceptibility. Nine previously known polymorphisms rs17102080, rs28363192, rs2295465, rs28363218, rs61239976, rs118091259, rs150315374, rs3188812, and rs28910278 in *RAD54L* were detected.

We further genotyped the *RAD51D* c.576+1G>A mutation with Taqman real-time PCR in an additional 297 patients from breast and/or ovarian cancer families (including 281 probands with breast cancer, 13 with ovarian cancer, and three with breast and ovarian cancer), 337 breast cancer and four breast and ovarian cancer patients with one first degree relative affected with breast cancer, 32 breast cancer patients with one first degree relative affected with ovarian cancer, 10 patients with both breast and ovarian cancer, 849 unselected breast cancer patients, 541 unselected ovarian cancer patients, and 1287 healthy female population controls from the Helsinki region. All the familial breast, ovarian, or breast and ovarian cancer patients had previously tested negative for *BRCA1/2* mutations. The mutation was also genotyped in 691 breast cancer patients, 1094 prostate cancer patients (including 905 unselected and 189 familial cases), and 815 healthy population controls from the Tampere region of Finland, and 980 colorectal cancer patients (including 158 familial cases) from south-eastern Finland. All samples were genomic DNA isolated from peripheral blood, except the colorectal cancer samples which were genomic DNA isolated from blood or normal mucosa and 207 of the ovarian cancer samples which were tumour DNA. The datasets are described in more detail in supplementary material and methods. This study was carried out with informed consent from the patients and with permission from the respective ethics committees.

Results

When combining mutation screening of the whole coding region of *RAD51D* and genotyping of the c.576+1G>A mutation, the mutation was found in 1/344 breast cancer families ($p=0.38$, compared to population controls) but in 3/105 breast and ovarian cancer families ($p=0.0016$) (table 1). Two of the breast and ovarian cancer families had two ovarian cancer cases, and one family had one ovarian cancer case; two probands had breast cancer and one ovarian cancer. Among the unselected ovarian cancer patients the mutation was found in 3/541 patients ($p=0.08$). One of the unselected patients is also included in the set of breast and ovarian cancer families, one patient has no breast or ovarian cancer family history, and for the third patient the cancer family history is not known. Combined, the c. 576+1G>A mutation was detected in five out of 707 patients with a personal or family history of ovarian cancer ($p=0.024$). Among the breast cancer cases from Tampere (691 unselected cases) and Helsinki (1414 breast cancer cases without family history of ovarian cancer) combined, two mutations were found (2/2105 vs 1/2102 controls, $p=1$). Altogether, the mutation was found in seven cases and in one control aged 26 years. No mutations were found among the prostate or colorectal cancer cases.

Additional genotyping was done in relatives of the mutation carriers by sequencing the *RAD51D* exon 6. Only one family member with invasive ovarian cancer and one with invasive breast cancer had available samples for sequencing and they were both found to carry the mutation. One patient with *in situ* breast cancer was mutation negative. Two out of

five healthy males and four out of nine healthy females (aged between 42 and 72 years) were carrying the mutation.

To investigate whether the mutation represents a founder mutation in Finland we also undertook haplotype analysis for the mutation carriers by sequencing eight single nucleotide polymorphisms (SNPs)—rs797993, rs936656, rs8075760, rs8077720, rs3736143, rs8069929, rs9303682, and rs4795064—within and around *RAD51D* spanning 95221 bp (supplementary table 2). Ten individuals with available genomic DNA samples from four mutation carrier families were included in the haplotype analysis. All the studied mutation carriers shared the same haplotype. This suggests that the *RAD51D* c.576+1G>A mutation is a Finnish founder mutation and the families have a common ancestry.

The identified mutation is a G to A substitution of the first base after the *RAD51D* exon 6. We used Human Splicing Finder (<http://www.umd.be/HSF/#>) and MaxEntScan (http://genes.mit.edu/burgelab/maxent/Xmaxentseq_scoreseq.html) to predict the effect of the mutation on the splice site. The mutation is predicted to disrupt the 5' donor splice site of exon 6 and the MaxEntScan splice site score decreases from 9.60 to 1.42 with the mutation. This may lead to skipping of adjacent exon (s), inclusion of intron(s) or usage of cryptic splice sites and ultimately to a truncated protein.

We further carried out loss-of-heterozygosity (LOH) analysis for four tumours from mutation carriers to see whether the tumours have lost the wild-type allele. DNA isolated from paraffin embedded tumour tissues was amplified and sequenced using primers specific for *RAD51D* exon 6. All three ovarian tumours studied showed pronounced reduction of the wild-type allele whereas the breast tumour did not show LOH (supplementary figure 1). This is in agreement with the observed association with ovarian cancer and supports the pathogenic nature of the mutation.

The mean age of breast cancer diagnosis among the mutation carriers was 45.7 years (range 35.7–61.4 years) which is younger than among all the mutation negative breast cancer patients (56.5 years) ($p=0.13$) or among the familial (55.0 years) ($p=0.17$) or sporadic breast cancer patients (58.0 years) ($p=0.09$). The mean age of ovarian cancer diagnosis was 66.5 years (range 53.6–79 years), slightly higher than in the general population (62 years).¹² The breast tumours were of ductal histology and the ovarian tumours were serous. The breast tumours were of varying grade but no information on grade or stage of the ovarian tumours was available.

Discussion

The identified founder mutation in the *RAD51D* gene allowed us to assess the role of *RAD51D* not only in large series of unselected breast and ovarian cancer patients but also in other common cancers which have not previously been studied. Mutations in breast cancer susceptibility genes may increase the risk of other cancer types besides breast and ovarian cancer. An example of this is the moderate penetrance breast cancer susceptibility gene *PALB2* which also confers an increased risk of pancreatic cancer¹³ and *BRCA2* mutations which increase the risk of prostate cancer.¹⁴ Ovarian cancer is also present in Lynch

syndrome that predominantly predisposes to colorectal cancer.¹⁵ We did not identify any *RAD51D* c.576+1G>A mutations in the genotyping of prostate and colorectal cancer patients. However, screening of the whole coding region would be needed for an in-depth analysis of the role of *RAD51D* in other cancer types.

As *RAD51D* mutations are very rare, it is difficult to make precise risk estimates even for the breast-ovarian cancer families or ovarian cancer cases. We found the founder mutation c.576+1G>A in 2.9% of the patients with a family history of breast and ovarian cancer, with an OR of 37.82 (95% CI 3.90 to 366.91, $p=0.0016$, compared to population controls) (table 1). Among all the patients with either personal or family history of ovarian cancer the OR was 9.16 (95% CI 1.07 to 78.56, $p=0.024$) and among unselected ovarian cancer patients it was 7.17 (95% CI 0.74 to 69.10, $p=0.08$). The frequency among the breast cancer cases in the Helsinki and Tampere series combined was similar to the population controls (0.1% vs 0.05%, $p=1$). Altogether, our data and the results by Loveday *et al*⁵ indicate *RAD51D* as a moderate penetrance ovarian cancer susceptibility gene. Loveday *et al*⁵ detected *RAD51D* mutations in eight of 911 (1%) breast-ovarian cancer families, with a higher prevalence in families with more than one case of ovarian cancer. They further estimated that the relative risk of ovarian cancer for *RAD51D* mutation carriers was 6.30 (95% CI 2.86 to 13.85) whereas the breast cancer risk was not statistically significantly elevated (1.32, 95% CI 0.59 to 2.96). These results also suggest the clinical significance of *RAD51D* mutation testing in families with ovarian cancer patients.

Similar to our previous results on *RAD51C*, the association of the *RAD51D* mutation c.576+1G>A with familial breast and ovarian cancer appears to be driven by the high risk of ovarian cancer with no elevated risk for breast cancer *per se*. Also the presence of LOH in all the studied ovarian tumours but not in the breast tumour is in line with this. The presence of multiple breast cancer cases in the mutation carrier families suggests that there might be other unidentified mutations in other genes which confer the breast cancer risk in these families or modify the risk conferred by the *RAD51D* mutation. The earlier age of breast cancer diagnosis observed in the *RAD51D* carriers would be in line with this. Furthermore, it is possible that there are mutation specific risk effects on different cancer types such as are seen in *BRCA2* where the risk of breast or ovarian cancer is known to vary by mutation position.¹⁶ Different types of mutations or mutations located in different positions might confer varying cancer risks also in *RAD51C* and *RAD51D*. Notably, the founder mutation we detected as well as all the pathogenic mutations detected by Loveday *et al*⁵ are predicted to result in protein truncation.

In conclusion, we did not identify mutations in the *RAD54L* gene among the breast and breast-ovarian cancer families studied, and in-depth analysis in larger datasets will be needed to assess fully the role of *RAD54L* in cancer susceptibility. However, we identified one recurring deleterious splicing mutation among Finnish breast and ovarian cancer patients in the *RAD51D* gene. The shared haplotype among the mutation carriers suggests a founder effect in the Finnish population. Our results on the founder mutation in extensive series of familial and unselected breast, ovarian, prostate, and colorectal cancer patients suggest that *RAD51D* is primarily a moderate penetrance susceptibility gene for ovarian cancer. Our data, together with those of Loveday *et al*⁵ are consistent with mutations in

RAD51D accounting for a likely modest proportion of the unexplained genetic predisposition to ovarian cancer, with also clinical significance for the carriers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1
Frequencies of the *RAD51D* mutation c.576+1G>A in different patient groups by family history and the risk ratios

	Total	GG	%	GA	%	p Value	OR (95% CI)
Helsinki-control	1287	1286	99.9	1	0.1		
Familial BC	344	343	99.7	1	0.3	0.38	3.75 (0.23 to 60.10)
Familial BC+OC	105	102	97.1	3*	2.9	0.0016	37.82 (3.90 to 366.91)
Familial OC	8	8	100	0	0	1	
Two affected BC	475	475	100	0	0	1	
Two affected BC+OC	63	63	100	0	0	1	
Sporadic BC	595	595	100	0	0	1	
Unselected BC	849	848	99.9	1*	0.1	1	1.52 (0.09 to 24.28)
Unselected OC	541	538	99.4	3*	0.6	0.08	7.17 (0.74 to 69.10)
Any OC	707	702	99.3	5	0.7	0.024	9.16 (1.07 to 78.56)
CRC	980	980	100	0	0	1	
Tampere-control	815	815	100	0	0		
Tampere-BC	691	690	99.9	1	0.1	0.46	
Tampere-prostate	1094	1094	100	0	0	1	

Familial BC includes breast cancer cases with strong family history of breast cancer. Familial BC+OC includes 90 breast, six breast and ovarian, and nine ovarian cancer cases with strong family history of breast and ovarian cancer. Two affected BC includes breast cancer cases with one first degree relative affected with breast cancer. Two affected BC+OC includes breast cancer cases that have one first degree relative affected with ovarian cancer or cases with both breast and ovarian cancer. Any OC includes all cases with personal or family history of ovarian cancer. GG=c.576+1GG, GA=c.576+1GA.

* The mutation positive unselected breast cancer patient and one of the mutation positive unselected ovarian cancer patients are also included in the group of familial breast and ovarian cancer patients.

BC, breast cancer; CRC, colorectal cancer; OC, ovarian cancer.