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SHORT COMMUNICATION

Mutation analysis of the gene encoding the PALB2-binding protein MRG15 in *BRCA1/2*-negative breast cancer families

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MRG15 is a recently identified member of the BRCA multiprotein complex, essential for the maintenance of the genome integrity and DNA repair. The functional relationship between PALB2 and MRG15 makes *MRG15* a strong candidate breast cancer susceptibility gene. We screened affected probands from 232 *BRCA1/2*-negative breast cancer families for mutations in *MRG15*. We identified seven previously unreported variants but *in silico* analyses revealed that none of these variants appears to modify the function of MRG15. Thus, it seems unlikely that any constitutional changes in *MRG15* confer an increased risk for breast cancer.

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DESCRIPTION

Proteins encoded by the breast cancer susceptibility genes *BRCA1* and *BRCA2* and their partners form several networks.[1] One such protein complex is responsible for the maintenance of the genome by repairing damaged DNA and preventing harmful gene rearrangements.[2,3] Deleterious germ-line mutations in *BRCA1* and *BRCA2* are mainly associated with increased risks for breast and ovarian cancers, but pancreas and prostate cancers are also observed at increased frequency in *BRCA2* carriers. Nonetheless, most cases of these cancers are not attributable to mutations in these genes. To date, known protein partners of BRCA1 and BRCA2 have been investigated to determine possible associations between their genetic variants and an increase in the risk of developing cancers.[4] One example is PALB2, which interacts with both BRCA1 and BRCA2 proteins, and multiple different constitutional mutations have been found to be associated with an elevated risk of breast and pancreatic cancers.[5–7] These and other studies show the importance of investigating additional genes from the BRCA network as potential breast cancer susceptibility genes.

Recently, MRG15 (also known as MORF4L1), a new BRCA complex-interacting protein, has been characterized. It participates in the response to double-strand breaks by recruiting the BRCA complex to sites of damaged DNA.[8] MRG15 is encoded by a gene located on 15q24. It is a 41.5 kDa chromodomain protein that directly binds to PALB2.[9] This interaction is essential for homologous recombination regulation during DNA repair. This functional relationship with PALB2 means that *MRG15* is a candidate breast cancer susceptibility gene. Consequently, we have formally investigated this hypothesis by screening germ-line DNA from a set of *BRCA1/2* mutation-negative breast cancer cases for potential cancer-related genetic alterations.

Approval to perform the study was obtained from the local ethical review boards. DNA from blood of affected index cases of 232 breast or breast/ovarian cancer families was screened for *MRG15* mutations. All families were negative for the relevant *BRCA1/2* founder mutations in the Finnish (n=148), French-Canadian (n=45) and Ashkenazi Jewish groups (n=9) and/or were negative for *BRCA1/2* mutations by full sequencing. The average age at diagnosis of the index case was 48.12 years (range 19–89 years) and 225 of 232 (97%) probands presented with breast cancer (including three men), with the remainder presenting with ovarian cancer. A total of 179 families (77%) had three or more affected individuals, 44 families (19%) had two affected individuals and nine index cases (4%) presented with either breast cancer $<$30 years (n=3) or bilateral breast cancer (n=6), with no family history.

Mutation screening of the coding and exon–intron boundaries of the gene was carried out by a combination of high-resolution melting mutation scanning analysis[10] and DNA-sequencing analysis using an

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Table 1 Primer information

Exon	Size of the PCR fragment (bp)	Forward primer (5′–3′)	Reverse primer (5′–3′)
1	574	TCAAATCCCCGCTTTACCAAC	GAATTGCCATCCGCAATGC
2	277	CCCGTCCTCATTTTTCCTAATTT	GGCAACTCTCTTACTGAGGCAAC
3	309	AGGCCACCATAAAGCTTGTGTAG	CAAATGATTCACCTTTGCTTCAA
4	279	AGGCTCAATTCTGCACCTGG	ATGATTTCCCACCTCTGACCTC
5	276	GAGAAATCCAGTCTTGCCTCTTG	TTATAGGCAGGGCAGCCAAC
6	301	TCCTAATGGAATCAGGCTGACA	GACCTGATACCTGAGGAATGGG
7	244	CTGTGAACTTAACCCTGATAAAGGG	CACTGCTGTGAGACTCCTATTTGAG
8	465	GTTCGGTAGAGCTCTTCATGAAAGT	GGGTAGGTAAATCTGGAGCTACAGG
9	351	TCCCTAGTGCCTCTAAAAGTCAGC	CTGGTAGTAGTTCTAAAGTGTTCAGCCT
10	421	CCATTGTAAGTTGGGAACTGTCTG	GCCAACTCCTGTGTCAAAGCA
11	421	CCCTGCTTTCATATGCCCTTTA	AAAATGGGAGTCATGTTTTGGAA
12	337	GGAATTGGTGGTGTTTGCTCA	TGAGACTCCCATGTGTTTGCAC
13	1037	CCTGAATTGTCATTATGTGTGGTTT	GCGTCTCATTCCATAAAGGCC

Table 2 Variants detected in *MRG15*

Sequence variant	Protein change or localization	No. of time observed	Additional information
c.40+42 G>T	Intron 1–2	52	rs8034658
c.87+65 T>C	Intron 2–3	3	Non-referenced
c.88-88 T>G	Intron 2–3	52	rs17243470
c.272+35 C>T	Intron 4–5	7	rs16970187
c.441-67insAA	Intron 6–7	2	Non-referenced
c.441-26 T>C	Intron 6–7	3	rs4778952
c.467-79 T>A	Intron 7–8	18	rs10519215
c.572 A>G	N191S	1	rs73477676
c.658-144 T>C	Intron 9–10	1	Non-referenced
c.658-133 T>C	Intron 9–10	1	Non-referenced
c.658-4 A>G	Intron 9–10	24	rs1836556
c.675 C>A	A225A	42	rs1435163
c.719 A>G	K240R	18	rs61734867
c.746+32 A>G	Intron 10–11	1	rs74024473
c.746+38 A>G	Intron 10–11	1	rs56816206
c.919+12 A>G	Intron 11–12	2	rs16970207
c.920-45 C>A	Intron 11–12	1	Non-referenced
c.1004+95 T>A	Intron 12–13	11	rs10519216
c.1005-185 T>A	Intron 12–13	1	Non-referenced
c.1005-43 T>C	Intron 12–13	60	rs12438433
c.1089+161 G>C	3′UTR	1	Non-referenced

ABI 3130 XL DNA analyzer (Applied Biosystems, Foster City, CA, USA). Primer information is summarized in Table 1. Electropherograms were aligned using Lasergene-SeqMan software (DNASTAR, Madison, WI, USA) and every exon was screened for variants by comparing with MRG15 reference sequence (NC_000015.9, range 79165171–79190074).

In total, we identified 21 variants: 17 intronic changes, 3 coding SNPs and 1 variant localized in the 3′UTR (Table 2). Six out of the 17 intronic variants plus the one localized in the 3′UTR were referenced neither in dbSNP nor Ensembl SNP databases. These seven variants were each detected in not more than 1% of the patients indicating they could be rare and potentially harmful variants (Table 2). To gain further insight into possible negative effects of these non-referenced variants, we performed *in silico* analyses using accurate splicing prediction software: BDGP,[11] NetGene 2 server[12,13] and GENSCAN.[14] None of these intronic changes were predicted to impact on the splicing of the pre-mRNA. Analysis of the 3′UTR, using TargetScanHuman 5.1 software,[15] revealed that the site where the variant in the 3′UTR lies is not a known target of miRNAs. Consequently, it is very unlikely that this variant has any effect on the regulation of *MRG15* mRNA expression.

In conclusion, we found no evidence that mutations in *MRG15* strongly predispose to breast cancer. Given our relatively small sample size, it remains possible that rare, or very rare, variants in this gene will be found to increase the risk for breast cancer. Moreover, sequencing of other novel genes whose products interact with BRCA proteins and their partners should be undertaken as members of this network are some of the most important contributors to the inherited susceptibility to breast cancer.

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1 Narod, S. A. & Foulkes, W. D. BRCA1 and BRCA2: 1994 and beyond. *Nat. Rev. Cancer* **4,** 665–676 (2004).
2 Chen, J., Silver, D. P., Walpita, D., Cantor, S. B., Gazdar, A. F., Tomlinson, G. *et al.* Stable interaction between the products of the BRCA1 and BRCA2 tumor suppressor genes in mitotic and meiotic cells. *Mol. Cell* **2,** 317–328 (1998).
3 Tutt, A. & Ashworth, A. The relationship between the roles of BRCA genes in DNA repair and cancer predisposition. *Trends Mol. Med.* **8,** 571–576 (2002).
4 Campeau, P. M., Foulkes, W. D. & Tischkowitz, M. D. Hereditary breast cancer: new genetic developments, new therapeutic avenues. *Hum. Genet.* **124,** 31–42 (2008).
5 Rahman, N., Seal, S., Thompson, D., Kelly, P., Renwick, A., Elliott, A. *et al.* PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat. Genet.* **39,** 165–167 (2007).
6 Jones, S., Hruban, R. H., Kamiyama, M., Borges, M., Zhang, X., Parsons, D. W. *et al.* Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* **324,** 217 (2009).
7 Erkko, H., Xia, B., Nikkila, J., Schleutker, J., Syrjakoski, K., Mannermaa, A. *et al.* A recurrent mutation in PALB2 in Finnish cancer families. *Nature* **446,** 316–319 (2007).
8 Hayakawa, T., Zhang, F., Hayakawa, N., Ohtani, Y., Shinmyozu, K., Nakayama, J. *et al.* MRG15 binds directly to PALB2 and stimulates homology-directed repair of chromosomal breaks. *J. Cell Sci.* **123,** 1124–1130 (2010).
9 Sy, S. M., Huen, M. S. & Chen, J. MRG15 is a novel PALB2-interacting factor involved in homologous recombination. *J. Biol. Chem.* **284,** 21127–21131 (2009).
10 Reed, G. H. & Wittwer, C. T. Sensitivity and specificity of single-nucleotide polymorphism scanning by high-resolution melting analysis. *Clin. Chem.* **50,** 1748–1754 (2004).
11 Reese, M. G., Eeckman, F. H., Kulp, D. & Haussler, D. Improved splice site detection in Genie. *J. Comput. Biol.* **4,** 311–323 (1997).
12 Hebsgaard, S. M., Korning, P. G., Tolstrup, N., Engelbrecht, J., Rouze, P. & Brunak, S. Splice site prediction in Arabidopsis thaliana pre-mRNA by combining local and global sequence information. *Nucleic Acids Res.* **24,** 3439–3452 (1996).
13 Brunak, S., Engelbrecht, J. & Knudsen, S. Prediction of human mRNA donor and acceptor sites from the DNA sequence. *J. Mol. Biol.* **220,** 49–65 (1991).
14 Burge, C. & Karlin, S. Prediction of complete gene structures in human genomic DNA. *J. Mol. Biol.* **268,** 78–94 (1997).
15 Lewis, B. P., Burge, C. B. & Bartel, D. P. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* **120,** 15–20 (2005).