ONLINE MUTATION REPORT

Absence of constitutional *H2AX* gene mutations in 101 hereditary breast cancer families

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Several genes involved in the DNA damage response and in maintaining genomic stability have emerged as breast cancer susceptibility genes. These include *BRCA1* and *BRCA2*, as well as other genes with smaller contributions to breast cancer aetiology, such as *TP53*, *CHEK2*, and *ATM*. Germline mutations in *BRCA1* and *BRCA2* increase sensitivity to DNA damage and decrease cellular capacity to repair double strand DNA breaks through homologous recombination.¹⁻³ Interestingly, DNA damage induces activation of ATM (ataxia-telangiectasia mutated), which performs a central role in relaying signals that orchestrate DNA repair.⁴ People with heterozygous nonsense mutations in *ATM* appear to display increased susceptibility to breast cancer.⁵⁻⁷ Besides rapidly phosphorylating *BRCA1*,⁸ activated ATM also phosphorylates p53 and *CHEK2* (*CHK2*, *hCDS1*), which have been implicated in breast cancer predisposition. Germline mutations in *TP53* or *CHEK2* cause Li-Fraumeni syndrome, a multiple cancer phenotype syndrome, which features early onset breast cancer.⁹⁻¹⁰ Recently, it was found that germline mutations in *CHEK2* also increase the relative risk for breast cancer outside the Li-Fraumeni syndrome.¹¹⁻¹² Given the emerging relationship of impaired DNA damage response and breast cancer susceptibility, we hypothesised that other genes in this pathway might be candidate cancer susceptibility genes.

Histone *H2AX* (*H2AFX*, OMIM 601772) is such a candidate gene. *H2AX* is a minor variant of the highly conserved histone H2A that is part of the histone octamer in the core of the nucleosome.¹³ It differs from H2A by having a longer carboxy-terminal tail that contains an SQE motif, a consensus site for phosphorylation by PI3K related kinases such as ATM, ATR, and DNA-PK. Following DNA damage, ATM phosphorylates *H2AX* at serine 139 (part of the SQE motif)¹⁴ and phosphorylated *H2AX* (γ -*H2AX*) seems to localise specifically at sites of damage.¹⁵⁻¹⁶ More importantly, several proteins involved in DNA repair including *BRCA1*, *BRCA2*, *Rad51*, and *Mre11* are recruited to sites of γ -*H2AX*.¹⁶ Two other independent lines of evidence derived from model organisms support the notion that *H2AX* plays an important role in the DNA damage response and in chromosomal stability. Phosphorylation of S129 of *Saccharomyces cerevisiae* H2A (homologous to S139 in human *H2AX*) is necessary for efficient processing of DNA repair and is proposed to cause alteration of chromatin structure that facilitates repair.¹⁷ Mice lacking *H2AX* are sensitive to radiation, are growth retarded, and their cells display high levels of ionising radiation induced chromosomal instability.¹⁸⁻¹⁹ Thus, although the exact function of *H2AX* is still unknown, it is clear that it plays a role in the DNA damage response.

To establish if germline *H2AX* mutations are present in breast cancer families, DNA samples were obtained from 101 unrelated breast cancer patients.

METHODS AND RESULTS

Each of these patients was from a family with three or more cases of breast cancer. These families were selected because

Key points

- Several genes whose products participate in the cellular response to DNA damage have emerged as breast cancer susceptibility genes. These genes include *BRCA1*, *BRCA2*, *ATM*, *CHEK2*, and *TP53* and germline mutations in these genes increase the predisposition to breast cancer.
- We hypothesised that other genes in the DNA damage response pathway might also be breast cancer susceptibility genes. The gene for histone *H2AX* is such a candidate. Its product is an early substrate of ATM kinase activity following DNA damage. Phosphorylated *H2AX* (γ -*H2AX*) colocalises with DNA breaks, and other proteins involved in DNA repair such as *BRCA1*, *Rad51*, and *Mre11* are recruited to γ -*H2AX* sites.
- We screened subjects from 101 families with hereditary breast cancer by direct sequencing of *H2AX* coding sequence. We did not detect any mutations or sequence variants in this sample, suggesting that it is unlikely that germline mutations in *H2AX* play a major role in hereditary breast cancer.

they have previously been tested for the presence of germline mutations in *BRCA1* and *BRCA2* using the protein truncation test (PTT) and no mutations were found. There were, on average, 4.2 cases of breast cancer per family (range 3 to 11) with an average of 3.0 cases of breast cancer in first degree relatives per family (range 2 to 9). Seventeen of the families also contained cases of ovarian cancer. For each family, a single patient affected with breast cancer was studied, with a mean age of diagnosis of 47 years (range 24 to 72 years).

The *H2AX* gene contains a single exon with 432 nucleotides. The coding sequence of the *H2AX* gene was evaluated by direct sequencing of a 561 bp fragment amplified using the following primers: 5'-CGTCTGTTCTAGTGTGAGC-3' and 5'-TGAGGGCGGTGGTGGCCCTTAA-3'. No mutations or sequence variants were found in the 101 patients.

DISCUSSION

Our results suggest that germline *H2AX* mutations are unlikely to be common in families with familial breast cancer. Furthermore, the absence of polymorphic variation in this gene precludes the possibility that missense variants within the coding region of *H2AX* are associated with breast cancer risk in the population as a whole. It is, of course, possible that rare disease causing mutations in *H2AX* exist, and these might be uncovered in a larger study.

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REFERENCES

- Venkitaraman AR**. Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* 2002;**108**:171-82.
- Moynahan ME**, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. *Mol Cell* 1999;**4**:511-18.
- Moynahan ME**, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol Cell* 2001;**7**:263-72.
- Zhou BB**, Elledge SJ. The DNA damage response: putting checkpoints in perspective. *Nature* 2000;**408**:433-9.
- Teraoka SN**, Malone KE, Doody DR, Suter NM, Ostrander EA, Daling JR, Concannon P. Increased frequency of ATM mutations in breast carcinoma patients with early onset disease and positive family history. *Cancer* 2001;**92**:479-87.
- Swift M**, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med* 1991;**325**:1831-6.
- Concannon P**. ATM heterozygosity and cancer risk. *Nat Genet* 2002;**32**:89-90.
- Cortez D**, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. *Science* 1999;**286**:1162-6.
- Bell DW**, Varley JM, Szydlowski TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF, Birch JM, Li FP, Garber JE, Haber DA. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science* 1999;**286**:2528-31.
- Malkin D**, Li FP, Strong LC, Fraumeni Jr JF, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, Friend SH. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Science* 1990;**250**:1233-8.
- Meijers-Heijboer H**, van den OEA, Klijn J, Wasielewski M, de Snoo A, Oldenburg R, Hollestelle A, Houben M, Crepin E, Veghel-Plandsoen M, Elstrodt F, van Duijn C, Bartels C, Meijers C, Schutte M, McGuffog L, Thompson D, Easton D, Sodha N, Seal S, Barfoot R, Mangion J, Chang-Claude J, Eccles D, Eeles R, Evans DG, Houlston R, Murday V, Narod S, Peretz T, Peto J, Phelan C, Zhang HX, Szabo C, Devilee P, Goldgar D, Futreal PA, Nathanson KL, Weber B, Rahman N, Stratton MR. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nat Genet* 2002;**31**:55-9.
- Vahteristo P**, Bartkova J, Eerola H, Syrjäkoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Aittomäki K, Heikkilä P, Halli K, Blomqvist C, Bartek J, Kallioniemi OP, Nevanlinna H. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. *Am J Hum Genet* 2002;**71**:432-8.
- Redon C**, Pilch D, Rogakou E, Sedelnikova O, Newrock K, Bonner W. Histone H2A variants H2AX and H2AZ. *Curr Opin Genet Dev* 2002;**12**:162-9.
- Burma S**, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J Biol Chem* 2001;**276**:42462-7.
- Rogakou EP**, Boon C, Redon C, Bonner WM. Megabase chromatin domains involved in DNA double-strand breaks in vivo. *J Cell Biol* 1999;**146**:905-16.
- Paull TT**, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr Biol* 2000;**10**:886-95.
- Downs JA**, Lowndes NF, Jackson SP. A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* 2000;**408**:1001-4.
- Bassing CH**, Chua KF, Sekiguchi J, Suh H, Whitlow SR, Fleming JC, Monroe BC, Ciccone DN, Yan C, Vlasakova K, Livingston DM, Ferguson DO, Scully R, Alt FW. Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. *Proc Natl Acad Sci USA* 2002;**99**:8173-8.
- Celeste A**, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A. Genomic instability in mice lacking histone H2AX. *Science* 2002;**296**:922-7.