# The mismatch repair gene *hPMS2* is mutated in primary breast cancer

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**Abstract.** Mismatch repair (MMR) genes play a fundamental role in the correction of replication errors and their mutation leads to cancer development. In the present study we have analyzed the *hPMS2* MMR gene for mutation using 20 primary breast cancers and seven breast tissues obtained from areas adjacent to breast cancer. For this purpose we have used cDNA sequence analysis and Western blotting using the specific antibody against the amino-terminal domain E-19. In primary breast cancers we found that the *hPMS2* gene had 9 missense mutations [codons: 513 (by change of Ser x Asp) in 14 tumors, 520 (Ala x Val) in 8 tumors, 573 (by change of Thr x Ser in 19 tumors), 578 (by change of Arg x Leu in 9 tumors), 587 (by change of Ser x Asp in 7 tumors), 590 (by change of Ile x Leu in 12 tumors), 598 (by change of Gln x His in 5 tumors), 601 (by change of Ser x Leu in 13 tumors), 608 (by change of Ala x Ser in 9 tumors. Nine out of 20 breast cancers had a non-sense mutation in nucleotide 1862 by changing Adenine by Thymine (AAG x TAG), which corresponded with a change in codon 613 by a change of Lys by stop codon. This non-sense mutation is responsible for the premature truncation of the protein *hPMS2*, which is reflected in the Western blotting by two bands, one corresponding with the wild-type form (100 kDa) and a lower one (75 kDa) corresponding with the truncated form of the *hPMS2* MMR protein. This truncated protein and the mutations in the *hPMS2* gene were also detected in two samples of normal-appearing tissue adjacent to their corresponding cancerous lesion. Altogether the present report demonstrates that primary breast cancers harbor mutations in this MMR gene and that normal-appearing breast tissue adjacent to the primary lesion also harbors the same mutations before the neoplastic process is manifested.

## Introduction

New insight into the mechanisms of cancer initiation has been the discovery of genetic instability of the abundant highly polymorphic short nucleotide repeat sequences known as microsatellite instability (MSI). MSI is a manifestation of errors in DNA mismatch repair, and has been found in many cancers, including those of the breast (1-15). Direct evidence for the association of MSI and mutated mismatch repair genes is derived from biochemical studies *in vitro* of human tumor cell lines demonstrating that mutated MMR genes are unable to efficiently repair heteroduplex DNA fragment (16). In humans six genes have been demonstrated to be involved in MMR [the *MutS* homolog: *hMSH2 (2p16)*, *hMSH6 (7p22)* (G/T binding protein, *HMSH6*) and *hMSH3*; and the *MutL* homolog: *hMLH1 (3p21)*, *hPMS2 (7p22)* and *hPMS1 (2q 31-33)*] (17,18). *hMSH2* and *hMSH6* have been shown to interact with each other in the h*Mut*Sα heterodimer that binds to heteroduplex DNA (19-22). The exact role of the *Mut*L homolog in human cells is still unclear. Recently it has been shown that a heterodimer of *hPMS2* and *hMLH1* is capable of restoring mismatch repair activity in human cancer cells (23). Deficiencies in DNA mismatch repair (MMR) have been found in hereditary colon cancers (hereditary non-polyposis colon cancer, HPNCC) (24) as well as sporadic cancers, illustrating the importance of MMR in maintaining genomic integrity. Mutations in the MMR genes in human breast epithelial cell lines have been associated to a premature protein truncation in hPMS2 protein (25,26).

These findings have led us to hypothesize that defective DNA mismatch repair (MMR), a putative mechanism underlying the instability of microsatellite DNA, may play a role in the process of breast tumorigenesis. Toward this purpose we have evaluated the expression of the hPMS2 MMR protein and mRNAs in human breast tumor tissue and using normal breast tissue as a control. In the present study we report a premature protein truncation due to a non-sense mutation in the hPMS2 gene in 55% of the breast tumor tissues studied. These results suggest that the hPMS2 MMR gene could be involved in DNA repair defect in human breast tumors.

## Materials and methods

*Human breast tissue*. Primary breast cancer tissue from 20 patients and aliquots of seven normal breast tissues from areas not involved by the primary tumors were obtained from the Fox Chase Cancer Center's tumor bank (Tables I and II).

*cDNA sequencing of the hPMS2 MMR gene*. Total RNA from the frozen samples described above was isolated using the

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Table I. Histology of control breast tissues.

| Sample no. | Histology | Age | Associated pathology |
|---|---|---|---|
| Normal 1 | Lob 2 and 3 in regression, lymphocyte infiltration, predominant dense stroma | 41 | *In situ* duct carcinoma, nuclear grade III, with necrosis (comedo carcinoma) |
| Normal 2 | Few ducts in a predominant fatty stroma | 63 | No evident tumor |
| Normal 3 | Few ducts in a predominant fatty stroma, remnants of Lob 1 | 69 | High grade *in situ* and invasive ductal carcinoma showing extensive intraductal component (90% of tumor volume) |
| Normal 4 | Few ducts in a predominant fatty stroma | 76 | Left breast, two invasive duct carcinomas, grade III (3.0 cm each); right breast, no evident residual tumor. Ductal hyperplasia |
| Normal 5 | Few ducts in a predominant fatty stroma, remnants of Lob 1 | 61 | Atypical medullary carcinoma (3.0 cm) |
| Normal 6 | Few ducts in a predominant fatty stroma, remnants of Lob 1 | 74 | Left breast, foci of residual severely atypical ductal hyperplasia; right breast, infiltrating ductal carcinoma, grade III |

Table II. Histology of invasive breast carcinomas.

| Sample no. | Histology | Age | Histological grade |
|---|---|---|---|
| Tumor 1 | Invasive ductal carcinoma | 52 | 3 |
| Tumor 2 | Invasive ductal carcinoma with strong inflammatory reaction | 82 | 3 |
| Tumor 3 | Invasive lobular carcinoma | 56 | 3 |
| Tumor 4 | Invasive ductal carcinoma | 45 | 3 |
| Tumor 5 | Invasive ductal carcinoma | 57 | 3 |
| Tumor 6 | Invasive ductal carcinoma | 65 | 3 |
| Tumor 7 | Invasive ductal carcinoma with strong inflammatory reaction | 71 | 3 |
| Tumor 8 | Invasive ductal carcinoma | >90 | 3 |
| Tumor 9 | Invasive ductal carcinoma | 39 | 3 |
| Tumor 10 | Invasive ductal carcinoma with minimal inflammatory reaction | 56 | 3 |
| Tumor 11 | Invasive lobular carcinoma | 57 | 3 |
| Tumor 12 | Invasive ductal carcinoma with small foci of cribiform ductal carcinoma | 56 | 3 |
| Tumor 13 | Invasive ductal carcinoma | 43 | 3 |
| Tumor 14 | Invasive ductal carcinoma | 50 | 3 |
| Tumor 15 | Invasive ductal carcinoma | 68 | 3 |
| Tumor 16 | Invasive ductal carcinoma | 84 | 3 |
| Tumor 17 | Invasive ductal carcinoma | 81 | 3 |
| Tumor 18 | Invasive ductal carcinoma | 51 | 3 |
| Tumor 19 | Invasive ductal carcinoma | 42 | 3 |
| Tumor 20 | Invasive ductal carcinoma | 68 | 3 |

classic phenol purification method (27). The oligonucleotide sense primer sequence for the hPMS2 MMR gene was 5'-ACTCCAGAACCAAGAAGGAGCG-3' and the antisense sequence was 5'-GTTGAGAGTCTGAGGTGCTATGAGC-3'. Total RNA (1 $\mu$g) was directly amplified by one-step RT-PCR (Gibco, Life Technologies, Inc.). cDNA was synthesized at 50˚C for 30 min. The PCR amplification cycles consisted of denaturalization at 94˚C for 1 min, 35 cycles of denaturalization at 94˚C for 30 sec, annealing at 62˚C for 30 sec and extension at 68˚C for 2 min, and a final elongation at 68˚C for 10 min. RT-PCR products were separated on a 1.5% agarose gel (containing 1 $\mu$g/ml ethidium bromide) and visualized with ultraviolet light. The quantity and integrity of the messenger transcripts of this mismatch repair gene were indirectly analyzed as cDNAs. The GADPH gene was used as a reference for normalization of the level of expression.

The RT-PCR product was subjected to sequencing in an automatic DNA sequencer (CEQ 8000, Beckman Coulter) using the same primers employed to amplify the template. The nucleotides and peptide sequences were analyzed using SeqWeb Version 1.1, for use with the GCG Version 10. SeqWeb is a web-based sequence analysis software suit for molecular biology research. SeqWeb is http://deneb.fccc.edu, a web-based interface to a subset of the GCG programs.

*Western blot analysis*. Western blot analyses were carried out to study hPMS2 MMR gene expression at the protein level. Western blots were prepared according to a standard protocol (28). Briefly, proteins were extracted from the breast tissues and electrophoretically separated and transferred to nitrocellulose membranes. Membranes were blocked and hybridized to the polyclonal anti-human antibody hPMS2 (E-19; Santa Cruz Biotechnologies, Inc., Santa Cruz, CA). Horseradish peroxidase-conjugated goat anti-mouse and anti-rabbit IgG (Amersham, Arlington Heights, IL), were used as the secondary antibody. Enhanced chemiluminescence system (Amersham, Arlington Heights) was used for the final immunoblot detection.

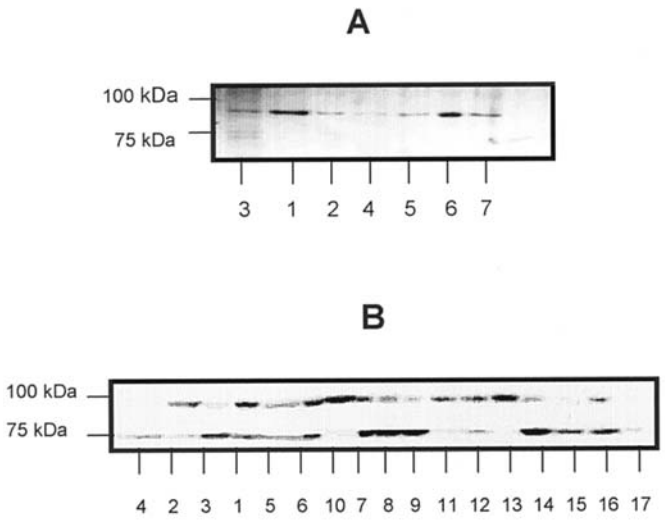


Figure 1. hPMS2 protein expression in breast tumors. (A) Western blot corresponding to the expression of hPMS2 protein in the control breast tissues. (B) hPMS2 protein expression in the invasive ductal carcinomas.

Table III. hPMS2 missense mutations from control breast tissues.

| Codon no. | AAC change | Nucleotide change | Nucleotide no. | Frequency |
|---|---|---|---|---|
| 502 | Asp x Ala | GAT x GAA | 1530 | 1/7 |
| 509 | Pro x Thr | CCA x CAA | 1551 | 1/7 |
| 510 | Asp x Glu | GAT x GAA | 1553 | 1/7 |
| 524 | Pro x Gln | CCA x CAA | 1595 | 1/7 |
| 526 | Asp x Asn | GAC x AAC | 1603 | 1/7 |
| 540 | Pro x Thr | CCT x ACT | 1642 | 1/7 |
| 589 | Ser x Thr | AGT x ACG | 1785-86 | 1/7 |
| 653 | Cys x Gly | TGT x GGT | 1981 | 1/7 |
| 673 | Phe x Leu | TTT x TTG | 2043 | 1/7 |
| 679 | Ile x Leu | ATT x TTG | 2061 | 1/7 |

## Results

*hPMS2 protein expression in breast tumors*. The hPMS2 protein was expressed in all of the control breast tissues as a band of 100 kDa (Fig. 1A). The sample numbers 1 and 6 showed a weak band corresponding to a 75 kDa of the hPMS2 truncated form. Most of the invasive ductal carcinomas (samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 14, 15, 16, and 17) clearly expressed the truncated form with a molecular weight of 75 kDa (Fig. 1B). Samples 4 and 17 do not express the wild-type form of hPMS2.

*Mutational analysis of hPMS2*. We found 10 missense mutations in the hPMS2 gene in the six unaffected breast tissue in codon numbers 502 (Asp x Ala), 509 (Pro x Thr), 510 (Asp x Glu), 524 (Pro x Gln), 526 (asp x Asn), 540 (Pro x Thr), 589 (Ser x Thr), 653 (Cys x Gly), 673 (Phe x Leu) and 679 (Ile x Leu) (Table III). In primary breast cancer we

Table IV. hPMS2 missense mutations from invasive breast carcinomas.

| Codon no. | AAc change | Nucleotide change | Nucleotide no. | Frequency |
|---|---|---|---|---|
| 503 | Ser x Ala | TCT x GGT | 1531-32 | 4/20 |
| 506 | Phe x Ser | TCT x GGT | 1542-43 | 1/20 |
| 507 | Ser x Cys | AGC x TGC | 1544 | 2/20 |
| 511 | Thr x Asn | ACG x AAC | 1557-58 | 2/20 |
| 523 | Ser x Thr | TCC x ACC | 1591 | 2/20 |
| 526 | Asp x Glu | GAC x GAA | 1603 | 1/20 |
| 554 | Asn x His | AAC x CAC | 1685 | 1/20 |
| **563** | **Arg x Gln** | **CGA x CAA** | **1712** | **7/20** |
| 572 | Ala x Thr | GCA x ACA | 1739 | 1/20 |
| 582 | Lys x Glu | AAA x GAA | 1765 | 3/20 |
| 583 | Glu x Lys | GAA x AAA | 1768 | 3/20 |
| 594 | Gln x Ter | CAA x TAA | 1799 | 1/20 |
| 596 | Leu x Val | TTA x GTT | 1805 | 1/20 |
| 597 | Thr x Ser | ACT x TCC | 1813, 1815 | 3/20 |
| 598 | Gln x His | CAG x CAC | 1818 | 2/20 |
| 598 | Gln x Pro | CAG x CCA | 1817-18 | 3/20 |
| 599 | Asp x Pro | GAT x CCT | 1819-20 | 1/20 |
| 602 | Ala x Gly | GCC x GGC | 1829 | 2/20 |
| 604 | Gln x Ter | CAG x TAA | 1834, 1836 | 2/20 |
| **608** | **Ala x Ser** | **GCT x TCC** | **1847-49** | **8/20** |
| 618 | Leu x Arg | CTG x CGG | 1877 | 1/20 |
| **624** | **Ser x Ala** | **TCT x GCT** | **1895-96** | **7/20** |
| 625 | Leu x Ter | TTA x TAA | 1899 | 2/20 |
| 644 | Asn x Ile | AAT x ATT | 1955 | 2/20 |
| 651 | Arg x Lys | AAG x AGG | 1976 | 5/20 |
| 652 | Ile x Leu | ATT x TTG | 1978 | 1/20 |

Table V. hPMS2 polymorphisms in invasive breast carcinomas.

| Codon no. | AAc change | Nucleotide change | Nucleotide no. | Frequency |
|---|---|---|---|---|
| 508 | Ile x Leu | ATC x CTC | 1547 | 3/20 |
| **513** | **Ser x Asp** | **AGT x GAT** | **1561-62** | **14/20** |
| **519** | **Tyr x Cys** | **TAT x TGT** | **1580** | **8/20** |
| **520** | **Ala x Val** | **GCG x GTG** | **1583** | **8/20** |
| **573** | **Thr x Ser** | **ACC x TCC** | **1742** | **19/20** |
| **579** | **Arg x Leu** | **CGT x CTT** | **1758** | **9/20** |
| 584 | Glu x Lys | GAA x AAA | 1771 | 5/20 |
| 589 | Ser x Asp | AGT x GAT | 1784-85 | 5/20 |
| **592** | **Ile x Leu** | **ATT x CTT** | **1793** | **12/20** |
| 600 | Met x Leu | ATG x CTT | 1822,1824 | 4/20 |
| **601** | **Ser x Leu** | **TCA x TTA** | **1826** | **13/20** |
| **613** | **Lys x Ter** | **AAG x TAG** | **1861** | **7/20** |
| 629 | Ile x Leu | ATA x TTA | 1909 | 6/20 |
| 633 | His x Leu | CAT x CTT | 1922 | 5/20 |
| 653 | Cys x Gly | TGT x GGT | 1981 | 4/20 |

found 26 missense mutations in the hPMS2 gene, from codon number 503 to 652 (Table IV). Twenty-three of these mutations were found in less than 5 out of 20 primary breast cancer (<25%), however 3 missense mutations located in codon numbers 563 (change of Arg x Gln), 608 (change of Ala x Ser) and 624 (change of Ser x Ala) were observed in more than 7 out of 20 patients (>35%) (Table IV). Also we observed 3 non-sense mutations located in codon 594 by a change of Gln to a stop codon, codon 604 by a change of Gln to a stop codon, and in codon 625 by a change of Leu to a stop signal (Table IV).

*Polymorphisms of the hPMS2 gene*. In the hPMS2 gene we detected 13 changes that could be called polymorphisms because they were detected in the controls as well as in the invasive breast carcinoma tissues (Table V). Seven of these changes were detected in more than 35% of the tissues, and these changes were in codons 513 (Ser x Asp), 519 (Tyr x Cys), 520 (Ala x Val), 573 (Thr x Ser), 579 (Arg x Leu), 592 (Ile x Leu), 601 (Ser x Leu) and 613 (Change Lys x Stop codon) (Table V).

## Discussion

It is well known that the activation of oncogenes, loss or inactivation of repressor genes and impairment of mismatch-repair function are involved in the development of solid tumors. Whereas a number of reports suggest that MSI does not play a significant role in the pathogenesis of breast cancer (29-32), there are recent publications showing defects or deficiency in DNA mismatch repair genes that lead to replication errors with the instability in microsatellite markers not only in colon cancer (33-35) but also in breast tumors (5, 36-40). We report a mismatch repair defect in the hPMS2 gene in primary breast cancer. In the protein expression assay we observed a different profile in the invasive breast carcinomas compared with the controls. The truncated band in the control tissues 1 and 7 was very weak, however the hPMS2 form of 75 kDa was strong in most of the tumor tissues. Also the wild-type hPMS2 form was observed in invasive tumor numbers 1-3, 5-14 and 16. These results suggest that the truncated form of the hPMS2 MMR protein coexists with the wild-type form in the tumor tissues in different amounts depending on the tissue. In tumor numbers 3, 4, 7-9, and 14-17 the truncated form is predominant (>50%), however in tumors 1, 2, and 10-13 the hPMS2 wild-type form is predominant. Conversely, in the control breast tissue hPMS2 wild-type protein expression was the most predominant form. Supporting these data is the finding that hPMS2 protein expression was totally lost during transformation of human breast epithelial cells (25,26), in prostate cancer (41), and in *Pms2*$^{-/-}$ mice (42). Thirty percent of hPMS2 cDNA products of the Vaco481 colon cancer cell line have shown a G-T mutation at codon 279 within exon 8, which convert GGA (Gly) to a premature TGA stop codon (43).

In the mutational analysis of the hPMS2 gene, 12 invasive breast carcinomas out of 20 showed a non-sense mutation located between codon numbers 594 and 625 and the protein expression analysis revealed a truncated form in 14 out of 20 tumors. The truncated form of approximately 75 kDa in the Western blotting corresponds to approximately 625 amino acids. This indicates that the non-sense mutation detected would cause the protein to be shorter. In the breast tissue not involved with tumor we also observed the non-sense mutation in the hPMS2 gene in two samples at codon 613, changing Lys (AAG) by stop codon (TAG), corresponding to the truncated forms expressed by a weak band in the Western blotting.

The presence of a stop signal in our samples has also been reported in colorectal cancer and in HNPCC type 4 (44). However, the G to A transition that has been reported in codon 20, which resulted in an arginine to glutamine change that codified the stop signal in 3 of 18 HNPCC patients, has been interpreted to represent a polymorphism rather than a functional mutation (45).

Our study indicates that primary breast cancer presents an increased number of mutations in the hPMS2 gene compared with the control non-involved breast tissue. The mutation phenotype for the hPMS2 protein could include changes in codons 563 (change of Arg x Gln), codon 608 (change of Ala x Ser), codon 613 (change of Lys x Stop) and codon 624 (change of Ser x Ala) that were observed in more than 40% of the tumor tissues. Defects in hPMS2 MMR protein expression could be responsible for the LOH and MMR deficiency in primary breast tumors.

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