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MMTV prevalence in breast cancer samples in Romania- do major geographical differences exist in the world population?

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Abstract
Breast cancer, although the most frequently diagnosed malignant tumour in humans, has a less clear aetiology compared to other frequent cancer types. Mouse-mammary tumour virus (MMTV) is involved in breast cancer in mice and dogs and might play a role in the aetiology of some breast cancers in humans, since it has been identified in 20-40% of breast cancer samples in Western Europe, USA, Australia and some other parts of the world population. The purpose of our study was to identify MMTV DNA sequences in breast tissue samples from breast cancer patients operated in our regional centre from Romania. We have selected 75 patients with non-metastatic breast cancer treated with curative intent and searched with PCR the MMTV-like DNA sequence in the breast cancer tissue and normal breast tissue obtained from the same patients. Since none of the examined samples was positive for MMTV-like target sequences on PCR we could not prove that MMTV plays a role in the aetiology of breast cancer in our patient group. This

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finding is similar to publications of other geographically related research groups and might be due to the fact that only the *Mus musculus domesticus* mouse species was proven to carry infectious MMTV, but not the *Mus musculus musculus* species, which is specific to South-Eastern Europe (including Romania) and some parts of Asia.

Background

In 2020, female breast cancer has surpassed lung cancer as the most commonly diagnosed cancer, with an estimated 2.3 million new cases (11.7%), being also the leading cause of mortality due to cancer in female patients around the world. Among women, breast cancer accounts for 1 in 4 cancer cases and for 1 in 6 cancer deaths. Incidence rates are 88% higher in transitioned countries than in transitioning countries with the highest incidence rates (>80 per 100,000) in Australia/New Zealand, Western Europe (Belgium has the world's highest incidence), Northern America, and Northern Europe and the lowest rates (<40 per 100,000) in Central America, Eastern and Middle Africa, and South-Central Asia. [1]

Although there are various risk factors known for breast cancer, only about 10% of the cases have a precise genetic cofactor. Breast cancer associated gene 1 and 2 (BRCA1 and BRCA2) are two anti-oncogenes located on chromosome 17q21 and 13q12, respectively, and both encode tumour suppressor proteins. Totally, about 20-25% of hereditary breast cancers and 5-10% of all breast cancers are caused by BRCA1/2 mutations. [2] Family history of breast cancer is another major risk factor. [3] Women with a family history of breast cancer (two or more cases in women younger than 50 years or three or more cases at any age) who do not present BRCA mutations are approximately 11 times more likely to develop breast cancer. [4] Exogenous hormone use, such as contraceptives [5], ovulation-stimulating drugs [6] and some schedules of menopausal hormone replacement therapy [7] might also increase the risk of breast cancer. Other risk factors include obesity, alcohol consumption [8], radiation exposure or nulliparity. [2]

One retrovirus that has been used for many years for the study of cancer pathogenesis in mice is the Mouse Mammary Tumour Virus (MMTV). It was discovered in 1936 as a milk-transmitted agent. The agent was then demonstrated to have reverse transcriptase activity, similar to other retroviruses, and hormone-responsive elements in the viral genome that enhance viral replication during pregnancy. [9] In 1989 Harold Varmus was awarded the Nobel Prize for the observation that the insertion of MMTV proviral genome in host DNA resulted in the activation on proto-oncogenes.

The majority of breast cancers in mice are caused by MMTV. [10]

Several studies examined the association between MMTV and human breast cancer. A major breakthrough came in 1972 with the identification of RNA in human breast cancer that was homologous to MMTV RNA. [11] Prior to the widespread use of PCR, MMTV-related sequences in human breast cancer cells were identified by hybridization techniques. Using hybridization methods Szakacs & Moscinski identified in DNA sequences homologous to the entire MMTV provirus using LTR- long terminal repeat, *gag*, *pol* and *env* probes in 7 (13%) of 52 human breast cancers. [12] However, it was difficult to distinguish MMTV gene sequences from those of the human endogenous retrovirus

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(HERV). HERV gene sequences are very similar to MMTV and may be the remnants of MMTV viruses that have become integrated into the human genome over millennia [13]. This problem was overcome by the Beatriz Pogo group by their identification of MMTV envelope gene sequences which were unique to MMTV. [14].

Using PCR techniques directed at a 660 bp highly conserved portion of the MMTV-env gene with only 16% homology to the prototype HERV-K10 human endogenous retrovirus, Wang et al. were able to demonstrate MMTV-env specific sequences in 38.5% of the 314 breast carcinomas and in 6.9% of the 29 breast fibroadenoma samples, compared to only 1.8% of 107 samples of normal breast reduction mammoplasty tissues. [15] A series of similar studies using PCR primers and nested primers were then conduced in an attempt to correlate the presence of the MMTV specific 660 bp env sequence with mammary tumourigenesis. A meta-analysis published in 2019 analyzed 20 studies reported in 17 publications in which PCR was used to detect MMTV specific region identified by Wang et al; 11 studies showed a positive correlation between the presence of the indicative MMTV signal and breast tumour tissue at the $p < 0.01$ significance level. To further investigate the presence of a subfragment of the highly conserved MMTV *env* region, laser microdissection techniques were used to study breast cancer epithelial cells followed by real-time PCR analysis. [16] MMTV was identified in 40 (82%) of 49 ductal carcinoma in situ specimens and 7 (35%) of 20 invasive ductal carcinoma specimens compared to no identification in 20 normal breast specimens from reduction mammoplasty.

The aim of our study was to identify MMTV *env* viral sequences in surgical breast cancer tissue samples in Romania, to verify if in this part of South-Eastern Europe there is a possibility that some breast cancers are related to MMTV infection.

Methods

We conducted a retrospective study in which we analyzed the presence of the MMTV env gene sequence in human breast cancer samples. The samples were collected from the tissue archive of the Oncological Institute of Cluj-Napoca. They were formalin-fixed paraffin-embedded breast tissue samples obtained from either mastectomies or lumpectomies of patients diagnosed with invasive ductal carcinoma between 2003 – 2011. The patients selected for the study did not undergo neoadjuvant chemo- or radiotherapy.
For each patient, a pathologist (R.B.) examined the tissue samples obtained surgically and selected one tissue sample that contained tumour tissue characteristic for invasive ductal carcinoma and one sample that contained normal peri-tumour tissue. The formalin-fixed paraffin-embedded tissue samples were each sectioned at 10 micrometers and 5-6 sections were put into a tube, two tubes for each tumour tissue and two tubes for each normal tissue, therefore 4 tubes corresponding to each patient.

DNA was extracted from deparaffinised sections of each paraffin block using PureLink® Genomic DNA Kit (Invitrogen), K182001, as instructed by the manufacturer.

The PCR reaction for the selected samples was performed with Thermo Scientific Phusion High-Fidelity PCR Master Mix according to the recommended protocol from a 200 ng total concentration of DNA. The

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condition used for polymerase chain reaction was 98 °C / 30s for initial denaturation, 98 °C/10s, 61 °C/30s and 72 °C/30s for denaturation annealing and extension and 72 °C/10min., 4 °C/hold for final extension in a Veriti™ 96-Well Thermal Cycler (ThermoFisher Scientific). Positive and negative controls were included in each run.

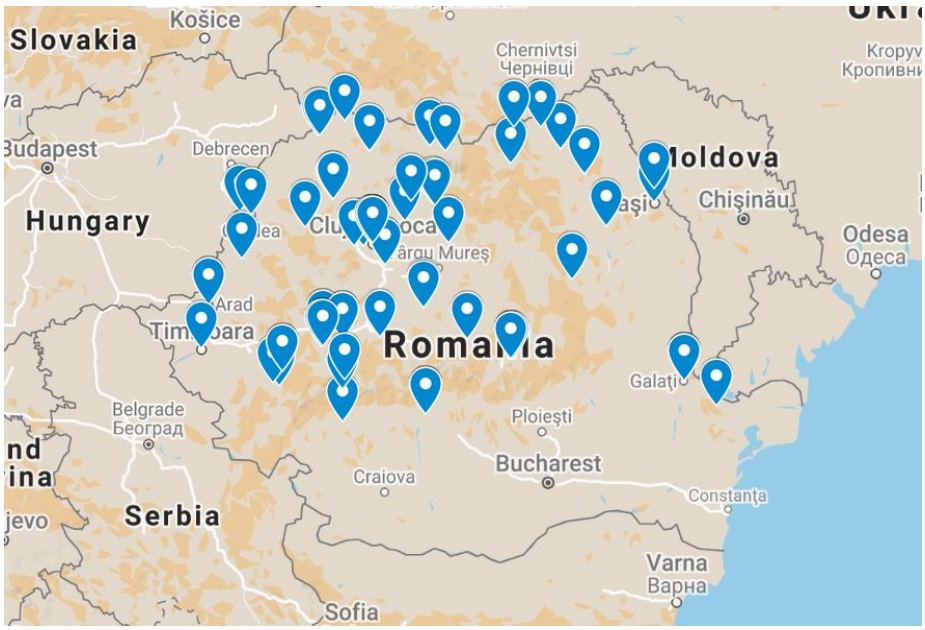
For the electrophoresis a 3% agarose gel was used with a ThermoFisher Scientific GeneRuler Low Range DNA ladder. A 104 base pair oligonucleotide was ordered based on the gene bank MMTV-like virus envelope protein [ACCESSION #: GU109516] and was used as a positive control. The primer used was MMTV env gPr73 [Mouse mammary tumour virus] forward, 5′-GATGGTATGAAGCAGGATGG-3′ and reverse, 5′-CCTCTTTTCTCTATATCTATTAGCTGAGGTAATC-3′.

Results

1. Patients

We selected 75 patients with ages between 32 and 77 years old; 25 patients underwent modified radical mastectomy and 50 patients were treated with lumpectomy. The characteristics of the patients and the descriptors of the tumours are summarized in Table 1. Patients were selected from various regions of Romania, so that a geographical distribution of the virus in Romania could be studied. The geographical distribution of the patients is shown in Figure 1.

Figure 1. The geographical distribution of patients selected for the study.



Map data ©2021 GeoBasis DE/BKG (©2009), Google, Inst. Geogr. Nacional, MapaGIsrael

Table 1. Patients' characteristics

Variable		Number	%
Mean age (years)		54.29	
Stage	IA	17	22.67
	IIA	31	41.33
	IIB	11	14.67

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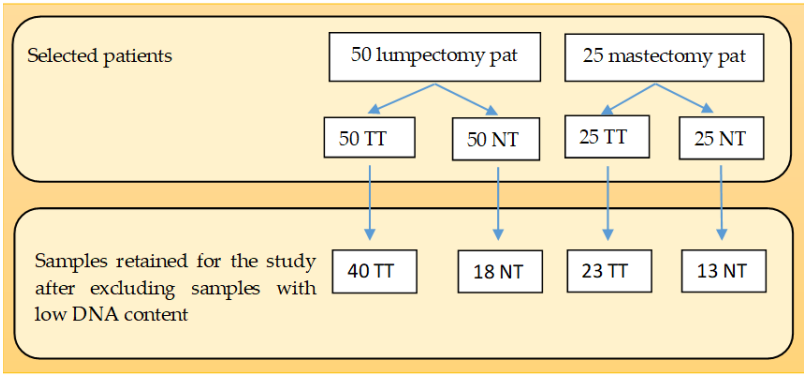
	IIIA	5	6.67
	IIIB	3	4
	IIIC	8	10.66
Histologic grade (Nottingham score)	I	13	17.33
	II	23	30.66
	III	39	52
Estrogen receptors	Positive	58	77.33
	Negative	17	22.66
Progesterone receptors	Positive	53	70.66
	Negative	22	29.33
HER-2 receptors	Positive	29	38.66
	Negative	46	61.33
Triple negative		16	21.33

2. PCR results

The samples with an insufficient quantity of tissue or a very low concentration of DNA were excluded from the study, as shown in supplementary Tables 1 and 2. More precisely, for some cases, when realizing 10 μm sections for obtaining either tumour or normal tissue we could not obtain at minimum 5 sections for each tube, so these samples had to be excluded. From the lumpectomy group, out of 50 tumour tissue samples, 48 remained for further analysis and out of these 48 samples 40 had sufficient quantity of DNA. Out of 50 normal tissue samples, in 32 cases there was sufficient tissue, but only 18 were kept in the study as samples with proper DNA content. From the mastectomy group, out of 25 tumour tissue samples, 23 had an optimal concentration of DNA, and out of 20 normal tissue samples with sufficient material only 13 were kept in the study for further investigations, for the same reasons. (Fig. 2) Therefore, after DNA extraction, in the lumpectomy group, there were 40 tumour samples and 18 normal tissue samples, while in the mastectomy group there were 23 tumour samples and 13 normal tissue samples.

As a final result, **none** of the examined samples was positive for MMTV-like target sequences on PCR.

Figure 2. Samples retained for PCR after excluding samples with low tissue and DNA content.



Abbreviations: TT=tumour tissue, NT=normal tissue, pat=patients

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Discussion

Breast cancer is both an ancient disease, first described by the Egyptians [17], and a disease of civilization, since its incidence has increased in the last century.

In mice, MMTV can be transmitted **horizontally** as an infectious particle containing viral RNA or **vertically** in the murine germline as an endogenous proviral DNA genome. MMTV replicates in the mothers' mammary glands where the infectious B-type particles accumulate in milk. When the nurslings ingest the virus, it invades the gut-associated lymphoid tissues and infects B- lymphocytes. To aid viral replication, MMTV uses a superantigen, encoded by the *orf* gene in the viral long terminal repeat (LTR) region, to activate specific T-lymphocyte subsets. This serves to stimulate viral replication as well as clonal amplification of T lymphocytes, which provides a vehicle for viral passage from the gut-associated tissue to the breast. Although viral replication is maximal in the breast epithelium, hormone stimulation is still required to activate the corticosteroid response elements in the retroviral LTR. During pregnancy, viral replication is increased, which leads to hyperplasic nodule formation that can eventually lead to tumour formation. [18] MMTV can also be transmitted to offspring as proviral DNA in a Mendelian fashion. MMTV does not contain an oncogene, but it has been shown that proviral DNA can integrate close to cellular proto-oncogenes and enhance their activity. [19]

But what is MMTV's role in human breast cancer on the globe? Our results are similar with the results of a series of studies that could not detect the presence of the MMTV in human breast cancer samples. Tabriz et al. [20] evaluated the same MMTV-like target sequence as us by RT-PCR using the same primers, and did not detect any virus particle in breast cancer tissues of 40 Iranian women. Perzova et al. [21] analyzed the prevalence of MMTV env sequences in 66 samples of FFPE or snap-frozen biopsies of breast tissue (US patients) by PCR and took supplementary measures to prevent carry-over contamination with murine DNA; they concluded that the MMTV copy numbers detected in the breast cancer specimens were too low to be derived from a monoclonally integrated expanded tumour cell sample. Fukuoka et al. [22] investigated in Japan 46 breast cancer patients and 3 patients with benign mammary tumours and used PCR and Southern blot hybridization; they could not detect the MMTV *env* gene-like sequence in any of the samples tested.

However, there are several reports on the MMTV env gene in human breast cancer samples. [15], [23], [24], [25] Additionally Johal et al. found MMTV like virus sequences by PCR in 5% (4/91) of breast milk samples from healthy lactating women from Australia, [26]. Furthermore, MMTV-like sequences were found in the saliva [27] and peripheral blood lymphoid cells [28], suggesting that viral transmission in humans is similar to that seen in mice, occurring via breastfeeding and infection of mucosa-associated lymphocytes before reaching the breast tissue.

The contrast between the different outcomes of the studies may indicate that there is a regional virus epidemiology. Our study is the first one in Romania and even South-Eastern Europe which analyzes the prevalence of MMTV virus infection in human breast tissue. Based on our sample the results indicate that breast cancer is not likely to be related to the MMTV

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infection in Romania. The negative result is most likely explained by the transmission vectors of the virus and the geographic distribution of *Mus musculus sp. domesticus* – the native MMTV reservoir. It has been proposed that a higher prevalence in some areas of *Mus musculus domesticus* in the vicinity of humans, which is the species of mouse that carries the infectious MMTV, is correlated to a higher prevalence of human breast cancer. [29] *M. m. domesticus* seems to be less present in South-Eastern Europe, where *M. m. musculus* is the predominant species [30]. In relationship to MMTV this variability of species needs to be further explored, but we found no reports of naturally occurring MMTV infection in *M. m. musculus*. Callahan et al [31] studying several feral mouse species stated that *M. m. musculus* specimens from Czechoslovakia did not carry MMTV, although all mice species studied had small fragments of inserted MMTV which is explained by the "accumulation of evolutionarily divergent MMTV-alpha insertions into the mouse germ line". Ford et al [24] could not identify MMTV in first-generation Australian-Vietnamese women, in comparison to Caucasian-Australian woman (42.2%). It is known that in South-Eastern Asia the dominant mouse species is not *M. m. domesticus*, but *M. m. castaneus*.

Furthermore, even *Mus musculus sp. domesticus*, also known as "western house mouse", since its spread to Europe from Asia in the postglacial period [32], clustered in somewhat different local populations [33], and local subpopulations may have different rates of infection with MMTV, which might explain the variable positivity of MMTV in Western countries, ranging 20-40%. Another reason for the variability of the rate of association of MMTV to human breast cancer is the presence of a possible intermediary host, the dog. Laumbacher et al [34] studied dog-human contact epidemiologically in Bavaria, Germany and found that more than twice the number of breast cancer patients kept dogs permanently in the last 10 years leading up to the diagnosis compared to control individuals (37.7% vs. 14.8%, p=0.0000003, relative risk 3.5).

Because breast cancer is more prevalent among women of higher socio-economic status, it has been hypothesized that this may be due to a late exposure to MMTV infection from mice, as compared to infection during early life among girls of low socio-economic status and hence early immunity. [35]

As human breast cancer progresses, the MMTV viral load increases but it falls in late-stage invasive breast cancer. [16] The lack of identification of MMTV in advanced breast cancer may be due to the breakdown of cell physiology, but our patient sample consisted of early stage, non-metastatic breast cancer patients.

Another explanation for the divergent results regarding the MMTV infection in humans might be that some positive results were due to carry-over contamination of human samples with previously amplified murine MMTV DNA. Rodent DNA might be present in the building's walls and ventilation systems, most likely as small particulate matter. [21] We consider this scenario very unlikely in all of the MMTV-positive studies, although might have had happened in some, taking into account the extreme measures taken to avoid contamination by most of the laboratories.

While it is clear that the association between MMTV infection and human breast cancer is still a debatable topic, most results indicate that a

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correlation cannot be ruled out. Proving that MMTV is an etiologic agent for human breast cancer will probably require non-PCR based traditional retrovirology techniques such as virus isolation and determination of monoclonal integration in tumour cells. This would certainly be a breakthrough in oncology and would open the gates to the prevention of breast cancer through (1) hygiene measures, (2) counselling of those less than 5% of MMTV-infected women to find alternatives to breastfeeding and (3) the development of a vaccine. If in Western countries and Australia the involvement of MMTV in breast cancer probably occurs in 20-40% of patients, such measures would lead to a proportional reduction of newly diagnosed breast cancer cases in about one or two generations. MMTV-related breast cancers tend to be less aggressive as well. [36]

Other virus infections have been proposed as well as causative agents of breast cancer; the most studied is bovine leukaemia virus (BLV). [37]

Breast cancer remains one of the most mysterious human cancers, since most cases cannot be linked to specific risk factors.

Conclusions

We have shown that MMTV is not present in our region in breast tissue samples obtained from breast cancer patients. The same result was achieved by other geographically related research groups. Only *Mus musculus domesticus* mouse species was proven to carry infectious MMTV, thus our results can be explained by the variable local distribution of different mice species.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

Conceptualization, Z.F., B.O.T..; methodology, Z.F., B.O.T., L.R.; formal analysis, L.R., R.B.; resources, Z.F., D.T.E, R.B., M.G.; data curation, Z.F., B.O.T., R.B., L.R.; writing—original draft preparation, Z.F., B.O.T., L.R.; writing—review and editing, Z.F.; visualization, Z.F., B.O.T., L.R.; supervision, I. B-N.; project administration, I. B-N.; funding acquisition, Z.F., B.O.T. All authors have read and agreed to the published version of the manuscript.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. (2021) **Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries** *CA Cancer J Clin***. 71**, 209-249. https://doi.org/10.3322/caac.21660
2. Momenimovahed Z, Salehiniya H. (2019) **Epidemiological characteristics of and risk factors for breast cancer in the world** *Breast Cancer: Targets and Therapy* **11***,* 151-164. https://doi.org/10.2147/BCTT.S176070
3. Thakur P, Seam RK, Gupta MK, Gupta M, Sharma M, Fotedar V. (2017) **Breast cancer risk factor evaluation in a Western Himalayan state: A case–control study and comparison with the Western World**. South Asian J Cancer **06**, 106–109. https://doi.org/10.4103/sajc.sajc_157_16
4. Metcalfe KA, Finch A, Poll A, Horsman D, Kim-Sing C, Scott J, et al. (2009) **Breast cancer risks in women with a family history of breast or ovarian cancer who have tested negative for a BRCA1 or BRCA2 mutation** *Br J Cancer* ***100*****,** 421–425. https://doi.org/10.4103/sajc.sajc_157_16
5. Marchbanks PA, McDonald JA, Wilson HG, Folger SG, Mandel MG, Daling JR, et al. (2002) **Oral Contraceptives and the Risk of Breast Cancer** *N Engl J Med*. **346,** 2025–2032. https://doi.org/10.1056/NEJMoa013202
6. Brinton LA, Scoccia B, Moghissi KS, Westhoff CL, Althuis MD, Mabie JE, et al. (2004) **Breast cancer risk associated with ovulation-stimulating drugs** *Hum Reprod*. **19**, 2005–2013. https://doi.org/10.1158/1055-9965.
7. Collaborative Group on Hormonal Factors in Breast Cancer (2019) **Type and timing of menopausal hormone therapy and breast cancer risk: individual participant meta-analysis of the worldwide epidemiological evidence** *Lancet* **394**, 1159-1168. https://doi.org/10.1016/S0140-6736(19)31709-X
8. Miller ER, Wilson C, Chapman J, Flight I, Nguyen AM, Fletcher C, et al. (2018) **Connecting the dots between breast cancer, obesity and alcohol consumption in middle-aged women: Ecological and case control studies** *BMC Public Health*. **18,** 460. https://doi.org/10.1186/s12889-018-5357-1
9. Schiller JT, Lowy DR (2014) **Virus infection and human cancer: An overview** *Recent Results in Cancer Research* vol. **193,** 1–10. https://doi.org/10.1007/978-3-642-38965-8_1
10. Hennighausen L. (2000) **Mouse models for breast cancer** *Oncogene* **19**, 966–967. https://doi.org/10.1038/sj.onc.1203346
11. Axel R, Schlom J, Spiegelman S (1972) **Presence in human breast cancer of RNA homologous to mouse mammary tumour virus RNA** *Nature*. **235,** 32–36. https://doi.org/10.1038/235032a0
12. Szakacs JG, Moscinski LC. (1991) **Sequence homology of deoxyribonucleic acid to mouse mammary tumour virus genome in human breast tumours** *Ann Clin Lab Sci*. **21**, 402–412. PMID: 1664195.
13. Salmons B, Lawson JS, Günzburg WH (2014) **Recent developments linking retroviruses to human breast cancer: Infectious agent, Enemy within or both?** *J Gen Virol*, **95,** 2589–2593. https://doi.org/10.1099/vir.0.070631-0.
14. Melana SM, Picconi MA, Rossi C, Mural J, Alonio LV, Teyssié A, Holland JF, Pogo BGT (2002) **Detection of murine mammary tumour virus (MMTV) env gene-like sequences in breast cancer from Argentine patients** *Medicina (B Aires)* **62**, 323-327. PMID: 12325488
15. Wang Y, Holland JF, Bleiweiss IJ, Melana S, Liu X, Pelisson I, et al. (1995) **Detection of Mammary Tumour Virus ENV Gene-like Sequences in Human Breast Cancer** *Cancer Res*. **55**, 5173-5179. PMID: 7585568
16. Mazzanti CM, Al Hamad M, Fanelli G, Scatena C, Zammarchi F, Zavaglia K, et al. (2011) **A mouse mammary tumour virus env-like exogenous sequence is strictly related to progression of human sporadic breast carcinoma** *Am J Pathol*. **179**, 2083-2090. https://doi.org/10.1016/j.ajpath.2011.06.046
17. Freeman MD, Gopman JM, Salzberg CA (2017) **The evolution of mastectomy surgical technique: from mutilation to medicine** *Gland Surgery* **7**, 308–315. https://doi.org/10.21037/gs.2017.09.07.
18. Mason A (2000) **Is there a breast cancer virus?** *Ochsner J*. **2**, 36–39. PMID: 21765660
19. Morris DW, Dutra JC. (1997) **Identification of a MMTV insertion mutation with the coding region of the Fgf-3 protooncogene** *Virology* **238**, 161–165.
20. Tabriz HM, Zendehdel K, Shahsiah R, Fereidooni F, Mehdipour B, Hosseini ZM (2013) **Lack of detection of the mouse mammary tumour-like virus (MMTV) env gene in**

iranian women breast cancer using real time PCR *Asian Pacific J Cancer Prev*. **14**, 2945–2948. https://doi.org/10.7314/apjcp.2013.14.5.2945

21. Perzova R, Abbott L, Benz P, Landas S, Khan S, Glaser J, et al. (2017) **Is MMTV associated with human breast cancer? Maybe, but probably not** *Virol J*. **14**. https://doi.org/10.1186/s12985-017-0862-x
22. Fukuoka H, Moriuchi M, Yano H, Nagayasu T, Moriuchi H. (2008) **No association of mouse mammary tumour virus-related retrovirus with Japanese cases of breast cancer.** *J Med Virol***. 80**, 1447–1451. https://doi.org/10.1002/jmv.21247.
23. Etkind P, Du J, Khan A, Pillitteri J, Wiernik PH (2000) **Mouse Mammary Tumour Virus-like ENV Gene Sequences in Human Breast Tumours and in a Lymphoma of a Breast Cancer Patient** *Clin Cancer Res*. **4**, 1273-1278. https://pubmed.ncbi.nlm.nih.gov/10778951/
24. Ford CE, Tran D, Deng Y, Ta VT, Rawlinson WD, Lawson JS (2003) **Mouse Mammary Tumour Virus-like Gene Sequences in Breast Tumours of Australian and Vietnamese Women** *Clin Cancer Res*. **9**, 1118-1120. https://pubmed.ncbi.nlm.nih.gov/12631616/
25. Al Dossary R, Alkharsah KR, Kussaibi H (2018) **Prevalence of Mouse Mammary Tumour Virus (MMTV)-like sequences in human breast cancer tissues and adjacent normal breast tissues in Saudi Arabia**. *BMC Cancer*. **18,** 1–10. https://doi.org/10.1186/s12885-018-4074-6
26. Johal H, Ford C, Glenn W, Heads J, Lawson J, Rawlinson W. (2011) **Mouse mammary tumour like virus sequences in breast milk from healthy lactating women**. *Breast Cancer Res Treat* **129**, 149–155. https://doi.org/10.1007/s10549-011-1421-6
27. Mazzanti CM, Lessi F, Armogida I, Zavaglia K, Franceschi S, Al Hamad M, et al. (2015) **Human saliva as route of inter-human infection for mouse mammary tumour virus**. *Oncotarget* **6**, 18355–18363. https://doi.org/10.18632/oncotarget.4567
28. Naushad W, Ayub S, Sadia H, Ahmad Joyia F (2017) **Significant correlation of MMTV (Mouse mammary tumour virus) LTR gene with hormone receptor status in peripheral blood samples of breast cancer patients from North Pakistan.** *Int J Biosci* **10**, 399–405. http://dx.doi.org/10.12692/ijb/10.3.399-405
29. Stewart THM, Sage RD, Stewart AFR, Cameron DW (2000) **Breast cancer incidence highest in the range of one species of house mouse, Mus domesticus**. *Br J Cancer*. **82**, 446–51. http://dx.doi.org/10.1054/bjoc.1999.0941
30. Maloy S., Hughes K., Eds (2013) **Brenner's Encyclopedia of Genetics**, **2nd ed**. (Amsterdam: Elsevier) pp. 582–5. DOI: 10.1016/B978-0-12-809633-8.06558-4. https://www.elsevier.com/books/brenners-encyclopedia-of-genetics/maloy/978-0-12-374984-0
31. R Callahan, W Drohan, D Gallahan, L D'Hoostelaere, M Potter (1982) **Novel class of mouse mammary tumour virus-related DNA sequences found in all species of Mus, including mice lacking the virus proviral genome** *Proceedings of the National Academy of Sciences* **79**, 4113-4117; https://doi.org/10.1073/pnas.79.13.4113
32. Rajabi-Maham H, Orth A, Bonhomme F. (2008) **Phylogeography and postglacial expansion of Mus musculus domesticus inferred from mitochondrial DNA coalescent, from Iran to Europe** *Mol Ecol*. **17**, 627-641. https://doi.org/10.1111/j.1365-294X.2007.03601.x
33. García-Rodríguez O, Hardouin EA, Hambleton E, Monteith J, Randall C, Richards MB, Edwards CJ, Stewart JR (2021) **Ancient mitochondrial DNA connects house mice in the British Isles to trade across Europe over three millennia** *BMC Ecol Evol*. **21**, 9. https://bmcecolevol.biomedcentral.com/articles/10.1186/s12862-021-01746-4
34. Laumbacher B, Fellerhoff B, Herzberger B, Wank R. (2006) **Do dogs harbour risk factors for human breast cancer?** *Med Hypotheses* **67**, 21-26. https://doi.org/10.1016/j.mehy.2006.01.016.
35. Lehrer S, Rheinstein PH (2019) **The virology of breast cancer: viruses as the potential causative agents of breast tumourigenesis** *Discov Med*. **27**, 163–166. https://www.discoverymedicine.com/Steven-Lehrer/2019/03/virology-of-breast-cancer-viruses-as-potential-causative-agents-of-breast-tumorigenesis/
36. de Sousa Pereira N, Akelinghton Freire Vitiello G, Karina Banin-Hirata B, et al. (2020) **Mouse Mammary Tumour Virus (MMTV)-Like Sequence in Brazilian Breast Cancer Samples: Implications in Clinicopathological Parameters in Molecular Subtypes** *International Journal of Environmental Research and Public Health* **24**. https://doi.org/10.3390/ijerph17249496..

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37. Buehring GC, Shen HM, Jensen HM, Jin DL, Hudes M, et al. (2015) **Exposure to Bovine Leukemia Virus Is Associated with Breast Cancer: A Case-Control Study** *PLOS ONE* **10**, e0134304. https://doi.org/10.1371/journal.pone.0134304

Supplementary Materials:

Table S1: Mastectomy samples, DNA extraction results.
Abbreviations: TT=tumoural tissue, TN=normal tissue.

#	Sample ID	DNA Conc.	Unit	260/280	260/230	DNA (200ng)	H2O
1	1M TN	2	ng/µl	1.36	0.87	100.00	-76.00
2	2M TN	5.8	ng/µl	1.55	1.38	34.48	-10.48
3	3M TN	39	ng/µl	1.68	2.26	5.13	18.87
4	4M TN	0.2	ng/µl	-2.53	-0.05	1000.00	-976.00
5	6M TN	9.7	ng/µl	1.53	1.19	20.62	3.38
6	7M TN	-1.5	ng/µl	3.79	0.18	-133.33	157.33
7	8M TN	6.4	ng/µl	1.69	1.66	31.25	-7.25
8	9M TN	44.9	ng/µl	1.71	2.57	4.45	19.55
9	10M TN	15.9	ng/µl	1.72	2.59	12.58	11.42
10	11M TN	1.1	ng/µl	1.81	-0.39	181.82	-157.82
11	12M TN	17.9	ng/µl	1.8	3.4	11.17	12.83
12	15M TN	5.9	ng/µl	1.91	-16.54	33.90	-9.90
13	16M TN	17	ng/µl	1.75	1.05	11.76	12.24
14	17M TN	33.1	ng/µl	1.81	2.68	6.04	17.96
15	18M TN	54.5	ng/µl	1.75	2.28	3.67	20.33
16	19M TN	11.6	ng/µl	1.72	2.34	17.24	6.76
17	21M TN	46.7	ng/µl	1.76	2.5	4.28	19.72
18	23M TN	9.2	ng/µl	1.56	0.75	21.74	2.26
19	24M TN	8.7	ng/µl	1.55	1.76	22.99	1.01
20	25M TN	20.6	ng/µl	1.71	2.73	9.71	14.29
1	1M TT	25.2	ng/µl	1.48	0.59	7.94	16.06
2	2M TT	3.9	ng/µl	1.63	22.87	51.28	-27.28
3	3M TT	390.5	ng/µl	1.81	2.14	0.51	23.49
4	4M TT	2.9	ng/µl	1.23	-1.35	68.97	-44.97
5	5M TT	11.1	ng/µl	1.79	2.78	18.02	5.98
6	6M TT	6.9	ng/µl	1.48	0.81	28.99	-4.99
7	7M TT	94.1	ng/µl	1.76	1.62	2.13	21.87
8	8M TT	25.9	ng/µl	1.73	3	7.72	16.28
9	9M TT	90.2	ng/µl	1.72	2	2.22	21.78
10	10M TT	39.6	ng/µl	1.63	1.64	5.05	18.95
11	11M TT	234.1	ng/µl	1.67	2.15	0.85	23.15
12	12M TT	566.6	ng/µl	1.81	2.15	0.35	23.65
13	13M TT	158.4	ng/µl	1.77	1.66	1.26	22.74
14	14M TT	698	ng/µl	1.79	1.92	0.29	23.71
15	15M TT	79	ng/µl	1.77	2.13	2.53	21.47
16	16M TT	32.5	ng/µl	1.7	1.84	6.15	17.85
17	17M TT	10.1	ng/µl	1.81	6.42	19.80	4.20
18	18M TT	190.7	ng/µl	1.76	1.98	1.05	22.95
19	19M TT	102.6	ng/µl	1.72	2.03	1.95	22.05
20	20M TT	44.7	ng/µl	1.75	2.54	4.47	19.53
21	21M TT	46	ng/µl	1.73	2.21	4.35	19.65

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22	22M TT	399.6	ng/µl	1.77	2.05	0.50	23.50
23	23M TT	11.7	ng/µl	1.64	1.2	17.09	6.91
24	24M TT	24.1	ng/µl	1.76	2.09	8.30	15.70
25	25M TT	70.8	ng/µl	1.69	1.74	2.82	21.18

Table S2: Lumpectomy samples, DNA extraction results.
Abbreviations: TT=tumoural tissue, TN=normal tissue.

	Sample ID	ADN Conc.	Unit	260/280	260/230	DNA (200 ng)	H2O
1	1S TN	49.4	ng/µl	1.71	2.07	4.05	19.95
2	2S TN	100.2	ng/µl	1.76	2.42	2.00	22.00
3	3S TN	-0.9	ng/µl	-0.63	0.3	-222.22	246.22
4	4S TN	16	ng/µl	1.53	3.88	12.50	11.50
5	6S TN	3.2	ng/µl	0.89	19.84	62.50	-38.50
6	7S TN	3.5	ng/µl	0.84	-2.58	57.14	-33.14
7	8S TN	7	ng/µl	1.16	-1804.79	28.57	-4.57
8	12S TN	1.2	ng/µl	0.47	-0.61	166.67	-142.67
9	13S TN	12.6	ng/µl	1.44	3.05	15.87	8.13
10	14S TN	0.1	ng/µl	0.06	-0.05	2000.00	-1976.00
11	16S TN	28.2	ng/µl	1.69	2.96	7.09	16.91
12	19S TN	152	ng/µl	1.78	2.24	1.32	22.68
13	20S TN	22.4	ng/µl	1.55	2.02	8.93	15.07
14	22S TN	-0.6	ng/µl	-0.31	-0.07	-333.33	357.33
15	25S TN	-2.2	ng/µl	-4.05	0.78	-90.91	114.91
16	26S TN	2.7	ng/µl	0.77	-1.51	74.07	-50.07
17	29S TN	128.2	ng/µl	1.77	2.64	1.56	22.44
18	30S TN	2.8	ng/µl	1.49	-0.64	71.43	-47.43
19	31S TN	1.4	ng/µl	2.05	-0.2	142.86	-118.86
20	32S TN	5.2	ng/µl	1.82	-1.07	38.46	-14.46
21	34S TN	6.9	ng/µl	1.63	19.18	28.99	-4.99
22	35S TN	34.1	ng/µl	1.68	3.35	5.87	18.13
23	38S TN	4.7	ng/µl	1.83	-0.81	42.55	-18.55
24	39S TN	6.8	ng/µl	1.74	-2.63	29.41	-5.41
25	40S TN	15.6	ng/µl	1.71	-17.58	12.82	11.18
26	41S TN	1.5	ng/µl	1.74	-0.21	133.33	-109.33
27	42S TN	136.2	ng/µl	1.56	2.33	1.47	22.53
28	43S TN	15.1	ng/µl	1.56	11.59	13.25	10.75
29	46S TN	5.7	ng/µl	1.87	-1.22	35.09	-11.09
30	47S TN	8.6	ng/µl	1.69	-2.2	23.26	0.74
31	48S TN	169.5	ng/µl	1.65	2.31	1.18	22.82
32	50S TN	12.6	ng/µl	1.82	-7.08	15.87	8.13
1	1S TT	120.7	ng/µl	1.69	2.28	1.66	22.34
2	2S TT	107.2	ng/µl	1.72	2.21	1.87	22.13
3	3S TT	5.9	ng/µl	1.75	-19.58	33.90	-9.90
4	4S TT	91.9	ng/µl	1.69	2.4	2.18	21.82
5	5S TT	23.2	ng/µl	1.89	2.78	8.62	15.38
6	6S TT	43.7	ng/µl	1.76	1.87	4.58	19.42
7	7S TT	7.3	ng/µl	1.81	2.89	27.40	-3.40

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8	8S TT	24.8	ng/µl	1.57	1.33	8.06	15.94
9	9S TT	9.5	ng/µl	1.61	1.41	21.05	2.95
10	10S TT	6.1	ng/µl	1.74	0.87	32.79	-8.79
11	11S TT	131.3	ng/µl	1.76	2.03	1.52	22.48
12	13S TT	25.7	ng/µl	1.8	2.38	7.78	16.22
13	14S TT	10.3	ng/µl	1.81	1.33	19.42	4.58
14	15S TT	13	ng/µl	1.82	2.66	15.38	8.62
15	16S TT	71.5	ng/µl	1.8	2.41	2.80	21.20
16	17S TT	38.6	ng/µl	1.77	2.62	5.18	18.82
17	18S TT	12.4	ng/µl	1.84	2.42	16.13	7.87
18	19S TT	10.7	ng/µl	1.89	2.22	18.69	5.31
19	20S TT	24.6	ng/µl	1.84	2.79	8.13	15.87
20	21S TT	6.1	ng/µl	1.68	1.15	32.79	-8.79
21	22S TT	25.9	ng/µl	1.73	2.02	7.72	16.28
22	23S TT	17.5	ng/µl	1.96	2.9	11.43	12.57
23	24S TT	31.3	ng/µl	1.76	2.41	6.39	17.61
24	25S TT	28.1	ng/µl	1.63	1.04	7.12	16.88
25	26S TT	23.4	ng/µl	1.7	2.52	8.55	15.45
26	27S TT	12.3	ng/µl	1.78	2.16	16.26	7.74
27	28S TT	5.1	ng/µl	1.6	2.6	39.22	-15.22
28	29S TT	11.1	ng/µl	1.91	3.5	18.02	5.98
29	30S TT	4.2	ng/µl	1.94	3.28	47.62	-23.62
30	31S TT	13.1	ng/µl	1.71	2.1	15.27	8.73
31	32S TT	15.2	ng/µl	1.79	2.25	13.16	10.84
32	33S TT	68.4	ng/µl	1.65	0.9	2.92	21.08
33	34S TT	5	ng/µl	1.49	1.75	40.00	-16.00
34	35S TT	7.3	ng/µl	1.45	0.67	27.40	-3.40
35	36S TT	10.6	ng/µl	1.79	3.5	18.87	5.13
36	37S TT	4.1	ng/µl	1.69	84.43	48.78	-24.78
37	38S TT	27.4	ng/µl	1.74	2.73	7.30	16.70
38	40S TT	13.6	ng/µl	1.64	2.33	14.71	9.29
39	41S TT	92.4	ng/µl	1.63	2.36	2.16	21.84
40	42S TT	60.2	ng/µl	1.55	2.3	3.32	20.68
41	43S TT	45.6	ng/µl	1.57	2.32	4.39	19.61
42	44S TT	0.6	ng/µl	0.94	-0.2	333.33	-309.33
43	45S TT	9.7	ng/µl	1.62	6.66	20.62	3.38
44	46S TT	18	ng/µl	1.65	3.66	11.11	12.89
45	47S TT	223.4	ng/µl	1.58	1.64	0.90	23.10
46	48S TT	58.8	ng/µl	1.58	2.11	3.40	20.60
47	49S TT	29.4	ng/µl	1.64	1.89	6.80	17.20
48	50S TT	31.4	ng/µl	1.67	2.33	6.37	17.63