Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

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Effect of SMAD4 gene polymorphism on breast cancer risk in Bangladeshi women

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Abstract

Background Breast cancer, one of the most prevalent cancer types among women worldwide as well as in Bangladesh, is the leading cause of cancer death in women throughout the globe. The risk of breast cancer development was found to be associated with genetic polymorphism according to several studies. As a convenient prognostic marker, a biomarker helps to identify disease progression, can lead to an effective therapeutic strategy, development of prognostic marker is very important for any cancer to initiate treatment strategy early to increase the possibility of the success rate of the treatment along with reduction of the treatment cost. This study aims to establish the correlation between polymorphism of SMAD4 rs10502913 and risk of breast cancer development in Bangladeshi women. This study was conducted on 70 breast cancer patients and 60 healthy volunteers through blood sample collection followed by DNA separation between the intervals of August 2019–October 2019. The collected DNA sample was arranged for the RFLP analysis of a PCR amplified fragments followed by gel electrophoresis. The obtained data was analyzed by structured multinomial logistic regression model.

Results Obtained different fragment size after gel electrophoresis indicated different genotypes in this experiment. Our findings demonstrated that mutant homozygous A/A genotype, plays a significant role in breast cancer development among Bangladeshi women ($P=0.006$, OR = 4.9626, 95% CI = 1.9980–12.3261) compared to the reference homozygous G/G genotype. Moreover, heterozygous G/A genotype was also found to be significantly associated with the risk of breast cancer development ($P=0.0252$, OR = 2.6574, CI = 1.1295–6.2525). Considering the A/A genotype and G/A genotype combined, it also indicates a strong association of breast cancer development in Bangladeshi women ($P=0.008$, OR = 3.5630, CI = 1.6907–7.5068).

Conclusion Our study indicated a novel association between SMAD4 (rs10502913) polymorphism and increased risk of breast cancer development in Bangladeshi women.

Keywords Breast cancer, Polymorphism, SMAD4, Women, Bangladesh

1 Background

Cancer is a disease in which a group of untypical cells grows uncontrollably without adhering to the standard rules of cell division [1]. Regular healthy cells are constantly subject to signals that decide whether the cell should divide, differentiate into another cell or die. But the division of cancer cells does not correspond to these signals, resulting in uncontrolled growth and proliferation. When this proliferation is allowed to continue and spread, cancer develops. Almost 90% of cancer-related deaths are due to tumor spreading—a process called

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metastasis [2]. Cancer genetics can predict the networks and pathways that contribute to tumor development and the mechanism of cancer genes promoting tumor evolution [3]. One of the most common invasive cancers in females worldwide is breast cancer [4]. In the beginning, breast cancer starts with a lump with or without other manifestations [5]. As with other types of cancer, breast tumors can be benign or malignant, the benign type is not life-threatening, can usually be removed, and does not spread to other parts of the body [6]. However, malignant breast tumors are life-threatening and can invade surrounding tissues. It can metastasize to other parts of the body via the lymphatic system (lymphatic vessels and lymph nodes) such as the liver and bone [7].

According to the estimation of web-based global cancer statistics platform, GLOBOCAN, in 2020, worldwide female breast cancer has overtaken lung cancer and become the most commonly diagnosed cancer with an estimation of 2.3 million new cases [8]. At the end of 2020, breast cancer was the world's most prevalent cancer as 7.8 million breast cancer survived women were found in the past 5 years [9]. Breast cancer is responsible for the most frequent cause of death in different regions of the world [4]. The peak age of breast cancer patients in Asia as well as in Bangladesh is around 40–50 years while in western countries the peak age is around 60–70 years [10, 11]. According to GLOBOCAN estimation of 2020, in Bangladesh, 13,028 new breast cancer cases have been diagnosed in 2020, the most prevalent cancer type among females with an occurrence rate of 19% of the total female cancers [12].

Variation at single position of DNA is termed as Single nucleotide polymorphicm (SNP). According to multiple studies, SNPs are associated with breast density and the risk of breast cancer development [13, 14]. SMAD4, a gene located on chromosome 18q21.1, is a downstream mediator of transforming growth factor-beta (TGF-β) and plays a role in regulating cellular function such as proliferation, differentiation, etc. [15]. Both alleles of the gene are inactivated in pancreatic carcinomas, but their role in the tumorigenesis of other cancer is still unknown [16]. A study suggested that SMAD4 might be a potential prognostic marker for the early detection of breast cancer [17]. An earlier study indicated that SMAD4 has a role to play in the regulation of the initiation, progression and prognostic outcome of breast cancer [18]. TGF-β, an inhibitory signaling molecule, generally binds with TGF-β receptor and via SMAD signaling pathway inhibits cell growth [19]. SMAD4 is a candidate for the tumor-suppressor gene [20]. In estrogen receptor-α positive breast cancer cells, SMAD4 can induce programmed cell death by TGF-β-mediated inhibition of ERα estrogenic transcription activity in tumor samples [21]. In recent years, studies on SNPs have increased due to their use to predict certain disease susceptibility. Gene transcription and translation as well as necessary protein synthesis can be altered due to SNPs [22].

Till date, according to our knowledge, no study has been conducted to assess the risk of SMAD4 gene polymorphism and breast cancer risk in Bangladeshi women. The aim of this study is to investigate the potential involvement of the SMAD4 gene polymorphism at position rs10502913 in Bangladeshi women to detect breast cancer in the early stage so that patients can be unclogged in terms of cure and cost.

2 Methods

2.1 Study population and sample collection

The case–control study was conducted on seventy breast cancer patients (case) and sixty healthy female volunteers (control). Two groups, case and control, were matched by means of age. The healthy volunteers were subjected to physical examination to mark them as control group. Breast cancer patients have been chosen from the National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh, between the intervals of August 2019–October 2019. Ethical approval was taken before approaching to patients from Ethical Approval Committee of Medical Oncology Department of NICRH, Dhaka, Bangladesh. Details of ethical approval procedure is outlined in Supplementary Information (Additional file 1). Patients had been histologically diagnosed with breast cancer according to the TNM staging device accustomed by American Joint Committee of Cancer (AJCC). Demographic traits and lifestyle factors were acquired through interviews by means of skilled nurses in presence of professional physicians. The study was performed in accordance with the statement of Helsinki and its subsequent revisions [23]. Breast cancer patients or their respective caregivers filled out a consent form after being informed about the study protocol. The blood sample (3 mL) was collected from the patient and control using the EDTA tubes and was stored at − 80 °C until further use and the freeze–thaw cycle was avoided.

2.2 DNA extraction and genotyping

DNA was extracted by chemical method mentioned by Daly et al. [24]. DNA was isolated from the blood samples of the patients and the healthy volunteers and kept at − 20 °C temperature till analysis was done. Amplification of the SNP of interest for this study was performed by Polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) technique to genotype the SMAD4 rs10502913 polymorphism [25–27]. Thermostable Taq DNA polymerase, isolated enzyme from *Thermus aquaticus*, was used to automatize the specific

Table 1 Name of the allele, sequence of the designed primer with their size and melting point

Nos.	Allele	Primer sequence	M.T (°C)	Size (bp)
1.	rs10502913FP	5′-GGGGTTGGTTGTCACTGCAG -3′	56	20 bp
2.	rs10502913 RP	5′-GGCCACCAATCCACCAAACC -3′	56	20 bp

FP, forward primer; RP, reverse primer; M.T, melting temperature

DNA sequence amplification repetitively during the PCR technique. The forward primer and reverse primer were designed by following the rule of the National Center for Biotechnology Information (NCBI). Primer details are given in Table 1.

The produced fragment was isolated and kept in a thermostat chamber overnight at 37 °C then digested by the restriction enzyme *HpyCH4IV* (source: *Helicobacter pylori*). Gel electrophoresis (with 2% agarose) was performed to visualize extracted DNA fragments after the digestion and ethidium bromide was used to stain the fragments. The restriction enzyme, Taq DNA polymerase and the primers were procured from New England BioLabs Inc., USA. Table 2 represents the patient group and control group with clinicopathological features.

2.3 Statistics

A statistical multinomial logistic regression model was used to evaluate the association between SMAD4 polymorphism and breast cancer risk in the studied Bangladeshi women. p value less than 0.05 was considered statistically significant with a 95% confidence interval. The statistical analysis was performed by the statistical software SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA).

3 Results

This population-based case–control study was conducted to demonstrate the prevalence of SMAD4 gene polymorphism in the Bangladeshi population of breast cancer patients and normal healthy controls. We conducted the study through 70 breast cancer patients with a number of 60 controls.

The splitting of the normal functioning allele G provided 46 bp and 206 bp sized fragments for SMAD4 rs10502913 polymorphism. The cleaving of the polymorphic allele A provided 46 bp, 206 bp and 252 bp sized fragments (Fig. 1). Different sized DNA fragments got after digestion with the restriction enzyme and it has been presented in Table 3.

The result has been depicted through classified the genotype of patient and control group into four; the heterozygous genotype, mutant homogygous genotype and combination of both were scrutinized against the reference genotype. In the case of SMAD4 rs10502913 the G/G homozygous genotype showed statistically lower frequency in breast cancer patients if it is compared to healthy controls volunteers. In this analysis, we set this GG genotype as a reference value to compare other data. The percentage frequency of G/A heterozygous genotype in the studied breast cancer cases was found in 34.28% with a p value of 0.0252. The G/A genotype showed an odds ratio of 2.6574 and the 95% confidence Interval was in the range between 1.1295 and 6.2525. Again, in the case of mutant homozygous A/A genotype the patients were found to have a frequency of 41.24%. With an odds ratio of 4.9626. The p value was found 0.0006 with a 1.9980–12.3261 range of 95% confidence interval. Moreover, considering both genotypes G/A and A/A which

Table 2 Clinicopathological characteristic of the breast cancer patients and controls

Characteristics	rs10502913 ($n=70$) (%)	Controls ($n=60$) (%)
Age, year		
≤47	43 (61.42%)	41 (68.33%)
>47	27 (38.58%)	19 (36.70%)
Dwelling		
Urban	18 (25.71%)	43 (71.66%)
Rural	52 (74.29%)	17(28.34%)
Menstrual status		
Pre-menopause	36 (51.42%)	28 (46.66%)
Post-menopause	34 (48.58%)	32 (53.34%)
Parity		
0–7	62 (88.57%)	58 (96.66%)
>7	8 (11.43%)	12 (3.34%)
Contraception		
Oral pills	51 (72.85%)	42 (70%)
Others	8 (11.43%)	14 (23.33%)
None	11 (15.72%)	4 (6.66%)
Family history of cancer (first degree relatives)		
Yes	45 (64.28%)	24 (40%)
No	25 (35.72%)	36 (60%)
Stage of cancer		
IA-IB	2 (1.28%)	–
IIA	33 (47.14%)	–
IIB	32 (45/71%)	–
IIIA-IIIB	3 (4.28%)	–
Tumor grade		
I	9 (12.85%)	–
II	53(75.71%)	–
III	8 (11.43%)	–

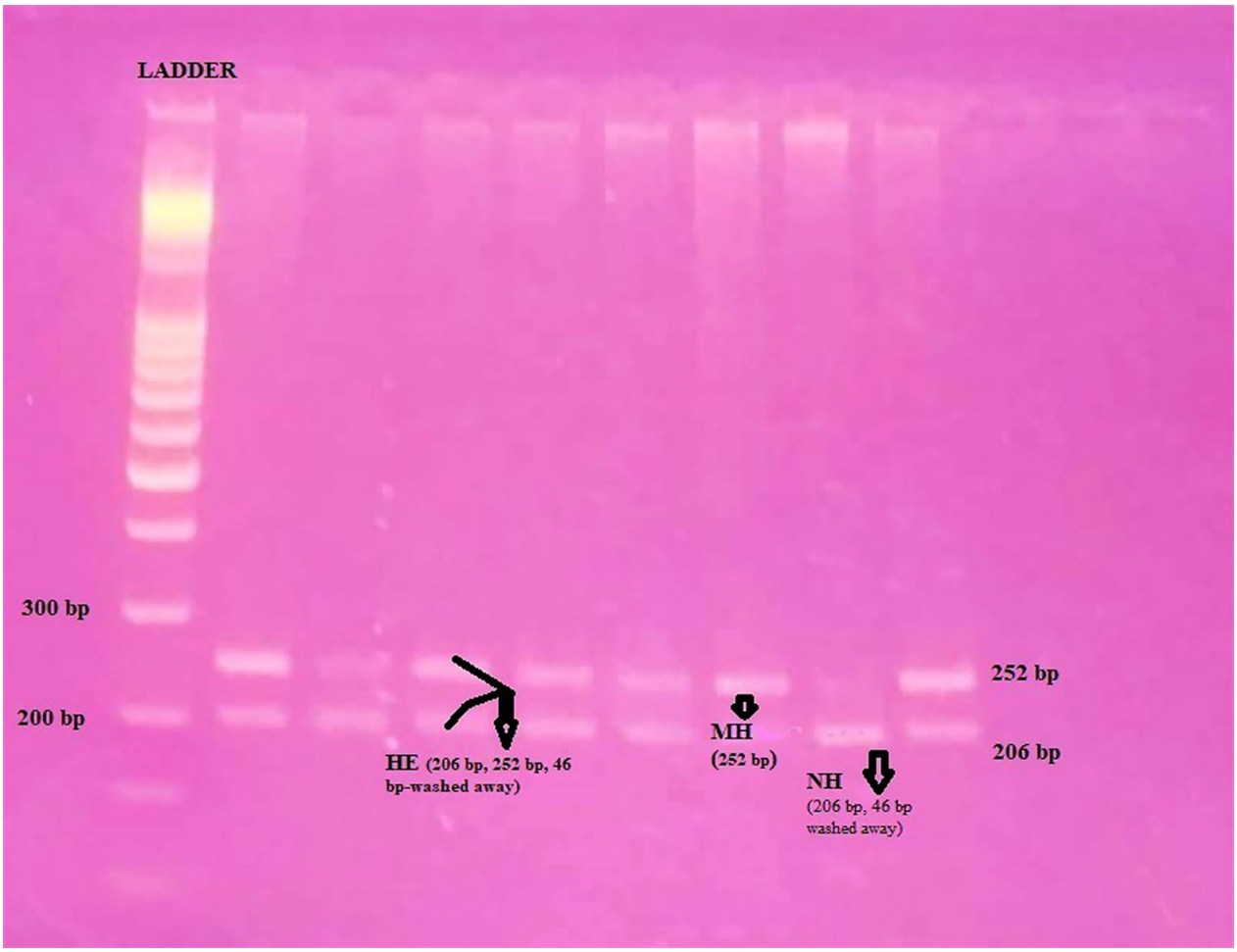

Fig. 1 Restriction enzyme (HpyCH4IV) digestion fragments of SMAD4 gene polymorphism (2% agarose gel). Normal Homozygous (NH) has bands of 206 bp and 46 bp (washed away), Heterozygous has bands of 206 bp, 252 bp and 46 bp (washed away), Mutant Homozygous (MH) has band of 252 bp

Table 3 Name of the allele, PCR product size, and restriction enzyme, length of the expected and observed fragments on digestion for SMAD4 Gene rs10502913

Allele	PCR product size (bp)	RE	Digestion conditions	Genotypes	Fragment patterns with number of base pairs
SMAD4	252	HpyCH4IV	37 deg	NH	46, 206
				HE	46, 206, 252
				MH	252

are deviated from normal genotype the odds ratio was found to be 3.5630 with p value of 0.0008 and the confidence Interval was in the range between 1.6907 to 7.5068 (Table 4).

4 Discussions

This study shows that the carriers of the G/A genotype population were found to have 2.6574 times more risk of developing breast cancer compared to the normal genotype. The carriers of the A/A genotype population have 4.9626 times more risk and G/A and A/A genotypes combined has 3.4630 times more risk to develop breast cancer. Heterozygous (G/A), mutant homozygous (A/A) along combination both of these genotypes were found to be significantly associated with breast cancer development in the studied population group of Bangladeshi women. The shortcomings of the current study is that the function of gene of interest related to breast cancer development has not been evaluated in vivo and in vitro.

Science is blending on regular basis successfully to set up the innate and nuclear reason of various complex disorders corresponding to certain sorts of carcinomas. In

Table 4 SMAD4 Gene rs10502913 polymorphisms in breast cancer patients and control subjects

Genotype	Cases $N=70$ (%)	Controls $N=60$ (%)	Odds ratio	95% CI	*p* value
GG	17 (24.28%)	32 (53.33%)	–	–	–
GA (heterozygous)	24 (34.28%)	17 (28.33%)	2.6574	1.1295 to 6.2525	0.0252
AA (mutant homozygous)	29 (41.42%)	11 (18.33%)	4.9626	1.9980 to 12.3261	0.0006
GA + AA	53 (75.71%)	28 (46.66%)	3.5630	1.6907 to 7.5068	0.0008

explicit, SNPs have been productive in genome-based contemplates perceiving the genetic defenselessness of malignant growths [28]. The foundation of positive connection between polymorphism in genomic DNA and the possibility of tumor development has empowered various cover in looking for after development consideration in disease science but such associations can vary considerably between various ethnic populations [29]. A study suggested that breast cancer is associated with lower expression of the SMAD4 gene [30]. As earlier studies suggested that SMAD4 rs10502913 was associated with the development of colon cancer and gastric cancer, we targeted the alleles for its previous association with cancer [31, 32]. Worldwide, there was no study conducted to find out the relationship between mutation at position rs10502913 of SMAD4 gene and risk of development of breast cancer. SNPs can be positioned in the promoter region, exon or intron. SMAD4 rs10502913 is an intronic variant functional SNP [33]. Intronic SNPs are responsible for splicing and change the long noncoding RNAs in terms of function and binding [34]. A study found that an intronic SNP was related to the risk of breast cancer development [35]. As an intronic variant SNP, SMAD4 rs10502913 might be associated with the risk of breast cancer development.

SMAD4 rs10502913 polymorphic variant was found to be responsible for colorectal cancer and missense mutation was observed in the MH2 domain of SMAD protein [16, 32]. Mostly Arg361 region and Pro356 region were found to be affected due to this mutation [36]. Missense mutation generally renders the protein to be less effective. Earlier studies found that missense mutation is associated with the risk of breast cancer development [37, 38]. Upon binding with the DNA, SMAD4 protein regulates particular gene that is responsible for cellular proliferation [39]. So disruption of the function of this protein might be associated with the risk of breast cancer development.

A study with colorectal cancer in the Polish population found that in colorectal cancer patients the SMAD4 rs10502913 G/G genotype (60%) was predominant, followed by heterozygous GA (35%) and mutant homozygous A/A (5%) [40]. But in the case of samples from breast cancer patients in Bangladesh from this study, it was found that SMAD4 rs10502913 A/A mutant homozygous genotype is predominant with an occurrence of 41.42%, followed by heterozygous G/A genotype (34.28%). In case of SMAD4 rs10502913 G/G genotype, it was found only 24.28% occurrence. Previous studies have suggested that different ethnic backgrounds and different geographic populations may act as factor of incidence of cancer as well as treatment efficacy and prognosis [41, 42]. Furthermore, race factor was found to be a major differentiator of risk of breast cancer development [43]. Due to differences in cancer nature and geographical location, the genotypes involved in cancer development were found to be different though same the SNP was found to be involved.

As there was no previous study for Bangladeshi women regarding breast cancer development associated with the mutated SMAD4 gene, this study can help us to find a novel correlation. It has been observed that, if breast cancer can be diagnosed at an earlier stage, the treatment cost would be much lower compared to detection in the late stage [44]. So developing a potential early prognostic marker will help us to treat the patient at the right time with a significant reduction in the cost. In our study, we have illustrated the impact of a distinctive SNP on the development of breast cancer. The primary SNP of interest was SMAD4 gene rs10502913. Polymorphism in this SNP was found to have a significant correlation with breast cancer risk in Bangladeshi women.

5 Conclusion

SMAD4 gene rs10502913 allele polymorphism identification can be a possible promising diagnostic marker for breast cancer in Bangladeshi women. Further studies are needed to corroborate the findings of the research.

Abbreviations

Bp	Base pair
GLOBOCAN	Global cancer observatory
SNP	Single nucleotide polymorphism
PCR–RFLP	Polymerase chain reaction-restriction fragment length polymorphism
TGF-β	Transforming growth factor beta
EDTA	Ethylene diamine tetra acetic acid
MH2	MAD homology 2

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-023-00347-y.

Additional file 1. Details of ethical approval procedure.

Acknowledgements
The authors are grateful to the patients, their families, caregivers, volunteers, nurses, physicians, collaborators, and scientists of the National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh. The authors would also like to thank the Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Bangladesh to provide lab facilities and other opportunities to carry out the research work.

Author contributions
MR: interpretation of data and conduction of the project. MRI: design of the project. MNHA: compilation and revision. MNU: data collection. SAS: data collection. NAN: conception of the project. MSI: conception of the project. All authors read and approved the final manuscript.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Ethical approval was taken before approaching to patients from Ethical Approval Committee of Medical Oncology Department of NICRH, Dhaka, Bangladesh. Ref no. NICRH/Ethics/2019/469. Consent form was filled up by the participants, mention in the Supplementary Information (SI).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 8 June 2022 Accepted: 10 January 2023
Published online: 18 January 2023

References

1. Hejmadi M (2010) Introduction to cancer biology. In: Introduction to cancer biology, p 7
2. Paulmurugan R (2012) Introduction to cancer biology. In: Molecular imaging probes for cancer research, World Scientific Publishing Co., pp 3–27. https://doi.org/10.1142/9789814293686_0001
3. Lach RP, Adams DJ (2013) Cancer genetics. In: Brenner's encyclopedia of genetics, 2nd edn, pp 416–419. https://doi.org/10.1016/B978-0-12-374984-0.00190-X
4. Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M et al (2019) Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. Int J Cancer 144:1941–1953. https://doi.org/10.1002/ijc.31937
5. Omondiagbe DA, Veeramani S, Sidhu AS (2019) Machine learning classification techniques for breast cancer diagnosis. IOP Conf Ser Mater Sci Eng 495:012033. https://doi.org/10.1088/1757-899X/495/1/012033
6. Sharma GN, Dave R, Sanadya J, Sharma P, Sharma KK (2010) Various types and management of breast cancer: an overview. J Adv Pharm Technol Res 1:109–126
7. Rahman M, Mohammed S (2015) Breast cancer metastasis and the lymphatic system. Review 2015:1233–1239
8. 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
9. GLOBOCAN. Breast cancer 2021. https://gco.iarc.fr/. Accessed 28 May 2021
10. Leong SPL, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS et al (2010) Is breast cancer the same disease in Asian and Western Countries? World J Surg 34:2308–24. https://doi.org/10.1007/S00268-010-0683-1
11. Afroz S, Rahman SS, Hossain MM (2017) A study survey on risk factors associated with breast cancer in Bangladeshi population. J Cancer Sci Ther 09:463–467. https://doi.org/10.4172/1948-5956.1000460
12. GLOBOCAN (2020) Cancer incidence in Bangladesh, vol 745. https://gco.iarc.fr/today/data/factsheets/populations/50-bangladesh-fact-sheets.pdf
13. Sartor H, Brandt J, Grassmann F, Eriksson M, Czene K, Melander O et al (2020) The association of single nucleotide polymorphisms (SNPs) with breast density and breast cancer survival: the Malmö Diet and Cancer Study. Acta Radiol 61:1326–1334. https://doi.org/10.1177/0284185119900436
14. Graves KD, Peshkin BN, Luta G, Tuong W, Schwartz MD (2011) Interest in genetic testing for modest changes in breast cancer risk: implications for SNP testing. Public Health Genomics 14:178–189. https://doi.org/10.1159/000324703
15. Massagué J (2012) TGFβ signalling in context. Nat Rev Mol Cell Biol 13:616–630. https://doi.org/10.1038/nrm3434
16. Woodford-Richens KL, Rowan AJ, Gorman P, Halford S, Bicknell DC, Wasan HS et al (2001) SMAD4 mutations in colorectal cancer probably occur before chromosomal instability, but after divergence of the microsatellite instability pathway. Proc Natl Acad Sci USA 98:9719–9723. https://doi.org/10.1073/pnas.171321498
17. Liu NN, Xi Y, Callaghan MU, Fribley A, Moore-Smith L, Zimmerman JW et al (2014) SMAD4 is a potential prognostic marker in human breast carcinomas. Tumor Biol 35:641–650. https://doi.org/10.1007/s13277-013-1088-1
18. Liu N, Yu C, Shi Y, Jiang J, Liu Y (2015) SMAD4 expression in breast ductal carcinoma correlates with prognosis. Oncol Lett 10:1709–1715. https://doi.org/10.3892/ol.2015.3442
19. Hata A, Chen YG (2016) TGF-β signaling from receptors to smads. Cold Spring Harb Perspect Biol 8:1. https://doi.org/10.1101/cshperspect.a022061
20. Devereux TR, Anna CH, Patel AC, White CM, Festing MFW, You M (1997) Smad4 (homolog of human DPC4) and Smad2 (homolog of human JV18-1): candidates for murine lung tumor resistance and suppressor genes. Carcinogenesis 18:1751–1755. https://doi.org/10.1093/carcin/18.9.1751
21. Li Q, Wu L, Oelschlager DK, Wan M, Stockard CR, Grizzle WE et al. (2005) Smad4 inhibits tumor growth by inducing apoptosis in estrogen receptor-positive breast cancer cells. https://doi.org/10.1074/jbc.M505071200
22. Robert F, Pelletier J (2018) Exploring the impact of single-nucleotide polymorphisms on translation. Front Genet 9:1–11. https://doi.org/10.3389/fgene.2018.00507
23. World Medical Association (2013) Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA J Am Med Assoc 310:2191–2194. https://doi.org/10.1001/jama.2013.281053
24. Daly AK, Monkman SC, Smart J, Steward A, Cholerton S (1998) Analysis of cytochrome P450 polymorphisms. Methods Mol Biol 107:405–422. https://doi.org/10.1385/0-89603-519-0:405
25. Ota M, Seki T, Nomura N, Suglmura K, Mizuki N, Fukushima H et al (1991) Modified PCR-RFLP method for HLA-DPB1 and -DQA1 genotyping. Tissue Antigens 38:60–71. https://doi.org/10.1111/j.1399-0039.1991.tb01882.x
26. Rivu SF, Apu MNH, Shabnaz S, Nahid NA, Islam MR, Al-Mamun MMA et al (2017) Association of TP53 codon 72 and CDH1 genetic polymorphisms with colorectal cancer risk in Bangladeshi population. Cancer Epidemiol 49:46–52. https://doi.org/10.1016/j.canep.2017.05.005
27. Shahdaat Bin Sayeed M, Nazmul Hasan Apu M, Tabassum Munir M, Uddin Ahmed M, SafiqulIslam M, Maksumul Haq M et al (2015) Prevalence of CYP 2C19 alleles, pharmacokinetic and pharmacodynamic variation of clopidogrel and prasugrel in Bangladeshi population. Wiley Online Libr 42:451–7. https://doi.org/10.1111/1440-1681.12390
28. Erichsen HC, Chanock SJ (2004) SNPs in cancer research and treatment. Br J Cancer 90:747–751. https://doi.org/10.1038/sj.bjc.6601574

29. Botstein D, Risch N (2003) Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nat Genet 33:228–237. https://doi.org/10.1038/ng1090
30. Woo JS, Chung MS, Paik SS (2019) Clinicopathological significance of SMAD4 expression in breast cancer. J Breast Dis 7:52–8
31. Wu DM, Zhu HX, Zhao QH, Zhang ZZ, Wang SZ, Wang ML et al (2010) Genetic variations in the SMAD4 gene and gastric cancer susceptibility. World J Gastroenterol 16:5635–5641. https://doi.org/10.3748/wjg.v16.i44.5635
32. Slattery ML, Herrick JS, Lundgreen A, Wolff RK (2011) Genetic variation in the TGF-β signaling pathway and colon and rectal cancer risk. Cancer Epidemiol Biomarkers Prev 20:57–69. https://doi.org/10.1158/1055-9965.EPI-10-0843
33. NCBI. rs10502913 - SNP 2021. https://www.ncbi.nlm.nih.gov/snp/?term=rs10502913. Accessed 14 July 2021
34. Xiong HY, Alipanahi B, Lee LJ, Bretschneider H, Merico D, Yuen RKC et al (2015) The human splicing code reveals new insights into the genetic determinants of disease. Science (80-) 2015:347. https://doi.org/10.1126/SCIENCE.1254806
35. Zhou J, Nagarkatti PS, Zhong Y, Creek K, Zhang J, Nagarkatti M (2010) Unique SNP in CD44 intron 1 and its role in breast cancer development. Anticancer Res 30:1263–1272
36. Sarshekeh AM, Advani S, Overman MJ, Manyam G, Kee BK, Fogelman DR et al (2017) Association of SMAD4 mutation with patient demographics, tumor characteristics, and clinical outcomes in colorectal cancer. PLoS ONE 12:1–14. https://doi.org/10.1371/journal.pone.0173345
37. Scott RJ, Meldrum CJ (2005) Missense mutations in cancer predisposing genes: can we make sense of them? Hered Cancer Clin Pract 3:123. https://doi.org/10.1186/1897-4287-3-3-123
38. Tavtigian SV, Chenevix-Trench G (2014) Growing recognition of the role for rare missense substitutions in breast cancer susceptibility. Biomark Med 8:589–603. https://doi.org/10.2217/bmm.13.143
39. NIH. SMAD4 gene. Medlineplus (2020). https://medlineplus.gov/genetics/gene/smad4/. Accessed 14 July 14 2021
40. Wosiak A, Wodziński D, Michalska K, Pietrzak J, Kordek R, Balcerczak E (2021) Assessment of the role of selected smad3 and smad4 genes polymorphisms in the development of colorectal cancer: preliminary research. Pharmgenomics Pers Med 14:167–178. https://doi.org/10.2147/PGPM.S281958
41. Huang Q, Baudis M (2020) enabling population assignment from cancer genomes with SNP2pop. Sci Rep. https://doi.org/10.1038/s41598-020-61854-x
42. Jing L, Su L, Ring BZ (2014) Ethnic background and genetic variation in the evaluation of cancer risk: a systematic review. PLoS ONE 9:97522. https://doi.org/10.1371/journal.pone.0097522
43. Chlebowski RT, Chen Z, Anderson GL, Rohan T, Aragaki A, Lane D et al (2005) Ethnicity and breast cancer: factors infl uencing differences in incidence and outcome. J Natl Cancer Inst 2005:97. https://doi.org/10.1093/jnci/dji064
44. Blumen H, Fitch K, Polkus V (2016) Comparison of treatment costs for breast cancer, by tumor stage and type of service, vol 9

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