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A Pilot Study of Detecting rs920778 and rs1899663 Genetic Variants of *HOTAIR* in a Cohort of Sporadic Breast Cancer Patients in Sri Lanka

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ABSTRACT

Background: Some functional single nucleotide polymorphisms (SNPs) on the *HOTAIR* gene are potent to elevate breast cancer risk where rs920778 and rs1899663 are associated with the expression and function of the *HOTAIR* gene, and these SNPs were detected in the current study.

Methods: A cohort of nine sporadic cancer patients and five healthy controls were screened for selected *HOTAIR* variants using direct sequencing.

Results: Among the patients, seven showed the TT genotype, with the other 2 showing the CT genotype for rs920778 C>T, while two healthy controls showed TT, with two others showing CT, and the remaining cases showed the CC genotypes. Among the cohort, the rs1899663 variant was not found in any patients and healthy controls.

Conclusion: In the study, the T allele of rs920778 was prominent among the sporadic breast cancer in the studied cohort, while only a wild allele (G) of rs1899663 was more prominent in the patients and healthy controls.

INTRODUCTION

LncRNAs are noncoding RNA species with more than 200 nucleotides in length, involved in regulating gene expression at epigenetic, transcriptional, and post-transcriptional levels. Dysregulation of lncRNA expression can cause malignancies such as breast, liver, colon, and lung cancer and leukaemia.[1] HOX transcript antisense intergenic RNA (HOTAIR) is one of the lncRNAs with an oncogenic impact on developing different cancers, especially breast, gastric, pancreatic, colorectal, hepatocellular, lung, and cervical cancer.[2]

Some studies on SNP analysis in the *HOTAIR* gene have found a relationship between SNPs and BC susceptibility. The functional single nucleotide polymorphisms (SNPs) located in the intronic region of the gene (rs920778, rs4759314, and rs1899663) are involved in the overexpression of HOTAIR relating to the development and progression of some cancers, including estrogen-dependent cancers such as breast cancer and gastrointestinal cancer in the Asian population.[3] The current study focused on detecting *HOTAIR* functional genetic variants in sporadic breast cancer patients who acquired mutations in genes during their life, representing the majority of breast cancer diagnosed when compared to hereditary breast cancers.

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METHODS

Sample Processing

Ethical approval was obtained from the Ethical Review Committee (ERC), Faculty of Medicine, University of Colombo, Sri Lanka (EC/22/085) to reuse the extracted DNA for the current study. Genomic DNA was extracted from surgically excised

tissues of sporadic breast cancer patients (previously collected for a Doctor of Philosophy study done by Manoharan, 2020 under the ethical approval of EC/14/160, using an AllPrep® DNA/RNA/Protein Mini Kit (Cat. No./ ID: 8000, Qiagen).[4] Nine DNA samples from sporadic breast cancer patients and 5 DNA samples from healthy individuals were used.

Analysis of selected variants in HOTAIR

Two primer sets were designed to amplify the regions containing the two selected SNPs using the online NCBI/ Primer-BLAST software. Then PCR products were outsourced for sequencing to Genelabs Medical, Colombo, Sri Lanka. The sequencing results were analyzed using BioEdit® and Mutation Surveyor® v4.0.10 by aligning with the Human *HOTAIR* NCBI Reference Sequence: NC_000012.12 at the National Centre for Biotechnology Information (NCBI).

RESULTS

Sequencing results of HOTAIR rs920778 C>T and rs1899663 G>T

Direct sequencing was carried out to detect the rs920778 C>T and rs1899663 G>T in nine sporadic breast cancer and 5 healthy samples. Tables 1 and 2 summarize the status of the genotype of each SNP in the patient with tumur features and healthy individual cohort.

Table 1. Mutation status of *HOTAIR* rs920778 and rs1899663 in a) sporadic breast cancer patients with tumur features

Sample No	Age at collection	Tumur features	*HOTAIR* SNP analysis	
			rs920778 C>T	rs1899663 G>T
1	72	T2N0MX	TT	GG
2	46	Ductal carcinoma, T1-2N1M0	TT	GG
3	48	Ductal carcinoma	TC	GG
4	61	Consistent with ductal carcinoma	TT	GG
5	60	Invasive ductal carcinoma, NG-2, T4N2MX	TC	GG
6	61	N/A	TT	GG
7	69	Invasive ductal carcinoma, NG-2, T3N0MX, C5	TT	GG
8	62	Invasive ductal carcinoma, NG-1, T4bN0MX	TT	GG
9	50	Ductal carcinoma C5	TT	GG

T1, T2, T3, T4- TNM staging (Tumour), N0, N1, N2- TNM staging (nodes), MX, M0- TNM staging (Metastasis), T1-2N1M0-Triple-negative breast cancer, NG-Nuclear grade, C5- Malignant (classification in Fine Needle Aspiration cytology report), N/A: Not Available

DISCUSSION

It was understood that these two variants were reported to be involved in the overexpression of HOTAIR concerning the development and progression of estrogen-dependent cancers.[3] A study done on Indian pre-menopausal women by Rajagopal *et al*. (2022) reported that TC and CC genotypes of rs920778 increased BC risk [5] while the study on the southeast Iranian population found a significant association between rs920778 and BC.[6]

Table 2. Mutation status of HOTAIR rs920778 and rs1899663 in healthy individuals

Healthy Individual no.	*HOTAIR* SNP analysis	
	rs920778 C>T	rs1899663 G>T
H1	TT	GG
H2	TT	GG
H3	TC	GG
H4	CC	GG
H5	TC	GG

In addition, this pattern was observed in the study done on the Turkish population, which showed the CC genotype of HOTAIR rs920778 was associated with an elevated risk of BC and clinicopathological characteristics of the tumour.[7] Conversely, a population-based study in China reported that the T allele of rs920778 significantly increased BC risk.[8] In addition, a meta-analysis done by Xu *et al*. (2019) supported the fact that individuals in Asian populations with either the T allele or TT genotype of rs920778 C>T had a significantly increased cancer risk in digestive and gynecological cancers. [9] In the current study, TT and TC genotypes were prominent in the cohort of sporadic breast cancer samples with an allele frequency of T = 0.89 and a C = 0.11, showing the fact that TT and TC genotypes may have a possible association with breast cancer risk in the studied cohort. These results are aligned with the results of a study on the Chinese population which showed an association between T allele and breast cancer risk. When considering the hormone receptor status of the patients in the studied cohort, based on the available information, four patients (sample no: 5, 6, 7, and 8) have ER+ PR+ HER2 – status, where three of them showed the TT genotype and the remaining cases showed the TC genotype. Out of the three patients with TT genotype, sample 7 had

invasive ductal carcinoma-NG - II, T3N0Mx, and sample 8 had invasive ductal carcinoma NG - I, T4bN0Mx, whereas sample 6 tumour feature was not available. Sample 5 with the TC genotype for rs920778 had invasive ductal carcinoma- NG-2, T4N2Mx. The patients with available data for receptor status showed invasive ductal carcinoma with estrogen-positive. Thus, it was found that elevated levels of HOTAIR expression can be seen in aggressive ductal carcinoma *in situ (*DCIS) and estradiol (E2) induces HOTAIR expression in an estrogen receptor-dependent manner.[10] In addition, elevated HOTAIR expression showed poor prognosis in estrogen-positive tumours.[11] Furthermore, the studied healthy control showed allele frequency of wild allele (C) of 0.4 while altered allele frequency (T) of 0.6, aligned with the results of the 1000 Genome project concerning the South Asian population, where the C allele frequency was 0.445 and the T allele frequency was 0.555. The results are more supported by the Global allele frequency of 0.4519 for C and 0.5481 for T in the 1000 Genome project. Therefore, the involvement of genotype (TT/TC) of rs920778 in the BC risk of the studied patients was significant. Further studies are needed to confirm the association with a larger cohort on a case-control basis. With respect to rs1899663G>T SNP in a cohort of sporadic breast cancer patients and healthy controls, none of the cases or controls showed the mutant allele (T).

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available upon request from the corresponding author.

ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka (EC-14-160) before sampling, collected for a PhD study. A cohort of already collected samples from the previous study was reused for the current study. Additional ethical clearance was obtained from the Ethics Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka, under EC-22-085 to be reused.

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REFERENCES

1. Jin H, Du W, Huang W, Yan J, Tang Q, Chen Y, et al. lncRNA and breast cancer: Progress from identifying mechanisms to challenges and opportunities of clinical treatment. *Mol Ther Nucleic Acids.* 2021;25:613-37. doi: 10.1016/j.omtn.2021.08.005.
2. Hajjari M, Salavaty A. HOTAIR: an oncogenic long non-coding RNA in different cancers. *Cancer Biol Med*. 2015;12(1):1-9. doi:10.7497/j.issn.2095-3941.2015.0006.
3. Lv Z, Kou C, Chen N, Jia L, Sun X, Gao Y, et al. Single Nucleotide Polymorphisms in HOTAIR Are Related to Breast Cancer Risk and Prognosis in the Northeastern Chinese Population. *Front Oncol.* 2021;11:706428. doi:10.3389/fonc.2021.706428.
4. Manoharan V, Karunanayake EH, Tennekoon KH, De Silva S, Imthikab AIA, De Silva K, et al. Pattern of nucleotide variants of TP53 and their correlation with the expression of p53 and its downstream proteins in a Sri Lankan cohort of breast and colorectal cancer patients. *BMC Cancer*. 2020;20(1):72. doi:10.1186/s12885-020-6573-5.
5. Rajagopal T, Seshachalam A, Akshaya RL, Rathnam KK, Talluri S, Jothi A, et al. Association of HOTAIR (rs920778 and rs1899663) and NME1 (rs16949649 and Rs2302254) Gene Polymorphisms With Breast Cancer Risk in India. *Gene*. 2020;762:145033. doi: 10.1016/j.gene.2020.145033.
6. Hassanzarei S, Hashemi M, Sattarifard H, Hashemi SM, Bahari G, Ghavami S. Genetic polymorphisms of HOTAIR gene are associated with the risk of breast cancer in a sample of southeast Iranian population. *Tumour Biol.* 2017;39(10):1010428317727539. doi:10.1177/1010428317727539.
7. Bayram S, Sümbül AT, Batmacı CY, Genç A. Effect of HOTAIR rs920778 polymorphism on breast cancer susceptibility and clinicopathologic features in a Turkish population. *Tumor Biol*. 2015;36(5),3863–70. doi: https://doi.org/10.1007/s13277-014-3028-0.
8. Yan R, Cao J, Song C, Chen Y, Wu Z, Wang K, et al. Polymorphisms in lncRNA HOTAIR and susceptibility to breast cancer in a Chinese population. *Cancer*

Epidemiol. 2015;39(6):978–85. doi:10.1016/j.canep.2015.10.025.
9. Xu T, Zhou Y, Zhang Y, Yang C, Yang H, Zhu S. Association between HOTAIR polymorphisms and cancer risk : a meta-analysis based on twenty-one case-control studies. *J BUON*. 2019;24(1):354-67.
10. Abba MC, Fabre ML, Lee J, Tatineni P, Kil H, Aldaz CM. HOTAIR Modulated Pathways in Early-Stage Breast Cancer Progression. *Front Oncol*. 2021;11:783211. doi:10.3389/fonc.2021.783211.
11. Tang Q, Hann SS. HOTAIR: An Oncogenic Long Non-Coding RNA in Human Cancer. *Cell Physiol Biochem*. 2018;47(3):893-913. doi:10.1159/000490131.

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