Association of *Retn (Rs3219175)* Polymorphism with Oscc Progression and Development - An *In Silico* Study

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Abstract

Oral squamous cell carcinoma (OSCC) is more common with 90% of malignant neoplasm in oral cavity. Multiple factors leading to OSCC like genetic alterations, chronic exposure, micro environmental carcinogens, premalignant disease like oral epithelial dysplasia, mitochondrial membrane damage and viruses infections like HPV (human papillomavirus). *RETN* gene belongs to the family of mouse resistin-like genes. Human genetic variations are found to influence inter-individual variation observed in disease susceptibilities, drug response, response to an infectious disease. At the molecular level these sequence variations influence gene expression, protein production, function, disease susceptibility in particular individuals. The present *in silico* study determines the possible association of *RETN* polymorphism with that of oral squamous cell carcinoma in different populations. In this study the *RETN* gene polymorphism *rs321975* was analysed in comparison to the global frequencies. The present study attempts to identify the possible reason for the selection of "A" allele in the south asian population. Furthermore, genotyping analysis has to be performed in a south Indian population to ascertain the association between *RETN* polymorphism (*rs3219175*) and OSCC.

Keywords: *Polymorphism; oral squamous cell carcinoma; RETN; variant*

Introduction

Oral squamous cell carcinoma (OSCC) is more common, which accounts for 90% of malignant neoplasm in oral cavity. Worldwide, oral cancer accounts for 2% - 4% of all cancer cases, tongue as a more common sub-type[1,2]. In some regions, the prevalence of oral cancer is higher, 45% in India & 10% in Pakistan [3,4].The field cancerization has been explained as in altered telomeres and unbalanced allelic loci seen in both tumors and surrounding histologically normal tissues at distances of least 1 cm from the visible tumor margins results in the occurrence of multiple primary cancers in the head and neck region and recurrence following complete excision of the original tumor[5,6]. Multiple factors rising OSCC which are genetic alterations, chronic exposure, micro environmental carcinogens [7], premalignant disease to oral cancer can occur with alteration seen in the stromal tissue[8]. Oral epithelial dysplasia also has a malignant transformation potential [1], mitochondrial membrane damage leads to apoptosis[9,10] and viruses like HPV (human papillomavirus) also plays an important role in oral cancer[11].Numerous risk factors associated with oscc are chronic inflammation, alcohol and tobacco consumption, betel quid chewing and viral infections [12]. It is known that tumour cells on course of development undergo molecular alterations in cellular molecules including DNA, RNA, and proteins which could be attributed to the inherent biological properties of the

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cancer cell[131415]. The survival rate has reduced due to its diagnosis in the advanced stage[16]. There is a need for molecular prognostic factors to detect patients with high risk of oral cancer recurrence and treatment failure. The upregulation genes were seen involved in extracellular matrix degradation and epithelial mesenchymal transition, downregulation seen in detoxification pathways[17,18]. Expression of multinucleated giant cells shows host immune response[19,20,15].

Latest reports from the International Agency for Research on Cancer (IARC) with ICD-10 code = coo-06 : lip, oral cavity in 2018 reports shows incidence around the world is 3.54,864 and in India it is 119,992. Prevalence of 9,13,514 reported around the world and In India it is around 2,65,225. Mortality rate reported around the world is 1,77, 384 and in India around 72,616 cases. The *RETN* gene belongs to the family of mouse resistin-like genes. Hormone released suppresses insulin ability which stimulates glucose uptake in adipose cells. Studies have shown links between obesity and diabetes. It also helps promote chemotaxis in myeloid cells - gene Cards[12,21]. Single nucleotide polymorphisms (SNP) is common DNA sequence variation which influences gene expression, protein production, function, disease susceptibility in high risk individuals[7,12,21,22]. Resistin levels of plasma correlates with the inflammatory markers and coronary artery calcification, and plays an important role in the measure of coronary atherosclerosis[22–24]. Kumar et al, studied genetic variations of *RETN* associated with greater risk with various diseases like metabolic syndrome and colon cancer[22,23]. A study reported that upregulation of resistin gene expression in human breast cancer tissues[25]. The present *in silico* study determines the selection of *RETN* SNPs and its possible association with oral squamous cell carcinoma.

Materials and Methods

One of the regulatory region variants of the *RETN (*gene was selected for the study based on the literature mining process. The minor allele frequency was found to be 0.08 (A allele). Since oral cancer is a disease that is more predominant in patients with a chronic history of using smoking and smokeless tobacco use such as pan, gutka etc., genetic variants which were more closely related to environmental and habitual factors were identified in the gene and selected. As most of these factors are known to affect the inflammatory pathways leading to disease a study carried out by Yang et al was selected for comparison. The authors declared that the AA homozygous state of *RETN* polymorphism *(rs3219175)* presented a high risk of advanced tumor size when compared to GG homozygous genotype. The Ensembl database was used to acquire the frequency data of the variant in different populations. The data presented by Yang et al., was selected as a reference population (Taiwanese), which was used for further comparisons. Gene expression analysis would provide clues on the putative role or *RETN* gene in HNSCC. Hence, gene expression profile for different grades of tumor was analysed using the UALCAN database in the HNSC dataset. Survival curve analysis based on the Kaplan-Meier method was also investigated for differential expression of RETN within the patient group.[26,27] .

Results and Discussion

The phenotype database from ensembl database for *rs3219175* polymorphism showed 92% of "G" and 8% of "A" allele polymorphism reported, represented in figure 1. Individual allele frequencies of different populations are represented in figure 2. Analysis of allele frequency among different population like African (G=87% , A=13%), East Asian (G=82% , A=18%), South Asian (G=98% , A=2%) and European (G=99% , A=1%) was compared with global population (G=92% , A=8%). Allele frequency which is observed in the East Asian population showed 82% of "G" allele and 18% of "A" allele within different sub-populations such as Chinese Dai in Xishuangbanna, China(CDX), Han Chinese in Beijing, China (CHB), Southern Han Chinese(CHS), Japanese in Tokyo, Japan(JPT), Kinh in Ho Chi Minh City, Vietnam (KHV). Allele frequency observation in South-Asians population showed 98% of "G" allele frequency and 2% of "A" allele frequency which is compared among the sub-population of BEB (Bengali from bangladesh), GIH (Gujarathi Indians from Houston), ITU (Indians Telugu from UK), PJL (Punjabi from Lahore), STU (Sri Lankan Tamil from UK) among which "A" allele in higher in BEB and "G" allele is high in STU population (data not shown). To derive a more accurate picture on the deviations observed for the polymorphism in different populations, we compared the allele frequency from the Yang et al., (Taiwanese

population) study of the data acquired from the Ensembl database. The allele frequency comparison was almost similar (Figure 3 and 4). But when comparing the frequency of alleles between the south Asian and the ancestral population which is the African population, we observed marked differences in the allele frequency. The "A" allele frequency was higher in African and east Asian population when compared to other groups. Furthermore, the A allele was negatively selected in other populations such as American, south Asian and European. A negative selection of the allele in the population may be indicative of the fact that it could be deleterious in some way, but the reason for such deviation has to be analysed to derive a strong conclusion.

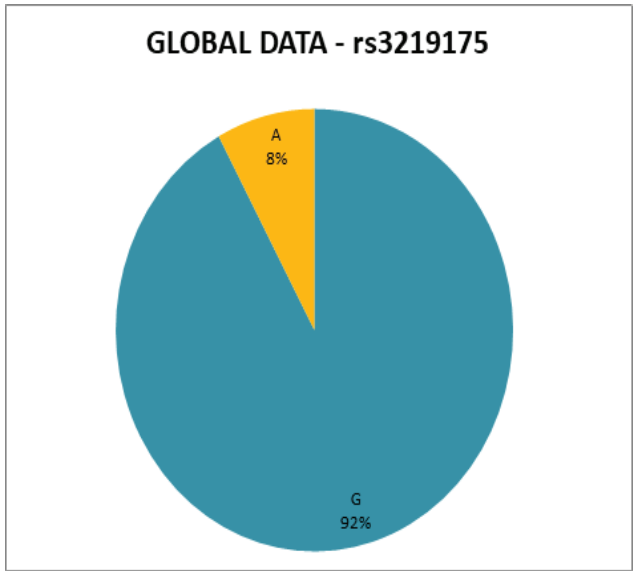


Figure 1: The global phenotype data from Ensembl database for *rs3219175* polymorphism (Yellow color denotes "A" allele frequency = 8%; Blue color denotes "G" allele frequency = 92%)

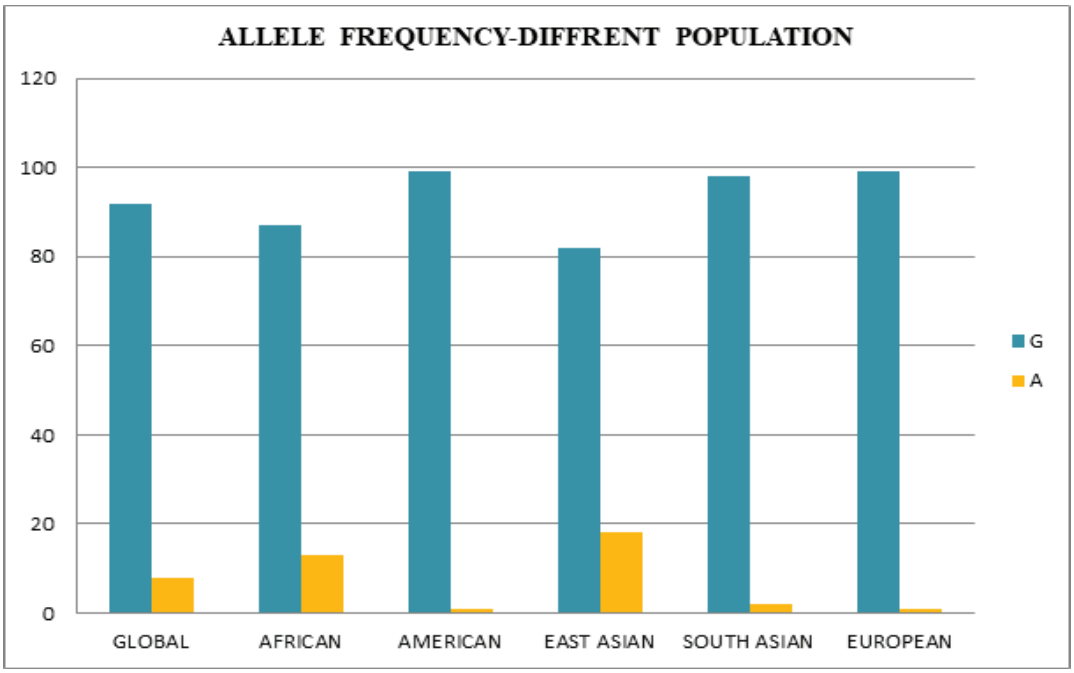


Figure 2: Analysis of allele frequency for *rs3219175* polymorphism among different populations. Yellow color denotes "A" allele frequency; Blue color denotes "G" allele frequency. [Global G - 92% and A- 8%; African - G-87% and A-13%; American -G-99% and A-1%; East Asian - G-82% and A-18%; European - G-99% and A-1%; South Asian - G-98% and A-2%].

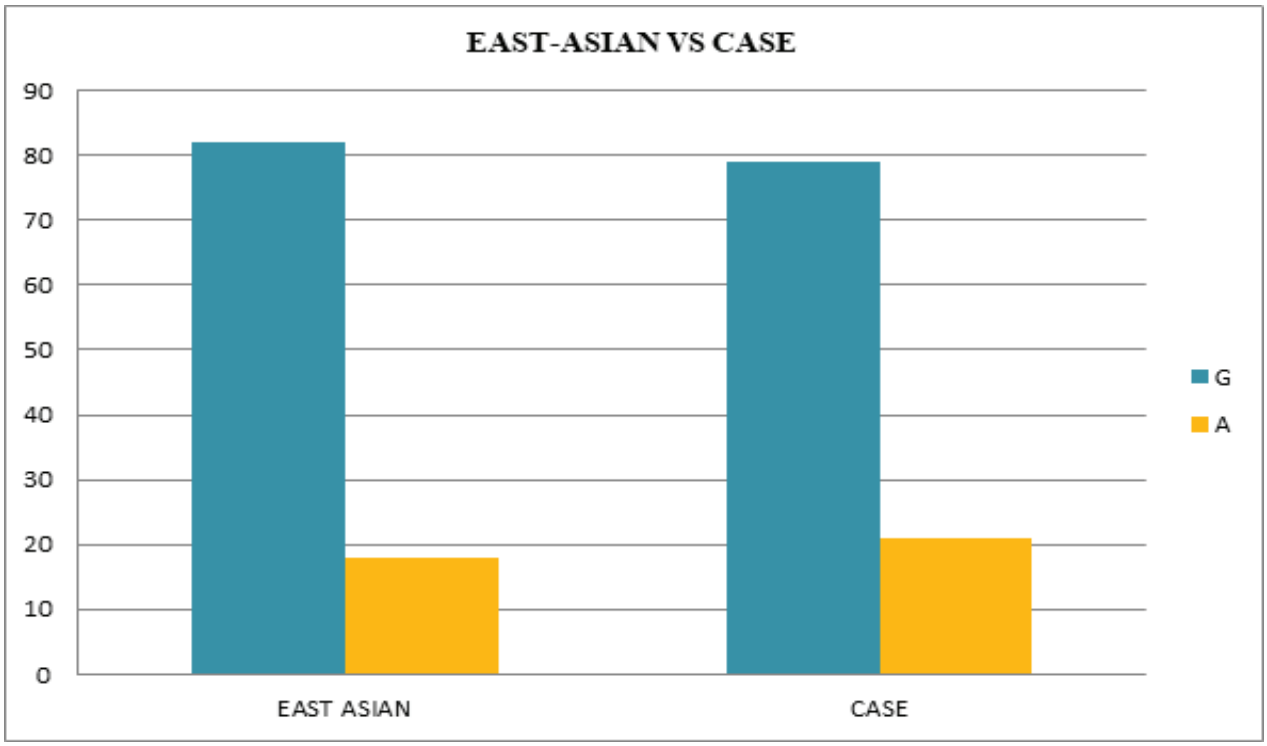


Figure 3: Comparison of *rs3219175* allele frequency case data from research study by Yang et al (G = 78% and A = 22%) with East Asian population (G = 82% and A = 12%). Yellow color denotes "A" allele frequency; Blue color denotes "G" allele frequency.

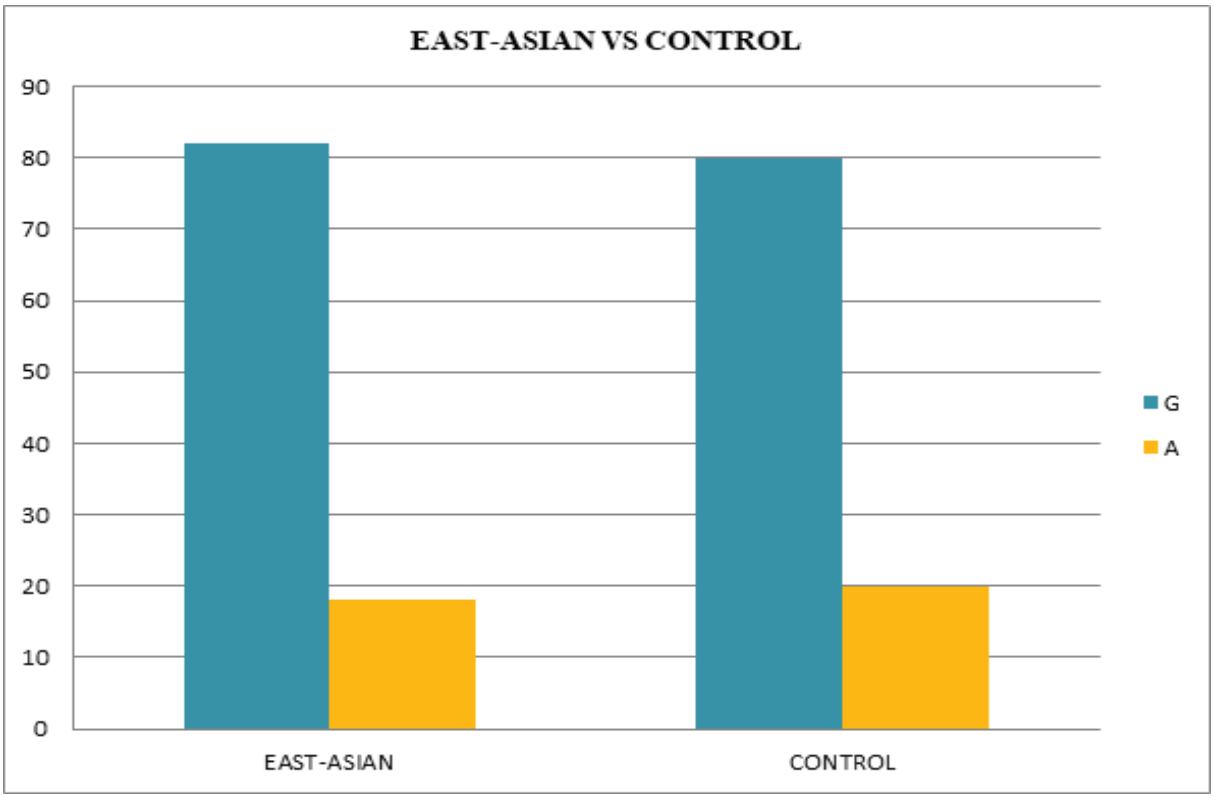


Figure 4: Comparison of *rs3219175* allele frequency of control data (G = 80% and A = 20%) from the research study by Yang et al with East Asian population (G = 82% and A = 12%). Yellow color denotes "A" allele frequency; Blue color denotes "G" allele frequency.

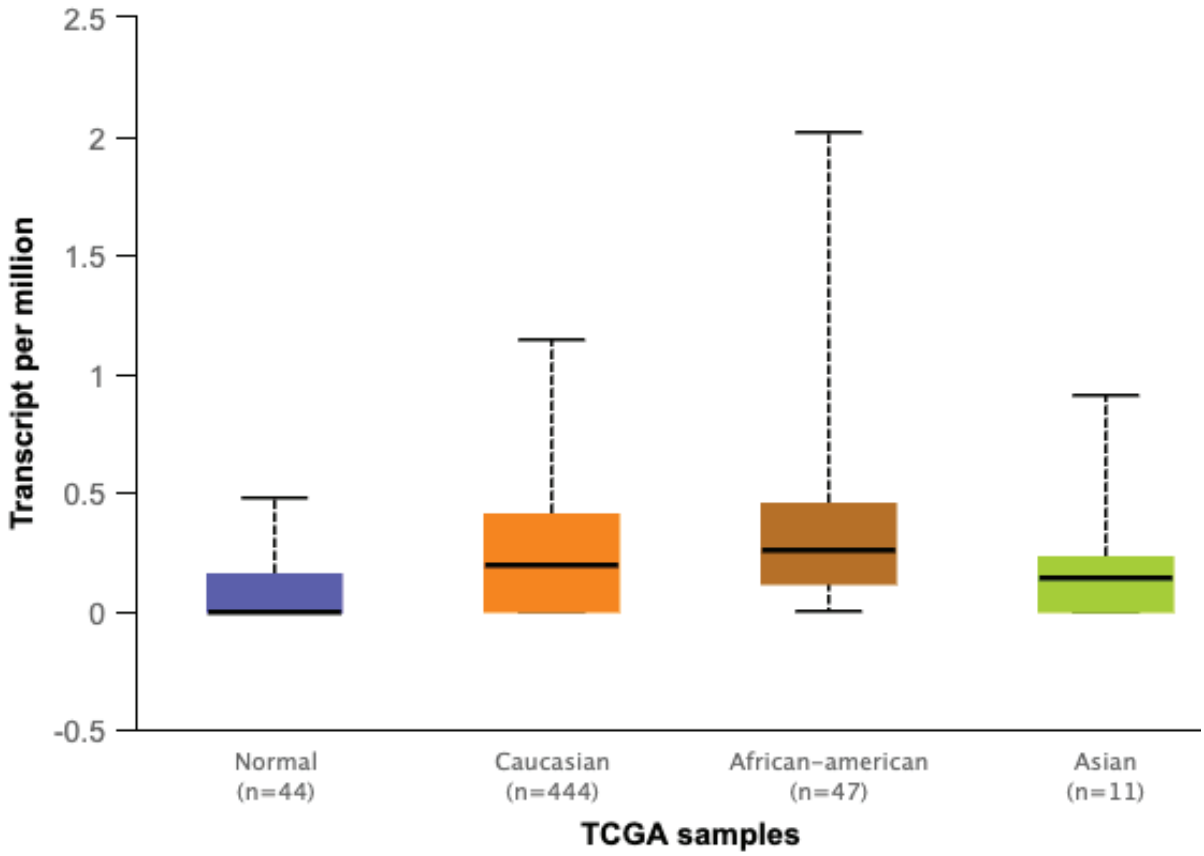
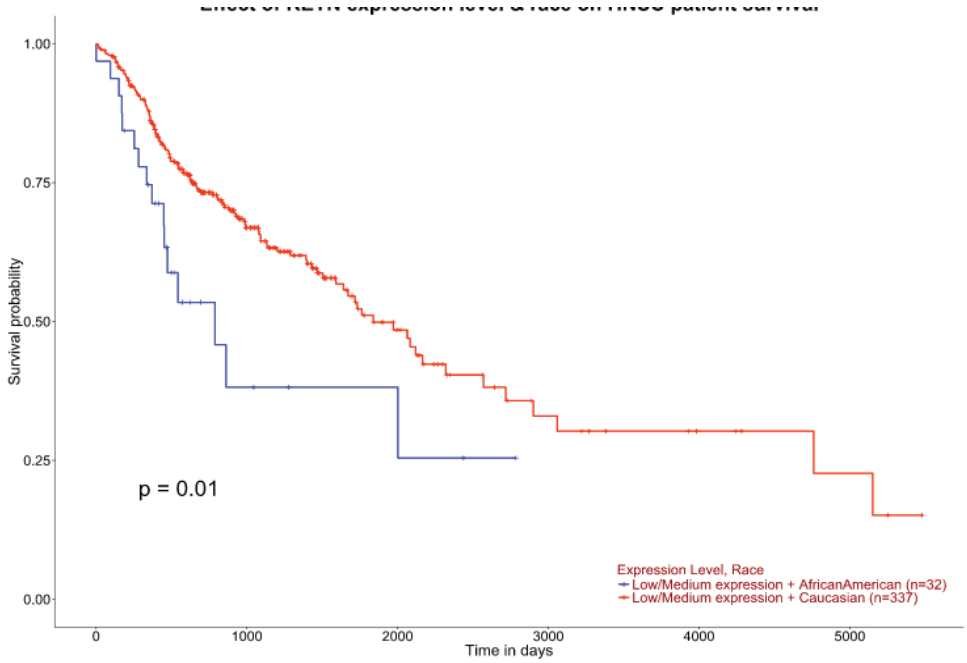
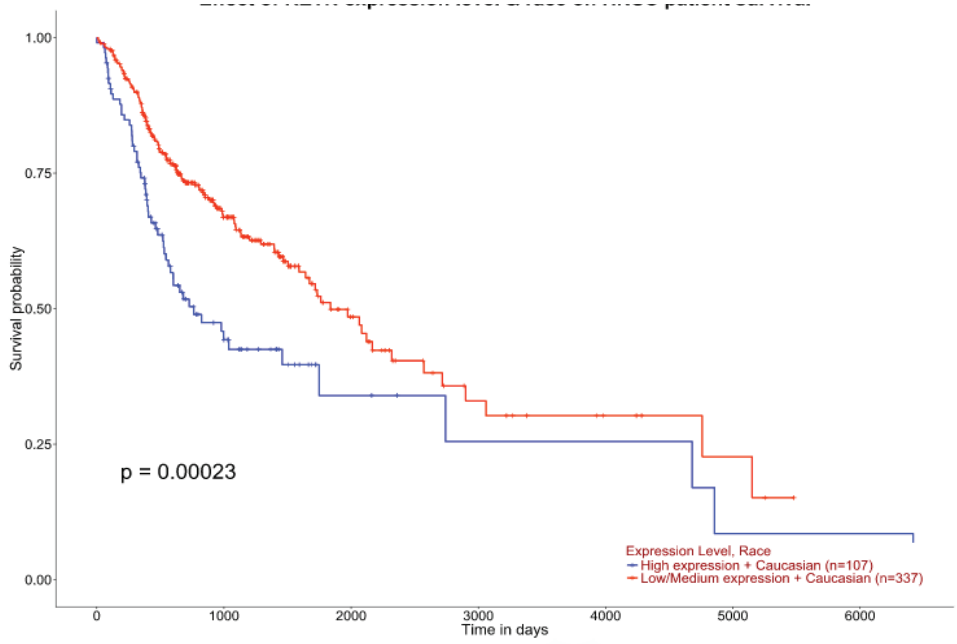


Figure 5: Expression of *RETN* in HNSC based on the patient's race. Blue color denotes normal population; Orange color denotes Caucasian population; brown color denotes African-American population; green color denotes Asian population. The comparison of gene expression between different races returned significant values between the group African-American vs Asian ($p=4.4 \times 10^{-02}$).



(a)



(b)

Figure 6: (a) Comparison of low/medium *RETN* gene expression between African American and Caucasian patients produced a significant p value of 0.01; (b) Comparison of high and low/medium *RETN* gene expression between Caucasian patients produced a significant p value of <0.01.

In addition, expression of the *RETN* gene found to differ significantly between the African American population and Asian population (Figure 5). When analysing the gene expression pattern between groups, low/medium level expression between African American and Caucasians returned a significant p value of 0.01, wherein low/medium gene expression in African Americans corroborated with poor survival of HNSC patients. Interestingly, we identified a significant difference between high and low/medium expression in Caucasian patients, where high level expression resulted in poor survival of these patients (Figure 6). These results provided preliminary evidence on the pivotal role of the *RETN* gene in the progression of HNSC. It is a known fact that nature tries to eliminate anything that is not in use[28,9]. Resistin is an adipokine that is associated with obesity, inflammation and various study cancer.[7] Resistin levels may play a role in pathogenesis of cancer cachexia[7,12]. Upregulation of serum Resistin has been detected in OSCC patients[7]. Online databases are an excellent source for identifying differential gene expression, which is a popular source for exploration of gene expression in diseases. The modern biomedical investigators can genetically profile diseases with this organised online database. There are various tools with basic designs available which are needed in experimenting *in silico* studies[7,29]. The "A" allele frequency is dramatically increased in the ancestral and east Asian population which creates an inquisitiveness to analyze the gene and the polymorphism to draw an association between the gene and the disease phenotype. The negative selection of "A" allele especially in the south Asian population is of our interest.

Conclusion

The present in silico study has provided preliminary data regarding the population wide-analysis of the *RETN* polymorphism selected. The deviation in allele frequency clearly indicates that the gene is evolving and is under constant process of natural selection. As a thumb rule, the alleles which are known to be deleterious are negatively selected, whereas a beneficial allele is positively selected in a population. The observation here denotes that the A allele could be a deleterious allele, and may confer risk of tumor progression in OSCC patients. Further experimental evidence are warranted to provide firm evidence on the association of *RETN* gene mutations or variations with that of OSCC.

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