RESEARCH ARTICLE

Editorial Process: Submission:10/24/2024 Acceptance:03/22/2025

Adenosine Triphosphate - Binding Cassette Transporter A1 gene polymorphism (rs1800977 and rs2230806) analysis in Oral Premalignant Disorder of South Indian Cohort

Usha Subbiah*, Kaniha Sivakumar

Abstract

Objective: To evaluate the association between the *ABCA1* upstream variant rs1800977 (G/A) and the missense variant rs2230806 (C/T) with oral premalignancies such as oral submucous fibrosis and leucoplakia. **Methods:** The rs1800977 and rs2230806 of *ABCA1* were analysed using PCR-RFLP in 350 subjects including OSMF, leukoplakia and healthy controls matched with age, sex, and chewing or smoking habitual factors. The allele frequency distribution and genotypic association for homozygous wild, heterozygous and variant for the diseased groups and healthy control were examined. The influence of rs1800977 and rs2230806 on mRNA secondary structure, gene-gene and protein-protein interactions was examined. **Results:** The prevalence of chewers and smokers was 67% and 58% in OSMF and leukoplakia respectively, compared to 48% among chewers in the control group. The rs1800977 variant AA had a genotype distribution of 20% for OSMF and 18% for leukoplakia, while the TT genotype of rs2230806 was 10% for OSMF and 17% for leukoplakia. The allele frequencies for both diseases were 0.35 and 0.36. The odds ratios (OR) for the heterozygous CT genotype were 2.45 (CI: 1.37-4.37, P=0.00) and 1.81 (CI:1.05-3.11, P=0.04), indicating significant associations. In silico tools indicated the variants of rs2230806 and rs2230806 had lower free energy compared to the wild type suggesting variants were more stable than the wild type on mRNA. **Conclusion:** The CT genotype of the missense SNP rs2230806 was associated with an increased risk for the disease and could serve as a valuable marker for assessing the prognosis of oral premalignancies.

Keywords: ATP binding cassette subfamily A member 1- Single nucleotide polymorphism

Asian Pac J Cancer Prev, **26 (3)**, 1009-1016

Introduction

Oral cancer is the most prevalent type of cancer in India, accounting for 8% of global cases and representing 30%–40% of all head and neck cancers [1]. Oral potentially malignant disorder (OPMD) is a condition impacting the oral mucosa that carries an elevated risk of oral cancer. The OPMD prevalence in India is increased by the use of smoking and chewing such as areca nuts, gutkha, pan masala, flavour-infused supari and different forms of tobacco consumption [2]. OSMF and leukoplakia are OPMDs that carry the risk of malignant transformation [3].The overall prevalence of OSMF is about 4.47% and leukoplakia is about 1.5 to 4.3% worldwide [4].Genetic, environmental, social, and behavioural factors are involved in the predisposition and development of some cancers. Strong evidence from molecular epidemiology supported the involvement of these factors in cancer [5]. The genetic makeup influences an individual's susceptibility to various types of cancer [6]. The likelihood of developing cancer is linked to polymorphisms in DNA repair genes and common single nucleotide polymorphisms (SNPs) [7]. Besides chromosomal aberrations, SNP also contributed to the risk of developing oral cancer [8]. SNP association studies for leukoplakia, erythroplakia and OSMF were related to lifestyle risk exposures such as tobacco, betel quid and alcohol [9, 10] . Hence the identification of SNP-based biomarkers as a prognostic model is essential for the early diagnosis of premalignancies. Adenosine triphosphate-binding cassette transporter A1 (*ABCA1*), a 220 kDa export protein belongs to the ATP-binding cassette (ABC) family. This gene is highly polymorphic, located on human chromosome 9 (9q31.1), that encode an integral membrane protein of 2,261 amino acids [11]. Its primary function is regulation of cholesterol efflux and phospholipids [12]. Studies reported that inflammation regulates ATP-binding cassette transporter A1 (*ABCA1*) as its expression is suppressed by proinflammatory cytokines [13] and involved in the biogenesis of high-density lipoprotein cholesterol, and its structural changes and

*Human Genetics Research Centre, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Pallikaranai, Chennai, Tamil Nadu, India. *For Correspondence: ushas@sbdch.bharathuniv.ac.in*

protein expression altered metabolic disturbances [14].

ABCA1 has a dual role in cancer and exerts anticancer effects by facilitating cholesterol efflux, decreasing cholesterol accumulation in cells, and influencing anticancer activities [15]. Epidemiological and experimental evidence suggested that *ABCA1* contributed to the progression of certain cancers. *ABCA1* expression in cancerous breast and prostate tissues was associated with cancer progression. It was identified as a cooperative response genes, with non-mutant genes synergistically downregulated by various cancer gene mutations during malignant cell transformation [14]. Mutations in ABC1 genes were responsible for 21 monogenic diseases, while polymorphism in these genes was linked to susceptibility to complex diseases. Additionally, ABC transporters were crucial in drug bioavailability and mediated multidrug resistance in cancer [16]. The genetic polymorphism of *ABCA1* was associated with susceptibility to Type 2 diabetes [17] and coronary heart disease [18]. However, no studies have investigated the susceptibility of *ABCA1* polymorphism rs1800977 upstream variant (G>A) and rs2230806 missense variant C>T) in oral premalignancy subjects. Hence the present study aimed to identify the genetic association of these two SNPs in OSMF and leukoplakia with habitual factors among the south Indian cohort.

Materials and Methods

Study subjects

The case-control study comprised 350 participants and was designed to achieve a power analysis of 80%. The subjects were categorized into three distinct groups: (i) Oral submucous fibrosis alone, (ii) Leukoplakia alone, and (iii) healthy control subjects who did not have a history of pre-malignant or malignant lesions. The study was approved by Sree Balaji Dental College & Hospital Ethical Committee (SBDCH/IEC/23-08/40, dated 5/10/2023) and written informed consent was collected from the participants. The study was conducted from November 2023 to July 2024. The study participants were age-matched between 30-65, including OSMF (125), Leukoplakia (125), and Healthy control (100). Inclusion criteria for the study group comprised of clinically and histopathologically proven OSMF and leukoplakia and control with age and sex-matched consenting healthy subjects with and without chewing or smoking habits. Exclusion criteria included cases with other systemic illnesses, haematological diseases, previous malignancies, pregnant women, previously treated or under the course of treatment of OSMF and leukoplakia, skin diseases, and autoimmune disorders were excluded from the study.

Genomic DNA Isolation

Genomic DNA was isolated from whole blood using a standard salting-out method. Initially, 1 ml of whole blood was mixed with 1.5 ml of RBC lysis buffer, vortexed for 20 seconds, and incubated at 37°C for 15 minutes with occasional inversion to ensure thorough mixing of the cells. The samples were centrifuged at 13,000 rpm for 10 minutes, and the supernatant was discarded. This process was repeated 2 to 3 times to remove excess red blood cells from the pellet. It is important to break down the pellet by vortexing with the RBC lysis buffer to ensure the removal of residual red blood cells from the white blood cells. Next, 500 µl of nucleic lysis buffer was added to the pellet and vortexed vigorously for 10 seconds. Then, 30 µl of 10% SDS was added, vortexed for another 10 seconds, and incubated at 37°C for 15 minutes, 110 µl of 7.5 M ammonium acetate was added, vortexed for 20 seconds, and centrifuged at 13,000 rpm for 5 minutes. The supernatant (approximately 800 µl) was transferred, and ice-cold isopropanol was added, followed by overnight incubation. The next day, the samples were centrifuged at 13,000 rpm for 15 minutes. The DNA pellet was washed with 300 µl of 70% ethanol, the pellet was air dried and 100 µl of nuclease-free water was added to dissolve the DNA. The quality of the DNA was assessed using 1% agarose gel electrophoresis, and its concentration was measured with a Quantus Fluorometer (Promega, USA).

Genotyping

Upstream variant rs1800977 (G/A) and Missense variant rs2230806 (C/T) of *ABCA1* were selected using the NCBI-SNP database. Oligonucleotide primers were designed using the Prime3 software tool. Amplification of *ABCA1* rs1800977 and rs2230806 gene was performed using ABI Veriti 96 well thermal cycler (Thermo Fisher Scientific, India). Reaction mixture included were DNA (100ng) 4 µl, Forward Primer: TCCTACCCCTTGACAAGCCT and Reverse Primer: CGTGCTTTCTGCTGAGTGAC, 1 µl each, Ampliqon Master Mix 10 µl. The optimized PCR condition as follows: Initial Denaturation 95℃/5 min, 35 cycles of Denaturation 94℃/30 sec, Annealing 55℃ /30 sec and Extension72℃/1 min, final extension of 72℃/5 min. The amplified PCR product of *ABCA1* (rs1800977) was visualized using 1% agarose gel electrophoresis.

Amplification of the *ABCA1* (rs2230806) was performed using ABI Veriti 96 thermocycler. Reaction mixture included were DNA (100ng) 2µl, Forward Primer: CCTCACATTCCGAAAGCATTA, Reverse Primer: AAAGACTTCAAGGACCCAGCTT, 1 µl each, Ampliqon Master mix 10 µl. The optimised PCR conditions were as follows: Initial Denaturation 95°C/5 min, 35 cycles of Denaturation 94°C/30 sec, Annealing 64°C /30 sec and Extension72°C/1 min, final extension of 72°C/5 min. The amplified PCR product of rs2230806 of *ABCA1* was visualized using 1% agarose gel electrophoresis.

Restriction Enzyme Digestion

To detect the *ABCA1* rs1800977 the PCR products (10 µl) were incubated with the restriction enzyme of BSMA1 (New England BioLabs) (3 Units) overnight at 37▫C. The digested products were electrophoresed on a 2.5% agarose gel stained with ethidium bromide. The genotypes were 333 bp representing the genotype fragments of 333 bp, 199 bp, and 134 bp.

Similarly, the PCR product (10 µl) of rs2230806 (*ABCA1*) was digested with the ECoN1 enzyme (New England BioLabs) overnight at 37°C for 4 hours followed

by heat shock at 65°C, 30mins. The digested products were electrophoresed on a 2.5% agarose gel stained with ethidium bromide. The genotypes were 309 bp representing the genotype fragments of 309 bp, 184 bp, and 125 bp. The primers and enzymes used for rs1800977 and rs2230806 (*ABCA1*) are listed in Table 1.

Statistical analysis

Epi Info software version 7.0 (Centre for Disease Control and Prevention, Atlanta, Georgia) was used to analyse the Odds ratios with a 95% confidence interval, and the chi-square tests were performed for SNP analysis. The Pearson chi-square goodness-of-fit test was used to analyse the differences in genotype and allele frequencies between cases and control of study subjects and whether the genotype prevalence distribution deviated from the Hardy-Weinberg equilibrium (HWE). The odds ratio with statistical significance was used to calculate the risk attributed to the genotype, with statistical significance $p < 0.05$.

Computational prediction of variants on mRNA

The effect of wild-type, upstream transcript variant rs1800977 and rs2230806 of *ABCA1* on the secondary structure of mRNA of *ABCA1* was determined by the mRNA structure prediction tool RNA-fold (http://rna.tbi.univie.ac.at/). The structural stability of the wild-type and variant mRNA was examined by comparing their minimum free energy (MFE) and secondary structures.

Gene-Gene interaction

Gene MANIA was utilized to examine gene-gene interactions, co-expression, co-localization, pathways, and protein domain similarities, to predict how the input genes interact with numerous other genes [19]

Protein-protein interaction

Protein-protein interactions (PPI) are crucial for understanding the functional relationships among proteins within a cell. The STRING database (version 11.0; https://string-db.org/) was used to obtain the PPI network information for the *ABCA1* protein. PPI network showed direct or indirect links between known proteins and other proteins [20].

Results

Demographic analysis of the study subjects

The prevalence of OSMF and leukoplakia with habitual factors such as chewing and smoking status were categorised in Table 2. Among individuals with OSMF, 67% were chewers, while 32% were non-chewers. For leukoplakia, 58% were chewers and 42% were non-chewers. In the healthy control group, 48% were chewers and 52% were non-chewers. This indicated that chewers were more prone to OSMF than leukoplakia and the percentage of healthy control chewers was lesser when compared to chewers of diseased condition. Conversely, the prevalence of non-chewers in the healthy control group was high (52%) when compared to those with diseased conditions. Regarding smoking, 63% of OSMF patients

Table 1. *ABCA1* SNP Genotyping and Restriction Digestions Details

ABCA1 Variants	Primer Sequence	Restriction enzyme	PCR product size	Restriction Fragments size
rs1800977 G/A	F:5'- TCCTACCCCTTGACAAGCCT -3' R: 5'- CGTGCTTTCTGCTGAGTGAC -3'	BSMA1	333 bp	333bp, 199bp,134bp
rs2230806 C/T	F:5'- CCTCACATTCCGAAAGCATTA -3'R:5'- AAAGACTTCAAGGACCCAGCTT-3'	ECoN1	309bp	309bp, 184bp,125bp

ABCA1, ATP-binding cassette transporter; A1, PCR-Polymerase Chain Reaction

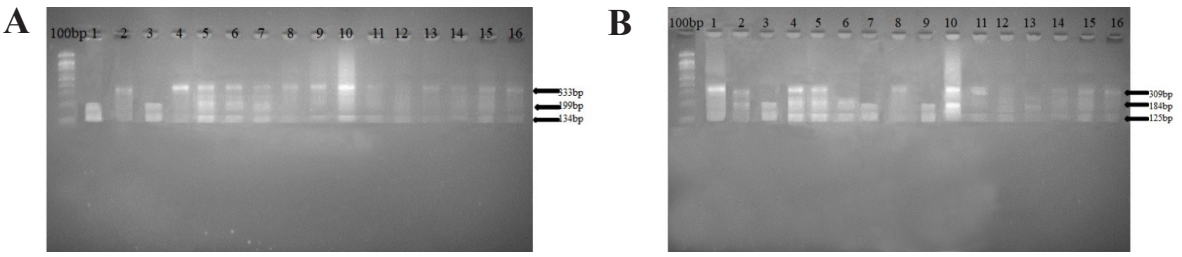

Figure 1. A.The PCR-RFLP results from *ABCA1* (rs1800977) polymorphism. B. The PCR-RFLP results from *ABCA1* (rs2230806) polymorphism.

Table 2. Habitual Factors of the Study Subjects

Factors	OSMF (N=125)	Leukoplakia (N=125)	Healthy control (N=100)
Chewer	(84) 67%	(72) 58%	(48) 48%
Non-Chewer	(41) 32%	(53) 42%	(52) 52%
Smoker	(79) 63%	(82) 66%	(67) 67%
Non-smoker	(46) 37%	(43) 34%	(33)33%

OSMF, Oral submucosis fibrosis.

were smokers, while 66% of leukoplakia patients were smokers. Non-smokers made up a smaller proportion in both conditions (37% for OSMF and 34% for leukoplakia. The difference in smoking status between healthy controls was minimal, with 67% smokers and 33% non-smokers.

The age distribution for homozygous wild-type, heterozygous, and variant alleles of SNPs rs1800977 and rs2230806 in OSMF, leukoplakia, and healthy controls, ranging from 30 to 65 years, is detailed in Tables 3 and 4. For the disease risk variant allele A/A of rs1800977, the average age distribution was 53.84±5.55 for OSMF, 53.5±8.05 for leukoplakia, and 52.5±8.29 for healthy controls. The age distribution showed only minor differences among the homozygous wild-type, heterozygous, and variant alleles in the study subjects. For the rs2230806 variant T/T, the average age was 56.46±7.22 for OSMF and 53.71±7.41 for leukoplakia. The prevalence of OSMF and leukoplakia in males was 81% and 79%, respectively, compared to 44% and 49% in females . This indicates a higher disease prevalence in males compared to females. In the healthy control group, males constituted 62% and females 38%, with the age ranging from 30 to 65 years.

Genotyping of ABCA1 gene

To investigate the risk association of diseased conditions, we analyzed the *ABCA1* upstream variant rs1800977 (G/A) and the missense variant rs2230806 (C/T). Representative RFLP images of these genetic variants are shown in Figures 1A and 1B. The restriction fragment for rs1800977 homozygous wild ancestral allele G/G at 199bp, 134 bp, and heterozygous carrier G/A at 333bp, 199bp and 134 bp, and diseased variant A/A at 333 bp. Meanwhile for rs2230806 homozygous wild C/C at

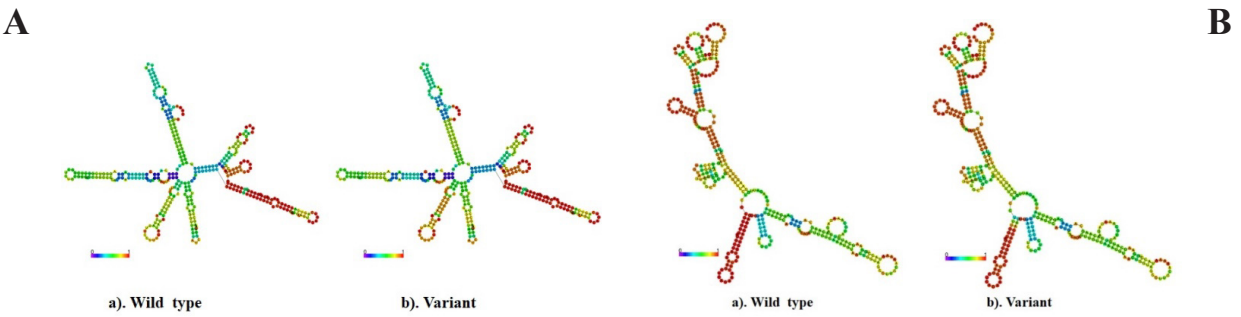

Figure 2. A. Impact of wild-type and variant rs1800977 on mRNA structure. B. Impact of wild-type and variant rs2230806 on mRNA structure

Table 3. Age Distribution of rs1800977alleles in OSMF, Leukoplakia and Control Groups

Age		OSMF				Control		
(30-65)		(N=125)				(N=100)		
rs1800977	G/G	G/A	A/A	p-value	G/G	G/A	A/A	p-value
Mean±SD	53.69±6.91	54.40±5.61	53.84±8.55	0.69	54.03±6.55	55.15±6.70	52.5±8.29	0.82
Age		Leukoplakia				Control		
(30-65)		(N=125)				(N=100)		
rs1800977	G/G	G/A	A/A	p-value	G/G	G/A	A/A	p-value
Mean±SD	52.41±8.04	55.72±6.19	53.5±8.05	0.05	54.03±6.55	55.15±6.70	52.5±8.29	0.82

OSMF, Oral submucosis fibrosis.

Table 4. Age Distribution about rs2230806 alleles in OSMF, Leukoplakia and Control

Age		OSMF				Control		
(30-65)		(N=125)				(N=100)		
rs2230806	C/C	C/T	T/T	p-value	C/C	C/T	T/T	p-value
Mean±SD	53.17±7.15	55.10±7.63	56.46±7.22	0.1	53.69±5.52	51.38±8.36	54.57±7.65	0.34
Age		OSMF				Control		
(30-65)		(N=125)				(N=100)		
rs2230806	C/C	C/T	T/T	p-value	C/C	C/T	T/T	p-value
Mean±SD	53.71±6.82	54.17±6.63	53.71±7.41	0.79	53.69±5.52	51.38±8.36	54.57±7.65	0.34

OSMF, Oral submucosis fibrosis.

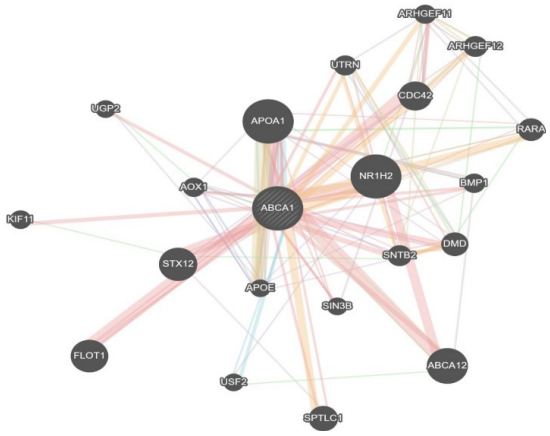

Figure 3. Gene-Gene Interaction Analysis of *ABCA1*

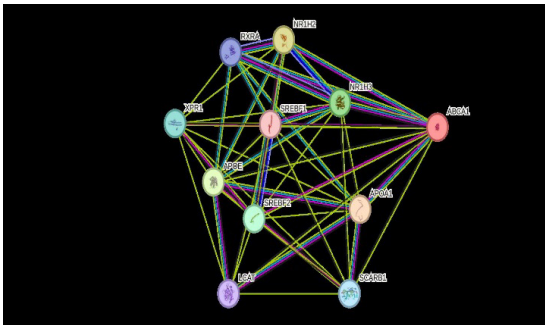

Figure 4. The Protein-Protein Interaction Network by *ABCA1* Protein Using STRING Software.

184bp and 125bp, heterozygous carrier C/T 309 bp¸184 bp and 125 bp and the diseased variant T/T for rs2230806 was at 309 bp.

Genetic association of ABCA1 rs1800977 and rs2230806 with the disease

The genotype distribution of the *ABCA1* SNP (rs1800977) in the study subjects was investigated (Table 5). The study observed that 63 patients (50%) with OSMF were GG (wild-type) genotype carriers, 56 (49%) with leukoplakia were GG carriers, and 54(54%) in the control group were GG carriers. GA carriers included 37(29.6%) with OSMF, 44(35%) with leukoplakia, and 32 (32%) in the control group. The variant AA (mutant) carriers were 25 (20%) in OSMF, 22 (18%) in leukoplakia, and 14 (14%) in the control group. The respective minor allele frequency distribution of the subjects was listed (Table 5).

The genotype distribution of the *ABCA1* SNP rs2230806 is shown in Supplementary Table 6. OSMF with CC (wild-type) genotype carriers were 47 patients (37%), 59 (47%) with leukoplakia were, and 55 (55%) in the control group were CC carriers. CT carriers included 65 (52%) with OSMF, 45 (36%) with leukoplakia, and 31 (31%) in the control group. TT (mutant) carriers were 13 (10%) in OSMF, 21 (17%) in leukoplakia, and 14 (14%) in the control group (Supplementary Table 6). The allelic frequency distribution for rs2230806 was 0.36 and 0.35 for the diseased variant groups compared to a control allele frequency distribution was 0.30.

The genetic association of *ABCA1* SNP rs1800977 was calculated using an odds ratio (OR>1) with a 95% confidence interval (CI) for OSMF, leukoplakia and healthy controls is tabulated in Supplementary Table 7. The diseased groups were compared with the control group, and both diseased groups OSMF vs leukoplakia were also compared. The homozygous wild genotype GG was a reference group. The OR(>1) with the CI(95%) was 0.99(0.54-1.79) with non-significant P=1for OSMF vs control and the diseased variant in OSMF vs control was

Table 5. Genotype and Alele Frequency Distribution of *ABCA1* G/A rs1800977 SNP in Study Subjects

Phenotype/Genotype	OSMF (n=125)	Leukoplakia (n=125)	HealthyControl (n=100)
Homozygous			
wild/GG	(63)50%	(56)49%	(54)54%
Heterozygous /GA	(37)29.6%	(44)35%	(32)32%
Variant/ AA	(25) 20%	(22)18%	(14)14%
Allele distribution	OSMF	Leukoplakia	Healthy Control
A	0.35	0.36	0.3
G	0.65	0.64	0.7

OSMF, Oral submucosis fibrosis.

1.5(0.72-3.23), P=0.35, 1.5(0.7-3.26), P= 0.38 and 1.01 (0.5-1.98), P=1.00 with an insignificant P value (>0.05) when compared with the diseased group with the control, and between the diseased group. This indicated that the variant allele A/A was not associated with the risk of both diseases as they were not satisfying OR and P-value when compared with the healthy individual and also between the diseased group of the South Indian cohort.

The genetic association for rs2230806 was analysed for the diseased groups and healthy controls were represented in Supplementary Table 8. The CC genotype was considered as the reference genotype. All 3 groups were compared as mentioned earlier. The results of our study showed that the OR> 1 with the CI for the heterozygous disease carrier genotype in OSMF vs control and both diseased groups (OSMF vs leukoplakia) was significant with 2.45(1.37-4.37), P=0 and 1.81(1.05-3.11), P= 0.04. Interestingly, heterozygous carrier genotype CT for the disease was significant with a p-value< 0.05 in the OSMF vs control group and between OSMF vs leukoplakia. but was not significant in leukoplakia vs control. This indicated that variant genotype TT was not associated with risk for both diseases but the heterozygous "CT" genotypes exhibited as disease carriers for OSMF and leukoplakia.

Impact of wild-type and variant on mRNA structure

The minimum free energy of the wild-type of rs1800977 was found to be -155.90 kcal/mol and the variant was found to be -156.90 kcal/mol. However, the free energy of the thermodynamic ensemble was found to be -162.19 kcal/mol for the wild-type and -163.13 kcal/mol for the variant rs1800977. The variant had lower free energy compared to the wild type indicating that the variant was more stable than the wild type (Figure 2A).

The minimum free energy of the wild type of rs2230806 was found to be -90.10 kcal/mol and the variant was found to be -88.90 kcal/mol. However, the free energy of the thermodynamic ensemble for the wild type was -98.03 kcal/mol and the free energy of the thermodynamic ensemble for the variant rs2230806 was -96.40 kcal/mol. The variant had lower free energy compared to the wild type indicating that the variant was more stable than the wild type Figure 2B.

Gene-gene interaction

The functional interaction network of gene *ABCA1* was constructed using GeneMANIA. The physical contact was 77.64% and the genes involved were ATP Binding Cassette Subfamily A Member 12 (*ABCA1*2), Serine Palmiyoyltransferase Long Chain Subunit 1 (SPTLC1), SIN3 Transcription Regulator Family Mmeber B (SIN3B), Apolipoprotein E (APOE), Syntaxin 12 (STX12), Flotillin 1 (FLOT1), Kinesin Family Member 11 (KIF11), Aldehyde Oxidase 1 (AOX1), UPT-Glucose Pyrophosphorlase 2 (UGP2), Apolipoprotein 1 (APOA1), Nuclear Receptor Subfamily 1 Group H Member 2 (NR1H2), Syntrophin Beta 2 (SNTB2),Dystrophin (DMD), Bone Morphogenic Protein 1 (BMP1), Cell Division Cycle 42 (CDC42), Utrophin (UTRN). The other related details were 8.01% co-expression, 3.63% co-localization, 2.87% genetic interaction, 0.60% shared domains, 1.88% pathway and 5.37% predicted genes were shown in Figure 3.

Protein-protein interaction

The STRING server result revealed that *ABCA1* protein interacts with ten proteins including Apolipoprotein A1(APOA1), Scavenger Receptor Class B Member 1 (SCARB1), Lecithin- Cholesterol Acyltransferase (LCAT), Sterol Regulatory Element Binding Transcription Factor 2 (SREBF2), Apolipoprotein E (APOE), Sterol Regulatory Element Binding Transcription Factor 1 (SREBF1), Xenotropic and Polytropic Retrovirus Receptor 1 (XPR1), Retinoid X Receptor Alpha (RXRA), Nuclear Receptor Subfamily 1 Group H Member 3 (NR1H3), Nuclear Receptor Subfamily 1 Group H Member 2 (NR1H2) proteins had direct interaction which was illustrated in Figure 4.

Discussion

From our investigation, the prevalence of OSMF and leukoplakia was above 58% in south Indian subjects who had chewing or smoking habits. The habitual factors were not subcategorised as they were unaware of the chewing substances including areca nut or betel quid with or without tobacco and subtypes of tobacco smoke. Hence the study participants' habitual factors were generally categorised as chewers and smokers, and from the disease prevalence analysis, males were at higher risk for the disease than females when compared to control, which indicated that males had high disease prevalence because of chewing and smoking habits as reported [21] . Venka et al. reported that 80% of patients with oral leukoplakia consumed tobacco in various forms, including pan and chewing tobacco, with higher rates in males (94.5%) compared to females (53.3%) in Chengalpattu, Tamil Nadu [22].

ABCA1 polymorphisms were associated with ulcerative colitis and linked to colorectal carcinoma, highlighting the role of ABC transporters in promoting tumor-related inflammation [23]. Our analysis found that the *ABCA1* rs1800977 polymorphism was not significantly associated with OSMF or leukoplakia in a South Indian cohort. Furthermore, another study indicated no significant relationship between genotype and lipid concentration [11]. The T allele of the rs1800977 polymorphism was identified as a protective factor against T2DM (24) as it was associated with a reduced risk of T2DM in a Chinese Han population, despite no significant link with lipid levels. The loss-of-function mutation in *ABCA1* impaired insulin secretion. Additionally, the T allele of the rs1800977 polymorphism was associated with higher HDL cholesterol levels. Recent studies investigated the relationship between the *ABCA1* rs1800977 polymorphism and T2DM risk and the T allele of the rs1800977 polymorphism was linked to a reduced risk of T2DM among Turkish patients, while increasing the risk among Malaysians. However, clinical and biochemical characteristics related to this polymorphism did not show significant differences

between case and control groups and reported that this SNP increased the risk of T2DM among Malaysians. The clinical and biochemical characteristics of this polymorphism did not reveal any differences between the case and control groups [24]. From the in silico analysis, the *ABCA1* SNP rs1800977 variant was found to be more stable than the wild type. The *ABCA1* gene interacted physically with 16 other genes, including key components of the ATP binding cascade such as *ABCA12, SPTLC1, SIN3B, APOE, STX12, FLOT1, KIF11, UGP2. SPTLC1* and *ABCA1* physically interact with each other. It also interacted with genes related to neuronal cell functions (CDC42), regulated muscle function (DMD), cardiac physiological hypertrophy (UTRN), and contributes to metabolic disorders (SNTB2). Additionally *ABCA1* protein- protein analysis revealed that Apo lipoprotein E helps transport cholesterol and other fats in blood and brain [25] the SCARB1 gene plays a crucial role in the reverse cholesterol transport pathway, and the LCAT gene responsible for cholesterol metabolism [26]. It is widely recognized that this gene plays a crucial role in the transport of cholesterol and phospholipids across cell membranes.

Tang, F. reported that the *ABCA1* gene variant was linked to variations in cardio-metabolic traits among patients with gestational diabetes. This variation included obesity, reduced insulin secretion, elevated blood glucose levels, and abnormal lipid parameters [27, 28]. Additionally, inhibition of *ABCA1* affected the invasion capabilities of melanoma cells by altering focal adhesion dynamics [29]. Specifically, loss of *ABCA1* function reduced cellular motility by disrupting the formation of active focal adhesions through interference with phosphorylated focal adhesion kinases and integrin β3 clustering. Furthermore, *ABCA1* activity influenced the plasma membrane melanoma cells and increased cholesterol content disrupted the cellular organization and inhibited focal adhesion of the cell [29]. However, no studies have yet linked this SNP to cancer, and our research found that the heterozygous carrier of rs2230806 showed a significant association with oral premalignancies such as oral submucous fibrosis (OSMF) and leukoplakia. Our study concluded that *ABCA1* genotype rs2230806 was associated with an increased risk of OSMF and leucoplakia with the habit of chewers and smokers of the south Indian subjects. The predisposition of oral premalignancies, with special reference to OSMF and leucoplakia, was explored and these functional rs2230806variants could serve as a potential prognostic marker for oral cancer risk during clinical treatment regimen. Further studies with larger and more diverse sample cohorts could provide additional evidence on the association of this SNP with premalignancy disorders.

Author Contribution Statement

All authors contributed to the study conception and design. Material preparation, collection and analysis were performed by Usha Subbiah. Draft of the manuscript was written by Usha Subbiah. Conceptualization: Kaniha Sivakumar; Methodology: Kaniha Sivakumar; Writing - Original Draft, Review & Editing, Investigation : Usha Subbiah; Supervision : Usha Subbiah. All authors read and approved the final manuscript.

Acknowledgements

Authors wish to thank The DST-FIST (Ref. No.SR/FST/College-23/2017), Government of India, New Delhi, India, for funding research equipment facilities to Sree Balaji Dental College and Hospital, Pallikaranai, Chennai, Tamil Nadu.

Ethical Declaration

The study was supported by (BIHER/RDF2022/1010, dated 27/05/2022), Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

Availability of data and materials

All data analysed during this study are included in this article.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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