Polymorphism of IL-17A Related with Otitis Media

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Abstract
Background: Otitis media (OM) is characterized as an infective and inflammatory state affecting the mucosa of the middle ear. Suppurative OM occurs when there is inflammation accompanied by discharge and perforation of the tympanic membrane. Methods: We genotyped 60 patients with otitis media, specimens taken from 30 patients, and 30 healthy people . Filling an EDTA tube with 2mL of blood, for the purpose of a molecular study for IL-17A. Blood specimens were saved under -20°C in frozen stat until uses. Results: A current study, highlight on the frequency of risk allele A in both heterozygous (GA) and Homozygous (AA) in SNP rs2275913 based on the Odd Ratio. The results shown lower in genotype GA was OR= 0.57(0.18 - 1.72) with p value 0.3, and the allele frequency was lower in with A allele. Conclusion: The Otitis media may be not correlated with polymorphism of IL17A but, this think was impossible based on the data of previous researches , which emphasized the pioneer of IL17A in Otitis media infection.
Keywords: Interleukin-17A, otitis media

Introduction

Otitis media (OM) is a condition indicated by inflammation and infection of the middle ear, specifically the mucosa of the middle ear. Suppurative OM occurs when this inflammation is accompanied by a perforation in the tympanic membrane and discharges. It can be either acute or chronic. This common childhood infectious disease can cause various long-term complications, which include sensorineural and conductive hearing loss (1).
The most frequent complication of otitis media is the rupture of the eardrum, which occurs due to increased pressure caused by the accumulation of fluid in the middle ear. Symptoms experienced by patients may include ear pain, hearing loss, discharge from the ear, ringing in the ears, or dizziness (2).
IL-17, an inflammatory protein, plays a role in controlling multiple cytokines and chemokines that attract neutrophils to areas of inflammation. IL-17A has been shown to have a defensive effect against various microorganisms, particularly bacteria and fungi that exist outside of cells, in the context of infection. In the case of otitis media with effusion, IL-17 acts as an inflammatory protein (3,4).
Among the IL-17 family members, IL-17A has a strong association with human health and disease. Cells involved in blood cell formation, including Th17 cells, Tc17 cytotoxic T cells, γδ T cells, natural killer cells, ILC3 innate lymphoid cells, and "natural" Th17 cells, are responsible for producing IL-17A (5,6).

Materials and Methods

Samples Collection: Sixty samples of blood were collected, comprising 30 samples from patients and 30 samples from healthy individuals. A total of three milliliters of blood was collected and directly injected into an EDTA tube. For transportation from the hospital to the laboratory, a cold box was utilized, ensuring that the samples were preserved at a temperature of -20°C in a deep freeze. These samples were intended for use in molecular research. According to the manufacture's instruction, The DNA was extracted and purified using the FAVORGEN kit. The primer for IL- 17A is F:AATCAAGGTACATGACACCAG and R:TTAGCCCCAATATAGCTATCTT with product size 648 bp. The PCR conditions for this primer is Pre denaturation 95°C for 5 min and 30 cycles with following steps (Denaturation 95°C for 30 sec, Annealing 56°C for 60 sec and Extension 72°C for 60 sec) finally Final extension 72°C for 5 min. Afterwards, the amplified DNA fragments obtained through PCR were identified using agarose gel electrophoresis, and the visualization of these fragments was achieved by staining with Ethidium bromide. The molecular size marker DNA (100-bp DNA ladder) was used to determine the size of the PCR products.
Detection by Sequencing Analysis: The 60 PCR products (30 patients and 30 control) of sequencing primers IL-17A were directly sequenced by Macrogen Company (Korea). The PCR product sequencing outcomes from different samples were altered, aligned, and checked next to the corresponding sequences in the reference database using BioEdit, a software for editing sequences (version 7.1, DNASTAR, Madison, WI, USA). The differences found in each sequenced sample were assigned numbers in the PCR amplicons and their positions in the reference genome.
Statistical analysis: The statistical analysis was done using SPSS (version 22). The numerical results were presented as the average and standard deviation, while the qualitative data was expressed as frequency and percentage. To compare two groups, an independent-sample t-test was used, and to determine any relationship between ordinal and nominal variables in this study, a chi-square test was used. A P-value of ≤ 0.05 was considered statistically significant.

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Results

The polymorphism of IL-17A gene was done for 60 samples of human blood DNA (30 patients, and 30 healthy people). Site of targeted partial sequence covering the SNP rs2275913 was occurred on Chromosome 6. Figure (1) shows the validity of SNP rs rs2275913. The results shown success the primer pair efficiency to amplification region 52186008 & 52186691as target DNA region of IL17A included SNP :rs2275913 G>A.

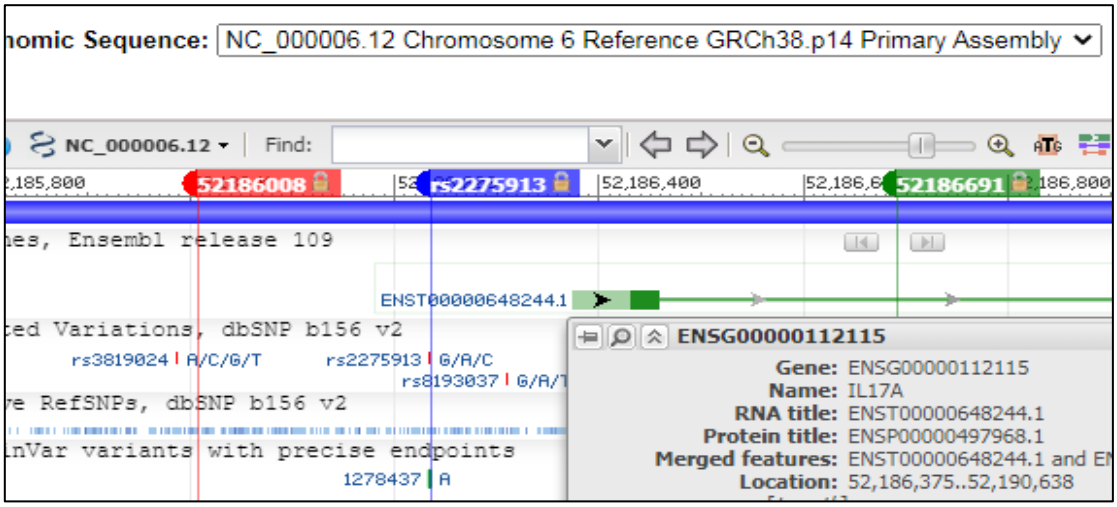

Figure (1): The targeted region of partial sequence of IL17A amplified by primer pair F1 covering the SNP rs2275913.

The primer pair for the gene of IL-17A was successfully amplified the target under amplification. The PCR products of IL-17A gene shown 648 bp. Figure (2) for patients and Figure (3) for control showed agarose gel electrophoresis of PCR products of IL-17A pair primer.

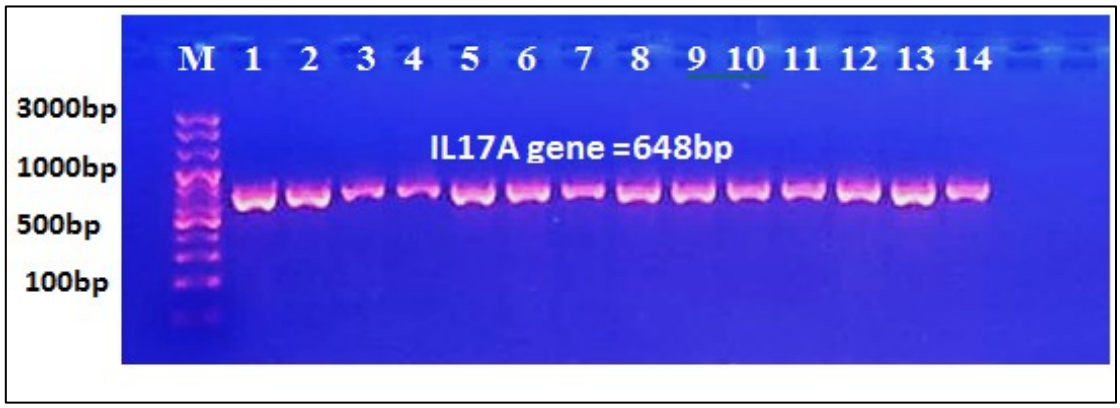

Figure (2): Gel electrophoresis Profile of PCR products of target of IL10 shown 648bp for 20 PCR product of IL17A of Otitis media. 1-14 patient samples, M=molecular marker first step100bp.

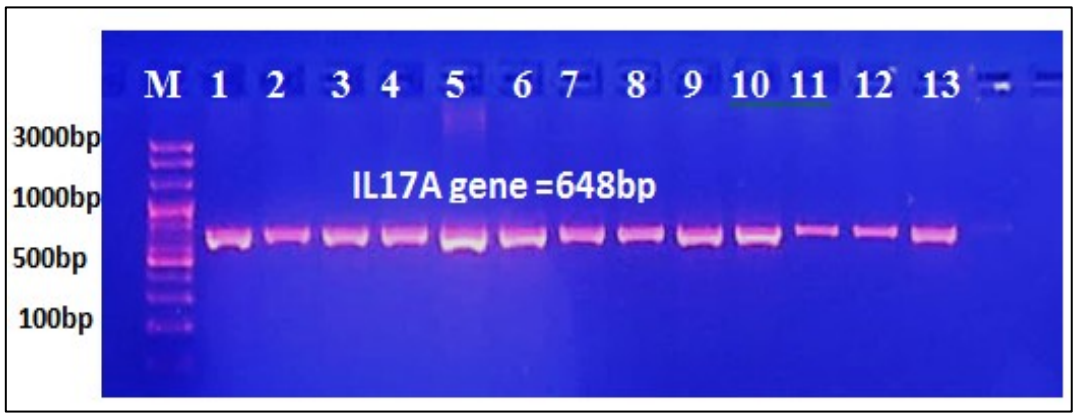

Figure (3): Gel electrophoresis Profile of PCR products of target of IL10 shown 648bp for 20 PCR product of IL17A of Otitis media. 1-13 control samples, M=molecular marker first step100bp.

The result shown in Figure (4) represent amplification the targeted region of partial sequence of IL17A amplified by primer pair F1 and R1 covering the SNP rs2275913. While Figure (5) shows the amplicone sequence region with flanking primers, it also shows the location of SNP of IL17A under study.

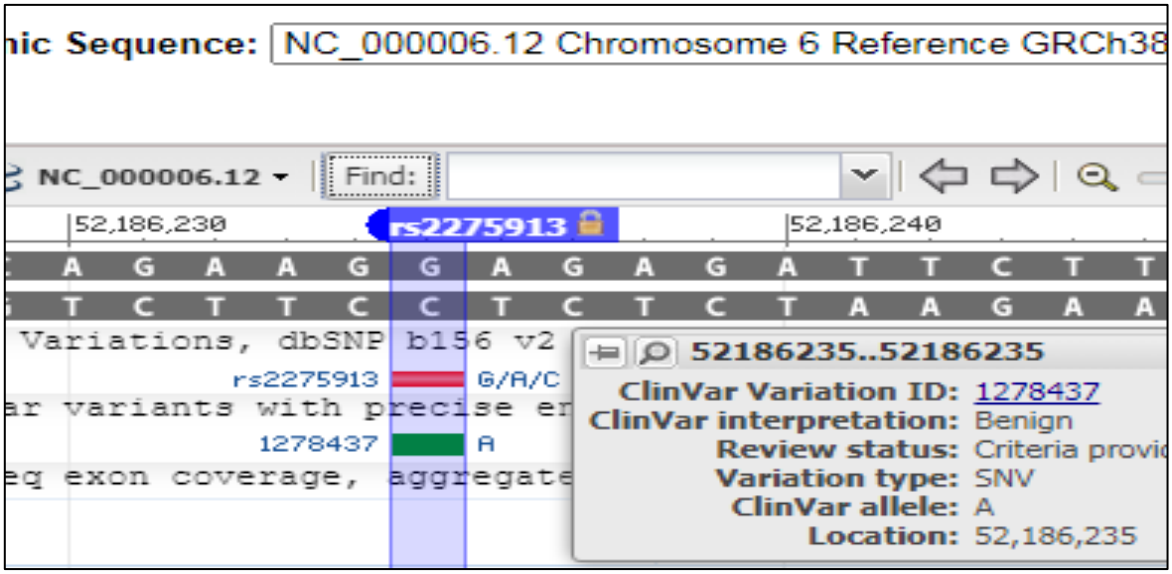

Figure (4): Amplification of targeted SNP of IL17A.

Figure (5): Amplicone sequence region with flanking primers

When comparing the chromatograms of SNP rs2275913, based on the facilities of Snap gene software, the wild type was shown black single peak which termed as homozygous wild allele GG, while the heterozygous mutant allele which shown overlapped two peak black and green on to indicated converted wild allele G to A allele, the substitution mutant allele A stead of wild allele G and shown one green peak termed by homozygous mutant allele AA (Figure 6).

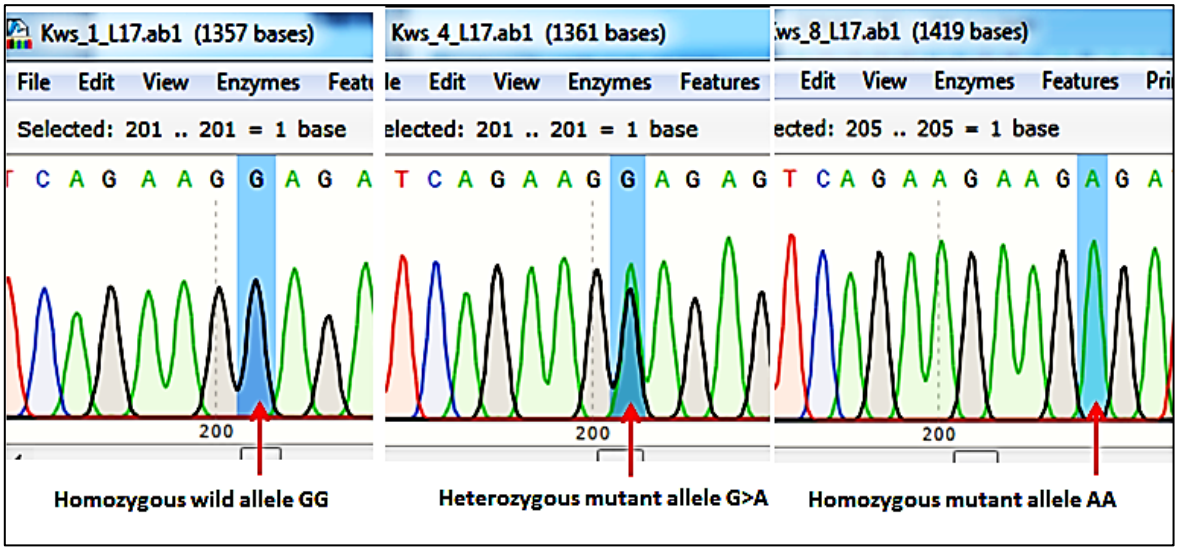

Figure (6): The site of Heterozygous allele GA of SNP rs2275913 G>A on IL17A was located on 52186236 chromosome 6, the allele G mutant to allele A, in combination images of NCBI IL17A partial sequence site, Multiple alignment sequence and chromatogram.

The SNP rs2275913 allele and genotype frequencies showed no critical varieties between OTS media patients and control. The subsequent SNP (rs2275913) was seen to have three genotypes (GG, GA, and AA) in OT patients, and control were noticed. These genotypes were identified with two alleles; G and A. A combination SNP site on chromosome and sites on patient sequence were performed to illustrated SNP validity. The results of multiple alignment of chromatograms of patients with Otitis media in Figure (7), while of control is in Figure (8).

The genotype GA and AA were shown low distribution in patients undergo Otitis media and control group for each, the values of Odd Ratio(OR) were support that A allele in both GA and AA was not far correlated with disease under interest and considered as risk allele. The Odd Ratio was lower in genotype AA OR= 1(0.13-7.6) with no significance p value 1, and the allele frequency was lower in with A allele 11(18.3%) in patient group with low value of OR=0.67(0.28-1.6), p. value = 0.3, while the allele frequency 15(25%) in control group, This SNP shown low frequent and not been Otitis media disease to be correlated risk allele (Table 1).

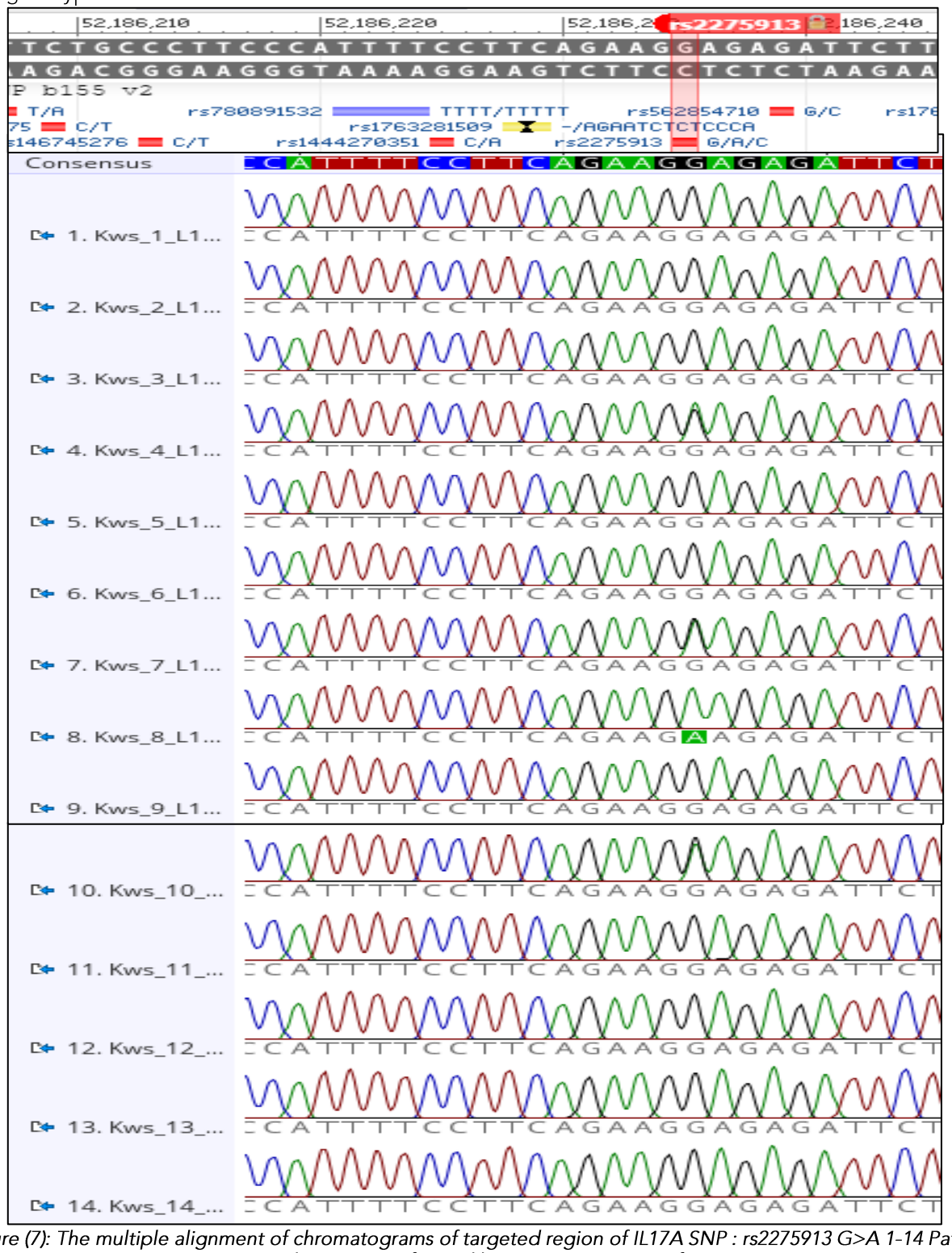

Figure (7): The multiple alignment of chromatograms of targeted region of IL17A SNP : rs2275913 G>A 1-14 Patient group. alignment performed by Geneious prime software.

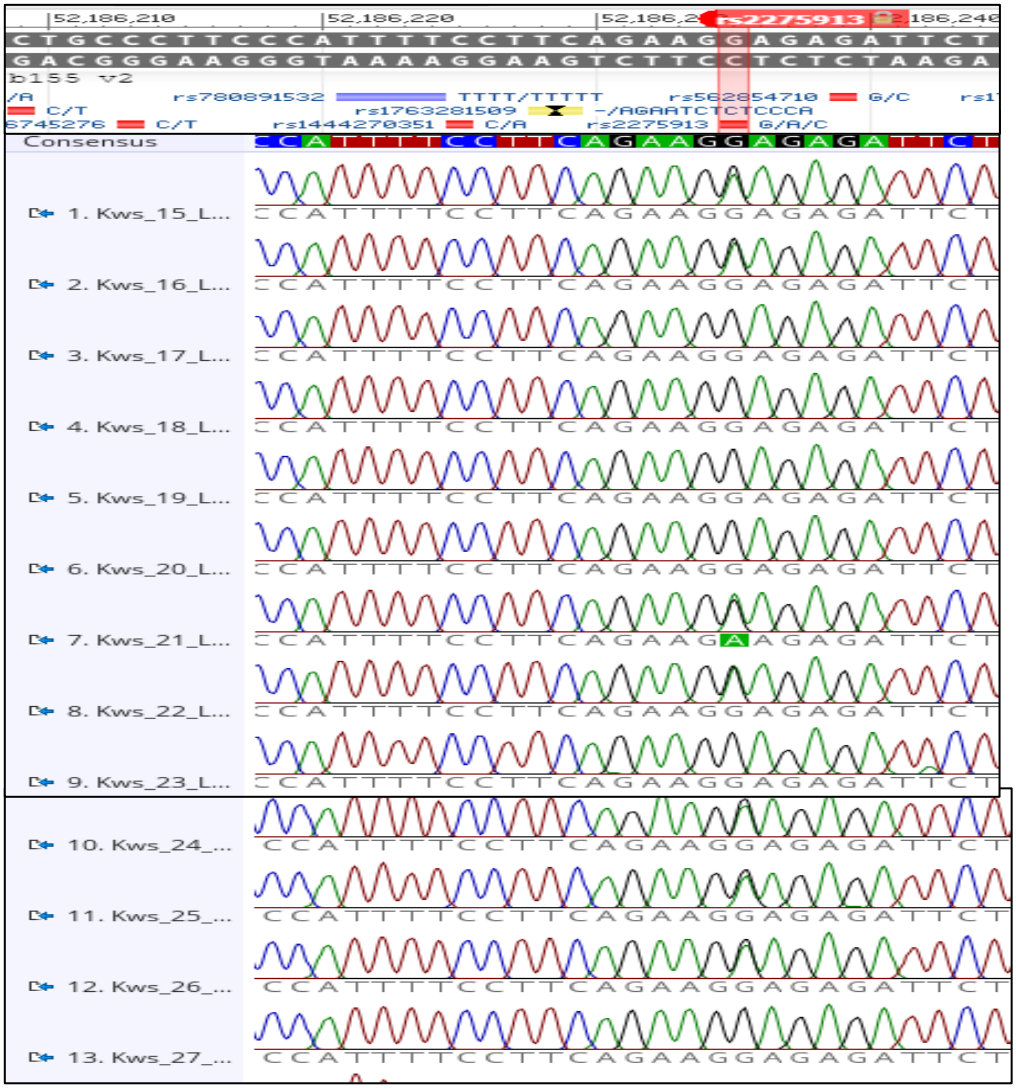

Figure (8): The multiple alignment of chromatograms of targeted region of IL17A SNP : rs2275913 G>A 1-13 control group. alignment performed by Geneious prime software.

Table (1): Genotypes distribution and allele frequency of wild type allele and mutant allele of SNP: rs2275913 G>A, OR values and p values for Otitis media infection in patients and control groups.

rs2275913 G>A		Case N=30	Control N=30	OR (95%CI)	P-value
genotypes	GG	21 (70%)	17 (56.7%)	Reference group	
	GA	7 (23.3%)	11 (36.7%)	0.57(0.18 - 1.72)	0.3
	AA	2 (6.7%)	2 (6.7%)	1 (0.13 - 7.6)	1
Allele % Frequency	G	49(81.7%)	45(75%)	1.48(0.61 - 3.51)	0.3
	A	11(18.3%)	15(25%)	0.67 (0.28-1.6)	0.3

Discussion

The potential modifiers of an individual's susceptibility to decreased ear function are genes that play a role in the inflammatory process. One important cytokine involved in the inflammation of human organs is IL17A. These candidate genes, which interact in a complex manner, have the potential to modulate the risk of inflammatory disease.

This study highlights of Otitis media as important disease and attempt to evaluate the role of IL17A polymorphism and Otitis media disease. The disease of Otitis media infection was one of the common disease, and the severe disease that affects immunocompromised patients is caused by significant pathogens. The ensuing disease's pathology and immune responses have not been adequately described by microbial infections. A current study, highlight on the frequency of risk allele A in both heterozygous (GA) and Homozygous (AA) in SNP rs2275913 based on the Odd Ratio. The results shown lower in genotype GA was OR= 0.57(0.18 - 1.72) with p value 0.3, and the allele frequency was lower in with A allele Table (1).

Many pathogenic and opportunistic microbes were isolated from patients undergo ear infection and considered one of the most impact infections, the isolated bacterial and fungal species were consistent with results of Getaneh et al., (2021). Getaneh and his team isolated and identified Staphylococcus aureus (27.9%), Proteus spp. (20.8%), Streptococcus spp. (10%), and Pseudomonas spp. (8.92%) were the main isolates. More than 45% of isolates, with 50.9% of Gram-negative and 37.3% of Gram-positive, were multidrug-resistant. (7).

IL-17 has been found to play a crucial role in the development of OVA-induced OME in rats, as discovered by Zhang et al. (2022). Inflammatory responses induced by IL-17 through the Notch signaling pathway contribute to the condition. Targeting IL-17 may prove to be an effective therapeutic approach for OME (8).

Fungal diseases have emerged as a significant cause of morbidity and mortality, as highlighted by Szmuilowicz and Young in 2019. This emphasizes the need for more effective antifungal treatments. IL-17 and Th17 cells are crucial for providing broad immunity against extracellular microorganisms, particularly in protecting against superficial candidiasis caused by Candida albicans (2).

IL-17A, a member of the IL-17 receptor family, is a potent inflammatory cytokine that is produced by activated T cells. This cytokine plays a key role in orchestrating immune responses and modulating various physiological processes. When IL-17A is released at the site of infection, it triggers a cascade of events that lead to the production of multiple inflammatory molecules, chemicals, antimicrobial peptides, and proteins. These molecules work together to mount a robust defense against invading pathogens and promote tissue repair (9).

The impact of IL-17A on host defense and immune regulation cannot be overstated. Its ability to stimulate the production of antimicrobial peptides and proteins helps to bolster the body's natural defenses against infections. Moreover, IL-17A is involved in maintaining the integrity of barriers in the skin and intestine, thereby preventing the entry of harmful microorganisms into the body (9).

In addition to IL-17A, another member of the IL-17 family, IL-17C, also plays a crucial role in immune defense. Like IL-17A, IL-17C promotes protective antimicrobial responses and helps to maintain the integrity of skin and intestinal barriers (10). Recent studies have also shed light on its role in protecting peripheral sensory neurons during reactivation of Herpes simplex virus and promoting skin inflammation during infection (11,12).

The significance of IL-17 responses becomes even more apparent when considering their role in combating fungal and bacterial pathogens. IL-17-mediated responses are particularly effective in defending against extracellular pathogens such as Candida, Cryptococcus, Klebsiella, and Staphylococcus. In fact, defects in the Th17 or IL-17 signaling pathway can result in severe mucocutaneous Candida infections, highlighting the critical role that IL-17 plays in fungal immunity (13,14).

Conclusion

Final finding of this study conduction, the IL17A considered an important interleukin defense and estimation other interleukins for defense, the rs2775913G>A was validated in patients group, but the OR was low than one value to be significant. The justification of this issues may correlated with two cases, first the Otitis media may be not correlated with polymorphism of IL17A but, this think was impossible based on the data of previous researches , which emphasized the pioneer of IL17A in Otitis media infection. The second caused may correlated with limitation of patient sample size may not supported Or values. In general the conducted results highlight on determined the role of polymorphism of IL17A with an otitis media.

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