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Investigation of Il-18 Gene Polymorphisms In Chronic Otitis Media: A Case-Control Study

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ABSTRACT

Chronic otitis media (COM) is associated with impairments in language development and communication, poor quality of life, and severe complications. It includes chronic inflammation and immunologic mechanisms. IL-18 also is involved in chronic inflammation and inflammatory conditions. This study aims to examine the association of IL-18 gene -137 G/C and -607 C/A polymorphisms with COM.

The study included 195 COM patients and 185 healthy individuals. DNA isolation was performed according to the Poncz method and polymorphisms were identified by polymerase chain reaction (PCR). The COM group was divided into two groups according to presence/absence of cholesteatoma.

Genotype frequencies for IL-18 -137 G/C polymorphism were 42.56% GG, 45.13% GC, and 12.31% CC in the COM group, and 42.70%, 43.78%, and 13.52% in the control group (p=0.88, p=0.73). For IL-18 -607 C/A, frequencies were 41.54% CC, 37.44% CA, and 21.02% AA in COM, compared to 41.62%, 32.97%, and 25.41% in controls (p=0.58, p=0.48). No significant differences were found, but -137 CC and -607 AA genotypes were associated with reduced COM risk.

Although no statistically significant association was found, -137 CC and -607 AA genotypes may have a protective role in COM. These findings highlight the potential role of genetic factors in COM pathogenesis, contributing to future diagnostic and therapeutic strategies.

Keywords: Chronic otitis media, IL-18, Polymorphism, Inflammation

Introduction

Chronic otitis media (COM) is an important health concern affecting both developed and developing countries. As a leading contributor to hearing loss globally, it results in considerable medical costs. Although the etiopathogenesis of COM has not been fully elucidated, it is accepted to be a chronic inflammatory disease. In the literature, it has been reported that COM may be an autoimmune disease, and factors such as osteoclastic activity, cytokines, endotoxins, and chronic inflammation play a role in the chronicization process. Factors such as tube dysfunction, allergy, and nasopharyngeal and paranasal infections have also been reported to facilitate the development of COM (1,2,3). The disease can be associated with cholesteatoma, a histologically benign but clinically aggressive lesion. The presence of cholesteatoma is a key factor in determining the course of treatment. It is characterized by the unusual growth of squamous epithelium in other areas of the temporal bone that are typically not covered by this tissue (4,5).

IL-18 plays a critical role in the regulation of inflammation, especially in chronic inflammatory diseases. It amplifies inflammation by stimulating the production of proinflammatory cytokines, such as TNF-α, IL-1β, and IFN-γ (6,7).

IL-18 expression is increased in chronic inflammatory diseases, autoimmune diseases, various cancers, and many infectious diseases. Recently, many studies have been conducted on IL-18 gene polymorphisms, and many data on IL-18 polymorphisms and the development, severity, and prognosis of diseases were shared (8). It has been shown that especially -137 G>C and -607 C>A polymorphisms of the IL-18 gene increase both gene expression and serum levels of IL-18 and therefore may be associated with diseases in which IL-18 levels are increased. Studies have shown that these polymorphisms influence IL-18 expression and contribute to functional differences in its associated signaling pathways (9,10).

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Pathophysiologically, COM includes chronic inflammation and immunologic mechanisms. IL-18 also is involved in chronic inflammation and inflammatory conditions. Therefore, it is reasonable to hypothesize that IL-8 may be associated with COM and is worth investigating. Accordingly, this study aims to investigate the association of IL-18 gene -137 G/C and -607 C/A polymorphisms with COM.

Materials and Methods

The present study included 195 patients who were subjected to surgery for COM. The diagnosis was established through a combination of medical history, physical examination, otomicroscopic evaluation, radiological assessments, and pure-tone audiometry. Approval for the study was granted by the Van Yüzüncü Yıl University Non-Interventional Ethics Committee on 27/10/2015 under the decision number 08**.** Patients were provided with information about the study, and their informed consent was obtained. Patients were evaluated in detail in terms of COM and appropriate surgical procedures were planned and performed.

Patients with COM were divided into two groups according to the presence/absence of cholesteatoma. Of 195 COM patients, 96 had cholesteatoma and 99 had no cholesteatoma. The control group included 185 healthy volunteers who were admitted to our hospital and did not have any hereditary, acquired, or chronic disease. Five mL of peripheral blood from patients and controls was collected in EDTA tubes and stored at +4°C until analysed.

DNA Isolation: A variety of protocols have been developed for the extraction, isolation, and purification of human genomic DNA. DNA isolation from whole blood samples was carried out using the Poncz method (11).

Polymerase Chain Reaction (PCR) Application: Polymorphisms in the -137 and -607 regions of the IL-18 gene were determined by PCR. The primers used in the study were selected from the research articles of Karakaş-Çelik et al. (12). Forward and reverse primers were used to determine polymorphisms in IL-18 gene by PCR. Thermal Cycler (Techne, Cambridge, UK) was used for the reaction. The forward and reverse primer sequences for the determination of IL-18 -137G/C, -607C/A polymorphism are shown in Table 1. PCR products obtained as a result of cutting were run on 2% agarose gel (Agarose Basica LE, Prona) and evaluated.

Statistical Analysis: The SPSS Statistics for Windows software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the data. Frequencies and percentages were determined for all parameters. A post-hoc power analysis indicated that the current sample size was adequate for the statistical analyses conducted, with a low risk of failing to detect significant associations due to insufficient power. The relationships between the frequencies of these parameters in the patient and control groups were investigated with the chi-square test. Odds ratios (OR) were calculated. A value of 0.05 was chosen as the error level. A p-value less than or equal to this threshold was considered to indicate that "the relationship between the parameters was statistically significant".

Results

The mean age of the patient group (n=195, M/F:1.22) was 49.6±16.26 years (range: 12-72 years). The mean age of the patients without cholesteatoma (n=99, M/F:1.41) was 47.4±18.13 (range: 14-72), while the mean age of the patients with cholesteatoma (n=96, M/F:1.04) was 51.7±19.45 (range: 12-67). The mean age of the control group (n=185, M/F:1.13) was 48.63±19.62 (range: 16-74).

As a result of this study, for the -137 G/C polymorphism of IL-18 gene, 83 (42.56%), 88 (45.13%), and 24 (12.31%) of 195 patients with COM had GG, GC and CC genotypes, respectively. Among 185 healthy controls, 79 (42.70%), 81 (43.78%), and 25 (13.52%) had GG, GC, and CC genotypes, respectively.

Genotyping was also performed according to the presence/absence of cholesteatoma in patients with COM. Accordingly, the genotypes of 99 COM patients without cholesteatoma were 41 GG (41.41%), 43 GC (43.33%) and 15 CC (15.16%). Among 96 COM patients with cholesteatoma, 42 (43.75%), 45 (46.88%) and 9 (9.37%) had GG, GC, and CC genotypes, respectively

Genotype frequencies of all groups were compared by multiple regression model with reference to the control group. It was concluded with 95% confidence that the GC genotype increased susceptibility to COM by 1.0341-fold and the CC genotype decreased it by 1.09-fold; however, these results were not statistically significant ($p>0.05$) (Table 2).

Similarly, it was concluded with 95% confidence that the GC and CC genotypes increased

Table 1: Primers Used For Determination of IL-18 -137G/C and -607C/A Polymorphism by PCR

Polymorphisms	Forward Primer Sequence	Reverse primer sequence	PCR product
IL-18 −137G/C	5'-ATG CTT CTA ATG GAC TAA GGA -3'	5'-GTA ATA TCA CTA TTT TCA TGA ATT-3'	131 bp
IL-18 -607C/A	5'-GCC CTC TTA CCT GAA TTT TGG TAG CCC TC-3'	5'-AGA TTT ACT TTT CAG TGG AAC AGG AGT CC-3'	171 bp

Table 2: Genotype Distributions, Allele Frequencies and Odds Ratios of IL-18 -137G/C, -607C/A Polymorphism In Patients With Chronic Otitis Media (COM) and Healthy Controls

Polymorphisms	Genotype / Allele	COM group (n=195)	Control group (n=185)	Odds ratio [95% CI]	p value
IL-18 −137G/C	GG	83 (% 42,56)	79 (% 42,70)	1 (Reference GG)	*p= 0.880 **p=0.736
	GC	88 (% 45,13)	81 (% 43,78)	1,0341 [0,6718-1,5916]	
	CC	24 (% 12,31)	25 (% 13,52)	0,9137 [0,4822-1,7316]	
	G	254 (% 65,13)	239 (% 64,59)	1 (Reference G)	p= 0.880
	C	136 (% 34,87)	131 (% 35,41)	0,9769 [0,7252-1,3159]	
IL-18 -607C/A	CC	81 (% 41,54)	77 (% 41,62)	1 (Reference CC)	*p= 0.580 **p=0.480
	CA	73 (% 37,44)	61 (% 32,97)	1,1376 [0,7171-1,8047]	
	AA	41 (% 21,02)	47 (% 25,41)	0,8293 [0,4919-1,3980]	
	C	122 (% 63,54)	215 (% 58,11)	1 (Reference C)	p= 0.215
	A	70 (% 36,46)	155 (% 41,89)	0,7959 [0,5557-1,1398]	

* Statistical comparison of GG and GC genotype distributions
** Statistical comparison of GG and CC genotype distributions
p: chi-square test

susceptibility to COM without cholesteatoma by 1.0229 and 1.1561-fold, respectively, but this increase was not statistically significant (p>0.05) (Table 3). Furthermore, it was concluded with 95% confidence that the GC genotype increased susceptibility to without cholesteatoma by 1.045-fold, while the CC genotype decreased it by 1.47-fold; however, this was also not statistically significant (p>0.05) (Table 4).

The association of allele frequencies of this polymorphism with the groups was evaluated by chi-square analysis. It was concluded with 95% confidence that the presence of C allele decreased the susceptibility to COM by 1.02 fold (Table 2), increased the susceptibility to COM without cholesteatoma by 1.0655 fold (Table 3) and decreased the susceptibility to COM with cholesteatoma by 1.12 fold (Table 4). However, it was not statistically significant (p>0.05).

For the -607C/A polymorphism of the IL-18 gene, among 185 individuals in the control group, 77 individuals had CC (41.62%), 61 individuals had CA (32.97%) and 47 individuals had AA (25.41%) genotypes. In the COM group of 195 individuals, 81 individuals had CC (41.54%), 73 individuals had CA (37.44%) and 41 individuals had AA (21.02%) genotypes. In the COM without cholesteatoma group, 36 of 99 individuals had CC (36.37%), 41 had CA (41.41%) and 22 had AA (22.22%) genotypes. In the COM whit cholesteatoma group, out of 96 individuals, 45 individuals had CC (46.88%), 32 individuals had CA (33.33%) and 19 individuals had AA (19.79%) genotypes.

It was concluded with 95% confidence that CA and AA genotypes increased and decreased the susceptibility to COM by 1.1376 and 1.2 times, respectively. However, it was not statistically

Table 3: Genotype Distributions, Allele Frequencies, and Odds Ratios of IL-18 -137G/C, -607C/A Polymorphism In Patients With Chronic Otitis Media (COM) Without Cholesteatoma (C) and Healthy Controls

Polymorphisms	Genotype / Allele	COM without C (n=99)	Control group (n=185)	Odds ratio [95% CI]	p value
IL-18 −137G/C	GG	41 (% 41,41)	79 (% 42,70)	1 (Reference GG)	*p= 0.935 **p=0.701
	GC	43 (% 43,43)	81 (% 43,78)	1,0229 [0,6031-1,7348]	
	CC	15 (% 15,16)	25 (% 13,52)	1,1561 [0,5499-2,4305]	
	G	125 (% 63,13)	239 (% 64,59)	1 (Reference G)	p= 0.736
	C	73 (% 36,87)	131 (% 35,41)	1,0655 [0,7442-1,5254]	
IL-18 -607C/A	CC	36 (% 36,37)	77 (% 41,62)	1 (Reference CC)	*p= 0.205 **p=0.990
	CA	41 (% 41,41)	61 (% 32,97)	1,4376 [0,8214-2,5161]	
	AA	22 (% 22,22)	47 (% 25,41)	1,0012 [0,5265-1,9037]	
	C	113 (% 57,07)	215 (% 58,11)	1 (Referans C)	p= 0.810
	A	85 (% 42,93)	155 (% 41,89)	1,0434 [0,7360-1,4793]	

*Statistical comparison of GG and GC genotype distributions
**Statistical comparison of GG and CC genotype distributions
p:chi-square test

Table 4: Genotype Distributions, Allele Frequencies, and Odds Ratios of IL-18 -137G/C, -607C/A Polymorphism In Chronic Otitis Media (COM) Patients With Cholesteatoma (C) and Healthy Controls

Polymorphisms	Genotype / Allele	COM with C (n=96)	Control group (n=185)	Odds ratio [95% CI]	p value
IL-18 −137G/C	GG	42 (% 43,75)	79 (% 42,70)	1 (Reference GG)	*p= 0.870 **p=0.370
	GC	45 (% 46,88)	81 (% 43,78)	1,0450 [0,6198-1,7619]	
	CC	9 (% 9,37)	25 (% 13,52)	0,6771 [0,2897-1,5825]	
	G	129 (% 67,19)	239 (% 64,59)	1 (Reference G)	p= 0.540
	C	63 (% 32,81)	131 (% 35,41)	0,8910 [0,6161-1,2886]	
IL-18 -607C/A	CC	45 (% 46,88)	77 (% 41,62)	1 (Reference CC)	*p= 0.710 **p=0.260
	CA	32 (% 33,33)	61 (% 32,97)	0,8976 [0,5106-1,5780]	
	AA	19 (% 19,79)	47 (% 25,41)	0,6917 [0,3620-1,3216]	
	C	122 (% 63,54)	215 (% 58,11)	1 (Reference C)	p= 0.215
	A	70 (% 36,46)	155 (% 41,89)	0,7959 [0,5557-1,1398]	

* Statistical comparison of GG and GC genotype distributions
** Statistical comparison of GG and CC genotype distributions

significant (p>0.05) (Table 2). It was concluded with 95% confidence that CA and AA genotypes increased the susceptibility to COM without cholesteatoma by 1.4376 and 1.0012 fold, respectively. However, this increase was not statistically significant (p>0.05) (Table 3).

It was concluded with 95% confidence that CA and AA genotypes decreased the susceptibility to COM with cholesteatoma by 1.11 and 1.44 fold, respectively. However, this reduction was not statistically significant (p>0.05) (Table 4). It was concluded with 95% confidence that the presence of the A allele of this polymorphism decreased the susceptibility to COM by 1.09-fold (Table 2), increased the susceptibility to COM without cholesteatoma by 1.0434-fold (Table 3), and decreased the susceptibility to COM with cholesteatoma by 1.25-fold (p>0.05) (Table 4).

Discussion

Numerous studies in the literature have explored the etiopathogenesis of COM. However, despite these studies, the phases of COM development and its progression to cholesteatoma remain controversial. The effects of cytokines in COM

have especially been the center of interest. This study is one of the first studies investigating the association of IL-18 polymorphisms with COM. Previously, Liu et al. (13) reported a significant negative correlation between IL-18 in effusion of otitis media and the duration of disease.

In our study, CC genotype reduced the risk of disease by approximately 9% in COM patients. In COM with cholesteatoma patients, CC genotype reduced the disease risk by 32.5%. When allele frequency was analyzed, C allele reduced the risk of disease by 2.5% in COM patients. In COM with cholesteatoma patients, C allele reduced the risk of disease by 11%. When the results were analyzed, it was noteworthy that the risk of disease increased in patients with heterozygous polymorphism at position -137 of the IL-18 gene, while the risk of disease decreased in homozygous polymorphism.

At location -607 of the IL-18 gene, the AA genotype was associated with an approximately 17% reduced risk of disease in COM patients. In COM patients with cholesteatoma, the CA genotype reduced the risk of disease by 2% and the AA genotype reduced the risk of disease by 31%. In COM patients, the A allele frequency reduced the risk of disease by 8.5%. In COM patients with cholesteatoma, the A allele reduced the risk of disease by 20.5%.

Within this context, the observation that -137 CC and -607 AA genotypes played a protective role in our study may be explained by the fact that these genotypes attenuate the inflammatory response by reducing IL-18 expression. In some studies, it has been reported that IL-18 polymorphisms may regulate the severity of inflammation and this may affect the clinical course of the disease (10,14,15).

It is known that inflammation is more severe in COM patients with cholesteatoma and this leads to complications such as bone destruction (16). In the literature, it has been reported that the levels of proinflammatory cytokines such as TNF-α, IL-1, and IL-6 present in cholesteatoma are associated with acceleration of bone destruction (17). In another study, TNF-α, IL-1α, and epidermal growth factor levels of COM patients with cholesteatoma were found to be higher compared to COM patients without cholesteatoma (18). In our study, the lower frequency of -137 CC and -607 AA genotypes in the patients with cholesteatoma may be explained by the suppressive effect of these genotypes on inflammation. These findings indicate that more studies are required to understand the role of IL-18 polymorphisms in developing cholesteatoma.

No statistically significant relationship was observed between polymorphisms at the -137 G/C and -607 C/A positions of the IL-18 gene and COM. However, the -137 CC and -607 AA genotypes may have a protective effect against the disease. These findings align with the regulatory role of IL-18 in inflammation and immune response (6,19). The more severe course of inflammation in COM patients with cholesteatoma may cause the protective effect of these genotypes to become more pronounced.

Recent studies have shown that IL-18 polymorphisms yield varying results across different populations. For instance, a meta-analysis by Chen et al. (10) demonstrated that the IL-18 -137 G/C polymorphism is linked to autoimmune diseases, although the strength of this association may differ across populations. Similarly, another study reported that environmental and racial differences may affect the results of genetic studies (20). The differences in these studies may be explained by the different genetic characteristics of populations and the fact that the etiopathogenesis of diseases is influenced by many factors.

The current study has some advantages and disadvantages. It has high originality because there is very limited data on the research topic. Therefore, on the other hand, the depth of the discussion may be limited and the validity of the findings should be retested by future studies. In addition, the case-control design of the study is limited to revealing the causal relationships between the investigated factors.

Our findings raise the possibility that IL-18 polymorphisms may play an influential role in the regulation of inflammation and that genetic factors may play a role in the treatment of COM. However, it should be kept in mind that the effects of IL-18 polymorphisms on the disease may differ between populations and may interact with environmental factors, and these findings should be supported by studies in different populations and larger sample groups. More comprehensive studies investigating the effects of IL-18 and other cytokines on COM are needed.

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