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Exploring Gene Polymorphisms Associated with Otitis Media through Variable Number Tandem Repeats (VNTR) Region

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Abstract

Background: Otitis is an inflammation and infection of the inner lining of the middle ear. The problem known as suppurative otitis media if there are droplets and holes in the tympanic membrane due to this inflammation and infection of the mucous membrane. The present study focused on genes potentially associated with otitis media in 60 subjects clinically diagnosed with OM The study focuses on two genes, MUC5B and IL-1RN, mutations assessed through variable number tandem repeat (VNTR) regions.

Methods: Variable numbers of tandem repeats (VNTR) regions were used to genotype 60 patients with otitis media and 30 healthy individuals for genotypic polymorphisms for the MUC5B and IL-1RN genes through variable number tandem repeat (VNTR) regions Blood samples were collected from participants, and three milliliters were placed directly into EDTA tubes. Subsequently, PCR products were detected by agarose gel electrophoresis and visualized by ethidium bromide staining. The size of amplified DNA fragments was determined by comparison with a 100-bp DNA ladder molecule size marker.

Results: This study presents the association of gene polymorphisms with otitis media by genotyping a total of 60 patients as OM, while 30 individuals were noted to be healthy. This was to determine genotypic polymorphisms for the two genes, that is, MUC5B and IL1RN, within the VNTR region. The results showed that the carriers of allele 2 in the genotypic position had nearly a twofold increased risk of developing otitis media.

Conclusion: Polymorphism of the MUC5B and IL-1RN genes can be responsible for the severity of the inflammatory process in the middle ear, complicated by otitis media or hearing loss.

Introduction

Otitis generally refers to conditions of infection and inflammation of the middle ear [1], with acute ear infections, effusion otitis (OME), and chronic ear infections includes AOM, an acute form, OME can be acute or chronic , CSOM It was a chronic condition. AOM and OME are more common in children, especially in younger age groups, due to their immature immune system, a frequent viral infection of the upper respiratory tract [2,3].

Acute otitis media is described as an infection of the middle ear and is considered the second most frequent diagnosis in children visiting the emergency department after upper respiratory conditions [4]. Chronic otitis media is described as the chronic inflammation of the middle ear and mastoid cavity and has been described by so many terminologies, some of which include chronic suppurative otitis media, chronic active mucosal otitis media, chronic otomastoiditis, and chronic tympano-mastoiditis [5].

Mucins can be classified into membrane-associated MUC and secretory MUC based on their characteristics [6]. These proteins are heavily glycosylated. The viscosity of middle ear effusions is primarily influenced by mucins. Excessive production of these highly viscous mucins may impair mucociliary clearance in the middle ear, contributing to pathological conditions such as chronic otitis media and hearing loss [7].

More than 20 genes of this family are described, including at least one human (MUC) and one murine. A couple of them, MUC5AC and MUC5B, correspond to the significant polymeric mucin glycoproteins found in the mucus of airway secretions. There has been a hypothesis that the MUC5B protein is a significant mucin of chronic otitis media [8]. At the same time, MUC5AC was reported as expressed at the level of RNA in the middle ear epithelium under normal conditions [9,10].

Discovery of such variability in the anti-inflammatory IL-1RA, otherwise known as IL-1RN, VNTR—variable number tandem repeat—polymorphism (rs2234663), led to speculations of the same on the IL-1RA protein expressions [11]. This IL-1RN polymorphism is founded in intron two and consists of 86 bp repeats but does not cause any change in the amino acid sequence of the IL-1RA protein. However, these repetitive sequences might hold functional importance owing to their capacity to act as binding sites for transcription factors [12].

Methods

Samples collection

Three milliliters of blood were collected, whereby 60 specimens of blood were obtained from a total of 30 patients and 30 individuals in good health. The blood was introduced directly into EDTA tubes. For the purpose of molecular investigations, the samples were transported from the hospital to the laboratory using a refrigerated container and subsequently conserved in a deep freeze set at -20°C.

DNA extraction and Molecular study

Following the manufacturer's instructions, DNA extraction and purification were conducted using the FAVORGEN kit. The primers for the MUC5B gene VNTR and IL-1 RN VNTR are provided in Table 1.

Primers	Primer sequence (5' → 3')	Product size
MUC5B gene / VNTR [7]	F: AGTGTGCAGTGACTGGCGAG R: CTAGAGTTGCAGGTGGCAGG	Allele 9 repeats = 692 bp Allele 8 repeats = 633 bp Allele 6 repeats = 515 bp
IL-1RN/ VNTR [13]	F: CTCAGCAACACTCCTAT R: TCCTGGTCTGCAGGTAA	Allele 1= 410 bp (four repeats) Allele 2= 240 bp (two repeats) Allele 3= 530 bp (five repeats) Allele 4=325 bp (three repeats) Allele 5= 595 bp (six repeats)

Table 1: PCR primers employed to amplify MUC5B gene VNTR and IL-1 RN VNTR in Otitis Media Patients and Healthy Individuals.

After the reaction was done, the amplification products were examined using 2% agarose gel electrophoresis and visualized by staining with bromide ethidium. Amplified DNA fragments were compared according to the migration of the DNA marker 100 bp.

Statistical analysis

Data were analyzed using version 22 of SPSS. Quantitative data were presented in the form of mean and standard deviation and qualitative data in frequency and percentage of the report. The independent-sample t-test was used to compare both groups, and the possible relationship between the ordinal or nominal variables about the current study was determined using the chi-square test. Significance was inferred if the p-value was $\leqslant 0.05$.

Results

Demographic characteristics of patients

A total of 74 patients were enrolled in the study. The demographic characteristics of patients are presented in Table 3, and those of control individuals are shown in Table 3. The study included 74 patients of various ages and genders, exhibiting otitis media symptoms. The maximum patient count (26) was observed in the 20-40 years age range, followed by less than 20 years (24) and more than 40 years (24).

Table 2 illustrates that the samples had a mean age (SD) of 33.47 (18.84) years, with a majority (35%) aged between 20-40 years. In terms of gender distribution, 44.5% were male, and 55.5% were female. Regarding residency, over three-quarters (75.7%) were from urban areas. The results showed a higher prevalence of ear infection among females than males, without a clear explanation for gender predilection in ear infections. The study found that 75.7% of patients resided in urban areas, while 24.3% lived in rural areas.

The study enrolled 74 patients with otitis media, with a relatively even distribution across age groups (less than 20 years, 20-40 years, and more than 40 years). Females exhibited a slightly higher prevalence of ear infection compared to males. The majority of the patients resided in urban areas. Despite observing a higher frequency of ear infections in females, the study did not identify a definitive reason for this gender difference.

Demographic characteristics		Frequency	Percent
Age	Less than 20	24	32.6
	20-40	26	53
	More than 40	24	32.4
	Total	74	100
Mean ± SD		33.47 ± 18.84	
Min-Max		1-72	
Gender	Male	33	44.5
	Female	41	55.5
	Total	74	100
Residence	Rural	18	24.3
	Urban	56	75.7
	Total	74	100

Table 2: Demographic characteristics of Otitis Media Patients and Healthy Individuals.

Genotypic polymorphism of MUC5B gene

Genotypic polymorphism of the MUC5B gene was evaluated through PCR using primers designed to amplify the entire variable numbers of tandem repeats (VNTR) region. Agarose gel electrophoresis of PCR products for the MUC5B primer from both otitis media patients and control individuals is presented in Figure 1

Table 4 presents the frequencies of MUC5B gene polymorphism genotypes and alleles. In this study, three genotypes of the MUC5B gene polymorphism were identified among patients with otitis media and control individuals. The genotypes 6/8, 8/8, and 8/9 had frequencies of 16.7%, 66.6%, and 16.7% in patients, and 16.7%, 76.7%, and 6.6% in controls, respectively. The most common genotype in both groups was 8/8, with frequencies of 66.6% in patients and 76.7% in controls.

The allele frequency of allele 8 was notably high, accounting for 83.4% in patients and 88.3% in controls. The other two alleles (6 and 9) had frequencies of 8.3% each in patients, and in controls, they were 8.3% and 3.3% respectively. The results suggest that carriers of allele 9 face an increased risk of developing otitis media. No significant difference was detected at a significance level of $P \leq 0.05$ between the patients and controls.

This study investigated the association between genotypic polymorphism of the MUC5B gene and otitis media susceptibility. Three genotypes were identified (6/8, 8/8, and 8/9), with 8/8 being the most common in both patients and controls. While allele 8 was dominant in both groups, allele 9 potentially carries a slightly increased risk for otitis media. However, statistically significant differences in genotype or allele frequencies between otitis media patients and controls were not observed. Further studies with larger sample sizes might be needed to definitively assess the role of MUC5B polymorphisms in otitis media development.

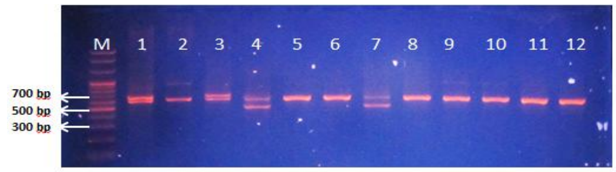

Figure 1: Agarose Gel Electrophoresis of PCR Products for MUC5B Gene Polymorphism in Patients (Lanes 1-6) and Control (Lanes 7-12). Lane M = Molecular Marker 100 bp. Lanes 1 and 3: Heterozygous pattern 1 (Allele 9 = 692 bp and Allele 8 = 633 bp). Lanes 4 and 7: Heterozygous pattern 2 (Allele 8 = 633 bp and Allele 6 = 515 bp). Lanes 2, 5, 6, 8-12: Homozygous (Allele 8 = 633 bp).

MUC5B Genotype		Genotypes frequency (%) Patients n=30 (%)	Control n= 30 (%)	χ^2 value	Sig.
MUC5B	6/8	5 (16.7)	5 (16.7)	1.495	0.474
	8/8	20 (66.6)	23 (76.7)		
	8/9	5 (16.7)	2 (6.6)		
Allele	Alleles frequency (%)				
	6	5 (8.3)	5 (8.3)	1.373	0.503
	8	50 (83.4)	53 (88.3)		
	9	5 (8.3)	2 (3.3)		

Table 3: Comparison of genotypes of (*MUC5B*) gene between patients and control groups.

Genotypic polymorphism of IL-1 RN gene

Frequency of IL-1RN gene polymorphism genotypes and alleles is shown in Table 4. Four genotypes of IL-1RN gene polymorphism were identified among patients with otitis media, whereas two genotypes were observed in control individuals. The genotype frequencies in patients were: 1/1 (60.0%), 1/2 (16.7%), 1/3 (16.7%), and 2/2 (6.6%). In controls, the frequencies were: 1/1 (88.0%) and 1/3 (12.0%). The most prevalent genotype in both groups was 1/1 (60.0% in patients and 88.0% in controls). The allele frequency of allele 1 was notably high, at 76.7% in patients and 96.0% in controls. Allele 3 had a lower frequency of 8.3% in patients and 4.0% in controls. Allele 2 was detected only among patients, with a frequency of 15.0%. This reveals a nearly twofold increased risk for developing otitis media in allele two carriers.

The current study achieved a statistically significant association between the polymorphism of the interleukin-1 receptor antagonist gene and susceptibility to otitis media. Genotypes with allele 2 were significantly more in numbers concerning the otitis media group as compared to the control.

Furthermore, allele 2 was exclusively present among the group of patients and may therefore be implicated for the etiology of the disease. homozygotes may have an approximate 1.9-fold increased risk of otitis media compared with this allele. However, available data are limited, further nuanced analysis is needed to clarify a better understanding of the functional consequence of IL-1RN gene polymorphisms needed for one to know their role in the pathogenesis of otitis media.

IL-RN Genotype		Genotypes frequency (%)			
		Patients n=30 (%)	Control n= 25 (%)	χ^2 value	Sig.
IL-RN	1/1	18 (60.0)	25 (83.3)	4.079	0.253
	1/2	5 (16.7)	0		
	1/3	5 (16.7)	5 (16.7)		
	2/2	2 (6.6)	0		
Allele	Alleles frequency (%)				
	1	46 (76.7)	55 (91.7)	7.194	0.027*
	2	9 (15.0)	0		
	3	5 (8.3)	5 (8.3)		

* Significance level at $P \leq 0.05$.

Table 4: Comparison of genotypes of (*IL-1RN*) gene between patients and control groups.

Discussion

Otitis is characterized by inflammation and infection of the membrane lining the middle ear. The presence of inflammation, as well as drainage and perforation of the tympanic membrane, can increase the incidence of otitis media (OM) which can present as an acute and chronic condition. This condition has the potential to result in lasting complications, such as conductive and sensorineural hearing impairments [14].The current investigation is centered on MUC5B and IL-1RN polymorphisms, delving into the significance of variations and alterations in genes associated with innate immunity.

The higher prevalence of ear infections in women is consistent with the findings of Aleneji et al., and Yadava [15,1 However, these results differ from those reported in other studies. The study of Addas et al., [17] and Osazuwa et al., [18] reported that ear infections were more common in men. However, there are no well-documented genetic or physiological differences between the sexes in hearing-related issues [19].

In contrast, another study demonstrated that the prevalence of the illness was higher among communities characterized by inadequate personal hygiene, substandard housing conditions, and limited literacy levels. This discrepancy may be ascribed to the fact that a significant portion of the subjects in this examination were affiliated with a particular urban demographic subgroup, which experienced superior living conditions in comparison to others. This assertion was corroborated by investigations carried out among urban communities by Ahmed & Akaiduzzaman [20], as well as Uddin et al., [21]. Comparable results are also documented by Biswas et al., [22] and Anggraeni et al., [23].

MUC5B is classified in the group of secreted gel-forming mucins. Chromosome 11 is a gene located on band p15.5, which resides in a 400 kb segment of genomic DNA along with MUC6, MUC2, and MUC5AC, which also secretes gel-forming mucins Major properties of in mucus and mucins sequences are often and differ among different mucins. Detailed MUC5B genomic and cDNA sequences provide a valuable framework for investigating the developmental regulation of a wide range of mucins [26]. Ahn and colleagues identified seven variable numbers of tandem repeats (VNTRs; minisatellites) within the complete MUC5B region. These minisatellites may serve as markers for paternity mapping and DNA fingerprinting [25].

Our findings align with those of Ubell et al., who also did not observe significant differences between otitis media patients and controls regarding the size of MUC5B genes determined through polymerase chain reaction [7]. This could be attributed to the relatively small sample size of patients. Recent studies propose that targeted treatment strategies focused on MUC5B might be beneficial for addressing otitis media with effusion (OME) [8,27]. MUC5B stands out as the primary mucin in conditions like cystic fibrosis, chronic obstructive pulmonary disease, and middle ear effusion and mucosa during OME [28, 29].

Genes that control the production of cytokines are commonly identified as strong candidates in the development of chronic otitis media (COM), as well as various chronic inflammatory and autoimmune human conditions. Numerous research studies have utilized the IL-1 RN VNTR primer as indicators of host immune regulation of immunity. Although many investigations have suggested a link between the IL-1 gene cluster and a predisposition to specific inflammatory diseases, only a limited number have specifically investigated otitis media [31]. Our findings are consistent with those of Zivkovic et al., who investigated the relationship between chronic otitis media and variations in genes within the IL-1 RN gene associated with innate immunity and inflammatory processes. The carriage of this allele has been found in their study to be positively associated with chronic otitis media [32]. The genotypes of IL-1RN-VNTR 1/2 and 2/L were significantly higher in another study in patients with cutaneous melanoma (43.6% and 45.1%, respectively) compared to healthy subjects [33].

In conclusion, our study established an association between polymorphisms in the MUC5B gene and IL-1 RN gene with the severity of otitis media and hearing loss. Testing for these polymorphisms could support early identification of disease progression and identify patients who may benefit from targeted antagonist therapy into their treatment regimen. This indicates that targeted treatment strategies based on specific genes could hold potential benefits for managing OME.

Author Contributions

Kawther: Refinement of study design , supervision and technical support.
Safa Amer: Sample collection, laboratory work and writing.

Conflict of Interest

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

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