International Journal of Pediatric Otorhinolaryngology 87 (2016) 1–4

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: http://www.ijporlonline.com/

FCN2 c.772G>T polymorphism is associated with chronic adenoiditis and/or tonsillitis, but not −4 A>G and −602 G>A

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ARTICLE INFO

Article history:
Received 1 February 2016
Received in revised form
10 May 2016
Accepted 12 May 2016
Available online 20 May 2016

Keywords:
Ficolin 2
Genotype
Polymorphism
Chronic adenotonsillitis

ABSTRACT

Objective: Ficolins are complement activating peptides that play a role in the initial host defense against infectious pathogens. In the present study, we investigated the relationship between single nucleotide polymorphisms (SNPs) in the ficolin 2 gene (FCN2) and chronic adenotonsillitis in pediatric cases.
Study Design: Case-control study.
Methods: A total of 101 pediatric patients diagnosed with chronic adenotonsillitis and 100 healthy children were enrolled in the study. Genotypes of FCN2 promoter SNPs − 602 G>A and −4 A>G, and the exonic SNP c.772G>T were determined by light SNP assay after realtime PCR analysis using genomic DNA samples obtained from peripheral blood samples of all participants.
Results: Of the 101 chronic tonsillitis patients, 38 were girls and 63 were boys; the mean age was 5.2 ± 2.3 years. The c.772G>T SNP frequency was significantly higher in chronic adenotonsillitis cases compared to the control group ($p = 0.00$); however, no significant difference was determined at positions −602 G>A or −4 A>G ($p > 0.05$).
Conclusions: The FCN2 c.772G>T genotype appears to be associated with predisposition to chronic adenotonsillitis in the pediatric age group. This nucleotide change is likely to influence the level of gene expression and contribute to the development of disease.

1. Introduction

Innate immunity is the initial defense system against infections, involving proteins of the complement system [1], which play a role in the recognition of microorganisms, immune complexes, and damaged host cells, as well as in opsonization, invasion, and phagocytosis [1,2]. Lectins are proteins, including mannose-binding lectin (MBL), ficolin, and MBL-related serine proteases [2,3], that bind specifically to carbohydrates on the surface of cells and organelles. The lectin pathway is one means of activating the complement system, thereby contributing to the first step in fighting infection and initiating inflammatory processes.

Ficolins are homologous, soluble molecules. Three different ficolin genes have been identified in humans: ficolin 1 (FCN1), FCN2, and FCN3. FCN2 (Gen Bank accession no. NG_011649, Synonymous with L-ficolin, ficolin/P35, and hucolin) is localized on chromosome 9q34 and contains eight exons and seven introns [4,5]. Ficolin-2 recognizes structures containing N-acetyl-D-glucosamine and triggers the complement system by activating the lectin pathway, binding to lipoteichoic acid on Gram-positive bacterial surfaces [6]. Indeed, L-ficolin has been shown to bind to many serotypes of Streptococcus pyogenes and Streptococcus agalactiae [6–10].

Tonsils and adenoids are found at the entrance of the respiratory and digestive system and are part of the group of lymphoid tissues known as the Waldeyer's ring. They have strategic importance in the development of the first-step defense mechanism against microorganisms and other antigenic substances. In addition to their antigen-specific primary immune response, they also generate a secondary immune response [11]. Group A beta-hemolytic

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http://dx.doi.org/10.1016/j.ijporl.2016.05.017
0165-5876/

streptococci (S. pyogenes) are the major bacterial agents in chronic adenotonsillitis, but they are not found in normal flora [12,13]. Chronic infections of adenoid and tonsil tissues are one of the most common indications for adenotonsillectomy surgery; however, the pathophysiology of this inflammation remains unclear [13,14].

Serum ficolin-2 concentrations are known to vary among individuals [10], although it is not known whether this difference is associated with genetic polymorphisms and their effect on innate immunity. Therefore, the present study investigated FCN2 polymorphisms in pediatric patients undergoing adenoidectomy and tonsillectomy for chronic adenotonsillitis.

2. Materials and methods

This case–control study involved 201 pediatric patients under 18 years of age who were enrolled between January 2012–September 2014. The patient group was comprised of 101 pediatric patients with the diagnosis of recurrent adenotonsillitis who underwent either a total tonsillectomy, a tonsillectomy with adenoidectomy, or an adenoidectomy. The control group was comprised of 100 healthy volunteers without a history of recurrent respiratory tract infections or adenotonsillectomy. The study was approved by the ethics committee and Institutional Review Board of Baskent University (IRB no. KA11/87), and written informed consent was obtained from all of the participants and/or their parents/guardians.

The decision to perform each tonsillectomy was made according to the diagnostic criteria for tonsillectomy defined by the American Otolaryngology and Head–Neck Surgery Academy [15]. Tonsillectomies were performed in patients with a history of: 1) 7 episodes of tonsillitis during the last year; 2) 5 episodes of tonsillitis in the last consecutive two years; or 3) 3 episodes of tonsillitis in the previous three years complicated by fever and a poor response to adequate antibiotic therapy. To determine whether to perform an adenoidectomy, patients who described upper respiratory tract infections that occurred 4–5 times per year combined with nasal congestion, snoring, sleeping with their mouths open, irritable sleeping, and sleep apnea during infection-free periods were examined by flexible fiberoptic nasopharyngoscopy and/or direct adenoid x-ray. Both nasal passages, choanal patency, and obstructions caused by adenoid tissue in the nasopharynx were examined, and adenoidectomies were performed in the patients with 75% and above choanal obstruction.

We excluded all patients with proven immunodeficiency, diabetes mellitus, renal failure, known malignancy or other diseases proven in pathological studies. All of the patients and volunteers were Turkish.

2.1. Genotyping

Briefly, 200 μl of whole blood was added to a 1.5 ml Eppendorf tube with 200 μl binding buffer and 40 μl proteinase K, and incubated at 70 °C for 10 min. Then 100 μl of isopropanol was added to the mixture and mixed well. The mixture was loaded into a High Pure Filter Tube and centrifuged for 1 min at 8000 g. Following the three washing steps, the gDNA was eluted in 100 μl elution buffer. Five milliliters of venous blood was collected from each subject. The genomic DNA was extracted from the peripheral blood samples using the High Pure PCR Template Preparation Kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. Patient genotypes for the three FCN2 single nucleotide polymorphisms (SNPs) −602 G>A (rs3124953) and −4 A>G (ss32469537), and c.772G>T (NM-004108.2) (rs7851696) were determined using real-time LightSNiP assays (Roche Applied Science) according to the manufacturer's instruction. Genomic data were acquired and analyzed using the LightCycler 480 II real-time PCR system (Roche Applied Science).

2.2. Statistical analysis

Calculations were performed using SPSS statistical software (version 17.0; SSPS Inc, Chicago, IL). The genotypes of the patients and the controls were analyzed by the chi-squared test. The level of significance was set at $p < 0.05$.

3. Results

Our patient group consisted of 101 consecutive patients; 63 (62.3%) were male, and 38 (37.7%) were female (mean age, 5.2 ± 2.3 years; range, 1.5–14 years). The controls were 100 healthy volunteers; 42 (42%) were male, and 58 (58%) were female (mean age, 9.1 ± 3.12 years; range, 4–16 years). There was a significant age difference between the patient and control groups ($p < 0.05$), but no significant gender difference ($p > 0.05$) (Table 1). A total of forty-four patients (43.5%) underwent both tonsillectomy and adenoidectomy, seven (6.93%) underwent tonsillectomy, and 50 (49.5%) underwent adenoidectomy (Table 1).

The FCN2 genotype distributions and allele frequencies of the patients and the controls are shown in Table 2. The FCN2 −602 G>A and −4 A>G genotype frequencies did not differ significantly between the patient and control groups ($p = 0.651$ and $p = 0.317$, respectively). However, the frequency of −4 A>G alleles was significantly higher in the control group than the patient group ($p < 0.05$), and the frequency of −602 G>A alleles was significantly higher in the patient group than the control group ($p < 0.05$).

The distribution of the c.772G>T genotypes and the frequency of the G alleles were significantly higher in the patient group than the control group ($p < 0.05$; $p < 0.05$, respectively).

Comparing the 1- only tonsillitis, 2- only adenoiditis and 3- adenotonsillitis groups, there were not statistically significant differences in the C.772G>T allele between these 3 groups ($p = 0.887$, $p = 0.252$, $p = 0.845$).

4. Discussion

In the present study, we investigated the role of SNPs −602 G>A, −4 A>G, and c.772G>T, which are localized in the promoter and the exonic region, and their functional properties in chronic adenotonsillitis. While there were no differences between the patient and control groups in terms of genotype frequencies at promoter SNPs −602G>A and −4 A>G, the number of variant alleles at position c.772G>T, localized in FCN2 exon 8, was significantly higher in the patient group. In the patient group, the G allele occurred more frequently than in the control group. The GG genotype was observed in 64.4% of the children in the patient group. Whereas in the control group, the TT and GT genotypes occurred more frequently. The frequency of the T allele was higher in the control group than the patient group (46%vs 24.8%).

FCN2 contains a number of clinically important polymorphisms involving promoter, exonic, and intronic regions [10,16,17]. To date, 14 polymorphisms have been identified in the promoter region, and 22 have been identified in the coding regions [18]. Differences in the polymorphisms have been noted among different ethnic groups and geographic patterns. Studies in healthy Danish and German Caucasian individuals have identified high SNP frequencies in the FCN2 promoter (−986 (A>G, rs3124952), −602 (G>A, rs3124953), −557 (A>G, rs3811140), −64 (A>C, rs7865453), and −4 (A>G, rs17514136)), and exonic regions (exon 3, rs4520243; +6359 C>T, exon 8, rs17549193 (Thr236Met); +6359 C>T, and exon 8, rs7851696 (Ala258Ser); c.772G>T) [10,16,18]. A study involving European