International Journal of Pediatric Otorhinolaryngology 88 (2016) 153–156

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

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

Lactoferrin gene polymorphisms in Italian patients with recurrent tonsillitis

Luisa Zupin [a, *], Francesco Angelelli [b], Domenico Leonardo Grasso [c], Sergio Crovella [a, c]

[a] Department of Medicine, Surgery and Health Sciences, University of Trieste, Trieste, Italy
[b] Department of Life Science, University of Trieste, Trieste, Italy
[c] Institute for Maternal and Child Health, IRCCS "Burlo Garofolo", Trieste, Italy

ARTICLE INFO

Article history:
Received 31 May 2016
Accepted 2 July 2016
Available online 6 July 2016

Keywords:
Recurrent tonsillitis
Innate immunity
Lactoferrin
Genetic polymorphisms

ABSTRACT

Introduction: Recurrent tonsillitis is an oral pathology characterized by inflammation of tonsils. The disease susceptibility depends upon environmental and host factors, specifically the innate immune response, the first line of host defence could play an important role. Among innate immunity members, lactoferrin, known for its antimicrobial properties, was previously correlated with the risk of oral pathology as periodontitis and dental caries.
Methods: 89 Italian children presenting recurrent tonsillitis and 95 healthy children were genotyped for two *LTF* non-synonymous polymorphisms, called Thr29Ala and Arg47Lys, in order to investigate their potential role in recurrent tonsillitis susceptibility.
Results: no different allele, genotype and haplotype frequency distributions were detected comparing patients and controls.
Conclusion: data from the current study indicate that *LTF* polymorphisms might not be involved in recurrent tonsillitis development in our Italian population. However, since the importance of lactoferrin in oral immunity has been previously assessed, further studies should be necessary to unravel the potential role of *LTF* genetic variants in oral cavity.

1. Introduction

Recurrent tonsillitis is an inflammation of tonsils caused by pathogens, microbes and foreign materials present in the mouth: tonsils, the local immune tissues in oral cavity, are able to furnish an unspecific response to pathogens but they could also be affected by infections [1]. Recurrent tonsillitis are defined as at least five episodes of inflammation of the tonsils within one year, during which Group A beta hemolytic streptococci were recovered at least three times [2].

The multifactorial aetiology of recurrent tonsillitis depends on environmental and host factors [3,4]; the immune response of oral cavity is known to play an important role in the disease susceptibility, especially the molecules of innate immune system, the first line of defence at mucosal level [1,5].

Among all members of innate immune system, lactoferrin (LF), also known as lactotransferrin, belonging to the transferrin family, may play an important role in oral immune response [6]. Indeed salivary levels of LF are elevated in inflammatory diseases, like periodontitis [7]. LF is an 80 kDa glycosylated and multifunctional protein of 692 amino acids [8–10]; for its capability of binding iron, LF is also known as a metal iron chelator [11].

LF has bacteriostatic and bactericidal activity against Gram-negative and Gram-positive bacteria [12,13]: LF exerts its antibacterial action by binding to lipopolysaccharides (LPS) of Gram-negative bacteria [14], or attaching its N-terminal region to the cell walls of fungi and bacteria causing membrane perturbation and leakage of intracellular components [15,16].

LF is encoded by *LTF* gene (chromosomal localization: 3p21.3): two non-synonymous polymorphisms at exon 1, namely Thr29Ala and Arg47Lys (rs1126477 and rs1126478, respectively), result in two amino-acid substitutions in the mature protein [17], possibly affecting the protein antimicrobial functionality [18]. The two polymorphisms have not been previously studied in susceptibility towards recurrent tonsillitis and no study investigated their influence on protein expression, however some publications correlated the two genetic variants with other oral pathologies such as

* Corresponding author. Department of Medicine, Surgery and Health Sciences, University of Trieste, Via dell'Istria 65/1, Trieste 34137, Italy.
E-mail address: luisa.zupin@burlo.trieste.it (L. Zupin).

http://dx.doi.org/10.1016/j.ijporl.2016.07.002
0165-5876/

periodontitis [18–20] and caries [21,22], thus corroborating the choice of *LTF* polymorphisms analysis in the current study.

All this considered in our study the distribution of *LTF* Thr29Ala and Arg47Lys polymorphisms was investigated in a children population from North-East Italy in order to unravel if these genetic variants could be associated with the susceptibility towards recurrent tonsillitis development.

2. Patients and methods

2.1. Study population

Eighty-nine Italian children (43 males, 46 females, mean age 6, range 4–11, European origin) with recurrent tonsillitis were enrolled at the Ear, Nose Throat Unit Department of the Institute for Maternal and Child Health IRCCS "Burlo-Garofolo" (Trieste, Italy). Recurrent tonsillitis was defined as tonsillitis occurring more than five times in one year with the presence of Group A beta hemolytic streptococci observed at least three times [2]. Written and informed consents were given by parents of enrolled children; all procedures have been performed in accordance with the ethical standards of the 1975 Declaration of Helsinki (7th revision, 2013) and approved by the IRCCS "Burlo-Garofolo" Ethical Committee (RC03/04, L1055, protocol number 118/10). DNA from 95 healthy control individuals, with no reported episode of tonsillitis, was already available from previous studies (45 males, 50 females, mean age 8, range 6–12 enrolled in a previous screening program for celiac disease in schools "Good as Rice").

2.2. LTF genotyping

Genomic DNAs of children with recurrent tonsillitis were extracted from peripheral whole blood using the EZ-1 Blood Extraction Kit and the EZ1 workstation for automated purification of nucleic acids (Quiagen, Hilden, Germany). DNAs of controls were already available at our laboratories.

Thr29Ala and Arg47Lys (rs1126477 and rs1126478, respectively) *LTF* (National Center for Biotechnology Information Reference Sequence: NC_000003), functional polymorphisms, were analyzed using C___9698511_10 and C___9698521_10 TaqMan SNPs genotyping assays on the ABI7900HT Real Time PCR platform (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.), following manufacturer's instructions.

Allelic discrimination was performed both manually and automatically with the SDS detection software (Thermo Fisher Scientific).

2.3. Statistical analysis

Allele and genotype frequencies of *LTF* Thr29Ala and Arg47Lys polymorphisms were calculated by direct counting, haplotype frequencies were computed with Arlequin software, version 3.1.0 [23]. The Fisher's exact test was used for pairwise comparison of allele, genotype and haplotype frequencies using contingency tables as appropriate, and only p-values <0.05 were considered statistically significant. The statistical analyses were performed employing R software version 3.1.3 [24]. P-value for linkage disequilibrium analysis was calculated using the permutation test with the EMB algorithm, Arlequin [23] whereas D′ and r^2 measures were computed with SNPstats [25]. *Post hoc* power calculations were performed through G*Power software version 3.1.9.2 [26].

(*post-hoc* χ^2 test: effect size w = 0.21, α error probability = 0.05, Df = 3; *post-hoc* Fisher Exact test: tails = two, α error probability = 0.05).

3. Results

The distribution of *LTF* Thr29Ala and Arg47Lys polymorphisms' frequencies was in Hardy Weinberg Equilibrium (HWE) in children with recurrent tonsillitis and healthy controls.

No statistically significant differences in allele and genotype *LTF* polymorphisms frequencies were detected when comparing children with recurrent tonsillitis and controls (Table 1) even when dominant, recessive and overdominant genetic model were employed (data not shown). Among the subjects analyzed the Thr29Ala G allele resulted more frequent in healthy controls (77%) than in patients (72%); the Arg47Lys A allele was more frequent in healthy controls (74%) than in children with recurrent tonsillitis (67%).

The two polymorphisms were in linkage disequilibrium (D' = 0.90, r^2 = 0.64, $p < 0.05$ in patients and D'>0.99, r^2 = 0.85, $p < 0.05$ in controls) and combined to form three major haplotype (GA, AG and GG). When comparing subjects with recurrent tonsillitis and healthy individuals no statistically significant differences were found, although a trend was observed, since GA haplotype was more frequent among healthy controls and GG haplotype was more frequent among patients with recurrent tonsillitis (p = 0.09) (Table 1).

The power analysis, performed in order to prevent type II statistical errors related to absence of association due to low number of individuals enrolled, showed a high power (0.86) for haplotype analysis using *post-hoc* χ^2 test (non-centrality parameter $\lambda = 12.75$; critical $\chi^2 = 7.81$) and a less power for AG (0.09) and GG (0.37) haplotypes frequency distribution using *post-hoc* Fisher Exact test (actual $\alpha = 0.03$).

4. Discussion

LF is a component of innate immunity present in the saliva at concentrations of 1–7 μgr/ml [27]. The importance of LF in the contest of oral mucosa environment was previously assessed: LF has been reported as involved in susceptibility to periodontitis [28], caries [21], and its ability to prevent HSV-1 replication has been described [29]. Moreover, in the context of tonsils anatomical district, Stenfors et al. showed how human LF is able to coat bacterial pathogens on the tonsillar surfaces of patients with acute pharyngotonsillitis possibly activating the phagocytosis process [30].

Recently, *LTF* genetic variants have been investigated in patients, both adults and children, with caries [31], in individuals experiencing dental implant loss [32], in the susceptibility to coronary artery stenosis [33], to nasopharyngeal carcinoma [34] and to dyslipidaemia [35]. In the study of dyslipidaemia by Moreno-Navarrete et al. [35] the circulating LF concentration and *LTF* polymorphisms, in spite of not being associated, were both related to the plasma lipid profile.

Nevertheless, the biological effects of these two polymorphisms in the human body *in vivo* or in cellular model *in vitro* were not explored so far.

In this study two non-synonymous polymorphisms located at exon 1 of *LTF* gene were investigated in the context of susceptibility towards recurrent tonsillitis development. No differences in allele and genotype frequencies were detected for *LTF* Thr29Ala and Arg47Lys polymorphisms between patients and controls. The frequencies of our population from North East Italy were comparable to those reported in the HapMap project [36] for the European populations.

The same negative results were also observed for haplotype combinations of the two polymorphisms, although GG haplotype resulted more frequent among patients respect to GA haplotype more represented among controls, however this comparison did