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Research Article

Molecular Genetic Study on VNTR-polymorphism of Two Cytokine Genes Antagonist of the Receptor of Interleukin 1 (rs2234663) and Interleukin 4 (rs8179190) Associated with Dental Caries in Children

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Abstract

In this study, we investigated VNTR polymorphisms in intron 2 of the *IL1RN* gene (rs2234663) and intron 3 of the *IL4* gene *(rs8179190)* related to the development of caries in school children (N = 196) with mixed bite dentition from Krasnodarskii Krai. The genotypes *A1/A1* and *L/L* (*L*-"long" alleles) (*rs2234663*) provided resistance to the most intensive form of dental caries. The groups of children with DFC (decompensated form of caries) were significantly different from the pooled group SFC (subcompensated form of caries) and CFC (compensated form of caries) (and healthy children) for two genotypes: *A1/A1 (OR = 0.37, p = 0.015, 95% CI: 0.17-0.84)* and L/L (*OR = 0.38. p = 0.020, 95% CI: 0.17-0.88)*. In locus *rs8179190* (IL4), a rare allele with a single repeat (70 bp)-*P3* (113 bp) was observed. For VNTR polymorphisms of *rs8179190,* association with the most intensive form of caries was found only for two-locus

genotypes with *rs2234663.* The results suggested that the studied cytokine markers and the ratio of the pro-inflammatory to anti-inflammatory cytokines might be involved in the development of the most intensive form of caries.

Keywords
Dental caries; children; cytokine; Krasnodarskii Krai; IL1RN (rs2234663); IL4 (rs8179190); VNTR; association

1. Introduction

Genome-wide association studies (GWAS) often lack large samples that are necessary to associate molecular genetic markers with diseases, including markers with a relatively weak effect [1]. Population-based associative studies for determining the role of individual molecular genetic markers in disease development help elucidate the role of genetic markers in the development of diseases.

Dental caries is a chronic infectious multifactorial disease that occurs when oral bacteria penetrate the tooth enamel and dentin. The main causative agent of dental caries is *Streptococcus mutans,* which causes carious lesions. Fermentable sugars facilitate the development of the disease. Genetic and environmental factors and the resistance of teeth influence the formation of lesions, which depends on the duration of exposure to disease-causing factors [2]. The risk of dental caries is predetermined by the genetic and immune peculiarities of an individual.

During the progression of dental caries, microorganisms trigger inflammation in the tooth pulp, and during the development of dental caries, immunocompetent cells produce immune responses [3]. Cytokines are produced by activated macrophages and monocytes and serve as important mediators of infection, inflammation, and immune response. Cytokines control inflammatory responses to bacterial infections [4] and are associated with the inflammation of soft and hard tissues [5]. Moreover, they are involved in the development and progression of dental caries [6, 7].

The role of cytokines in the pathogenesis of dental caries is not clear; however, the components of *S. mutans* facilitate the production of pro-inflammatory cytokines [3]. Protein I/II of *S. mutans* is important for the colonization of tooth surfaces and stimulates the synthesis of pro-inflammatory cytokines, e.g., IL-1β, by monocytes [8]. During the formation of caries, cytokine expression, including that of pro-inflammatory cytokines, increases multiple times in the odontoblast layer at the periphery of dental pulp [9].

The interleukin 1 system protects against microbial colonization, infection, and malignant transformation. Interleukin 1 (IL-1) is a pro-inflammatory cytokine encoded by the *IL-1* gene localized at 2q13-21. The gene of the receptor antagonist of interleukin 1 *(IL1RN)* encodes a protein that belongs to the interleukin cytokine family 1 and is a competitive inhibitor that regulates inflammation induced by interleukin 1 [3, 10-12].

A variable number tandem repeat (VNTR) with a subunit of 86 bp (base pairs) (*rs2234663)* is present in intron 2 of the *IL1RN* gene and leads to the formation of six alleles: “short-S” alleles with one *(VNTR/6)* and two repeats (VNTR⁄2) and “long” (long-L alleles (VNTR⁄L) with 3-6 repeats [13]. The pro-inflammatory immune response of homozygous individuals with VNTR⁄2/VNTR⁄2 was

stronger and more prolonged compared to the response of individuals with other VNTR genotypes [11]. Therefore, the role of the VNTR⁄2 allele of the *IL1RN* gene was studied in various diseases [11, 12, 14]. The VNTR⁄2 allele of IL1RN is a risk allele in the development of sepsis [12]. Several studies [4, 15-18] have investigated the role of VNTR polymorphism *(rs2234663)* of the *IL1RN* gene and the polymorphic variants of other cytokines in the development of caries with deep tooth lesions and the peculiarities of its course, as well as, in the development of other dental diseases, especially periodontitis. *ILB (rs1143643)* was found to be associated with tooth lesions in deep caries [17].

Interleukin 4 (IL-4) is an anti-inflammatory cytokine. The interleukin 4 gene *(IL4)* is located on chromosome 5 (q23-31). Intron 3 of the *IL4* gene includes 70-bp tandem repeats of a VNTR or a minisatellite repeat (rs8179190) that is associated with the level of gene expression [19]. VNTR polymorphism in *IL4* generates two alleles, one with a deletion of 70 bp (with two repeats) and the other with insertion of 70 bp (with three repeats); they are designated as *P1* and *P2* [19, 20].

The *P1* allele induces higher expression of the gene *IL4* than the *P2* allele, considering that the *P2/P2* genotype was associated with a lower concentration of IL-4 [20]. The relationship of VNTR polymorphism of *IL4* was studied with immune and autoimmune diseases and also with the diseases of the oral cavity (periodontitis and recurrent aphthous stomatitis) [20-24].

In this study, we investigated VNTR polymorphisms in intron 2 of the *IL1RN* gene (rs2234663) and intron 3 of the *IL4* gene *(rs8179190)* associated with the development of caries in school children with mixed bite dentition.

2. Materials and Methods

2.1 Sampling

Children of school age (N = 196) living in Krasnodar Krai were studied in the children's regional clinical hospital and the dental clinic of the Kuban State Medical University (KubMU). Buccal epithelium scraps from the oral cavity (in TAE buffer) and blood samples of some individuals (10% of the total group) were collected in special tubes with EDTA. All samples were stored at 4°C.

The main inclusion criterion in the study was that the school children must be living in the city of Krasnodar. The exclusion criteria were:

- the presence of somatic pathology on the day of observation;
- living outside Krasnodar;
- the presence of alimentary imbalance;
- the presence of allergic diseases

In total, 196 school children participated in the study. They were living in Krasnodar, and over 90% of these children were Russian. These children had regular access to professional preventive and therapeutic dental care. The fluoride content in the drinking water of the region studied was 0.42-0.54 mg/L. In the city and suburbs, there was only agricultural production and no industrial production. The region was environmentally prosperous. In this study, either the children or their parents provided answers to a questionnaire on food preferences and the devices used to maintain oral hygiene, the nutrition of the child, dietary habits, demographic characteristics, and environmental factors to elucidate the key factors and rectify alimentary behavior. All children used toothbrushes and toothpaste recommended for their age. Most children (77.5%) brushed their teeth twice a day regularly; the remaining children brushed their teeth once a day regularly and twice a day occasionally. Some children consumed carbonated drinks (46.5%), chocolates (34.2%),

biscuits (28.6%), and milk products (38.0%). The quality of oral hygiene was evaluated by the simplified Green-Vermilion index (OHI-S). The estimate of the index was 1.13 for the sample studied, with a dispersion of 0.99-1.35. We performed a pilot study on the effect of genetic markers on the development of caries. Further studies might be performed on the effect of factors, such as diet and hygiene, on the development of caries.

A dental examination was performed as a preventive examination or upon admission to a medical and preventive institution for all children. The dental examination was performed according to the recommendations of the WHO. Children were categorized by age based on the periods of development of biting: 0-6 years-temporary bite, 7-12 years-mixed bite, and 13-17 years-permanent bite. In all children, the degree of the carious process was determined. Less than 15% of the participating children were healthy. The intensity of the carious process was evaluated by evaluating the total kpu/KPU index (caries/fillings/off-change tooth lost). According to the obtained kpu/KPU values, children in the samples were divided into the following groups: compensated form of caries (CFC), with index values 0-3; subcompensated form of caries (SFC), with index values 4-5; decompensated form of caries (DFC), with index values ≥6 [25].

2.2 DNA Extraction

Genomic DNA was isolated from biological samples following a standard procedure using reagents from "Izogen" (Moscow) according to the manufacturer's instructions. For performing molecular genetic studies, minisatellite (VNTR) markers of the *IL1RN* gene *(rs2234663)* and the *IL4* gene *(rs8179190)* were selected.

2.3 PCR Analysis

The VNTR polymorphism of the *IL1RN* gene *(rs2234663)* was evaluated by polymerase chain reaction (PCR), the conditions are described in a different study [14]. For PCR amplification, "Izogen PCR reagent kits" (Moscow) were used. The polymorphic region of the gene was amplified using direct and reverse primers 5'-CTCAGCAACACTCCTAT-3' and 5'-TCTGGTCTGCAGGTAA-3', respectively. Primers were synthesized at "Litekh" (Moscow). The PCR protocol used was as follows: denaturation at 95°C for 5 min for 30 cycles; denaturation at 94°C for 30 s, annealing of primers at 57°C for 45 s, elongation at 72°C for 1 min, and final elongation at 72°C for 10 min on a device from MJ Research (USA). The PCR products were visualized in a 2%-agarose gel that was stained with ethidium bromide (Figure 1a).

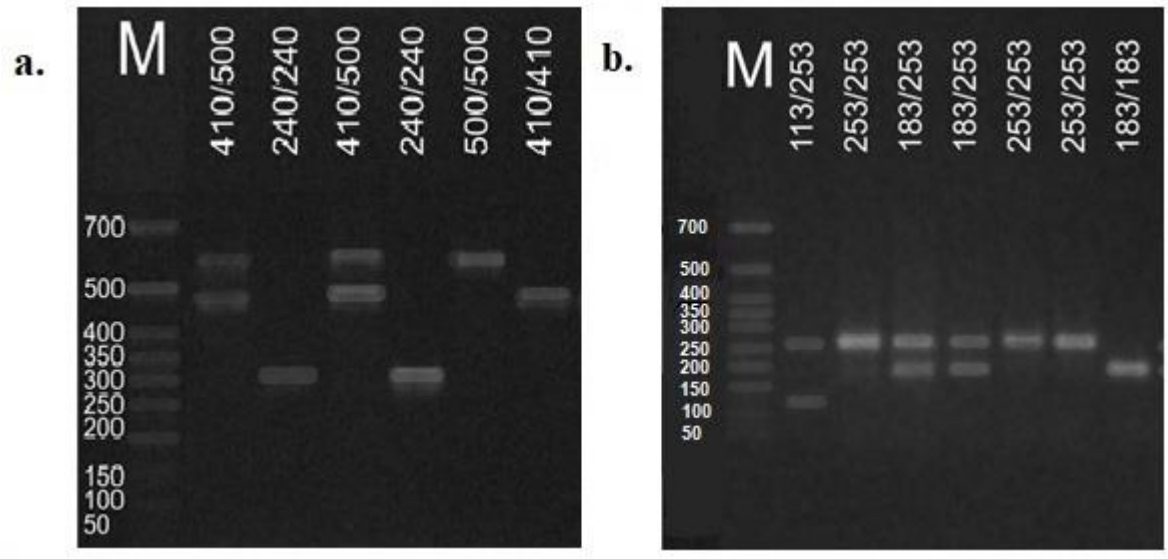

Figure 1 Electrophoresis was performed to determine VNTR polymorphisms of the *IL1RN* gene *(rs2234663)* (a) and of the *IL4* gene *(rs8179190)* (b) in a 2%-agarose gel. A molecular weight marker (M) is shown on the left of Figures 1a and 1b, and the numbers indicating bands are in bp. The remaining lanes show the results of the VNTR polymorphism analysis by determining the locus with different genotypes for the selected samples. The numbers at the top of the figure indicate the lengths of the identified alleles in bp. (a) The alleles 240 bp (A2), 410 bp (A1), and 500 bp (A4) are shown, which differ by the presence of tandem repeats in 86 bp, as found in another study [13]. (b) The alleles 183 bp (P1), 253 bp (P2), and 113 bp (P3) (rare allele) are shown, differing by the presence of tandem repeats in 70 bp.

The expected spectrum of polymorphic variants of the *IL1RN* gene was represented by the following fragments: a fragment of 410 bp corresponding to the *IL1RN1 (A1)* allele *(*containing four repeats of 86 bp); a fragment of 240 bp-IL1RN2 (A2) (two copies); a fragment of 325 bp-*IL1RN3 (A3)* (three copies); a fragment of 500 bp-*IL1RN4 (A4)* (five copies); a fragment of 585 bp-*IL1RN 5 (A5)* (six copies), and a fragment of 155 bp-*IL1RN6 (A6)* (one copy). In our sample, only alleles of *IL1RN1, ILRN2,* and *ILRN4* were present. Alleles with two and four copies are the most common in populations [13].

To study 70 bp VNTR polymorphism of the IL-4 gene (rs8179190), PCR was performed following the method described in another study [20]. PCR-amplification was performed with direct and reverse primers: F5' AGGCTGAAAGGGGGAAAGC-3' and R5'-CTGTTCACCTCAACTGCTCC-3', respectively. The primers were synthesized at "Litekh" (Moscow). The PCR protocol used was as follows: denaturation at 95°C for 5 min for 30 cycles; denaturation at 94°C for 30 s, annealing of primers at 58°C for 45 s, elongation at 72°C for 1 min, and final elongation at 72°C for 10 min on a device from MJ Research (USA). The PCR products were visualized in the same way as we visualized the PCR products of *IL1RN*. For allele *P1,* the PCR product was 183 bp long, and for allele *P2,* it was 253 bp long. In the studied sample, a minor allele in 113 bp-*P3* with one copy of the subunit in 70 bp was found (Figure 1b). For the reliability of typing and reproducibility, the method for each genotype included internal controls.

2.4 Statistical Analysis

For statistical analyses, Statistica 6.0 and WinPepi algorithms were used for determining OR (odds ratio). A 95% confidence interval (CI) of OR was determined for $p < 0.05$ by performing Fisher's exact test (two-tailed for two distributions). Using the algorithms of the Statistica program, the allele frequencies, the Hardy-Weinberg equilibrium, and the estimated expected and observed heterozygosity were determined, and the comparison of samples was performed using the G criterion.

2.5 Ethics Statement

All procedures performed in the study involving people complied with the ethical standards of the institutional and/or national committee for research ethics and the 1964 Declaration of Helsinki and its subsequent changes or comparable standards of ethics. Informed voluntary consent was obtained from all participants. The authors stated that they had no conflict of interest.

The study was approved by the ethics committee of the KubMU, as mentioned in protocol No. 63 of May 21, 2018.

3. Results

The average age of children with CFC (and healthy children) was 12.08 ±0.38, with SFC was -11.66 ±0.46, and with DFC was -10.19 ±0.54. The average age of children in the three groups suggested mixed bite dentition, which allowed us to perform comparative studies.

The results of the analysis of the studied VNTR polymorphisms *IL1RN (rs2234663)* and the gene *IL4 (rs8179190*) in the total group of the studied children are presented in Table 1. The characteristic frequency profiles of the studied VNTR polymorphisms and estimates of observed and expected heterozygosity were detected in children from Krasnodarskii Krai. In the studied sample, the Hardy-Weinberg equilibrium was found at both VNTR loci, which allowed us to consider the association of two VNTR polymorphisms with caries in the subgroups that differed in the intensity of caries.

Table 1 The distribution of the polymorphic variants of the VNTR gene *IL4 (rs8179190)* and the VNTR gene *IL1RN (rs2234663)* in children from Krasnodarskii Krai.

Children (total)							
VNTR	Genotype	No	Fo	Allele frequency	Ne	$\chi 2$	Heterozygosity parameters
rs8179190	*P1/P1*	14	0.0714	P1 = 0.2500 ±0.0219	12.25	0.7513, d.f. = 3 p > 0.05	He = 0.3788 ± 0.0220, Ho = 0.3622 ±0.0343, D =-0,0437 ± 0.0703, td = 0.4065, p > 0.05
	P1/P2	70	0.3571		73.25		
	P2/P2	111	0.5664	P2 = 0.7474 ±0.0219	109.50		
	P1/P3	0	0.25				
	P2/P3	1	0.75				
	P3/P3	0	0.000	P3 = 0,0026 ±0.0025			
	Σ	196	1.000		ne = 1.6098 ±0.0220		

Children (total)

VNTR	Genotype	No	Fo	Allele frequency	Ne.	χ2	Heterozygosity parameters
rs2234663	*A1/A1*	121	0.7160	PA1 = 0.8373 ±0.0201	118.47	5.3996, d.f. = 3 p > 0.05	He = 0.2776 ±0.0283, Ho = 0.2426 ±0.0330, D =-0.1262 ±0.0810, td = 0.8056, p > 0.05
	A1/A2	35	0.2071		41.03		
	A2/A2	7	0.0414	PA2 = 0.1450 ±0.0192	3.33		
	A1/A4	6	0.0355	PA4 = 0.0178 ±0.0072	5.02		
	A2/A4	0	0		0.87		
	A4/A4	0	0		0.05		
	Σ	169	1.0000		ne = 1.3843 ±0.0283		

Note: No denotes the observed number of genotypes, Ne denotes the theoretically expected number of genotypes, Fo denotes the observed frequency of phenotypes, He denotes the expected heterozygosity, Ho denotes the observed heterozygosity, d.f. is the number of degrees of freedom, D = (Ho-He)/He; ne is the effective number of alleles.

Tables 2 and 3 show the results of the analyses of the studied VNTR polymorphisms IL1RN (rs2234663) and the IL4 gene (rs8179190), respectively, for groups of children with different degrees of activity of tooth caries. A comparison of the group with DFC was performed with the combined group with SFC and CFC (and healthy children). In the samples with different intensities of caries, the Hardy-Weinberg equilibrium was observed at both VNTR loci. No significant differences were found for VNTR IL4 (rs8179190) and VNTR IL1RN (rs2234663) in two groups of children with DFC and a pooled group of children with SFC and CFC (and healthy children) (Tables 2 and 3). The group with DFC showed the highest reduction in the observed level of heterozygosity compared to the values of expected heterozygosity for both loci studied relative to the differences in the values of observed and expected heterozygosity in the combined group of children with SFC and CFC (and healthy children) (Tables 2 and 3). There were no differences in the distribution of the genotypes *A1/A1* and *L/L* between the CFC (and healthy children) and SFC groups, or the distribution of the total spectrum of the genotypes. This confirmed the validity of the pooling groups with SFC and CFC (and healthy children) when performing comparative analyses with the DFC group. Independently, the SFC and CFC (and healthy children) groups differed from the DFC group based on the distribution of the markers studied (Figure 2).

Table 2 The distribution of polymorphic variants of VNTR *IL4 (rs8179190)* in children from Krasnodarskii Krai, based on the degree of activity of caries.

Children with DFC							
VNTR	Genotype	No	Fo	Allele frequency	Ne	χ^2	Heterozygosity parameters
rs8179190	*P1/P1*	3	0.0546	P1 = 0.1909 ±0.0375	2	0.9508,	He = 0.3235 ±0.0469,
	P1/P2	15	0.2727		16.80		

	P2/P2	36	0.6545	P2 = 0.8000 ±0.0381	35.20	*d.f.* = 3	Ho = 0.2909 ±0.0612, D =-0.1007 ± 0.1363, td = 0.4220, p > 0.05
	P1/P3	0	0		0.19	p > 0.05	
	P2/P3	1	0,0182	P3 = 0.0091 ±0.0090	0.80		
	Σ	55	1.0000		ne = 1.4781 ±0.0469		
United group of children with SFC and CFC (and healthy)							
VNTR	Генотип	No	Fo	Allele frequency	Ne	χ^2	Heterozygosity parameters
rs8179190	*P1/P1*	11	0.0780	P1 = 0.2730 ±0.0265	10.51	0.0428, *d.f.* = 1 p > 0.05	He = 0.3970 ±0.0241, Ho = 0.3901 ±0.0411, D =-0.0174 ±0.0835, td = 0.1452, p > 0.05
	P1/P2	55	0.3901		55.98		
	P2/P2	75	0.5319	P2 = 0.7270 ±0.0265	74.51		
	Σ	141	1.0000		ne = 1.6583 ±0.0241		

Children with DFC were compared to a pooled group of children with SFC and CFC (and healthy children) by genotype: G = 2,9909, d.f. = 3; p > 0.05.
Children with DFC were compared to a pooled group of children with SFC and CFC (and healthy children) by genotype: P2/P2, G = 2.4321, d.f. = 1; p > 0.05; OR = 1.67, p = 0.149, 95% CI: 0.84-3.38.

Table 3 The distribution of polymorphic variants of the VNTR *(rs2234663)* of the *IL1RN* gene in children from Krasnodarskii Krai, based on the degree of activity of caries.

Children with DFC							
VNTR	Genotype	No	Fo	Allele frequency	Ne	χ2	Heterozygosity parameters
rs2234663	A1/A1	22	0.5500	PA1 = 0.7250 ±0.0499	21.03	2.1099 d.f. = 3 p > 0.05	He = 0.4112 ±0.0499, Ho = 0.3500 ±0.0754, D =-0.1489 ±0.1452, td = 0.6775, p > 0.05
	A1/A2	12	0.3000		14.50		
	A2/A2	4	0.1000	PA2 = 0.2500 ±0.0484	2.50		
	A1/A4	2	0.0500	PA4 = 0.0250 ±0.0175	1.45		
	A2/A4	0	0		0.50		
	A4/A4	0	0		0.03		
	Σ	40	1.0000		ne = 1.6985 ±0.0499		
United group of children with SFC and CFC (and healthy)							

VNTR	Genotype	N.O.	F.O.	Allele frequency	N.E.	$\chi 2$	Heterozygosity parameters
rs2234663	A1/A1	99	0.7674	PA1 = 0.8721 ±0.0208	98.11	1.9230 d.f. = 3 p > 0.05	He = 0.2266 ±0.0320, Ho = 0.2093 ±0.0358, D =-0.0762 ±0.0960, td = 0.2966, p > 0.05
	A1/A2	23	0.1783		25,29		
	A2/A2	3	0.0233	PA2 = 0.1124 ±0.0197	1.63		
	A1/A4	4	0.0310	PA4 = 0.0155 ±0.0077	3.49		
	A2/A4	0	0		0.45		
	A4/A4	0	0		0.03		
	Σ	129	1.0000		ne = 1.2930 ±0.0320		

Children with DFC were compared to a pooled group of children with SFC and CFC (and healthy children) by genotype: G = 1.3021, d.f. = 2; p > 0.05.

Children with DFC were compared to a pooled group of children with SFC and CFC (and healthy children) by genotype A1/A1: G = 6,5496; p < 0.05, d.f. = 1; OR = 0.37, p = 0.015, 95% CI: 0.17-0.84.

Children with DFC were compared to a pooled group of children with SFC and CFC (and healthy children) by genotype L/L: G = 5.905, d.f. = 1; p < 0.05, OR = 0.38. p = 0.020, 95% CI: 0.17-0.88.

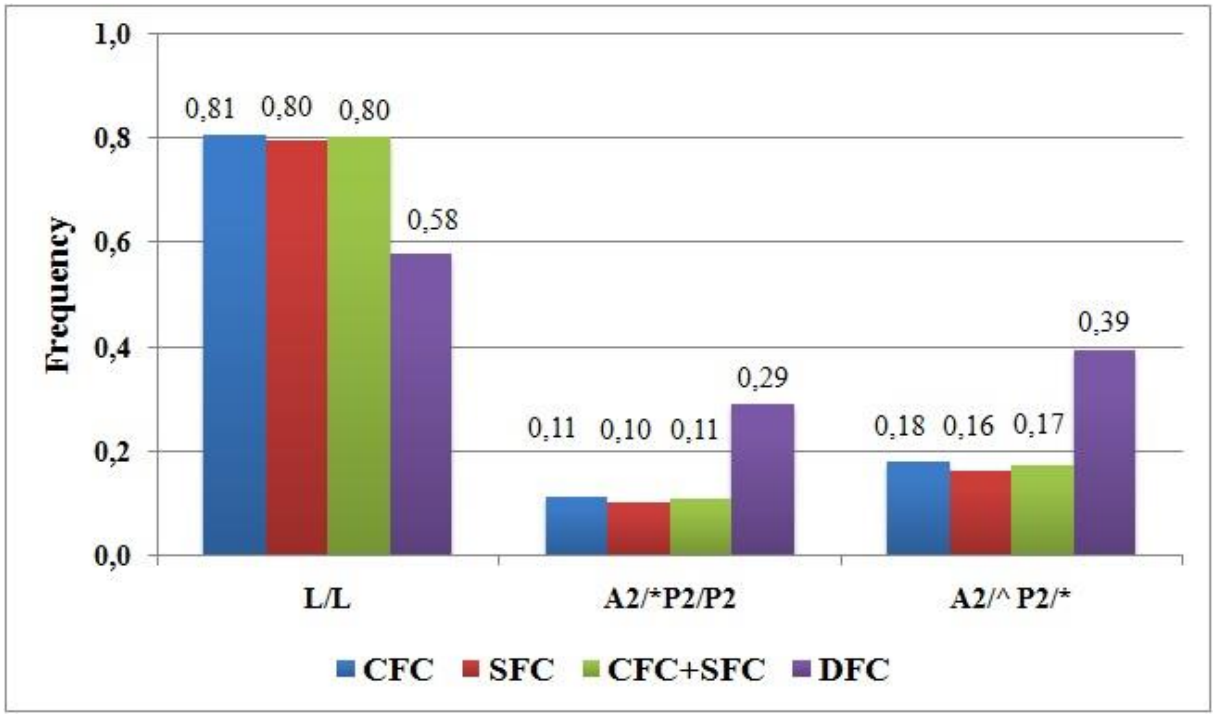

Figure 2 The distribution of *L/L* genotypes (rs2234663; VNTR IL1RN) (*L* - includes alleles *A1* and *A4)*, as well as selected two-locus genotypes (rs2234663, VNTR IL1RN) (rs8179190, VNTR *IL4*) in groups of children with DFC-decompensated form of caries (N = 40), SFC-subcompensated form of caries (N = 51), CFC-compensated form of caries (and healthy children) (N = 78), and combined group CFC (and healthy children) and SFC (N = 129).

The group of children with SFC was similar to the group with CFC (and healthy children) and significantly different from the group with DFC based on the same spectrum of markers of the studied cytokine genes (Figure 2).

The groups of children with DFC and the combined group of children with SFC and CFC (and healthy children) had a significant difference in the presence of the genotype with two "long" alleles L/L (rs2234663), to which the identified alleles A1 and A4 belong (OR = 0.38, p = 0.020, 95% CI: 0.17-0.88). Thus, the L/L genotype provided resistance to the most intensive form of dental caries. A similar statistically significant association was found for the genotype A1/A1 (Table 3).

Figure 2 shows the distribution of genotypes based on *rs2234663, rs2234663* and *rs8179190*, for which significant differences were found between the group of children with DFC and the pooled group of SFC and CFC (and healthy children).

In the three groups, the frequency distribution of double loci genotypes based on *rs2234663* and *rs8179190* was determined, which revealed the prevailing genotypes *A1/A1 P2/P2* and *A1/A1 P1/P2* with frequencies of 43.1% and 30.6% in the group of children with CFC (and healthy children), 47.0% and 22.5% in the group of children with SFC, as well as, 39.5% and 13.2% in the group of children with DFC.

Significant differences were found for the presence of the total group of genotypes homozygous for *P2* and containing at least one *A2* allele, including *A1/A2 P2/P2* and *A2/A2 P2/P2*, between groups of children with DFC and a combined group of children with SFC and CFC (and healthy children) (*OR = 3.38, p = 0 .010; 95% CI: 1.22-9.17*). Significant differences were also found for the presence of genotypes with at least one P2 allele based on rs8179190 and with one A2 allele ("short") based on rs2234663 between groups with DFC and a combined group with SFC and CFC (and healthy children) (*OR = 3.11, p = 0.007; 95% CI: 1.27-7.42*). The genotypes mentioned make the most intensive caries (decompensated form) susceptible.

4. Discussion

The distribution of polymorphic variants of VNTR of the two cytokine genes *IL1RN* and *IL4 i*n the groups of children with DFC, SFC, and CFC (and healthy children) suggested that these markers play a key role in the development of the most intensive caries (decompensated form of caries).

The results of our analysis showed that homozygotes by *L/L* alleles by the VNTR gene IL1RN act as the genotypes that provide resistance to the most intensive form of caries; genotypes of *A1/A2 P2/P2* and *A2/A2 P2/P2,* and genotypes with at least one *P2* allele (*rs8179190*) and one *A2* allele ("short") (*rs2234663*) mediate susceptibility. According to a study [13], the transcriptional activity of IL1RN increases with the number of repeats. The identified associations for VNTR *IL1RN L/L* with DFC resistance were consistent with the results of previous studies, i.e., they generated higher concentrations of IL1RN. According to the results of previous studies, a low concentration of ILRN1 was correlated with caries in combination with a high concentration of IL-1β [4].

Components of *S. mutans* can stimulate monocytes to produce pro-inflammatory cytokines such as IL-1β and IL-α [26]. IL-1ra (IL1RN) can inhibit the activity of IL-1β and IL-α [11, 12]. IL-1β is correlated with caries [27]. In a study [4], the infection rate of *S. mutans* was positively correlated with the level of IL-1β concentration in saliva and negatively correlated with the concentration of IL-1ra. Regarding the immune process involved in the development of caries, the results suggested

that a high concentration of IL-1β and a low concentration of IL-1ra contribute to the development of dental caries, which is associated with the colonization of the oral cavity by *S. mutans* [4].

In our study, the associations with intensive caries for VNTR I*L4* were found only for two-locus genotypes with the participation of the alleles of VNTR IL1RN, which suggested that the ratio between the studied pro-inflammatory and anti-inflammatory cytokines plays a role in the development of caries. According to a study [23], inflammatory diseases can occur with the excessive production of pro-inflammatory cytokines or insufficient production of anti-inflammatory cytokines. Therefore, prolonged inflammation might support the development of caries.

Studies on cytokines can help in the diagnosis and treatment of diseases of the oral cavity. Cytokine level can serve as a diagnostic parameter in the treatment of chronic and acute dental diseases. VNTR *IL4* was found to be significantly associated with periodontitis [22, 23], brucellosis [21], and other diseases, indicating the role of this cytokine in the immunomodulation of the immune response to the infectious agents studied. Our results suggested an association of VNTR *IL4* with high carious lesion activity. According to a study [27], VNTR *IL4* in intron 3 might be a functional polymorphism, as it probably influences the mRNA splicing process, generating different splicing variants.

Our results suggested probable differences in the mechanism of the development of caries with different lesion activity. These results might provide additional information related to the findings of the study on the development of deep carious tooth defects [17] and the involvement of VNTR polymorphisms of the cytokine genes *IL1RN (rs2234663)* and *IL4 (rs8179190)* associated with the development of highly active carious lesions. Further studies on the associations between genetic markers of cytokines (as potential biomarkers of a disease) and dental caries with cytokine spectrum expansion need to be conducted with more individuals to understand the role of cytokines in the development of caries and identifying genetic markers of cytokine genes that contribute to the development of caries. Recent studies on the association of SNPs of cytokine genes with the development of caries revealed SNPs of the *IL1B* gene *(rs1143627)* and *IL1A (rs17561)* as potential biomarkers of the disease [28, 29]. Studies on children with congenital malformations of the orofacial area, e.g., clefts of the lips and the palate, accompanied by various defects of dental development and other pathologies, e.g., with malformations of the central nervous system, are of particular interest. A low level of hygiene is associated with the pathologies mentioned [2]. Therefore, consideration of the immune and immunomodulatory mechanisms of the immune response leading to the development of caries in these groups is relevant.

The development of caries is a very complex process, and further studies are required to identify all the factors and biomarkers for predicting the course of the disease for urgent prevention and treatment of caries, accounting for the individual characteristics of the patients and the earlier stages of the disease [30]. We conducted a pilot study to determine the impact of certain genetic markers on the development of caries. Further studies might investigate the influence of environmental, dietary, and demographic factors on the development of the disease.

5. Conclusions

The genotypes A1/A1 and L/L (rs2234663; 500 bp or 410 bp) can mediate resistance to the most intensive caries. Also, two-locus genotypes (rs2234663 and rs8179190) (A1/A2 P2/P2 and A2/A2

P2/P2) and the genotypes containing A2 (“short”) (rs2234663) and P2 (rs8179190) alleles can mediate susceptibility to the most intensive caries.

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Author Contributions

Udina Irina – the main author, writing of paper, DNA extraction, organizer of the study, Vasiliev Yurii and Volobuev Vladimir, and Gulenko Olga – dental examination, buccal scrapes collecting, Gracheva Alesya – molecular laboratory work, typing of samples, paper editing.

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Competing Interests

The authors state that they have no conflict of interest.

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