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Cytokines' cascade changes in children with hearing loss depending on gap junction protein beta 2 (C.35delG) and interleukin 4 (C-590T) genes polymorphism

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Abstract
The cytokines profile changes depending on genes mutations of connexin 26 (GJB2) (rs80338939) and interleukin 4 (IL-4) (rs 2243250) in children of Bukovina (West Ukraine) with sensorineural (SNHL) or conductive hearing loss (CHL) / deafness were evaluated. An imbalance of the immune response in children with SNHL characterized by inhibition of cellular Th1 immunity with Th2 humoral activation: genetically caused by low production of TNF-α and IL-1β in C-allele carriers of the IL-4 gene and low synthesis of IL-1β with high or normal synthesis of anti-inflammatory IL-4, IL-10 and IL-13 cytokines in mutant 36delG-genotype carriers of CJB2 gene and IL-4 hyperproduction in TT-genotype carriers of the IL-4 gene. CHL course in children is associated with an increase of TNF-α production (TT-genotype of IL-4 gene, p=0.02), which, however, was not enough to stimulate the synthesis of IL-1β, but caused direct compensatory hyperproduction of IL-4 (in T-allele carriers of IL-4 gene and in Non-del-allele carriers of CJB2 gene, p=0.002) with low production of IL-10 and IL-13 (in Non-del-allele carriers of CJB2 gene by 15.55% and 36.34%, $p<0.05$), confirming the presence of acute inflammation with cellular (mainly) and humoral (less) immune response activation.

Keywords: GJB2 (c.35delG), IL-4 (C-590T) genes, hearing loss, deafness, cytokines, immunity.

1. Introduction
WHO highlights serious threat posed by exposure to recreational noise: 1.1 billion people at risk of hearing loss due to the unsafe use of personal audio devices and exposure to damaging levels of environmental sound [1]. Besides this, the WHO estimates 1 child birth with a deafness per 1000 of newborns with normal hearing, and mild or moderate degree hearing loss is found in 1-2% of infants [2]. Resent research from different countries (USA, Canada, Mexico, Britain, Denmark, and Japan) concluded that the rate of congenital hearing loss constitutes from 0, 8 to 15, 5 per 1000 of newborns [3]. In addition, during the first three years of life hearing loss is found in 2-3 more children from this thousand. Hearing loss in children contrary to the adult's results in retardation of speech development, intellectual formation disorders, especially when hearing loss and deafness occur in newborns or in early prelinguistic age. Hearing impairment in the adults is no less problem occurring in 5-14% at the age of 45-64, and in 30% - after 65, and in 50% at the age of 80. Hearing loss developing in the adults is of a multifactorial etiology, and genetic factors occupy an important place among them. Genetic forms of hearing loss can be inherited by autosomal-dominant, autosomal-recessive, X-linked, mitochondrial, and mixed types. Autosomal-recessive hearing loss is the most frequent in general – they constitute more than 70% of all the congenital nonsyndromic hearing impairment [3]. Autosomal-recessive forms of neurosensory (sensorineural) deafness are usually the most severe and almost in all the cases are due to cochlear defects. More than 50% of autosomal-recessive forms of hearing loss are due to mutations in *CJB2* gene (gap junction protein, beta 2), localized in the chromosome 13q11-q12 in position 19659614-19665037 [4]. CJB2 codes Connexin 26 (Cx26) – a membranous protein forming the canal with six subunits. Two semi-canals of adjacent cells (so-called gap junction) forms the connection which provides a passive diffusion of electrolytes, secondary messengers and metabolites (small molecules with the size of 1kDa and less) between the cells' cytoplasm ensuing a local audio homeostasis.
Among the other causes of hearing loss with possible genetic determinant there are chronic

inflammatory diseases of the middle and external ear which accompany by the immune system disorders. Numerous studies demonstrate the immune response dependence from cytokine genes allele state and associate with corresponding cytokines production level [5-7], disease course, complications appearance and specific immune response. However, the questions of association of certain and combined gene mutations of CJB2 and cytokines with the lymphocyte-macrophage system function in children with hearing loss remain underestimated, requiring further studies with the aim of early diagnostics, prognostics and treatment.

Consequently, the aim of our study was to analyze the cytokines cascade regulatory mechanisms depending on genes mutations of connexin 26 (GJB2) (rs80338939) and interleukin 4 (IL-4) (rs2243250) in children with sensorineural (SNHL) or conductive hearing loss (CHL).

2. Materials and Methods

2.1. Compliance with bioethics

Study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics of the Ministry of Health of Ukraine. Patients' Examination Cards and Patients' Informed Consent Forms were approved by the Biomedical Ethics Commission of Bukovina State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). All enrolled subjects were studying in the Municipal "Chernivtsi Special boarding school №2" for children with hearing impairment (Chernivtsi, Ukraine) during 2013-2015 y.y. Genetic bench study performed in the laboratory of Medical Biology and Genetics Department of Bukovina State Medical University. After screening (matching inclusion/exclusion criteria) 102 children with hearing loss / deafness were selected for further examination. The control group included 60 practically healthy individuals who had no hearing impairment and inflammatory diseases at any location during the last 6 months; without reliable differences of sex and age with study group.

2.2. Inclusion / Exclusion criteria

Inclusion criteria. Children of 8-18 years old with sensorineural (SNHL) or conductive hearing loss (CHL) / deafness: severe deafness - hearing loss ≥71 dB, moderate hearing loss (III-IV degree) - hearing loss 41-70 dB; no exacerbation of possible chronic out of ear inflammatory disease; voluntary consent sign by children's parents to participate in the study.

***Exclusion criteria*:** We excluded patients younger than 8 y.o. and older than 18 y.o.; hearing impairment ≤41 dB; treating with antibiotics at the moment of inclusion; presenting symptoms of acute inflammatory diseases in non-ENT organs, or 6 months earlier had such kind of disorders; subjects with anatomical impaired ear canal patency; psychological disorders.

2.3. Diagnosis of sensorineural or conductive hearing loss / deafness

Screening of children and distribution into groups depending on conductive hearing loss types was performed according to the International Recommendations (National US Institute on Deafness and Other Communication Disorders, World Health Organization) [8,9], as well as current Orders of the Ministry of Public Health of Ukraine (Ukrainian protocols "Children's otolaryngology") [10,11]. The diagnosis of sensorineural or conductive hearing loss (SNHL, CHL) was made on the basis of the acting national and international recommendations criteria [8-11]: on the basis of otoscope, speech audiometry (spoken and whispered speech), tone audiometry (air and bone conduction), camerton (tuning fork) evaluation, tympanometry. Processus mastoideus, paranasal sinuses in two projections and chest X-rays' examination was performed additionally in case of necessities.

2.4. Cytokines level investigation

The tumor necrosis factor α (TNF-α), IL-1β, IL-4, IL-10 and IL-13 levels in pg/ml where detected in blood plasma by Immuno-enzyme method (ELISA) with the set of reagents of "Vector Best" (RU, ISO certificates 9001, 13485). Informing of immune system determined by the immunological disorders degree (IDD) for each indicator: IDD = (patient index /control group indicator - 1) x 100%. Negative ("-") index was testified as immunodeficiency, a "+" testified as immune system hyperactivity. The value of the result within 1-33% interpreted as IDD I grade, 34-66,7% - II degree, more than 66,7% - IIIrd degree.

2.5. Genotyping of the CJB2 (c.36delG) and IL-4 (C-590T) polymorphisms

Alleles of the polymorphic areas of CJB2 (c.36delG) and IL-4 (C-590T) genes were studied by means of Genomic DNA extraction from the peripheral blood leukocytes using the "DNA-sorb-B" test system, with primers specific to the genes' alleles (Table 1). Amplified polymorphic locus was detected by polymerase chain reaction (PCR) on “Amply-4L” amplificatory according to the manufacturer’s protocol. The PCR products were digested overnight by restriction endonucleases MvaI for non-35delG-allele+ of CJB2 gene and AvaII for C-allele+ of IL-4 gene ("Thermo Scientific", USA) at 37 °C. The PCR products (for CJB2 gene: non-35delG - 60, 29 bp, 35delG – 89 bp; for IL-4 gene: TT genotype - 195 bp, CC – 177 and 18 bp, CT – 195, 177 and 18 bp) were separated by horizontal electrophoresis on 3% agarose gels, stained with 4 μl of ethidium-bromide and visualized by in the presence of molecular mass ladder (50-1000 bp) using a UV transilluminatior (Nyxtechnic, USA).

Table 1: Primer sequences, restriction enzymes and allele, genotypes calling for GJB2 and IL-4 SNPs

Genes, SNP	Restriction enzyme	Primers	Primer sequences (5’-3’)	Fragments size, bp
CJB2 gene c.36delG (rs80338939)	MvaI (BstNI)	Forward	CTTTTCCAGAGCAAACCGCCC	non-35delG: 60, 29 bp; 35delG: 89 bp
		Reverse	TGCTGGTGGAGTGTTTGTTCAC	
IL-4 gene C-590T (rs 2243250)	AvaII	Forward	TAAACTTGGGAGAACATGGT	CC: 177, 18bp; CT: 195, 177, 18 bp; TT: 195 bp
		Reverse	TGGGGAAAGATAGAGTAATA	

2.6. Statistical analysis

Statistical analysis was performed using Statistica 7.0 (StatSoft Inc, USA) software. Reliability of the data for independent quantitative sampling was calculated using Student's t-test (if distribution by Kolmogorov-Smirnov and W-Shapiro-Wilk test was close to the normal) and U-test *Wilcoxon-Mann-Whitney* (in case of irregular distribution), analysis of qualitative data (categorical variables) – by odds ratio (OR), with 95% confidence interval (CI) using a chi-square test (χ^2) (df=1). P values <0.05 were considered statistically significant.

3. Results and Discussions

Prospective Study included 102 children with hearing impairment: 68 with SNHL and 34 with CHL, among them 36 (35.29%) girls and 66 (64.71%) boys. The age of patients vary from 8 to 18 (on the average 13.90 ± 3.11 y.o.). The control group included practically healthy children (n=60) of the following gender distribution: girls - 22 (36.67%), boys - 38 (63, 33%) (χ^2<1,0, p>0.05), who had no hearing loss of inflammatory diseases of any location during the last 6 months.

SNHL and CHL course in children is associated with a decreased concentration of IL-1β in the peripheral venous blood plasma by 36.06% and 29.53%, increasing of IL-4 in 1.69 (p<0.05) and 2.68 times (p=0.013) and different changes of TNFα content (increases in CHL children, reduces in SNHL cases), IL-10 and IL-13 (contrary, it increases in SNHL children and decreases in CHL subjects) (Table 2). The cytokines content level considered as: low (lower quartile of the control group) for the IL-1β less than 23 pg/ml, for TNFα - ≤15 pg/ml, IL-4 -≤4,95 pg/ml, IL-10 and IL-13 - ≤15 pg/mlл and ≤28 pg/ml respectively; high (upper quartile of the control group) for TNFα more than 32 pg/ml, for IL-1β -≥60 pg/ml, IL-4 - ≥45 pg/ml, IL-10 and IL-13 - ≥25,96 pg/ml and ≥38 pg/ml, respectively.

Table 2: Cytokines' plasma content in children with hearing loss, M±S.D.

Cytokines	Control	Sensorineural hearing loss	IDD	Conductive hearing loss	IDD
IL-1β, pg/ml	33.22±7.91	21.24±4.23	-II	23.41±5.31	-I
TNFα, pg/ml	29.01±6.38	22.85±6.56	-I	38.38±5.98 p_1=0.048	+I
IL-4, pg/ml	24.61±7.25	41.56±7.95 p<0.05	+III	65.88±14.28 p=0.013 p_1=0.037	+III
IL-10, pg/ml	22.57±7.36	40.21±9.33	+III	14.37±3.49 p_1=0.031	-II
IL-13, pg/ml	30.45±4.82	32.52±6.06	+I	14.37±2.11 p<0.001 p_1=0.018	-II

Notes: 1. IDD - Immunological Disorders Degree; SNHL - sensorineural hearing loss; CHL - conductive hearing loss. 2. p – reliability of differences concerning control group; p_1 – reliability of differences concerning children with sensorineural hearing loss / deafness (SNHL).

In SNHL children decreased pro-inflammatory IL-1β and TNFα production was found in 45 (66.18%) and 36 children (52.94%) against the anti-inflammatory IL-4 level increasing in 32 (47.06%) and IL-10 – in 36 (52.94%) children, with no reliable IL-13 level increase – in 28 (41.18%) persons (Table 3). In CHL children the TNFα and IL-4 production increasing was found in 16 (47.06%) and 12 (35, 29%) persons, on the basis of decreased IL-10 and IL-13 production - in 18 (52.94%) and 34 (100%) children respectively.

Table 3: Levels of cytokines production in children with hearing loss

Cytokines		Control group	Low production, n (%)	High production, n (%)	Normal production, n (%)
IL-1β	SNHL, n	33.22±7.91	45 (66.18)	4 (5.88)	19 (27.94)
	CHL, n		20 (58.82)	4 (11.76)	10 (29.41)
TNFα	SNHL, n	29.01±6.38	36 (52.94)	16 (23.53)	16 (23.53)
	CHL, n		12 (35.29)	16 (47.06)	6 (17.65)
IL-4	SNHL, n	24.61±7.25	8 (11.76)	32 (47.06)	28 (41.18)
	CHL, n		8 (23.53)	12 (35.29)	14 (41.18)
IL-10	SNHL, n	22.57±7.36	11 (16.18)	36 (52.94)	21 (30.88)
	CHL, n		18 (52.94)	0	16 (47.06)
IL-13	SNHL, n	30.45±4.82	16 (23.53)	28 (41.18)	24 (35.29)
	CHL, n		34 (100.0)	0	0

Note. SNHL - sensorineural hearing loss; CHL - conductive hearing loss.

The analysis of genotype distribution of IL-4 gene (C-590T) and Connexin gene 26 CJB2 (c.35delG) has been conducted to define the role of genetic constituent in cytokine production changes (Table 4). Among the SNHL children with a low TNFα production dominate CC- and CT-genotypes carriers of IL-4 gene over those with TT-genotype: 22.06% and 26.47% versus 4.41% (χ^2=34.0, p<0.001), and over C-allele carriers with a high TNFα production – 8.82% and 14.71% (χ^2=5.23, p=0.022 and χ^2=3.52, p=0.05, respectively). The synthesis of IL-1β in SNHL children was analogical: a low production was found more often in C-allele carriers of IL-4 gene, than in mutant T-allele homozygotes (by 33.82% and 29.41% vs 2.94%, χ^2=55.83, p<0.001). Low IL-1β levels were produced more often by the mutant 36delG-genotype carriers of CJB2 gene, than Non-del-carriers by 30.35% (χ^2=8.91, p=0.003). Reliable differences in a low production of TNFα depending on the allele condition of CJB2 gene (c.35delG) in SNHL children were not found. On the contrary, the low TNFα and IL-1β production persons prevailed over those with a high one: for TNFα for Non-del carriers – by 16.18% (χ^2=5.39, p=0.02) and for 36delG-genotype carriers by 13.24% (χ^2=4.29, p=0.038) respectively, for IL-1β – by 27.94% (χ^2=19.13, p<0.001) and 32.35% (χ^2=23.36, p<0.001), respectively. The SNHL children more often presented normal or compensatory IL-4 hyperproduction in 88.23% persons, with higher frequency in TT-genotype vs C-allele carriers by 30.55% (p=0.038). No reliable differences in the IL-10 and IL-13 high

production depending on IL-4 gene allele condition were found. On the contrary, the CJB2 gene: a relatively high and normal IL-4, IL-10 and IL-13 productions were found in SNHL children deletion genotype (36delG) carriers by 36.27% (χ^2=12.95, p<0.001), 36.76% (χ^2=13.07, p<0.001) and 40.99% (χ^2=9.76, p=0.002) respectively.

In CHL children (Tables 3, 4) was found a greater part of high TNFα production children (prevailed among the TT-genotype carriers by 29.45%, p=0, 02). The number of CHL children with a high/normal IL-4 level prevailed over those with a low level 3.25 times (χ^2=19.06, p<0.001): in T-allele carriers 32.25% vs 15.0% with CC-genotype of IL-4 gene (χ^2=3.81, p=0.05). In CHL children with a high TNFα production was accompanied by a low IL-10 and IL-13 synthesis, without reliable differences between genotypes of IL-4 gene. However, it should be noted, that among the individuals with a high and normal IL-1β production were exclusively persons with Non-del-allele of CJB2 gene (100%). More frequently in CHL children the TNFα and IL-4 increased / normal synthesis and low IL-10 and IL-13 production were found in the Non-del-allele carriers, than in the 36delG-genotype carriers: for TNFα – 31.25% vs 5.26% (χ^2=9.52, p=0.002), for IL-4 – 35.94% vs 7.89% (χ^2=9.87, p=0.002), for IL-10 – 23.44% vs 7.89% (χ^2=5.22, p=0.022), for IL-13 – 46.87% vs 10.53% (χ^2=14.18, p<0.001).

Children with SNHL suffered with comorbid chronic non-obstructive and obstructive upper and lower respiratory tract diseases more often than those with CHL: 86.76% vs 44.12% (χ^2=18.89, p<0.001); additionally, SNHL children presented more often comorbid gastro-intestinal pathology, endocrine system and vestibular disorders, pathology of the central and peripheral nervous systems.

Table 4: The genotypes distribution of IL-4 gene C-590T polymorphism and GJB2 gene c.35delG polymorphism depending on the cytokine production level

Groups		Low production, n (%)		High production, n (%)		Normal production, n (%)	
		SNHL	CHL	SNHL	CHL	SNHL	CHL
TNFα, pg/ml		**≤15 pg/ml**		**>32 pg/ml**			
Gene IL-4	CC, n=40	15 (22.06)	5(14.71)	6 (8.82)	5(14.71)	8 (11.76)	1 (2.94)
	CT, n=50	18 (26.47)	7(20.59)	10 (14.71)	6(17.65)	4 (5.88)	5(14.71)
	TT, n=12	3 (4.41)	0	0	5(14.71)	4 (5.88)	0
Gene GJB2	Non-del, n=64	20 (29.41)	10(29.41)	9 (13.23)	15(44.12)	5 (7.35)	5(14.71)
	35delG, n=38	16 (23.53)	2 (5.88)	7 (10.29)	1 (2.94)	11(16.18)	1 (2.94)
IL-1β, pg/ml		**≤23 pg/ml**		**≥60 pg/ml**			
Gene IL-4	CC, n=40	23 (33.82)	7(20.59)	2 (2.94)	3 (8.82)	4 (5.88)	1 (2.94)
	CT, n=50	20 (29.41)	12(35.29)	0	1 (2.94)	12 (17.65)	5(14.71)
	TT, n=12	2 (2.94)	1 (2.94)	2 (2.94)	0	3 (4.41)	4(11.76)
Gene GJB2	Non-del, n=64	21 (30.88)	16(47.06)	2 (2.94)	4(11.76)	11(16.18)	10(29.41)
	35delG, n=38	24 (35.29)	4(11.76)	2 (2.94)	0	8 (11.76)	0
IL-4, pg/ml		**≤4.95 pg/ml**		**≥45 pg/ml**			
Gene IL-4	CC, n=40	3 (4.41)	5(14.71)	8 (11.76)	2 (5.88)	18 (26.47)	4(11.76)
	CT, n=50	5 (7.35)	3 (8.82)	17 (25.0)	5(14.71)	10 (14.71)	10(29.41)
	TT, n=12	0	0	7 (10.29)	5(14.71)	0	0
Gene GJB2	Non-del, n=64	5 (7.35)	7 (20.59)	17 (25.0)	12(35.29)	12 (17.65)	11(32.35)
	35delG, n=38	3 (4.41)	1(2.94)	15 (22.06)	0	16 (23.53)	3 (8.82)
IL-10, pg/ml		**≤15 pg/ml**		**≥25.96 pg/ml**			
Gene IL-4	CC, n=40	8 (11.76)	6(17.65)	4 (5.88)	0	17 (25.0)	5(14.71)
	CT, n=50	2 (2.94)	8(23.53)	27 (39.71)	0	3 (4.41)	10(29.41)
	TT, n=12	1 (1.47)	4(11.76)	5 (7.35)	0	1 (1.47)	1 (2.94)
Gene GJB2	Non-del, n=64	7 (10.29)	15(44.12)	20 (29.41)	0	7 (10.29)	15(44.12)
	35delG, n=38	4 (5.88)	3 (8.82)	16 (23.53)	0	14 (20.59)	1 (2.94)
IL-13, pg/ml		**≤28 pg/ml**		**≥38 pg/ml**			
Gene IL-4	CC, n=40	7 (10.29)	11(32.35)	13 (19.12)	0	9 (13.23)	0
	CT, n=50	7 (10.29)	18(52.94)	13 (19.12)	0	12 (17.65)	0
	TT, n=12	2 (2.94)	5(14.71)	2 (2.94)	0	3 (4.41)	0
Gene GJB2	Non-del, n=64	9 (13.23)	30(88.24)	12 (17.65)	0	13 (19.12)	0
	35delG, n=38	7 (10.29)	4(11.76)	16 (23.53)	0	11 (16.18)	0

Note. SNHL - sensorineural hearing loss; CHL - conductive hearing loss.

The evidence concerning cytokine imbalance between the type 1 T-lymphocyte-helpers (Th1) and type 2 (Th2) detects the direction of the immune response disorders [12-14]. In current research the SNHL is associated with TNFα concentration decrease, that indicates a lower Th1 activity and T-lymphocytes and macrophages function reduce (inhibition of the cellular type immunity response), and caused a low IL-1β production. In its turn, IL-1β is a factor of activation, growth and maturation of T- and B-lymphocytes, NK-cells, fibroblasts, endothelial cells; it interacts with Th2 and induces the synthesis of IL-3, IL-4, IL-5, IL-6, IL-8, IF-γ, expression of IL-2 receptors, increases secretion of antibodies by B-lymphocytes, causes chemotaxes of macrophages, neutrophils, promotes their migration through the vessels endothelium into the inflammatory focus, where it activates synthesis of cytokines, prostaglandins, collagen and fibronectin, acute phase proteins (C-reactive, etc.), presents a pyrogenic action [15-17]. On the contrary, we found compensatory IL-4 content production (the B-lymphocytes growth factor) via alternative ways (is excreted in general by Th2) and increased number of individuals with a high IL-10 concentration (known as the Th1 cells activity inhibitor) and IL-13 in the blood plasma (mostly the product of Th2). IL-10 promotes the humoral response via stimulating B-lymphocytes, mast cells and inhibits cellular

immune response. IL-13 stimulates the B-cells growth, but it inhibits the differentiation of Th1 with deviation for Th2 formation; it inhibits macrophage response and synthesis of pro-inflammatory cytokines (IL-1, IL-2, IL-6, IL-8, TNF-α), that was observed in our study. IL-13 also promotes the production of Ig and switching antibody synthesis for IgE. The cytokine cascade changes explain partially the high comorbid pathology frequency of the upper and lower respiratory tract, endocrine system disorders etc. in SNHL children [16]. Thus, the obtained results in SNHL patients indicate a decreased activity of nonspecific anti-infectious immune defense factors, high body reactivity as well as increased risk of additional allergic mechanisms of immune response. At the same time, there is an insufficient cellular immune response activity to eliminate possible pathogen, high probability of primary chronic course of any comorbid infectious disease increasing the risk of bacterial complications or allergic reactions.

In our research the CHL course in children is associated with increased production of TNF-α (in the TT-genotype carriers of IL-4 gene, p=0,02), which although, was not sufficient for the stimulation of IL-1β synthesis, but caused a direct compensatory hyperproduction of IL-4 (in the T-allele carriers of IL-4 gene and the Non-del-allele carriers of CJB2 gene, p=0,002), as a compensatory cytokine of an early immune response to inhibit cellular immunity chain against a low IL-10 and IL-13 production (more often in the Non-del-allele carriers of CJB2 gene, than in those with 36delG-genotype by 15,55% and 36,34%, respectively, $p<0,05$). Thus, our results indicate the presence of an acute inflammatory process in CHL children with activation of cellular (mostly) and humoral (less) immune response.

4. Conclusion

Immune response imbalance in SNHL children is characterized by cellular Th1 immunity system inhibition and Th2: hereditary caused TNF-α and IL-1β low production in the C-allele carriers of IL-4 gene, as well as low IL-1β synthesis and high or normal anti-inflammatory cytokines IL-4, IL-10 and IL-13 synthesis in the mutant 36delG-genotype carriers of CJB2 gene, and excessive IL-4 production in the TT-genotype carriers of IL-4 gene. CHL in children develops with both cellular (mostly) and humoral (less) immune response activation followed by increased synthesis of TNF-α (in the TT-genotype carriers of IL-4 gene) and compensatory anti-inflammatory IL-4 excessive production (in the T-allele carriers of IL-4 gene and Non-del-allele carriers of CJB2 gene), low IL-10 and IL-13 levels (in the Non-del-allele carriers of CJB2 gene), that indicate the presence of acute inflammatory process.

In perspective we plan to analyze the association of haplotypes of GJB2 (rs 80338939) and IL-4 (rs 2243250) genes with risk factors of hearing loss and immunogram indexes changes.

Conflict of Interest: None declared.

5. Acknowledgement

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