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NEW PROGNOSTIC MARKERS OF HEARING IMPAIRMENT IN CHILDREN: GENE-GENE INTERACTION AND APPROXIMATION MODELS

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Abstract

Objective: to evaluate the gene-gene interaction, assess the risks and develop some approximation models of hearing loss / deafness occurrence in children, depending on the genes polymorphism gab junction B2 (*GJB2,* rs80338939), and interleukin-4 (*IL-4*, rs 2243250) and other risk factors.

Materials and methods: Study included 102 children with hearing impairment: 68 with sensorineural (SNHL) and 34 with conductive hearing loss (CHL), among them 36 (35.29%) girls and 66 (64.71%) boys. The patients' age vary from 8 to 18 yo (on the average 13.90±3.11 yo). Diagnosis set by otorhinolaryngology (ENT) methods: ENT examination, computer audiometry, impedancemetry, tympanometry. The control group included 60 practically healthy children: 22 girls (36.67%), 38 boys (63.33%). Polymorphism of *GJB2* (rs80338939) and *IL-4* (rs 2243250) genes was studied by polymerase chain reaction method. Risk assessed by Relative Risk, Odds Ratio and 95% Confidential intervals.

Results. The combination of 35delG / TT, as well as Non-Del / TT and 35delG/ TC genotypes in the genome is associated with a high risk of hearing loss in general children population (from 0.932 to 1.432; OR=19.5; p=0.003), as well as the appearance SNHL (from

0.765 to 1.765), stronger than the combination of unfavorable homozygotes TT / 35DelG - 1.765. The combination of homozygotes for the wild allele of both genes (especially CC / Non-Del) is associated with a low risk of deafness: hearing loss in general -1,068, for SNHL - 0,908, for CHL -0,750 ($p<0,01$), for CC / 35delG combination, or TC / Non-Del: in general - 0.068 -, and for SNHL -0.235 and -0.11 respectively, ($p>0.05$).

Infectious diseases in anamnesis (meningitis, measles, mumps, or rubella) increases the likelihood of CHL by 9.41 times (OR=12.0; p=0.007). Concomitant chronic non-obstructive and obstructive upper and lower respiratory tract diseases increase the risk of both SNHL and CHL in children regardless of age: for SNHL 3.75-7.81 times (OR=6.50-10.9; $p\leq0.028$-0.01), for CHL – 4.29-8.75 times (OR=6.19-12.9; $p\leq0.03$-0.009). The revealed dependence of the indicators is best described by the logit-regression approximating models with high multiple correlation coefficient ($R^2=0.9761$); low standard error of the model estimation ($\varepsilon=0.1114$); connection criterion F=124.2; degrees of freedom df=7.43 ($p<0.001$).

Conclusions: Genes polymorphism's *GJB2* (rs80338939) and *IL-4* (rs 2243250) and their interactions are new prognostic markers of hearing impairment in children. Approximating models describe the likelihood of SNHL and CHL in observed population.

Key words: sensorineural; conductive hearing loss; deafness; genes *GJB2*; IL-4; markers; approximation; models; prognosis; children.

Introduction

Nowadays 466 million people are estimated to be living with hearing loss (6.1% of the world's population). More than 5% - 360 million people (328 million adults and 32 million children), suffer from severe hearing loss [1-3]. Such hearing loss is understood as hearing loss in the better hearing ear, which exceeds 40 dB in adults and 30 dB in children. Hearing loss means if persons are not able to hear as well as someone with normal hearing, meaning hearing thresholds of 20 dB or better in both ears. It can be mild, moderate, moderately severe, severe or profound, and can affect one or both ears. Major causes of hearing loss include congenital (non-syndromic sensorineural) or early onset childhood hearing loss, chronic middle ear infections (conductive hearing loss), noise-induced hearing loss, age-related hearing loss, and ototoxic drugs that damage the inner ear. More than 1 million young people (12-35 years) are at risk for hearing loss due to recreational exposure to the loud sound. Approximately one out of three people over the age of 65 suffers from severe hearing loss. The highest prevalence of this condition in this age group is observed in South Asia, the

Pacific region of Asia and in sub-Saharan Africa. 750 million USD is the overall annual cost of unaddressed hearing loss globally [1-3].

Despite the fact that the main congenital or acquired causes of hearing loss are sufficiently studied, but the influence of risk factors such as implementing, causal, moderating factors, mediators, independent, synergistic, antagonistic, etc., in the development of pathology continues to be studied [1-6]. In addition, the degree of hearing loss (from a mild decrease to complete deafness) depends on the strength of the pathogenic factor, the duration of its action, as well as the number of adverse factors. Such predictors can be a direct cause of hearing loss, or contribute to its implementation. One of such possible factors is the gene's polymorphism's of gap junction protein Beta 2 (GJB2, p.35delG, rs80338939) it encodes the connexin 26, a transmembrane protein involved in cell-cell attachment of almost all tissues and gene Interleukin 4 (IL-4, C-590T, rs 2243250). However, the contribution of each potential factor in the development of sensorineural or conductive hearing loss (SNHL, CHL), including mentioned above genes, needs further research.

Therefore, **the objective** of the study was to evaluate the gene-gene interaction, assess the risks and develop some approximation models of hearing loss / deafness occurrence in children, depending on the genes polymorphism *GJB2* (rs80338939), *IL-4* (rs 2243250) and other risk factors.

Material and methods

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). After screening (matching inclusion/exclusion criteria) 102 children with hearing loss / deafness were selected for further examination. All criteria had been presented in our former research [7-13]. 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. Diagnosis set by otorhinolaryngology (ENT) methods: ENT examination, computer audiometry, impedancemetry, tympanometry.

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 specific primers [10-14]. Amplified polymorphic locus was detected by polymerase chain reaction (PCR) on "Amply-4L" amplificator 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 in 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).

Statistical analysis was performed using Statistica 7.0 (StatSoft Inc, USA) software. To study the gene-gene interactions and build a model of its influence on the appearance of a certain type of deafness in children in the population the Multifactory Demension Reduction method (MDR 3.0.2) with the calculation of prediction potentials was used to evaluate gene-gene interaction influence on healing loss. The analysis of qualitative data (categorical variables), epidemiological risk factors was evaluated 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.

Results and discussion

In children with SNHL, the risk of deafness increases 10-fold in the presence of *35delG*-genotype of the *GJB2* gene (OR=19.0; 95%CI OR: 5.42-66.62; p<0.001). Whereas the Non-del allele carrier on the contrary is protective and makes the chances of SNHL developing the lowest in the surveyed population (OR=0.53; 95%CI OR: 0.02-0.18; p<0.001). The analyzed *IL-4* gene polymorphism (rs2243250) is not associated with the risk of SNHL in the population.

Allelic variants of the *GJB2* gene (rs 80338939) are not a risk factor for CHL. Whereas the *TT*-genotype of the *IL-4* gene increases marginally the probability of CHL in the population 4.41 times (OR=3.5; 95% CI OR: 0.95-27.35; p=0.053) and, conversely, in *CC*-genotype carriers there are low chances of CHL developing (OR=0.42; 95% CI OR: 0.17-1.01; p=0.0497).

According to the MDR method's results, the best models of gene-gene interaction depending on the deafness type with the highest cross-validation consistency are shown in Table 1. The reproducibility of the one-factor model involving the *GJB2* gene was 80% (8 / 10) with high accuracy in predicting the SNHL risk in children - 75.24%, for other models, reproducibility was 100% (10/10). However, for CHL, the one- and two-component models had lower accuracy (50.45% and 59.91%, respectively) with 100% reproducibility, while for the two-factor model (both genes *GJB2, IL-4*) the permutation test did not confirm its

probability. Cross-validation Testing T-statistic (CV-TT) was the highest for SNHL and deafness in general (both types) predicting in children in a model that included both *GJB2* and *IL-4* genes likewise (CV-TT=8.16 and 11.34, respectively).

Table 1

Models of gene-gene interaction depending on the deafness types and in general among the surveyed

Deafness type	Genes combination in prognostic model	Model reproduce-bility	Cross-validation Testing	Model accuracy,%	OR [95%CI OR]; p
SNHL	*GJB2*	8/10	4.24	75.24	*OR*=5.96 [5.82-6.13]; p=0.016
	GJB2, IL-4	10/10	8.16	79.17	*OR*=8.80 [8.63-8.94]; p=0.022
CHL	*IL-4*	10/10	4.62	50.45	*OR*=15.0 [14.2-15.7]; p=0.018
	GJB2, IL-4	10/10	3.27	59.91	*OR*=1.14 [0.45-1.97]; p>0.05
Totally	*IL-4*	10/10	5.83	57.72	*OR*=3.89 [3.11-4.83]; p=0.044
	GJB2, IL-4	10/10	11.34	72.73	*OR*=19.5 [18.6-20.3]; p=0.003

Note. SNHL – sensorineural hearing loss; CHL – conductive hearing loss; *OR* - Odds Ratio); *95%CI OR* - Odds Ratio Confidence Interval.

To assess the complex effect of the studied gene loci on the deafness occurrence in the population, as well as to predict its risk, a graphical model of gene interaction was created using the MDR method. Figure 1 shows a combination of genotypes of the analyzed genes, where the *Non-del* variant (no mutation) of the *GJB2* gene is marked as "0", the homozygous *35delG* variant as "1". Regarding the *IL-4* gene: *CC*-genotype - "0", *TC*-genotype - "1", *TT*-genotype - "2". The given graphical classification model had a prognostic accuracy of 72.73% on the tested sample (Testing Balanced Accuracy) with Cross-validation Consistency 11.34, 100% reproducibility (10/10). It was found that the combination of homozygotes for the minor alleles of both genes (dark gray cells in Figure 1) and *35delG* homozygotes of the *GJB2* gene with the *TC*-genotype of the IL-4 gene is associated with a high risk of hearing loss in the examined population of children (from 0.932 to 1.432). Instead, the combination of wild-type homozygotes of both genes (*CC* / *Non-Del*), or at least one of them (*CC* / *35delG*, or *TC* / *Non-Del*), is associated with a low risk of deafness (from -1,068 to -0,068).

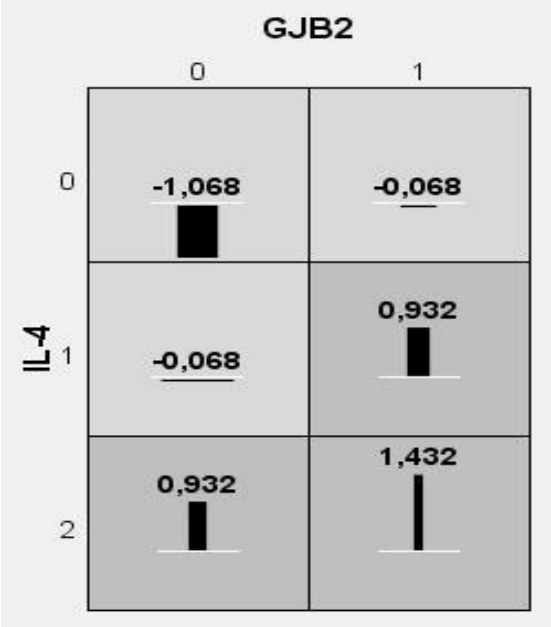

Fig. 1. A combination of polymorphic variants of genes *GJB2* (*c.35delG*) and *IL-4* (*C-590T*), which causes a high (dark gray cells) and low (light gray cells) risk of hearing loss. *IL-4* 0, 1, 2 - polymorphic variants of the gene *IL-4* CC, TC, TT; *GJB2* 0, 1 - polymorphic variants of the *GJB2* gene: 0 - *Non-Del*, 1 - *35delG* variant.

The circular graph of cluster analysis results' of gene-gene interaction modeling by MDR method is shown in Figure 2.

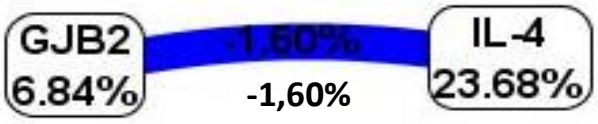

Fig. 2. Cluster analysis of gene-gene interaction modeling by Multifactory Demension Reduction method (MDR)

The nature of interlocus interaction between genes was non-synergistic, but at the level of "independent effects" of influence (-1.60%). The entropy fraction of the studied polymorphism of each gene relative to the case-control status was 6.84% for the *GJB2* gene and 23.68% for the *IL-4* gene, and testified to the high significance of the latter and its significant influence on deafness appearance in the population. The classification ability of the created two-component model for children with SNHL and CHL, despite 100% reproducibility (10/10), confirmed the probability only for the two-factors model for SNHL (OR=8.80; p=0.022) with an accuracy of 79.17%, testing Cross-validation Consistency 8.16:

- the combination of homozygotes for the minor allele of both genes (*35delG* / *TT*), as well as *Non-Del* / *TT* and *35delG* / *TC* variants in the genome are associated with a high risk of hearing loss in the general population of examined children (from 0.932 to 1.432; OR=19.5; p=0.003), as well as the SNHL appearance (from 0.765 to 1.765), the highest score – for unfavorable homozygotes combination of *TT* / *35DelG* – 1.765;

- combinations of homozygotes for the wild allele of both genes are associated with a low risk of deafness: stronger in *CC* / *Non-Del* combination carriers (-1.068 for deafness in

general, -0.908 – for SNHL, -0,750 – for CHL, p≤0.005), less in *CC* / *35delG*, or *TC* / *Non-Del* variants carriers (-0.068 – in general, -0.235 and -0.11 – for SNHL, p>0.05).

We found that infectious diseases in anamnesis (meningitis, measles, mumps, or rubella) increased the likelihood of CHL 9.41 times (OR=12.0; p=0.007). Psychoneurological disorders recorded in SNHL children 1.25-3.36 times more common, than in CHL persons (p≤0.05-0.001), but they were not a risk factor, as well as endocrine pathology. Concomitant pathology: chronic non-obstructive and obstructive diseases of the upper and lower respiratory tract (CNPD, COPD) increased the risk of both SNHL and CHL in children regardless of age: for SNHL 3.75-7.81 times (OR=6.50-10.9; p≤0.028-0.01), for CHL – 4.29-8.75 times (OR=6.19-12.9; p≤0.03-0.009); a slightly stronger effect of CNPD on the hearing impairment formation was observed in children under 12 years old (OR=10.5; p=0.026 and OR=12.9; p=0.009), and lower respiratory tract COPD increased the SNHL risk 6.35 times (OR=10.3; p=0.01) in children over 12 yo.

Nonparametric correlation analysis by Kendall Tau (τ) and Gamma (γ) coefficients showed a close connection between SNHL and a family history burden of hearing impairment in both parents, or separately (p≤0.003-0.007), as well as in relatives (p=0.0007). In addition, the development of SNHL is associated with chronic tonsillitis (p<0.001) and causes a language barrier (p<0.001). Regarding CHL, the only statistically significant strong connection of its development was observed in the presence of chronic otitis, or their exacerbation (γ=0.76, p<0.001). Regression analysis of correlations by Kendall Tau (τ) and Gamma (γ) coefficients showed a direct strong association of SNHL with chronic diseases of the upper and lower respiratory tract, stronger upper respiratory tract (γ=0.92; p<0.001) and CNPD of lower respiratory tract (γ=1.0; p=0.003), also with concomitant pathology of the gastrointestinal tract, cardio-vascular system, eyes, endocrine and nervous systems, vestibular disorders. Also, a strong association of the SNHL development in children has been established with maternal infections during pregnancy, low body weight, or the asphyxia at birth appearance; severe jaundice at birth was also moderately directly correlated with SNHL. CHL in the subjects moderately correlated with an infectious children diseases history (meningitis, measles, mumps, or rubella).

As a result of the performed log-regression multifactor analysis of SNHL development, the logit-regression model described the best of obtained indicators dependence (multiple correlation coefficient R^2=0.9761; standard error of model estimation is low ε=0.1114; high connection criterion F=124.2; degrees of freedom df=7.43; p<0.001). The adequacy of the model is evidenced by the residues histogram approximation to the normal

distribution. The dependence of the SNHL and CHL development in children, taking into account the analyzed risk factors, can be approximated by the log-regression equations:

$$Y_{SNHL} = 0{,}01 + 0{,}94{*}X_{\text{Speech disorders}} - 0{,}24{*}X_{\text{Congenital chromosomal pathology}} - 0{,}10{*}X_{\text{Otitis}} - 0{,}10{*}X_{\text{Low birth weight}};$$

$$Y_{CHL} = 0{,}992 - 0{,}91{*}X_{\text{Speech disorders}} + 0{,}24{*}\ X_{\text{Congenital chromosomal pathology}} + 0{,}10{*}X_{\text{Otitis}} + 0{,}10{*}X_{\text{Infectious diseases history}},$$

where X – factor sign of pathology; SNHL - sensorineural hearing loss; CHL - conductive hearing loss.

Conclusions: The combination of *35delG* / *TT*, as well as *Non-Del* / *TT* and *35delG* / *TC* genotypes in the genome is associated with a high risk of hearing loss in general the population of examined children (from 0.932 to 1.432; p=0.003), as well as the occurrence of SNHL (from 0.765 to 1.765), especially for *TT* / *35DelG* combination (1,765).

Infectious diseases in anamnesis (meningitis, measles, mumps, or rubella) increase the likelihood of CHL 9.41 times (OR=12.0; p=0.007). Concomitant chronic non-obstructive and obstructive diseases of the upper and lower respiratory tract increase the risk of both SNHL and CHL in children regardless of age: for SNHL 3.75-7.81 times as much, for CHL - 4.29-8.75 times ($p<0.01$).

The appearance of SNHL or CHL in the child population can be approximated by logit regression models, based on the multifactor regression analysis, with a high coefficient of multiple correlation $R^2=0.9761$ ($p<0.001$).

Conflict of Interest

The authors declare no conflict of interest.

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