


Functional Polymorphism of *MMP9* and *BDNF* as Potential Biomarker of Auditory Neuroplasticity in Prelingual Deafness Treatment With Cochlear Implantation—A Retrospective Cohort Analysis

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Abstract

Genetic biomarkers of neuroplasticity in deaf children treated with cochlear implantation (CI) might facilitate their clinical management, especially giving them better chances of developing proficient spoken language. We investigated whether carrying certain variants of the genes encoding matrix metalloproteinase *MMP9* and neurotrophin brain-derived neurotrophic factor (*BDNF*), involved in synaptic plasticity, can be taken as prognostic markers of how well auditory skills might be acquired. Association analysis of functional *MMP9* rs3918242 and *BDNF* rs6265 variants and the child's auditory development measured at CI activation and 1, 5, 9, 14, and 24 months post CI activation with LittLEARS Questionnaire (LEAQ) was conducted in a group of 100 children diagnosed with DFNBI-related deafness, unilaterally implanted before the age of 2 years. Statistical analysis in the subgroup implanted after 1 year of life ($n = 53$) showed significant association between *MMP9* rs3918242 and LEAQ scores at 1 month ($p = .01$), at 5 months ($p = .01$), at 9 months ($p = .01$), and at 24 months ($p = .01$) after CI activation. No significant associations in the subgroup implanted before 1 year of life were observed. No significant associations between the *BDNF* rs6265 and LEAQ score were found. Multiple regression analysis ($R^2 = .73$) in the subgroup implanted after 1 year of life revealed that *MMP9* rs3918242 was a significant predictor of treatment outcome. In conclusion, C/C rs3918242 *MMP9* predisposes their deaf carriers to better CI outcomes, especially when implanted after the first birthday, than carriers of C/T rs3918242 *MMP9*.

Keywords

neuronal plasticity, language development, *MMP9*, *BDNF*, congenital deafness

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Variability of Cochlear Implantation Outcome

Congenital deafness leaves the auditory system of a rapidly developing child, essentially unstimulated (Kral, 2013). This lack of neurosensory input to the developing auditory cortex means that the auditory system becomes progressively less capable of acquiring functional competency and so the child steadily loses the ability to develop spoken language (Kral, 2013, Kral et al., 2016). Cochlear implants (CIs) make it possible to avoid this loss, and they are now widely accepted as standard therapy in the treatment of deafness. However, despite the undeniable success of the method, it is far from perfect, and there is vast variability in auditory, speech, and language outcomes among pediatric CI users (Colletti et al., 2011; de Raeve, 2010; Kral et al., 2016; Levine et al., 2016; Niparko et al., 2010). Many deaf-born children cope exceptionally well with the limited sensory stimulation delivered to the auditory system by a CI, and a large fraction of them reach almost age-appropriate milestones in speech and language development (Kral et al., 2016; May-Mederake, 2012; Niparko et al., 2010). At the same time, however, some implanted children, despite considerable effort at rehabilitation, lag behind their normal-hearing and implanted peers and never achieve satisfactory proficiency in speech, language, and verbal communication (de Raeve, 2010; Kral et al., 2016; Niparko et al., 2010). Numerous studies have been undertaken to identify the factors that influence speech and language formation after cochlear implantation at a very early age (Houston et al., 2012; Leigh et al., 2013; Levine et al., 2016; Niparko et al., 2010). The findings suggest that the age at implantation is the largest factor contributing to auditory development in very young patients. Still, other factors have also been proposed to explain some implanted children's language deficits, including etiology of deafness, developmental delay, age at onset of deafness, age at diagnosis of hearing impairment, involvement and participation in rehabilitation programs, and various environmental factors, such as the parents' educational status (Abdurehim et al., 2017; Angeli et al., 2011; de Raeve, 2010; Eppsteiner et al., 2012; Kral et al., 2016; Leigh et al., 2013; Levine et al., 2016; Oziębło et al., 2020; Park et al., 2017; Reinert et al., 2010; Shearer et al., 2017). Data of etiologic diagnosis of congenital deafness show that up to 60% of cases have a genetic background. In half of them, two pathogenic recessive variants are detected in *GJB2* and *GJB6* genes in *DFNB1 locus*. They are expressed in membranous labyrinth (encode connexin 26 and connexin 30, respectively), and these mutations affect the function of the organ of Corti and as a consequence lead to deafness (Oziębło et al., 2020). There are some hypotheses proposed to explain the exact

mechanism leading to *DFNB1*-related deafness, such as loss of function of ion channels or malfunction of supporting cells; however, these do not give a full understanding of the phenomenon (Chen et al., 2014; Kikuchi et al., 2000; Oziębło et al., 2020). In this context, there appears to be a need to identify a possible marker that may help to identify children, whose prognosis for auditory skills development is not promising. This would allow clinicians to focus on these children's specific needs and formulate individually tailored intervention programs giving the best possible outcomes.

Contributing Role of Molecular Factors

Recent years have brought us new insights into the neural underpinnings of a child's capacity to respond to sensory stimulation delivered to the cortex, both at a system and molecular level. However, to date, no major biochemical or genetic biomarker of speech and language acquisition, relevant to the treatment of deafness with CI, has yet been investigated or proposed (Hunter et al., 2017; Kral, 2013; Kral et al., 2016, 2019). In this context, the brain is treated as a system in a dynamic balance between the range of neural activity and the sensory excitation coming from the environment (Herholz, 2013; Kral et al., 2016, 2019). A regular inflow of sensory stimuli triggers neuronal connectivity and increases the potential for synapse formation in processes involving both cell-to-cell and cell-to-extracellular matrix interactions (Reinhard et al., 2015). Certain physiological conditions involving pathological neuronal plasticity—for example in diseases of the central nervous system or addictions, as well as experimental animal data—suggest that there is a molecular regulation of neuroplasticity in the human auditory system (Ethel & Ethel, 2007; Holtmaat & Caroni, 2016; McGregor et al., 2018; Reinhard et al., 2015; Rybakowski et al., 2009). Synaptic dynamics relies on a variety of processes, including long-term potentiation and long-term depression. Both of these are regulated by multiple molecular cascade mechanisms involving matrix metalloproteinases (MMPs), such as matrix metalloproteinase-9 (MMP9) and neurotrophin brain-derived neurotrophic factor (BDNF; Bekinschtein et al., 2011; Beroun et al., 2019; Ethel & Ethel, 2007; Hariri et al., 2003; Nagy et al., 2006; Reinhard et al., 2015; Vafadari et al., 2016; Wang et al., 2008).

MMP9 is an extracellularly acting endopeptidase, which exerts multiple effects on the structure and function of excitatory synapses, in particular affecting dendritic spine maturation; it also cleaves components of extracellular milieu in neural tissue, such as cell adhesion molecules and neurotrophins, as well as promoting synaptogenesis (Ethel & Ethel, 2007; Reinhard et al., 2015; Vafadari et al., 2016). In synaptogenesis, BDNF plays a

significant role in long-term potentiation and is one of the factors maintaining the sensitivity of postsynapses for high-frequency stimulation on hippocampal neurons (Hariri et al., 2003). Both *MMP9* and *BDNF* have their functional variants, being the result of single nucleotide polymorphisms (SNPs), for example, *MMP9* (rs3918242), known to affect the gene expression, and *BDNF* (rs6265), known to affect the protein function (Cheeran et al., 2008; McGregor et al., 2018; Rybakowski et al., 2009). In general, every gene has two copies, that is, two alleles, each inherited from a parent. In the human genome at a nucleotide position numbered rs3918242 within the *MMP9* gene, a person can have either two cytosines (C/C) that correspond to two reference alleles or a cytosine and a thymine (C/T) that correspond to a reference allele and an alternative allele or two thymines (T/T) that correspond to two alternative alleles. C/C genotype of *MMP9* rs1839242 results in significantly lower transcriptional activity of the gene, whereas C/T and T/T genotypes result in high transcriptional activity of the gene. As a result, the excitatory synapses might be affected. For the Polish population, it has been reported that carriers of the less transcriptionally active C/C allele are more prone to schizophrenia than the C/T allele carriers (Rybakowski et al., 2009). It has been shown that carriers of the Val/Val *BDNF* variant (rs6265) display higher potential for plasticity in motor cortex than carriers of the Val/Met variant (Cheeran et al., 2008).

Knowing the role of *MMP9* and *BDNF* in neuronal plasticity in other physiological and pathological conditions, it is, therefore, possible that *MMP9* and *BDNF*, and their polymorphisms, may play a role in the plasticity of the auditory system in congenital deafness treatment and thus influence auditory development. For this reason, we set out to investigate the possible role of the variants of *MMP9* and *BDNF* in the process of acquiring auditory competency after cochlear implantation. We designed an association study looking at the associations between rs1839242 in *MMP9* and rs6265 in *BDNF* and auditory development, as measured by the parental LittEARS Auditory Questionnaire (LEAQ) score in a group of deaf infants and toddlers with identified causal variants in the *DFNB1* locus.

Aim of the Study

We aimed to investigate whether carrying a certain set of variants of *MMP9* and *BDNF* can act as a potential prognostic marker of auditory development following cochlear implantation to treat deafness in infants and toddlers. To identify the maximum possible involvement of *MMP9* and *BDNF* in neuroplasticity in the auditory pathway after cochlear implantation, we focus on a genetically very homogenous group of children with

DFNB1-related deafness. The children had no known environmental risk factors for deafness or for auditory deficits. We wanted to test the hypothesis that carrying a specific combination of variants of *MMP9* and *BDNF* predisposes young CI users to better functional outcomes after cochlear implantation. To our knowledge, there is currently no data that has demonstrated a role for *MMP9* and *BDNF* in the neuroplasticity of the human auditory system.

Material and Methods

Study Design, Participants, and Ethical Approval

We performed a retrospective cohort study involving participants diagnosed with bilateral hearing loss who underwent cochlear implantation in Institute of Physiology and Pathology of Hearing in Warsaw, Poland, between 2009 and 2017. Inclusion criteria were congenital bilateral profound sensorineural hearing loss, confirmed by auditory brainstem response, diagnosed at birth, the presence of pathogenic variants in the *DFNB1* locus, and cochlear implantation before the second birthday. Exclusion criteria were presence of environmental risk factors, such as chronic concomitant diseases, prematurity, use of ototoxic drugs, asphyxia, and history of viral infection during pregnancy. Following activation of the patient's speech processor, parents or caregivers followed instructions of auditory-verbal therapy. During all follow-up intervals, the children were clinically assessed, including for auditory development, and had their speech processor fitted. A group of 170 patients met the inclusion criteria, and out of them precisely 100 patients had completed six auditory development measures (LEAQ scores) at following intervals at CI activation, 1, 5, 9, 14, and 24 months afterward. Demographic data were obtained from all of them. These participants were also classified into two subgroups: CI activation before 1 year old and CI activation above 1 year old. The study was designed and conducted according to the Declaration of Helsinki and the study protocol. It was reviewed and approved by the Bioethics Committee of the Institute of Physiology and Pathology of Hearing (nr IFPS:KB/13/2015). Parents or caregivers of all participants gave written informed consent.

Etiology of Deafness

DFNB1 testing was performed according to recommendations of the European Molecular Quality Network on DNA samples isolated from peripheral blood using a standard procedure (Hoefsloot et al., 2013).

Auditory Development Assessment

Participants were assessed for their auditory development by the LittleEARS Auditory Questionnaire (LEAQ) that is designed to assess auditory development in very young children (Weichbold et al., 2005). LEAQ consists of 35 questions with a yes or no answer. The total score is the number of yes answers. The LEAQ has been validated in more than 20 languages, as well as in children using cochlear implants (Coninx et al., 2009; García Negro et al., 2016; Geal-Dor et al., 2011; Obrycka et al., 2009, 2017; Wanga et al., 2013). Baseline and follow-up audiological data were obtained from all 100 participants.

Genotyping

The *MMP9* polymorphism rs3918242 (NM_004994.2:c.-1590C>T) was genotyped using the polymerase chain reaction (PCR) restriction fragment length polymorphism method. The genomic region encompassing rs3918242 was amplified using forward 5'GCCTGGCACATAGTAGGCC3' and reverse 5'CTTCCCTAGCCAGCCGGCATC3' primers (Oligo IBB PAN, Warsaw, Poland) under the following conditions: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 35 s, annealing at 62°C for 30 s and extension at 72°C for 45 s, and a final elongation at 72°C for 5 min. Next, 10 µL of PCR products were digested overnight with 10 units of PaeI restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C. After digestion, the DNA fragments were separated by agarose gel electrophoresis and visualized with the DigiDoc-It Imaging System (UVP LCC, Upland, CA, USA). The allele containing reference variant C was represented by the DNA band of size 435 bp, and the allele containing alternative variant T was represented by bands of sizes 188 and 247 bp.

The *BDNF* polymorphism rs6265 (NM_170735.5:c.196G>A) was genotyped using ABI Custom TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA) and a real-time PCR system (Viia7, Thermo Fisher Scientific). The accuracy of genotyping was confirmed by Sanger sequencing in randomly selected samples. The results were 100% concordant.

Statistical Analyses

Paired Comparisons Methodology. The mean LEAQ scores were compared between patients with C/C and C/T *MMP9* genotypes as well as between patients with Val/Val and Val/Met *BDNF* variants using a Welch two-sample *t* test (if test assumptions were met) or a Wilcoxon rank-sum test. These comparisons were made for all tested intervals, that is, at a time at CI activation, and at 1st, 5th, 9th, 14th, and 24th month

after CI activation. All computations were made using the R language in version 3.5.3 (2019). Differences were considered statistically significant at p value $\leq .05$.

Modeling Methodology. Repeated-measures and repeated-events data have a hierarchical structure that can be analyzed using multilevel models (Steele, 2008). Considering the longitudinal aspect of the study design, linear mixed-effects (LME) model with a random intercept were used as they provide an effective way to incorporate within-subject and between-subject variation and the correlation structure of longitudinal data (Davidian & Giltinan, 1995; Diggle et al., 2002; Fitzmaurice et al., 2004).

Predictor variables included in models were sex, *BDNF* rs6265 and *MMP9* rs3918242 genotypes, follow-up interval, and age at CI activation. We also report R^2 for models; we calculate it by creating a simple linear model, where we predict actual LEAQ values with our multilevel model predictions; R^2 can range between 0 and 1 and gives an estimate of how much variance in LEAQ scores can be explained by our models. LME models allow assigning additional parameters (random effects) on a group level in addition to those normally seen in simple linear regression models. In our modeling, we decided to add an additional constant (intercept) for each participant (individual intercepts). A constant is the expected value of the dependent variable when the values of all the independent variables are zero. An individual intercept is an additional constant that differs for each patient in the data set (in our particular situation individual intercept accounts for the fact that participants are not on the same LEAQ level at the onset of the experiment). R language in version 3.5.3 (2019) and libraries: lme4, blme, stargazer were used (Bates et al., 2015; Chung et al., 2013; Hlavac, 2018). Reported p values for models were obtained with lmerTest package using Satterthwaite approximation (Kuznetsova et al., 2017). Differences were considered statistically significant at $p \leq .05$.

Explainer Methodology. Some linear models can be quite complex. For this reason, we applied DALEX algorithms to simplify findings and explain models (Biecek, 2018) by computing variable importance (<https://uc-r.github.io/dalex#pdp>). Variable importance is estimated with the following algorithm:

1. The loss function (a measure of how well model fits the data, in our case root mean square error [RMSE]) is first calculated for a model under inspection (full model—on Figure 1 denoted as red reference line);
2. The response variable (LEAQ) is permuted across all measurements, hence breaking all correlations with

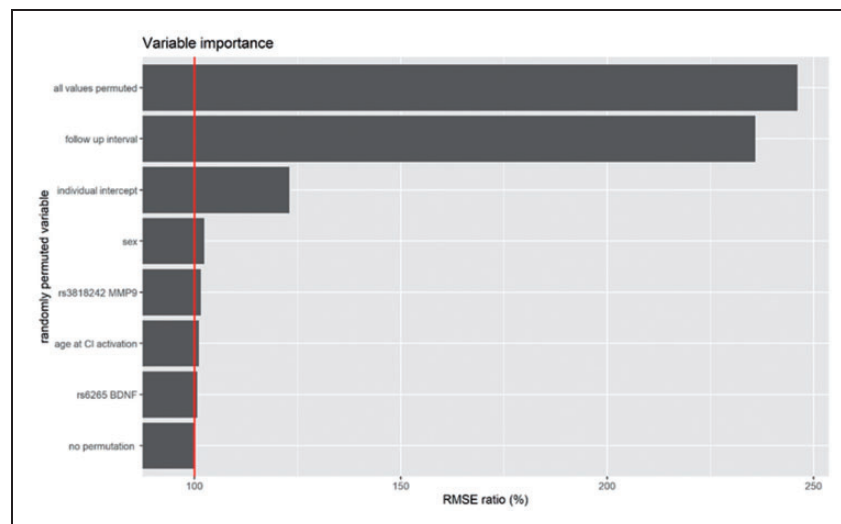


Figure 1. Interpretation of the Regression Model for All Tested Patients Showing a Rank of Particular Variables in the Model. The x axis presents the ratio (%) of RMSE for the model with randomly permuted values of one variable to RMSE for the model for which variable values are not changed.

RMSE = root mean square error; MMP9 = matrix metalloproteinase-9; CI = cochlear implant; BDNF = brain-derived neurotrophic factor.

variables, the loss function is computed again, and this value constitutes a baseline;

3. For all variables used in modeling, values are randomized (breaking its association with LEAQ), then loss function computed.

The ratio of RMSE for the model with randomly permuted values of one variable to RMSE for a model for which variable values are not changed was calculated for all predictor variables as well as for individual intercepts and was visualized by horizontal bar charts.

Contributions of particular variables were also analyzed at the level of individual predictions and visualized using breakDown algorithm. breakDown plots present variable attributions, that is, the decomposition of the model's prediction into contributions of different explanatory variables. The underlying idea of breakDown plots is to present the contribution of an explanatory variable to the model's prediction by computing the shift in the expected value of Y while fixing the values of other variables (<https://pbiecek.github.io/ema/breakDown>). The modelStudio library (Baniecki & Biecek, 2019) was used for interactive exploration of the model.

Results

Sample Demographics and Auditory Development

Out of initial 170 implanted children with pathogenic variants of the DFNB1 locus, 100 (43 girls and 57 boys) had a complete set of LEAQ scores at all tested

intervals and were included in the analysis. The mean age at CI activation was 12.6 months (min = 6.2; max = 21.6; $SD = 3.3$). There were 47 children (47%) who had their CI activated before their first birthday and 53 children (53%) afterward. All children were implanted with a multichannel device and became regular CI users. All participants were of Caucasian origin.

Genotyping

Genotype distributions were in agreement with the Hardy–Weinberg equilibrium in the cohort group. *BDNF* genotype Val/Met was found in 32 cases (32%), Val/Val genotype in 68 cases (68%), allele numbers were Val = 168 (84%), Met = 32 (16%). Prevalence of *MMP9* variants in the cohort group was C/T 28 cases (28%) and C/C in 72 cases (72%); allele frequencies were C = 172 (86%), T = 28 (14%).

Association Analyses

In the whole tested group, patients with the C/C *MMP9* genotype and Val/Met in *BDNF* had higher LEAQ scores during the complete follow-up period. Still, we did not identify significant associations between the analyzed genotypes and LEAQ score at any of the tested intervals (data not shown).

However, the analysis performed in subgroups showed statistically significant differences in LEAQ score between patients with C/C and C/T *MMP9* genotypes implanted after 1 year old. (Tables 1 and 2).

MMP9 rs3918242 was associated at a significant level with LEAQ scores in the subgroup of children implanted

Table 1. Association of Auditory Development Measures (LEAQ) Over All Follow-Up Intervals (0–24 Months) With Variants of *MMP9* and *BDNF* in the Subgroup CI Activation Above 1 Year Old.

	<i>MMP9</i> rs3918242		<i>BDNF</i> rs6265	
	Mean LEAQ score (SD) C/C/C/T	<i>p</i> value	Mean LEAQ score (SD) Val/Val/Val/Met	<i>p</i> value
Time at CI activation	9.1 (9.3) / 4.7 (4.7)	.237	6.4 (6.9) / 10.5 (10.3)	.288
1st month after CI activation	13.8 (7.8) / 7.7 (7.4)	.011*	10.9 (7.9) / 14.1 (8.2)	.177
5th month after CI activation	22.4 (6.3) / 16.4 (7.3)	.011*	19.9 (6.6) / 22.1 (7.8)	.301
9th month after CI activation	28. (5.2) / 21.6 (7.7)	.012*	25.6 (6.1) / 27.3 (7.5)	.172
14th month after CI activation	31.7 (3.6) / 29.2 (5.5)	.217	30.6 (4.2) / 31.7 (4.5)	.190
24th month after CI activation	33.6 (2) / 31.6 (3.8)	.018*	33.1 (2.8) / 33.1 (2.7)	1.000

Note. *MMP9* = matrix metalloproteinase-9; *BDNF* = brain-derived neurotrophic factor; LEAQ = LittlEARS Auditory Questionnaire; CI = cochlear implant; $p < 0.05$.

Table 2. Association of Auditory Development Measures (LEAQ) Over All Follow-Up Intervals (0–24 Months) With Variants of *MMP9* and *BDNF* in the Subgroup CI Activation Before 1 Year Old.

	<i>MMP9</i> rs3918242		<i>BDNF</i> rs6265	
	Mean LEAQ score (SD) C/C/C/T	<i>p</i> value	Mean LEAQ score (SD) Val/Val/Val/Met	<i>p</i> value
Time at CI activation	3.6 (5.3) / 2.1 (3.3)	.267	3.4 (5.3) / 2.4 (3.1)	.786
1st month after CI activation	8.1 (4.7) / 9.8 (5.9)	.384	9.2 (5.1) / 6.9 (4.6)	.149
5th month after CI activation	19.3 (6.7) / 21.7 (3.4)	.142	19.5 (6.7) / 21.2 (3.9)	.278
9th month after CI activation	25.5 (5.6) / 28.3 (2.6)	.112	25.8 (5.5) / 27.5 (3.7)	.474
14th month after CI activation	30 (4.7) / 31.5(3.1)	.480	30.1 (4.8) / 31.2 (3)	.811
24th month after CI activation	33 (3.5) / 33.6 (1.8)	.804	32.9 (3.4) / 33.8 (2)	.574

Note. *MMP9* = matrix metalloproteinase-9; *BDNF* = brain-derived neurotrophic factor; LEAQ = LittlEARS Auditory Questionnaire; CI = cochlear implant.

after 1 year of life at 1, 5, 9, and 24 months follow-up intervals (Table 1). In these intervals differences between mean LEAQ score for C/C and C/T genotypes, *MMP9* are the greatest (13.8 points vs. 7.7 at 1 month follow-up interval, 22.4 points vs. 16.4 at 5 months follow-up interval, 28 vs. 21.6 at 9 months follow-up, and 33.6 vs. 31.6 in 24 months follow-up). For *BDNF* rs6265 variants, there were no statistically significant differences in all tested follow-up intervals (Table 1).

However, for the younger group, no significant associations between tested SNPs and LEAQ scores were found (Table 2).

Linear Regression Model

To build a multiple regression model, each patient's observations were broken down into single measurements of language development (LEAQ score) from CI activation to 24 months follow-up interval, which gave altogether the set of 600 observations. The model revealed that a significant predictor of auditory development outcome (LEAQ scores), apart from the follow-up interval, is the *MMP9* rs3918242 genotype, and the sex of patients (Table 3). The average R^2 for the model was

Table 3. Multiple Regression Summary for LEAQ Score for the Whole Analyzed Group.

Predictors	Dependent variable: Results—regression coefficients
Follow-up interval (month) (SE)	1.109 $p = 2e-16$ (0.032)
rs3918242 <i>MMP9</i> (SE)	-1.861 $p = .045$ (0.919)
rs6265 <i>BDNF</i> (SE)	-1.224 $p = .169$ (0.885)
Sex (SE)	-1.913 $p = .024$ (0.835)
Age at CI activation (SE)	0.006 $p = .134$ (0.004)
Constant (SE)	11.294 $p = 39e-08$ (1.826)
Observations	600
Log Likelihood	-2,015.289
Akaike Inf. Crit.	4,046.578
Bayesian Inf. Crit.	4,081.735

Note. p values of the impact predictors. $R^2 = .73$. *MMP9* = matrix metalloproteinase-9; *BDNF* = brain-derived neurotrophic factor; CI = cochlear implant; SE = standard error.

.73, indicating that it could explain a considerable level of response variation. The p values of the predictor variables and their associated impact on global outcome scores are shown in Table 3.

To give a clinical interpretation of the meaning of significant predictors, the explainer methodology, as described earlier, was used. For *MMP9* rs3918242, it shows that an average patient who is a carrier of a C/C variant has an estimated LEAQ score in any follow-up interval equal to 21.60 points. In contrast, for a carrier of a C/T variant, it is 19.74 points. It means that a patient with C/C genotype will, on average, at any follow-up interval score 1.86 points higher than a patient with C/T genotype. Another significant predictor in the model is sex of patients. An implanted girl will score on average 22.17 points in LEAQ in any follow-up interval, and an implanted boy will on average score 20.26 points in LEAQ in any follow-up interval, that is, 1.91 points lower.

Using the calculated ratio of RMSE for the model with randomly permuted values of one variable to RMSE for the unchanged model, the impact of a single variable on the entire model was established. The follow-up interval is the most important predictor of auditory development, followed by individual intercept, sex, *MMP9* genotype, and age at CI activation. There is only a marginal impact of *BDNF* variants and age at CI activation on the accuracy of the tested model (Figure 1).

Following the results of paired comparisons, analogically to the whole study group, we have built the multiple regression model for the subgroup implanted after 1 year old, on the set of 318 observations. The average R^2 for the model was .73, and the only significant predictor of auditory development outcome (LEAQ scores), apart from the follow-up interval, is the *MMP9* genotype. The p values of the predictor variables and their associated impact on global outcome scores are shown in Table 4.

Explainer methodology used for the regression model for children implanted after 1 year old showed that patient carrying *MMP9* C/C genotype on average scores 23.10 points in LEAQ, while patient carrying *MMP9* C/T genotype scores 18.53 points in LEAQ—a difference of 4.57 LEAQ points in any analyzed interval.

For patients implanted after 1 year old RMSE ratio analysis revealed that the follow-up interval is the most important predictor of auditory development, followed by individual intercept, *MMP9* genotype, and sex. There is no impact of *BDNF* variants or age at CI activation on the accuracy of the tested model (Figure 2).

Application of breakDown algorithm and modelStudio transformed our model into an interactive tool for prediction of LEAQ score for an individual patient after taking into account the contribution of

Table 4. Multiple Regression Summary for LEAQ Score for Children Implanted After 1 Year Old.

Predictors	Dependent variable: Results—regression coefficient
Follow-up interval (month) (SE)	1.029 $p = 2e-16$ (0.043)
rs3918242 <i>MMP9</i> (SE)	-4.561 $p = .002$ (1.380)
rs6265 <i>BDNF</i> (SE)	-0.945 $p = .468$ (1.294)
Sex (SE)	-2.081 $p = .097$ (1.231)
Age at CI activation (SE)	0.008 $p = .375$ (0.009)
Constant (SE)	12.180 $p = 2e-16$ (4.2)
Observations	318
Log Likelihood	-1,061.364
Akaike Inf. Crit.	2,136.727
Bayesian Inf. Crit.	2,163.062

Note. p values of the impact of predictors in the group of patients implanted after 1 year old. $R^2 = .73$. *MMP9* = matrix metalloproteinase-9; *BDNF* = brain-derived neurotrophic factor; CI = cochlear implant; SE = standard error.

the analyzed factors. breakDown plots show how do the contributions attributed to individual explanatory variables change the mean model's prediction to yield the actual prediction for a particular single observation. Figure 3, with a breakDown plot, shows an example of this approach for a selected patient (Figure 3). Inclusion of the analyzed predictors increased the initial LEAQ score of 21.078 to 25.664.

Discussion

This study investigated possible associations between the presence of functional variants of *MMP9* and *BDNF* and the outcomes of cochlear implantation. To extract the significance of molecular factors in the auditory development in our study design, we selected a large group of etiologically homogenous pediatric CI users. These children were implanted early on when their auditory and language development was still plastic. We used detailed longitudinal observations of auditory development of children with complete sets of LEAQ scores throughout the follow-up (at activation and 1, 5, 9, 14, and 24 months after CI activation). Moreover, to minimize the effect of factors known to interfere with the auditory development, we excluded all children with comorbidities, either congenital or acquired during pregnancy or after birth, as well as any preterm children. Our patients received universal neonatal screening, were

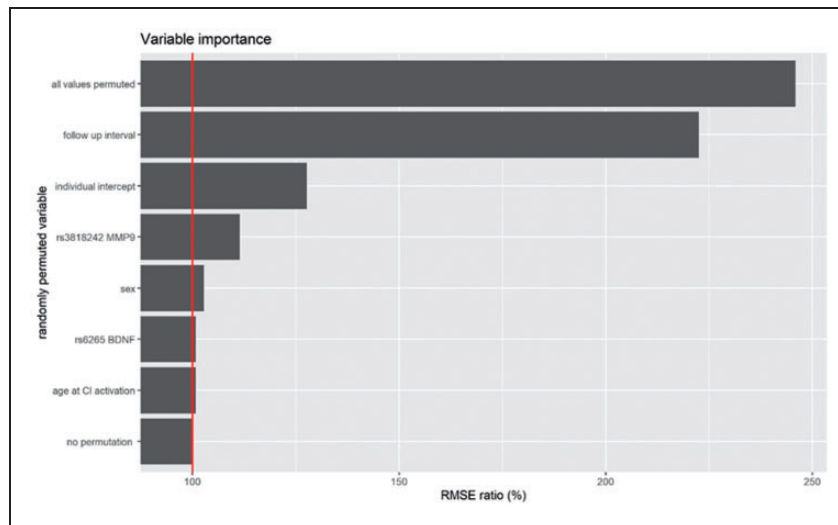


Figure 2. Interpretation of the Regression Model for Patients Implanted After 1 Year Old Showing a Rank of Particular Variables in the Model. The x axis presents the ratio (%) of RMSE for the model with randomly permuted values of one variable to RMSE for the model for which variable values are not changed.

RMSE = root mean square error; MMP9 = matrix metalloproteinase-9; CI = cochlear implant; BDNF = brain-derived neurotrophic factor.

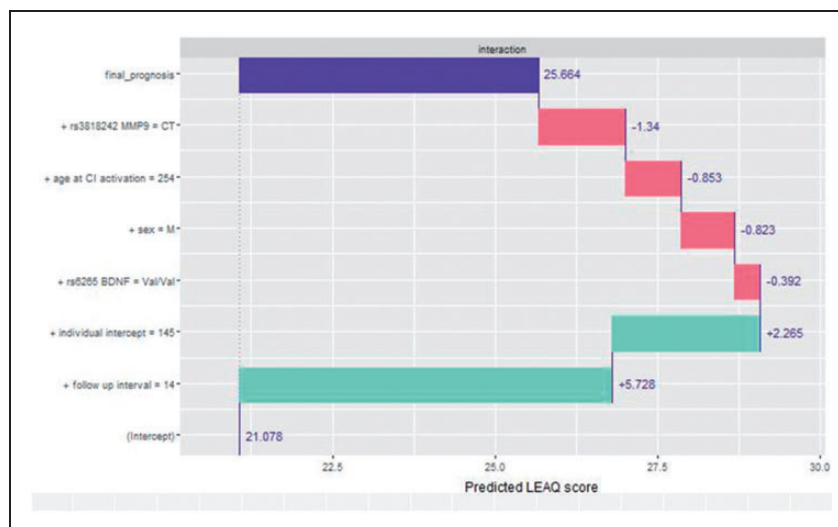


Figure 3. Contribution of Particular Variables in Predicting of LEAQ Score for a Selected Patient. The green and red bars indicate, respectively, positive and negative changes in the mean predictions (contributions attributed to explanatory variables). The final predicted contribution is shown in blue.

MMP9 = matrix metalloproteinase-9; CI = cochlear implant; BDNF = brain-derived neurotrophic factor; LEAQ = LittEARS Auditory Questionnaire.

diagnosed with DFNB1-related, bilateral profound sensorineural hearing loss, were implanted before the age of 2, and were enrolled in the same rehabilitation program following activation of their speech processor. In addition, following clinical practice and literature data and also to diminish the influence of age at CI activation, known to significantly shape CI outcome, the cohort was divided into two subgroups (activation before or after 1 year of age), and analyses were performed separately

(Leigh et al., 2013; Levine et al., 2016; May-Mederake et al., 2010; Niparko et al., 2010).

MMP9 rs3918242 Significance

The significance of *MMP9* rs3918242 contribution to the language acquisition measurements scores (p value $< .05$) is repeatable across almost all follow-up intervals in subgroup implanted after 1 year old.

We have not found any significance of *BDNF* rs6265 in auditory development, and for this reason, we focus our discussion on *MMP9* rs3918242. Here, we demonstrate the significance of the statistical relation between this SNP and auditory development measurements (LEAQ scores) in congenitally deaf children who have been treated with cochlear implantation. Table 1 reveals that, over the whole older group, the lower LEAQ score at the end point of observation was strongly associated with this functional variant of *MMP9* rs3918242. The applied regression models revealed a significant impact of *MMP9* rs3918242 on LEAQ score. The influence of *MMP9* rs3918242 on LEAQ score is more considerable in the older group. In the group of patients implanted after 1 year old, the average estimated difference in LEAQ score between a child with a C/C genotype and a child with a C/T genotype is 4.57 points, which corresponds with 5.8 months of delay in auditory development after cochlear implantation (Obrycka et al., 2009). In the younger group (implanted before 1 year old), a general lack of effect of *MMP9* rs3918242 may have several explanations. One possibility is that fast auditory development in these children is driven by some additional factors which steadily decrease with age, and so the role of *MMP9* variants, seen in the older group, does not come to the fore (Houston et al., 2012; May-Mederake, 2012). Another possible explanation corresponds to Kral & Sharma (2012) and Kral et al.'s (2019) experiments with congenitally deaf and normally hearing cats that there are significant differences between mean synaptic activity in the primary auditory cortex of normally hearing cats and implanted cats indicating a delay in synaptogenesis in animals hearing via the implant. Our results would suggest that cortical synaptogenesis in response to CI stimulation before and after 1 year old may appear with the engagement of different molecular mechanisms. Implantation before 1 year old may allow normal neuronal and molecular machinery to develop, whereas in later implantation, when synaptogenesis is already delayed, it is not possible and physiological processes are replaced by some new, activated processes involving *MMP9*. This would support the clinical indication of cochlear implantation before the first birthday (Houston et al., 2012; Leigh et al., 2013; Levine et al., 2016). It should be also considered that other MMPs, such as *MMP-2*, may play a more significant role during early development, overshadowing the role of *MMP9*. Such suggestion is, in fact, supported by animal models (Szklarczyk & Kaczmarek, 2005). As has been documented, *MMP9* is involved in numerous other physiological and pathological conditions, such as psychiatric, neurodegenerative, and vascular disorders, and it is possible that at some age, in some groups of patients, *MMP9* function could be altered by one of these coexisting, ongoing involvements. In such

cases, the contribution of a particular SNP in *MMP9* may remain undetected by statistical analysis (McGregor et al., 2018; Rybakowski et al., 2009; Zhang et al., 2015). It is documented that children implanted before 1 year of life during the initial period after CI activation have rapid growth of their performance (May-Mederake, 2012; May-Mederake et al., 2010). Observations on linguistic development of children who received a CI before 2 years old by Houston et al. (2012) indicate that the younger group (implanted up to 1 year old) shows better word learning skills than older group. The authors postulate that possible mechanisms underlying this difference may be based on the fact that children who got access to auditory cues earlier in life seem to have better developed cognitive processing, such as sensory integration (Houston et al., 2012; Kral et al., 2016). LEAQ score is a measure of auditory development, not capable of capturing and differentiating these particular elements of the process. Given this fact, it is possible that differences in the statistical influence of *MMP9* rs3918242 on cochlear implantation outcome between the two age groups shown in our results allow us to conclude that *MMP9* rs3918242 may not be involved in neuroplasticity mechanisms underlying cognitive processes in children implanted before 1 year old, but it can be involved in neural mechanisms underlying these processes in the older group.

In the regression model for the whole studied group, the most prominent role, understandably, is attributed to the follow-up interval, which defines the amount of time (in number of months) a patient has been using a CI; in other words, it reflects the duration of hearing experience (Table 3, Figure 1). It should be emphasized that this variable also includes the biological age of an implanted child, which increases from one measurement to the next and is the strongest factor influencing auditory and language development after cochlear implantation (Geers et al., 2003; Niparko et al., 2010). The second position in the ranking is the intercept associated with an individual patient. This value reflects the sum of all patients' individual, internal, and external, though unspecified features assumed to shape language development. Next are sex and *MMP9* rs3918242 genotype (Figure 1). Sex of patients has already been indicated as a significant predictor by Geers et al. (2003) who reported results of their study on a group of 181 of children implanted before 5 years old. They showed that female sex predisposes a deaf child to better language outcomes at 4 to 5 years after implantation. However, it is difficult to compare or extrapolate results from these two groups, as their cohort was far less homogenous than ours in terms of etiology of deafness, comorbidities and existence of other biological factors, such as prematurity or contributing medical history during pregnancy. Ching et al. (2013) also reported that female sex

predisposes hearing-impaired children with early diagnosis to better auditory outcomes after treatment in a 3-year time frame (Ching et al., 2013). A number of reports on language development after cochlear implantation have been published, but due to a diversity of outcome measures across languages, lack of etiological homogeneity and a wide range of age at implantation in the groups, it is difficult to directly compare the results (Leigh et al., 2013; Levine et al., 2016; Niparko et al., 2010). For example, Niparko et al. (2010) reported results of a multicenter study of language development in CI children implanted before 5 years old. They divided the cohort according to age at which children underwent implantation: before the 18th month of life, between the 18th and 36th month, and after 36th month. The authors did not find sex as a significant contributing factor for auditory development after CI treatment in any of these subgroups. It is interesting to note that in our cohort, sex as a predictor lost its significance when the analysis was focused only on the older group (Table 4, Figure 2). Age at CI activation did not reach the significance level in our model. The range of age at CI activation in our cohort is relatively short and may correspond to the range of age of implantation of the youngest group of children—implanted before 18th month of life reported by Niparko et al. (2010). Although the significance of sex influence on LEAQ score is not surprising, the almost equivalent power of *MMP9* variants indicates that there is a hitherto unknown factor that has the capacity to control early auditory development (Figure 1). Using the interactive tool (modelStudio transformed), one can sum up a contribution of particular variables introduced to the model into the final score of a single auditory development measurement (LEAQ) (Figure 3). Here, we present just a single example of a child's LEAQ measurement, but such a model is available for each child's LEAQ measurements, showing the evolving contribution of particular elements to the final score.

Although to our knowledge no data so far have been published on the specific involvement of *MMP9* rs3918242 in synaptic plasticity, there are indirect lines of evidence supporting such a notion (Stefaniuk et al., 2017). Nevertheless, further investigation considering pathway analyses may be warranted to elucidate the role of this variant, and other SNPs, in auditory neuroplasticity after CI. In our study, we did not examine the serum level of the protein. However, it is possible that *MMP9* rs3918242 may have an influence on it, and subsequently, *MMP9* serum level may influence the LEAQ scores.

Limitations

Although LEAQ is a widely used tool for assessing language development in infants and toddlers, it is still, as a

parental questionnaire, burdened with a high degree of subjectivity. We are also aware that an association study would be more valuable if it was broadened to include a larger number of tested SNPs. The other limitation of the current study is its retrospective nature, where exposure to environmental factors or outcome assessment cannot be directly controlled. We were unable to eliminate the impact of the child's environment, that is, the psychosocial influence of the family, and of parental/maternal education and motivation, which is one of the major contributing factors to neurodevelopment (Niparko et al., 2010). Finally, our sample was limited only to a Caucasian (Polish) population, so it is uncertain whether this finding is repeatable elsewhere. Further analyses performed on a larger cohort should be performed to confirm this pattern of results.

Perspective

The significance of our findings is limited, given that longitudinal testing was done only up to 24 months of follow-up, and there was no strict control of certain independent variables known to affect CI performance. Extension of the observation period up to an age when more reliable tests for assessing speech understanding could be used would certainly add considerable value and verify the significance of the current result. Another research option that would significantly contribute to current findings is panel test widening by adding more known SNPs in both genes, as well as tests of *MMP9* and *BDNF* serum levels in analogically completed cohort group. It would be extremely valuable to explore if presence of more active T allele in *MMP9* rs3918242 corresponds to higher serum level of *MMP9* and in any way corresponds to language outcome.

Nevertheless, although extreme caution must be used, particularly because of the limitations of LEAQ, our findings can provide clinical value. The identification of increased risk of poor language development by identification of the *MMP9* rs3918242 C/T variant indicates that early remediation might be appropriate, such as conducting strategies of earlier implantation and more intensive educational intervention.

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Data Accessibility Statement

The data sets are analyzed during this study are accessible on reasonable request.

Declaration of Conflicting Interests

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