ISSN: 2083-389X
eISSN: 2084-3127

© J Hear Sci, 2022; 12(3): 47–53
DOI: 10.17430/JHS.2022.12.3.5

NEUROPLASTICITY AFTER COCHLEAR IMPLANTATION AS ASSESSED BY THE PLASMA LEVEL OF MMP-9 AND ITS GENETIC POLYMORPHISMS: A PROSPECTIVE STUDY OF CONGENITALLY DEAF CHILDREN

Monika Matusiak[1A-G], Dominika Oziębło[2B-E], Monika Ołdak[2C-E], Emilia Rejmak[3B], Leszek Kaczmarek[3ADG], Henryk Skarzynski[1B]

[1] Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, Warsaw, Poland
[2] Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland
[3] Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Warsaw, Poland

Corresponding author: Monika Matusiak, Oto-Rhino-Laryngology Surgery Clinic, Institute of Physiology and Pathology of Hearing, Mochanckiego 10, 02-042 Warsaw, Poland; email: m.matusiak@ifps.org.pl

Contributions:
A Study design/planning
B Data collection/entry
C Data analysis/statistics
D Data interpretation
E Preparation of manuscript
F Literature analysis/search
G Funds collection

Abstract

Background: If it was possible to assay biomarkers of neuroplasticity it might facilitate clinical management of deaf implanted children by identifying those among them who are at risk of speech and language rehabilitation failure. *MMP9* is a proteinase involved in neuroplasticity underlying different clinical conditions in human.

Material and methods: This was a longitudinal, prospective cohort study of 61 congenitally deaf children who underwent cochlear implantation. We investigated the genetic variants of matrix metalloproteinase 9 (*MMP9*) and plasma levels of MMP-9 that have been implicated in neuroplasticity after cochlear implantation. Auditory development was assessed by using the LittlEARS Questionnaire (LEAQ) at three follow-up points and the plasma level of MMP-9 was measured at implantation.

Results: There was a significant negative correlation between MMP-9 plasma level at implantation and LEAQ score at 18 months follow-up ($p < 0.05$). Two clusters of good and poor CI performers could be isolated based on this correlation. The prevalence of genetic variants of MMP9 – rs3918242, rs20544, and rs2234681 – in the good performers cluster was different to their prevalence in the poor performers cluster.

Conclusions: The study showed that children born deaf who have an MMP-9 plasma level of less than 150 ng/ml at cochlear implantation have a reasonable chance of attaining a high LEAQ score after 18 months of speech and language rehabilitation. It appears that MMP-9 plasma level at cochlear implantation is a promising prognostic marker for CI outcome.

Keywords: neuroplasticity • MMP-9 • cochlear implant • congenital deafness

NEUROPLASTYCZNOŚĆ PO WSZCZEPIENIU IMPLANTU ŚLIMAKOWEGO – POZIOM MMP-9 W PLAZMIE I JEJ POLIMORFIZM FUNKCJONALNY – WYNIKI BADANIA KOHORTOWEGO PROSPEKTYWNEGO DZIECI Z WRODZONĄ GŁUCHOTĄ

Streszczenie

Wprowadzenie: Wytypowanie biomarkerów neuroplastyczności w leczeniu głuchoty wrodzonej pozwoliłoby na zidentyfikowanie dzieci obarczonych ryzykiem niepowodzenia rehabilitacji słuchu i mowy po wszczepieniu implantu ślimakowego. MMP-9 jest proteinazą o udokumentowanej roli w procesach neuroplastyczności leżących u podłoża wielu stanów patologicznych i fizjologicznych w organizmie człowieka.

Materiał i metody: Przeprowadzone zostało podłużne, prospektywne badanie kohortowe 61 dzieci z głuchotą wrodzoną, leczonych za pomocą wszczepienia implantu ślimakowego. Celem badania było zbadanie występowania wariantów genetycznych matrix metaloproteinazy 9 (*MMP9*) oraz poziomów MMP-9 w osoczu dzieci włączonych do badania oraz ocena rozwoju słuchowego przy użyciu kwestionariusza

LittlEARs (LEAQ). Badanie LEAQ wykonano w trzech interwałach czasowych, badanie poziomu MMP-9 w osoczu wykonano podczas wszczepienia implantu ślimakowego.

Wyniki: Potwierdzono istnienie statystycznie istotnej ujemnej korelacji pomiędzy poziomem MMP-9 zmierzonym w osoczu podczas wszczepiania implantu ślimakowego a wynikiem LEAQ po 18 miesiącach korzystania z implantu ślimakowego ($p < 0.05$). Na tej podstawie wyróżniono dwie grupy użytkowników implantu ślimakowego – uzyskujących dobre wyniki i uzyskujących słabe wyniki słuchowe po 18 miesiącach rehabilitacji słuchu i mowy. Występowanie każdego z wariantów genetycznych *MMP9:* rs3918242, rs20544, rs2234681 z grupie użytkowników z dobrymi wynikami różni się od ich występowania w grupie z słabymi wynikami słuchowymi.

Wnioski: W badaniu wykazano, iż u dzieci z głuchotą wrodzoną, u których poziom MMP-9 mierzony w osoczu podczas wszczepienia implantu ślimakowego był niższy niż 150 ng/ml, mogą uzyskać wysoki wynik LEAQ po 18 miesiącach rehabilitacji słuchu i mowy. Wskazuje to, że poziom MMP-9 w osoczu mierzony podczas wszczepienia implantu ślimakowego jest dobrym czynnikiem prognostycznym funkcjonalnych wyników implantacji ślimakowej w głuchocie wrodzonej.

Słowa kluczowe: neuroplastyczność • MMP-9 • implant ślimakowy • wrodzona głuchota

Introduction

Cochlear implantation is a routine treatment for congenital deafness, and overall has very good results [1,2]. But after 30 years of treating childhood deafness in this way, and despite steady advancements in cochlear implant (CI) technology, it has been realised that there was still a large unexplained variance between CI users in their speech and language outcomes. Some implanted children develop auditory skills at a rate equal to their hearing peers, whereas others fail to do so despite strong efforts at speech and language rehabilitation [1–3]. If it was possible to have a tool that could predict, at the pre-implant diagnosis, which patients were at risk of a poor cochlear implantation outcome, that would be of considerable benefit in managing childhood deafness [1–6].

The sensory cortex requires a constant inflow of neural excitation to acquire its modality-specific abilities [7,8]. Delivery of electrical pulses to spiral ganglion nerve fibres by a cochlear implant allows the auditory cortex to develop [3]. Physiologically, it involves synaptic plasticity, which is the ability to modify the strength and efficacy of cortical synapses [7,8]. This, in turn, involves cascades of molecular regulation. In the light of recent research, a key player among many molecules connected with synaptic dynamics is matrix metalloproteinase-9 (MMP-9) [9–12]. MMP-9's role in neuroplasticity has been proven in experiments in which long term potentiation (LTP) has been shown to play a critical role [13–15].

In a previous study of the longitudinal observation of a cohort of 61 congenitally deaf children, we looked for correlations between the plasma levels of MMP-9 (measured at cochlear implantation) and the results of speech and language rehabilitation some 18 months later [16]. We performed a similar study on a cohort of 40 children with DNFB1-related deafness [17]. In both studies, we found a significant negative association between the plasma level of MMP-9 at implantation and language acquisition outcome [17]. We have also found a trend, although not quite significant, between the mean values of MMP-9 plasma levels (measured at cochlear implantation) in carriers of different genetic variants of rs3918242 of *MMP9*, which suggest that C/C carriers have lower plasma levels of MMP-9 than C/T carriers [17]. Substantiating this, the literature reports that the C/C genotype leads to transcriptional activity of the gene [18].

In our work, we used the parental questionnaire LittlEARs (LEAQ) to assess auditory development. For the DFNB1-related deafness group, we have reported that an MMP-9 serum level of 150 ng/ml, measured at cochlear implantation, can serve as a benchmark to differentiate two clusters of good and poor performers [19]. Patients who score better in LEAQ after 18 months of CI use start with an MMP-9 plasma level lower than 150 ng/ml, while those who scored poorly in LEAQ had MMP-9 plasma levels higher than 150 ng/ml. Moreover, for the same subgroup with DFNB-1 related deafness, we identified a significant association between the genetic variants rs3918242 *MMP9* and LEAQ at 18 months, indicating that carriers of C/C are predisposed to better language outcome after 18 months of CI use [17].

Given the fact that, in the DFNB-1 related deafness subgroup, an MMP-9 plasma level at implantation of 150 ng/ml suggests a better CI outcome, we thought it would be interesting to see if the same level of MMP-9 provides a similar prognostic value in a bigger, etiologically non-homogenous group of deaf children. It would also be interesting to study the distribution of genetic variants of *MMP9* in two clusters of good and poor performers after cochlear implantation.

In the current study we aimed to verify the hypothesis that, in a group of children with congenital deafness, an MMP-9 plasma level of 150 ng/ml, measured at implantation, can serve as a biomarker to separate good and poor CI performers some 18 months later. A second hypothesis was that the percentages of carriers of functional variants of the *MMP9* gene in the two clusters would not be equal.

Material and methods

Study design and participants

The study was conducted at the Institute of Physiology and Pathology of Hearing, Warsaw, Poland, between December 2016 and December 2019. We enrolled 70 infants and toddlers with congenital deafness who did not have any documented environmental risk factors, such as concomitant disease, severe prematurity, or history of viral infection during pregnancy. All enrolled patients had successfully undergone cochlear implantation with full insertion of the array and had their speech processor activated before the age of 2. Children had hearing thresholds equal to or above 80 dB in auditory brainstem responses (ABRs), and were implanted

with the same type of device by the same surgeon. After the procedure, parents or caregivers were instructed in auditory/verbal therapy according to the same rehabilitation protocol. All children followed the same observation program, which consisted of measurements at the time of implantation of MMP-9 plasma level (MMP-9_0) and of C-reactive protein (CRP). Observations were performed longitudinally and consisted of 3 time points: at cochlear implantation and at 8 and 18 months after CI activation, when auditory development measures were assessed using the LittlEARS Auditory Questionnaire (LEAQ_0, LEAQ_8, LEAQ_18). Of the group of 70 enrolled children, 2 were excluded due to parental withdrawal from participation in the protocol, 6 had elevated CRP levels, and 1 child was diagnosed with autism spectrum disorder after CI activation. Altogether, LEAQ and plasma protein data from 61 children were analysed. All children were of Caucasian origin. The study was designed and conducted according to the Declaration of Helsinki and was reviewed and approved by the Bioethics Committee of the Institute of Physiology and Pathology of Hearing (no. IFPS: KB/13/2015). Parents or caregivers of all children gave written informed consent.

Auditory development assessment

To assess auditory development the LittlEARS Auditory Questionnaire (LEAQ) was used in our study [20]. LEAQ consists of 35 questions with a "yes" or "no" answer, with a final score made up of the total number of "yes" answers. The questionnaire has been widely validated in many languages [21–26].

Genotyping

The *MMP9* polymorphisms rs3918242, rs2234681 and rs20544 were genotyped as described previously [17].

Plasma sample collection

Blood samples were collected on heparin as an anticoagulant. After sampling, tubes were centrifuged for 15 min at 1400 g. Next, plasma was obtained, aliquoted, and stored at −80°C for further analysis. Total protein content was measured with a BCA protein assay kit (Thermo-Scientific) following the manufacturer's protocol.

MMP-9 plasma level

The levels of MMP-9 in the plasma samples were measured using commercially available specific enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Inc., Minneapolis, USA) according to the manufacturer's protocol. The MMP-9 ELISA kit specificity is 82 kDa active MMP-9, and its sensitivity 0.156 ng/ml. A total of 30 µg/µl of protein from each plasma sample was diluted 70-fold with calibration diluent from the assays and analyzed in duplicate. The optical density of wells was determined at 450 nm using an automated microplate reader (Sunrise Microplate Absorbance Reader).

Statistical analyses

Correlation analysis methodology. The correlations between MMP-9 plasma levels measured at cochlear implantation and LEAQ scores collected at three time points were analyzed using a Pearson test (if test assumptions were met) or a Spearman test (if not). Beforehand, a Shapiro-Wilk test of normality was made in order to check assumptions. All variables for which the correlation was tested were normalized using the min–max scaling method. Correlation was considered statistically significant at a p-value ≤ 0.05. All computations were made using R version 3.6.3 (2020).

Clustering methodology. The PAM (Partitioning Around Medoids) algorithm was used for data partitioning, and variables used for clustering were scaled [27]. After the clustering process, paired comparisons were done.

Proportion analysis methodology. Proportions of observations having the same genotypes were analysed using a two-proportions Z-test. Prior to the tests, a Shapiro-Wilk test of normality was made in order to check the normality assumption and the numbers of observations in each cluster were verified (each of the clusters needed to contain at least 5 elements).

Results

Sample demographics

The study group's demographics was described in detail in our first paper [16]. Briefly, the group of 61 children contained 28 girls (46%) and 33 boys (54%) and the mean age at CI activation was 411 days (min = 208; max = 739; SD = 135). All subjects were implanted with the Med-El Synchrony CI and became regular CI users. All participants were of Caucasian origin.

Auditory development

In the study cohort the mean value of LEAQ_0 was 6.8 (min = 0, max = 28, SD = 7.6), the mean value of LEAQ_8 was 27.0 (min = 7, max = 35, SD = 6.4), and the mean value of LEAQ_18 was 32.5 (min = 22, max = 35, SD = 3.2).

Genotyping

Distributions of genotypes were in the Hardy-Weinberg equilibrium in the study group and was reported in detail in our first report [16]. Briefly, in the study group genotype distribution was as follows: for rs3918242 of *MMP9* gene, the C/C genotype was found in 43 cases (70%) and the C/T genotype in 18 cases (30%). For rs20544 of *MMP9* gene, the C/C genotype was found in 16 cases (26%), the C/T genotype in 29 cases (48%), and the T/T genotype in 16 (26%) cases. For rs2234681 of *MMP9* gene, the < 20/< 20 genotype was found in 17 cases (28%), the < 20≥ 20 genotype in 34 cases (56%), and the ≥ 20/≥ 20 genotype in 10 cases (16%).

MMP-9 plasma levels

In the study group the mean value of protein plasma level for MMP-9_0 was 236.9 ng/ml (min = 31.1, max = 769.7, SD 135.6).

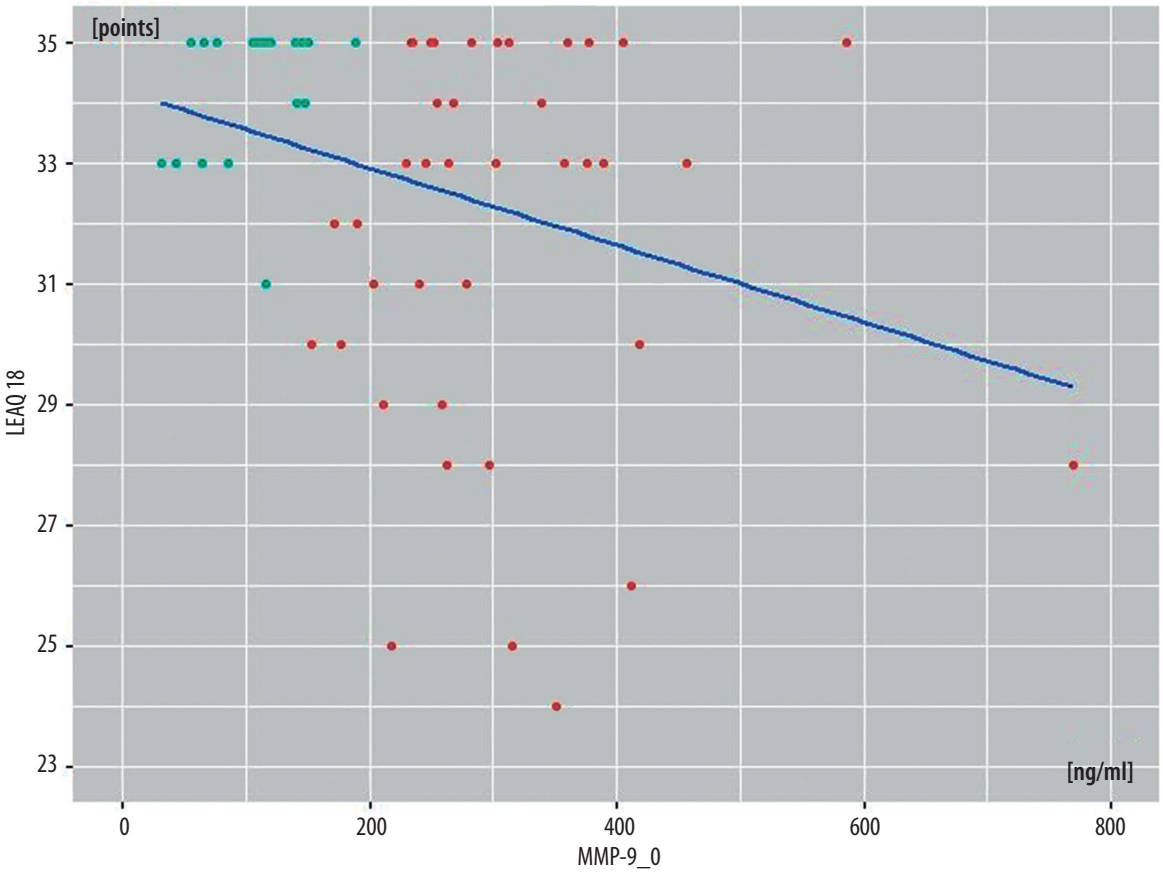

Figure 1. Two clusters of good (cyan circles) and poor (red circles) performers

Table 1. Distribution of genotypes of *MMP9* between both clusters of the study group

Genotypes	Number of patients (%)	
	Cluster 1 ($n = 21$)	Cluster 2 ($n = 40$)
MMP9 rs3918242		
C/C	17 (80.9%)	26 (65%)
C/T	4 (19.1%)	14 (35%)
MMP9 rs2234681		
< 20 / < 20	6 (28.5%)	11 (27.5%)
< 20 / > 20	12 (57.3%)	22 (55.0%)
≥ 20 / ≥ 20	3 (14.2%)	7 (17.5%)
MMP9 rs20544		
C/C	5 (23.8%)	11 (27.5%)
C/T	10 (47.6%)	19 (47.5%)
T/T	6 (28.6%)	10 (25.0%)

Correlation analyses and clusters

Testing for correlations in the 61-child study group showed that there was a significant correlation between the serum level of MMP-9 measured at cochlear implantation (MMP-9_0) and the auditory development measured after 18 months of CI use (LEAQ_18) ($p < 0.05$, rho = –0.25), as already reported [17]. Based on the correlation, using the clustering methodology, two clusters of good and poor performers were isolated (**Figure 1**) [19].

The cluster of good performers (cluster 1) comprised 21 children, whereas the cluster of poor performers (cluster 2) comprised 40 children. Mean age at CI activation in cluster 1 was 13.5 months (min = 7.8, max = 21.2, SD = 4.6) and in cluster 2 it was 13.7 months (min = 6.9, max = 24.6,

SD = 4.5). In cluster 1, children who had their CI activated before 1 y.o. formed 52% (11/21) of the group, while the children who had their CI activated after 1 y.o. made up 48% (10/21) of the group. In cluster 2, children who had their CI activated before 1 y.o. formed 45% (18/40) of the group, while the latter made up 55% (22/40).

Distribution of genotypes of *MMP9* in clusters

In the study group distribution of genotypes of *MMP9* between both clusters is presented in **Table 1**.

Testing for comparisons of genotype frequencies of *MMP9* did not meet the methodological criteria due to too few genotypes in the study group.

Discussion

The current study was preceded by a retrospective analysis [16], also conducted in the Institute of Physiology and Pathology of Hearing in Warsaw, which showed results of research on the involvement of functional variants of the *MMP9* gene in neuroplasticity after cochlear implantation. To gain a broader view of the role of these genes and their products in molecular regulation of synaptic plasticity after implantation, we conducted a longitudinal observational cohort study of implanted children. To gauge the prognostic value of MMP-9 plasma level measured at cochlear implantation for functional outcomes after treatment, we focused on the subgroup of congenitally deaf but otherwise heathy children, with the same genetic etiology of their hearing impairment [19]. As a result, based on the significant correlation between pre-implant plasma level of MMP-9 and results of auditory development after 18 months of speech and language rehabilitation, two clusters of good and poor CI performers were isolated using the PAM algorithm, as already reported [19].

Following the aforementioned results in the etiologically homogenous group of implanted children we aimed to verify the assumption that 150 ng/ml of MMP-9 plasma level is also a predictive value for a larger group of children with different etiologies of deafness. Clustering in the study group of 61 implanted children repeats the already reported result, showing that children who start with an MMP-9 plasma level lower than 150 ng/ml have better odds for good speech and language rehabilitation outcome, despite genetic etiology of deafness [19]. Note that the study was designed to assess the prognostic value of plasma measurements of MMP-9 on the functional outcome of cochlear implantation in prelingual deafness; it does not directly show the specific, molecular activity of the protein in neuronal remodelling following implantation.

Polymorphism selection

We selected polymorphisms according to their documented role in other conditions involving neuronal plasticity, like depression, bipolar disease, or schizophrenia [18,28–30]. Rs3918242 of *MMP9* is the best studied polymorphism located in the promoter region of the *MMP9* gene, with a reported role in schizophrenia and bipolar disease [18,29]. The C to T substitution at −1562 bp leads to increased transcription activity of the gene [8,29]. The other polymorphism located in the promoter region is the rs2234681 of *MMP9*, which is a microsatellite repeat of (CA)n, also closely related to transcriptional activity of the promoter. The rs20544 of *MMP9* is a relatively recently described polymorphism, but has already well documented involvement in clinical symptoms such as delusions in schizophrenia [29].

Distribution of gene functional variants

The distribution of functional variants of the tested genes in both clusters showed that, in cluster 1 (good performers), carriers of the C/C variant of rs1839242 *MMP9* made up 81% of all cases, while carriers of the C/T variant made up 19%. The mean age at CI activation in both clusters was almost the same, and we did not see any greater representation of younger or older participants in any cluster – contrary to the C/C genotype of rs1839242 of *MMP9* carriers, who outnumbered C/T carriers in cluster 1. Likewise, in the already reported analysis of DFNB1-related deafness (the group of 40 children), mean age at CI activation was almost equal in each cluster [19]. This observation is in line with our previous results of the retrospective group of CI children with DFNB1-related deafness, aged 2 y.o., where we saw that rs1839242 of *MMP9* was a significant predictor of auditory development (based on a multiple regression model and lack of significance for age at CI activation as a predictor in the model) [16]. This finding should also be read in the context of prevalence of genetic variants of this polymorphism in the study group, where carriers of the C/C variant of rs39181242 of *MMP9* were most prevalent (75% frequency), whereas in the cluster of good performers the figure was 81%. Comparable data in the literature show that, in the normal hearing population, the C/C variant of rs39181242 of *MMP9* is present with frequencies ranging from 72.0% to 81.2%, whereas the C/T variant is present with a frequency of 16.9% to 26.0% [18,31]. Of course, one needs to keep in mind that our sample was relatively small compared to the control groups of the cited authors, which involved hundreds of subjects.

Each of the other two polymorphisms, rs20544 and rs2234681, is represented in our material by three functional variants. This makes interpretation of their prevalence more difficult, especially in the smaller cluster 1. However, in general, frequencies of their functional variants in both clusters are close to each other. Population data for rs2234681 is not available in databases, nor in the literature. For rs20544, Łepeta et al. report that, in the healthy population, carriers of the C/C variant were present with a frequency of 18.8%; carriers of C/T variant, 48.9%; and carriers of T/T variant, 32.3% [30]. This distribution pattern is close to the frequencies of variants reported in our material.

Limitations

Undoubtedly, a weakness of this study is the subjective character of the parental questionnaire, LEAQ. Another factor difficult to control is the effect of the child's environment – family support, parental involvement, and motivation to support the child's efforts in rehabilitation. Also a longer observation period, with more frequent follow-ups, would add considerable value, particularly if it

included an age at which more credible, or possibly objective, tests for language development could be done. Unavoidably, our results need to be verified in larger studies focusing on how the MMP-9 protein affects the neuronal reorganisation within auditory and language pathways of the brain following cochlear implantation in congenital deafness. Increasing the number of participants by performing large-scale, multicentre studies would throw more light onto how general our findings are. Increasing the number of tested polymorphisms of *MMP9* would also help us to deepen our understanding of the role of MMP-9 protein in neuronal plasticity after implantation.

Acknowledgements

The authors would like to thank to all participants and their parents or caregivers for cooperation in this study and the Board of Directors of the Institute of Physiology and Pathology of Hearing in Warsaw for their support.

Funding

This paper represents independent research funded by the National Centre of Science – grant nr UMO 2014/13/D/NZ5/03337. ER and LK were supported by the BRAINCITY MAB/2018/10. The "Center of Excellence for Neural Plasticity and Brain Disorders: BRAINCITY" project is carried out within the International Research Agendas Programme of the Foundation for Polish Science, co-financed by the European Union under the European Regional Development Fund.

Conflict of interests statement

The authors declare that there are no conflict of interests.

References

1. Kral A, O'Donoghue G. Profound deafness in childhood. N Eng J Med, 2010; 363: 1438–50.
2. Kral A, Kronenberger WG, Pisoni DB, O'Donoghue GM. Neurocognitive factors in sensory restoration of early deafness: a connectome model. Lancet Neurol, 2016; 15(6): 610–21.
3. Niparko J, Tobey EA, Thal DJ, Eisenbrg LS, Wang NY, Quittner AL, et al. Spoken language development in children following cochlear implantation. JAMA, 2010; 303(15): 1498–506.
4. Houston DM, Stewart J, Moberly A, Hollich G, Miyamoto RT. Word learning in deaf children with cochlear implants: effects of early auditory experience. Dev Sci, 2012; 15: 448–61.
5. Dettman SJ, Pinder D, Briggs RJS, Dowell RC, Leigh JR. Communication development in children who receive the cochlear implant younger than 12 months: risks versus benefits. Ear Hear, 2007; 28 (Suppl 2): 11S–18S.
6. Fallon JB, Irvine DRF, Shepherd RK. Neural prostheses and brain plasticity. J Neural Eng, 2009; 6: 065008.
7. Kral A. Auditory critical periods: a review from system's perspective. Neurosci, 2013; 247: 117–33.
8. Holtmaat A, Caroni P. Functional and structural underpinnings of neuronal assembly formation in learning. Nature Neurosci, 2016; 19: 1553–62.
9. Reinhard SM, Razak K, Ethell I. A delicate balance: role of *MMP9* in brain development and pathophysiology of neurodevelopmental disorders. Front Cell Neurosci, 2015.
10. Beroun A, Mitra S, Michaluk P, Pijet B, Stefaniuk M, Kaczmarek L. MMPs in learning and memory and neuropsychiatric disorders. Cell Mol Life Sci, 2019; 76: 3207–28.
11. Vafadari B, Salamian A, Kaczmarek L. MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J Neurochem, 2016; 139 (Suppl 2): 91–114.
12. Ethel IM, Ethel DW. Matrix metalloproteinases in brain development and remodeling: synaptic functions and targets. J Neurosci Res, 2007; 85: 2813–23.
13. Nagy V, Bozdagy O, Matynia A, Balcerzak M, Okulski P, Dzwonek J, et al. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. J Neurosci, 2006; 26: 1923–34.
14. Nagy V, Bozdagy O, Huntley WH. The extracellular protease matrix metalloproteinase-9 is required by inhibitory avoidance learning and required for long term memory. Learn Mem, 2007; 14: 655–64.
15. Bozdagy O, Nagy V, Kwei KT, Huntley GW. In vivo roles for matrix metalloproteinase-9 in mature hippocampal synaptic physiology and plasticity. J Neurophysiol, 2007; 98: 334–44.
16. Matusiak M, Oziębło D, Obrycka A, Ołdak M, Kaczmarek L, Skarżyński P, Skarżyński H. Functional polymorphism of M*MP9* and *BDNF* as a potential biomarker of auditory plasticity in prelingual deafness treatment with cochlear implantation: a retrospective cohort analysis. Trends Hear, 2021; 23312165211002140.
17. Matusiak M, Oziębło D, Ołdak M, Rejmak E, Kaczmarek L, Skarżyński PH, Skarżyński H. Prospective cohort study reveals MMP-9, a neuroplasticity regulator, as a prediction marker of cochlear implantation outcome in prelingual deafness treatment. Mol Neurobiol, 2022; 59(4): 2190–203.
18. Rybakowski JK, Remlinger-Molenda A, Czech-Kucharska A, Wojcicka M, Michalak M, Losy J. Increased serum matrix metalloproteinase-9 (MMP-9) levels in young patients during bipolar depression. J Affect Disord, 2013; 146: 286–9.
19. Matusiak M, Oziębło D, Ołdak M, Rejmak E, Kaczmarek L, Dobek D, et al. MMP-9 plasma level as a biomarker of cochlear implantation outcome: results of a prospective, longitudinal study of congenitally deaf children [preprint]. Available at https://doi.org/10.21203/rs.3.rs-1395711/v1. Accessed 2022 Sept 19.
20. Weichbold V, Tsiakpini L, Coninx F, D'Haese P. Development of a parent questionnaire for assessment of auditory behaviour of infants up to two years of age. Laryngo-Rhino-Otol, 2005; 84: 328–34.
21. Obrycka A, Padilla Garcia JP, Pankowska A, Lorens A, Skarżyński H. Production and evaluation of a Polish version of the LittlEars questionnaire for the assessment of auditory development in infants. Int J Pediatr Otorhinolaryngol, 2009; 73: 1035–42.
22. Coninx F, Weichbold V, Tsiakpini L, Autrique E, Bescond G, Tamas L, et al. Validation of the LittlEARS Auditory Questionnaire in children with normal hearing. Int J Pediatr Otorhinolaryngol, 2009; 73: 1761–68.
23. Geal-Dor M, Jbarah R, Meilijson S, Adelman C, Levi H. The Hebrew and the Arabic version of the LittlEARS Auditory Questionnaire for the assessment of auditory development: results in normal hearing children and children with cochlear implants. Int J Pediatr Otorhinolaryngol, 2011; 75: 1327–32.

24. Wanga L, Sun X, Liang W, Chen J, Zheng W. Validation of the Mandarin version of the LittlEARS Auditory Questionnaire. Int J Pediatr Otorhinolaryngol, 2013; 77: 1350–54.
25. García Negro AS, Padilla García JL, Sainz Quevedo M. Production and evaluation of a Spanish version of the LittlEARS Auditory Questionnaire for the assessment of auditory development in children. Int J Pediatr Otorhinolaryngol, 2016; 83: 99–103.
26. Obrycka A, Lorens A, Padilla JL, Piotrowska A, Skarżyński H. Validation of the LittlEARS Auditory Questionnaire in cochlear implanted infants and toddlers. Int J Pediatr Otorhinolaryngol, 2017; 93: 107–16
27. Kaufman L, Rousseeuw PJ. Finding Groups in Data: An introduction to cluster analysis. Wiley Series in Probability and Statistics, New York: Wiley; 1990.
28. Xia Q-R, Zhang C, Liang J, Xu Y-Y, 2019. The association of functional polymorphism of matrix metalloproteinase-9 gene (rs3918242) with schizophrenia: a meta-analysis. Int J Psychiatry Clin Pract, 23(3): 207–14.
29. McGregor N, Thompson N, O'Connell KS, Emsley R, van der Merwe L, Warnich L. Modification of the association between antipsychotic treatment response and childhood adversity by *MMP9* gene and its variants in a first-episode schizophrenia cohort. Psychiatry Res, 2018; 262: 141–8.
30. Łepeta K, Purzycka KJ, Pachulska-Wieczorek K, et al. A normal genetic variation modulates synaptic MMP-9 protein levels and the severity of schizophrenia symptoms. EMBO Mol Med, 2017; 9(8): 1100–6.
31. Demacq C, de Souza AP, Machado AA, Gerlach RF, Tanus-Santos JE. Genetic polymorphism of matrix metalloproteinase (MMP-9) does not affect plasma MMP-9 activity in healthy subjects. Clin Chim Acta Mar, 2006; 365(1–2): 183–7.