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ORIGINAL ARTICLE

Advancement in singing ability using The YUBA Method in patients with cochlear implants

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Abstract

Conclusion. Although overall improvement was not so dramatic due to a lack of retention, session by session advancement of matching pitch for targeted MIDI (Musical Instrument Digital Interface) sound was predominantly obvious. It was proved that The YUBA Method worked to improve singing ability for patients with cochlear implants. *Objectives.* This study sought to verify whether or not the Yuba theory and method improved the singing ability of patients with cochlear implants. *Subjects and methods.* Based on diagnosis, the instructor experimented to improve matching pitch of singing for three patients with cochlear implants using The YUBA Method. The mean fundamental frequencies and standard deviation of singing were then compared with before and after instructions to patients. The instruction was given for over 40 days at the University of Tokyo Hospital. *Results.* For each patient, the mean fundamental frequencies of their singing approached the mean MIDI specified frequencies as references for tests done in all three songs. Overall, the SD between fundamental frequencies of their singing and reference MIDI sounds became smaller.

Keywords: *Vocal folds, falsetto voice, vocal register shock, natural voice, vocal functional physiology, Yuba theory, tone deafness, music education, speech therapy*

Introduction

It is known that people with cochlear implants tend to sing off-key, monotonously, and flat [1]. Earlier results are the work of two separate research groups using the same unique technological singing voice method, The YUBA Method. One group tested 60 normal hearing subjects and found that the main effect of treatment was at the $p < 0.0001$ significance level [2,3]. Another group's results indicated a clear improvement in the singing ability of eight children with cochlear implants through the same method at the $p < 0.02$ significance level. The mean fundamental frequencies of their singing approached the mean MIDI specified frequencies as references. Secondly the deviation between fundamental frequencies of their singing and reference MIDI sounds decreased [4].

There are varying factors among the subjects that influence their singing ability, i.e. device and program of cochlear implants, the inner shape of the cochlea and the condition of the hair cells, musical background, implantation circumstances, cause of hearing loss or strong bradyacusia, and age at implantation. Therefore, it is necessary to evaluate the development of singing skills in individual cases rather than trying to examine their singing by standardizing the subjects' conditions.

In this study, we gave subjects several courses of individual singing voice instruction to teach them whole songs, while gradually increasing the degree of difficulty, so as to understand their singing ability.

This study asserts that if cochlear implant patients can recognize falsetto and natural voices, their singing ability, specifically pitch adjustment, can improve with the application of The YUBA Method. This method facilitates the subjects' intrinsic laryngeal muscular activities, which control their vocal folds, resulting in improved ability in matching pitch.

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With regards to the mechanism of pitch adjustment and pitch control in voicing, pitch is determined by the number of vibrations of the vocal folds. The main factor determining pitch is the function of intrinsic laryngeal muscles that control the length and thickness of vocal folds and the opening and closing of the glottis. Breathing muscles which adjust expiratory pressure can also influence pitch [5]; however, the influence of intrinsic laryngeal muscles is much stronger [6]. In the intrinsic laryngeal muscles there are specific functions of muscles such as the cricothyroid muscle which stretches the vocal folds and the closing muscle group which closes the glottis (interior thyroarytenoid muscle, posterior thyroarytenoid muscle, lateral cricoarytenoid muscle, transverse muscle, oblique muscle) [7–9]. In the production of the falsetto voice the cricothyroid muscle is more active than the closing muscle group, and in the production of the natural voice the closing muscle group is more active [10].

The contraction of the cricothyroid muscle narrows the distance between the cricoid cartilage and the thyroid cartilage; this results in stretching of the vocal folds. The number of vibrations increases not only because of the contraction of the cricothyroid muscle, but also because of the increase in expiratory pressure which elevates the subglottic air pressure [5–9]. The contraction of the closing muscle group closes the glottis, and as the cricothyroid muscle relaxes, the vocal folds are shortened. The number of their vibrations decreases not only because of the contraction of the closing muscle group but also because of decreasing expiratory pressure, which lowers the subglottic air pressure [5–9].

The mechanism for lowering pitch has not been fully revealed [10] in speech physiology. However, past experiments succeeded in lowering pitch by decreasing expiratory pressure, resulting in production of the natural voice [11,12].

Subjects and methods

Three young outpatients of the University of Tokyo Hospital were given therapeutic vocal instruction by The YUBA Method. Table I presents relevant information regarding the patients.

This study was conducted at a language training laboratory in the University of Tokyo Hospital. The mean laboratory base level during the treatment was around 40 dB. The patients were treated four times between 1 April and 13 May 2006. The patients did not have any medical illness that would affect singing performance (i.e. flu, cold, cough) during the treatment.

Subjects were each given copies of three musical figures and three songs on separate sheets, totaling six sheets. We used a Sony PCM-D1 hard disk recorder to record singing data, instructions, and singing diagnosis, and an electric piano for melody and harmony accompaniment.

The nursery songs chosen for use in this study are well known to most Japanese people, including the subjects. We started out at an easy level and gradually worked up to higher levels. Song 1 'Frog chorus' and song 2 'Twinkle, twinkle little star' have an interval within the major 6th. Song 2 is a little more difficult than song 1, because in song 1, they can sing along with the scale, but song 2 has two jumps in the melody and is much longer than song 1.

Table I. Profile of patients.

Subject	Gender	Age	Device	Medical history	Speech perception test score	Musical background
Case 1	Female	23	Med-El Combi 40+	(Congenital bradyacusia) Hearing aid from 1 year 8 months until implantation of CI Cause of anacusis: vestibular aqueduct dilatation CI: since 20 years 3 months	Single note: 7% Single word: 20% Short sentence: 18% Post surgery time period to test: 1/2 years	No prior singing or instrument experience except school standard lessons. Violin lessons from 5 to 9 years
Case 2	Male	20	Cochlear Nucleus 22	(Congenital bradyacusia) Cause of anacusis: cold Hearing aid until 11 years CI: since 13 years 1 month	Single note: 60% Single word: 80% Short sentence: 88% Post surgery time period to test: 4 years	No prior singing or instrument experience except school standard lessons
Case 3	Male	20	Cochlear Nucleus 22	(Acquired anacusis) Age of loss: 9 years 4 months Cause of anacusis: Mycoplasma pneumoniae infection CI: since 10 years 5 months	Single note: 45% Single word: 72% Post surgery time period to test: 1 year	No prior singing or instrument experience except school standard lessons

Song 3, 'Sea', is much more difficult than the other two because it has a wider range and skips more frequently. The tonal range of the songs for a male voice is 131–294 Hz, and for a female voice is 262–587 Hz. To ensure consistency due to differences in vocal range, the key was adjusted according to individual vocal range.

Yuba theory and method

The Yuba theory is based on the 'Vocal functional physiology', which deals with the anatomic function of voicing in humans. This theory was originated by Toru Yuba, who has also named this field. It is mainly about controlling intrinsic laryngeal muscular activities, which in turn control the movement of the vocal folds in an effective and original way.

A part of this theory and method comes from the studies of Cornelius L. Read [13], based on the vocal pedagogy of eighteenth century Italy. The Yuba theory is rooted in the belief that humans do not possess any vocal organ. Instead, humans utilize the larynx, which is part of the digestive and respiratory systems, to produce voice. From the practical perspective of vocal functional physiology, humans only have two registers – falsetto and natural. Intrinsic laryngeal muscles are skeletal muscles responsible for making these two registers. However, Toru Yuba considers intrinsic laryngeal muscles as being simultaneously both *semi-voluntary* and *semi-involuntary muscles*. This is because producing falsetto voice or, more importantly, coordinating two registers to eliminate *vocal register shock* cannot be easily controlled like any skeletal muscle, i.e. flexing the arm by the biceps or moving the fingers. Basically, the singing voice is developed by strengthening and coordinating the functions making the two registers – falsetto and natural voices. The frequency of the vibration of the vocal folds determines pitch. We can control and train the intrinsic laryngeal muscles by imitating a modeled voice and at the same time correct motor-related off-key singing. The method based on this theory has facilitated the voicing correction of many off-key singers.

In this treatment The YUBA Method mainly focuses on: producing the falsetto and natural voice; singing both registers independently; interchanging between both registers smoothly; and coordinating the singing of the two registers. The YUBA Method also develops basic abilities for singing by following these essential steps: teaching subjects how to sing a one octave scale, arpeggio, and jump.

The three ways of targeting the pitch adjustment in singing with The YUBA Method are as follows. (1) In the case of the pitch being too low, it is possible to raise the pitch by balancing via instruction with the falsetto voice and increasing air pressure. (2) In the case of the pitch being too high, it is possible to lower the pitch by balancing via instruction with the natural voice and decreasing air pressure. (3) In the case of being off-pitch due to the register shock, it is necessary to coordinate the natural and falsetto voice registers.

Treatment procedure

First, we interviewed each patient to get their basic information: relevant medical and musical background, mental and physical health, and motivation for learning to sing.

In the singing diagnosis, we played the melody of the first two bars of 'Frog chorus' (song 1) as an introduction to each patient and then allowed them to sing the whole song from the first to the eighth bar a cappella (without accompaniment). The first four bars of 'Twinkle, twinkle little star' (song 2) were played as an introduction and the patients sang the complete song from the first to the twelfth bar a cappella. The last four bars of 'Sea' (song 3) were played as an introduction and the patients were asked to sing it from the first to the eighth bar a cappella. We diagnosed their singing acuity comparing their performance before and after each instruction. For one subject (case 2), we adjusted the key of song 1 and 2 to solve the problem of vocal range; the original key was too low for the subject to sing, therefore we raised the key for five semi-tones.

At the beginning of the instruction the instructor produced samples of both falsetto and natural voice several times, letting the subjects listen, while each time explaining to them about the difference in tone quality, i.e. 'This is the falsetto voice, that is the natural voice', to give them a clear concept of both voices. Secondly, for their falsetto voice instruction we made them imitate an owl call sounding like 'whoo', or a dog howling 'whoo', in a breathy high pitched voice. For their natural voice instruction, they produced 'ah—', in a less breathy, low pitched voice. These are vocal abilities necessary for elevating and lowering pitch. Third, we instructed them how to sing a one octave scale, a one octave arpeggio and a one octave jump in this order, until each sound pattern was sung nearly in tune. We instructed them to keep in tune by adjusting their generation of the falsetto voice and the natural voice, and adjusting their expiratory pressure according to the singing conditions of each subject. Lastly, we asked them to sing the same songs to compare their performance before and after instruction.

Method of measurement and analysis

We used the data recorder to compare the fundamental frequencies of the subjects' singing voices with the frequencies of each reference MIDI sound by time series to examine the accuracy of their singing. In this study, we used the frequencies of MIDI signals as the correct reference frequencies. We statistically processed the sequential differences of the vocal frequencies of the subjects' singing before and after the singing voice instruction. We eliminated most of the recording noise, adjusted the volume range per data, and presented the frequencies of each musical note by MIDI for ease of computer processing. For their vocal frequencies, we selected and adopted the fundamental frequencies of their singing voices to the exclusion of overtones. We then calculated the differences between the fundamental frequencies of their singing and the frequencies of the MIDI signals per sample.

Here, we use the frequency measurement unit 'cent', to compare each example of sampling data over MIDI reference frequencies. The mean ($\bar{x}$) of fundamental frequencies in each subject's singing is calculated by dividing the sum of x by the number of data (x) through singing and SD as a standard deviation with respect to x in each subject's singing. In spectrum analysis of the data, we used sampling period 1/86(Sec.) and 4096 points for the frequency resolution.

Data were processed as follows. We did not accept as voice data that which did not correspond to MIDI data. Therefore, the obtained data are somewhat short compared with the actual time series. The statistic for data analysis in this situation is made only when the data for both groups correspond.

In statistical processing, we compared each mean ($\bar{x}$) of singing of all cases and their SDs before and after the singing voice instruction. The changes of mean pitch of each subject's singing were indicated by the former ($\bar{x}$) and the change of the width of sound deviation were indicated by the latter (SD). We determined whether or not the SD of the frequencies was improved through these statistical tests.

We evaluated means, SDs, and p value (probability by Student's t test) in every singing sample of each case in the following way. As an example, we explain the procedure for song 1 of case 1. We used the same procedure for other songs and other cases.

1. We collected all fundamental frequency data in a singing session that correspond to some MIDI note frequency (e.g. C note). As for song 1, its MIDI tone sequences are like C, D, E, F, E, D, C, . . ., where each tone is included multiplicatively. In this case, for example, we collected data of the first C and of the seventh C as same group data (e.g. C is included eight times in song 1). Then we evaluated the mean of a singing fundamental frequency in a song for C from the data with the 'cent' unit.
2. By the same process described above, we can evaluate all means of singing fundamental frequencies that should be equal to each reference MIDI tone frequency in the song. We calculated the mean for each voice corresponding to MIDI's six notes, from C to A, in song 1.
3. We applied this processing to song 1–B1 [singing data before instruction (first day)] and song 1–A4 [singing data after instruction (fourth, last day)]. Then we obtained the mean of all fundamental frequency data in a singing session as for before and after instruction.
4. Using data from (3), we tested the difference of mean of fundamental frequencies of a song between before and after instruction. Here we used Student's paired t test, with $\alpha=0.05$.
5. We also evaluated mean of SDs of a song with the same processing as described above using SDs that correspond to each MIDI tone frequency in a song. The value of each SD corresponds to scattering measure of each singing session in this study.
6. We applied the same procedure to song 1, song 2, and song 3 for every case (1, 2, and 3). As well as simple statistical analysis, we calculated quantitatively the variation between the results and the target MIDI frequencies.

Results

Here we use following abbreviations. B1, before instruction [1st time]; A1, after instruction [1st time]; A2, after instruction [2nd time]; A3, after instruction [3rd time]; A4, after instruction [4th time]. Table II shows mean ($\bar{x}$), $\pm$SD [unit: cent] and p (T <t) before and after the singing voice instruction. p (T <t) is probability by Student's t test with $\alpha=0.05$ to B1 and A4 pair and All. 'All' are evaluated by simple average of all cases or songs. A function of statistical data processing in MS-EXCEL was used.

Figure 1 shows means ($\bar{x}$) of fundamental frequency of voice of singing for three songs (1, 2, and 3) for each case. Figure 2 shows the absolute values of mean ($\bar{x}$) by gray bar and $\pm$SDs by vertical bold line for every song for each case.

Table II. Mean ($\bar{x}$). ±standard deviation (SD) [cent] and p (T <t).

Song	Parameter	Case 1			Case 2			Case 3			All
		B1	A4	p	B1	A4	p	B1	A4	p	p
Song 1	$\bar{x}$	−317.4	−2.1	0.0058	609.4	−19.3	0.0002	−258.9	−7.1	0.0005	0.0022
	±SD	272	219.7	0.0588	264.4	211.5	0.0399	127.9	150.2	0.1307	0.0765
Song 2	$\bar{x}$	−382	−183.3	0.0266	447.1	−204.2	0.0018	−339.8	−64.9	0.0008	0.0097
	±SD	256.9	247.4	0.0008	305.2	207.1	0.9893	153.3	148.2	0.0108	0.3336
Song 3	$\bar{x}$	−739.6	−452.8	0.0026	178.1	128.9	0.3858	−749.4	−221.5	0.0027	0.1304
	±SD	261.8	272.4	0.0469	289.8	270.7	0.7409	145.6	210.2	0.0014	0.2631

Average pitch after the singing voice instruction was improved

As shown in Table II, we obtained a level of significance of 5% p (T < = t) = {0.002 (song 1), 0.01 (song 2), 0.13 (song 3)} by t test for $\bar{x}$ from averaging all cases. As a result, we could conclude that the average of frequencies of all subjects' singing was improved after instruction, because the average pitch after the instruction approached the best value (0). We found that the average of frequencies of all subjects' singing was improved remarkably by a few periods of instruction. Figures 1 and 2 show the same behaviors.

Scattering of pitch (SD) was improved slowly by instructions

From the table, we found that the average pitch of singing of all cases was improved soon after each instruction, although the SDs of singing were improved slightly. Overall, the SD between fundamental frequencies of their singing and reference MIDI sounds became smaller by instruction, although the pace of the improvement of scattering of pitch was slow. We found that improvement for the scattering of pitch needs continuous instruction. This shows the difficulty in curing the scattering of pitch of singing. Figure 2 illustrates this statement.

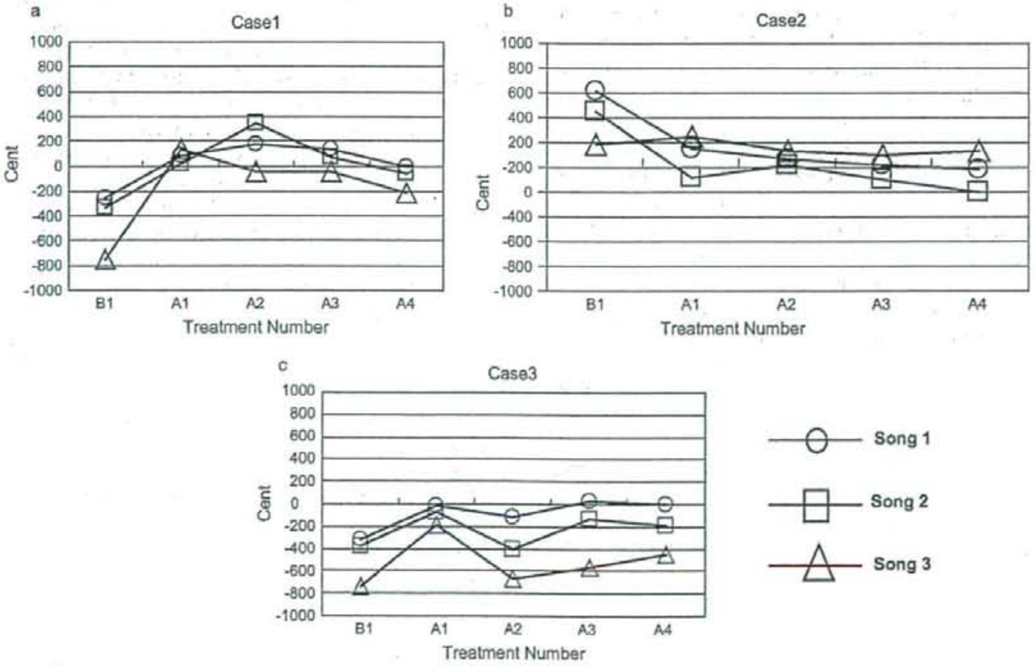

Figure 1. Means ($\bar{x}$) of singing in (a) case 1, (b) case 2, and (c) case 3 for every song (1, 2, and 3). B, before instruction; A, after instruction.

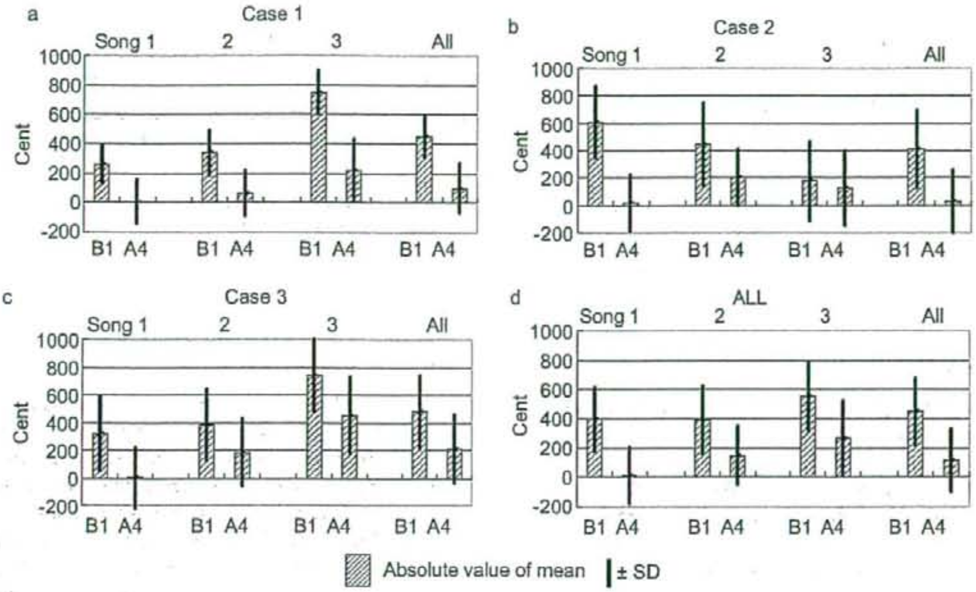

Figure 2. Means and SDs of singing for three songs (1, 2, and 3); (a) case 1, (b) case 2, (c) case 3, (d) all. B, before instruction; A, after instruction.

Discussion

It is known that people with cochlear implants tend to sing off-key, monotonously, and flat. Former results have been the work of two separate research groups using The YUBA Method. One subject group in Hawaii tested 60 elementary students attending the normal level of hearing classes and found that the main effect of treatment was at the $P<0.0001$ significance level. In 2004 the latter group tested eight pediatric patients with cochlear implants and found a clear improvement in their singing ability and that the main effect of treatment was at the $p<0.02$ significance level.

This latest research method was improved to provide more of a wide spectrum of results with the patients. This time we conducted the research at a patient's individual level, with four exposures over 40 days as opposed to an individual exposure. We also had the patients sing three whole songs instead of four bars of one song, to provide a larger base of data to process for the results. The three sample songs were deliberately picked for varying levels of difficulty and singing patterns for the same reason. The songs were also chosen because they are well known, to maximize realism within the research. An instructor's voice and electric piano sound were used as reference sounds to clarify the pitch and melody for the patients.

Based on earlier research, we expected that the mean fundamental frequencies of their singing would approach the mean MIDI specified frequencies as references for this study. Also, we thought that the deviation between fundamental frequencies of their singing and reference MIDI sounds would decrease. We expected that these factors would remain constant despite the longer period of exposure and variation in level of difficulty.

Initial consultations with the three patients showed that they could almost distinguish between falsetto and natural voices. Building on this ability, the application of The YUBA Method allowed them to control their laryngeal muscular activities, as demonstrated in their improved ability to produce both the falsetto and natural voices; to sing a one octave scale, arpeggio, and jump guided by an electric piano; to sing the three songs a cappella; and to raise or lower pitch in the rendition of songs. After every instruction, the singing ability, particularly matching pitch, of each patient was diagnosed.

Before instruction, the first patient (case 1) sang monotonously and lower than the targeted pitch. After receiving instructions regarding the falsetto voice, the patient sang higher than the targeted pitch, resulting in off-key singing. Furthermore, she had vocal register shock when shifting from the natural to the falsetto voice and vice versa. When instructed to coordinate the two registers, the improvement in her singing pitch was apparent despite not being able to erase the vocal register shock completely.

The second patient (case 2) sang monotonously and higher than the targeted pitch at the outset, thereby exhibiting a narrow vocal range manifested in singing a higher key when asked to sing in a low note and vice versa. Based on this diagnosis, instructions were given regarding the two registers to expand his vocal range and to introduce the concept of high and low pitch. In addition, the key of songs 1 and 2 was changed from C to F to adapt to the patient's high-pitched male voice. Although the patient still found difficulty in producing definite falsetto and natural voices, he showed an expansion of his vocal range, with a tendency to produce the falsetto and natural voices when prompted.

Originally, the third patient (case 3) sang monotonously and lower than the targeted pitch. After receiving instructions, he was able to produce the falsetto voice. However, he could not produce the falsetto voice constantly until the end of the fourth instruction. Diagnosis revealed an improvement in his singing pitch but his pitch still registered below the target.

If the second and third patients (cases 2 and 3) produced two registers constantly, there would be a better improvement of matching pitch for their singing.

Science is relentlessly in pursuit of improving human quality of life through research. Science is finding that the human body, while fallible, may be repaired and updated through technology. As medical research technology allows us to repair our 'hardware' (the body) through surgery, prosthetic parts, pacemakers, cochlear implants, etc., we invariably have to update the 'software' (technological method) simultaneously. This unique technological method will serve to better the quality of life for the hearing disabled.

In this study, we were able to improve significantly the singing abilities of people with cochlear implants by The YUBA Method. This is an example of how technology developed by research has compensated for the inadequacy of the body's hardware.

In the case of acquired deafness it is known that hearing and speaking abilities can be regained quickly and certainly after implanting an artificial cochlea. This means that even if people lose their hearing ability through some disorders such as senile hearing loss, disease, or accidents, they can regain the pleasure of verbal communication.

We felt that the results of this research were encouraging; however, two uncontrollable factors contributed negatively to our work. First we were unable to use headphones during the experiment due to the subjects' medical condition. Consequently, our results were recorded with electric piano accompaniment and voice simultaneously, making it more difficult to analyze than in the case of normal-hearing subjects. Second, we believe that the various psychological profiles of the subjects detracted from our results and analysis. They were affected during the recording by the performance anxiety based on both prior teasing and having to sing in front of several adult persons in the laboratory. We believe that for these two reasons our results have been adversely affected and we wish to minimize these two factors as much as possible in future work.

This unique technological method can be used as an alternative method for speech therapy rehabilitation. Intonation is positively affected via this method over a larger vocal range than traditional speech therapy alone.

We conjecture that the patients might improve matching pitch better with accompaniment than unaccompanied.

Conclusion

Although overall improvement was not so dramatic due to a lack of retention, session by session advancement of matching pitch for targeted MIDI sound was predominantly obvious. It was proved that The YUBA Method worked to improve matching pitch for the singing of patients with cochlear implants. At this research stage, we cannot conclude with absolute certainty that this technology will work with all people with cochlear implants. However, we are positively encouraged by the results of this, our second study. The same technological method used in an earlier study had similar results. Therefore we feel confident that this unique technological voice method works well.

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Hearing profile and MRI myelination of auditory pathway in Pelizaeus–Merzbacher disease

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Abstract

Conclusions. This study showed that delayed auditory pathway myelination is common in Pelizaeus–Merzbacher disease (PMD), but this delay does not necessarily indicate poor hearing function. *Objective.* PMD is a rare recessively inherited X-linked leukodystrophy characterized by defective central nervous system myelination owing to a mutation in the proteolipid protein gene (*PLP*). The aims of this study were to evaluate the hearing function and auditory brain response (ABR) findings of patients with PMD and relate these findings to MRI-assessed myelination in the central auditory pathway. *Patients and methods.* We retrospectively studied eight male pediatric patients with PMD. Serial auditory examinations included audiometry, behavior audiometry, distortion product otoacoustic emission (DPOAE), and ABR. MRI-assessed myelination in the auditory pathway was evaluated in the PMD patients and in 23 normal young children as a control group. *Results.* Audiometry showed normal to moderate hearing impairment and the hearing threshold improved with age and became almost normal over time. DPOAEs positivity and only ABR wave I or waves I and II were found in all the patients. MRI showed delayed myelination in all the patients and the auditory pathway was myelinated up to the inferior colliculus in four cases and up to the medial geniculate body in four cases. Serial MRIs showed no progression in myelination. No clear relation was found between hearing threshold and MRI-assessed myelination in the auditory pathway.

Keywords: *ABR, DPOAE, progression, audiometry*

Introduction

Pelizaeus–Merzbacher disease (PMD) is a recessively inherited X-linked leukodystrophy caused by a mutation in the proteolipid protein gene (*PLP*) on chromosome Xq 22. *PLP* mutations result in dysmyelination, i.e. a lack of properly formed myelin, and in this respect PMD is different from other leukodystrophies. The neuropathologic characteristics of PMD are (i) a low reduction in the number or absence of myelin sheaths in large areas of the white matter, predominantly in the periventricular regions; (ii) well-preserved neurons and axons; and (iii) relatively preserved islets of myelin giving the white matter a patchy 'tigroid' appearance without active demyelination. A strong relation between the degree of dysmyelination and clinical severity was found [1–3].

The classification suggested by Seitelberger [4,5] according to clinical and pathologic features is now divided into two subtypes, namely the classic and connatal forms. The onset of the classic form occurs in the first year of life with muscular hypotonia, nystagmus, and delayed motor development. Tigroid dysmyelination is observed on neuropathologic investigation. In contrast, patients with the rare and more malignant connatal form show little developmental progress. Severe neurologic symptoms include feeding problems, stridor, and marked spasticity resulting in multiple contractures. Pathologic examination shows a complete lack of myelination throughout the brain.

Cognitive delay in early childhood and a delay or difficulty in speaking are commonly observed in PMD patients. In the first case report by Pelizaeus,

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he noted that 'sight and hearing were undisturbed and the patient always understood spoken language' [1]. In the classic form of PMD, most patients acquire some degree of language skill, which may even approach normal levels; however, the speech is dysarthric and language output speed is usually slow. In addition, they also have some degree of cognitive disability. On the other hand, patients with the connatal form of PMD usually exhibit poor growth and develop very limited language skills [5]. Previous studies seldom addressed hearing function and the results from those that did showed variability. Some reports presented patients with severe to extremely severe sensorineural hearing disturbance [6,7], whereas some presented patients with good auditory perceptions [8–11].

Abnormal auditory brainstem responses (ABRs) presenting as the absence of later components after wave I or wave II are common neurophysiological characteristics in PMD patients [10–12]. It has been suggested that these abnormal ABR findings and hearing impairment can be attributed to the dysmyelination of the central auditory pathway. However, a systemic myelination study focusing on the central auditory pathway had seldom been carried out.

In this study, we applied magnetic resonance imaging (MRI) as a method for assessing the dysmyelination of the central auditory pathway in PMD. We aimed to (a) document the various aspects of hearing profiles and ABR findings in PMD patients, (b) evaluate the myelination of the central auditory pathway by MRI, and (c) determine the relation between the hearing function and ABR findings with myelination milestones in the central auditory pathway.

Patients and methods

Patients

We retrospectively studied eight male pediatric patients with PMD. Mean age ± SD at study entry was 4.75 ± 4.1 years. The diagnosis of PMD was confirmed on the basis of clinical symptoms, MRI features, ABRs, and a mutation analysis of the *PLP* gene; all the patients had the classic form of PMD. Some of the patients had already participated in previous studies with our group [11]. The patients' clinical data are shown in Table I.

Hearing assessment

Audiometry and speech test. Conditioned orientation reflex (COR) audiometry or pure tone audiometry was conducted to evaluate hearing. Serial audiometry was found in three patients. Speech discrimination score was evaluated in one patient (patient 7) using monosyllables and three-syllable words. Other speech tests include a Japanese version of the Illinois Test of Psycholinguistic Abilities (ITPA) or the picture vocabulary test (PVT) was administered to four patients (patients 2, 3, 5, and 7).

Distortion product otoacoustic emissions (DPOAEs). DPOAEs were recorded and analyzed using an ILO-88 OAE dynamic analyzer system. Patients were tested inside an electrically shielded sound-attenuating room.

Auditory brainstem response (ABR). We tested all the patients in the supine position in an electrically shielded sound-attenuating room. Silver disk electrodes were placed on each patient's forehead referenced to the mastoid tip on the test side and connected to the ground on the opposite mastoid tip. We used click stimuli. Each click stimulus was presented for 1 cycle of a 3000 Hz sine wave. Monaural headphones (TDH-39) delivered 2000 clicks at a rate of 10 clicks/s. The stimulus intensity was 85 dB nHL. An on-line computer averaged, displayed, and recorded the data. The patients were sedated with trichloryl chloride during ABR recordings.

MRI assessment of auditory pathway

Nine MRI scans of the eight patients were evaluated. Every patient underwent MRI at 1.5 T; in each case the T2-weighted images were obtained using spin-echo sequences. Sections were perpendicular to the long axis of the brain and were 5–7 mm thick. To distinguish the effect of age-related myelination changes in the MRI results, we also examined the auditory pathway by MRI in normal young children ($n = 23$, aged from 0 months to 2 years) who were determined to be clinically healthy on the basis of MRI results and the clinical course observed by pediatricians and used as control groups. T2-weighted images were selected for several reasons. T2-weighted images provided information on degrees of myelination [13,14], and yielded more reliable graded judgments of myelin [15]. It is generally agreed that T1-weighted images are more useful in the first 6–8 months of age to image myelin, and T2-weighted images are more useful after 6 months of age [16,17].

The images were examined for signs of myelination in the central auditory pathway both in gray matter (i.e. cochlear nucleus, superior olive, and medial geniculate body) and white matter (i.e.

Table I. Clinical characteristics of patients.

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Present age/sex	20/male	24/male	9/male	7/male	21/male	7/male	23/male	8/male
Age of symptom onset	1 month	2 weeks	1 month	7 months	5 months	1 month	7 months	3 months
First sign	Nystagmus, head shaking	Nystagmus, head shaking	Nystagmus, motor retardation	Nystagmus, head tremor	Nystagmus, motor retardation	Nystagmus, head tremor	Nystagmus, head tremor	Nystagmus, motor retardation
Nystagmus	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal	Horizontal
Seizures	−	−	−	−	−	−	−	−
Stridor	−	−	−	−	−	−	−	−
Muscular hypotonia	+	+	+	+	−	−	+	+
Spasticity	+	+	+	+	+	+	+	+

cochlear nerve, lateral lemniscus, inferior colliculus branchium, and auditory radiation). Myelination was considered to be present in white matter if the signal intensity was hypointense in unmyelinated white matter on T2-weighted spin-echo MR images. Myelination was considered to be present in gray matter structures if the signal intensity was hypointense in the cortex on T2-weighted spin-echo MR images, as described by Barkovich [18]. The identity of the structures was confirmed by consulting a neuroanatomy textbook [19] and a previous study on young children [18]. Moreover, the tigroid appearance of preserved myelin and cerebral and cerebellar atrophies were evaluated.

Results

Hearing functions

Audiometry and speech test. Figure 1 shows the audiometry results of the eight patients. Hearing threshold was essentially within the normal range for two of the eight cases (patients 4 and 7). The remaining six cases showed moderate hearing loss (Table II). The average hearing threshold was 29.1 dB ± 4 dB. The audiograms showed a flap-type hearing impairment in seven patients, and a steeply down-sloping configuration in patient 3. The serial audiograms showed improvements in the hearing threshold and showed normal hearing (patients 4 and 7) or mild hearing impairment (patient 1) over time.

The speech discrimination score (patient 7) showed a good result (90% on both sides at 30 dB). However, receptive language delays were found among patients 2, 3, 5, and 7. On the auditory subtests of the ITPA, the score of patient 2 fell within the 2–3 year age level at the age of 4 years; that of patient 3 fell within the 5 year age level at the age of 6 years; that of patient 7 fell within the 5–6 year age level at the age of 16 years. On the picture vocabulary test (PVT), the score of patient 5 fell within the 4–5 year age level at the age of 11 years.

DPOAE. DPOAEs were within normal range in all the patients. DP-grams showed that all DP levels were higher than the noise floor levels at all the frequencies, the range being 0–17 dB.

ABRs. Both wave I and wave II were well delineated but the subsequent components were absent in patients 1–4; only wave I was recorded and all the subsequent components were absent in patients 5–8 (Table II).

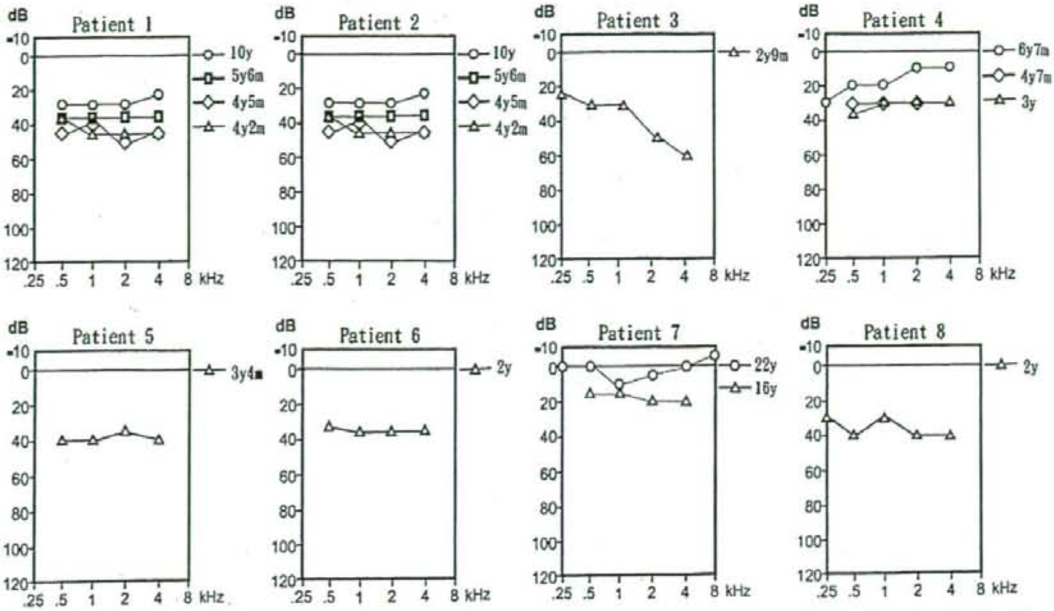

Figure 1. Audiograms of PMD patients. Serial audiograms showed improvements in hearing threshold and showed normal hearing (patients 4 and 7) or mild hearing impairment (patient 1) over time.

MRI assessment of auditory pathway

MRI conducted at a median age of 2 revealed a delay in myelination in all the patients (Figure 2, Table III). Myelination process only reached the inferior colliculus brachium (patients 1, 2, 6, and 8) or medial geniculate body (patients 3, 4, 5, and 7); it was not found in the auditory radiation. The second MRI scan in patient 3 showed no improvement in myelination. Morphological abnormalities including cerebral or cerebellar atrophy were visualized in two patients (patients 4 and 5). Tigroid appearance was visualized in all the patients.

In the mature newborns control group, the cochlear nerve, cochlear nucleus, and superior olivary nucleus were myelinated. The lateral lemniscus, inferior colliculus brachium, and medial geniculate body were myelinated at approximately 3 months; the auditory radiation was myelinated at approximately 15 months.

Discussion

This study revealed that patients with the classic form of PMD had normal to moderate hearing threshold impairment and receptive language delays were common among these patients. There was a tendency for hearing threshold to improve with age and become almost normal over time. This progression has never been reported. Some reports showed improvements in other clinical symptoms over time, particularly in female cases [20], but most of the neurological symptoms do not improve in male patients. Several authors have stated that the myelination of different tracts in the nervous system might express functional maturity [10,14], and that there seems to be a relation between delayed myelination and delayed hearing function development.

In all the patients, the DPOAEs showed positivity and ABR showed wave I only or waves I and II only; this may indicate functioning hair cells, spiral ganglion cells, and auditory nerves, with pathology affecting the central auditory pathway in the brainstem. The absence of waves III–V may denote desynchronized conduction, which may be associated with hearing loss.

Neuroradiological scanning offers a convenient diagnostic tool and yields results that correspond well to neuropathological findings. By MRI, normal postnatal myelin formation is monitored by an inversion of T1- and T2-weighted signals, which correspond to an increase in the levels of myelin lipid constituents with a concurrent decrease in tissue water content. MRI can also show a hypomyelination pattern, i.e. the reversal of white matter signal intensity on T1- and T2-weighted images. MRI thus allows an in vivo evaluation of myelination milestones [15–18]. For the first time, we applied MRI to investigate the myelination of the auditory pathway in PMD patients. Our results showed that irrespective

Table II. Hearing profiles and MRI auditory pathway myelination in PMD patients.

Parameter	Patient 1		Patient 2		Patient 3			Patient 4		Patient 5		Patient 6		Patient 7		Patient 8	
Hearing profile	Left	Right	Left	Right	Left	Right		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Audiometry (last visit)	27 dB	27 dB	37.5 dB	37.6 dB	35 dB	36 dB		18.8 dB	17.5 dB	39 dB	39 dB	35 dB	35 dB	3.3 dB	5 dB	36 dB	36 dB
DPOAE	NA	NA	NA	NA	Normal	Normal		Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
ABR																	
Wave I	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged		Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged
Wave II	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged		Prolonged	Prolonged	–	–	–	–	–	–	–	–
Wave III	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–
Wave IV	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–
Wave V	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–
MRI findings																	
Scan age	2 years 6 months		1 year		1 year 6 months		2 years	10 months		2 years 6 months		3 years		17 years		7 months	
Morphological abnormalities																	
Cerebrum atrophy	Normal		Normal		Normal		Normal	Normal		Normal		Normal		Normal		Normal	
Cerebellum atrophy	Normal		Normal		Normal		Normal	Normal		Normal		Normal		Normal		Normal	
Tigroid appearance	Cetrum semiovale		Cetrum semiovale		Cetrum semiovale		Cetrum semiovale	Cetrum semiovale		Cetrum semiovale		Cetrum semiovale		Cetrum semiovale		Cetrum semiovale	
Auditory radiation	–		–		–		–	–		–		–		–		–	
Medial geniculate body	+/–		–		▨		▨	▨		▨		–		▨		–	
Inferior colliculus brachium	▨		▨		▨		▨	▨		▨		▨		▨		▨	
Lateral lemniscus	▨		▨		▨		▨	▨		▨		▨		▨		▨	
Superior olive	▨		▨		▨		▨	▨		▨		▨		▨		▨	
Cochlear nucleus	▨		NA		▨		▨	▨		▨		▨		▨		NA	
Cochlear nerve	▨				▨		▨	▨		▨		▨		▨			

+, indicates myelinated regions, defined as hypointensity on T2-weighted images; –, indicates nonmyelinated regions, defined as hyperintensity on T2-weighted images; +/–, indicates transition between the two values. Dark gray shading indicates regions showing hypointensity on T2-weighted images, thus indicating myelinated regions; light gray shading indicates incomplete or partial myelination. NA, not available for evaluation.

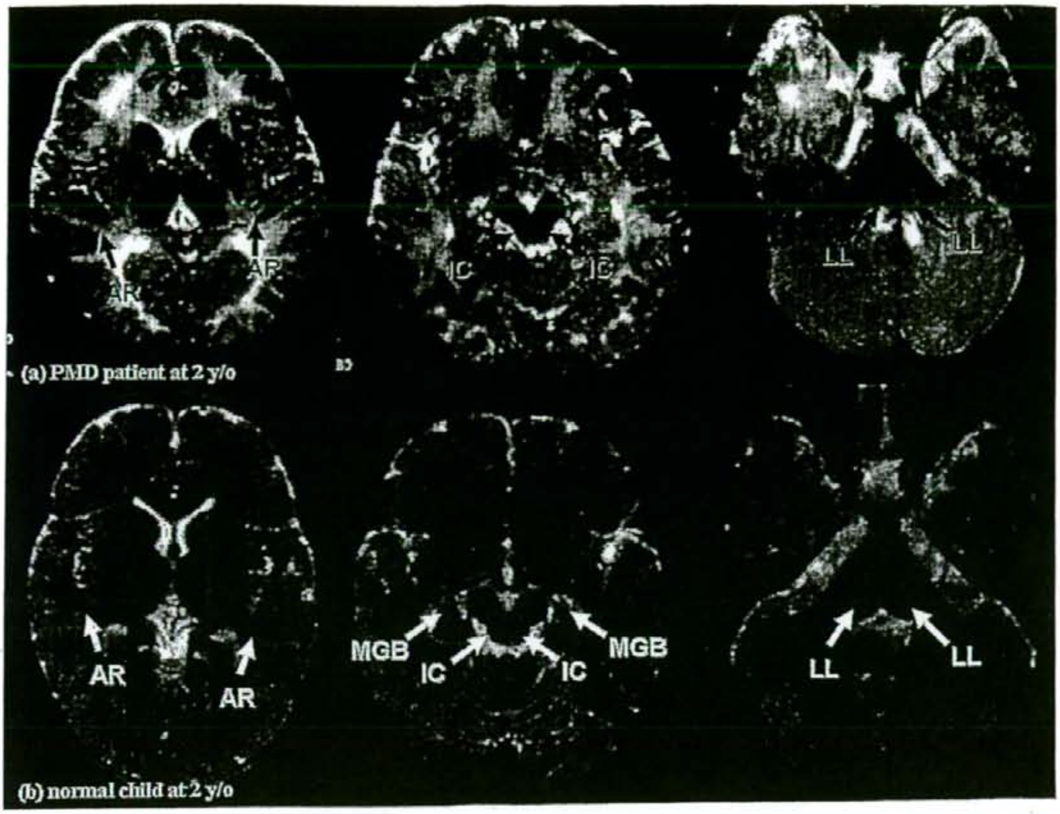

Figure 2. (a) The upper row shows that T2-weighted magnetic resonance imaging (MRI) results of patient 3 at age 2 resemble those of a normal neonate, suggesting that the arrest of myelination occurs soon after birth in PMD. (b) The lower row shows the normal myelination of a young child at age 2. Myelination process reached the auditory radiation. AR, auditory radiation; MGB, medial geniculate body; IC, inferior colliculus brachium; LL, lateral lemniscus.

of the degree of hearing impairment, all the patients showed delayed auditory myelination. Myelination only reached the inferior colliculus brachium or medial geniculate body; it did not reach the auditory radiation. There seems to be no clear relation between hearing threshold and myelination milestones. Also,

Table III. MRI auditory pathway myelination in normal young children (control group).

MRI findings								
Scan age	0 months	3 months	6 months	9 months	1 year	1 year 3 months	1 year 6 months	2 years
Number of subjects	3	3	3	3	4	3	2	2
Auditory pathway myelination								
Auditory radiation	–	–	–	–	+/–	+	+	+
Medial geniculate body	+/–	+	+	+	+	+	+	+
Inferior colliculus brachium	+/–	+	+	+	+	+	+	+
Lateral lemniscus	+/–	+	+	+	+	+	+	+
Superior olive	+	+	+	+	+	+	+	+
Cochlear nucleus	+	+	+	+	+	+	+	+
Cochlear nerve	+	+	+	+	+	+	+	+

+, indicates myelinated regions, defined as hypointensity on T2-weighted images; –, indicates nonmyelinated regions, defined as hyperintensity on T2-weighted images; +/–, indicates transition between the two values. Dark gray shading indicates regions showing hypointensity on T2-weighted images, thus indicating myelinated regions; light gray shading indicates incomplete or partial myelination. NA, not available for evaluation.

no relation was found between ABR findings and MRI-assessed myelination of the auditory pathway. By comparing the MRI results in PMD patients with the normal myelination milestones in the control group, we found that the myelination process of the auditory pathway in PMD seemed to cease between the ages of 0 and 6 months, was limited to the brainstem, and left the cerebral white matter unmyelinated. The second MRI scan in one patient also showed the absence of any progression in myelination. This is consistent with previous studies showing that an arrest of myelination occurs before or soon after birth in PMD [3,4,21–23]. Some reports, particularly in female cases, showed that myelination was slowly improving parallel to the ABR findings [20]. In our studies, we are unable to confirm whether the myelination is arrested or still progressing in the auditory pathway, since MRI was carried out only once on seven of the eight subjects and twice on one subject. Also the young as well as older subjects show lack of myelination irrespective of age.

Neuropathologically, PMD has been characterized by the lack of myelination in the central nervous system. In the classical form of PMD, patchy myelin deficits were observed in the cerebral white matter, particularly in the centrum semiovale. These preserved myelin islets have no association with neuroanatomical structures, such as fiber pathways and systems, but are frequently found perivascularly, sometimes as zones of preserved myelin along stretches of blood vessels, thus giving rise to the characteristic tigroid appearance [2–5]. The cerebellar white matter was less seriously affected, although it still showed the tigroid appearance. The brainstem and spinal cord showed almost normal myelination or sometimes only modest and focal decrease in the staining intensity of myelinated fibers. Cranial nerves, spinal roots, and peripheral nerves are normally myelinated [2,4,5,23,24]. However, toHhe connatal form of PMD is characterized by the total lack of myelination in the cerebrum, cerebellum, and brainstem, with the spinal roots and cranial nerves being normally myelinated [4,5,23–25]. Our MRI results are consistent with the pathological findings of classic PMD; however, to our knowledge there have been no pathologic descriptions of the auditory pathway of PMD in previous studies.

This raises the question of how patients with PMD perceive sound stimuli because they lack myelination past the inferior colliculus or medial geniculate body in the auditory pathway. We postulate that axons in the auditory pathway are sufficiently preserved to conduct the sound stimulus even though myelin is essentially absent in the subcortical white matter. These observations are compatible with those of the visual pathway in PMD patients in previous studies. Those patients retained pupillary light reflexes despite visual impairment and optic atrophy. The interpretation was that axons in the optic nerves are sufficiently preserved to conduct the light stimulus even though myelin is essentially absent [24]. Moreover, previous histological studies of the classic form of PMD showed a nearly normal axon density and a normal number of nerve cells in the brainstem and a mildly reduced number of axons and nerve cells in the affected subcortical white matter [4,5,23,24].

In this study, we demonstrated that delayed auditory pathway myelination is common in PMD, but it does not necessarily indicate poor hearing function. The serial audiometry suggested that hearing threshold improves over time. The ABR showed absence of later waves, which may indicate some asynchrony of firing at brainstem level. However, MRI does not show abnormal patterns in the corresponding structures of the brainstem. We may conclude that MRI does not show the same aspects of neural function as ABR and hearing threshold. Further studies should be conducted to clarify the pathogenesis and observe the long-term prognosis of hearing function in this disease. Finally, despite the rarity of PMD, the PLP mutations in humans and animals have generated enormous interest due to their importance for our understanding of myelination and myelin repair.

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Early myelination patterns in the central auditory pathway of the higher brain: MRI evaluation study

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KEYWORDS
Myelination;
MRI study;
Medial geniculate body;
Auditory radiation;
Splenium of the corpus callosum

Summary

Objective: The purpose of this study was to use magnetic resonance imaging (MRI) to investigate the early myelination patterns of the central auditory pathway and then compare the data with past histological research. We observe the MRI signal intensity of the central auditory pathway and clarify the time course difference between MRI and previous histological research studies.

Methods: A total of 192 infants ranging in age from −4 to 224 corrected postnatal weeks were included in the study. Images were obtained using a 1.5 T MR unit. We chose three sites (medial geniculate body, auditory radiation, and splenium of the corpus callosum) of the central auditory pathway for analysis. Three cross sections were obtained perpendicular to the long axis of the brain and used to analyze the signal changes of the T1- and T2-weighted MRI by employing a region-of-interest (ROI) methodology that was corrected for postnatal age.

Results: At 10 corrected postnatal weeks, the medial geniculate body showed myelinated intensity changes on T2-weighted images. Auditory radiation showed myelinated intensity changes at 19 corrected postnatal weeks on the T1-weighted images and at 24 corrected postnatal weeks on the T2-weighted images. The splenium of the corpus callosum showed myelinated intensity changes at 16 corrected postnatal weeks on T1-weighted images and at 24 corrected postnatal weeks on T2-weighted images.

Conclusions: As compared to the histological literature, the MRI documented signal intensity changes caused by myelination occurred approximately 3 weeks later for the medial geniculate body, 7–24 weeks later for the auditory radiation and 7–15 weeks later for the splenium of the corpus callosum. Since myelination is a process that occurs

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gradually, substantial changes of the myelin sheath makeup, a loss of water and the addition of lipids are more required in order to be detectable by MRI than myelin staining of histological study.

1. Introduction

We have previously reported the early myelination patterns of the brainstem auditory pathway using MRI and clarified the time course difference that has been reported to occur between MRI and histological research data [1]. In the current study, we attempted the same study for the central auditory pathway of the higher brain. The higher brain structures of the central auditory pathway are the medial geniculate body, auditory radiation and splenium of the corpus callosum. Histologically, the degree of myelination in the central auditory pathway of the higher brain has been studied from the viewpoint that it is a maturation parameter of the auditory system during early childhood [2–5]. In 1920, Flechsig [2] reported that myelination occurred at 7 weeks in the medial geniculate body, and at 9 weeks in both the auditory radiation and splenium of the corpus callosum. As reported by Yakovlev and Lecours [3] the auditory radiation exhibited myelination at 0 weeks while there was myelination at 16 week in the splenium of the corpus callosum. MRI signal intensity changes provide considerably more information about the myelination process than other methodologies, [6–16] and thus, MRI is quite useful when attempting to evaluate central nervous system myelination. During the progress of myelination, MRI signal intensities change from low to high for the T1-weighted imaging, and from high to low for the T2-weighted imaging [17–20]. Therefore, we used MRI to investigate the signal intensity of the central auditory pathway in infants as they age and then compared our results with previous histological research studies.

2. Patients and methods

2.1. Patients

Among subjects who underwent brain MRIs between 2000 and 2005 at the University of Tokyo Hospital, we examined a total of 192 neonates, infants and small children (98 males, 94 females), with a mean age of 8.7 weeks (range, −4 to 224 corrected postnatal weeks, with minus weeks indicating a premature infant (Fig. 1). Subjects were examined by MRI due to suspicion of a brain disorder and asphyxia, hyperbilirubinemia, low birth weight, muscular dystrophy, epilepsy, mental retardation, chromosome aberration, spasm, head injury. And anomalies, infarcts, or hemorrhages and brain lesions or severe central nervous system malformations in the central auditory pathway, myelination disorder pointed out by the radiologist were excluded from the study. Deaf children that became clearly by following inspection were also excluded.

2.2. Imaging methods

Imaging was performed using 1.5 T MR units (Magnetom Vision 1.5 T, Siemens, Germany; Signa Excite HD 1.5 T, GE, USA). T1-weighted images were obtained using spin-echo or inversion recovery sequences and T2-weighted images were obtained using spin-echo sequences. All sections were perpendicular to the long axis of the brain and were 5–7 mm thick. Repetition time (TR) was 300–600 ms in the T1-weighted images and 3000 ms in the T2-weighted images. Echo time (TE) was 10–20 ms in the T1-weighted images and 70–100 ms in the T2-weighted images.

2.3. Evaluation methods

MRI has been reported to be useful for evaluating myelination in the central nervous system [6]. With MRI, it is possible to observe the internal structures of the brain [21]. When there are changes in the signal intensity associated with myelination on T1- and T2-weighted images, these changes are indicative of the lipid and water content changes that accompany the developing myelin [8,22]. For the T1-weighted imaging, the signal intensity progresses from hypo- to hyperintensity when myelination occurs and becomes distinguishable as a hyperintense area. With T2-weighted imaging, the signal intensity progresses from hyper- to hypointensity when myelination occurs and becomes distinguishable as a hypointense area [16–18]. However, as the surrounding tissue develops and begins to display similar signal intensities to those seen for the nucleus or tracts (continued progress of the myelination in the surrounding white or gray matter), they become difficult to distinguish from the surrounding tissue on MRI, which is a phenomenon referred to as blurring [6].

For the present study, 1152 MRI images were examined (192 cases, 6 slices by T1- and T2-

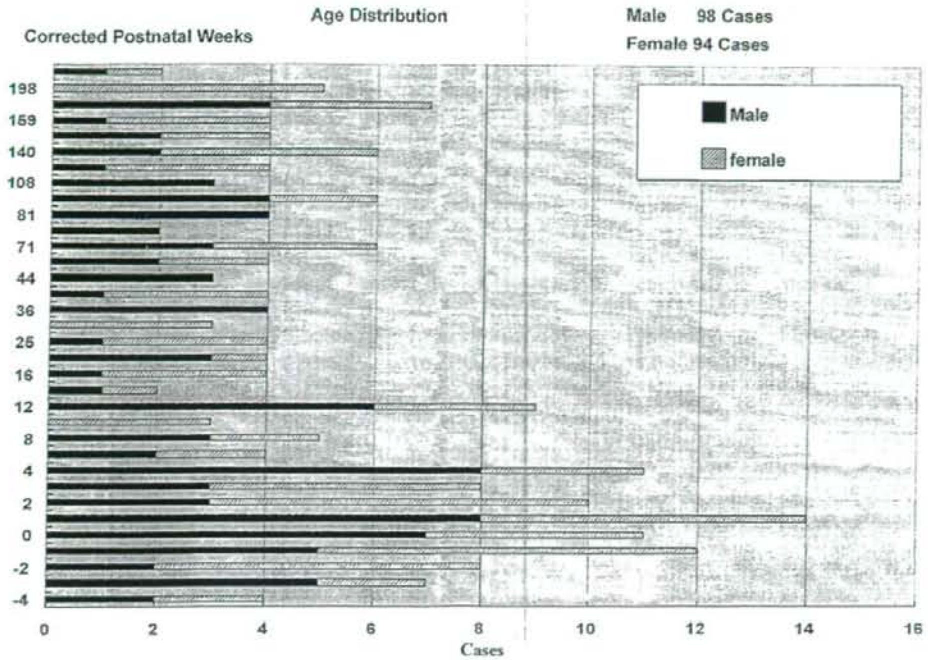

Fig. 1 Histogram shows the age distribution of MRI. We examined a total of 192 neonates, infants and small children (98 males, 94 females), with a mean age of 8.7 weeks (range, −4 to 224 corrected postnatal weeks, with minus weeks indicating a premature infant.

weighted imaging). We analyzed the myelination progress patterns at three different points (medial geniculate body, auditory radiation, and splenium of the corpus callosum) in accordance with a region-of-interest (ROI)-based analysis.

2.4. Depiction of ROIs (Fig. 2)

ROIs were located in the central auditory pathway and in the areas that surrounded each point of interest. The identification of the structure was confirmed by consulting a fetal neuroanatomy textbook [23] along with data from a previous study on term neonates [15]. Fig. 2(1) indicates the medial geniculate body (arrow) and the surrounding white matter (arrowhead) in a slice of the brainstem taken at the thalamus. Fig. 2(2) indicates the auditory radiation (arrow) and the surrounding white matter (arrowhead). Fig. 2(3) indicates the splenium of the corpus callosum (arrow) and the surrounding gray matter (arrowhead).

2.5. ROI-based analysis

Counts for each ROI were measured using Centricity Web-J software (GE Yokogawa Medical System Co. Ltd., Tachikawa, Japan). Signal intensity ratio (SIR) was defined as a difference noted between the ROI value at each assessment site of the central auditory pathway and the corresponding surrounding tissue.

SIR was calculated as follows: (ROI value of assessment site − the ROI value of surrounding tissue/ROI value of the assessment site) × 100. For example, if the ROI value of the medial geniculate body was 1000 and the ROI value of surrounding tissue was 800, SIR = (1000 − 800/1000) × 100 = 20. The SIR value of both sides was measured for each evaluation part, with the average value then computed.

2.6. Statistical analysis

We analyzed SIR in relation to corrected postnatal weeks. Corrected postnatal weeks (CPW) are required when working with premature born infants. The adjustment is calculated by subtracting the number of months that the child was born prematurely from the current age. For example, if a child is born at 28 gestational weeks (3 months before due date), the imaging done at the age of 9 months should yield values consistent with a 6-month-old child (9 minus 3). Corrected postnatal weeks were calculated according to the definition set by the University of Washington (UW)'s home-

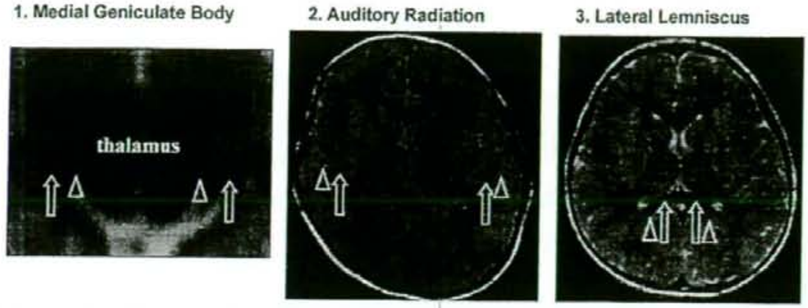
1. Medial Geniculate Body 2. Auditory Radiation 3. Lateral Lemniscus

Fig. 2 Depiction of ROIs (T2-weighted images). Arrow indicates each point of the central auditory pathway (medial geniculate body, auditory radiation and splenium of the corpus callosum), with arrowhead indicating the surrounding gray or white matter. The identification of the structure was confirmed by consulting a fetal neuroanatomy textbook along with data from a previous study on term neonates. (1) Medial geniculate body (arrow) and surrounding white matter (arrowhead). (2) Auditory radiation (arrow) and surrounding gray matter (arrowhead). (3) Splenium of the corpus callosum (arrow) and surrounding gray matter (arrowhead). After pointing these structures we did region-of-interest (ROI)-based analysis using Centricity Web-J software (GE Yokogawa Medical System Co. Ltd., Tachikawa, Japan).

page entitled, "Gaining and growing: Calculating corrected age [24].

SIR was calculated for each of the images as previously discussed in the above ROI-based analysis section, with SPSS ver. 14.0 software for Windows (SPSS 14.0 Japan) used for the analyses. Using Excel for Windows (Microsoft, USA), mean SIR was calculated using the same corrected postnatal weeks (CPW), with the number of postnatal weeks plotted on the *X*-axis and SIR plotted on the *Y*-axis. For the T1-weighted imaging, positive high SIR values are indicative of myelination, while for T2-weighted imaging, negative low SIR values are indicative of myelination. A lower SIR for the T1-weighted imaging and a higher SIR for the T2-weighted imaging after the myelination period indicates the occurrence of blurring.

Subsequently, we then determined the number of weeks for which the comparison of the average value for each section exhibited a significant difference when using Sigma Stat for Windows software version 3.5 (Systat Software, CA, USA). One-way factorial ANOVA was used to investigate the difference between the average SIR values among each section, followed by Bonferroni's multiple comparison tests for mutual comparison. A p-value less than 0.05 denoted the presence of a significant difference.

3. Results

The medial geniculate body, which includes the gray matter nuclei, did not exhibit any significant differences throughout all of the CPW for the T1-weighted imaging ($p > 0.05$). However, significant differences were observed between the <10 CPW and the 10–48 CPW cases. Additionally, significant differences were noted between the 10–48 CPW and the >48 CPW cases for the T2-weighted imaging ($p < 0.05$) (Fig. 3).

The auditory radiation, which includes the white matter tract, displayed significant differences between the <19 CPW and the >19 CPW cases ($p < 0.05$). There were significant differences noted in the T2-weighted images between the <24 CPW and the >24 CPW cases ($p < 0.05$) (Fig. 4).

The splenium of the corpus callosum, which is a white matter tract, displayed significant differences between the <16 CPW and >16 CPW cases for the T1-weighted imaging ($p < 0.05$). For the T2-weighted images, significant differences were noted between the <24 CPW and >24 CPW cases ($p < 0.05$) (Fig. 5).

4. Discussion

4.1. MRI study of myelination and compare with histological study

Numerous research groups [6,8,12–15,20,25–27] have used MRI to document central nervous system changes corresponding to myelination in the developing neonate and infant. However, most of these radiological reports did not examine or report data on the central auditory pathway. In some studies that did examine one part of the central auditory pathway (Table 1), Counsell et al. [28] used 1.0 T MRI to analyze the medial geniculate body in 26 preterm infants with a median fetal age of 28 weeks. They examined myelination-associated T2-weighted