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Abstract

Objective: While investigators have reported that patients with *GJB2*-associated deafness and cochlear implants have preferable language development, the mechanisms of this phenomenon remains unknown. The goal of the present study was to assess higher brain functions of patients with *GJB2*-related and *GJB2*-unrelated deafness as a method of evaluating language development. **Methods:** Eight children with cochlear implants were subjected to genetic testing for *GJB2* and underwent the Raven colored progressive matrices test, Rey's auditory verbal learning test, Rey's complex figure test, the standardized language test for aphasia, the picture vocabulary test, and the standardized comprehension test for abstract words **Results:** Three children were diagnosed with *GJB2*-related deafness, and five children were diagnosed with *GJB2*-unrelated deafness. All three *GJB2*-related cases demonstrated normal range higher brain functions and fair language development. By contrast, one *GJB2*-unrelated case showed a semantic disorder, another demonstrated a visual cognitive disorder with dyslexia, and another had attention deficit-hyperactivity disorder. **Conclusions:** Children with *GJB2*-unrelated deafness showed a high frequency of heterogeneous disorders that can affect proper language development. This difference between children with *GJB2*-related and *GJB2*-unrelated deafness may account for the improved language development in children with *GJB2*-related deafness and cochlear implants. Further, genetic diagnosis of the non-syndromic hearing loss represents a useful tool for the preoperative prediction of outcomes following a cochlear implant procedure.

1. Introduction

Cochlear implants can produce tremendous functional benefit in children with severe-to-profound deafness. However, language performance after cochlear implantation varies widely from child to child. Thus, identification of the parameters that predict improved language performance after implantation would be of great utility for the preoperative counseling of children who are candidates for cochlear implants[1)].

While several groups[3)-9)] have reported excellent speech performance after cochlear implantation, we previously demonstrated that the improved language development in children with *GJB2*-related deafness after cochlear implant[2)] may be dependent on preferable cognitive abilities. Indeed, measurements of non-verbal developmental tests[2)] were higher in children with *GJB2*-related deafness than in those with *GJB2*-unrelated deafness. Further, several groups have demonstrated that hearing abilities were similar when comparing children with *GJB2*-related deafness and those with *GJB2*-unrelated deafness[5)10)11)].

Language development is highly associated with hearing ability in the case of prelingual deafness, but hearing ability is not the only predictive factor for language development. For example, recent studies[12)] demonstrated that partial brain damage may play a central role in language problems. Whether similar factors affect language development in the case of prelingual deafness cases is not clear. Thus, the goal of the present study was to assess higher brain functions of patients with *GJB2*-associated and non-*GJB2*-associated deafness as a potential method of evaluating and predicting language development.

2. Patients and Methods

2.1. Subject identification and DNA extraction

Of the 105 children who underwent cochlear implantation at Okayama University Medical School, 17 were presently engaged in elementary school education and were eventually enrolled in this study. Four children were excluded from this study because of apparent developmental problems (pervasive developmental disorder, n=2; severe mental retardation, n=2). Thus, eight school aged children with cochlear implants were included in the final analysis.

All eight children were diagnosed with profound deafness at 4-18 months of age and underwent cochlear implantation (Nucleus 21-channel cochlear implant, Cochlear Corp., Englewood CO, USA) at 3-6 years of age.

At the time of study, participant ages ranged from 7-11 years, and all children were currently engaged in elementary school with auditory-oral, or audiroty-verbal educations. All children previously received auditory-verbal or auditory-aural intervention at Kanariya Gakuen (Auditory center for hearing impaired children, Okayama), preoperatively with hearing aid and postoperatively with cochlear implant. Some of the patients had participated in previous studies with our group.[2)] In participating families, cellular samples were obtained from hearing-impaired children by brushing the oral mucosa with a Cytobrush™ (Medscand, Hollywood, CA). Genomic DNA was obtained by phenol/chloroform extraction and ethanol precipitation. DNA samples from 50 healthy children were obtained by the same procedure. Written informed consent was obtained from all participants.

2.2. Polymerase chain reaction and sequencing

First round polymerase chain reaction (PCR) was completed with a primer pair covering exon-2 in its entirety (primers A and B, Table 1), as previously described [18]. Briefly, each reaction contained 10 ng of genomic DNA, 2.0 pmol of each primer, 200 mM of each dNTP (Toyobo Inc., Osaka, Japan), 0.25 U of Taq DNA polymerase (Takara Shuzo Inc., Tokyo, Japan), and 1 ml of 10 μl/ buffer (Takara Shuzo Inc.) in a total volume of 10 ml. After the initial denaturation step at 94°C for 2 min, samples were amplified under the following thermal conditions: 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, for 25 cycles, with an additional extension time at 72°C for 10 min. PCR products were purified using the QIAquick™ PCR purification kit (QIAGEN Inc., Valencia, CA) and stored for later use in the following procedures.

Products obtained from the first PCR were used as the template for sequencing analysis. Sequence analysis was completed with the BigDye™ terminator cycle sequencing ready kit with Amplitaq™ DNA polymerase FS (ABI). PCR products were loaded and run on the ABI PRISM 373S Genetic Analyzer (ABI). Injection was performed at 15 kV for 12 s. Electrophoresis was performed at 2.8 kV for 18 h.

2.4. Audiological, Neuropsychological and linguistic evaluation

Pure-tone hearing thresholds by headsets (preoperative non-aided hearing level) and sound field hearing thresholds (postoperative, with cochlear implant) were evaluated. In addition, monosyllable speech perception tests were also conducted for all participants. Monosyllable speech sound that correspond to Japanese

Phonogram (Hira-gana) were presented from behind with presentation level at 70dBHL, and the children were asked to dictate these Hira-gana.

Raven colored progressive matrices test (RCPM) was used to evaluate non-verbal intelligence[13], and Rey's auditory verbal learning test (RAVLT) and Rey's complex figure test (RCFT) were used to evaluate visual-cognitive or auditory-cognitive abilities. The standardized language test for aphasia (SLTA) [14][15], which is widely used as a test battery for aphasia in Japan, was used to profile language problems according to the sub-classifications of writing, reading, speaking and listening abilities.

As linguistic evaluation, vocabulary was tested using the picture vocabulary test (PVT) and the standardized comprehension test for abstract words (SCTAW) [16]. For SCTAW, all questions were conducted under sound-presenting conditions. Questions were also repeated by the examinees to confirm that the SCTAW results were not affected by misheard words related to hearing loss.

All these tests were conducted at an acoustic chamber in Okayama University Medical School Hospital with trained speech and language therapists.

3. Results

Data are summarized in Tables 2 and 3. Among the eight children with cochlear implants, three had *GJB2*-related deafness. All three cases were 235delC homozygotes, which is the most frequent mutation found in the Japanese population. Five other children did not carry known deafness-causing *GJB2* mutations by sequencing of whole coding region of *GJB2*.

By audiological evaluation,

Eventually, the 8 children can divided into two groups; *GJB2* related deafness cases (3 cases) and *GJB2* unrelated cases (5 cases). Mean periods of hearing aid usage is 73 months in total, 79 months in *GJB2* related case and 69 months in *GJB2* unrelated cases, respectively. Although there is no statistically significant difference, slightly longer hearing aid usage in *GJB2* related cases may reflect the older ages at implantation in this group. i.e. Mean age of implantation is 8years 4month in *GJB2* related cases and 7 years and 7 months in *GJB2* unrelated cases. Audiological evaluations also revealed similar results in both cases. Hearing thresholds with cochlear implants between 500Hz to 4000Hz was 25 to 50 dB in all cases. Monosyllable speech perception was also demonstrated the similar results in both cases. These results were also summarized in table 1 (table1)

On the basis of tests evaluating higher brain functions, no apparent cognitive problems were present in children with *GJB2*-related deafness. By contrast, one child (case #6) with *GJB2*-unrelated deafness demonstrated poor visual-cognitive processing, as indicated by RCFT scores: 17 at copy, 4 at recall and 0 (impossible to write) at delayed recall. In addition another child (case #5) with *GJB2*-unrelated deafness displayed auditory cognitive problems, as indicated by good RCFT (36 at

copy, 18 at recall and 17 at delayed recall) and poor AVLT (6 at immediate recall, 9 at maximum recall and 6 at delayed recall) results. Another child (case #8) with *GJB2*-unrelated deafness had a very low SCTAW score; this child was able to response to only 4 out of 32 questions (-2SD level of normal control) despite the fact that the PVT score was not significantly affected. This problem did not appear to be related to severe hearing loss itself, as the child's normal-hearing brother showed a similar performance in these tests and was diagnosed with verbal learning difficulties. Further, evaluation of the brother with SPECT imaging showed localized reduction of blood flow in the temporal lobe (data not shown). Thus, three of five children with *GJB2*-unrelated deafness showed some degree of higher brain dysfunction that have been associated with learning difficulties.

SLTA scores in the three children with *GJB2*-related deafness were similar to those in 150 non-aphasic adults (normal control). Among the five children with *GJB2*-unrelated deafness, one child (case #4) demonstrated fair language development, showing no significant difference from the normal control participants. By contrast, the remaining four children all displayed some language difficulties. For example, one child (case #5) had low scores in auditory comprehension (30), sentence reading (40), and reading comprehension (50). Another child (case #6) had problems with kana-letter dictation and sentence dictation, indicating a developmental Kana-dyslexia disorder other than prelingual hearing impairment. Another child (case #7) had low sentence dictation score and lower auditory comprehension score, affecting sentence repetition and sentence dictation.

4. Discussion

Previous reports suggested that children with *GJB2*-related deafness had relatively better hearing or speaking ability than children with *GJB2*-unrelated deafness[19]. However, the present results demonstrated that children with *GJB2*-unrelated deafness may have associated deficits in higher brain functions that interfere with proper language development after cochlear implant, which may explain the difference in language performance. Learning difficulties (LD) combined with hearing impairment is a poor prognostic factor for language development in children with deafness[17]. In fact, 5-7% of students in programs for the deaf or hard-of-hearing have concomitant LD, making LD the single most frequent disorder in children with deafness [18].

In the present study, the most striking result was obtained with one child (case #8) who showed difficulty in understanding abstract words despite the absence of a pervasive developmental disorder or mental retardation. In addition, other tests, including the SLTA and RCFT, demonstrated that this child had almost preserved language ability in relation to her hearing peers. Her hearing sibling also complained of difficulty in learning Japanese, and similar testing of the sibling revealed deficits in the usage of abstract words, although other language ability, including non-verbal intelligence, was within normal range. These results suggest that a pure verbal semantic disorder was an independent cause of her language development deficit.

In case 6, a visual-cognitive disorder was identified by RCFT, and this visual problem was also independent from hearing loss. This child also showed developmental dyslexia/dysgraphia, probably caused by this visual-cognitive

disorder. Since written language plays a critical role in the education of the children with profound deafness, the child's difficulty in learning written language may have had a severe effect on her language development, as indicated by SLTA. Another child (case #5) demonstrated relatively good non-verbal intelligence by RCPM. However, poor vocabulary was revealed by PVT and SCTAW. Subsequent reverse numeration and phonological awareness tasks revealed very poor phonological awareness (data not shown), and the child was also diagnosed with attention deficit-hyperactivity disorder.

On the contrary, no apparent neurological deficit was observed in *GJB2* related cases. Interestingly, the results of audiological tests including monosyllable speech perception tests in *GJB2* unrelated cases were comparable to those of *GJB2* related cases. These results indicated that the different outcome as language development between *GJB2*-related and *GJB2*-unrelated cases was not caused by the different hearing ability after cochlear implant. We rather assumed that the prevalence of the higher brain function deficits is the major cause of this difference. Several different neurological deficits, such as dyslexia and dysgraphia, can cause difficulties in learning[20] and can result from disturbances in visual-spatial or auditory-phonetic cognitive processing[12]. Indeed, the presence of these cognitive deficits can easily be assumed to result in problems with language learning and development.

Higher brain function deficits can be diagnosed by the neuropsychologic tests used in this study. Further, the specific deficit observed in the SLTA can be explained by these test results. For example, visual learning problems were observed in case 6, who demonstrated dyslexic problems in SLTA, and the

problems with sentence repetition and dictation in case 8 may result from her difficulties with conservation of the sentence meaning. These facts suggest that the brain function tests used in this report may be of utility to identify specific cognitive problems and guide language education in school-aged children.

Despite their potential utility, the neuropsychological tests used in this study are too difficult to complete with very young children. For example, cochlear implantation is sometimes indicated at 12-18 months of age, and the majority of these tests cannot be applied to this age group. By contrast, genetic diagnosis, including *GJB2* status determination, may provide a useful prognostic factor for language development following cochlear implantation.

There are many other genetic mutations other than *GJB2* that are associated with deafness in the absence of other neurological problems. In the present study, one child (case #4) with *GJB2*-unrelated deafness had brain function and language development that was comparable to children with *GJB2*-related deafness. Identification of the genes for non-syndromic hearing impairment (i.e. the hearing loss is the single apparent neurological deficit for the children) and detailed evaluation of their brain functions may further increase our ability to understand the impact of therapeutic interventions in hearing-impaired children.

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References

〔1〕 Nikolopoulos TP, Dyar D, Archhold S, et al.,Development of spoken language grammar following cochlear implantation in prelingually deaf children, Arch Otolaryngol Head Neck Surg, 130(2004) 629-633.

〔2〕 Fukushima K, Sugata K, Kasai N,et al., Better speech performance in cochlear implant patients with GJB2-related deafness, Int. J. Pediatr. Otorhinolaryngol. 62(2002) 151-157.

〔3〕 Lustig LR, Lin D, Venick H, et al., GJB2 gene mutations in cochlear implant recipients: prevalence and impact on outcome, Arch Otolaryngol Head Neck Surg. 130(2004) 541-546.

〔4〕 Bauer PW, Geers AE, Brenner C, et al., The effect of GJB2 allele variants on performance after cochlear implantation, Laryngoscope 113(2003) 2135-2140.

〔5〕 Dahl HH, Wake M, Sarant J, Poulakis Z, Siemering K, Blamey P., Language and speech perception outcomes in hearing-impaired children with and without connexin 26 mutations, Audiol Neurootol., Sep-Oct;8(5)（2003） 263-268.

〔6〕 Green GE, Scott DA, McDonald JM et al., Performance of cochlear implant recipients with GJB2-related deafness, Am J Med Genet, 109(2002) 167-170.

〔7〕 Finsterer, J. and J. Fellinger, Nuclear and mitochondrial genes mutated in nonsyndromic impaired hearing, Int. J. Pediatr. Otorhinolaryngol, 69(2005)

621-647.

〔8〕 Sinnathuray, A.R, J. G. Toner, et al., Auditory perception and speech discrimination after cochlear implantation in patients with connexin 26 (GJB2) gene-related deafness, Otol Neurotol, 25(2004) 930-934.

〔9〕 Sinnathuray, A.R, J. G. Toner, et al., Connexin 26 (GJB2) gene-related deafness and speech intelligibility after cochlear implantation, Otol Neurotol, 25(2004) 935-942.

〔10〕 Matsushiro N, Doi K, Fuse Y, et al., Successful cochlear implantation in prelingual profound deafness resulting from the common 233delC mutation of the GJB2 gene in the Japanese, Laryngoscope, 112(2002) 255-261.

〔11〕 Cullen RD, Buchman CA, Brown CJ, et al., Cochlear implantation for children with GJB2-related deafness, Laryngoscope, 114(2004) 1415-1419.

〔12〕 Capellini SA, Padula NA, Ciasca SM, Performance of school children with specific reading disabilities in a remediation program, Pro, Fono., 16(2004) 261-274.

〔13〕 Uno A, Shinya N, Haruhara N et al., Raven’s Coloured Progressive Matrices in Japanese Children “As a Screening Intelligence Test for Children with Learning Disorder and Acquired Childhood Aphasia, The Japan Journal of Logopedics and Phoniatrics, 46(2005) 185-189.

〔14〕 Mimura M, Kato M, Kato M, Sano Y, Kojima T, Naeser M, Kashima H., Prospective and retrospective studies of recovery in aphasia. Changes in cerebral blood flow and language functions, Brain, 121(1998) 2083-2094.

〔15〕 Tamura A, Shichijo F, Matsumoto K., A study on simplification of the Standard Language Test of Aphasia (SLTA)., Tokushima J Exp

Med. ,43(1996)39-46.

〔16〕 Noriko Haruhara, Akira Uno, Masato Kaneko., Development of a Test to Assess Comprehension of Abstract Words in Aphasics, The Japan Journal of Logopedics and Phoniatrics, 45(2004)99-105.

〔17〕 Powers, Ann, et al., Learning disabled hearing-impaired students: are they being identified?, Volta Review, 89(1987) 99-105.

〔18〕 Powers, Ann, et al., The dilemma of identifying learning disabled hearing impaired students, Volta Review, 90(1988) 209-218.

〔19〕 Mesolella, M., G. Tranchino, et al., "Connexin 26 mutations in nonsyndromic autosomal recessive hearing loss: speech and hearing rehabilitation, Int. J. Pediatr. Otorhinolaryngol, 68(2004) 995-1005.

Iwata M, Neuronal circuits of reading and writing in Japanese language, Bull Acad Natl Med, 188(2004)667-673.

Figure Legends

Fig.1 Rey's complex figure test (RCFT) and Rey's auditory verbal learning test (RAVLT)

a) RCFT: Examinees are first asked to copy a nonsense, complex figure. Immediately after drawing the first figure, examinees are asked to recall and draw the same figure. Thirty minutes later, the examinees are again asked to recall the same figure (delayed recall). Points were assessed according to the degree of completion of each item or lines. Visual cognitive-learning ability was examined by this test.

b) RAVLT: Examinees are asked to repeat 15 words with high familiarity to school-aged children. Immediate recollection in random order was then asked and the number of the correct answers was marked. The same procedures are repeated five times, and the best score is recorded as thr Maximum Recollection Number. After completely memorizing 15 different words, the child is asked to recall the original 15 words. Thirty minutes later, the child is asked to recall the 15 words again (delayed recall).

Fig.2 An example of the standardized comprehension test for abstract words (SCTAW). After confirmation of correct hearing by repetition of the stimulus words, the examinee is asked to point one out of six alternatives. The six alternatives include one correct answer, two semantic mistakes, two phonological mistakes, and one irrelevant answer. A SCTAW set includes 15 different abstract words. An example of SCTAW (Kyou-ryoku: cooperation) is shown.

Fig.3 Summary of SLTA

Summary of SLTA scores of the eight cases: reading, hearing, speaking and writing ability was examined at the word and short sentence level.

Table.1 PCR primer

Table.2 Summary of GJB2 mutation, RCMT, PVT, SCTAW and additional diagnosis.

Table.3 Summary of RAVLT and RCFT

Each line of RAVL scores indicated as the first recall number / maximum recall number / delayed recall number. Each line of RCFT also indicated as copy/ immediate recall / delayed recall.

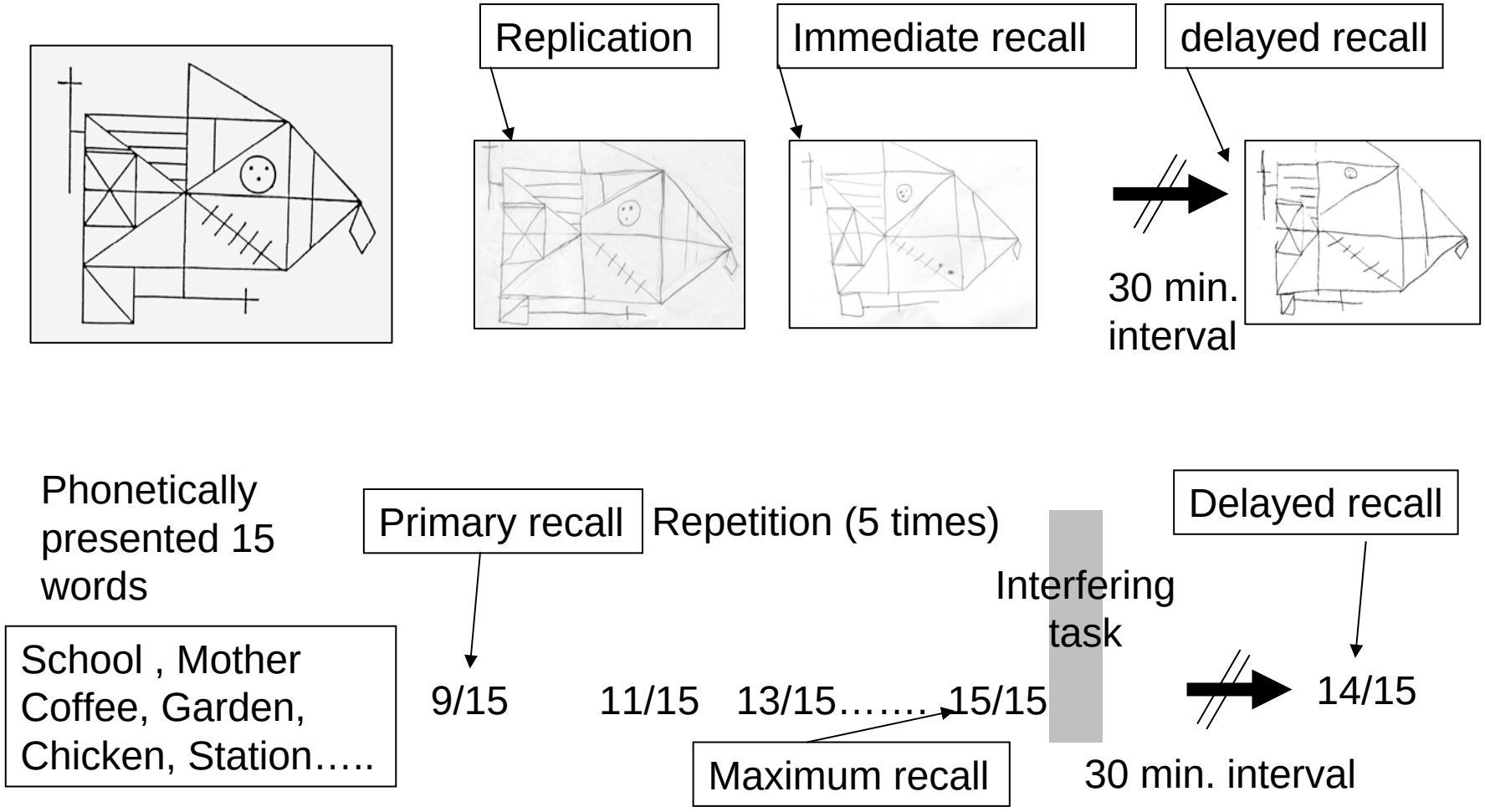

Fig.1 Visual-cognitive tests (RCFT) and Auditory-cognitive tests (RAVLT)

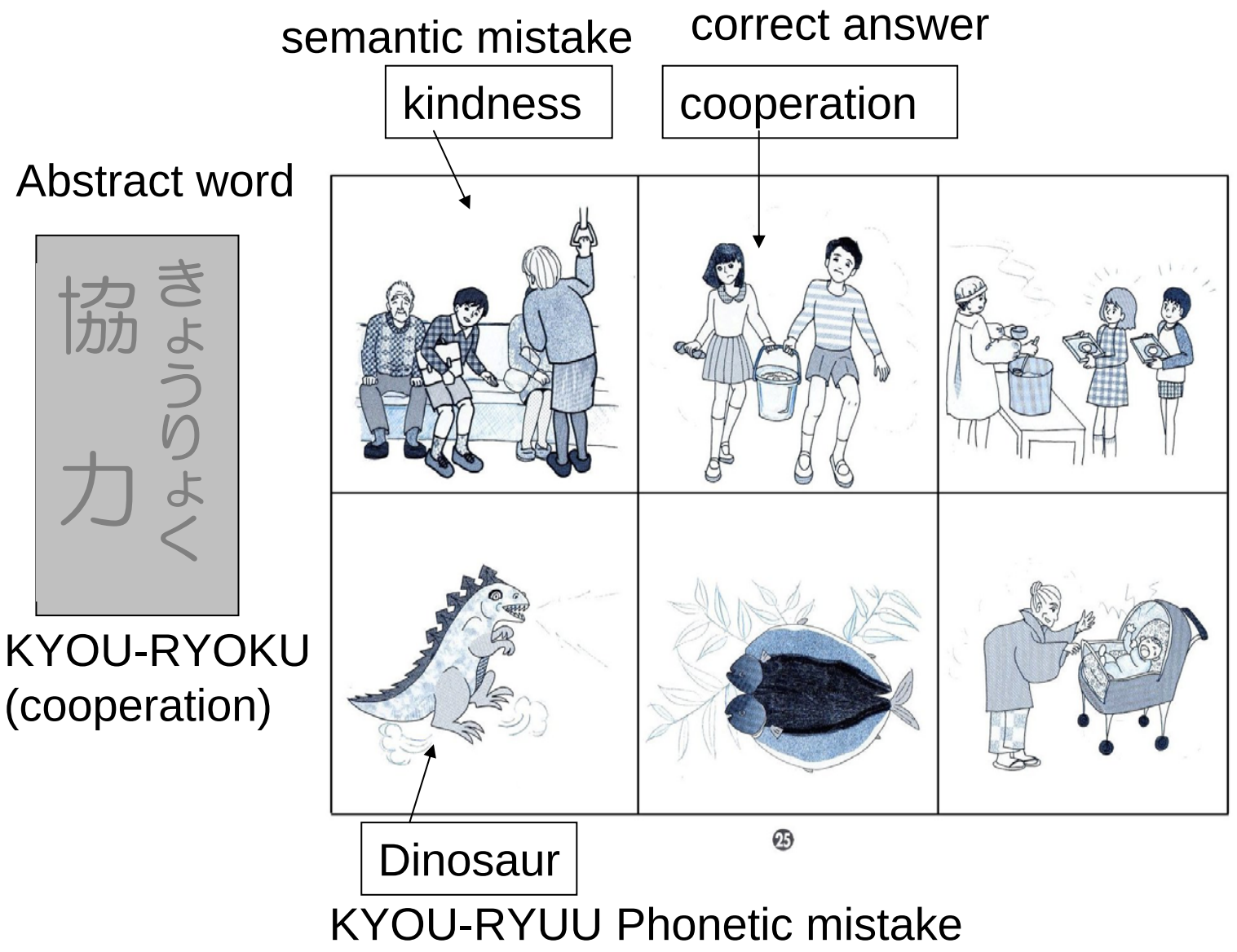

Fig. 2　An example of standardized comprehension test for abstract words (SCTAW)

Table.1 Summary of clinical background and GJB2 mutational status

Case	*GJB2* mutations	Monosyllable speech perception Upper: vowels Lower: consonant +vowel	Non-verbal intelligence (RCMT)	PVT	SCTAW upper Auditory lower visual	Diagnosis
1	235delC	96% 68%	36/36	61/68	28/32 31/32	Non-syndromic deafness
2	235delC	96% 66%	36/36	67/68	25/32 30/32	Non-syndromic deafness
3	235delC	98% 70%	34/36	53/68	22/32 22/32	Non-syndromic deafness
4	None	94% 70%	32/36	67/68	21/32 17/32	Non-syndromic auditory processing preference
5	None	90% 60%	32/36	16/68	unable to complete	Non-syndromic Auditory Cognitive problem
6	None	92% 68%	21/36	30/68	N/A	Congenital CMV infection Visual Cognitive disorder
7	None	94% 66%	16/36	48/68	N/A	Congenital leukodystrophy ADHD
8	None	98% 72%	35/36	18/68	0/32 4/32	Non-syndromic Semantic disorder

Table. 2 Summary of RAVLT and RCFT

Case	*GJB2* mutations	Reys AVLT score Primary/ Maximum/ Delayed	RCFT score Replication/ Immediate Recall/ Delayed Recall
1	235delC	11/ 15/ 15	36/ 34/ 32
2	235delC	9/ 15/ 15	36/ 32/ 34
3	235delC	6/ 15/ 15	35/ 27/ 28
4	None	9/ 15/ 14	36/ 27/ 21
5	None	6/ 9/ 6	36/ 18/ 17
6	None	8/ 14/ 11	17/ 4/ 0
7	None	2/ 4/ 1	8/ 4/ 0
8	None	2/ 7/ 4	36/ 36/ 34

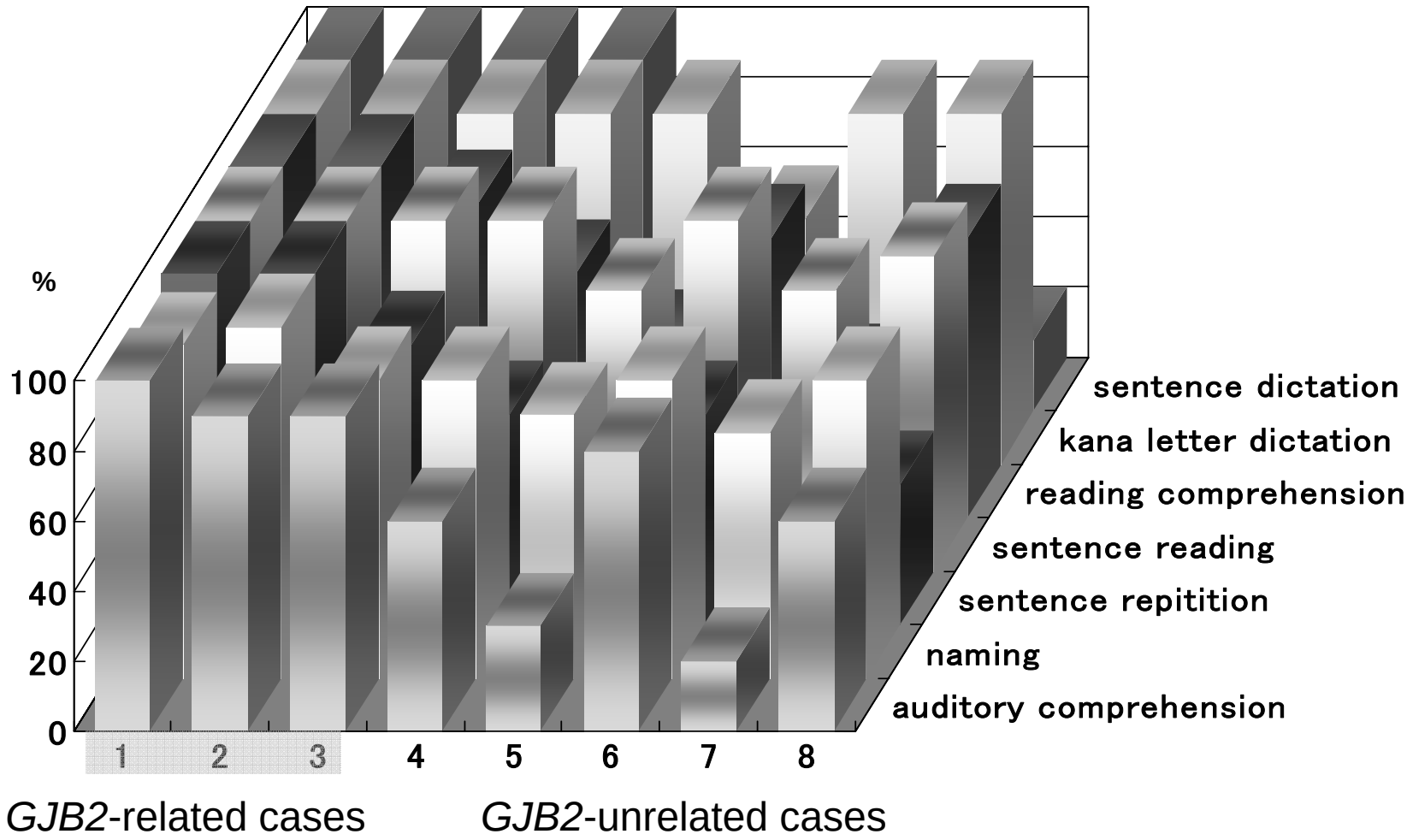

Fig.3 Summary of SLTA