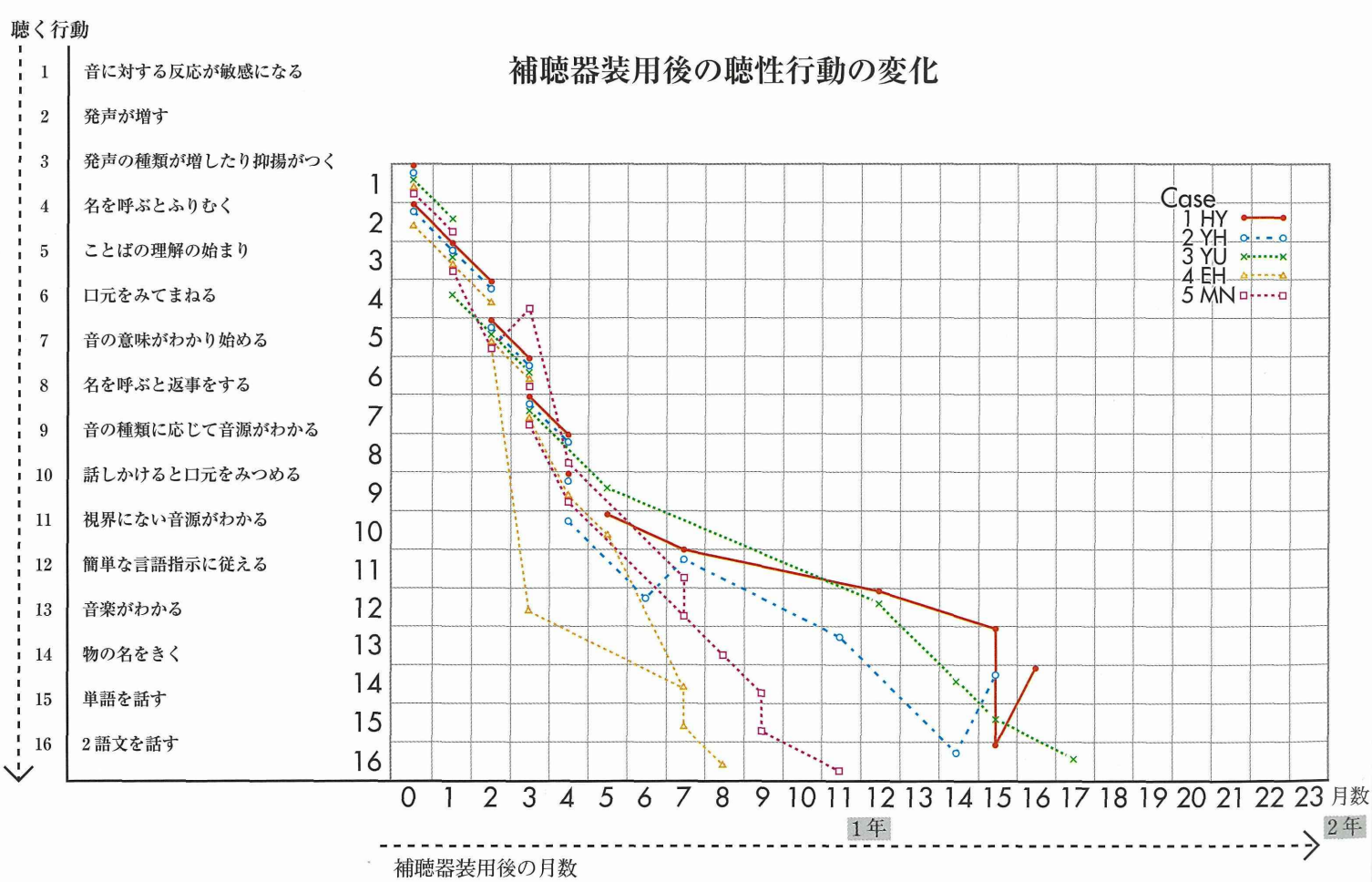
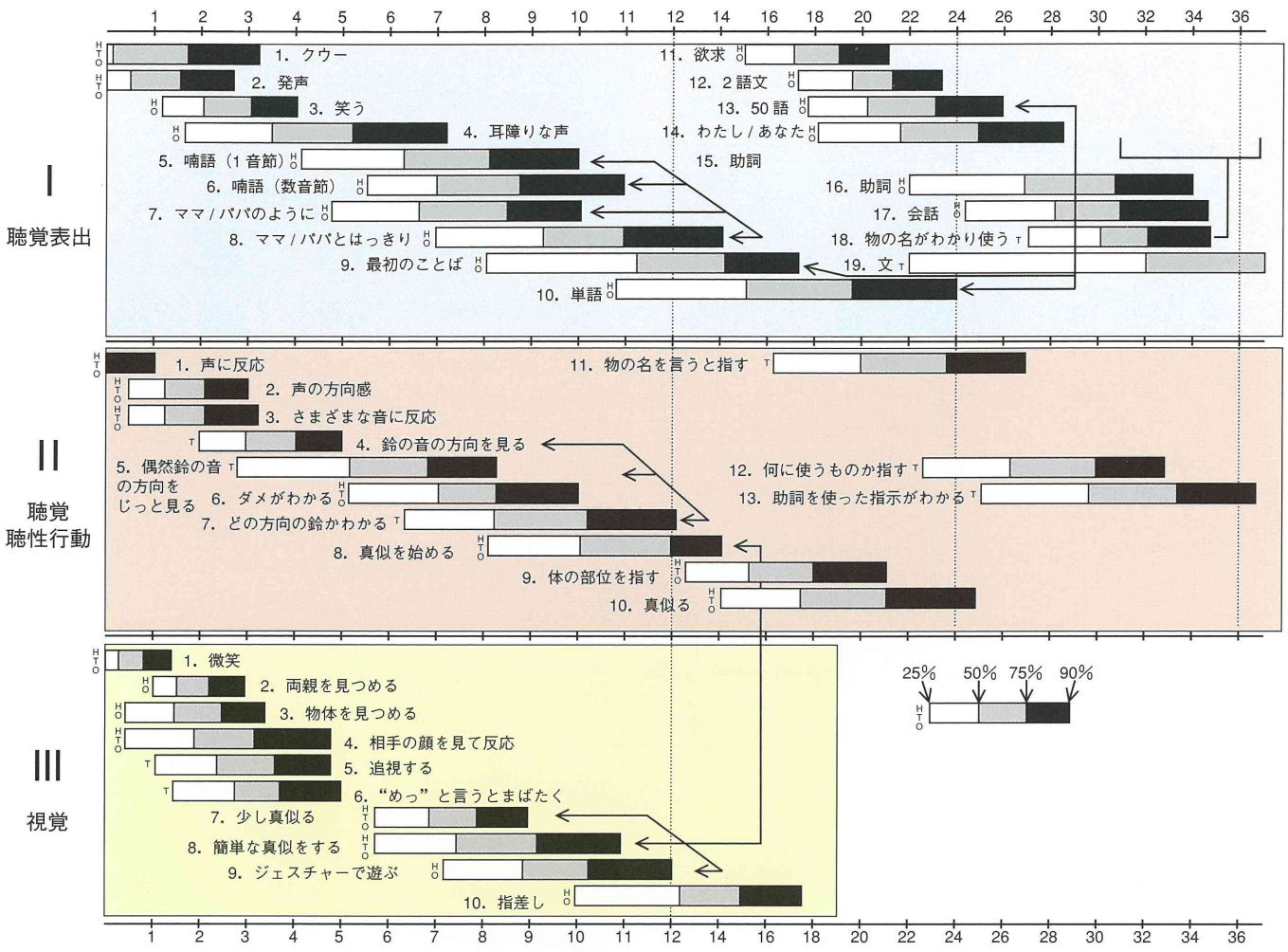
乳児の聴覚発達チェックリスト（田中ほか，1974）

月齢	番号	項　目
0か月児	1	突然の音にビクッとする（Moro 反射）
	2	突然の音に眼瞼をギュッと閉じる（眼瞼反射）
	3	眠っている時に突然大きな音がすると眼瞼が開く（覚醒反射）
1か月児	4	突然の音にビクッとして手足を伸ばす
	5	眠っていて突然の音に目をさますか、泣き出す
	6	眼が開いているときに急に大きな音がすると眼瞼が閉じる
	7	泣いているとき、または動いているとき声をかけると、泣き止むかまたは動作を止める
	8	近くで声をかける（またはガラガラを鳴らす）とゆっくり顔を向けることがある
2か月児	9	眠っていて急に鋭い音がすると、ビクッと手足を動かしたり、まばたきしたりする
	10	眠っていて、子どものさわぐ声や、くしゃみ、時計の音、掃除機などの音に眼をさます
	11	話しかけると、アーとかウーと声を出して喜ぶ（またはニコニコする）
3か月児	12	眠っていて突然音がすると眼瞼をピクッとさせたり、指を動かしたりするが、全身がビクッとなることはほとんどない
	13	ラジオの音、テレビのスイッチの音、コマーシャルなどに顔（または眼）を向けることがある
	14	怒った声や、やさしい声、歌、音楽などに不安そうな表情をしたり、喜んだり、またはいやがったりする
4か月児	15	日常のいろいろな音（玩具、テレビの音、楽器音、戸の開閉など）に関心を示す（振り向く）
	16	名前を呼ぶとゆっくりではあるが顔を向ける
	17	人の声（特に聞きなれた母親の声）に振り向く
	18	不意の音や聞きなれない音、珍しい音に、はっきり顔を向ける
5か月児	19	耳もとに目覚まし時計を近づけると、コチコチという音に振り向く
	20	父母や人の声、録音された自分の声など、よく聞き分ける
	21	突然の大きな音や声に、びっくりしてしがみついたり、泣き出したりする
6か月児	22	話しかけたり歌をうたってやると、じっと顔を見ている
	23	声をかけると意図的にサッと振り向く
	24	テレビやラジオの音に敏感に振り向く
7か月児	25	隣の部屋の物音や、外の動物のなき声などに振り向く
	26	話しかけたり歌をうたってやると、じっと口もとを見つめ、時に声を出して応える
	27	テレビのコマーシャルや、番組のテーマ音楽の変わり目にパッと向く
	28	叱った声（メッ！ コラッ！ など）や、近くで鳴る突然の音に驚く（または泣き出す）
8か月児	29	動物のなき声をまねるとキャッキャッといって喜ぶ
	30	きげんよく声をだしているとき、まねてやると、またそれをまねて声を出す
	31	ダメッ！ コラッ！ などというと、手を引っ込めたり、泣き出したりする
	32	耳もとに小さな音（時計のコチコチ音など）を近づけると振り向く
9か月児	33	外のいろいろな音（車の音、雨の音、飛行機の音など）に関心を示す（音のほうにはっていく、または見回す）
	34	「オイデ」、「バイバイ」などの人のことば（身振りをいれずにことばだけで命じて）に応じて行動する
	35	隣の部屋で物音をたてたり、遠くから名前を呼ぶとはってくる
	36	音楽を聞かせたり、歌をうたってやると、手足を動かして喜ぶ
	37	ちょっとした物音や、ちょっとでも変わった音がするとハッと振り向く
10か月児	38	「ママ」、「マンマ」または「ネンネ」など、人のことばをまねていう
	39	気づかれぬようにして、そっと近づいて、ささやき声で名前を呼ぶと振り向く
11か月児	40	音楽のリズムに合わせて身体を動かす
	41	「……チョウダイ」というと、そのものを手渡す
	42	「……ドコ？」と聞くと、そちらを見る
12~15か月児	43	隣の部屋で物音がすると、不思議がって、耳を傾けたり、あるいは合図して教える
	44	簡単なことばによるいいつけや、要求に応じて行動する
	45	目、耳、口、その他の身体部位をたずねると、指を指す

補聴器装用後の聴性行動の変化



初期の言語発達のマイルストーン　（Coplan MD, 1981 による）



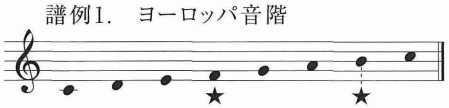
定型的な健聴児の音楽能力の発達

年齢	音楽能力の発達
生後 2 ヶ月	音楽に注意を向ける／音楽を聴くと静かになる
2〜6 ヶ月	喃語的歌：音楽に反応して反復動作をする：音源の方を向く
6〜12 ヶ月	自発的な歌：時おり音の高低や拍子が合う：反復動作が大きくなる
12〜18 ヶ月	音楽にあわせて踊る：歌詞に注意を向ける：習った歌の断片を歌う：さらにピッチが合うようになる
18〜24 ヶ月	ダンスの対手を探す：音楽にあわせて回る、行進する：自発的な歌に一定のリズムが付く：歌をまねることができる：ピッチよりは言葉が正確になる
2〜3 歳	話声に対して歌声を学ぶ：異なる高さや拍子で歌う：木琴で簡単なメロディを演奏する：歌いながら楽器を鳴らす：幾つかの楽器を聴き分ける
3〜4 歳	楽器を聴き分ける（音色）：歌いながらリズム楽器を伴奏する
4〜5 歳	大きな動き：想像上の歌や話を作る：自分のメロディを維持し始め、他の人の拍子に合わせる
5〜6 歳	拍子の認識：正確な音の高低でメロディを歌う：単純な楽器でメロディを演奏する：音楽を積極的に聴く
6〜7 歳	中心音階の認識が発達する：合唱を始める：歌声の音域が 5〜6 音の周辺に集中する：リズム記号を学ぶ
7〜9 歳	歌声の音域が広がる：音符の読み書きを始める：複雑な拍子や和音：音楽の好みが明らかになる

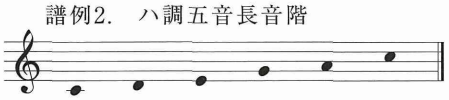
1 2 3 4 5 6 7 8 9 10

あいうえお
かきくけこ
さしすせそ
たちつてと
なにぬねの
はひふへほ
まみむめも
やゐゆゑよ
らりるれろ
わをん

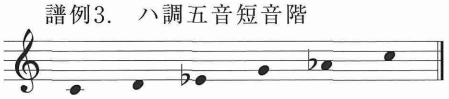
譜例1.　ヨーロッパ音階



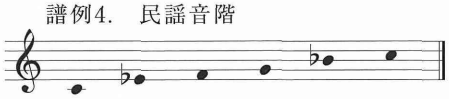
譜例2.　ハ調五音長音階



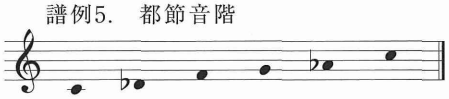
譜例3.　ハ調五音短音階



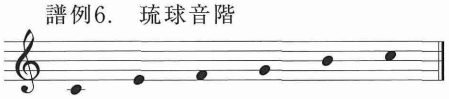
譜例4.　民謡音階



譜例5.　都節音階



譜例6.　琉球音階



かごめかごめ

遊戯歌〈千葉〉

かごめ　かごめ
かごの中の鳥は
いついつ出やる
夜明けの晩に
鶴と亀がすべった
うしろの正面だァれ

通りゃんせ

遊戯歌〈東京〉

通りゃんせ　通りゃんせ
ここはどこの細道じゃ
天神様の細道じゃ
ちいっと通してくだしゃんせ
ご用のない者通しゃせぬ
この子の七つのお祝いに
お札を納めにまいります
行きはよいよい　帰りは恐い
恐いながらも　通りゃんせ
通りゃんせ

開いた開いた

遊戯歌〈東京〉

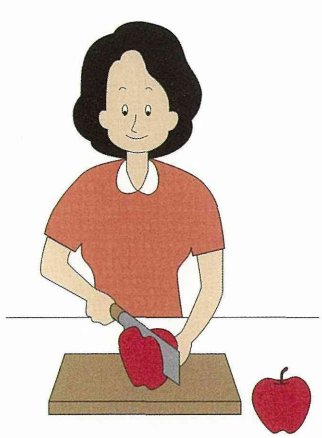
開いた　開いた
何の花が開いた
蓮華の花が開いた
開いたと思ったら
いつのまにかつぼんだ
つぼんだ　つぼんだ
何の花がつぼんだ
蓮華の花がつぼんだ
つぼんだと思ったら
いつのまにか開いた

ずいずいずっころばし

遊戯歌〈東京〉

ずいずい　ずっころばし
胡麻味噌　ずい
茶壺に追われて
トッピンシャン
抜けたら　ドンドコショ
俵の鼠が米食って　チュウ
チュウ　チュウ　チュウ
お父さんが呼んでも
お母さんが呼んでも
行きっこなし（よ）
井戸のまわりで
お茶碗欠いたのだァれ

何をしているところでしょうか？



平成23年度厚生労働科学研究費補助金（障害者対策総合研究事業（感覚器障害分野））
H22—感覚—一般—001
研究代表者　加我君孝

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
加我君孝、新正由紀子、内山勉、坂田英明	新生児聴覚スクリーニング	五十嵐隆	小児臨床ピクシス16	中山書店	東京	2010	55-59
加我君孝、新正由紀子、竹腰英樹、内山勉	先天性難聴児の喃語と音声と言語の発達	五十嵐隆	小児診療科ピクシス19	中山書店	東京	2010	193-199
加我君孝	二つの耳の不思議	日本学術協力財団	日学新書２ 感覚器[視覚と聴覚]と社会とのつながり―見るよろこび、聞くよろこび―	日本学術協力財団	東京	2011	136-155
加我君孝	正しい検査で適切な治療・療育へ	母子衛生研究会	母子保健ハンドブック2011	母子保健事業団	東京	2011	66-72
Hans J.Ten Donkelarr, Kaga K	The auditory system.	Clinical Nuero-anatomy	Hans J. ten Donkelarr	Springer		2011	305-29
		加我君孝	新耳鼻咽喉科学	南山堂	東京	2013	
		加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	
加我君孝	早期発見の歴史	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	2-5
加我君孝	どのように疑うか	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	6-7
竹腰英樹	ティンパノメトリー	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	8-11
加我君孝	気導ABR	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	29-34
坂田英明	骨導ABR	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	35-37

加我君孝	EABR(電気刺激聴性脳幹反応)	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	38-40
増田毅、加我君孝	チャープABR	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	41-44
新正由紀子	AABR(自動聴性脳幹反応)	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	45-48
坂田英明、浅沼聡	ABR、ASSRとオージオグラム	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	93-99
力武正浩、加我君孝	脳性麻痺	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	106-111
新正由紀子、加我君孝	盲聾児と髄膜炎	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	123-126
加我君孝	Auditory Neuropathyと Auditory Neuropathy Spectrum Disorders	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	127-131
加我君孝	脳幹障害	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	150-153
加我君孝	聴覚失認	加我君孝	新生児・幼小児の耳音響放射とABR	診断と治療社	東京	2012	166-168
加我君孝	聴力検査	日本発達障害学会	発達障害ハンドブック	金子書房	東京	2012	8-9

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kaga K, Fukushima K, Kanda Y, et al	Nationwide survey of pediatric cochlear implant in Japan.	7th Asia Pacific Symposium on Cochlear Implants and Related Sciences. International Proceedings.		69-71	2010
Kasai M, Kaga K et al:	Vestibular function of patients with profound deafness related to *GJB2* mutation.	Acta Otolaryngol	130	990-995	2010
Mizutarti K, Matsunaga T, Kaga K, et al	Vestibular dysfunction in a Japanese patient with a mutation in the gene *OPA1*.	Journal of Neurological Sciences.	293	23-28	2010

Rikitake M, Kaga K	Development of speech and hearing of two children with Pelizaeus-Merzbacherdisease presentin only waves I and II of the auditory brainstem response	Acta Otolaryngol	132	563-9	2012
Matsunaga T, Mutai H, Namba K, et al	Genetic analysis of PAX3 for diagnosis of moderate hearing loss	Acta Otolaryngol	132(11)	1160-7	2012
Matsunaga T, Mutai H, Kunishima S, et al	A prevalent founder mutatio n and genotype-phenotype correlations of OTOF in Japanese patientswith auditory neuropathy	Clin Gent	82	425-32	2012
加我君孝	聴覚障害	チャイルドヘルス	13(5)	25-28	2010
加我君孝、新正由紀子、竹腰英樹、内山勉	聞く・話す力の発達	チャイルドヘルス	13(12)	9-14	2010
加我君孝	重度難聴に対する人工内耳手術と聴覚脳幹インプラント	学術の動向.	15	60-64	2010
加我君孝、竹腰英樹、新正由紀子	細菌性髄膜炎－髄膜炎による聴覚障害および人工内耳手術－	JOHNS	26	1771-1779	2010
松永達雄	遺伝性感音難聴の研究	オーディオインフォ	8	17-19	2010
岡本康秀、松永達雄、坂田英明、加我君孝 小川郁 他	両側前庭水管拡大症の確実例とボーダーライン例の*SLC26A4*遺伝子変異および臨床所見の特徴	Audiology Japan	53 (2)	164-170	2010
加我君孝、竹腰英樹、新正由紀子、内山勉 他	新生児聴覚スクリーニング	小児科臨床	64(1)	52-55	2011
内山 勉	聴覚はどのように発達するのか？	JOHNS	28(3)	260-262	2011
内山 勉	言語はどのように発達するのか？	JOHNS	28(3)	263-266	2011
内山 勉	言語発達と臨界期	JOHNS	27(8)	1185-1189	2011
内山 勉	人工内耳装用児の療育開始年齢と早期療育効果との関係について	音声言語医学	52(4)	329-335	2011
松永辰雄	Auditory Neuropathyの遺伝子	Clinical Neuro-science	29(2)	1409-1411	2011
加我君孝	脳性麻痺に伴う感音性難聴の原因と補聴器・人工内耳による支援	重傷心身障害の療育	7(1)	9-17	2012

加我君孝	幼小児の難聴の医療の進歩の光と影~新生児聴覚スクリーニング後の最近10年の成果と課題~	チャイルドヘルス	15(10)	692-5	2012
加我君孝	中耳・内耳・中枢聴覚伝導路の発達	チャイルドヘルス	15(10)	696-700	2012
内山勉	聴こえと視力の二重障害児（盲ろう児）	チャイルドヘルス	10	43-5	2012
坂田英明	難聴の病態と診断の進め方	Medical Techonology	40	719-725	2012

Nationwide Survey of Pediatric Cochlear Implant in Japan

Kaga K.[1,2], Fukushima K.[1,3], Kanda Y.[1,4], Yamashita H.[1,5], Ito J.[1,6], Ichikawa G.[1,7]

[1] *Committee for Hearing-Impaired Infants and Children of the Oto-Rhino-Laryngological Society of Japan, Inc.*
[2] *National Institute of Sensory Organs, National Tokyo Medical Center*
[3] *Department of Otolaryngology and Head-Neck Surgery, Okayama University*
[4] *Kanda Hearing Clinic, Nagasaki*
[5] *Department of Otolaryngology, Head & Neck Surgery, Yamaguchi University*
[6] *Department of Otolaryngology, Head & Neck Surgery, Kyoto University*
[7] *Department of Otolaryngology, Head & Neck Surgery, Juntendo University*

Summary

The Committee for Hearing-Impaired Infants and Children of the Oto-Rhino-Laryngological Society of Japan conducted nationwide surveys of surgery for pediatric cochlear implant in 2005 and 2006 and compared problems of preoperation, operation and postoperative auditory verbal training between the two years. This survey clarified the present problems with regard to pediatric cochlear implants and revealed that more efforts are required to develop better hearing, speech and language skills of patients.

Introduction

To investigate data of pediatric cochlear implants in Japan and to identify current problems, we conducted nationwide questionnaire surveys for all hospitals responsible for cochlear implantation. Since 1994, when cochlear implantation was first covered by the government health insurance system in Japan, we have asked all hospitals to register patients' profiles with the Oto-Rhino-Laryngological Society of Japan. However, over the last several years, the number of pediatric cochlear implants performed has increased markedly and the registration system has been found insufficient to obtain detailed data. This is the first comprehensive report on our data on pediatric cochlear implantation in Japan.

©2009 by MEDIMOND s.r.l.

Methods

The Committee for Hearing-Impaired Infants and Children prepared the questionnaire regarding pediatric cochlear implantation in 2005. The questionnaire was sent to 94 hospitals, of which 79 hospitals (84%) sent it back. The questionnaire consisted of 3 sections concerning numbers of patients (3 questions), preoperative backgrounds (19 questions), operations (4 questions) and postoperative auditory training (10 questions). This questionnaire survey was conducted in 2005 and 2006 and changes were compared between these two years. The collected data were analyzed by the committee members. In this study, data of pediatric cochlear implantation in individuals below 6 years of age were analyzed and the results are reported here.

1. Number of patients

The numbers of pediatric cochlear implant were 189 among 399 patients (47.4%) in 2005 and 199 among 474 patients (42%) in 2006. The numbers of pediatric patients below 6 years of age were 156 (39.1%) in 2005 and 198 (41.8%) in 2006.

2. Preoperative background of patients below 6 years of age

1) Average age at operation: 47.7 months in 2005 and 37.6 months in 2006.

2) Average age at diagnosis of deafness: 12.9 months in 2005 and 14.3 months in 2006.

3) Average age at hearing aid fitting: 15.8 months in 2005 and 16.4 months in 2006.

4) How were hearing problems found?

a) Newborn hearing screening and refer: 36 patients (23.1%) in 2005 and 51 patients (25.8%) in 2006.

b) Passed newborn hearing screening but deafness found later:10 patients (6.4%) in 2005 and 16 patients (8.1%) in 2006.

c) Deafness was found without newborn hearing screening:69 patients (44.2%) in 2005 and 88 patients (44.4%) in 2006.

d) Deafness was found through one-and-a-half-year-old childrens' health examination system: 16 patients (10.3%) in 2005 and 12 patients (6.1%) in 2006.

e) Others: 25 patients (16%) in 2005 and 33 patients (16.7%) in 2006.

5) Prelingual age or postlingual age?

Numbers at prelingual age were 107 patients (68.6%) in 2005 and 141 patients (71.2%) in 2006 and numbers at postlingual age were 42 patients (26.9%) in 2005 and 46 patients (23.2%) in 2006.

6) Etiology of deafness (2005 vs. 2006):

a) Cytomegalovirus infection (5 and 11). b) Meningitis (5 and 4).

c) Waardenburg syndrome (4 and 3). d) Inner ear anomaly (5 and 5).

e) Congenital rubella (1 and 2). f) Mumps (1 and 2).

7) Gene abnormality: *GJB2* was detected in 7 patients in 2005 and 6 patients in 2006. *GJB2* (235 delc) was detected in 7 patients in 2005 and 0 patients in 2006.

8) Inner ear anomaly and cochlear nerve hypogenesis: Inner ear anomaly was present in 20 patients (12.8%) in 2005 and 26 patients (13.1%) in 2006 and cochlear

nerve hypogenesis was present in 4 patients (2.6%) in 2005 and 2 patients (1%) in 2006.

9) Auditory neuropathy: 4 patients (2.6%) in 2005 and 3 patients (1.5%) in 2006.

10) Double-handicapped children including these with cerebral palsy, mental retardation, developmental disorders and others: 25 patients (16%) in 2005 and 35 patients (17.7%) in 2006.

11) Another 9 questions were asked but the results are not described here.

3. Operations

1) Difficulties of electrode insertion: 7 patients (4.5%) in 2005 and 10 patients (5.1%) in 2006.

2) Type of device: Cochlear N24 was used in 151 patients (96.8%) in 2005 and 97 patients (49%) in 2006 and Cochlear N24 (Contour) was not used in 2005 but was used in 95 patients (48%) in 2006.

3) Complications including infection and transient facial palsy: 17 patients (10.9%) in 2005 and 9 patients (4.6%) in 2006.

4. Postoperative auditory training

a) Type of training: Auditory oral training was performed in 119 patients (76.3%) in 2005 and 171 patients (86.4%) in 2006. Visual language training was performed in 24 patients (15.4%) in 2005 and 20 patients (10.1%) in 2006.

b) Type of school: Deaf schools were attended by 101 patients (64.8%) in 2005 and 125 patients (63.1%) in 2006 and auditory oral centers for infants were attended by 29 patients (18.6%) in 2005 and 41 patients (20.7%) in 2006.

c) Average hearing level: 36.7dBHL in 2005 and 39.5dBHL in 2006.

d) Mapping: Performed by speech therapists only in 109 hospitals (69.9%) in 2005 and 145 hospitals (73.2%) in 2006. Performed with cooperation between speech therapists and otolaryngologists in 31 hospitals (19.9%) in 2005 and 28 hospital (14.1%) in 2006.

e) Others: Not described here.

Discussion and Conclusion

This nationwide survey clarifies current problems facing congenitally deaf infants and children. The first is the delayed discovery of congenital deafness in infants. The second is that surgeons are limited to only one company's device. The third is the limited numbers of postoperative auditory training preschools for patients. We need to make more efforts to achieve the best outcomes of pediatric cochlear implants.

Acta Oto-Laryngologica, 2010; 130: 990–995

informa
healthcare

ORIGINAL ARTICLE

Vestibular function of patients with profound deafness related to *GJB2* mutation

MISATO KASAI[1], CHIERI HAYASHI[1], TAKASHI IIZUKA[1], AYAKO INOSHITA[1], KAZUSAKU KAMIYA[1], HIROKO OKADA[1], YUKINORI NAKAJIMA[1], KIMITAKA KAGA[2] & KATSUHISA IKEDA[1]

[1]*Department of Otorhinolaryngology, Juntendo University School of Medicine, Tokyo and* [2]*National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan*

Abstract

Conclusion: GJB2 mutations are responsible not only for deafness but also for the occurrence of vestibular dysfunction. However, vestibular dysfunction tends to be unilateral and less severe in comparison with that of bilateral deafness. *Objectives:* The correlation between the cochlear and vestibular end-organs suggests that some children with congenital deafness may have vestibular impairments. On the other hand, *GJB2* gene mutations are the most common cause of nonsyndromic deafness. The vestibular function of patients with congenital deafness (CD), which is related to *GJB2* gene mutation, remains to be elucidated. The purpose of this study was to analyze the relationship between *GJB2* gene mutation and vestibular dysfunction in adults with CD. *Methods:* A total of 31 subjects, including 10 healthy volunteers and 21 patients with CD, were enrolled in the study. A hearing test and genetic analysis were performed. The vestibular evoked myogenic potentials (VEMPs) were measured and a caloric test was performed to assess the vestibular function. The percentage of vestibular dysfunction was then statistically analyzed. *Results:* The hearing level of all CD patients demonstrated a severe to profound impairment. In seven CD patients, their hearing impairment was related to *GJB2* mutation. Five of the seven patients with CD related to *GJB2* mutation demonstrated abnormalities in one or both of the two tests. The percentage of vestibular dysfunction of the patients with CD related to *GJB2* mutation was statistically higher than in patients with CD unrelated to *GJB2* mutation and in healthy controls.

Keywords: *Vestibular evoked myogenic potentials, caloric test*

Introduction

Since a correlation between the peripheral auditory and vestibular systems has been identified both anatomically and phylogenetically, a subgroup of children with congenital deafness (CD) may be associated with vestibular and balance impairments [1–3]. Interestingly, the vestibular disturbance in these children gradually disappears as they grow up, probably because of a compensatory mechanism of the central nervous system. However, there have been only a few reports that conducted a detailed analysis of the vestibular function in adults with CD.

CD has been reported in approximately one child per 1000 births [1]. In more than half of these cases, the disease is caused by gene mutation. In particular, mutation in the *GJB2* gene, which encodes Cx26 in the gap junction, is known to be a most common cause (up to 50% of such cases) [2,3]. Gap junction channels enable the neighboring cells to exchange small signaling molecules. Immunohistochemical studies have revealed that Cx26 exists not only in the cochlea but also in the vestibular organs [4]. K^+ cycling involving gap junction protein Cx26 in the vestibular labyrinth, which is similar to that in the cochlea, is thought to play a fundamental role in the endolymph homeostasis and sensory transduction [5]. These findings suggest that mutations in the *GJB2* gene may thus cause vestibular dysfunction.

Correspondence: Katsuhisa Ikeda MD PhD, 2-1-1 Hongo, Bunkyoku, Tokyo 113-8421, Japan. E-mail: ike@juntendo.ac.jp

(Received 18 October 2009; accepted 30 November 2009)

ISSN 0001-6489 print/ISSN 1651-2251 online © 2010 Informa Healthcare
DOI: 10.3109/00016481003596508

In this study, the relationship between *GJB2* gene mutation and vestibular dysfunction in adults with CD was investigated to confirm whether or not there are any abnormalities associated with the vestibular function.

Material and methods

Subjects

The subjects in this prospective study included 21 patients with CD and 10 healthy volunteers. The patients were excluded from the study if they were being treated with ototoxic drugs or if they had a cytomegalovirus infection, bacterial meningitis, external and middle ear pathological findings, or other risk factors for inner ear damage. No participants had syndromic deafness due to pigmentary retinopathy, nephropathy, goiter, or any other diseases. Patients with vestibular dysfunction due to head trauma, brain tumor, Menière's disease, or other conditions were also excluded from the study. All subjects underwent an otoscopic examination and were found to have a normal tympanic membrane. Audiometric testing was performed in a double-walled, sound-treated booth. All patients gave their informed consent in writing and the study was approved by the Ethics Committee of Juntendo University School of Medicine.

Genetic analysis

DNA was extracted from peripheral blood leukocytes of the subjects. The coding region of *GJB2* was amplified by PCR using the primers *GJB2*-2F 5′-GTGTGCATTCGTCTTTTCCAG-3′ and *GJB2*-2R 5′-GCGACTGAGCCTTGACA-3′. The PCR products were sequenced using the PCR primers and sequence primers *GJB2*-A 5′-CCACGC-CAGCGCTCCTAGTG-3′ and *GJB2*-B 5′-GAA-GATGCTGCTGCTTGTGTAGG-3′. These were visualized using an ABI Prism 310 Analyzer (PE Applied Biosystems, Tokyo, Japan).

Vestibular evoked myogenic potentials

The vestibular evoked myogenic potentials (VEMPs) were measured as described in a previous report [6]. Both sound stimuli of clicks (0.1 ms, 95 dBnHL) and short tone burst (500 Hz; rise/fall time, 1 ms, 95 dBnHL) were presented to each side of the ear through the headphones using a Neuropack evoked-potential recorder (Nihon Kohden Co. Ltd, Tokyo, Japan). The surface electromyographic activity was recorded with the patient in the supine position from symmetrical sites over the upper half of each sternocleidomastoid (SCM) muscle with a reference electrode on the lateral end of the upper sternum. During recording, the subjects were instructed to lift their head up or to turn the contralateral side to induce hypertonicity of the SCM. Thereafter, the electromyographic signals from the stimulated side of the SCM muscle were amplified.

Caloric test

The caloric test in the current study was performed as described elsewhere [7]. Briefly, 2 ml of ice-water (at 4°C) was irrigated in the external auditory meatus to induce a thermal gradient across the horizontal semicircular canal of one ear. The duration of horizontal and vertical nystagmus was recorded. The results were compared between the right and left ears.

Statistical analysis

The data are expressed as the mean ± SD. Statistical analyses were conducted using a non-repeated measures analysis of variance (ANOVA). Significant effects were further analyzed by post hoc multiple comparison tests using the Student-Newman-Keuls test. A value of $p < 0.05$ was considered to indicate statistical significance.

Results

Hearing test

The pure-tone averages of 0.5, 1.0, and 2.0 kHz are shown in Table I. The hearing impairments of CD patients ranged from severe (71–95 dB) to profound (>95 dB). The hearing levels of all controls were at the normal level (<30 dB; data not shown).

Genetic analysis

GJB2 mutations were found in nine CD patients (Table I). All three mutations have been described previously in association with deafness. Among these mutations, 235delC mutation was found in eight patients. One nonsense mutation (Y136X) and one frameshift mutation (176-191del) were also identified. In six patients with a homozygous *GJB2* mutation and one patient with a compound heterozygous

Table I. Results of hearing level, genetic analysis, and vestibular function of subjects with congenital deafness (CD)

Case no.	Hearing level (dB) Left	Right	Sex	Age (years)	Mutation in *GJB2*	VEMPs	Caloric test
Patients with *GJB2*-related CD							
1	86	98	M	26	Homo 235delC	Right decreased	Left CP
2	106	108	M	25	Homo 235delC	Right decreased	Normal
3	108	106	M	28	Homo 235delC	Right decreased	Normal
4	108	106	M	37	Homo 235delC	Normal	Right CP
5	100	106	M	32	Homo 235delC	Normal	Right poor/left CP
6	80	91	M	25	Homo 235delC	Normal	Normal
7	115	108	M	25	Y136X/235delC	Normal	Normal
Patients without *GJB2*-related CD							
8	98	98	F	24		Left decreased	Bilateral CP
9	98	115	M	26		Normal	Bilateral CP
10	97	97	M	20		Normal	Normal
11	111	108	M	31		Normal	Normal
12	100	104	F	34		Normal	Normal
13	98	95	M	21		Normal	Normal
14	91	91	M	24		Normal	Normal
15	99	101	F	26		Normal	Normal
16	99	95	F	23		Normal	Normal
17	80	68	M	27		Normal	Normal
18	96	95	M	27		Normal	Normal
19	85	73	M	23		Normal	Normal
Patients with heterozygous *GJB2* mutation							
20	73	100	M	25	Hetero 235delC	Normal	Normal
21	97	98	M	25	Hetero 176-191del16	Normal	Normal

CP, canal paresis; Poor, nystagmus was obviously weak.

mutation (case nos 1–7); their profound deafness was thought to be caused by a *GJB2* mutation. No *GJB2* mutation was identified in any of the controls.

Vestibular function

No patients or controls had any subjective symptoms of vertigo. Table I shows the results of the vestibular function in all CD patients. Abnormal responses of VEMPs and the caloric test in CD with a *GJB2*-related mutation were observed in three patients each (case nos 1–5). Three patients with a homozygous *GJB2* mutation showed asymmetrical responses in VEMPs (case nos 1–3). Three patients with a homozygous *GJB2* mutation showed asymmetrical responses in the caloric test (case nos 1, 4, and 5). One of them showed both VEMPs and the caloric test asymmetrical responses (case no. 1). One patient with a homozygous *GJB2* mutation and one patient with compound heterozygous *GJB2* mutation showed normal responses in both VEMPs and the caloric test (case nos 6 and 7). It is notable that five of the six patients with a homozygous 235delC mutation showed no abnormalities in either test. Two heterozygous patients (case nos 20 and 21) showed normal responses in both tests.

Two CD patients with no *GJB2* mutation exhibited abnormal findings for the vestibular tests (case nos. 8 and 9). One patient showed a unilateral reduction in VEMPs and bilateral canal paresis (case no. 8). Bilateral canal paresis was also observed in another patient (case no. 9).

All the controls with normal hearing showed normal responses in both the VEMPs and the caloric test (data not shown).

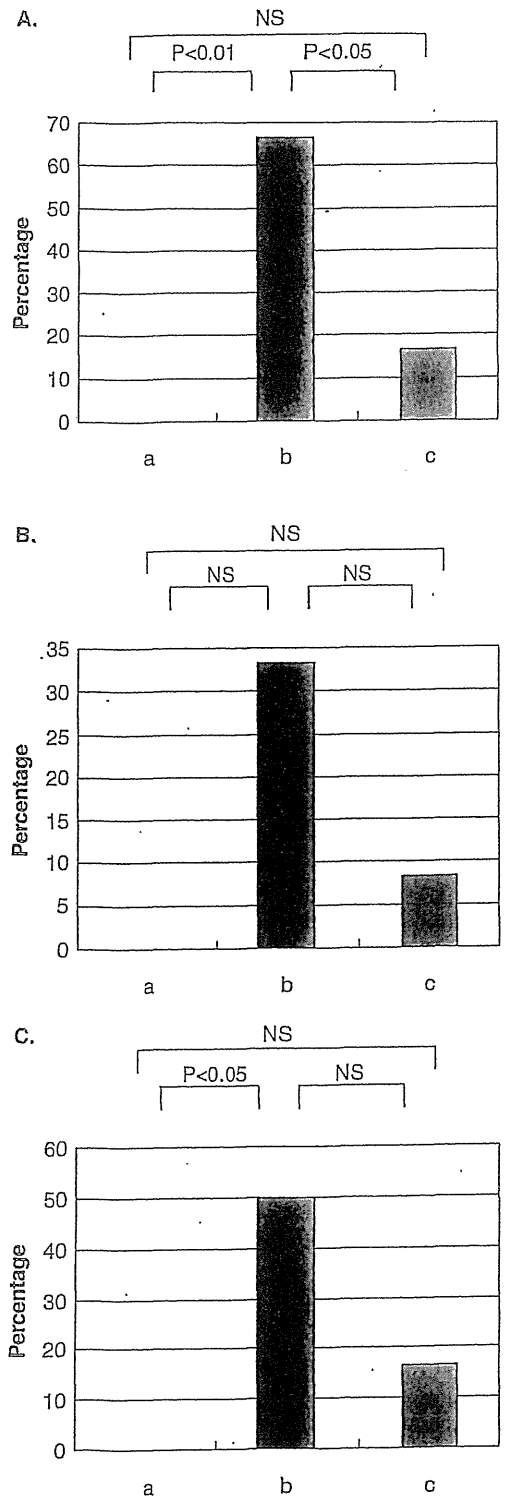


Figure 1. Comparison of the incidence of abnormality in the vestibular tests among the three groups. (A) Percentage showing abnormality in VEMPS and/or caloric test. (B) Percentage showing abnormality in VEMPs. (C) Percentage showing abnormality in the caloric test. a, Controls; b, *GJB2*-related CD subjects; c, CD subjects without *GJB2* mutations.

Statistical analysis of vestibular function in the three groups

Figure 1 shows a comparison of the controls, patients with CD related to a *GJB2* mutation, and those with CD without a *GJB2* mutation. The CD patients with *GJB2* heterozygous mutation were excluded from this statistical analysis, since their symptoms of hearing impairment are not necessarily caused by the *GJB2* mutation alone. Vestibular dysfunction showing an abnormality in VEMP and/or the caloric test significantly increased in patients with *GJB2*-related CD in comparison with those with CD without *GJB2* mutation ($p < 0.05$) and the controls ($p < 0.01$), whereas no difference was observed between CD without a *GJB2* mutation and the controls (Figure 1A). No differences in the incidence of abnormality in VEMPs were observed among the three groups (Figure 1B). The incidence of abnormalities in the caloric test in patients with *GJB2*-related CD differed significantly from that in the controls, but the other two comparisons were not significant (Figure 1C).

Discussion

In this study, vestibular tests were performed in CD patients with or without a *GJB2* mutation by measuring the VEMPs and using the caloric test. Only one report has previously investigated the vestibular function of patients with *GJB2*-related CD [8]. The authors noted that five of the seven patients showed no VEMP responses bilaterally and that only one case had a unilateral pathological response in the caloric test, which led to the conclusion that CD with a *GJB2* mutation is associated with severe saccular dysfunction. However, in the present study, there were no patients showing the absence of both VEMP and a caloric response. Todt et al. [8] showed the existence of *GJB2* mutations that do not cause CD (polymorphisms), thus suggesting a considerable bias. Furthermore, patients with low-grade hearing loss were included in their study. In contrast, all of the *GJB2* mutations detected in the present study are known to cause CD in the Asian population [9]. In addition, the present study included only patients with severe to profound hearing loss, which would therefore clarify the correlation between CD and *GJB2* mutations. Among the seven patients with *GJB2*-related CD, five (71.4%) showed abnormal responses in either or both tests. The incidence was apparently and significantly higher than that in patients with CD without a *GJB2* mutation (2/13: 15.4%). Moreover, the incidence in the controls significantly differed from that in patients with CD related to a *GJB2*

mutation but not in those with CD without *GJB2* mutation. Therefore, these findings support the hypothesis that *GJB2* mutations play a critical role in the disturbance of the vestibular function.

GJB2 mutations cause profound deafness and the associated mechanism has been discussed in several studies [10,11]. A recent study showed that *GJB2* is indispensable in the normal development of the organ of Corti and normal hearing on the basis of the study in *Gjb2* dominant-negative mutant mice [12]. Despite the widespread expression of Cx26 in both the cochlear and vestibular organs [4], the vestibular function impairment of the patients with a *GJB2* mutation is not as severe as the hearing dysfunction observed in the present study. Two hypotheses have been proposed to explain this inconsistency between hearing and balance function. One hypothesis is based on the fact that two temporal bone studies performed in patients with *GJB2*-related hearing impairment in the previous study revealed that one patient had mild vestibular hydrops and saccular degeneration, while another patient had a dysplastic neuroepithelium of the saccule [13,14]. This suggests that a *GJB2* mutation can cause morphological dysplasia in not an entire organ, but in part of the vestibular organs. This is contrast to the cochlea of these patients, which showed nearly total dysplasia of the organ of Corti. These histopathological studies support the results of the vestibular dysfunction of patients with *GJB2*-related CD in the present study. The other hypothesis is based on the presence of several connexins such as Cx26, Cx30 (encoded by *GJB6*), Cx31 (encoded by *GJB3*), and Cx32 (encoded by *GJB1*) in the inner ear. A previous study showed all of these connexins to be distributed in the vestibular organs [15]. Cx30 gene knockout mice had hair cell loss in the saccule, which was restored by the over-expression of the Cx26 gene [16]. Therefore, the specific loss of Cx30 causes vestibular dysfunction, which can be compensated by other types of connexins. The present clinical study in which a complete defect of Cx26 resulted in a definitive but partial dysfunction of vestibular end organs can be explained by the compensation of other connexins normally expressed in the vestibule. Further studies are required to clarify the relationship between connexins and the vestibular function.

Although there was a statistically significant difference in the objective examination of the vestibular function among patients with *GJB2*-related CD, those with CD without a *GJB2* mutation, and healthy controls, none of these subjects had any vestibular symptoms regardless of the presence or absence of a *GJB2* mutation. The peripheral vestibular dysfunction predicted in individuals with the *GJB2* mutation may be compensated by the central vestibular system in young patients with deafness, as shown in the present study. However, aging is known to affect both the peripheral and central vestibular system [17]. In patients with a *GJB2* mutation, the vestibular symptoms may progress with aging. Another problematic point regarding patients with CD related to *GJB2* mutations is cochlear implantation, which has been reported to cause vestibular dysfunction, such as a reduction of the caloric responses [18] and a decrease in the VEMP responses [19]. It is thought that the mechanical damage caused by the insertion of the electrode may induce vestibular dysfunction [20]. In the present study, four patients with *GJB2*-related deafness showed unilateral vestibular dysfunction, while only one of them had bilateral dysfunction. Therefore, it should be emphasized that the assessment of the vestibular function in patients with *GJB2*-related CD is important to determine which side of the ear should be selected to insert the cochlear implant.

Conclusions

A *GJB2* mutation is responsible not only for deafness but also for vestibular dysfunction. However, such vestibular dysfunction is likely to be unilateral and less severe in patients with a *GJB2* mutation than in those with bilateral deafness.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Morton NE. Genetic epidemiology of hearing impairment. Ann N Y Acad Sci 1991;630:16–31.

[2] Denoyelle F, Marlin S, Weil D, Moatti L, Chauvin P, Garabedian EN, et al. Clinical features of the prevalent form of childhood deafness, DFNB1, due to a connexin-26 gene defect: implications for genetic counselling. Lancet 1999;353:1298–303.

[3] Murgia A, Orzan E, Polli R, Martella M, Vinanzi C, Leonardi E, et al. Cx26 deafness: mutation analysis and clinical variability. J Med Genet 1999;36:829–32.

[4] Masuda M, Usami S, Yamazaki K, Takumi Y, Shinkawa H, Kurashima K, et al. Connexin 26 distribution in gap junctions between melanocytes in the human vestibular dark cell area. Anat Rec 2001;262:137–46.

[5] Wangemann P. K(+) cycling and its regulation in the cochlea and the vestibular labyrinth. Audiol Neurootol 2002;7:199–205.

[6] Jin Y, Nakamura M, Shinjo Y, Kaga K. Vestibular-evoked myogenic potentials in cochlear implant children. Acta Otolaryngol 2006;126:164–9.

[7] Yukiko S, Yulian J, Kimitaka K. Assessment of vestibular function of infants and children with congenital and acquired deafness using the ice-water caloric test, rotational chair test and vestibular-evoked myogenic potential recording. Acta Otolaryngol 2007;127:736–47.

[8] Todt I, Hennies HC, Basta D, Ernst A. Vestibular dysfunction of patients with mutations of Connexin 26. Neuroreport 2005;16:1179–81.

[9] Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, Van Later L, V, et al. GJB2 deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet 2003;112:329–33.

[10] Kudo T, Kure S, Ikeda K, Xia AP, Katori Y, Suzuki M, et al. Transgenic expression of a dominant-negative connexin26 causes degeneration of the organ of Corti and non-syndromic deafness. Hum Mol Genet 2003;12:995–1004.

[11] Cohen-Salmon M, Ott T, Michel V, Hardelin JP, Perfettini I, Eybalin M, et al. Targeted ablation of connexin26 in the inner ear epithelial gap junction network causes hearing impairment and cell death. Curr Biol 2002;12: 1106–11.

[12] Inoshita A, Iizuka T, Okamura HO, Minekawa A, Kojima K, Furukawa M, et al. Postnatal development of the organ of Corti in dominant-negative Gjb2 transgenic mice. Neuroscience 2008;156:1039–47.

[13] Griffith AJ, Yang Y, Pryor SP, Park HJ, Jabs EW, Nadol JB Jr, et al. Cochleosaccular dysplasia associated with a connexin 26 mutation in keratitis-ichthyosis-deafness syndrome. Laryngoscope 2006;116:1404–8.

[14] Jun AI, McGuirt WT, Hinojosa R, Green GE, Fischel-Ghodsian N, Smith RJ. Temporal bone histopathology in connexin 26-related hearing loss. Laryngoscope 2000;110:269–75.

[15] Forge A, Becker D, Casalotti S, Edwards J, Marziano N, Nevill G. Gap junctions in the inner ear: comparison of distribution patterns in different vertebrates and assessment of connexin composition in mammals. J Comp Neurol 2003;467:207–31.

[16] Qu Y, Tang W, Dahlke I, Ding D, Salvi R, Sohl G, et al. Analysis of connexin subunits required for the survival of vestibular hair cells. J Comp Neurol 2007;504: 499–507.

[17] Gazzola JM, Perracini MR, Gananca MM, Gananca FF. Functional balance associated factors in the elderly with chronic vestibular disorder. Braz J Otorhinolaryngol 2006;72:683–90.

[18] Buchman CA, Joy J, Hodges A, Telischi FF, Balkany TJ, Vestibular effects of cochlear implantation. Laryngoscope 2004;114:1–22.

[19] Ernst A, Todt I, Seidl RO, Eisenschenk A, Blodow A, Basta D. The application of vestibular-evoked myogenic potentials in otoneurosurgery. Otolaryngol Head Neck Surg 2006;135:286–90.

[20] Jin Y, Shinjo Y, Akamatsu Y, Ogata E, Nakamura M, Kianoush S, et al. Vestibular evoked myogenic potentials evoked by multichannel cochlear implant – influence of C levels. Acta Otolaryngol 2008;128:284–90.

Journal of the Neurological Sciences 293 (2010) 23–28

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Vestibular dysfunction in a Japanese patient with a mutation in the gene *OPA1*

Kunio Mizutari [a,b,*], Tatsuo Matsunaga [b], Yasuhiro Inoue [a], Hiroki Kaneko [c], Hirotaka Yagi [d], Kazunori Namba [b], Satoko Shimizu [e], Kimitaka Kaga [f], Kaoru Ogawa [a]

[a] Department of Otolaryngology, Keio University School of Medicine, Tokyo, Japan
[b] Laboratory of Auditory Disorders, National Tokyo Medical Center, Tokyo, Japan
[c] Department of Integrated Sciences in Physics and Biology, College of Humanities and Sciences, Nihon University, Tokyo, Japan
[d] VALWAY Technology Center, NEC Soft, Ltd., Tokyo, Japan
[e] Department of Ophthalmology, Teikyo University School of Medicine, Tokyo, Japan
[f] National Institute of Sensory Organs, National Tokyo Medical Center, Tokyo, Japan

ARTICLE INFO

Article history:
Received 6 November 2009
Received in revised form 10 March 2010
Accepted 19 March 2010
Available online 10 April 2010

Keywords:
OPA1
Vestibular dysfunction
Auditory neuropathy
Vestibular evoked myogenic potentials (VEMPs)
Caloric test
OPA1 predicted structure

ABSTRACT

OPA1 mutations are known to cause autosomal dominant optic atrophy (ADOA), and some types of *OPA1* mutations also cause auditory neuropathy. In the present study, we evaluated the vestibular dysfunction that accompanied auditory neuropathy in a patient with an *OPA1* mutation. A caloric test failed to elicit nystagmus or dizziness in either ear. Vestibular evoked myogenic potentials (VEMPs) in the right ear were characterized by a normal biphasic waveform. In contrast, no VEMPs were evoked in the left ear. Model building suggested that the *OPA1* mutation, p.R445H, indirectly distorts the catalytic structure of the GTPase reaction center and decreases GTPase activity. The patient complained of instability while walking or moving but thought these symptoms were caused by visual dysfunction. This is the first report of a detailed evaluation of vestibular dysfunction in a patient with an *OPA1* mutation. This case suggests that vestibular dysfunction may be involved in motor instability in patients with an *OPA1* mutation, even when patients do not complain of vestibular symptoms. Based on this case, we suggest that vestibular evaluation should be performed in auditory neuropathy patients carrying an *OPA1* mutation, even if the patients are free of symptoms of vestibular dysfunction.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Autosomal dominant optic atrophy (ADOA; OMIM #165500) is a dominantly inherited optic neuropathy resulting in progressive loss of visual acuity, color vision deficits, a centrocecal scotoma, and optic nerve pallor [1]. ADOA is the most common form of optic atrophy, with an estimated prevalence of 1 in 50,000 individuals [2]. Although several types of loci are known to cause ADOA, it has been reported that as many as 89% of cases may be associated with a mutation in the gene *OPA1* (3q28-29) [3]. *OPA1* encodes a dynamin-related GTPase that is located in the mitochondrial intermembrane space and plays a key role in controlling the balance of mitochondrial fusion and fission. In most cases, ADOA occurs without additional neurological symptoms. However, there are several known cases of optic atrophy associated with sensorineural hearing loss, and the Arg445His (p.R445H) mutation of *OPA1* has been reported in patients with ADOA and moderate progressive hearing loss [4]. In patients having the p.R445H mutation, progressive hearing impairment begins in childhood, and audiological examinations show features of auditory neuropathy, for which the primary lesion is located in the inner hair cells, the auditory nerve, or the synapses between them [4,5]. Recently, a detailed analysis of OPA1 protein expression in the inner ear was reported in rat, and OPA1 protein was detected in the inner hair cells, outer hair cells, and spiral ganglia in the cochlea, as well as the hair cells and ganglia in the vestibular organ [6]. Although there have been several reports of auditory function in patients with this *OPA1* mutation, the analysis of vestibular function has not yet been reported in any *OPA1* mutation. In this paper, we report the results of examinations for auditory and vestibular function in a patient who presented with both hearing impairment and vestibular dysfunction due to an *OPA1* mutation that leads to distortion of the catalytic structure of the OPA1 protein.

2. Materials and methods

2.1. Auditory function tests

2.1.1. Audiometric tests

The patient underwent standard pure-tone air- and bone-conducted audiometry (125–8000 Hz) and speech discrimination testing using an audiometer (AA-75, Rion Co., Tokyo, Japan) and the 67-S Japanese word list.

* Corresponding author. Department of Otolaryngology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, 160-8582, Japan. Tel.: +81 3 3353 1211; fax: +81 3 3353 1261.
E-mail address: tari@mbf.ocn.ne.jp (K. Mizutari).

0022-510X/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2010.03.014

2.1.2. DPOAEs

DPOAES were recorded and analyzed using the ILO-92 system (Otodynamics Ltd, Herts, UK). DPOAE primary tones f1 and f2 were presented at 70 dB SPL. The f2:f1 ratio was kept at 1.22, and the frequency of f2 was changed in one-third octave steps from 708 to 6299 Hz. The levels of 2f1–f2 DPOAE were recorded. DPOAE values were plotted on a DP-gram, which expresses the emission level as a function of the f2 frequency.

2.1.3. Auditory brainstem responses (ABRs)

ABRs were recorded using the Neuropack system (Nihon Kohden, Tokyo, Japan) with an electrode montage of vertex (CZ) to the ipsilateral (stimulated) ear lobe and ground to forehead (Fz). The amplifier band pass was 100–1000 Hz. Alternating-polarity click stimuli were presented monaurally at a rate of 20 Hz at 100 dB nHL. Average responses to 1024 clicks were collected in each of two experiments.

2.2. Vestibular function tests

2.2.1. Electronystagmography

The patient underwent an electronystagmography test battery consisting of spontaneous, optokinetic, positional, postural, and caloric-induced nystagmus recordings. Nystagmus was recorded using an electronystagmograph recorder (Rion, Tokyo, Japan). Caloric testing using 20 °C and ice-cold water (5 cm^3, 5 s) was used to irrigate the external auditory meatus to induce a thermal gradient across the lateral semicircular canal.

2.2.2. Vestibular evoked myogenic potentials (VEMPs)

The sternocleidomastoid (SCM) muscle was chosen as the target to record VEMPs using the Neuropack system (Nihon Kohden, Tokyo, Japan). Surface electromyographic activity was recorded from symmetrical sites over the upper half of each SCM, with a reference electrode over the sternal attachment site of the contralateral SCM. The patient was laid supine on a bed and asked to raise and orient his head contralateral to the tested ear to maximally activate the SCM ipsilateral to the stimulation. Responses to 200 short-tone bursts (105 dB nHL, 500 Hz) were recorded at 100-ms intervals over a band pass of 500–1500 Hz.

2.3. Neuroimaging studies

2.3.1. High-resolution computed tomography (HRCT)

The protocol for HRCT included scanning with a multi-slice computed tomography scanner (Sensation 64; Siemens Medical Solutions, Inc., Malvern, PA, USA). Images were acquired with direct axial sequences using a spiral scan procedure with a 1.0-mm collimation. Data were reconstructed with a slice thickness of 1.0 mm using a bone algorithm.

2.3.2. Magnetic resonance imaging (MRI)

The patient was scanned on a 1.5-T MRI machine (Signa EXITE 1.5T, General Electric, Fairfield, CT, USA) with surface and head coil. Axial three-dimensional fast imaging employing steady-state acquisition (FIESTA, repetition time, 9.3 ms/echo time, 3.3 ms; scan thickness 1.0 mm) was performed. The axial images were reconstructed in the oblique sagittal plane traversing the internal auditory canal (IAC), producing cross-sectional images that visualize the neural structures of the IAC.

2.4. Homology modeling of OPA1 and ligand fitting

The crystal structure of the GTPase domain of rat dynamin 1 (PDB ID: 2AKA) was used as a template in homology modeling because the GTPase domain of rat dynamin 1 is closely related to that of OPA1 in both function and structure (32% amino acid sequence identity). A program package for protein engineering and drug design, BIOCES[E] (NEC Corp., Tokyo, Japan) [7], was used for a series of molecular modeling. This package runs on an OCATANE2 (Silicon Graphics Inc., Fremont, CA, USA). The GTP molecule of Ras-GTP (PDB ID: 5P21) was fitted into the corresponding active site of the OPA1 model using DALI (http://ekhidna.biocenter.helsinki.fi/dali_server/) [8]. The p.R445H mutation structure was superimposed on the native structure (backbone atoms only) and displayed using UCSF Chimera (http://www.cgl.ucsf.edu/chimera/) [9].

3. Case report

The patient is a 28-year-old man who first presented with sudden optic atrophy at the age of 17 years. Clinical history of vision disorder and the result of genetic test have been reported [10]. In brief, he received a detailed examination for visual function at age 21. His best corrected visual acuity was 20/200 in both eyes. He had atrophy of the optic disks, central scotoma, and generalized bilateral dyschromatopsia. As a result, the patient was diagnosed with ADOA, and a genetic examination revealed a heterozygous G-to-A substitution in the second nucleotide of codon 445 in *OPA1*, resulting in an Arg-to-His amino acid substitution (p.R445H). He had no apparent family history of either optic atrophy or hearing impairment. At that time, he was also found to have a slight bilateral hearing impairment. The patient

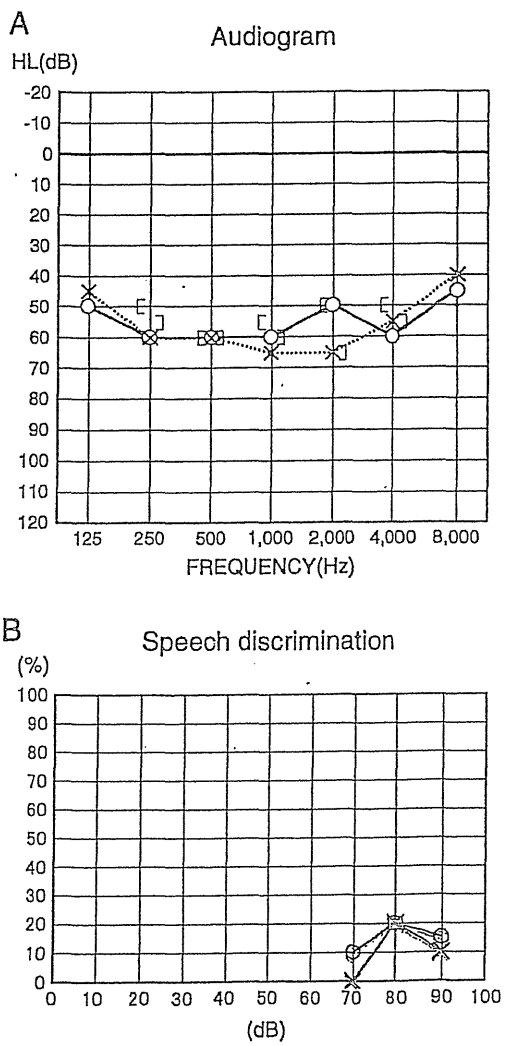


Fig. 1. Pure-tone (A) and speech (B) audiograms of a patient with an *OPA1* mutation. O = right air conduction hearing level; X = left air conduction hearing level; [=right bone conduction hearing level;] = left bone conduction hearing level.

developed progressive hearing impairment, and had particular difficulty understanding speech. He came to our department for a hearing evaluation at age 28. Although he did not initially complain of balance disorders, he stopped riding a bicycle at age 17 years because of difficulty controlling balance and also started to feel unsteady walking at that time. He thought the unsteadiness resulted from his visual dysfunction.

4. Results

4.1. Auditory function test results

Direct otoscopic observation revealed normal findings in both ears. A bilateral sensorineural hearing loss of approximately 60 dB was shown by pure-tone audiometry (Fig. 1A). The maximum speech discrimination scores were 20% in both ears (Fig. 1B), which were significantly worse than expected based on the results of pure-tone audiometry. Although no differences were observed between left and right ears, the patient reported better hearing discrimination in the right ear (Fig. 1). ABRs were absent bilaterally even at 100 dB nHL (Fig. 2A), but high-amplitude DPOAEs were present at all frequencies tested in both ears (Fig. 2B).

4.2. Vestibular function test results

No spontaneous, positioning, or pressure-induced nystagmus was found by electronystagmography. Neither 20 °C nor ice-water caloric stimulation of the labyrinth elicited nystagmus or dizziness in eithe ear (Fig. 3A). Short-tone burst-evoked VEMP analysis revealed biphasic VEMP waveform in the right ear; however, the latency c n23, which is the second wave of VEMP, was delayed. No VEMPs wer evoked in the left ear (Fig. 3B).

4.3. Neuroimaging studies

There were no abnormal findings by HRCT. In particular, no inner ea malformation or internal auditory canal stenosis was observed (Fig. 4/ D). By MRI, both the cochlear nerves and vestibular nerves wer detected from brainstem to the inner ear in both ears in axial FIEST slices (Fig. 4B, E). However, the diameter of the right cochlear nerve wa 0.82 mm whereas that of the left cochlear nerve was 0.69 mm, and th diameter of the right facial nerve was 1.06 mm whereas that of the le facial nerve was 1.02 mm in oblique sagittal reconstructions through th IAC (Fig. 4C, F). Thus, the cochlear nerves on both sides are considere hypoplasia according to reported criteria [11].

4.4. OPA1 predicted structure

The distance between Cα of R445 of OPA1 and the GTP bindin pocket is 18 Å (Fig. 5). The electric field around R445 is negativel charged due to its proximity to D450, D442, and E444. Unde physiological conditions, positively charged R445 is structurall stable, and thus the mutation p.R445H reduces the electrostati stability and indirectly distorts the structure of the GTPase catalyti

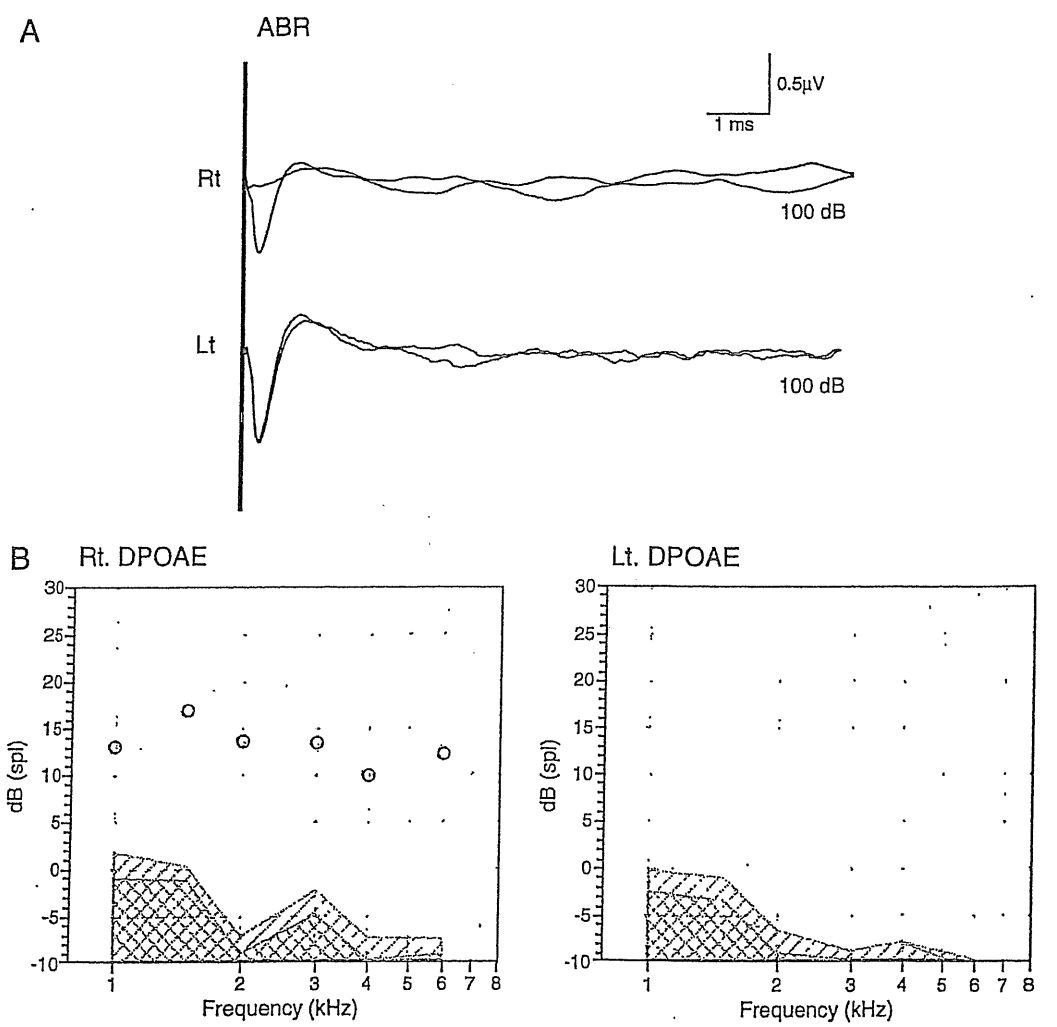


Fig. 2. (A) ABR tests revealed no ABR waveforms in this patient. (B) DPOAE recordings were normal for this patient. Residual noise levels are shown by the shaded area.

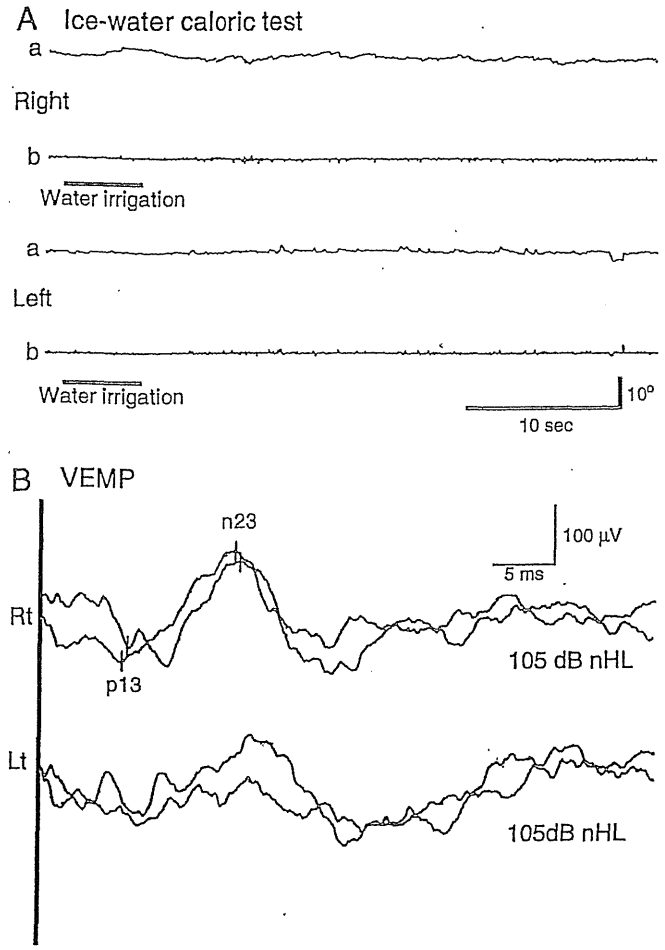


Fig. 3. (A) Horizontal record of electronystagmograph on ice-water caloric test. Time constants: a, 3.0 s; b, 0.03 s. No nystagmus were elicited in both side of ears. (B) Air-conducted VEMPs. Electromyographic responses of the right (Rt) and left (Lt) SCM to right ear stimulation. A biphasic VEMP waveform was revealed in the right ear; however, a latency of n23 was delayed. In contrast, no VEMPs were evoked in the left ear.

domain. In addition, salt bridges between R445 and D450 in the α3-helix and strong electrostatic interactions between R445 and D442/E444 are observed. The α3-helix is a key structure that constructs the common wire frame of the G-protein core fold [7,9]. Thus, the p.R445H mutation indirectly distorts the catalytic structure of the GTPase reaction center and decreases GTPase activity.

5. Discussion

Several reports have described hearing impairments associated with an *OPA1* mutation [4,12–16]. As with the case we present here, these hearing impairments were reported to result from auditory neuropathy. Common features in these patients include moderate hearing threshold elevation and a severe speech discrimination disability. No vestibular symptoms or function test results have yet been reported. To our knowledge, this is the first report of a detailed vestibular analysis in a patient with an *OPA1* mutation. Moreover, inner ear neuroimaging studies, including HRCT or 3-D MRI, have not yet been reported in patients with *OPA1* mutations. This report provides the first evidence of cochlear nerve atrophy in the IAC in a patient with an *OPA1* mutation.

OPA1 encodes a dynamin-related GTPase that is located in the mitochondrial intermembrane space and plays a key role in controlling the balance of mitochondrial fusion and fission [17]. Furthermore, release of cytochrome *c* from mitochondria and caspase-dependent activation of the apoptosis cascade have been observed in the down-regulation model of expression by RNA interference in HeLa cells [17]. The *OPA1* p.R445H mutation is reportedly associated with various neurological disturbances, including ataxia, peripheral neuropathy, ptosis, and cognitive impairment [18]. In cases involving the heterozygous p.R445H mutation, ADOAs associated with deafness have been reported [4], and these sensorineural hearing losses show audiological features compatible with auditory neuropathy. In normal rats, expression of OPA1 protein is seen in the inner hair cells, outer hair cells, and spiral ganglia in the cochlea, and in the vestibular hair cells and ganglia [6]. OPA1 protein expression has also been observed in membranous or submembranous compartments of vestibular ganglion cells and at the level of the calyx synapse, which typically envelopes type 1 hair cells in the vestibular epithelium [6]. Bilateral vestibular dysfunction in our present patient is probably caused by dysfunction of these parts of the vestibular organs.

An abnormality in the OPA1 protein may cause mitochondrial dysfunction, leading to insufficient energy production. Homozygous mutant mice are not viable and show impaired development as early E8.5. [19]. This study also reported that heterozygous mutants show a reduction in OPA1 protein level (about 50% compared with wild-type littermates) due to rapid degradation of the mutant polypeptide [19]. Skin fibroblasts obtained from patients carrying the heterozygous *OPA1* p.R445H mutation show hyperfragmentation of the mitochondrial network, decreased mitochondrial membrane potential, and an ATP synthesis defect [4]. Our three-dimensional structure study suggests that the p.R445H mutation reduces the electrostatic interactions and therefore the stability of the protein and indirectly distorts the structure of the GTPase catalytic center, thereby decreasing GTPase activity. According to these findings, we suggest that the OPA1 p.R445H mutation leads to severely insufficient energy production by decreasing GTPase activity in the mitochondria. This deficiency could, in turn, affect critical energy-dependent functions such as axoplasmic transport in both cochlear and vestibular nerve fibers as well as optic nerve fibers.

This patient had almost normal VEMP results in the right ear but no response in the left ear. Although the mechanisms underlying these different responses are unclear, asymmetrical hearing impairments have been reported in patients with the *OPA1* p.R445H mutation [12,13]. There was no response to caloric stimulation in either ear. The VEMP consists of myogenic potentials obtained as a response to tone-burst stimuli and is used to test the saccule and inferior vestibular nerve of the vestibular system. The caloric test, on the other hand, is used to evaluate the function of the lateral semicircular canals and the superior vestibular nerve [20]. In the right ear, there was no response in the caloric test but fare VEMPs. *OPA1* is expressed in sensory epithelia in both the saccule and the lateral semicircular canal [6]. Atrophy of the superior vestibular nerve was not detected by MRI scan. The mechanisms underlying different responses for the caloric test and VEMPs in the right ear are uncertain. In the present case, the patient reported slightly better hearing in the ear that also had good VEMP responses (the right ear). It is well established that ADOA is a progressive atrophy disease. If the main mechanism for nerve atrophy in ADOA is the same in both the eye and the inner ear, we speculate that nerve atrophy in the inner ear may develop gradually from the superior vestibular nerve to the inferior vestibular nerve in patients with the *OPA1* mutation. It has been reported that VEMPs are less affected than horizontal semicircular canal function during caloric testing in bilateral vestibulopathy [21]. We found only two reports with results of both caloric testing and VEMP analysis in auditory neuropathy patients with causes other than an *OPA1* mutation [20,22], and these revealed normal caloric responses and abnormal VEMPs in all patients ($n=4$) with auditory neuropathy. We revealed a different profile in a patient with auditory neuropathy due to an *OPA1* mutation. We speculate that the vestibule is also an organ that is sensitive to the mitochondrial dysfunction associated with the *OPA1* mutation.

In conclusion, we have presented a case of vestibular dysfunction accompanied with auditory neuropathy in a patient with an *OPA1*