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Research Report

Mutation of *OPA1* gene causes deafness by affecting function of auditory nerve terminals

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ABSTRACT

Autosomal dominant optic atrophy (DOA) is a retinal neuronal degenerative disease characterized by a progressive bilateral visual loss. We report on two affected members of a family with dominantly inherited neuropathy of both optic and auditory nerves expressed by impaired visual acuity, moderate pure tone hearing loss, and marked loss of speech perception. We investigated cochlear abnormalities accompanying the hearing loss and the effects of cochlear implantation. We sequenced OPA1 gene and recorded cochlear receptor and neural potentials before cochlear implantation. Genetic analysis identified R445H mutation in OPA1 gene. Audiological studies showed preserved cochlear receptor outer hair cell activities (otoacoustic emissions) and absent or abnormally delayed auditory brainstem responses (ABRs). Trans-tympanic electrocochleography (ECochG) showed prolonged low amplitude negative potentials without auditory nerve compound action potentials. The latency of onset of the cochlear potentials was within the normal range found for inner hair cell summating receptor potentials. The duration of the negative potential was reduced to normal during rapid stimulation consistent with adaptation of neural sources generating prolonged cochlear potentials. Both subjects had cochlear implants placed with restoration of hearing thresholds, speech perception, and synchronous activity in auditory brainstem pathways. The results suggest that deafness accompanying this OPA1 mutation is due to altered function of terminal unmyelinated portions of auditory nerve. Electrical stimulation of the cochlea activated proximal myelinated portions of auditory nerve to restore hearing.

1. Introduction

Autosomal dominant optic atrophy (DOA) is characterized by a slowly progressive bilateral visual loss beginning in childhood. There is temporal pallor of the optic disc, central vision loss (centrocaecal scotomas) and impairment of color vision (tritanopia). DOA is one of the most common forms of inherited optic neuropathy with an incidence of 1:12,000 to 1:50,000 (Votruba et al., 2003) and mutation of *OPA1* is one of the most common genetic causes. OPA1 protein is a dynamin-related GTPase, encoded by the nuclear genome, but localized to the inner membrane of the mitochondria, and is ubiquitously expressed (Alexander et al., 2000; Delettre et al., 2000; Misaka et al., 2002). The protein consists of a mitochondrial target signal (MTS), a transmembrane domain (TM), a presenilin-associated rhomboid-like protease site (PARL), and a dynamin/GTPase

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domain. More than 100 mutations have been identified, including missense, nonsense, deletion/insertion and splicing mutations (http://lbbma.univ-angers.fr/lbbma.php?id=9). The majority of the mutations cause truncation of the protein, suggesting that haploinsufficiency is the mechanism causing this condition. OPA1 mutations lead to fragmentation of mitochondria, decreased ATP production and increased oxygen reactive species (Tang et al., 2009; Lodi et al., 2004).

Optic nerve degeneration in *OPA1* mutations is considered to be secondary to their effect on retinal ganglion cells and not to involve rods and cones or bipolar cells (Votruba et al., 2003). Axons of ganglion cells within the retina are unmyelinated, of small diameter, and contain numerous mitochondria that provide energy for transmitting graded neural potentials. The disorder of visual function in OPA1 mutations has been suggested to originate in disordered function of the unmyelinated axons within the retina (Delettre et al., 2000). After the nerve fibers exit the retina through the lamina cribrosa, they become myelinated and nerve conduction is saltatory and energy efficient.

Some OPA1 mutations have additional clinical features including hearing loss, ataxia, and peripheral neuropathy (Amati-Bonneau et al., 2008; Chen et al., 2007; Hudson et al., 2008; Ke et al., 2006). The hearing loss has been characterized as sensorineural and specifically as auditory neuropathy by abnormal auditory nerve and brainstem responses (ABRs) in the presence of preserved otoacoustic emissions (OAEs) (Amati-Bonneau et al., 2005; Ke et al., 2006). OPA1 proteins have been localized in both inner and outer hair cells, auditory nerve terminals, and spiral ganglion cells (Chen et al., 2007) but the site(s) of abnormal function in the cochlea are not yet known. We have identified R445H mutation of the OPA1 gene in two subjects, mother and daughter, previously described as having optic and auditory neuropathies (Santarelli et al., 2008). Fig. 1 contains their pedigree and sequence trace of R445H mutation. We now show that the OPA1 mutation affects synchrony of neural discharges in unmyelinated dendrites of the auditory nerve while receptor potentials of hair cells were normal. Moreover, the myelinated portions of auditory nerve remain capable of responding to electrical stimulation from cochlear implants to restore both hearing and neural synchrony in auditory brainstem pathways.

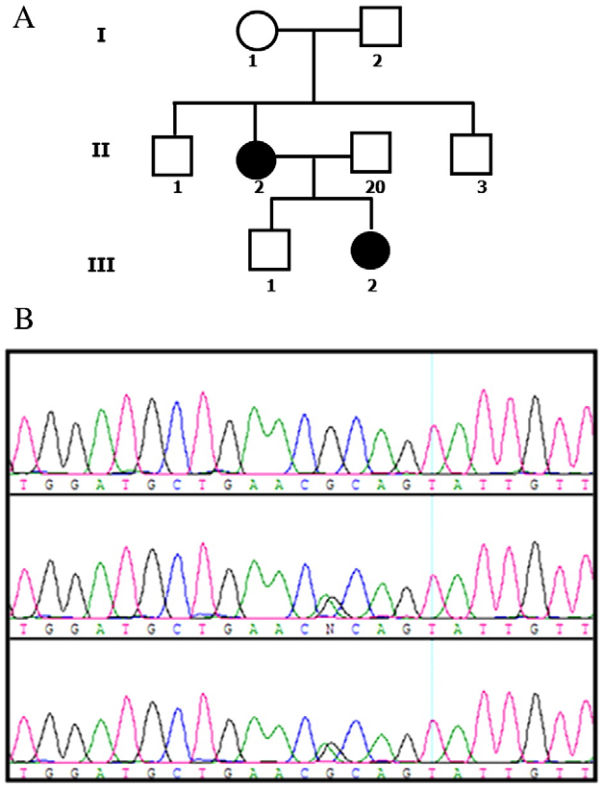

Fig. 1 – (A) Pedigree of OPA1 Family. Squares indicate males, circles indicate females, and filled symbols are the affected family members. (B) Sequence trace of R445H mutation detected in this family.

2. Results

2.1. *Audiological studies*

Pure tone thresholds were moderately elevated affecting predominantly low frequencies in the daughter (subject III-2) and high frequencies in the mother (subject II-2) (Fig. 2, Table 1). The audiogram is a measure of threshold and varies widely in AN (Sininger and Oba, 2001). In contrast, measures of auditory temporal processing such as gap detection threshold and speech perception can be related to the severity of the hearing disorder (Zeng et al., 2005). Speech recognition (vowels, words and sentences) was severely impaired (disyllabic words were not recognized, Table 1) beyond that expected for the degree of hearing loss. Acoustic but not non-acoustic middle ear reflexes were absent in both subjects. Fig. 2 shows the auditory measures. Cochlear outer hair cell receptor potentials (DPOAEs and CMs) were normal bilaterally in both subjects. In contrast auditory brain stem responses (ABRs) to high intensity clicks (125 dB p.e. SPL, 90 dB nHL) were absent unilaterally in both subjects whereas the other ear showed only low amplitude Wave V of delayed latency (7.5 ms II-2, 6.9 ms III-2, normal <6 ms). These results are consistent with abnormal synchrony of auditory nerve in the presence of normal receptor outer hair cell activities.

2.2. *Transtympanic ECochG*

Cochlear potentials recorded from a normal hearing control at 120 dB p.e. SPL and from the left ear (left ear) of both subjects with *OPA1* are in Fig. 3 as a function of stimulus intensity only in the OPA1 mutated subjects. In the control, summating potential (SP) begins at short latency after CM onset and is followed by the compound action potential (CAP) returning to baseline by 2.5 ms. In the *OPA1* subjects potentials were prolonged in duration without clear distinction between SP and CAP. Compared to control values, the cochlear potentials obtained from patients with R445H mutation in OPA1 were abnormally decreased in amplitude and prolonged in duration (Table 2).

A neural adaptation paradigm using rapid stimulus rates was performed to distinguish whether the abnormal cochlear potentials were generated by neural and/or receptor sources.

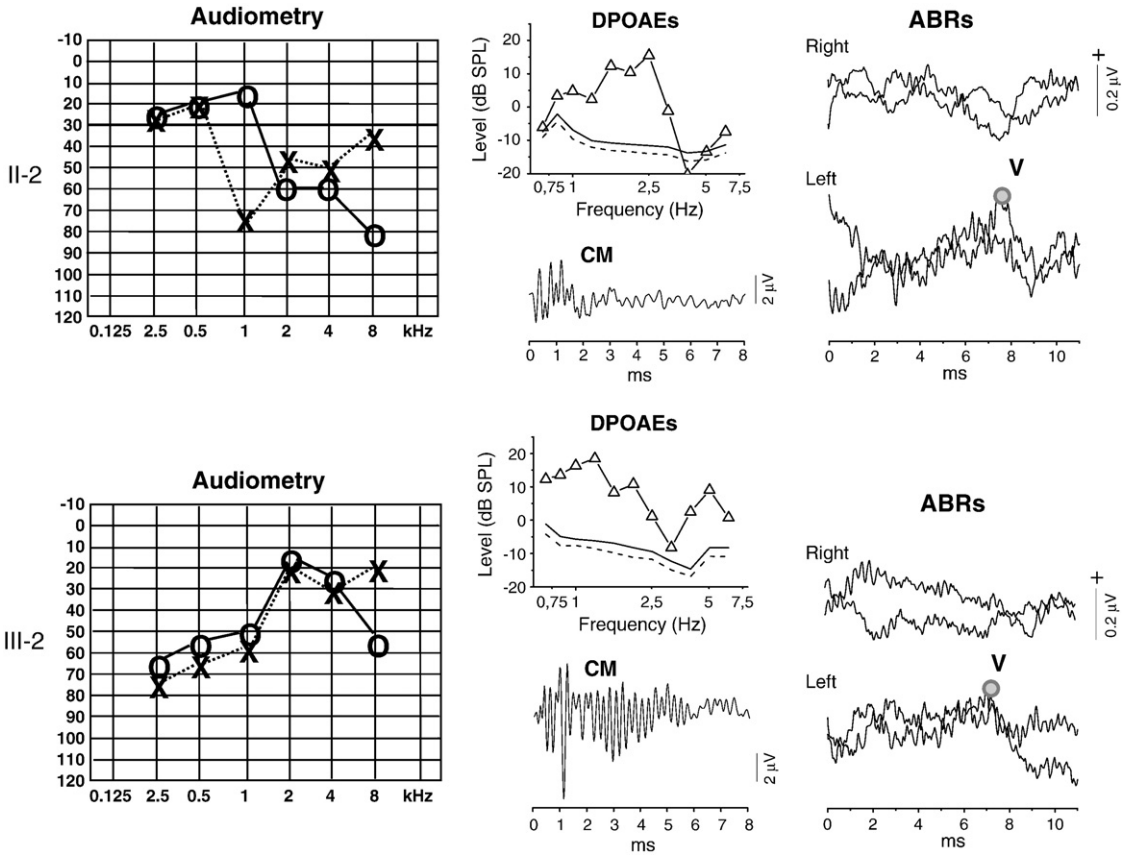

Fig. 2 – Audiometric test results of the OPA1 subjects. The left column contains audiograms (circles: right ear threshold; crosses: left ear threshold); the middle column contains cochlear receptor activities from the left ear as measured with distortion product otoacoustic emissions (DPOAEs) and cochlear microphonics (CMs), the latter recorded during electrocochleography; the right column contains ABRs to clicks at 125 dB p.e. SPL (corresponding to 90 dB nHL). Audiograms showed different patterns of hearing loss in the two subjects; the mother (II-2) had a moderate hearing loss affecting high frequencies symmetrical for both ears. The daughter (III-2) had a moderate low frequency hearing loss symmetrical for both ears. ABRs in both subjects showed no response to stimulation of the right ear and a delayed latency Wave V (filled circle, 7.5 ms II-2, 6.9 ms III-2, normal (<6 ms) to stimulation of the left ear.

The stimulus sequence consisted of an initial click followed after 15 ms by a train of 10 clicks with an inter-stimulus interval of 2.9 ms presented with a frequency of 5 Hz. ECochG potentials recorded from one control and both subjects with R445H mutation in response to the initial click (black line) and to the 11th and last click (gray line) of the stimulus sequence are displayed in Fig. 4 and values for both amplitude and duration are reported in Table 2. Rapid acoustic stimulation induced attenuation of cochlear neural potentials with little (Santarelli et al., 2008) or no change (Eggermont and Odenthal, 1977) in receptor potentials. Adaptation in a group of normal hearing controls was accompanied by a greater attenuation of CAP (68.27±2.31) than SP (27.19±3.64) while there was no change in duration of the cochlear potentials. The ECochG potentials from subjects with *OPA1* mutation decreased in both amplitude and duration during adaptation. The attenuation measured at the latency of the CAP in controls was within the range of the cochlear response attenuation calculated for controls (Table 2). The duration of the negative potential in OPA1 decreased in the adapted state to values seen in controls.

2.3. *Cochlear implantation*

Both patients received cochlear implants (Nucleus C124RE with Freedom processor), the daughter at the age of 22 years and her mother at the age of 51 years. Hearing thresholds were restored within 1 month of cochlear implant connection in both. Speech perception improved in the following months. Identification of vowels and disyllabic words that were close to chance before surgery increased markedly within one year of cochlear implantation (Table 1). Moreover, their inability to identify vowels in the presence of noise (SNR + 10) before cochlear implant surgery improved dramatically within 1 year of cochlear implant use. Speech recognition that was absent before implantation was improved dramatically within 1 year (Table 1).

3. Discussion

We studied a family with an OPA1 mutation affecting both optic and auditory nerves. We found abnormal auditory

Table 1 – Clinical and audiological data.

Subjects	II-2		III-2	
Audiology	AD/AS		AD/AS	
Hearing loss	Mild/Mod		Mild	
Slope	Falling		Rising	
PTA dB HL	40/50		35/40	
OAEs	N/N		N/N	
Stap. reflexes	ABS/ABS		ABS/ABS	
ABR	ABS/ABN		ABS/ABN	
Speech recognization (%)	Before	After	Before	After
Before/After Cochlear Implant				
Identification vowels (chance 25%), Normal 100%	25	95	30	100
Identification of vowels at SNR + 10, Normal 99±2%	0	50	0	95
Identification of disyllabic words (chance 25%) Normal	24	80	40	96
Recognition of disyllabic words, Normal 100%	0	50	0	75
Recognition of sentences Normal 100%	0	80	0	95
Gap threshold (ms), Normal 3.5±0.9 ms	164	NT	77	6
Neurology				
Ankle reflex	N		N	
Optic nerve	Atrophy		Atrophy	
Eye movements	N		N	
Vibration sense	N		N	
Muscle strength	N		N	
Gait	N		N	
Visual acuity	1/30		1/60	
Procedures				
NCV	N		N	
Caloric vestibular test velocity of slow phase nystagmus, Normal >15 degrees/s	6 degrees/s		NT	
Brain MRI	N		N	

AD/AS = right ear/left ear; Mod = moderate; PTA = pure tone average (0.5, 1, 2, 4 kHz); Speech % = percent of speech recognition; NT = not tested; CI = cochlear implant; OAEs = otoacoustic emissions; N = normal; Stap. Reflexes = acoustic reflexes of stapedius muscles; ABS = absent; ABR = auditory brainstem response; ABN = abnormal; Gap threshold = threshold in ms for detecting brief silent periods in noise; SNR = signal-to-noise ratio; NCV = nerve conduction velocity; VOG = video-oculography; MRI = magnetic resonance imaging.

brainstem and cochlear potentials consistent with disordered function of unmyelinated auditory nerve terminals, while receptor potentials were normal. Specifically, ECochG showed (1) hair cell receptor potentials to be normal consistent with normal transducer functions; (2) compound action potential of auditory nerve to be absent consistent with impaired synchronous activation of cochlear auditory nerve fibers; and (3) the appearance of low amplitude abnormally prolonged negative potential that adapts to rapid rates of stimulation consistent with neural generation. We suggest that the generators of this prolonged neural potential are the terminal unmyelinated segment of auditory nerve dendrites that are incompletely depolarized to reach threshold for generating action potentials at the first node of Ranvier, a site rich in Na channels (Hossain et al., 2005). The participation of efferent inhibitory activity of the olivocochlear bundle synapsing on auditory nerve terminals could play a role in attenuating the depolarization of auditory nerve terminal if their spontaneous activity were increased in this disorder. In OPA1 mutations, the visual loss is thought to be due to dysfunction of unmyelinated portions of the optic nerve in the retina while sparing the receptor rods and cones (Carelli et al., 2004). In the present study, electrical stimulation by cochlear implants restored hearing and normal synchronous activity in auditory brainstem pathways most likely by activating myelinated portions of auditory nerve. The quantity of mitochondria in unmyelinated auditory nerve afferents is greater for large than small fibers (Liberman, 1980), raising the possibility that the hearing disorder of OPA1 may be specific for those auditory nerve fibers rich in mitochondria.

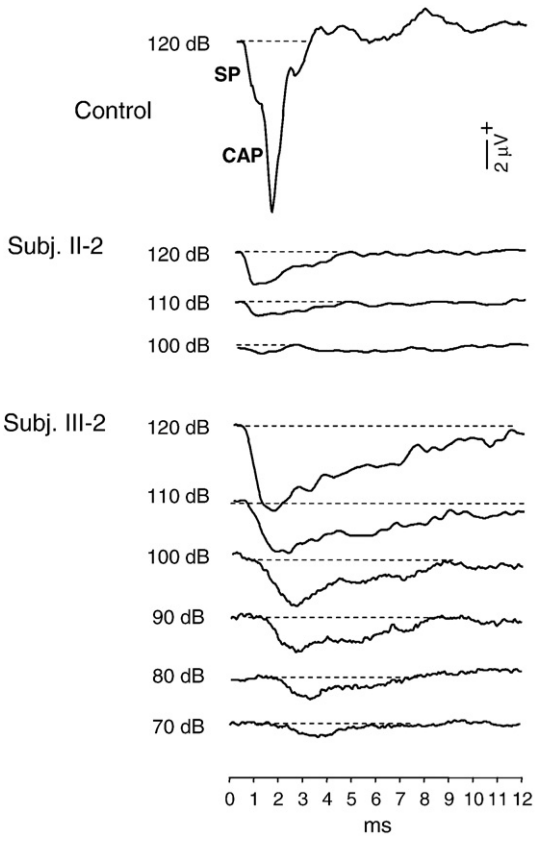

Fig. 3 – Cochlear potentials recorded by a transtympanic electrode from a control (upper trace) and OPA1 subjects to stimulation of the left ear. The potentials from a control in response to a high intensity click stimulus (120 dB p.e. SPL) show an initial negative summating potential (SP) followed by a biphasic compound action potential. The potentials return to baseline by 3 ms. Cochlear potentials from the left ears of OPA1 subjects are shown below in response to decreasing stimulus intensities from 120 dB p.e. SPL. A negative potential arises shortly after the stimulus onset. This is followed by a low-amplitude negative potential gradually returning to baseline by 4.5 ms for II-2 and 12 ms for III-2. A compound action potential (CAP) cannot be identified in OPA1. The horizontal dashed line indicates the baseline while time "0" refers to CM onset.

Table 2 – Cochlear potentials measures.

Subjects	Controls	II-2	III-2
	Mean ± St Err (min–max)	AD/AS	AD/AS
CM Amplitude at 120 dB (μV)	13.94 ± 10.69 (1.56–37.60)	7.60/4.49	10.04/9.83
SP-CAP threshold (dB SPL)	47.00 ± 1.60 (30.00–60.00)	100/100*	50/70*
SP-CAP onset at 120 dB (ms)	0.30 ± 0.03 (0.07–0.70)	0.32/0.30	0.37/0.42
SP-CAP amplitude at 120 dB (μV)	14.00 ± 1.69 (2.83–40.72)	1.83*/2.14*	1.76*/5.89
SP-CAP duration at 120 dB (ms)	2.51 ± 0.11 (2.29–2.73)	5.25*/4.52*	4.58*/12.04*
SP/CAP duration for click 11 at 110 dB (ms)	2.57 ± 0.09 (2.02–2.53)	2.55/2.17	2.78*/2.30
SP/CAP amplitude reduction by click 11 at 110 dB (%)	56.71 ± 2.23 (52.11–61.30)	48.0*/59.3	57.6/50.4

St Err = standard error; AD/AS = right ear/left ear; min–max = minimum–maximum value: values beyond normal range, SP-CAP refers to the whole cochlear response measured from the onset to the return to baseline.
* Measures beyond the range of controls.

Auditory neuropathy patients describe their hearing difficulty as affecting speech comprehension particularly in the presence of background noise. Hearing aids that benefit sensory hearing loss are typically not helpful. Physiological tests show absence or marked abnormality of auditory nerve and brainstem responses (ABRs) disproportionate to the pure tone threshold loss. Outer hair cell receptor functions reflected by measures of otoacoustic emissions or OAEs or cochlear microphonics (CMs) are normal. The site of auditory nerve involvement can vary (Starr et al., 2008) and include presynaptic disorders affecting neurotransmitter release at inner hair cell ribbon synapses (e.g., mutations of *OTOF*) (Santarelli et al., 2009) and postsynaptic disorders affecting both nerve loss and altered nerve conductions as in hereditary neuropathies (e.g., mutations of *MPZ*) (Starr et al., 2003).

The measures we made in *OPA1* subjects showed normal outer hair cell receptor activities, cochlear microphonics and otoacoustic emissions. In contrast, the compound nerve action potential was absent and was replaced by a low amplitude long-lasting (up to 12 ms, normals <3 ms) neural

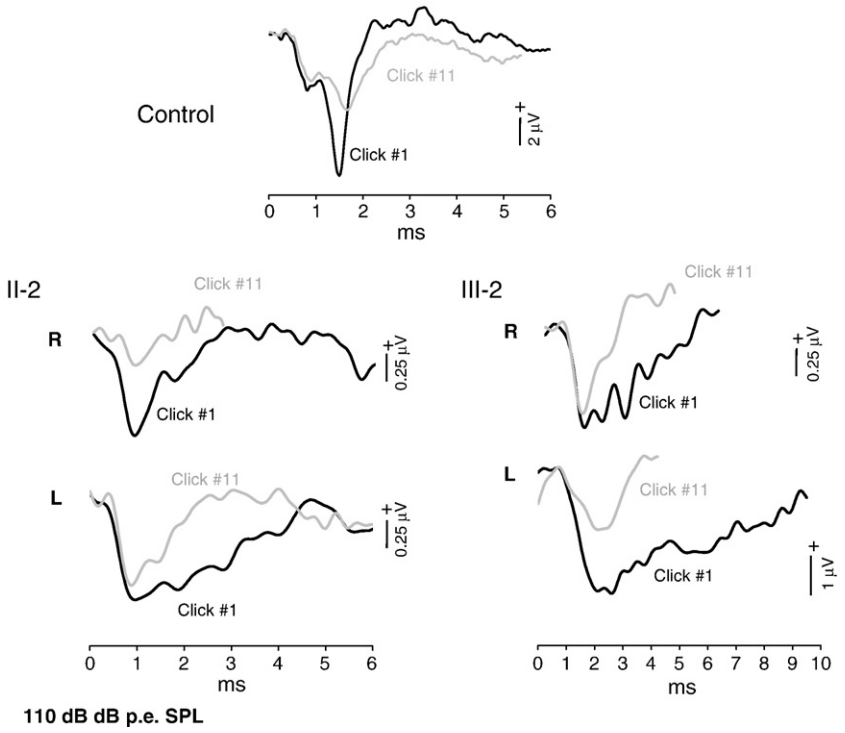

Fig. 4 – Adaptation of ECochG potentials accompanying rapid rates of stimulation. Cochlear potentials from a control and both OPA1 subjects were superimposed for the first (#1) and last (#11) stimulus (110 dB p.e. SPL) of the stimulus sequence, which consisted of an initial click followed after 15 ms by a train of 10 clicks with an inter-stimulus interval of 2.9 ms repeated every 191 ms. Adaptation changes for the control consisted of a delay in the CAP peak latency (0.3 ms) and a decrease of the CAP amplitude (61% attenuation), while the SP amplitude was only slightly attenuated (8%) and the duration of the whole cochlear potential prolonged by less than 0.5 ms. With adaptation, the negative cochlear potentials from both OPA1 subjects were shortened in duration to values close to that found in control and were attenuated in amplitude by an amount comparable for that calculated for the cochlear potentials in controls (see Table 2).

potential. Some synchronous neural activity must have been present in our subjects to account for the appearance of auditory brain stem pathway responses of delayed and reduced amplitude in two ears. The auditory nerve synchrony may have involved small numbers of fibers such that their associated field potentials were either too small to be distinguished from the sustained negativity, or action potentials may have developed proximally in the nerve and were undetected by the cochlear electrode. In support of a proximal site of origin for auditory nerve responses was the finding that the auditory nerve in this OPA1 disorder maintained an ability to respond to electrical stimulation from the cochlear implants resulting in normal ABR potentials and speech perception. Cochlear implants were an effective treatment for hearing impairments in these two subjects with *OPA1* mutation. It may be that proximal optic nerves also maintain abilities to respond to electrical stimuli that could be utilized for visual prostheses to restore vision.

The relationship between the benefits of cochlear implants and the site of hearing impairment varies both with the type of hearing disorder (sensorineural vs. AN) and the specific site of dysfunction. In "sensorineural deafness" the benefits of cochlear implants are unrelated to the numbers of surviving ganglion cells found at post mortem in temporal bone (Fayad et al., 1991; Nadol et al., 2001). In AN, cochlear implants benefit patients with OTOF mutations affecting pre-synaptic release of neurotransmitter from inner hair cell ribbon synapses (Rodriquez-Ballesteros et al., 2003). In contrast, cochlear implants are of minimal benefit (Miyamoto et al., 1999; Brookes et al., 2008) in AN due to genetic disorders causing degeneration of auditory ganglion cells and auditory nerve fibers. In the two subjects with OPA1 mutations presumed to affect the function of terminal dendrites of auditory nerve, the benefits of cochlear implants were excellent.

How OPA1 mutations cause auditory neuropathy is not understood. OPA1 is involved in ATP production, apoptosis, biogenesis of the mitochondria (Misaka et al., 2002; Olichon et al., 2002) and production of reactive oxygen species (Yarosh et al., 2008). Since OPA1 is highly expressed in mitochondria essential for providing the energy for conducting nerve impulses, the definition of disrupted auditory nerve activity is consistent with a post synaptic basis of the auditory nerve impairment. Nine different mutations have been reported to be associated with optic atrophy as well as hearing loss (Amati-Bonneau et al., 2008; Chen et al., 2007; Hudson et al., 2008; Ke et al., 2006), indicating a variety of abnormal proteins can participate in both visual and auditory impairments. The majority of the mutations are missense in the GTPase domain of OPA1, two of them are located in the coiled-coil C-terminal, which may be the GTPase effector domain (Amati-Bonneau et al., 2008). This group of missense mutations may be gain-function mutations, or mutations with dominant negative effects. Indeed, in the yeast system, over expression of OPA1 counterpart Mgm1p and Msp1p in the GTPase domain causes decreased variability of yeast, suggesting a dominant negative effect (Olichon et al., 2006). Recent studies also show that overexpression of OPA1 with missense mutations in the GTPase domain increase fragmentation of mitochondria and apoptosis in fibroblast cells and Hela cells (Olichon et al., 2007). This also suggests the dominant negative effect of missense mutations in the GTPase domain. Since hearing loss may not be R445H-specific, it is warranted to test clinical and subclinical sign of the hearing loss for the individuals with all patients with optic atrophy and OPA1 mutations.

Since nerve fibers of the cochlear and the retina are both highly energy-dependent, their function would become susceptibility to ATP deficiency or the negative consequences of their metabolism such as the production of reactive oxygen species (Carelli et al., 2004; Zanna et al., 2008). The time course of the clinical expression of optic and auditory nerve dysfunction can be delayed for many years. In our patients, hearing and visual loss only became apparent late in childhood or as young adults, suggesting that time is required to cause sufficient damage of the reactive oxygen species to produce symptoms (Yarosh et al., 2008). Recent studies of Opa1 mutations in Drosophila suggest that antioxidants can delay the expression of the retinal disorder. For instance, in heterozygous dOpa1 mutation, an age-dependent abnormality of retinal neural function is significantly delayed by treatment with antioxidants (Shahrestani et al., 2009), raising the possibility that such treatments may be of benefit as well in humans.

4. Experimental procedures

4.1. *Patients and methods*

Written informed consent for genetic analysis was obtained from patients in accordance with University of California, Irvine, Institutional Review Board (2005-4253) and for ECochG by the regional committee of Veneto for quality control of clinical and therapeutic procedures, CCHSA, Veneto Region (2007–2010). Diagnosis of optic atrophy was based on the family history, retinal examination, and clinical symptoms of vision loss. Neurological clinical examination for cognition, cranial nerve, motor, reflexes and peripheral sensory functions were performed. The pedigree is shown in Fig. 1A. Clinical information (Table 1) includes audiological studies, speech recognition, clinical neurology and neurological tests. Cochlear receptor potentials (summating potentials, SP), cochlear microphonics (CM) and auditory nerve compound action potentials (CAP) were recorded by transtympanic electrocochleography (ECochG) with an electrode placed on the promontory to define receptor and auditory nerve activity not apparent using far field ABR recoding methods (Doyle et al., 1998; Santarelli et al., 2008).

4.2. *Mutation analysis*

R445H mutation was identified in II-2 and III-2 in this family (Fig. 1B). DNA was purified from blood. OPA1 exons were PCR-amplified with exon-specific primers, and PCR products were purified with QIAquick columns following the manufacturer's manuals (Qiagen, CA). Each exon was sequenced in an Applied Biosystems 3100 automated sequencer in both directions using forward and reverse primers. Candidate sequence variants were identified by sequence aberration in forward and reverse traces.

4.3. *Clinical description*

Both mother and daughter experienced visual symptoms as children and bilateral optic atrophy was defined when they were 9 years of age. Speech comprehension problems were first experienced about the same age by the daughter and in the early 20s in the mother. Amplification by hearing aids was without benefit and speech comprehension depended on visual cues from lip reading. We examined them both in 2007 and found bilateral optic atrophy and reduced visual acuity. The neurological examination was otherwise normal (Table 1). Computerized tomography (CT) and magnetic resonance imaging (MRI, gadolinium infusion) scans of head and temporal bones (including internal acoustic canal) were normal. Conduction velocities of peripheral sensory and motor nerve functions were normal. Caloric tests of vestibular function in the mother revealed abnormally decreased slow phase of nystagmus for each ear (5, 7 /s, normal >15 /s) consistent with decreased peripheral vestibular sensitivity bilaterally. Pattern visual evoked potentials (PVEPs) in the daughter were normal in both latency (92.4 and 95.7 ms; normal values <100 ms) and amplitude 2.4 μV (right) and 3.0 μV (left).

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