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Title

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Permalink

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Journal

Hearing Balance and Communication, 9(2)

ISSN

2169-5717

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Publication Date

2011-06-01

DOI

10.3109/1651386x.2011.558764

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Peer reviewed

ORIGINAL ARTICLE

Presynaptic and postsynaptic mechanisms underlying auditory neuropathy in patients with mutations in the *OTOF* or *OPA1* gene

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Abstract

Objective: Our objective was to compare acoustically- and electrically-evoked potentials of the auditory nerve in patients with postsynaptic or presynaptic auditory neuropathy with underlying mutations in the *OPA1* or *OTOF* gene. **Study design:** Trans-stypanic electrocochleography (ECoChG) was recorded from two adult patients carrying the R445H *OPA1* mutation, and from five children with mutations in the *OTOF* gene. Cochlear potentials to clicks or tone-bursts were compared to recordings obtained from 16 normally hearing subjects. Electrically-evoked neural responses recorded through the cochlear implant were also obtained. **Results:** The cochlear microphonic (CM) was recorded from all subjects, with normal amplitudes. After cancelling the CM, cochlear potentials were of negative polarity with reduced amplitude and prolonged duration compared to controls in both groups of patients. Prolonged negative responses were recorded as low as 50–90dB below behavioural threshold in subjects with *OTOF* mutations whereas in the *OPA1* disorder the prolonged potentials were correlated with hearing threshold. A compound action potential (CAP) was superimposed on the prolonged activity at high stimulation intensity in two children with mutations in the *OTOF* gene while CAPs were absent in the *OPA1* disorder. Electrically-evoked compound action potentials (e-CAPs) were only recorded from subjects with *OTOF* mutations following cochlear implantation. **Conclusions:** The findings are consistent with abnormal function of distal portions of auditory nerve fibres in patients carrying the *OPA1* mutation whereas the low-threshold prolonged potentials recorded from children with mutations in the *OTOF* gene are consistent with abnormal neurotransmitter release resulting in reduced dendritic activation and impairment of spike initiation.

Key words: *electrocochleography, auditory brainstem responses, cochlear implant, electrically-evoked neural response*

Introduction

Auditory neuropathy (AN) is a disorder characterized by disruption of auditory nerve activity with preservation of outer hair cell (OHC) function (otoacoustic emissions, OAE, and/or cochlear microphonic, CM) (1). The disruption of auditory nerve discharge underlies both the absence of or profound alterations in auditory brainstem responses (ABRs) and severe impairment of speech perception. The impairment of auditory nerve function results from demyelination and axonal loss (post-synaptic AN) or lesions involving inner hair cells (IHCs) and/or the synapses with auditory nerve fibres (pre-synaptic AN) (1). The disorder has a wide range of aetiologies

(e.g. genetic, infectious, toxic-metabolic, immunological); however, in half the patients no aetiological factors can be identified (1,2). It occurs in all age groups (1) and may be present in isolation or associated with multisystem involvement (1,3,4).

Among non-isolated AN disorders, mutations in the *OPA1* gene are believed to cause disruption of auditory nerve discharge by affecting the unmyelinated portions of auditory nerve fibres (5). The *OPA1* gene encodes a mitochondrial GTPase involved in the regulation of oxidative phosphorylation (6), mitochondrial fusion (7), and apoptosis (8). Patients carrying *OPA1* mutations present with slowly progressive loss of visual acuity, with underlying atrophy of optic nerve fibres; two-thirds also show hearing

impairment possibly associated with the clinical picture of AN (5,9). Studies performed on an *OPA1*-deficient mouse model have shown that early lesions involve the terminal unmyelinated portions of retinal ganglion cells (10). Moreover, the restoration of speech perception after cochlear implantation in two patients with the R445H mutation has led to the hypothesis that the lesion must be localized to the distal portion of auditory nerve fibers (postsynaptic AN) (5).

Mutations of the *OTOF* gene result in a very homogeneous phenotype of prelingual, profound hearing loss associated with the absence or marked threshold elevation of ABRs and the presence of OAEs, which have been found in over 50% of subjects (11). The *OTOF* gene encodes otoferlin, a transmembrane protein belonging to the ferlin protein family, which contains several repeating C2 domains involved in calcium binding (12). Studies performed in mice indicate that otoferlin plays a crucial role in vesicle release at the synapse between IHCs and auditory nerve fibres by interacting with syntaxin1 and SNAP25 (13). In addition, otoferlin involvement in vesicle replenishment at the presynaptic membrane has been suggested (14). On the basis of these findings it can be hypothesized that a presynaptic disorder underlies AN in patients carrying *OTOF* mutations.

Abnormalities of auditory nerve fibre discharge underlain by presynaptic or postsynaptic AN cannot be evaluated by far-field recording techniques such as of ABR due to the low signal-to-noise ratio (15). Both receptor (cochlear microphonic, CM; summing potential, SP) and auditory nerve (compound action potential, CAP) activities can be recorded using a near-field recording technique such as transtympanic electrocochleography (ECoChG) (15). Our previous studies have shown that ECoChG recordings to click stimulation obtained from patients with *OPA1* (5) or *OTOF* (16) mutations consist of low-amplitude prolonged responses originating from abnormal activation of auditory dendrites.

In this study we compared the cochlear potentials to click or tone-burst stimulations in patients with postsynaptic or presynaptic AN, with underlying mutations of the *OPA1* or *OTOF* genes, respectively. In addition, electrically-evoked neural response recordings obtained through the cochlear implant were obtained.

Material and methods

Subjects

Two subjects carrying the R445H mutation in the *OPA1* gene, a mother (M) (47 years of age) and

daughter (D) (21 years of age), were affected by reduction in visual acuity associated with optic atrophy since the age of nine years. Speech comprehension difficulties were first experienced at about the same age by the daughter, but by the mother in her early twenties. At the time of evaluation, speech perception was severely impaired and relied on visual cues. The details of clinical, neurological and audiological evaluation are reported in a previous paper (5). Both patients underwent cochlear implantation (right side in the daughter, left side in the mother) resulting in restoration of speech perception (5).

Five subjects carrying the *OTOF* mutations were children (three male and two female, ranging in age from 12 to 20 months), and showed congenital profound hearing loss with absent ABRs and presence of OAEs. Three of the children, two brothers and a sister, were compound heterozygotes for mutations c.2732_2735dupAGCT and p.Ala964Glu; one subject was homozygous for mutation p.Phe1795Cys, and one was compound heterozygote for mutations c.1609delG in exon 16 and c.1966delC in exon 18 (16). Four of the children received a cochlear implant and showed almost normal language development within one year of cochlear implant use.

Electrocochleography (ECoChG)

ECoChG recordings were performed under general anaesthesia in children and local anaesthesia in adults. Recordings were obtained by means of a needle electrode placed on the promontory wall with the aid of an operating microscope.

Stimuli consisted of 0.1-ms rarefaction and condensation clicks, separately delivered in the sound field with a maximum intensity of 120dB p.e. SPL (corresponding to 90dB nHL, with reference to the psychoacoustical threshold of normally-hearing subjects). The stimulus was calibrated in the sound field by means of a Brüel and Kjaer 4165 microphone (mounted on an 800 B Larson-Davis sound level meter) placed at 1 m from the base of the polyurethane horn, corresponding to the distance of the patient's ear from the horn. The procedure of comparing the peak-to-peak click amplitude to the peak-to-peak amplitude of a 2-kHz tone was utilized to calibrate the click level (p.e. SPL). The stimulus paradigm consisted of an initial click, followed 15 ms later by 10 clicks with an inter-stimulus interval of 2.9 ms. This sequence was repeated every 191 ms. This particular stimulus paradigm was used to help distinguish CAP and SP potentials by taking advantage of different effects of adaptation induced by high stimulation rates on these responses (15,17).

Tone-bursts (1 ms rise-decay time, 10 ms plateau, 151 ms inter-stimulus interval) with a maximum

intensity of 100dB SPL were used in three patients, two carrying the *OPA1* mutation and one child with *OTOF* mutations.

The potentials were differentially amplified (50,000 times), filtered (5–8000 Hz) and digitized (25 μ s) for averaging (500 trials). The procedure of averaging the responses evoked separately by condensation and rarefaction clicks was applied to cancel the CM and extract the CAP with the superimposed SP. The resulting curve was subtracted from the potential evoked by condensation clicks to obtain the CM (15).

Cochlear potentials recorded from all patients were compared to the ECochG data previously collected from 16 normal hearing children (mean 3.7 years, range 3.5–6.5 years). They underwent ECochG testing for presumed cochlear deafness but proved to have normal cochlear function with normal thresholds when evoking neural and receptor potentials.

Latency was defined relative to CM onset in milliseconds (ms). Amplitude was computed relative to the period 1 ms before CM onset in microvolts (μ V). Values contained in the text indicate mean \pm standard error.

Since in the majority of patients the SP was followed by a slow neural potential without an identifiable CAP, we indicated the waveform obtained after CM cancellation as the SP-CAP complex, and defined the SP-CAP onset at the initial negative deflection arising from baseline and the SP-CAP end at the return to baseline (15). Analogously, both amplitude and duration changes during adaptation were evaluated on the SP-CAP as a whole and were compared to the corresponding values obtained from controls. Since the SP and CAP could not be identified separately, the amplitude attenuation during adaptation was calculated at the latency of the CAP in controls.

Electrically-evoked compound action potential (e-CAP) and auditory brainstem (e-ABR) recordings

The electrically-evoked compound action potential (e-CAP) was obtained by utilizing the Cochlear Corporation Custom Sound EP software. Stimulation consisted of a train of alternate polarity, biphasic, 25- μ s width per phase pulses presented at 80 Hz. Stimulation mode was MP1 + 2. The evoked electrical activity was recorded two electrodes apart.

e-ABRs were only tested on one patient with the *OPA1* mutation (D) by using biphasic pulses, 50 ms width per phase, which were presented at 35 Hz. Stimulation mode was MP1. Potentials were recorded from scalp electrodes (vertex to mastoid contralateral to the stimulated ear). Signals were differentially amplified (50,000 times), filtered (10–4000 Hz) and digitized (25 μ s) for averaging (1000 trials).

Results

Both adult patients carrying the *OPA1* mutation had a moderate hearing loss (pure tone average PTA threshold levels at 0.5, 1, 2 and 4 kHz were in the 41–70dB HL range according to the European Concerted Action Project on Genetics of Hearing Impairment, 1996).

ABRs were absent in the right ear and showed a delayed wave V in the left ear (wave V latency M-Left (L), 7.5 ms; D-L, 6.9 ms). OAEs dirrection products were obtained from both ears while ECochG recordings showed normal amplitudes of the CM (mean control amplitude at 120dB p.e. SPL $13.40 \pm 1.92 \mu$ V, mean values from *OPA1* patients $10.55 \pm 1.30 \mu$ V).

Cochlear potentials obtained at 120dB p.e. SPL after CM cancellation from the left ear of both *OPA1* patients are shown in Figure 1, together with the grand average of corresponding control recordings with 95% confidence limits (shadowed area). In controls, cochlear potentials show an initial fast SP deflection followed by the CAP which returns to baseline by 2.4 ms. The waveforms obtained in the *OPA1* disorder consisted of the SP appearing at the same latency as in controls, which was followed by a sustained negative potential returning to baseline by 12 ms. This response was identified at intensities close to the audiometric threshold (Figure 3).

In order to assess whether the prolonged responses were neural or receptor potentials, we used a neural adaptation paradigm aimed at distinguishing between neural and receptor potentials by taking advantage of the different effects of adaptation induced by high stimulation rates (15,17). In controls, the CAP amplitude as measured at 110dB from the first to the eleventh click, was attenuated by 70% ($68.3 \pm 2.3\%$) during adaptation whereas SP attenuation was much lower, averaging 27% ($27.2 \pm 3.6\%$) (compare the response to the first and eleventh click in the waveforms from controls reported in the left and middle upper panels in Figure 1). No change in response duration was found in controls (mean change from the first to the eleventh click at 110dB p.e. SPL -0.08 ± 0.10). In contrast, the potentials recorded from patients with the mutation in the *OPA1* gene were reduced in both amplitude (amount of attenuation at 110dB p.e. SPL for the right ear M, 8%; D 57%; amount of attenuation at 110dB p.e. SPL for the left ear M 59%; D 50%) and duration (change in duration at 110dB p.e. SPL for the right ear M 0.1 ms; D 2.3 ms; change in duration at 110dB p.e. SPL for the left ear M 1.9 ms; D 13 ms) after adaptation. The amount of response attenuation was within the range of the mean attenuation calculated for the whole SP-CAP complex in controls ($56.7 \pm 2.2\%$). However, recordings obtained from one ear (M-R) failed to show changes in either amplitude or duration after adaptation.

SP- CAP potentials from OPA1 patients

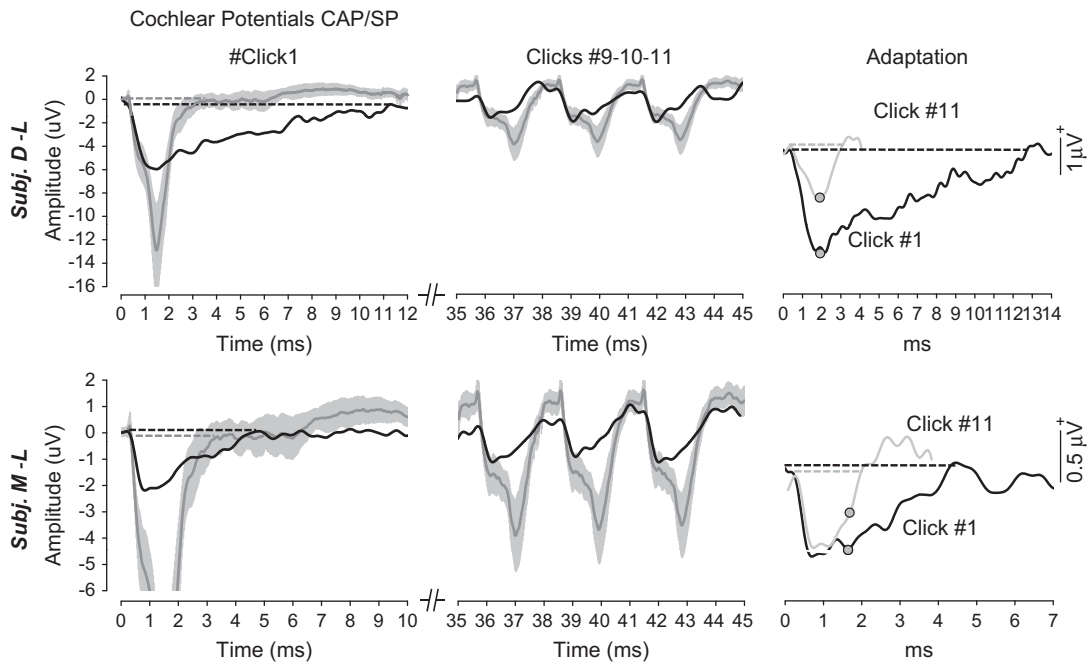


Figure 1. Cochlear potentials recorded from patients carrying R445H mutation in the *OPA1* gene in response to a high intensity click stimulus (120dB SPL). Recordings obtained from the left ear of mother (M-L) and daughter (D-L) are superimposed on the mean potentials obtained by averaging all control responses to the first (non-adapted responses, left graphs) and the last three (adapted responses, middle graphs) clicks of the stimulus sequence. The shadowed area refers to 95% confidence limits in controls. The ECochG responses recorded from the *OPA1* subjects show no CAPs and consist of the SP followed by a low-amplitude negative potential with increased duration compared to controls. On the right side the responses evoked by the first (black line) and eleventh (grey line) click of the stimulus sequence at 110dB SPL are superimposed. Changes in amplitude were measured at the latency of the CAP in controls (circles). The SP-CAP complex was decreased in both amplitude and duration after adaptation. The amount of response attenuation was comparable to that found in controls. The horizontal dashed lines extend from the SP-CAP onset to return to baseline; time '0' refers to CM onset in this and in the subsequent figures.

Cochlear potentials to tone-burst stimuli at frequencies ranging from 1 to 8 kHz were recorded from only one patient (mother, right ear). The waveforms obtained at 100dB p.e. SPL are superimposed on the corresponding traces recorded from one control in Figure 4 (left panel). CAPs were clearly identified in the control whereas in the patient with the *OPA1* mutation the recorded traces mainly consisted of a DC-coupled component, e.g. the SP.

CM potentials recorded from patients with *OTOF* mutations were of normal amplitude (mean control amplitude at 120dB p.e. SPL $13.40 \pm 1.92 \mu\text{V}$, mean values from *OTOF* patients $14.10 \pm 3.84 \mu\text{V}$).

ECochG waveforms obtained after CM cancellation followed three main patterns (Figure 2, upper panel). In the first pattern the SP (subjects 4, 5 right ear) was followed by a small CAP, and both responses were superimposed on a low-amplitude prolonged negative potential returning to baseline by 10–12 ms. The second pattern (subjects 2, 5 left ear) showed a normal SP that was followed by the sustained low-amplitude activity without a superimposed CAP.

Only a prolonged negative response peaking approximately at the same latency as the CAP in controls was identified in the third pattern (subject 3). Moreover, whatever the response pattern, the prolonged responses were identified as low as 60dB SPL, which is 90dB below behavioural threshold (Figure 3).

When comparing the response to the eleventh click with that of the first click of the sequence (Figure 2, lower panel), both amplitude (mean attenuation in amplitude $57.17 \pm 2.68\%$) and duration (mean change in duration $5.69 \pm 1.10 \text{ ms}$) of the adapted responses appeared reduced in all but one subject (subject 1). The amount of response attenuation was within the range of the mean attenuation calculated for the whole SP-CAP complex in controls ($56.7 \pm 2.2\%$).

Cochlear potentials evoked by tone-burst stimulation were recorded from both ears of one patient with *OTOF* mutations (subject 3). Waveforms obtained from the right ear at frequencies from 1 to 8 kHz are superimposed in Figure 4 (right panel) on the corresponding traces recorded from one control, the latter

SP-CAP potentials from OTOF patients

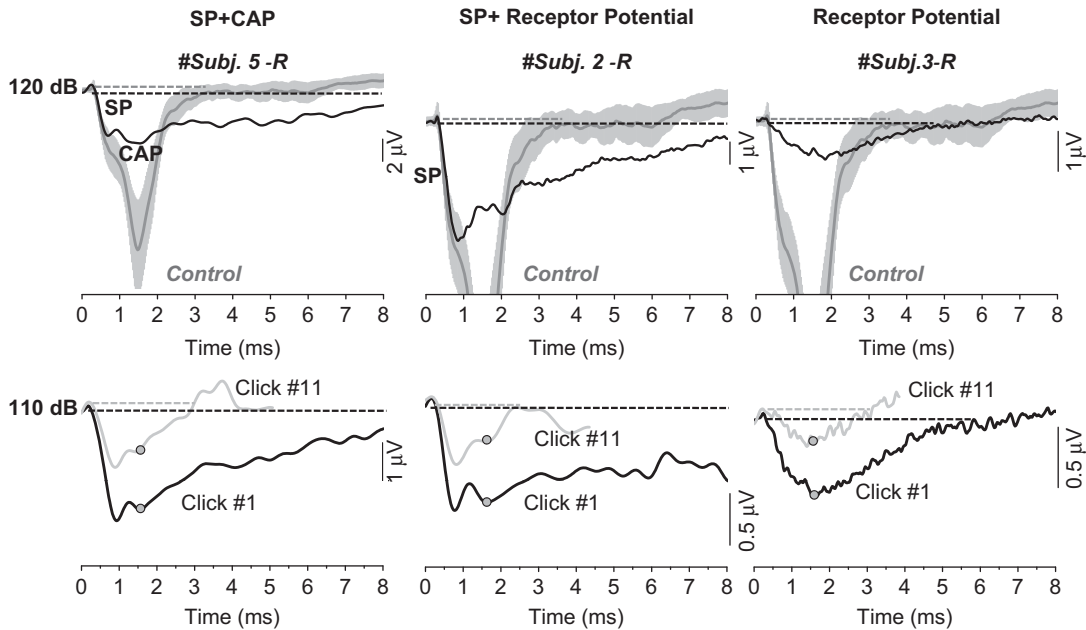


Figure 2. Cochlear potentials from patients with mutations in the *OTOF* gene. In the upper panel the responses recorded from three patients (black line) at 120dB p.e. SPL are superimposed on the mean potentials obtained by averaging all control responses (grey line). Three patterns of ECoChG waveforms can be identified. In the lower panel the responses evoked by the first (black line) and eleventh (grey line) click of the stimulus sequence at 110dB SPL have been superimposed. Changes in amplitude were measured at the latency of the CAP in controls (circles). Both amplitude and duration of the SP-CAP responses were markedly decreased after adaptation.

displayed on a different time scale. The potentials from the *OTOF* patient were of low amplitude, peaked about 1 ms later than the CAP in control, and were identified as low as 70–80dB p.e. SPL (motgh over).

Electrically-evoked compound action potentials (e-CAPs) were tested in all the implanted subjects through their cochlear implant (Figure 5). One example is reported in Figure 5 for *OPA1* (D-R) and *OTOF*

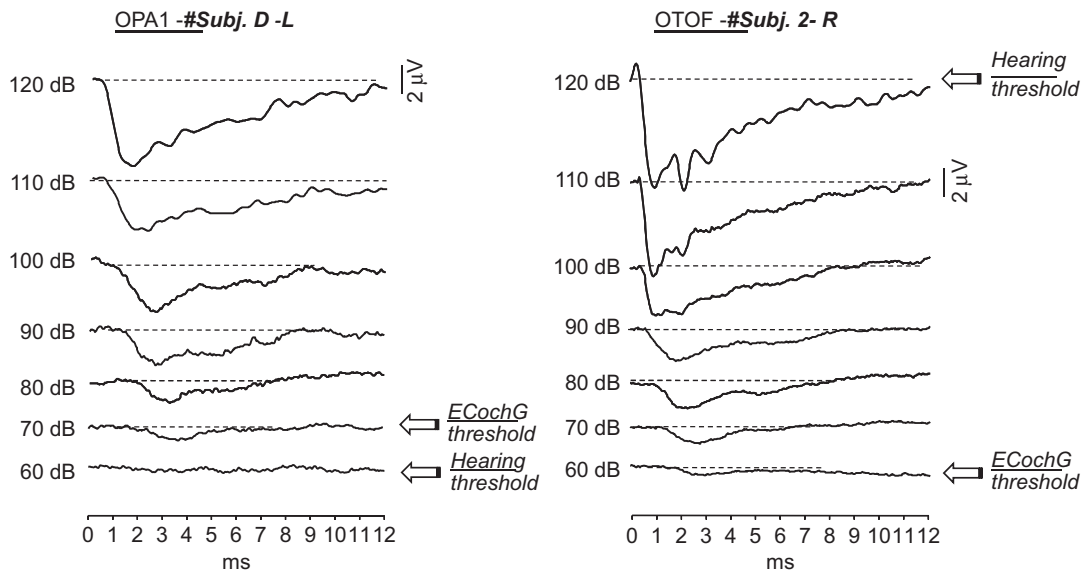


Figure 3. Cochlear potentials from patients with mutations in *OPA1* or *OTOF* gene at decreasing stimulation intensities. In both subjects the SP was followed by a slow negative potential returning to baseline by 12 ms. However, the threshold of this prolonged response was close to the hearing threshold in the patient with *OPA1* disorder whereas in subjects with *OTOF* mutations the sustained potential was identified 60dB below the audiometric threshold.

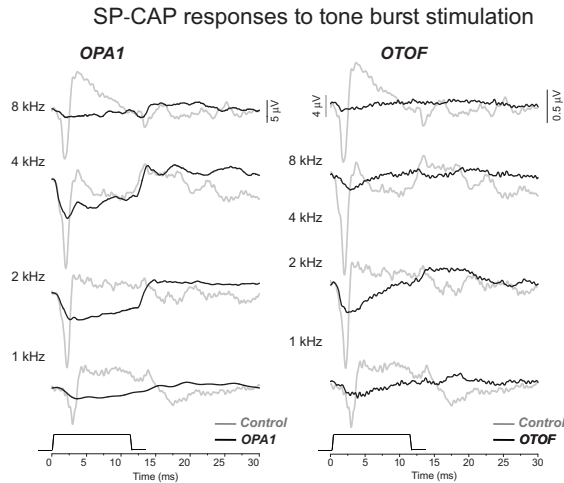


Figure 4. Cochlear responses evoked by tone-burst stimulation in *OPA1* and *OTOF* subjects at 100dB p.e. SPL. Waveforms from the *OPA1* subject (M-R) mainly consist of the SP while the responses recorded from the patient with *OTOF* mutations (subject 3, right) show low-amplitude negative potentials which peak 1 ms later than the CAP in control and slowly return to baseline.

disorders (subject 2, right ear). No electrically-evoked neural responses were recorded from the two subjects with the *OPA1* disorder whereas CAPs were obtained from all electrodes in subjects with mutations in the *OTOF* gene.

e-ABRs were tested in one patient with the *OPA1* mutation (D, electrode 11). Wave V was clearly identifiable in recorded waveforms (Figure 6).

Discussion

We recorded acoustic- and electrically-evoked cochlear potentials from patients with auditory neuropathy with underlying mutations in the *OPA1* or *OTOF* gene.

Detection of OAEs and CMs with normal amplitudes in all patients points to a preserved function of OHCs in both disorders. The ECoChG waveforms obtained after CM cancellation from both groups of patients appeared as negative deflections markedly prolonged in duration and reduced in amplitude compared to controls. The prolonged negative activity was sensitive to a neural adaptation paradigm consistent with a neural origin of the prolonged responses (15). Nevertheless, we found several differences between the two disorders, which possibly indicates that different mechanisms and sites of lesion underlie abnormal activation of auditory nerve fibres. In previous studies we hypothesized that the prolonged negative potentials recorded from patients with *OPA1* (5) or *OTOF* (16) mutations resulted from abnormal activation of auditory nerve terminals. Specifically, in patients with *OPA1* mutations disturbances of spike initiation and slowing of conduction velocity were believed to result from a lesion involving the auditory nerve fibres themselves (5). This hypothesis is consistent with studies on animal models showing that the early lesion in *OPA1* involves the terminal unmyelinated portions of optic nerve fibres (10). Moreover, the restoration of speech

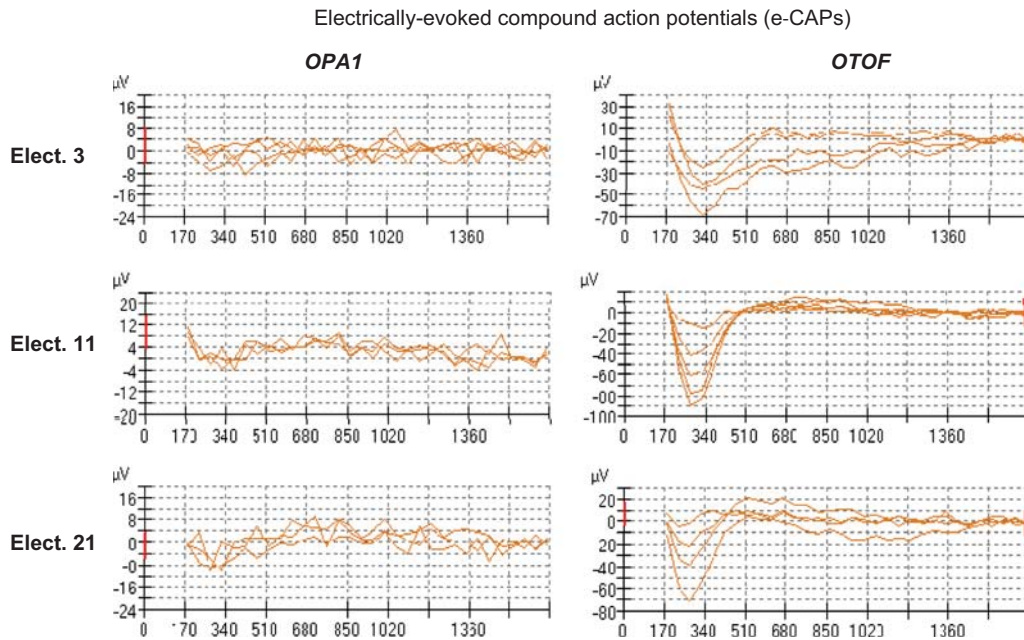


Figure 5. Electrically-evoked compound action potentials (e-CAPs) from two implanted patients (Nucleus C124RE with Freedom processor) with *OPA1* (D-R) or *OTOF* (subject 2, right ear) mutations. Individual waveforms recorded at increasing stimulation intensities (from 180 to 220 CL) by stimulating three different electrodes are displayed (basal 3, intermediate 10, apical 21). No responses were recorded from the *OPA1* subject whereas e-CAPs with typical morphology were obtained from the child carrying *OTOF* mutations.

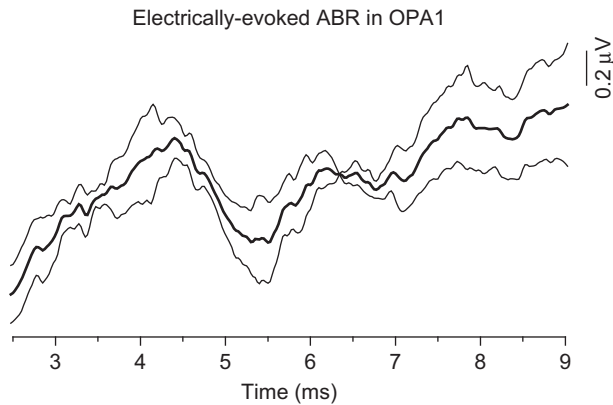


Figure 6. Electrically-evoked ABR from one patient with the *OPA1* mutation (D-R, electrode 11). The thick line results from averaging the two recorded waveforms.

perception after cochlear implantation suggests that the lesion should be localized to the auditory nerve dendrites since it could be by-passed by electrical stimulation through the cochlear implant (5). Further support to the hypothesis that the lesion should be confined to the distal portion of auditory nerve fibres is given by the findings reported in this study showing that no CAPs were recorded in response to electrical stimulation while ABR was restored after cochlear implantation.

The cochlear responses obtained from the right ear of one patient with *OPA1* mutation did not change either in amplitude or duration during adaptation. This result is consistent with the prolonged responses originating from receptor rather than neural sources (15). Accordingly, cochlear potentials to tone-burst stimulation recorded from the same ear consisted mainly of a DC-coupled response showing the same amplitude as the SPs in controls.

Mutations in the *OTOF* gene are likely to result in a presynaptic disorder since the product of this gene, otoferlin, is known to be involved in neurotransmitter release (13) and vesicle replenishment at the presynaptic site in IHCs (14). Analogous to patients with the *OPA1* mutation, prolonged negative responses were recorded from children with mutations in the *OTOF* gene.

However, Differently from patients with the *OPA1* mutation, the prolonged negative responses were recorded from children with mutations in the *OTOF* gene at intensities that were well below the hearing threshold (Figure 3). These potentials seem analogous to the dendritic responses recorded by Sellick et al. (18) from the scala tympani of guinea pigs after administration of tetrodotoxin to block neural spiking in terminal dendrites of auditory nerve fibres. Moreover, a CAP was superimposed on the prolonged negative activity at high stimulation intensity in two patients. On the basis of these findings it can

be hypothesized that the prolonged responses recorded at intensities below the hearing threshold may reflect local dendritic activation. This may be followed by spike initiation for some ears at high stimulation intensity leading to CAP recording whereas at low intensity, dendritic activation fails to evoke a synchronized neural activation. Abnormal dendritic activation, in turn, may result from reduction and alteration in timing of the neurotransmitter release. Therefore, unlike those patients with the *OPA1* mutation, auditory nerve terminals should retain their function in children with mutations in the *OTOF* gene. This hypothesis is further supported by the detection of electrically-evoked neural responses (e-CAPs), which were recorded through the cochlear implant from all patients with *OTOF* mutations.

Cochlear potentials evoked by tone-burst stimulation from the child with *OTOF* mutations showed the prolonged negative potential without a superimposed SP or CAP component. These prolonged potentials were of low amplitude, peaked 1 ms later than the CAP in controls, and were identified at intensities as low as 50–90dB below behavioural threshold. These responses may also result from the sum of local dendritic potentials, which fail to trigger spike initiation or synchronized activation of auditory fibres.

We conclude that in subjects with the *OPA1* mutation, lesions localized to the distal portion of auditory nerve fibres underlie AN, whereas abnormal synaptic release results in abnormal dendritic activation and disruption of auditory nerve discharge in children with mutations in the *OTOF* gene.

Acknowledgements

This work was supported in part by the University of Padua, and Treniso Hospital Spanish Ministerio de Educación y Ciencia.

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

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