Early subclinical cochlear dysfunction in myotonic dystrophy type 1

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Background: Myotonic dystrophy type 1 (DM1) is an autosomal-dominant inherited disorder clinically characterized by variable systemic manifestations. Among clinical features of the disease, 'precocious presbyacusis' has been previously reported. The underlying mechanism of this auditory impairment remains still poorly understood. Hearing is an active process located in the cochlea, where the outer hair cells (OHCs) play an important role in sound perception through a 'contractile' like movement resembling skeletal muscle fibers dynamics. OHCs status has not yet been investigated in DM1 patients. OHCs integrity can be assessed by measuring transient-evoked otoacoustic emissions (TEOAE), a non-invasive, repeatable, and objective quantitative tool.

Methods: We recruited 25 patients with a genetically confirmed diagnosis of DM1, and 28 age-matched control subjects. All of them underwent a routine audiological evaluation and TEOAE recordings.

Results: We detected a high prevalence of sensorineural high-frequency hearing loss (HFHL) in DM1 patients, significantly different if compared to control subjects. Interestingly, the accurate analysis of DM1 recorded data showed a marked impairment of TEOAE both in HFHL+ and unexpectedly in HFHL− group. Cochlear dysfunction was restricted to frequencies above 2000 Hz in the HFHL− group, but it extended to 1000 Hz in HFHL+ DM1 patients.

Conclusions: Our study indicates that cochlear impairment in DM1 is present, even in patients without evidence of hearing loss at a standard audiometric analysis. Hence, in the current clinical practice, an assessment of cochlear function by TEOAE recording may be useful in DM1 patients to identify precocious signs of cochlear dysfunction.

Introduction

Myotonic dystrophy type 1 (DM1) is an autosomal-dominant inherited disorder caused by an expanded [CTG]*n* repeat in the 3′ untranslated region of the gene encoding myotonic dystrophy protein kinase (DMPK) on chromosome 19q13. Clinically, it is characterized by variable systemic features, such as myotonia, distal weakness, precocious cataract, cardiac conduction abnormalities, and endocrine disorders. Besides common clinical features, a high prevalence of sensorineural high-frequency hearing loss (HFHL) at pure-tone audiometry has been reported in DM1 patients [1,2], implicating a precocious dysfunction of their auditory system. Considering that DM1 patients do not frequently report symptoms of hearing deficiency, audiological assessment has been rarely performed and hearing loss not pointed out. The underlying mechanism of this auditory impairment remains still poorly understood. Recently, a great interest has been given to evaluation of human auditory function in different nervous system and muscle diseases, including multiple sclerosis, myasthenia gravis, facioscapulohumeral muscular dystrophy, migraine and mitochondrial disorders, by focusing on the cochlea and its dynamics [3–7].

Hearing is an active mechanoelectrical transduction process located in the cochlea, where specialized elements, the outer hair cells (OHCs), are involved in stimulus amplification and sound-frequency selectivity. Interestingly, OHCs play this important role in sound perception through a 'contractile' like movement resembling skeletal muscle fibers dynamics [8]. OHCs functioning has not yet been investigated in DM1

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patients. Our study aims to evaluate the cochlear function and OHCs integrity in a sample of DM1 patients by measuring transient click-evoked otoacoustic emissions (TEOAE), a non-invasive, objective, and quantitative tool to assess OHCs responsiveness, to detect early subclinical manifestations of cochlear dysfunction.

Patients and methods

Twenty-five subjects, 11 women, 14 men (mean age 41.8 ± 11.1, ranging from 18 to 56 years) with a genetically confirmed diagnosis of DM1 were recruited. Genotype was classified according to CTG number in three classes: E1 class ranging from 50 to 200 CTG, E2 class from 200 to 1000, and E3 class from 1000 to 3000 [9]. Disease severity was assessed by means of the Muscular Impairment Rating Scale (MIRS), which is specifically designed for myotonic dystrophy [10]. Muscular weakness ranged from no muscular involvement (MIRS grade 1) to severe muscular impairment (MIRS grade 5). A second group of 28 subjects consisted of healthy volunteers, matched for age and gender (12 women, 16 men; mean age 41.4 ± 9.4 ranging from 25 to 60 years), with no history of neurological disorders. Details of the subjects enrolled are summarized in Table 1. Exclusion criteria were the following: age over 60 years, to minimize the prevalence of presbyacusis, previous history of otological or labyrinthine disorders, noise exposure, ototoxic drug consumption, diabetes, family history of hearing loss, evidence of acoustic neurinoma on magnetic resonance imaging. Before measuring audiometric pure-tone thresholds, an acoustic impedance test and otoscopic examination were performed in all subjects, to exclude possible middle ear diseases (e.g., otosclerosis, glue or tympanic perforation). All the patients had an intact ear drum and a type 'A' (normal) tympanogram.

Table 1 Demographic and clinical data of the groups of patients

Patients	Age at evaluation	CTG expansion class	MIRS score
DM1 total (n = 25)	41.8 ± 11.1	1.9 ± 0.7	2.9 ± 1.1
DM1 HFHL+ (n = 16)	46.6 ± 9.2	1.9 ± 0.7	3.1 ± 1.1
DM1 HFHL− (n = 9)	31.6 ± 7.5	1.7 ± 0.7	2.4 ± 0.9
Control (n = 28)	41.4 ± 9.4	–	–

Data in the table are expressed as mean ± SD.
HFHL+, DM1 patients with high-frequency hearing loss; HFHL−, DM1 patients without high-frequency hearing loss; MIRS, Muscular Impairment Rating Scale.

Hearing loss was calculated for each pure-tone frequency stimulation (from 125 to 8000 Hz) separately as the amount of threshold shift above the standard audiometric zero.

Transient click-evoked otoacoustic emissions are low-level audio-frequency sounds produced in response to a click stimulus by the active micro-movements of OHCs in the organ of Corti and simply detectable and measured from the external ear canal without requiring patient's cooperation. Click stimulus is used to elicit responses that are most robust in the middle frequency regions between 1000 and 4000 Hz [11]. TEOAE were acquired in normal subjects and DM1 patients with the ILO 292 Echoport system (Otodynamics Ltd, Hatfield, UK) in a silent room, with a stimulus level set to 90 dB SPL. The stimulus level measured in the ear canal of the subjects ranged from 85 and 90 dB SPL (mean 86.3 dB). For data acquisition, the 'Non-linear' paradigm was applied to remove the linear ringing artifact. During TEOAE acquisition, we reached a probe stability in the ear canal of more than 90% and we rejected data when the reproducibility was $< 70\%$. The reproducibility is correlated to a signal-to-noise ratio (SNR): a value of 100% corresponds to a SNR infinitely high, whilst a negative one is compatible with a SNR around zero. Noise reject level was set from 34 and 48 dB SPL (1–5 mPa). Applying MATLAB® (MATHWORKS©, MA, USA), a Fast Fourier Transform software was developed for TEOAE frequency analysis and another software was created to execute 1/3 octave analysis. All TEOAE data recorded were filtered between 600 and 6000 Hz and a frequency range between 800–5000 Hz was taken into account in our offline analysis. According to Probst *et al*. [12], all frequency bands with audiometric threshold higher than 30 dB HL were discarded for each ear. All procedures were carried out with the appropriate understanding and written consent of the subjects. The research protocol had been previously evaluated and approved by the Independent Ethical Committee of Policlinico Tor Vergata Foundation.

Statistical analysis

Considering the non-normal distribution of pure-tone audiometric data, non-parametric analyses were performed. Kruskal–Wallis test was applied to identify differences among the groups considered; when significant, the Mann–Whitney test was used to perform multiple comparison between DM1 and controls; a *post hoc* Bonferroni correction was applied to correct for multiple comparisons and considered $P < 0.016$ as statistically significant.

Depending on homogeneity of variance of the data, the amplitudes of TEOAE were compared using

one-way ANOVA and then applying Bonferroni *t*-test, a highly conservative *post hoc* analysis for multiple comparisons. Significance was determined by *P*-values of < 0.05. Pearson correlation test was used to evaluate the strength of association between clinical data and TEOAE. Calculations were performed with the statistical software SIGMASTAT version 3.5 (Systat Software Inc, Point Richmond, CA, USA).

Results

In the DM1 group, a threshold level above 20 dB HL in at least two of the frequency bands higher than 4000 Hz was found in 16 of 25 patients bilaterally (DM1 HFHL+), whilst nine patients had normal thresholds at all the standard audiometric frequencies (DM1 HFHL−). Controls revealed normal pure-tone thresholds at the lower and middle frequencies, with occasionally elevated thresholds above 20 dB HL at high-frequency bands in 10 subjects. A comparative statistical analysis between control group and DM1 patients revealed a significant difference ($P < 0.001$) in the frequency range of 3000–8000 Hz. Interestingly, DM1 HFHL+ subgroup confirmed the statistical data (Table 2). TEOAE were present in 48/50 ears in DM1 patients and in 52/56 ears in the control group. ANOVA testing reported a significant difference between DM1 patients with and without HFHL compared to controls. A *post hoc* analysis with highly conservative Bonferroni *t*-test revealed that DM1 HFHL+ differs from control at 1000 Hz ($t = 3.02$, $P = 0.007$), 1260 Hz ($t = 3.29$, $P = 0.003$), 1587 Hz ($t = 3.01$, $P = 0.007$), and 2000 Hz ($t = 2.29$, $P = 0.050$); surprisingly, DM1 HFHL− patients differ significantly from controls only at 2000 Hz ($t = 2.40$, $P = 0.037$) and 2520 Hz ($t = 2.40$, $P = 0.037$). No difference between DM1 subgroups and control subjects was detected below 1000 Hz or above 2520 Hz (Fig. 1). Considering the small number of DM1 subjects without hearing loss, the statistical power of the comparison test can be negatively influenced, although a progressive involvement of lower frequencies paralleling hearing loss may take place. A clinical and complete audiological follow-up study on the same patients during the course of the disease might likely better support this hypothesis. Moreover, in the DM1 group, no significant correlation between TEOAE levels and clinical data in terms of age at evaluation, CTG expansion class, or MIRS staging was found (Table S1).

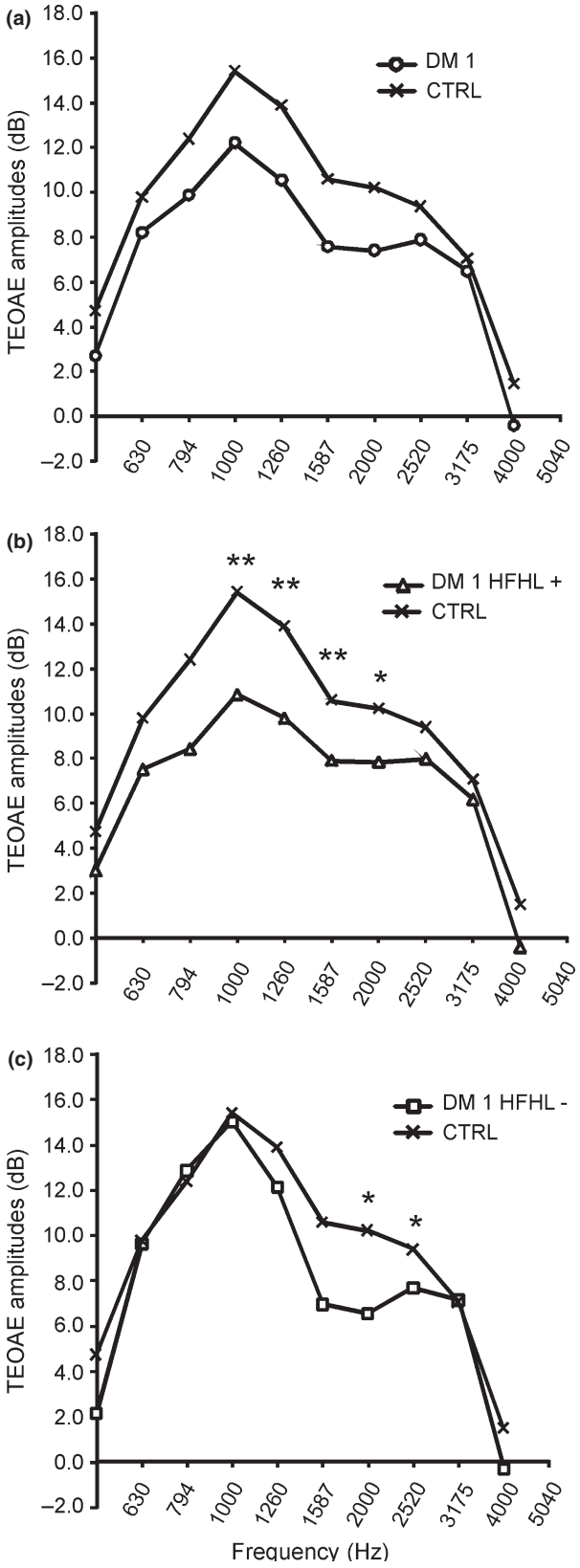
Discussion

High-frequency hearing loss in DM1 patients has been previously reported [1,2], but the underlying mechanism of this auditory failure remains still poorly understood.

Table 2 Pure tone audiometry thresholds and multiple comparison between each group of patients

Frequency (Hz)	125	250	500	750	1000	1500	2000	3000	4000	6000	8000
DM1 all	11.1 ± 2.6	11.5 ± 3.2	11.1 ± 3.4	11.1 ± 2.5	11.0 ± 2.8	11.5 ± 2.5	11.7 ± 3.1	14.8 ± 5.4	17.3 ± 7.9	25.1 ± 12.5	30.8 ± 18.2
DM1 HFHL+	11.0 ± 2.4	11.8 ± 3.6	11.7 ± 4.0	11.5 ± 3.0	11.5 ± 3.3	11.8 ± 2.8	12.0 ± 3.4	16.5 ± 5.6	20.3 ± 7.8	31.3 ± 10.2	39.3 ± 15.6
DM1 HFHL−	11.4 ± 3.1	10.7 ± 1.8	10.0 ± 0.1	10.0 ± 0.1	10.0 ± 0.1	10.7 ± 1.8	11.1 ± 2.1	11.1 ± 2.1	10.7 ± 1.8	11.8 ± 2.5	12.5 ± 4.3
CTRL	11.1 ± 2.4	11.3 ± 2.5	10.8 ± 2.2	10.7 ± 1.8	10.7 ± 2.4	10.5 ± 1.5	10.5 ± 1.5	11.2 ± 2.9	11.9 ± 4.3	15.6 ± 8.1	16.7 ± 10.1
DM1 all vs. CTRL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
DM1 HFHL+ vs. CTRL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
DM1 HFHL− vs. CTRL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Values are expressed as mean ± SD.
HFHL+, presence of high frequency hearing loss; HFHL−, absence of high-frequency hearing loss; n.s., not statistically significant.



However, a 'precocious' audiometric impairment in DM1 patients may hint an early dysfunction of their auditory system.

Although pure-tone audiometry is an useful test to assess and quantify hearing defect, it remains greatly influenced by patient's attention level and grade of collaboration, but also by technician's experience. Conversely, TEOAE recording is able to detect micromovements of a specific group of cochlear cells, named OHCs, thus representing a sensitive, non-invasive, repeatable, and objective quantitative tool to detect minute changes in cochlear integrity [11].

Our study confirmed the high prevalence of sensorineural hearing loss in DM1 patients showing a significant difference from healthy subjects. Moreover, it showed a marked impairment of TEOAE not only in HFHL+, but unexpectedly also in HFHL− patients suggesting an underlying subclinical cochlear dysfunction in this disease. Interestingly, when considering the mid-frequency region between 1000 and 4000 Hz, where TEOAE reach the highest sensitivity, the impairment was restricted to frequencies above 2000 Hz in the HFHL− group, but it extended to 1000 Hz in HFHL+ DM1 patients. Noteworthy, no significant correlation was found between TEOAE levels and patients' age, thus excluding a role of presbyacusis. Also, the observed lack of correlation between TEOAE and CTG repeats or MIRS staging indicates that the extent of cochlear injury is independent of disease severity in other tissues, in line with somatic mosaicism typical of DM1 [9].

The HFHL of DM1 patients may be due to the high 'contractile-like' response and metabolic demand of OHCs of the basal coil stimulated by high-frequency sounds and consequently more susceptible to noise damage, as previously demonstrated in mitochondrial diseases [13,14]. Nonetheless, in the present report, we described a dysfunction involving also

Figure 1 Frequency analysis of transient-evoked otoacoustic emissions (TEOAE) echo level in DM1 patients and control subjects. TEOAE mean amplitude levels recorded from ears of all DM1 patients and control group (CTRL) are shown in panel A. According to the presence (+) or not (–) of high-frequency hearing loss (HFHL) in pure-tone audiometry, DM1 patients were divided in two groups and their TEOAE data statistically compared with control ones respectively in panels B and C. Statistically significant differences between DM1 groups and CTRL are shown (*$P \leq 0.05$; **$P < 0.01$; one-way ANOVA analysis followed by a *post hoc* Bonferroni *t*-test for multiple comparison were performed).

lower frequencies, thus making possible to detect it through TEOAE recordings. Unfortunately, a reliable sensible audiological *in vivo* test specific for high-frequency range is still lacking.

An alteration of OHCs contractility and a failure of their tuning role may hinder neurotransmission along the auditory central pathway after stimulation of inner hair cells. We can speculate that an OHCs malfunctioning may be due to a somatic electromotility alteration that affects primarily voltage-dependent shape changes of OHCs [8]. Accordingly, as reported for other clinical signs in DM1, like myotonia, we can hypothesize that cochlear dysfunction may be linked to a misregulation of an alternative splicing of ionic channels [15–17], or cytoskeletal myosin proteins able to interact with membrane channels in OHCs [18].

Our results indicate that cochlear impairment in DM1 is common, even in normal-hearing patients. Hence, in the current clinical practice, an assessment of cochlear function by TEOAE recording may be useful in DM1 patients to identify precocious signs of cochlear dysfunction.

Large-scale audiological studies including also myotonic dystrophy type 2 and non-syndromic myotonias' patients are currently ongoing, firstly as a clinical screening evaluation, but also aimed to suggest possible pathogenetic mechanisms underlying hearing loss.

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Conflict of interest

Nothing to report.

Financial disclosure

The authors report no disclosures.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pearson's correlation coefficients between TEOAE responses and clinical data

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