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Value of short exercise and short exercise with cooling tests in diagnosis of recessive form of myotonia congenita (Becker disease) — are sex differences important?

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ABSTRACT

Introduction: In myotonia congenita (MC), activation with exercise or cooling can induce transient changes in compound motor action potential (CMAP) parameters, thus providing a guide to genetic analysis.

Material and methods: We performed the short exercise test (SET) and the short exercise test with cooling (SETC) in 30 patients with genetically confirmed Becker disease (BMC) to estimate their utility in the diagnosis of BMC.

Results: Although we observed a significant decrease in CMAP amplitude immediately after maximal voluntary effort in both tests in the whole BMC group, in men this decline was significantly smaller than in women, especially in SET.

Clinical implications/future directions: In men with a clinical suspicion of BMC, a small decrease in CMAP amplitude in SET together with a typical decline in SETC does not exclude the diagnosis of BMC. Our results show a sex-specific difference in chloride channel function in BMC, which needs further investigation.

Key words: myotonia congenita, Becker myotonia congenita, SET, SETC, CMAP

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Introduction

Myotonia congenita (MC) is the most common inherited skeletal muscle channelopathy characterised by myotonia, the impaired relaxation of skeletal muscle after voluntary contraction [1]. It is caused by loss of function of pathogenic variants in the *CLCN1* gene coding the chloride channel which plays an important role in stabilising the resting membrane potential [2, 3]. Chloride channel's dysfunction results in a relative depolarisation of the muscle membrane [2–4]. MC presents as either an autosomal recessive form (Becker MC, BMC) which tends to be clinically more severe than an autosomal dominant form (Thomsen MC, TMC) [1, 5]. In both forms, muscle stiffness improves with repeated activity, which is known as the 'warm-up' phenomenon [5, 6].

The milestone in clinical diagnosis of muscle channelopathies was the inclusion to diagnostic work-up of electrophysiological activation tests. Their basis is the analysis of changes in CMAP amplitude at various times after repetitive short (10 s) exercises and short exercises after cooling the muscle. This provides important information on changes in the number of active muscle fibres and their ability to depolarise and repolarise [7, 8]. These stimulation procedures were implied in two non-invasive electrophysiological tests: SET and SETC.

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Table 1. Clinical characteristic of all patients with Becker MC (n = 30) and men with BMC (n = 19)

Characteristics	Becker MC	Men with BMC
Number of patients	30	19
Gender (F:M)	11 : 19	0 : 19
Mean age at onset, yrs (range)	8.1 ± 4.2 (0.5–19)	9.0 ± 3.1 (2.5–14)
Mean age at examination, yrs (range)	29.4 ± 15.4 (4–61)	29.6 ± 16.8 (13–61)
Mean disease duration, yrs (range)	21.3 ± 16.8 (0–56.5)	20.6 ± 17.6 (3–54.5)

BMC — Becker myotonia congenita; CMAP — compound muscle action potential; MC — myotonia congenita

In both of them, decrease of CMAP amplitude of > 40% immediately after a maximal effort is thought to be indicative of chloride channelopathies, whereas reductions of between 10% and 40% are often difficult to interpret [7–9].

The purpose of our study was to estimate the utility of SET and SETC in the diagnosis of Becker disease (BMC).

Material and methods

We evaluated 30 patients (11 women and 19 men) aged 4-61 years with genetically confirmed BMC. All patients were diagnosed and treated at a single neuromuscular centre. The clinical characteristics of the patients are set out in Table 1.

The study protocol was approved by the Bioethical Committee at the Institute of Psychiatry and Neurology (IPiN No 3/2015). All the procedures were in accordance with the standards of the Committee on Human Experimentation at the Medical University of Warsaw and with the Helsinki Declaration of 1975.

Electrophysiological tests

Electrophysiological studies were performed using Keypoint, Medtronic Functional Diagnostics EMG in 2002––2018 during diagnostic process but prior to genetic confirmation of the disease. The SET and SETC were performed according to the protocol described by several groups [7–10]. All electrophysiological test results included were analysed by MN, who was blinded to diagnosis.

Standard techniques with surface electrodes were used. In the SET, the CMAP was recorded from the abductor digiti minimi (ADM) muscle. To prevent any decrease in CMAP amplitude and area by muscle warming, skin temperature was measured and maintained between 32 and 34°C. The ulnar nerve was stimulated 8 cm proximal to the recording electrode with supramaximal stimulation to obtain maximal, stable CMAP amplitudes. The patient was then asked to contract the ADM maximally (as strongly as possible) for 10 s. Immediately after effort, the patient was instructed to completely relax, and supramaximal stimulation was repeated (SET 0 s). After the end of exercise, CMAPs were measured at regular time intervals, once every 10 s for 50 s (SET 10 s, 20 s, 30 s, 40 s and SET 50 s). The same steps were repeated twice with 60 seconds between the beginning of two trials. In the second part of the test, the ADM was cooled to 20°C with a special cold gel compress (Nexcare ColdHot Maxi). The duration of cooling ranged from 7 to 15 min. After cooling, the protocol described above was repeated. The sites of stimulation and CMAP recording were marked with coloured markers to obtain a precise location for repeated testing of the CMAPs. This protocol, described earlier by Gawel et al. [11], is presented in Table 2.

Table 2. Protocol used in our EMG lab during SET and SETC performance in patients with BMC (as described in detail by Gawel et al. [11])

Steps in SET and SETC
1. CMAP recording in ADM with supramaximal stimulation
2. Maximal effort (1) in ADM (10 s)
3. CMAP recording immediately after effort — SET (1) 0 s
4. CMAP recording every 10 s for 50 s (10 s, 20 s, 30 s, 40 s, 50 s)
5. Maximal effort (2) in ADM (10 s) — from 50 s to 60 s
6. CMAP recording immediately after effort — SET (2) 0 s
7. CMAP recording every 10 s for 50 s (10 s, 20 s, 30 s, 40 s, 50 s)
8. Maximal effort (3) in ADM (10 s) — from 50 s to 60 s
9. CMAP recording immediately after effort — SET (3) 0 s
10. CMAP recording every 10 s for 50 s (10 s, 20 s, 30 s, 40 s, 50 s)
SETC protocol same as above after ADM cooling to 20°C

ADM — abductor digiti minimi; BMC — Becker myotonia congenita; CMAP — compound muscle action potential; SET — short exercise test; SETC — short exercise with cooling test

Statistical analysis

The data set was analysed using SAS 9.2. This allowed for descriptive analysis including: averages, standard deviations, medians, lower and upper quartiles. In the first step, correlations between variables were calculated using Spearman's correlation coefficients. Several non-parametric tests were used in the analysis, such as U-Mann-Whitney, Kruskal-Wallis (for many comparisons), and Wilcoxon (comparison of two dependent data samples). We also used the chi-square test to compare discrete data. Several multiple regression models were used.

In all statistical analyses, the level of significance was determined at the level of $p < 0.05$.

Results

SET results

The largest decrease (mean 23.4%) of CMAP amplitude in BMC patients was observed with the first stimulation immediately after a maximal effort. In the next two series of effort, the CMAP amplitude decline was less pronounced (Tab. 3). The normalisation of CMAP amplitude was rapid — in the 20 s response (SET 20s) the decrease was 5.5%, 4.3% and 2.1% in the consecutive series, with only a slight decline (from 2.64 to 1.04%) in the 50 s response (SET 50 s).

Table 3. Changes in CMAP amplitude obtained after SET and SETC immediately (0 s), and in 20 s and 50 s after a maximal effort in three consecutive series in patients with BMC (n = 30) and in male patients with BMC only (n = 19)

Test	CMAP absolute mean value (mV)	BMC (whole group)			CMAP absolute mean value (mV)	BMC (male patients only)		
SET Initial	10.58	Mean ± SD (%)	Min (%)	Max (%)	10.38	Mean ± SD (%)	Min (%)	Max (%)
SET (1) 0 s	7.76	–23.39 ± 27.54	–81	19	8.64	–16.20 ± 22.82	–73	19
SET (1) 20 s	9.96	–5.53 ± 11.54	–25	22	10.05	–3.33 ± 12.31	–24	22
SET (1) 50 s	10.29	–2.64 ± 9.32	–21	21	10.29	–1.13 ± 11.07	–21	21
SET (2) 0 s	9.63	–7.43 ± 13.95	–36	16	10.01	–3.53 ± 11.42	–21	16
SET (2) 20 s	10.14	–4.29 ± 13.17	–28	20	9.94	–4.53 ± 15.83	–28	20
SET (2) 50 s	10.37	–1.68 ± 10.11	–21	16	10.15	–2.31 ± 12.05	–21	21
SET (3) 0 s	9.95	–5.07 ± 14.69	–37	16	10.02	–4.00 ± 15.93	–31	16
SET (3) 20 s	10.31	–2.7 ± 12.32	–32	20	10.08	–2.53 ± 14.33	–32	20
SET (3) 50 s	10.40	–1.04 ± 10.35	–19	21	10.20	–1.20 ± 11.98	–19	21
SETC Initial	10.30				9.75			
SETC (1) 0 s	7.75	–22.07 ± 22.52	–63	17	7.84	–20.64 ± 20.67	–52	10
SETC (1) 20 s	9.80	–3.59 ± 15.48	–39	24	9.61	–2.21 ± 14.72	–39	16
SETC (1) 50 s	10.29	0.7 ± 13.04	–27	26	9.89	1.07 ± 14.21	–27	26
SETC (2) 0 s	8.95	–10.44 ± 21.96	–48	29	8.87	–8.36 ± 20.29	–45	25
SETC (2) 20 s	10.37	2.67 ± 14.21	–26	32	10.21	4.79 ± 11.73	–12	24
SETC (2) 50 s	10.59	4.63 ± 12.24	–24	34	10.24	5.00 ± 10.71	–11	26
SETC (3) 0 s	9.01	–9.22 ± 18.14	–45	34	8.62	–10.14 ± 16.32	–40	16
SETC (3) 20 s	10.30	2.56 ± 14.51	–30	32	9.92	2.07 ± 11.70	–15	22
SETC (3) 50 s	10.52	4.18 ± 14.48	–28	34	9.91	2.07 ± 12.12	–15	28

BMC — Becker myotonia congenita; CMAP — compound muscle action potential; SET — short exercise test; SETC — short exercise with cooling test

Interestingly, in the SET we observed a smaller (about 16%) decrease of CMAP amplitude immediately after effort (Table 3) in male patients with BMC than in females. Recovery of CMAP amplitude was similarly rapid in both groups (Fig. 1).

Only in eight patients (26.67%) was the reduction of CMAP amplitude in first stimulation > 40% and ranged between 41% and 81% (mean 61.4%). Five of them were female and two of them were siblings (a man and a woman) with the same mutation in chloride channel.

SETC results

In BMC patients, the SETC showed a significant decline of CMAP amplitude in the first stimulation immediately after a maximal effort (about 22%), similarly to SET. In the next two series, the reduction was half as much, but still more pronounced than in SET (Tab. 3). We observed a rapid CMAP amplitude recovery — in SETC 20s the decrease was only 3.6% and 2.7% in the first and second series, and in the third stimulation in fact we noticed a mild increase of CMAP amplitude, by 2.6%. In SETC 50 s, a slight increase of mean CMAP amplitude with a tendency to rise in every next series was noted (Fig. 1). Interestingly, in SETC, no sex differences were observed. In eight patients with the reduction of CMAP amplitude > 40% in first stimulation in SET, we observed also a decrease in SETC of mean 48.4% (range: 36–63%). Interestingly, in siblings with prominent CMAP amplitude reduction in SET and SETC, we observed different results: the sister had the largest CMAP amplitude decrease (80% in SET and 63% in SETC) but in her brother the decrease was much smaller in SET (43%) and similar in SETC (52%).

SET and SETC results within reference values for healthy controls were registered in eight patients (27%).

Discussion

In 73% of BMC patients of both sexes, we observed a significant decrease in CMAP amplitude immediately after effort in SET and SETC. But the range of reduction was lower than previously reported in BMC [7–9] and was 23.4% in SET and 22.1% in SETC. Only in 26.7% of our patients was the reduction higher than 40%. We noticed also statistically significant sex differences in response to effort. In men with BMC, a more than 20% decline in CMAP amplitude was seen only in SETC and not in SET. In BMC patients, the post-exercise decline in CMAP amplitude disappeared with repetitive trials, as expected.

The new findings in our study are sex differences in response to electrophysiological provocative tests among

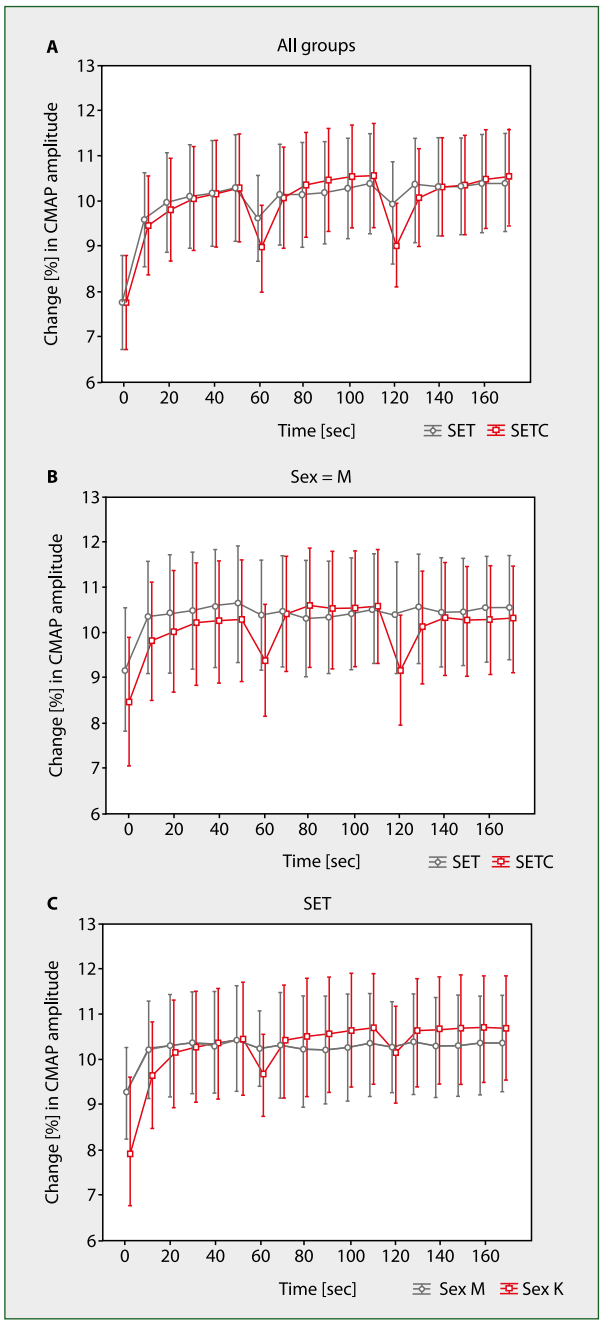

Figure 1. SET and SETC results (change (%) of CMAP amplitude) in BMC patients; **A.** Mean results of SET and SETC (change (%) of CMAP amplitude) in BMC group (n = 30); **B.** Mean results of SET and SETC (change (%) of CMAP amplitude) in male patients with BMC (n = 19); **C.** Comparison between SET results (change (%) of CMAP amplitude) in men and women with BMC. BMC — Becker myotonia congenita; CMAP — compound muscle action potential; SET — short exercise test; SETC — short exercise test with cooling

patients with BMC: in men, the decrease of CMAP amplitude was smaller than expected in SET and more distinct (> 20%) in SETC. Interestingly, we observed different results in siblings carrying the same mutation: in the sister, the most significant CMAP amplitude decrease was seen in SET and SETC. However, in her brother the decrease was only half as big in SET but very similar in SETC. We cannot exclude that sex-related factors might play an important role in the modulation of chloride channels function. In previously published reports, sex differences were not taken into consideration [7–9, 12]. On the other hand, sex hormones are natural ion channel regulators. This is seen in clinical practice, where women with MC tend to be less severely affected than men [1, 13]. In electrophysiological studies analysing myotonic discharges, male carriers of recessive mutations are more likely to have subclinical myotonia compared to women [13, 14]. It is hypothesised that different sex hormones could have different effects on ion channels. Fiahlo et al. have shown that ClC-1 channels are inhibited by testosterone and progesterone via most likely non-genomic sex hormone signalling pathway [13]. This could explain why symptoms caused by loss of function of ClC-1 channels are exacerbated when testosterone or progesterone are elevated. But the influence of hormones on muscle excitability *in vivo* is still an open question [15].

Our results in SET and SETC in BMC patients differ from those previously reported. These differences could be related to a larger study group or different pathogenic variants in the *CLCN1* in our patients.

In the work by Fournier et al. [7], in SET the upper limit of normal for CMAP amplitude decline at room temperature was established at 10%. The abnormal decrease in CMAP amplitude immediately after a maximal effort was observed in 5/6 (83%) MC patients. This observation allows the assignation of the pattern II response to exercise stimulation to patients with chloride channelopathies. This pattern was characterised by a significant decrease of CMAP amplitude (> 40%) in ADM immediately after exercise (range: –17% to –91%) with a rapid return to normal values within 20–40s. The post-exercise decline in CMAP amplitude disappeared with repetitive trials [7]. Most authors correlate this finding with the well-established clinical 'warm-up' phenomenon in MC patients [7–9, 12]. After cooling the ADM, the post-exercise reduction of CMAP amplitude in the first trial was not modified in BMC patients [8].

In further studies, reduction in CMAP amplitude was not as marked as described by Fournier et al. [9, 12]. In the study performed by Michel et al., in 80% of BMC patients a significant (>10%) decrease of CMAP amplitude was noted, but only in 30% did the reduction exceed 40%. [9] In the study conducted by Tan et al., a reduction > 20% was recognised as specific for MC: when amplitude-only reduction was measured, the specificity was 93%, and when concordant amplitude and area reduction was obtained, it was 100% (but with slightly less sensitivity, 72% instead of 78%) [12]. However, the authors noted that 3/11 BMC patients had negative SET and SETC results (information about sex was not given) [12].

Michel et al. linked different patterns of response in SET and SETC with the type of mutations or mutation

combinations in the *CLCN1* gene [9]. The reduction was seen in patients with mutations, which led to low or no expression of chloride channel or expression of specific missense or truncated channel subunits. Contradictory results in SET and SETC were noted in patients with the expression of F167L dimers only or mixed expression of mutated A313T subunits and wild type subunit. Information regarding the sex of patients was not given [9].

Our results in BMC patients are similar to the results obtained in patients with DM1 presented earlier in the paper by Gawel et al. [11]. In SET, the biggest reduction of CMAP amplitude (mean 25.62%) was observed in the first set immediately after maximal effort [SET (1) 0 s]. In the next two series, the CMAP amplitude decline was still about 10%. In SETC, the reduction of CMAP amplitude in SET (1) 0 s was similar (about 20%), but did not change in the next two sets. Sex analysis was not given, but the proportion of male patients was similar to this study (19/32 in DM1 group, and 19/30 in BMC group). A decrease in CMAP amplitude immediately after effort in DM1 patients has also been found in other studies [16–18].

The role of chloride channel dysfunction in the pathogenesis of DM1 seems to be clear. It is accepted that altered splicing of the chloride channel transcripts leads to myotonia [19, 20].

In conclusion, our study supports the utility of SET and SETC in the diagnostic work-up of patients with clinical myotonia. Our results suggest that decrease of CMAP amplitude in ADM of between 15% and 25% immediately after a maximal effort in the SET and SETC are also indicative of chloride channelopathies, especially when male patients are examined. In men with a clinical suspicion of BMC, a smaller than expected decrease in CMAP amplitude in SET, together with typical decline in SETC, does not exclude the diagnosis of BMC. The function of chloride channel in BMC might be modulated by sex-specific factors or mutation type, something which needs further investigation.

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