

Original article

Exercise test on the patients with normokalaemic periodic paralysis from a Chinese family with a mutation in the SCN4A gene

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Keywords: *exercise test; normokalaemic periodic paralysis; paramyotonia congenita; skeletal muscle sodium channelopathy*

Background Normokalaemic periodic paralysis (normoKPP) is characterized by transient and recurrent myoasthenia, and some patients also show muscle stiffness induced by cold exposure (paramyotonia congenita, PMC). It is caused by a mutation in the muscle voltage gated sodium channel alpha subunit (SCN4A) gene. Due to the diversity of the clinical manifestations of patients, it is difficult for clinicians to differentiate some of patients with atypical normoKPP from those who suffer from other periodic paralysis and nondystrophic myotonia. So far, for normoKPP there are almost no ways to assist definite diagnosis besides genetic screening. This research was designed to evaluate an exercise test (ET) in confirming the diagnosis of normoKPP and in assessing the therapeutic effectiveness of some drugs on this disease.

Methods ET, described by McMains, was performed on six subjects from a Chinese family, including four patients with overlapping disease of normoKPP and PMC caused by a mutation of SCN4A Met1592Val that is identified by genetic analysis and two normal control members. The change of compound muscle action potential (CMAP) was recorded. Besides the family, two patients were also tested during treatments with acetazolamide.

Results All patients showed a slight increase in CMAP immediately after exercise, followed by an abnormal gradual decline, which reached its nadir 25–30 minutes after exercise. CMAP amplitude dropped by more than 40% in patients but less than 23% in controls. In the patients who received pretreatment with acetazolamide, the change of CMAP amplitude was less than 28% and, at any fixed times, less than pretreatment values.

Conclusions The ET may be used as a predictive, easy and reliable method of diagnosing normoKPP under conditions without genetic screening help, and is an objective way to evaluate the therapeutic effectiveness. According to different response patterns, the ET may also be helpful in reducing the scope of genetic screening.

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Normokalaemic periodic paralysis (normoKPP) is characterized by transient and recurrent myasthenia interrupted by a complete recovery, and clinically resembles primary hypokalaemic periodic paralysis (hypoKPP). In contrast to hypoKPP, the serum potassium concentration is normal or slightly increased rather than decreased during an attack. Some patients also show myotonic stiffness under cold exposure conditions, paramyotonia congenita (PMC).^{1,2} A biomolecular study demonstrated that normoKPP is caused by a mutation in the muscle voltage gated sodium channel alpha subunit (SCN4A) gene,³ whereas hypoKPP has been linked to a variation in the skeletal muscle voltage-gated calcium channel 1 subunit (CLCN1).⁴ Because different portions of the Na⁺ channel serve different functions, mutations at different points in SCN4A will result in varied clinical manifestations that makes it usually hard to differentiate it from other nondystrophic myotonias and periodic paralysis. Furthermore, approximately 10% of hypoKPP is also caused by an SCN4A mutation, so the molecular diagnosis is necessary.

The mutation of SCN4A will cause a change in muscle membrane excitability, which can be recorded by electromyography (EMG). Since both muscle weakness

and stiffness may be triggered or alleviated by strenuous exercise, the use of exercise can be proposed as a provocative test to evaluate different types of dysfunctions of the skeletal muscles. In this study, we performed the exercise test (ET) described by Mc Mains et al. on four patients with normoKPP from a Chinese family carrying an SCN4A mutation (Met 1592Val), to determine if the ET could be used as a simple reliable diagnosis method, as well as assessing response during treatment. In addition, our study may be helpful for probing into the relationship between the phenotype and genotype of this rare “orphan disease” in Chinese.

METHODS

Patients

Totally six subjects were tested, including four patients and an unaffected family member, who were from a four

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generation kindred with fifteen members who suffered from severe homogeneous overlapping disease of normoKPP and PMC (Figure 1), and a normal spouse (female). The Met1592Val mutation of SCN4A was identified by genetic analysis (Figure 2). A summary of the clinical data for the six subjects is presented in Table 1.

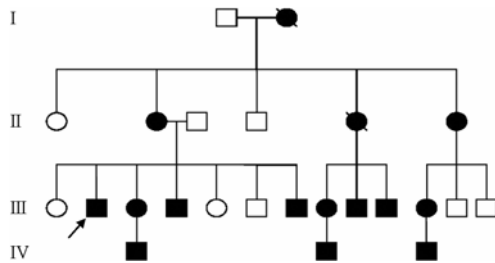


Figure 1. Family pedigree. The black symbols represent affected individuals; male is indicated by square, and female is indicated by a circle. The arrow indicates the proband.

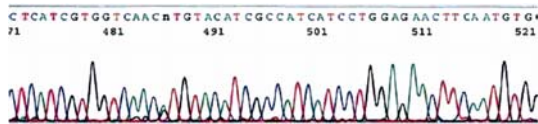


Figure 2. Sequencing of SCN4A E24: there is an A to G single base mutation at the position 486 in SCN4A E24.

Table 1. The clinical data of observed patients

Patient	Age (years)	Sex	PP	Met 1592 Val	PMC	Myotonic discharge	Duration	Frequency (mon)	RAE precipitate
III-2	60	M	+	+	+	+	7-10 d	3-4	+
III-3	54	F	+	+	+	+	3-28 d	2	+
III-5	52	F	-	-	-	-	None	None	-
IV-1	27	M	+	+	+	+	<24 h	2-3	+
IV-3	22	M	+	+	+	+	3 h-10 d	1	+
Spouse	50	M	-	-	-	-	None	None	-

PP: periodic paralysis; PMC: paramyotonia congenita; RAE: rest after exercise; M: male; F: female.

For all patients the onset of paralytic episodes occurred during their neonatal period and exhibited as difficulty in opening their eyes and gripping after crying. The episodes were frequent, lasting for several minutes to hours and were usually accompanied by muscle stiffness of the lower limbs. During childhood and adolescence, episodes were usually related to precipitating factors such as exercise, seasonal changes, fasting and nerve stress, eating pumpkin, tomato (esp. under hungry conditions) and muskmelon were also found to be important initiating factors in all of the family members. Oral potassium chloride 2 mmol/kg is not effective. Glucose solution intake can alleviate the paralysis at once, otherwise the symptoms gradually alleviate themselves within 1 to 2 hours, although, a complete recovery may take days to weeks. Cold exposure can trigger the focal myotonia, weakness of the eyelid and all the limbs, but patients recovered faster and more easily than when triggered by other factors.

During adulthood the frequency and severity of episodes

insidiously worsened as time passed. Diffuse inter-episode weakness, mainly in the proximal muscle, developed around the fourth to fifth decade and was progressive, with patients having difficulty in climbing stairs and walking and becomes a chronically progressive myopathy. All the female patients family of the experienced more frequent paralytic attacks during their period of pregnancy.

In this family there were two adult patients, III-3 and III-4, who showed a kind of paradoxical myotonia, typified by eyelid lag after repeatedly forcefully shutting and opening their eyes. All affected family members showed a positive response to acetazolamide treatment (50 mg, bid) in alleviating symptoms and a decreasing frequency of attacks. Additionally, most of adult patients showed gastrocnemial hypertrophy but no percussion myotonia. Serious fixed muscle weakness was evident in some family members after their fifties with obvious gastrocnemial atrophy. There is no cardiac arrhythmia in this family.

During paralytic episodes, excepting patient IV-1, serum potassium concentration went beyond the normal range (3.5-5.3 mmol/L), others remained within the normal range but were still higher than that of the preparalytic phase. The serum creatine kinase (CK) level was two or more times normal in all subjected patients after each attack and reached its highest level at 72 hours. Meanwhile the CK level of PM patients showed a sustaining rise as high as 2 fold of normal. Routine EMG shows myotonia discharge in all patients. The low amplitude and positive sharp waves were recorded from the gastronomies of patients III-3 and III-4. A muscle biopsy performed in the two patients showed a vacuolar myopathy (Figure 3).

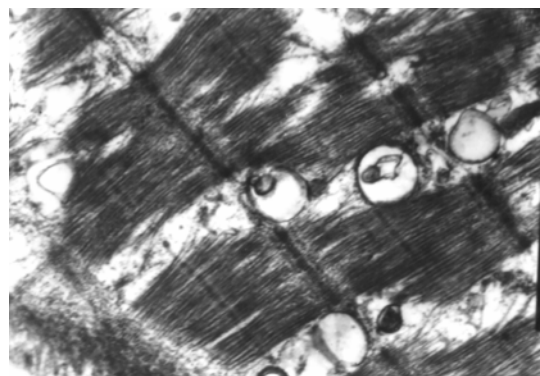


Figure 3. Electron micrograph of a muscle specimen of patient III-2 (original magnification $\times 15\,000$) showing myofibrillar separation, splitting multiple vacuoles were lined with a unit membrane.

Procedure and protocol

According to the protocols described by McManis et al⁵ the ET was performed during the period of inter-episodes under room temperature condition. The compound muscle

action potential (CMAP) was evoked by supramaximal nerve stimulation and recorded by skin electrodes. The electrical responses were recorded from right abductor after stimulation of the ulnar nerves at the wrist. CMAPs were first monitored before exercise every 30–60 seconds for 2–3 minutes to enable baseline stabilization. The patients were then asked to contract the muscle as strongly as possible in isometric condition for 5 minutes, with brief (3–4 seconds) rest every 15 seconds to prevent muscle ischemia. CMAPs were repeatedly recorded at 1-minute intervals, during exercise and recovery, for at least 30 minutes or until no further decrement is observed in the amplitude of CMAPs. The percentage of decrement was calculated by the following formula: $DP=(HAE-SAE)/HAE \times 100\%$.

Here, DP stands for the down percentage; HAE, the highest amplitude of CMAPs after exercise; SAE, the smallest amplitude of CMAPs. The DP of greater than 40% was considered abnormal.⁵ Hand grasping power were measured with a dynamometer at the beginning and the end of this test.

We also performed ET on patients III-2 and IV-3 during they received treatments with acetazolamide.

RESULTS

In control subjects a long-time exercise test just slightly decreased CMAP amplitude, <23%. While in patients the CMAP amplitude declined more than 40%, suggesting the presence of periodic paralysis (PP) (Table 2). All patients showed a slight and transient increase in CMAP amplitude after 2 to 5 minutes of exercise then a progressive decrease until reaching their nadir at 20–30 minutes. Taking patient III-3 as an example (Figure 4): the baseline is 8.5 mV and his baseline grasping power determined by dynamometer was 20 kg. After 3 minutes of voluntary contractions, its amplitude increased to 9.2 mV (8.1%). The CMAP amplitude then declined to the bottom, 4.6 mV (45.8%), over 30 minutes of exercise and remained stable for 35 minutes after exercise, and his grasping power had decreased to 15 kg.

Table 2. Amplitude changes of CMAP during ET

Change item	Patients				Controls	
	III-2	III-3	IV-1	IV-3	III-5	Spouse
During ET (%)	8.1*	7.6*	6.9*	4.0*	4.6*	0.4#
Lowest amplitude (%)	45.8#	44.3#	48.6#	53.8#	19.0#	22.3#
To lowest (min)	30	30	25	25	18	20

During ET: CMAP amplitude changes compared with basal level during ET; Lowest amplitude: CMAP amplitude changes at the lowest amplitude compared with basal level; *increase of amplitude change; #decrease of amplitude change.

During treatment with acetazolamide the change of CMAP amplitude in III-2 (Figure 5) and IV-1 were less than 28%, and the percentage of CMAP amplitude was reduced at any fixed time compared with the pretreatment values (Figure 6).

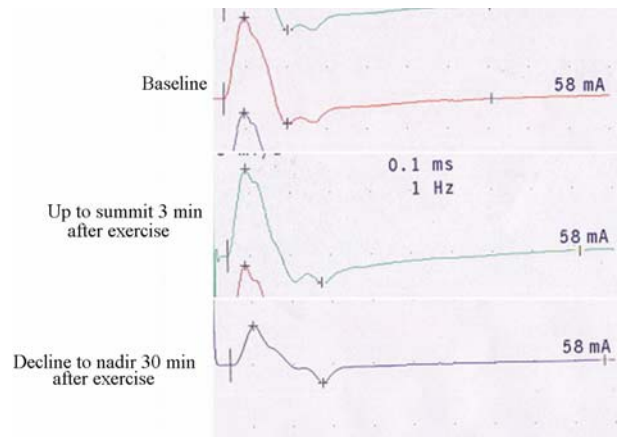


Figure 4. The changing course of CMAP amplitude in ET, patient III-3 (5 mv/D, 5 ms/D).

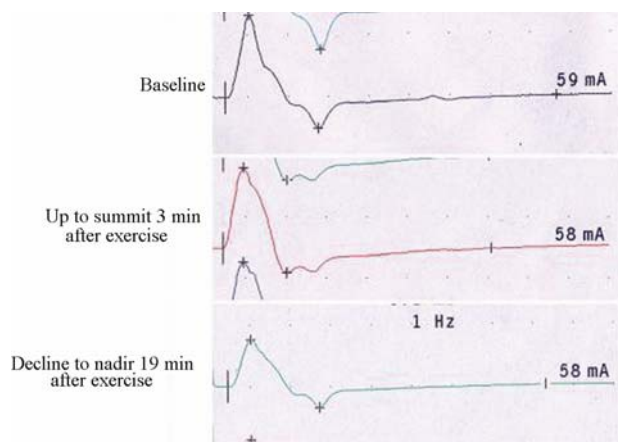


Figure 5. The changing course of CMAP amplitude in ET during treatment with acetazolamide, patient III-3 (5 mv/D, 5 ms/D).

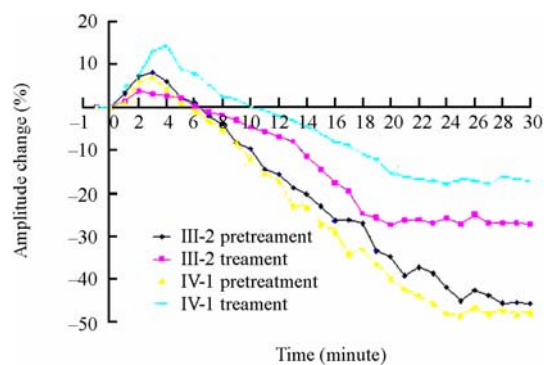


Figure 6. The effect of treatment with acetazolamide on CMAP amplitude in the III-2 and IV-1.

DISCUSSION

NormoKPP is characterized by episodic attacks of myasthenia, and some patients complain of muscle stiffness triggered by cold exposure. It is caused by mutations in the SCN4A gene on chromosome 13. In addition, hyperKPP, PC, fluctuating myotonia, permanent myotonia and acetazolamide-reactive myotonia are all due to mutations in SCN4A, the skeletal muscle sodium channelopathy.⁶⁻⁸ The clinical manifestations of this

disease are diverse and some patients also have overlapping symptoms.⁹ Therefore, clinicians usually find it difficult to clinically differentiate these diseases from other myotonic syndromes and periodic paralysis¹⁰ and there is almost no way to assist definite diagnosis besides genetic screening. In view of this, McManis et al⁵ in 1985 introduced an exercise test (ET) as a simple electrodiagnostic method to help in confirming clinical suspicion of periodic paralysis (PP).

Both the PP and nondystrophic myotonia originate from changes in excitability of the muscular cells, which can be monitored by recording the responses of the muscle to stimulation imposed on the nerve innervating the muscle. In the clinic exercise is known as a trigger which can aggravate or relieve the symptoms of patients.¹¹ So ET combined with electromyography can be used as a functional detective method to improve the diagnosis of these muscle disorders.^{12,13} ET includes the short-ET and long-ET. Researchers should choose different ET according to different clinical characteristics.¹⁴ The data demonstrate that a long-term repeat exercise resulted in a significant decrease in the CMAP amplitude in approximately 70%–80% of the patients with PP and in 17% and 33% of the patients with myotonia congenita (MC) and PC, respectively. While repeated exercises can relieve stiffness in patients with MC but not with PC; mild exercise can prevent or delay attacks of weakness, whereas intensive exercise can trigger or aggravate the attacks in PP.

By analyzing all results of ET it is easy to find most patients carrying the same mutation who shared a similar pattern of response to ET. Five main electrophysiological patterns could be defined.¹⁵⁻²⁰ Pattern I: seen mainly in PC patients, exhibited needle-EMG abundant myotonic discharges and immediate decrease of CMAP amplitude after long-exercise; Pattern II: was seen mainly in most of the MC patients with chloride channelopathy, also exhibited needle-EMG abundant myotonic discharges without evident change in CMAP amplitude after long-ET; Pattern III: found mainly in patients with the G1306A or I693T SCN4A mutation, showed almost the same response as pattern II to long-ET, but exhibited a different response to short-ET; Pattern IV: was reported in hyperKPP/normoKPP, characterized by an immediate increase and then immediate decrease in CMAP amplitude after exercise without, or rarely with, myotonic discharges; Pattern V, usually seen in hypoKPP patients, showed a delayed decrease in CMAP amplitude after long exercise without immediate change after short or long exercise.

The transient CMAP amplitude increase exhibited in pattern IV may pertain to a transient protective effect of acidosis in hyperKPP/normoKPP patients, as would be induced by exercise.

In this study, we investigated a Chinese family characterized by transient and recurrent episodes of

paralysis triggered by exercise and hunger with a normal or slightly increased serum potassium concentration and myotonia caused by cold exposure. A permanent myopathy can become apparent in most patients in their forties and fifties. The reaction to potassium loading is not obvious. Genetic analysis confirmed that this overlapping syndrome was caused by a Met1592Val mutation in the SCN4A gene. For this family the long-ET was performed. All effected subjects showed a slight CMAP increase immediately after exercise, followed by an abnormal gradual decrease. 25–30 minutes after the exercise test all the amplitudes of CMAP dropped more than 40% to the nadir.

In this study we selected a normal spouse and an unaffected number of these kindred as controls. The change of amplitude of their CMAP was less than 28%, which lessened the possibility that the abnormal CMAP changes are due to other genetic mutations in this family. That at least a 30% increase in amplitude of CMAP and (or) more than a 40% decrease confirm the diagnosis of PP. This response pattern of ET has been reported in some patients with overlaps of periodical paralysis and paramyotonia.^{16,21} Combined with the presence of myotonic discharges these EMG abnormalities resembled pattern IV. Accordingly, it is suggested that exercise was a provocative factor of attacks. The results of ET confirmed the results of genetic screening.

Jackson et al²² carried out the EMG study on patients with thyroxic hypoKPP and found abnormalities of CMAP. After thyroid ablation both the episode of PP and abnormal changes in CMAP were alleviated. For patients of this family we explored, acetazolamide can reduce the frequency of attacks and alleviate weakness and stiffness. Does ET have the same changes? To answer this question, we performed ET in patients while they were receiving treatment with acetazolamide and found that the change of CMAP amplitude was less than 28% and at any fixed time point less than pretreatment. It was the evident that acetazolamide can protect against the underlying pathophysiological derangement that ultimately led to the attacks of paralysis or myotonia, which is concordant with the clinical manifestation in patients. Therefore, we think the ET might be useful in objectively assessing the curative effect of some drugs.²³

Above all, our study indicated that the ET may be used as a predictive, easy and reliable tool of diagnosis PP by clinicians who cannot gain easy access to genetic screening. According to different response patterns, the ET may also be used as a guide for molecular diagnosis in clinical practice. In addition, by comparing the changes in CMAP between post treatment and pretreatment it is possible to adopt ET to evaluate the therapeutic effectiveness of some drugs.

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