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Cardiac autonomic function evaluation in pediatric and adult patients with congenital myasthenic syndromes

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Abstract

Cardiac autonomic dysfunction has been examined in myasthenia gravis but not in congenital myasthenic syndromes (CMS). We aimed to evaluate cardiac autonomic functions in genetically defined CMS. Patients diagnosed with and under treatment for CMS were reviewed for 24-hour cardiac rhythm monitoring. Heart rate variability (HRV) measures were defined as: SDNN, mean of the standard deviations for all R-R intervals; SDNNi, standard deviation of all R-R intervals in successive five-minute epochs; RMSSD, square root of the mean of squared differences between successive R-R intervals. Ten patients with mutations in the epsilon subunit of the acetylcholine receptor (AChRε) and five patients with mutations in the collagen-like tail of asymmetric acetylcholinesterase (ColQ) were included. Median age at evaluation was 17 (2.5–46) years. In the AChRε group, RMSSD values; and in the ColQ group, SDNN, SDNNi and RMSSD values were significantly lower than those of healthy subjects. This first extensive report examining HRV in CMS showed alterations in patients with ColQ mutations and, to a lesser extent, in the group with AChRε mutations. This might indicate an increased risk of cardiac arrhythmias. We suggest cardiological follow-up in CMS, and consideration of any potential cardiovascular effects of therapeutic agents used in management.

Keywords: Congenital myasthenic syndromes; Autonomic functions; Heart rate variability.

1. Introduction

Congenital myasthenic syndromes (CMS) are genetically inherited defects of the neuromuscular junction (NMJ) resulting in weakness and fatigability in skeletal, extraocular or bulbar muscles [1,2]. They are divided into three groups according to the site of the defect: presynaptic, synaptic, or postsynaptic, the latter being the most common. The nicotinic acetylcholine receptor (nAChR) in the human NMJ is a pentameric complex composed of four subunits: two alpha, one beta, and one epsilon (ε) or delta subunit [1,2]. Mutations in the ε subunit of the receptor (AChRε) and the collagenic tail of endplate acetylcholinesterase (ColQ) are frequent in the Turkish CMS population while rapsyn and downstream-of-kinase 7 (DOK7) have been observed more frequently from western Europe [3–5].

Autonomic dysfunction and cardiac problems have been examined in autoimmune myasthenia gravis but not in CMS except one pediatric report [6–9] where Caggiano et al. reported polysomnography results in five CMS patients. No significant variability in heart rate was observed: however, an age-matched control group, adult patients, and patients with AChRε mutations were not included and 24-hour ambulatory ECG (electrocardiography) monitoring was not performed [10].

The quantification of beat-to-beat variability in heart rate (heart rate variability, HRV), defined by changes in the time between beats represented by R-R intervals on ECG and analyzed by further methods, provides a non-invasive and easy

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way of studying cardiac autonomic function [11]. We report our evaluation of cardiac autonomic function in two groups of CMS patients in comparison with matched control groups.

2. Patients and methods

Clinical and laboratory data of patients diagnosed with CMS by clinical and genetic findings in Hacettepe University Faculty of Medicine, Neurology and Pediatric Neurology Department and Diyarbakır Children's Hospital, Pediatric Neurology Department between 1990–2017 were reviewed.

History, physical and neurological examination findings at the time of cardiac evaluation were noted. As no patient had cardiological symptoms or signs, clinical grading was based on motor function for the purpose of this study: (1) normal: no weakness, (2) mild: weakness causing no significant disability, daily activities carried out without assistance, (3) moderate: patient requires some assistance during daily activities, (4) severe: disability requiring wheelchair and continuous assistance.

Results of repetitive nerve stimulation (RNS) test, electrocardiography, echocardiography, and 24-hour cardiac rhythm monitoring were recorded. Patients' treatment was not changed or interrupted for these investigations. Results were compared with gender- and age-matched healthy control subjects' data from the same laboratory. Subjects with history of cardiac disease, diabetes mellitus, or malignancy were not included. Two groups of CMS: AChRε and ColQ mutation groups, were formed after exclusion of one patient with rapsyn gene mutation and one pregnant patient. This study was approved by the Ethics Committee.

2.1. Electrophysiological tests

Electrophysiological tests were done with Medtronic Keypoint (Medtronic A/S, Copenhagen, Denmark) EMG equipment using Ag-AgCl surface electrodes for recording.

2.2. Repetitive nerve stimulation (RNS) test

Twelve patients had RNS at 2, 3 and 5 Hz stimulating the facial and ulnar nerves and recording from the orbicularis oculi and abductor digiti minimi (ADM) muscles respectively. Also, 50 Hz-RNS could be performed in the ADM muscle in 10 patients. Ten pulses for 2, 3 and 5 Hz RNS and 50 pulses for 50 Hz RNS studies were given. After stimulation of the nerves, compound muscle action potential amplitude (CMAP) differences between the first (R1) and fourth (R4) responses were expressed as the percentage of the first response (R1-4). Decremental R1-4 responses $>10\%$ were accepted as abnormal [12].

2.3. Sympathetic skin response (SSR)

SSRs were examined in 11 patients. The active electrode was placed on the right palm and reference electrode, on the dorsum of the right hand. Left median nerve was electrically stimulated with intensity of 10–20 mA and duration of 0.5 ms using 0.1–2 Hz band filters.

2.4. Cardiac examination

All patients and control subjects were evaluated with transthoracic echocardiography (Vivid S5, General Electric Healthcare), 12-lead ECG and ambulatory ECG monitoring (EVO Holter monitoring system, SPACELABS Healthcare, Snoqualmie-USA). Patients' treatment was continued during the study (Table 1). A three-channel recorder was used for 24- hour ambulatory ECG monitoring. All recordings were analyzed by a pediatric cardiologist using Pathfinder SL software (SPACELABS Healthcare, Snoqualmie-USA). Normal NN, or R-R intervals, which cover time between beats are used to calculate heart rate, were identified. Artefacts and incompatible intervals were excluded from the calculation. Mean age, mean Holter ECG analysis times, and heart rate variability (HRV) measures defined as below were recorded for statistical comparison:

SDNN (ms), the mean of the standard deviations for all R-R intervals;

SDNNi (ms), standard deviation of all R-R intervals in successive five-minute epochs;

RMSSD (ms), root-mean square differences of successive R-R intervals, and triangular index of time-related HRV parameters.

2.4.1. Statistical analysis

These included descriptive statistics and comparison between groups. The normality of distribution of numerical variables was evaluated by the Kolmogorov–Smirnov or Shapiro–Wilk tests. Values for numerical variables were given as mean $\pm$ standard deviation or median (min-max), depending on the normality of distribution. For comparison between groups, the Mann-Whitney U test and Student's t-test were selected as appropriate, defining $p < 0.05$ as significant and <0.01, highly significant (Statistical Package for the Social Sciences program for Windows, version 16.0).

3. Results

Ten patients with AChRε mutations and five with ColQ mutations, all known as pathogenic, were included [3] (Table 1). Male/female ratio was 6/9. First symptoms had started in the newborn period in 7 patients, in infancy in 6 patients and early childhood in 2 patients. Median age at diagnosis was 2 years (range: 1 month-9 years). Median age at cardiac evaluation was 17 (2.5–46) years. Medications included pyridostigmine in all patients with AChRε and one with ColQ mutation, and salbutamol and/or 3,4 diaminopyridine in other patients with ColQ mutation (Table 1). Neurological examination revealed ptosis/ophthalmoplegia in all 15 patients, but no significant bulbar involvement. Two patients had mild and one had moderate limb/neck weakness. None of the patients reported any cardiac complaints such as syncope or palpitation; neither

Table 1
Patient characteristics including neurological findings and medication.

Patients with ColQ mutations											
Case no	Sex	Mutations	Age of onset	Age at diagnosis	Age at cardiac evaluation (years)	Ophthalmoparesis /Ptosis	Neck weakness	Limb weakness	Respiratory problems	Motor functional assessment at cardiac evaluation#	Medication
1	m	c.444G>A (p.W148X) homozygous	Newborn	9 months	13,5	+	+	–	–	Mild	3,4-DAP*
2	f	c.444G>A (p.W148X) homozygous	Newborn	4 years	17	+	+	+	-	Moderate	3,4-DAP/ salbutamol
3	f	c.444G>A (p.W148X) homozygous	Infancy	2 years	2,5	+	+	+	apnea	Mild	Salbutamol
4	m	c.444G>A (p.W148X) homozygous	Infancy	2 years.	2,5	+	–	–	Recurrent pneumonia	Normal	Anti-AChE**
5	f	c.444G>A (p.W148X) homozygous	Newborn	2 years	10,5	+	–	–	–	Normal	Salbutamol
Patients with AChR epsilon subunit mutations											
6	f	ε1206ins19 (c.1248_ 1266dup19) homozygous	Newborn	1 months	16,5	+	–	–	–	Normal	Anti-AChE
7	f	ε1206ins19 (c.1248_ 1266dup19) homozygous	Infancy	3 months	10	+	–	–	Shortness of breath	Normal	Anti-AChE
8	m	ε1206ins19 (c.1248_ 1266dup19) homozygous	Early childhood	3 years	46	+	–	–	–	Normal	Anti-AChE
9	m	ε70insG/εIVS7 +2T>C (c.130dupG/ c.802+2T>C) compound heterozygous	Newborn	2 years	34	+	–	–	–	Normal	Anti-AChE
10	f	ε70insG/εIVS7 +2T>C (c.130dupG/ c.802+2T>C) compound heterozygous	Newborn	2 years	30	+	–	–	–	Normal	Anti-AChE
11	m	ε1276delG (c.1336delG) homozygous	Newborn	5 years	39	+	–	–		Normal	Anti-AChE
12	m	ε1276delG (c.1336delG) homozygous	Young childhood	9 years	43	+	–	–	Shortness of breath	Normal	Anti-AChE
13	f	ε1276delG (c.1336delG) homozygous	Infancy	2.5 years	34	+	–	–	–	Normal	Anti-AChE
14	f	εIVS7+2T>C (c.802+2T>C) homozygous	Infancy	4 years	20	+	–	–	–	Normal	Anti-AChE
15	f	εIVS7+2T>C (c.802+2T>C) homozygous	Infancy	6 years	16	+	–	–	–	Normal	Anti-AChE

f: female, m: male, #see the text, *3,4-DAP: 3,4-diaminopyridine, **anti-AChE: anti Acetylcholinesterase.

Table 2
The results of the time domain analysis of heart rate variation (HRV) in patients with collagen-like tail of asymmetric acetylcholinesterase (ColQ) mutations and control subject.

	Colq patients	Control group	*P*
Age (years)	9 ± 6.4	9.4 ± 6.1	>0.05
Duration of analysis (h)	20.7 ± 2.4	19 ± 2.8	>0.05
SDNN	94 ± 21.4	143 ± 30.8	**<0.05**
SDNNi	46.2 ± 10.1	66 ± 10.5	**<0.05**
RMSSD	26.8 ± 4.6	46.2 ± 10.5	**<0.01 (0.006)**
Triangular index	26.6 ± 4.1	37.2 ± 12.4	>0.05

SDNN: mean of the standard deviations for all R-R intervals; SDNNi: standard deviation of all R-R intervals in successive five-minute epochs; RMSSD: square root of the mean of the squared differences between successive R-R intervals.

Table 3
The results of the time domain analysis of heart rate variation (HRV) in patients with epsilon subunit of the acetylcholine receptor (AChRε) mutations and control subject.

	Epsilon patients	Control group	*P*
Age (years)	28.1 ± 11.9	24.6 ± 12.6	>0.05
Duration of analysis (hours)	20.4 ± 3.3	18.5 ± 2.2	>0.05
SDNN	138.3 ± 23.2	147.7 ± 25.1	>0.05
SDNNi	59.3 ± 12.9	64.7 ± 7.1	>0.05
RMSSD	29.9 ± 10	39.1 ± 6.9	<0.05 (0,034)
Triangular index	34.5 ± 5.6	36.1 ± 5.1	>0.05

SDNN: mean of the standard deviations for all R-R intervals; SDNNi: standard deviation of all R-R intervals in successive five-minute epochs; RMSSD: square root of the mean of the squared differences between successive R-R intervals.

bradycardia nor tachycardia were detected during cardiac examination.

Echocardiograms and 24-hour ECG monitoring were within normal limits. The cardiac rhythm analysis did not show significant arrhythmia in any patients.

The results of the time domain analysis of HRV in patients with CMS and control subjects are shown in Tables 2 and 3. In the AChRε subunit group, RMSSD was significantly lower than healthy control subjects while SDNN, SDNNi and triangular index values, although lower, did not reach significance. In patients with ColQ mutations SDNN, SDNNi and especially RMSSD were significantly lower compared to control values.

Low frequency RNS revealed a decremental response in all patients who underwent this test, while no significant incremental response at 50 Hz RNS was observed in any patient. Baseline CMAP amplitudes were within normal limits. RNS findings are summarized in Table 4. SSR were in the normal range in all patients.

4. Discussion

Our study included CMS with AChRε and ColQ mutations which constitute the largest genetic groups among our CMS patients. AChRε mutations affect the expression and electrophysiology of the AChR. ColQ mutations prevent the collagen domain from associating with acetylcholinesterase, alter assembly of the triple-helical collagen domain, or reduce expression of the assembled enzyme.

The nAChR also has a role in the autonomic nervous system, where ganglionic nAChRs mediate fast excitatory transmission; at sympathetic, parasympathetic and sensory nerve endings, and the central nervous system, where neuronal nAChRs composed of α and β subunits exert a regulatory action. Neuronal nAChRs are also expressed on non-neuronal cells including glial, immune and endothelial cells while the ε subunit exists only in the NMJ [13]. ColQ may have other roles in non-muscle tissues like the brain and heart, as animal studies show its RNA being expressed in tissues with little or no acetylcholinesterase [14]. Our results indeed showed alterations in cardiovascular autonomic functions especially in patients with ColQ mutations.

Measurement of HRV assesses autonomic modulation of sino-atrial activity and is considered as a biomarker for cardiac autonomic dysfunction [15–17]. Attenuation in HRV has been demonstrated to be a potent marker of high cardiovascular risk. In this study we used the time-dependent parameters of HRV: SDNN, SDANN, RMSSD and triangular index as advised in the 1996 guideline of European Society of Cardiology and the North American Society of

Table 4
Repetitive nerve stimulation findings of available patients: %CMAP differences between the first and fourth responses at repetitive nerve stimulation.

Case no.	Orbicularis oculi				Abductor digiti minimi				
	First CMAP * amplitude(mV)	2 Hz	3 Hz	5 Hz	First CMAP * amplitude (mV)	2 Hz	3 Hz	5 Hz	50 Hz
1	3,3	−11	−16	−15	5,2	−23	−25	−25	−55
2	1,6	−24	−28	−29	4	−32	−39	−41	−64
5	1,2	−4	−8,7	−13	6,2	−9	−10	−11	−40
6	0,3	−30	−23	−23	7,6	−6	−9	−12	
7	1,2	−2	−10	−15	9,2	−12	−19	−3	3
8	0,2	−12	−17	−17	5,9	−27	−26	−24	7
9	1,6	−10	−14	−13	12,9	−4	−4	−2	18
11	1,2	−13	−16	−16	9,2	−4	−5	−1	2
12	0,6	−23	−36	−37	9	−7	−9	−11	15
13	0,9	−14	−22	−22	5,7	−5	−7	−9	8,9
14	2,1	−5	−3	−12					
15	2,9	−8	−19	−20					

* CMAP: compound muscle action potential amplitude.

Pacing and Electrophysiology [11]. These parameters reflect the overall flexibility of the autonomous nervous system. SDNN provides a measure of HRV in different states such as sleep, wake, and activity; RMSSD is associated with short-term, rapid changes in heart rate, and is correlated with vagus-mediated components of HRV [18]. The dominant component of the autonomic nervous system in cardiac atrioventricular and sinoatrial nodes is the parasympathetic system; it has negative chronotropic and negative inotropic effects [19]. The vagus nerve exerts its influence by release of acetylcholine at postganglionic muscarinic receptors and onto the sinoatrial node, and by inhibition of norepinephrine release presynaptically [18]. Our findings of reduced HRV parameters in the ColQ mutation group suggest dysfunction involving parasympathetic ganglia leading to relative sympathetic dominance. In particular, marked reduction of RMSSD in the ColQ mutation group suggests a long-term effect of the constant deficiency of acetylcholinesterase: this would result in prolonged activation and desensitization of the nAChRs. The absence of the compensatory role of butyrylcholinesterase, which is also anchored by ColQ at the NMJ, may contribute to the defect. In the with AChRε mutation, the HRV alterations cannot be explained by a direct effect of the mutation because the ε subunit of the AChR exists only in striated muscle fibers. Rather, an effect of medications or lifestyle, for instance, reduced physical activity can be considered, as the negative effect of sedentary lifestyle has been shown on HRV [18]. Normal results in SSR test, a widely used method for autonomic function, also support this possibility.

Medications can also affect parasympathetic function. A case of autoimmune myasthenia and arrhythmia was reported where anticholinesterase medications likely exacerbated a cardiac conduction defect [20]. Our patients were on treatment during cardiac assessment: the AChRε mutation patients were on pyridostigmine which has been shown to increase RMSSD while not affecting SDNN and SDANNi [21]. The reason for more pronounced HRV alteration in the ColQ mutation group might reside in the absence of anticholinesterase treatment in most patients of this group.

Reduced HRV predicts vulnerability to various arrhythmias resulting from autonomic dysfunction and has been found as an independent risk factor for sudden death in a large cohort [22,23]. Based on these findings, the risk of sudden cardiac arrhythmia may be envisaged to be increased in CMS. However, such cases are scarce in the literature. As HRV alterations occur in CMS, clinicians should pay additional attention to cardiac examination while using β2-mimetics whose administration has been associated with an increased risk of arrhythmia in asthma patients [24].

A limitation of this study is the absence of more detailed tests of the autonomic nervous system. Our evaluation was done only by routine clinical methods applicable to patients of all ages. The small number of patients limits statistical comparison in some groups; for instance, we had patients as young as 2.5 years old in the ColQ mutation subgroup whose SDNN values were lower than the other three adolescent patients. Nevertheless, our results may contribute to further studies relevant to clinical practice; in particular, studies on HRV before and during treatment are warranted.

5. Conclusion

Cardiological follow-up can be recommended for CMS patients because of a possibly increased risk of cardiac arrhythmia. While normal SSR findings argue against an abnormality of sympathetic pathways, a predisposition to arrhythmia warrants cardiological follow-up and caution with the use of any therapeutic agents in patients with CMS.

Compliance with ethical standards

This study was approved by Ethics Committee.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.02.004.

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