**CTG-repeat expansions in the DMPK gene do not cause takotsubo syndrome**

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**Abstract**

*Background:* Takotsubo syndrome and myotonic dystrophy type 1 are seemingly unrelated conditions. However we have noted remarkable similarities between two cases reported separately. In each case a patient with myotonic dystrophy type 1 suffered a takotsubo cardiomyopathy that was complicated by torsades de pointes and during coronary angiography ventricular tachycardia required defibrillation in both patients. This prompted us to hypothesise that myotonic dystrophy, or sub-pathologic *DMPK* trinucleotide repeat expansions, may be an underlying risk factor for takotsubo syndrome. We therefore examined the *DMPK* 3’ UTR repeat alleles in a cohort of takotsubo cases.

*Methods and Results:* We undertook cohort screening of sixty patients. Twenty eight were from a homogenous cohort of older women with takotsubo syndrome related to the Christchurch earthquakes and 32 were a heterogeneous group of sporadic takotsubo syndrome cases. PCR analysis found no additional individuals in these Christchurch cohorts carrying premutation or pathologic *DMPK* trinucleotide repeat expansions.

*Conclusions:* Unusual *DMPK* repeat expansions are not a widespread feature of takotsubo syndrome. An interaction between DMPK and the potassium channels implicated in torsades remains an intriguing possibility.

**Background**

Takotsubo syndrome (TTS) and myotonic dystrophy type 1 (MD1) are seemingly unrelated conditions. However we have noted remarkable similarities between two cases reported separately. One case is from Austria and the other from New Zealand. What is of particular interest is that not only did each patient have both conditions, they had identical unusual complications from their TTS. In each case the TTS was complicated by torsades de pointes and during coronary angiography ventricular tachycardia both patients required defibrillation. While the genetic basis for MD1 is well understood, the pathophysiology underlying TTS is uncertain and much debated. These cases therefore suggest an important interaction between the DMPK gene and arrhythmias in TTS.

*New Zealand case*

In 2006 a 37-year-old Caucasian woman presented to hospital as an emergency [1] because she had been found unconscious on the floor and cardiopulmonary resuscitation was briefly performed. On the arrival of the ambulance she was conscious and complaining of chest discomfort. She had earlier been told by her husband that he was leaving her – a revelation quite unforeseen by her. Twelve lead ECG showed T-wave inversion in leads V3-6 with a QTc of 530ms. Initial troponin T was 0.12 mcg/l (upper limit normal: 0.03 mcg/l). The next morning her heart rate was 57/min and systolic blood pressure ranged between 70-80mmHg. Echocardiography showed a classic takotsubo pattern with mid, distal and apical segment akinesis (figure 1). Ejection fraction was 20%. A clinical diagnosis of TTS was made. Noradrenaline infusion was commenced for haemodynamic support. Coronary angiography was normal but complicated by two episodes of ventricular tachycardia requiring emergency DC cardioversion. Antiarrhythmic or heart failure therapy was not commenced. Following the angiogram four further episodes of ventricular tachycardia/ventricular fibrillation required DC cardioversion and amiodarone infusion 1.2g over sixteen hours. Twenty-one hours later the patient had a torsades de pointes storm managed with DC cardioversion, intravenous magnesium, increased noradrenaline and temporary pacing wire insertion. She recovered and was discharged ten days later with a normal echocardiogram.

In 2012 the patient’s brother was diagnosed with MD1. On the basis of that diagnosis the patient herself underwent neurological evaluation and was found to have clinical features typical of MD1. Molecular genetic testing revealed a CTG trinucleotide expansion of 350 to 650 repeats on one allele and five on the other of the *DMPK* gene. She reported having had myotonic handgrip symptoms for ten years, and thus for four years prior to her presentation with takotsubo syndrome.

*Austrian case*

In 2012 a 47-year-old Caucasian woman presented for Wertheim surgery for removal of a leiomyoma of the uterus [2]. MD1 had been diagnosed at 20 years of age. Southern blot analysis revealed a heterogeneous *DMPK* CTG-repeat expansion of 1200-1900 repeats. During surgery thrombosis of the iliac veins was recognised requiring ligature of the left iliac vein and thrombectomy of the right iliac vein. Two days later in ICU she experienced four cardiac arrests due to ventricular fibrillation. She was successfully resuscitated each time. Transthoracic echocardiography revealed a classic takotsubo wall motion abnormality with an ejection fraction of 20%. Coronary angiography was normal. During coronary angiography she developed QT prolongation with recurrent torsades requiring defibrillation. Seventeen days following surgery she underwent tracheostomy because of difficulties weaning from the respirator. This tracheostomy was subsequently closed in August 2012. Follow up echo in May 2012 was normal. In January 2013 TTS recurred requiring transfer to an intensive care unit for non-invasive positive pressure ventilation. She was subsequently transferred to the intensive care unit where she died from sepsis and respiratory insufficiency. Autopsy revealed purulent pericarditis and bilateral purulent pneumonia. Cultures were negative.

**Methods**

*Genetic study*

In a case cluster of TTS following the Christchurch earthquakes of 2010 and 2011, we had collected DNA from a highly homogenous population of older women [3]. In addition, we also had collected samples from a highly heterogeneous group of sporadic TTS cases. To establish whether unusual *DMPK* trinucleotide repeat alleles occurred more commonly in TTS we undertook cohort screening of these samples. Informed consent was obtained from all subjects.

*Genotyping*

DNA was extracted from either peripheral blood or saliva samples by standard methods [4,5]. A total of 60 TTS samples were available for genetic analysis: 28 of these were earthquake related and 32 were sporadic cases. In addition, seven control samples known to have both a normal sized allele and a repeat expansion (ranging from 30 repeats to >125 repeats), as well as 40 samples from a healthy control population were also included. Oligonucleotide primers for PCR analysis (obtained from IDT, Singapore) were P1: 5’-CTTCCCAGGCCTGCAGTTTGCCCATC-3’; P2: 5’-AACGGGGCTCGAAGGGTCCTTGTAGC-3’; P4CTG: 5’-GGCGGTGGCGGCTGTTGCTGCTGCTGCTGC-3’; and P4CAG: 5’-GGCGGTGGCGGCTGTTGCCAGCAGCAGCAGCAG-3’. Analysis of the repeats was essentially as per Dryland et al, except that final concentrations used for each dNTP were 200mM, and for each primer 0.5uM [6]. For each sample, three PCRs were performed: a standard PCR using P1 and P2 primers which flank the repeat; a forward triplet repeat-primed PCR using P1 and P4CTG; and a reverse triplet repeat-primed PCR using P2 and P4CAG. PCR cycling was performed as per Dryland et al on an Eppendorf Mastercycler EP gradient S PCR machine. All PCR products were sized using a Shimadzu MultiNA Microchip electrophoresis system MCE®-202, using the manufacturer’s buffer and size standard for analysis of products up to 500bp (DNA-500 reagent kit, Shimadzu PN 292-27914-91).

**Results**

Repeat numbers were called on the electrophoresis data for all samples. Allele sizes in the TTS cohorts ranged from 5-32, with the majority of alleles displaying a repeat number of five, as seen in the general population. Where samples appeared to be homozygous, triplet repeat-primed PCR was carried out. This confirmed the presence of repeat expansions in the control samples, and in the sole Christchurch TTS case previously diagnosed with MD1 (case 13), and did not identify any additional penetrant CTG repeat expansion alleles in these samples (Table 1).

**Discussion**

MD1 is rare, occurring in approximately in 1:8000 of the population. TTS is also rare but is not so well characterised that incidence rates are known. What is well recognised is that TTS is a heterogeneous condition, not just in terms of the regional wall motion patterns that can be seen but also in the age of the patients and complications [7,8]. Most cases occur in elderly women and, whilst a wide range of complications are reported, in most patients there are none. The two cases discussed here are relatively young women who suffered unusual arrhythmic complications. The similarities between these two cases are striking. They suggest the possibility of a link between MD1 and TTS. This prompted us to hypothesise that MD1, or sub-pathologic *DMPK* repeat expansions, may be an underlying risk factor for TTS. We therefore examined the *DMPK* 3’ UTR repeat alleles in a series of 60 TTS cases.

Molecular analysis revealed no additional individuals in these Christchurch cohorts carrying premutation or pathologic DMPK trinucleotide repeat expansions. We therefore conclude that unusual DMPK repeat expansions are not a widespread feature of TTS, although where the two conditions co-exist this may result in a distinctive presentation. Whilst QT prolongation in TTS is relatively common, torsades is very rare, and did not occur in any of our 60 patients, other than the index case with MD1. Whether ventricular tachycardia in the New Zealand patient was due to noradrenaline infusion remains speculative but based upon the concept that TTS is due to stress, antiadrenergic rather than adrenergic substances should be used.

An interaction between DMPK and the potassium channels implicated in torsades remains an intriguing possibility. A link between MD1 and torsades has previously been suggested. Torsades was reported in a 53-year-old woman from Japan with MD1 [9]. In the family members related to that patient, QT prolongation was associated with high CTG repeats and MD1. This may occur through an interaction at the intercalated disc. Intercalated discs do not occur in skeletal muscle. They are an identifying feature of cardiac muscle, where their role is in synchronizing contraction. The gap junctions that allow action potentials to spread from cell to cell are a part of the intercalated discs. Both the delayed rectifier potassium channel that is implicated in torsades (Kv 1.5) and DMPK are located the gap junction of the cardiac muscle cell [10]. An interaction between these two might explain the association of torsades with MD1. This possibility is supported by the striking similarities in the patients reported here. Since MD1 is a RNA disease, it is also possible that any protein involved in excitation and impulse conduction could be dysfunctional due to haploinsufficiency.

In conclusion this study shows that CTG-repeat expansion does not seem to be causative for TTS. In MD1 patients, however, exposure to psychological or physical stress may trigger TTS. Cardiac complications of TTS may be more severe in MD1 than in patients without, since MD1 patients frequently develop cardiac conduction disease.

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**Table 1. *DMPK* 3’ UTR repeat allele genotypes.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sporadic cohort** | **DMPK triplet repeat1** | **Earthquake cohort** | **DMPK triplet repeat1** |
| Patient 1 | 11,11 | Patient 1 | 13,32 |
| Patient 2 | 11,11 | Patient 2 | 5,12 |
| Patient 3 | 5,12 | Patient 3 | 11,11 |
| Patient 4 | 5,5 | Patient 4 | 11,11 |
| Patient 5 | 5,11 | Patient 5 | 5,10 |
| Patient 6 | 5,5 | Patient 6 | 11,11 |
| Patient 7 | 5,12 | Patient 7 | 5,18 |
| Patient 8 | 5,12 | Patient 8 | 26,30 |
| Patient 9 | 11,13 | Patient 9 | 5,12 |
| Patient 10 | 5,14 | Patient 10 | 5,11 |
| Patient 11 | 5,12 | Patient 11 | 12,19 |
| Patient 12 | 5,5 | Patient 12 | 5,15 |
| Patient 13 | 5,Exp2 | Patient 13 | 5,12 |
| Patient 14 | 5,12 | Patient 14 | 11,11 |
| Patient 15 | 5,9 | Patient 15 | 5,10 |
| Patient 16 | 5,13 | Patient 16 | 5,11 |
| Patient 17 | 11,11 | Patient 17 | 12,12 |
| Patient 18 | 5,8 | Patient 18 | 5,10 |
| Patient 19 | 12,12 | Patient 19 | 10,13 |
| Patient 20 | 5,11 | Patient 20 | 5,12 |
| Patient 21 | 13,13 | Patient 21 | 5,9 |
| Patient 22 | 5,12 | Patient 22 | 5,11 |
| Patient 23 | 5,25 | Patient 23 | 2,25 |
| Patient 24 | 5,13 | Patient 24 | 11,21 |
| Patient 25 | 12,12 | Patient 25 | 13,13 |
| Patient 26 | 13,20 | Patient 26 | 5,12 |
| Patient 27 | 11,18 | Patient 27 | 5,11 |
| Patient 28 | 5,10 | Patient 28 | 5,12 |
| Patient 39 | 5,5 |  |  |
| Patient 30 | 5,11 |  |  |
| Patient 31 | 5,5 |  |  |
| Patient 32 | 5,5 |  |  |

1 *DMPK* 3’untranslated region CTG repeat alleles. Allele repeat numbers are approximate (+/- 1 repeat). Normal range 5-34 repeats; premutation range 35-49 repeats; expanded (fully penetrant) alleles > 50 repeats.

2Exp: expanded allele