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# Mutations in troponin T associated with Hypertrophic Cardiomyopathy increase $Ca^{2+}$-sensitivity and suppress the modulation of $Ca^{2+}$-sensitivity by troponin I phosphorylation☆

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ABSTRACT

We investigated the effect of 7 Hypertrophic Cardiomyopathy (HCM)-causing mutations in troponin T (TnT) on troponin function in thin filaments reconstituted with actin and human cardiac tropomyosin. We used the quantitative *in vitro* motility assay to study $Ca^{2+}$-regulation of unloaded movement and its modulation by troponin I phosphorylation. Troponin from a patient with the K280N TnT mutation showed no difference in $Ca^{2+}$-sensitivity when compared with donor heart troponin and the $Ca^{2+}$-sensitivity was also independent of the troponin I phosphorylation level (uncoupled). The recombinant K280N TnT mutation increased $Ca^{2+}$-sensitivity 1.7-fold and was also uncoupled. The R92Q TnT mutation in troponin from transgenic mouse increased $Ca^{2+}$-sensitivity and was also completely uncoupled. Five TnT mutations (Δ14, Δ28 + 7, ΔE160, S179F and K273E) studied in recombinant troponin increased $Ca^{2+}$-sensitivity and were all fully uncoupled. Thus, for HCM-causing mutations in TnT, $Ca^{2+}$-sensitisation together with uncoupling *in vitro* is the usual response and both factors may contribute to the HCM phenotype. We also found that Epigallocatechin-3-gallate (EGCG) can restore coupling to all uncoupled HCM-causing TnT mutations. In fact the combination of $Ca^{2+}$-desensitisation and re-coupling due to EGCG completely reverses both the abnormalities found in troponin with a TnT HCM mutation suggesting it may have therapeutic potential.



## 1. Introduction

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiomyopathy and is usually associated with mutations in sarcomeric proteins. A recent study has shown that 11% of the identified mutations are in the proteins of the thin filament; actin, tropomyosin, troponin I (TnI), troponin C (TnC) and troponin T (TnT) [1]. It is generally found that HCM-causing mutations result in a 2–3 fold higher myofilament $Ca^{2+}$-sensitivity compared to normal heart muscle and this has been proposed to be necessary to trigger the symptoms of HCM: a hyper-contractile phenotype, heart muscle hypertrophy, myocyte disarray and fibrosis [2], although the clinical manifestation of HCM is very variable, probably due to background genetic and environmental factors.

Mutations in the same sarcomeric protein genes are also associated with familial dilated cardiomyopathy (DCM), a disease characterised by a hypo-contractile phenotype with dilation of the heart chamber and thinning of cardiac muscle [3]. Whilst DCM clearly has a

*Abbreviations:* DCM, Dilated Cardiomyopathy; EGCG, Epigallocatechin-3-Gallate; HCM, Hypertrophic Cardiomyopathy; IVMA, *In vitro* Motility Assay; $k_{ACT}$, Rate of force development; $k_{REL}$, Rate of fast relaxation phase; $n_H$, Hill Coefficient; TnC, Troponin C; TnI, Troponin I; TnT, Troponin T; WT, wild-type.

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**Table 1**
Effect of the HCM related TnT K280N mutation on $Ca^{2+}$-sensitivity and phosphorylation dependence of $Ca^{2+}$-sensitivity using the TnT K280N patient sample (see Fig. 1). X = exchanged. All data rounded to 2 significant figures.

| | Experiment | $EC_{50}$, μM | | Ratio ($EC_{50}$ Test/Control) | n | p | TnI Phosphorylation | |
|---|---|---|---|---|---|---|---|---|
| | Test vs Control | Test | Control | | | | Test | Control |
| A | Donor vs K280N patient | 0.19 ± 0.015 | 0.20 ± 0.016 | ×0.99 ± 0.047 | 11 | 0.87 | 1.6 ± 0.060 | 1.4 ± 0.020 |
| B | K280N vs dpK280N patient | 0.14 ± 0.022 | 0.14 ± 0.024 | ×0.99 ± 0.020 | 6 | 0.54 | 1.4 ± 0.090 | 0.11 ± 0.10 |
| C | Donor vs dpDonor | 0.14 ± 0.030 | 0.050 ± 0.010 | ×3.1 ± 0.55 | 10 | 0.0040 | 1.6 ± 0.070 | 0.23 ± 0.070 |
| D | K280N patientXTnT vs dpK280N patientXTnT | 0.16 ± 0.035 | 0.088 ± 0.013 | ×1.8 ± 0.17 | 4 | 0.022 | 1.4 ± 0.12 | 0.090 ± 0.070 |

separate molecular mechanism from HCM, the simple hypothesis that $Ca^{2+}$-sensitivity is reduced in familial DCM (the opposite of HCM) has proven to be incorrect since changes of $Ca^{2+}$-sensitivity do not correlate with the phenotype [2,4,5]. Our investigations have established that a consistent feature of DCM-causing mutations in thin filament proteins is that the $Ca^{2+}$-sensitivity is not modulated by TnI phosphorylation, a process we have called uncoupling, and we have proposed that this is a disease-mechanism [4–6].

TnI is one of several substrates of protein kinase A (PKA). In normal heart, on adrenergic stimulation PKA is activated and TnI phosphorylation at Ser22 and 23 is increased. Phosphorylation of TnI decreases myofibrillar $Ca^{2+}$-sensitivity 2–3 fold and

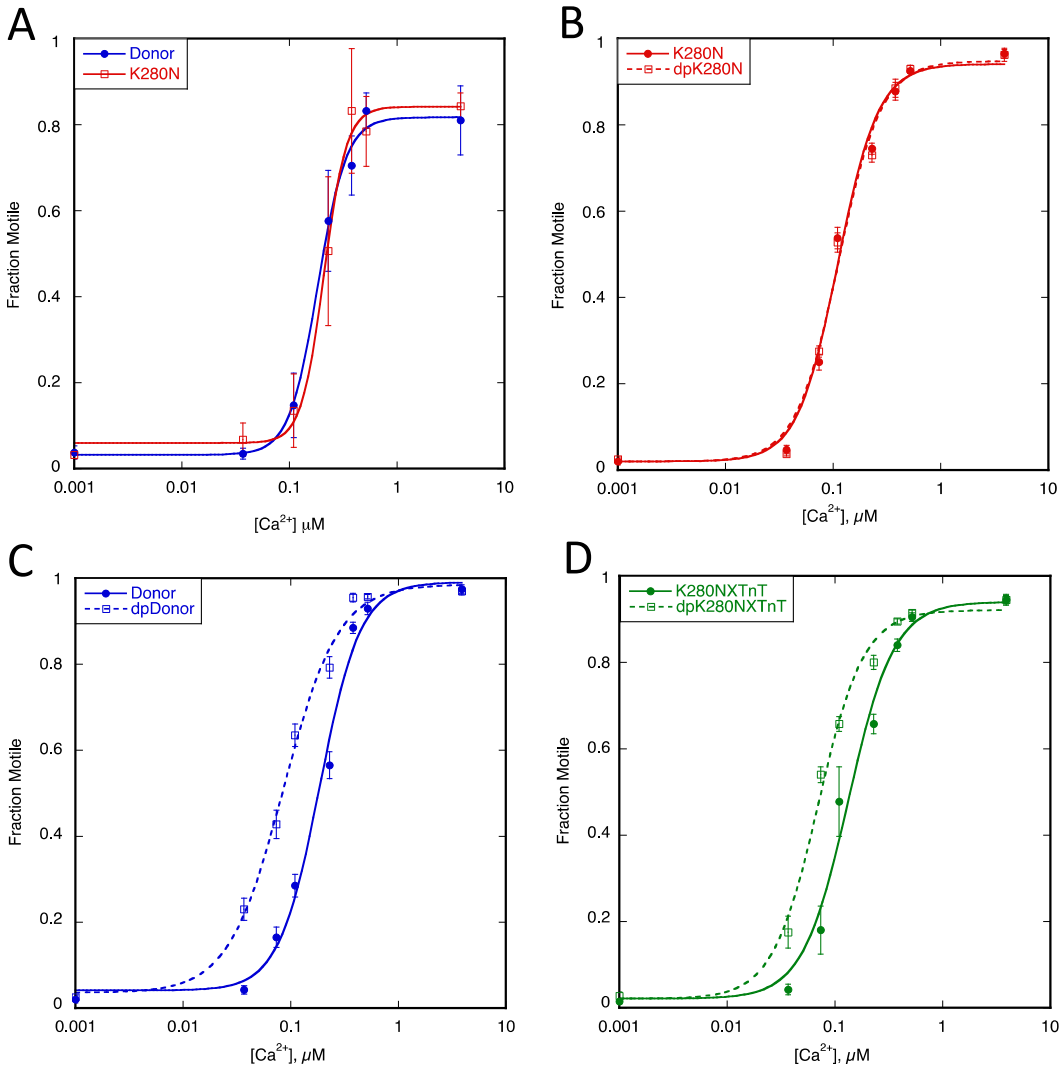


**Fig. 1. Effect of the HCM related TnT K280N mutation (patient sample)**. The fraction of filaments motile, measured in a paired experiment by IVMA, is plotted against [$Ca^{2+}$] for representative single experiments. The mean values of $EC_{50}$ from replicate experiments are shown in Table 1. Solid lines and points, phosphorylated troponin; dotted lines and open points, unphosphorylated troponin. Error bars represent SEM of four measurements of motility in the same motility chamber. Blue, native donor thin filaments; red, TnT K280N patient thin filaments; green, WT TnT exchanged into TnT K280N patient thin filaments. A. Donor and TnT K280N patient containing thin filaments: no change in $EC_{50}$ ($Ca^{2+}$-sensitivity) was seen. B. Effect of phosphorylation on thin filaments containing the TnT K280N mutation. The relationship of $Ca^{2+}$-sensitivity to TnI phosphorylation is uncoupled. C. Effect of phosphorylation on donor thin filaments. Normal relationship as phosphorylation increased $EC_{50}$. D. The difference in $Ca^{2+}$-sensitivity is restored when recombinant human cardiac TnT was exchanged into native TnT K280N patient thin filaments.