

Sudden cardiac death, particularly that which occurs following an episode of catecholaminergic polymorphic ventricular tachycardia, has been studied by a team in Cardiff, using fluorescence resonance energy transfer technology inside individual cells. Here, Susan Pearson discusses research into a condition that affects predominantly young children and adolescents.

New insights into sudden cardiac death

A breakthrough in understanding the causes of sudden cardiac death (SCD) in young people, which may provide new insights into unexplained SCD across all age groups, has just been published by a research team at Cardiff University. Based at the Wales Heart Research Institute, their study provides the first ever cell-based evidence that indicates exactly how mutation-linked defects of the heart cells' calcium release channels, the ryanodine receptors, release excess calcium, leading to potentially fatal tachycardia and arrhythmia.¹

Sudden cardiac death results from an abrupt and unexpected loss of heart function within minutes of the appearance of symptoms such as a rapid (tachycardic) or irregular (arrhythmic) heartbeat. Conservative estimates put the annual incidence of SCD in the Western world at approximately one case per 1000 people, or around 3500 in the UK, of all ages. The majority of these deaths will prove to be due to coronary heart disease, but a significant proportion ($\geq 4\%$) of cases show no evidence of any structural cardiac damage or drug exposure and so cannot be explained. Worryingly, a high percentage of this latter group includes young children and adolescents.

Catecholaminergic polymorphic ventricular tachycardia

The work at Cardiff has focused specifically on the mechanisms behind the disease known as catecholaminergic polymorphic ventricular tachycardia (CPVT). This is a condition, usually found in young people, in which polymorphic electrical abnormalities leading to a rapid heart beat (ventricular tachycardia) are triggered by stress (ie as a result of circulating catecholamines such as adrenaline and noradrenaline), and cases demonstrate a very distinctive electrocardiogram (ECG) fingerprint.

'In CPVT, polymorphic electrical abnormalities leading to a rapid heart beat are triggered by stress, and cases demonstrate a distinctive ECG fingerprint'

Although CPVT is often inherited, there is also substantial evidence to show that it can be caused by sporadic mutation occurring in offspring but not parents.

In 2000 and 2001, groundbreaking clinical studies on CPVT patients in Italy and Finland found that this abnormal ECG pattern correlates with mutations in the ryanodine receptor in the patient's cardiac cells.²⁻⁴ Dysfunction in ryanodine receptors has been implicated in heart failure for several years, but research has been based on older patients with no 'known' mutations. In these cases the disorder in this channel is

considered to be maladaptive (ie acquired over a lifetime).

The Finnish and Italian work was based on groups of individuals referred to national centres specialising in cases not easily diagnosed by standard methods. The patients typically would be very young and be suffering from stress-induced symptoms. However, the stress involved would be very wide-ranging (ie from extreme exertion to moderate or mild exercise, and even emotional stress).

As yet, no one knows the correlation between the degree of stress and the expression of the phenotype manifested by

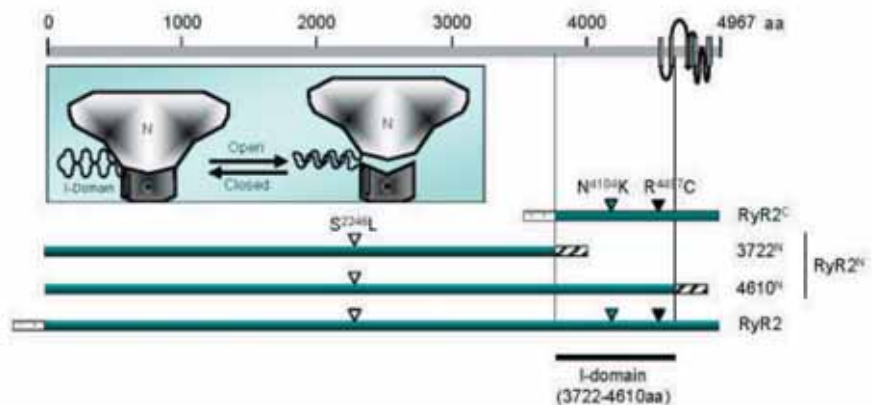


Fig 1. Schematic representation of RyR2 protein showing C and N termini. Reproduced from *Circ Res* 2006; **98**: 88-97, by kind permission.

the onset of symptoms. Studying the disorder is difficult as there is no forewarning in many cases, and the first episode may be severe enough to cause death.

Many of the patients studied were referred on the basis of having survived an episode of ventricular tachycardia that had no apparent cause. In many cases, a patient's family history would reveal parents and grandparents who had died of unexpected causes, indicating a genetic link. However, as the mutation is not necessarily passed on directly (hence the sporadic mutations already mentioned), the phenotype can be asymptomatic, with 30% of all people carrying disease-causing mutations in ryanodine receptors exhibiting no clinical symptoms.

These mutations provided the first clue that ryanodine receptors could be involved directly in arrhythmia and this gave researchers some idea of how dysfunction in the protein (ie mutation) could be amplified into clinical manifestation of arrhythmia.

Calcium studies in Cardiff

Research groups in Calgary in Canada and at Columbia University, New York, as well as the team in Cardiff, took the Finnish and Italian findings further, looking into these peculiar electrophysiological abnormalities.⁵⁻⁷ However, it was the Cardiff scientists who showed how calcium abnormalities following stressful stimulation of heart cells are tied in to the mutant channels, by becoming the first group to put recombinant DNA technology inside cardiac cells.

The group began by measuring every cell parameter possible (eg size, shape, how they contracted and the calcium transient that causes contraction) in wild-type cardiomyocytes. When they put DNA coding for these mutations into the cells, they found that activity remained completely normal while the cells were allowed to remain at

'Abnormal ECG pattern correlates with mutations in the ryanodine receptor in the patient's cardiac cells'

rest. However, when the cells were put under adrenergic stimulation, using the drug isoproterenol to mimic adrenalin-induced stressful activity or emotion, their cellular calcium signalling went haywire.

In wild-type cells the channel turns on and off in a controlled fashion, giving a highly coordinated calcium transient. In stimulated cells that express the mutations, this precise regulation appears to be lost and the resulting calcium transient is broadened, with the release of a far greater amount of calcium that overwhelms the cell.

As these channels are stimulated they should be controlled so that the channel peaks and troughs (opens and closes) with every depolarisation. However, in stress-stimulated mutant cells the group found that calcium peaks very quickly and drops slowly, giving a longer cycle. The lengthened calcium transient then is no longer synchronised with sodium and potassium exchange, which still peaks as normal. The group established this dysynchronous ionic flux, which causes the whole process to go awry, as the molecular basis of arrhythmia.

The Cardiff group then began to look at how this channel becomes faulty, in both physical and molecular terms, utilising for the first time established fluorescence resonance energy transfer (FRET) technology inside

living cells to look at ryanodine receptors. The FRET technique demonstrates the interaction between two proteins, or, to be more precise, two halves of a protein. Using FRET inside living cells, the team was able to look at how the two halves of the ryanodine molecule (RyR2), the calcium releasing pore and the regulatory domain, work together.

As the scale of these interactions is far too small to be resolved by light microscopy, biochemical techniques are used to probe the interaction. However, the various immunoprecipitation techniques, in which the physical interaction between interacting partners can be demonstrated, generally used to work out such interactions were not going to work in this case.

By George, they've got it!

British Heart Foundation lecturer Dr Christopher George, who led the research, explained: "We had already demonstrated the applicability of FRET to show how mutation affects these phenomena in the wild-type channel.⁸ We guessed that in a mouse, for example, this channel opens and closes 400–600 times a minute, giving us dynamic rather than static interactions. In this scenario, immunoprecipitation did not work, which is where FRET comes in. We tagged the domain embedded in the membrane (C terminus) with cloned, naturally fluorescent green fluorescent protein (GFP) obtained from jellyfish, and the regulatory domain that sits on top of the other domain (N terminus), which acts something like a safety valve, with a red sea anemone protein (Fig 1).

"The way FRET works is that when fluorescent moieties are close together (within about 10 nm) they swap energy. For example, if red comes close to green then what you begin to see is red, so you can look at the ratio of green to red. As green dims, red becomes brighter (Fig 2). This enabled us to monitor movement in the molecule dynamically. Using FRET inside living cells we were able to look at the two halves of the molecule effectively 'clapping' together. Then we were able to take this procedure apart in terms of time and localisation. This lets you see where it happens and gives you a very accurate trace of what is going on in the molecule."

Looking at where to go next, Dr George said: "The work done so far has been on single cells. Now we need to find out what happens in a whole muscle. Do the channels within cells all 'jitter' in unison, or do they do different things at different times? The next stage is to carry out this work in intact hearts to see how the organ behaves as a whole.

"Catecholaminergic polymorphic ventricular tachycardia is an extremely rare condition for which, as yet, there is no genetic test for affected families. What is emerging is that there appears to be no rhyme nor reason that determines which individuals in a family will have a mutation.

"The literature shows that at least 60 mutations associated with the disorder have been identified so far, but of these only 14

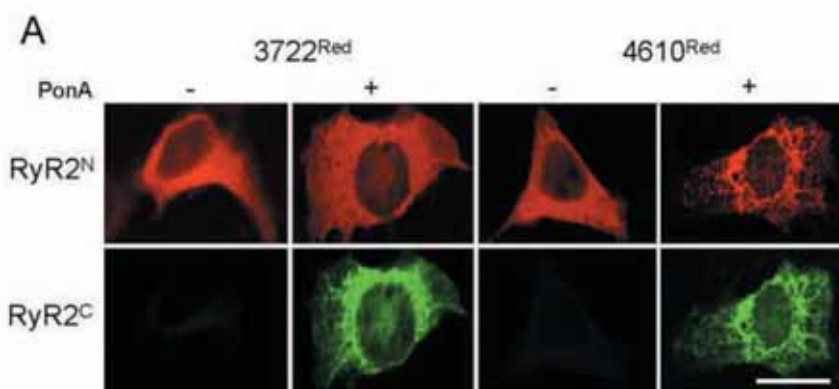


Fig 2. Sudden cardiac death-linked mutations do not perturb RyR2N/RyR2C interaction in resting cells. The intracellular localisation of eGFP-tagged RyR2C-RC and DsRed-tagged RyR2N-WT in the presence (+) or absence (-) of PonA induction was determined using confocal microscopy. PonA is a hormone used to trigger production of the calcium releasing (membrane-localised) pore. Scale bar represents 10 μ m. Reproduced from *Circ Res* 2006; **98**: 88–97, by kind permission.

‘Fluorescence resonance energy transfer technology was used inside living cells to look at the ryanodine receptors’

have been investigated more closely worldwide. At the moment, all we can say with any certainty is that the mechanism described here relates to only three of these. Of course, we need to look at all the other mutations, but this will take a huge amount of time. Now, there is also evidence that symptoms can occur in the absence of stress (eg while sleeping) and this introduces further confounding issues.

"However, we would argue that the way in which this channel goes wrong could be the same as dysfunctions in this channel in other disorders. We believe that we may be able to extrapolate this model to other diseases for which the end stage is SCD. Our data provide a reasonable rationale for explaining how an inappropriate response to a trigger translates into a physical instability."

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‘The FRET technique demonstrates the interaction between the calcium releasing pore and the regulatory domain of the ryanodine molecule’