Mutations in the ryanodine receptor 2 (RYR2) gene contribute to abnormal Ca^{2+} handling in cardiac myocytes, which, in turn, may produce various arrhythmias including catecholaminergic polymorphic ventricular tachycardia (CPVT). 1-3 CPVT is an inherited arrhythmia characterized by a normal electrocardiogram at rest and polymorphic ventricular tachycardia during stress and exercise, leading to a diagnosis of CPVT. 5

The serine-4153 residue in the ryr2 protein is conserved among all mammals including humans. This mutation occurs in a region widely regarded as a “hot spot” for CPVT mutations and is expected to alter the functional characteristics of the ryr2 channel, resulting in a gain of function. 10 Our analysis of published case reports, case series, and databases indicates that the S4153R mutation is novel. On these grounds and in combination with the distinct clinical features, it was argued that the mutation in the RYR2 gene was responsible for the ventricular and atrial arrhythmias in the patient. 5 However, Dzwieniel et al. 7 argued that the clinical and genetic evidence was insufficient to reach such a definitive conclusion. Here we characterized the effect of the S4153R mutation on...
spontaneous Ca\(^{2+}\) release in the human embryonic kidney 293 (HEK293) cell system and demonstrate that the S4153R mutation in the ryr2 protein produces abnormal Ca\(^{2+}\) handling.

**Methods**

For detailed methods, please see the Supplemental Appendix S1.

**Results**

S4153R mutation increases the propensity for store-overload-induced Ca\(^{2+}\) release

To determine whether the S4153R mutation is capable of affecting ryr2 channel function, we generated stable inducible HEK293 cell lines expressing the wild-type (WT) and mutant (SR) ryr2 channels. Cell lines showed comparable levels of protein expression (no significant difference; Fig. 1B). We used
these cell lines to assess properties of Ca\(^{2+}\) oscillations induced by progressively increasing the extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_e\)). Increasing [Ca\(^{2+}\)]\(_e\) resulted in Ca\(^{2+}\) oscillations in both WT and SR mutant cells (see representative oscillations for WT and S4153R in Fig. 1C). Plotting the fraction of oscillating cells (FOC) as a function of [Ca\(^{2+}\)]\(_o\) reveals that the propensity for Ca\(^{2+}\) oscillation increases in a concentration-dependent manner with an increase in [Ca\(^{2+}\)]\(_o\), (Fig. 1D). The S4153R mutant had half-activating concentration of 0.21mM, which was considerably lower than the half-activating concentration for WT cells (0.32mM), and the mutant had higher maximal activity (FOC\(_{max}\) = 0.924 for SR vs 0.772 for WT; Fig. 1D).

The S4153R mutation does not affect the Ca\(^{2+}\)-dependent activation of tritium-labelled ryanodine binding

Most of the gain-of-function CPVT-RYR2 mutations have little or no effect on tritium-labelled ryanodine binding.\(^8,9\) Consistent with these observations, we found that the mutation does not alter the Ca\(^{2+}\)-dependent activation of tritium-labelled ryanodine binding (Fig. 1E).

**S4153R mutation decreases the luminal Ca\(^{2+}\) threshold for store-overload-induced Ca\(^{2+}\) release**

The luminal Ca\(^{2+}\) threshold for store-overload-induced Ca\(^{2+}\) release (SOICR) was assessed by using the D1ER (a luminal Ca\(^{2+}\)-sensitive indicator protein) assay that monitors luminal Ca\(^{2+}\) levels in relation to maximal store level (F\(_{max}\); measured in the presence of tetracaine) and minimal store level (F\(_{min}\); measured in the presence of caffeine).\(^9\) Oscillation in response to 1mM to 2mM Ca\(^{2+}\)_o appeared in both WT and SR cells (see representative oscillations for WT and S4153R in Fig. 2A). S4153R had a significantly lower threshold of activation (WT, 87.6 ± 1.23%; S4153R, 80.9 ± 1.25%; P < 0.001, unpaired t test; Fig. 2B). There were no significant differences in fluorescence resonance energy transfer ratios F\(_{max}\) - F\(_{min}\) and ΔF (F\(_{max}\) - F\(_{min}\); Fig. 2C), that is, no change in maximal luminal Ca\(^{2+}\), minimal luminal Ca\(^{2+}\), and overall store capacity because of the mutation in RYR2.

**Discussion**

A number of RYR2 mutations (R176Q/T2504M, S2246L, N4104K, Q4201R, R4496C, V4653F, and N4895D) have been linked to ventricular tachycardia and sudden death.\(^1,4\) These mutations have been functionally characterized.\(^8,9\) All of them share a number of common changes, such as enhanced propensity for spontaneous Ca\(^{2+}\) release and reduced threshold for SOICR (Table 1). The newly found mutation (S4153R), due to a missense mutation of the RYR2 gene (A12457C), is located in a highly conserved region (amino acids 3778 to 4201)\(^4\) and exhibits the same characteristics of gain-of-function RYR2 mutation, albeit at more moderate degrees. For example, N4104K, R4496C, V4653F, and N4895D had half-maximal [Ca\(^{2+}\)]\(_o\) which was 3-fold to 4-fold lower, whereas S4153R had half-maximal [Ca\(^{2+}\)]\(_o\) only about 1.5-fold lower than WT. Similarly, reduction in threshold of activation by luminal Ca\(^{2+}\) for S4153R was considerably smaller in comparison with other characterized mutations (Table 1). Paradoxically, despite only modest alterations in RYR2 function, the S4153R mutation was found clinically to be associated with AF.\(^5\)

Atrial myocytes exhibit higher SR Ca\(^{2+}\) content and cellular Ca\(^{2+}\)-buffering capacity than do ventricular myocytes, which is consistent with enhanced SR Ca\(^{2+}\) reuptake via sarco/endoplasmic reticulum Ca\(^{2+}\) ATPase.\(^10\) Therefore, RYR2 mutations with modest reductions in luminal threshold will likely produce sufficient triggered Ca\(^{2+}\) release required for the formation of after-depolarizations in the atria resulting in paroxysmal AF. Composite heterozygote mutation M4109R/I406T has been reported to produce triggered AF

**Table 1. Comparison of changes in luminal threshold for different RYR2 mutations and their respective clinical manifestation**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Decrease in luminal threshold in relation to wild-type</th>
<th>Clinical manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R176Q/T2504M</td>
<td>14.1 ± 0.75%(^9)</td>
<td>CPVT(^9)</td>
</tr>
<tr>
<td>S2246L</td>
<td>17.7 ± 1.2%(^9)</td>
<td>CPVT(^1)</td>
</tr>
<tr>
<td>Q4201R</td>
<td>16.5 ± 1.1%(^9)</td>
<td>CPVT(^15)</td>
</tr>
<tr>
<td>V4653F</td>
<td>11.6 ± 0.85%(^8)</td>
<td>CPVT(^13)</td>
</tr>
<tr>
<td>S4153R</td>
<td>7.65 ± 1.4%</td>
<td>AF and CPVT(^15)</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; CPVT, catecholaminergic polymorphic ventricular tachycardia.
leading to ventricular fibrillation.\textsuperscript{11} Although the M4109R mutation was shown to affect Ca\textsuperscript{2+} handling,\textsuperscript{12} no data are available in regard to luminal Ca\textsuperscript{2+} sensitivity of the M4109R channels. Our results highlight a key role of the ryrr2 protein and altered Ca\textsuperscript{2+} cycling in AF.\textsuperscript{13} It is interesting that murine models harboring CPVT mutations have recently been shown to be susceptible to exercise- and stress-induced AF.\textsuperscript{14}

**Conclusion**

The novel RYR2-S4153R mutation exhibits typical properties of gain-of-function RYR2 mutations that have been linked to CPVT. The RYR2-S4153R mutation is the first functionally characterized mutation linked to both CPVT and AF and underscores the importance of Ca\textsuperscript{2+} dysregulation as a fundamental mechanism for both atrial and ventricular tachyarrhythmias.

**Acknowledgements**

P.Z. and F.H. contributed to this work equally.

**Funding Sources**

This research was funded by Canadian Institutes of Health Research and Heart and Stroke Foundation of Canada operating grants to S.R.W.C. and G.Y.O.

**Disclosures**

The authors have no conflicts of interest to disclose.

**References**


**Supplementary Material**

To access the supplementary material accompanying this article, visit the online version of the Canadian Journal of Cardiology at www.onlinecjc.ca and at http://dx.doi.org/10.1016/j.cjca.2012.12.019.