S4153R Is a Gain-of-Function Mutation in the Cardiac Ca\(^{2+}\)-Release Channel Ryanodine Receptor Associated With Catecholaminergic Polymorphic Ventricular Tachycardia and Paroxysmal Atrial Fibrillation

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**Abstract**

Mutations in ryanodine receptor 2 (RYR2) gene can cause catecholaminergic polymorphic ventricular tachycardia (CPVT). The novel RYR2-S4153R mutation has been implicated as a cause of CPVT and atrial fibrillation. The mutation has been functionally characterized via store-overload-induced Ca\(^{2+}\) release (SOICR) and tritium-labelled ryanodine ([\(^3\)H]ryanodine) binding assays. The S4153R mutation enhanced propensity for spontaneous Ca\(^{2+}\) release and reduced SOICR threshold but did not alter Ca\(^{2+}\) activation of [\(^3\)H]ryanodine binding, a common feature of other CPVT gain-of-function RYR2 mutations. We conclude that the S4153R mutation is a gain-of-function RYR2 mutation associated with a clinical phenotype characterized by both CPVT and atrial fibrillation.

**Résumé**

Les mutations du gène du récepteur de la ryanodine de type 2 (RYR2) peuvent causer une tachycardie ventriculaire polymorphe catécholaminergique (TVPC). La nouvelle mutation du RyR2-S4153R serait une cause de TVPC et de fibrillation auriculaire. La mutation a été fonctionnellement caractérisée par la libération de Ca\(^{2+}\) induite par une surcharge du stock calcique (SOICR : store-overload-induced Ca\(^{2+}\) release) et les analyses de liaison de la ryanodine marquée au tritium ([\(^3\)H]ryanodine). La mutation du S4153R a amélioré la propension à la libération spontanée de Ca\(^{2+}\) et a réduit le seuil de SOICR, mais n’a pas altéré l’activation de Ca\(^{2+}\) de la liaison [\(^3\)H]ryanodine, une caractéristique commune à d’autres mutations « gain de fonction » du RyR2 liées à la TVPC. Nous concluons que la mutation du S4153R est une mutation « gain de fonction » du RyR2 associée au phénotype clinique caractérisé par la TVPC et la fibrillation auriculaire.
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\(^{2+}\)]\(_o\)). Increasing [Ca\(^{2+}\)]\(_o\) 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\(_{\text{max}}\) = 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-\(R Y R 2\) 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\(_{\text{max}}\)) measured in the presence of tetracaine) and minimal store level (F\(_{\text{min}}\)) 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\(_{\text{max}}\)/F\(_{\text{min}}\) and ΔF (F\(_{\text{max}}\)−F\(_{\text{min}}\); Fig. 2C), that is, no change in maximal luminal Ca\(^{2+}\), minimal luminal Ca\(^{2+}\), and overall store capacity because of the mutation in \(R Y R 2\).

Discussion

A number of \(R Y R 2\) 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 \(R Y R 2\) gene (A12457C), is located in a highly conserved region (amino acids 3778 to 4201)\(^4\) and exhibits the same characteristics of gain-of-function \(R Y R 2\) 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 \(R Y R 2\) 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/endooplasmic reticulum Ca\(^{2+}\) ATPase.\(^10\) Therefore, \(R Y R 2\) 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/I4067T has been reported to produce triggered AF

**Figure 2.** Oscillations of luminal Ca\(^{2+}\) in response to rising extracellular Ca\(^{2+}\) concentration. (A) Representative oscillations of luminal Ca\(^{2+}\) for human embryonic kidney 293 (HEK293) cells expressing either wild-type (WT) \(r y r 2\) protein (left) or mutant (S4153R) \(r y r 2\) protein (right), expressed as a fraction between the lowest level of the Ca\(^{2+}\) stores (F\(_{\text{min}}\)) achieved in 2mM extracellular Ca\(^{2+}\) (Ca\(^{2+}\)\(_o\)) + 20mM caffeine (CAF) and maximum level (F\(_{\text{max}}\)) achieved in 1.5mM tetracaine (TET). (B) Activation threshold (A), termination threshold (T), and fractional release (F\(_{\text{R}}\)) for WT (white bars) and S4153R (SR, grey bars) cells. Measurements where done as shown in A (35 cells in 5 coverslips per group). * \(P < 0.05\) for unpaired \(t\) test. (C) Maximal level of fluorescence (F\(_{\text{max}}\)) for 1.5mM tetracaine, minimal level of fluorescence (F\(_{\text{min}}\)) for 2mM Ca\(^{2+}\)\(_o\) + 20mM CAF, and the difference between F\(_{\text{max}}\) and F\(_{\text{min}}\) (full release, ΔF), expressed as raw fluorescence resonance energy transfer (FRET) signal (au, arbitrary units). Error bars represent standard error of the mean.

Table 1. Comparison of changes in luminal threshold for different \(R Y R 2\) 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>
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<tbody>
<tr>
<td>R176Q/T2504M</td>
<td>14.1 ± 0.75%(^8)</td>
<td>CPVT(^7)</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(^1)</td>
</tr>
<tr>
<td>V4653F</td>
<td>11.6 ± 0.8%(^9)</td>
<td>CPVT(^1)</td>
</tr>
<tr>
<td>N4104K</td>
<td>8.65 ± 1.4%(^9)</td>
<td>AF and CPVT(^3)</td>
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AF, atrial fibrillation; CPVT, catecholaminergic polymorphic ventricular tachycardia.
leading to ventricular fibrillation. 11 Although the M4109R mutation was shown to affect Ca\(^{2+}\) handling, 12 no data are available in regard to luminal Ca\(^{2+}\) sensitivity of the M4109R channels. Our results highlight a key role of the \(r\)y\(r\)2 protein and altered Ca\(^{2+}\) cycling in AF. 13 It is interesting that murine models harboring CPVT mutations have recently been shown to be susceptible to exercise- and stress-induced AF. 14

**Conclusion**

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

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

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**Disclosures**

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**References**


**Supplementary Material**

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