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Alterations in the Interactome of Serine/Threonine Protein Phosphatase Type-1 in Atrial Fibrillation Patients

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Abstract

BACKGROUND—Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, yet current pharmacological treatments are limited. Serine/threonine protein phosphatase type-1 (PP1), a major phosphatase in the heart, consists of a catalytic subunit (PP1c) and a large set of regulatory (R)-subunits that confer localization and substrate specificity to the holoenzyme. Previous studies suggest that PP1 is dysregulated in AF, but the mechanisms are unknown.

OBJECTIVES—The purpose of this study was to test the hypothesis that PP1 is dysregulated in paroxysmal atrial fibrillation (PAF) at the level of its R-subunits.

METHODS—Cardiac lysates were coimmunoprecipitated with anti-PP1c antibody followed by mass spectrometry–based, quantitative profiling of associated R-subunits. Subsequently, label-free quantification (LFQ) was used to evaluate altered R-subunit–PP1c interactions in PAF patients. R-subunits with altered binding to PP1c in PAF were further studied using bioinformatics, Western blotting (WB), immunocytochemistry, and coimmunoprecipitation.

RESULTS—A total of 135 and 78 putative PP1c interactors were captured from mouse and human cardiac lysates, respectively, including many previously unreported interactors with conserved PP1c docking motifs. Increases in binding were found between PP1c and PPP1R7,

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APPENDIX For supplemental methods, tables, and a figure, please see the online version of this paper.

cold-shock domain protein A (CSDA), and phosphodiesterase type-5A (PDE5A) in PAF patients, with CSDA and PDE5A being novel interactors validated by bioinformatics, immunocytochemistry, and coimmunoprecipitation. WB confirmed that these increases in binding cannot be ascribed to their changes in global protein expression alone.

CONCLUSIONS—Subcellular heterogeneity in PP1 activity and downstream protein phosphorylation in AF may be attributed to alterations in PP1c–R-subunit interactions, which impair PP1 targeting to proteins involved in electrical and Ca^{2+} remodeling. This represents a novel concept in AF pathogenesis and may provide more specific drug targets for treating AF.

Keywords

atrial fibrillation; label-free quantification; mass spectrometry; PP1 regulatory subunits; protein phosphatase 1; proteomics

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia. Although current drugs improve the functional capacity and quality of life, many are proarrhythmic and some increase mortality (1), pointing to a lack in the understanding of AF pathogenesis. Various mechanisms contribute to structural, electrical, and Ca^{2+}-handling remodeling in AF, which provide a platform for AF pathogenesis (2,3). Recent studies have revealed that abnormal phosphorylation levels of various ion channels and Ca^{2+} transporters are causally associated with AF development (2,4–6). Although several studies have implicated enhanced CaMKII activity as a potential cause of increased protein phosphorylation in AF (2,7), it has remained unclear why there is great heterogeneity in protein phosphorylation in (2,5,6)

Protein phosphatases (PPs) play a key role in regulating the phosphorylation level of ion channels and Ca^{2+}-handling proteins in the heart (6). Serine/threonine protein phosphatase type-1 (PP1) is a major PP that is expressed ubiquitously in the heart where it has a wide range of cellular targets (6,8). The PP1 holoenzyme consists of a catalytic subunit (PP1c) and a large set of close to 200 regulatory subunits (R-subunits) (8,9). Because there are only a few different PP1c isoforms, all of which share a high degree of homology, the spatial and temporal specificity of PP1 for different targets is largely regulated by association with these R-subunits.

A number of studies have shown that the global expression and activity levels of PP1 are increased in patients with chronic AF, associated with inhomogeneous changes of protein phosphorylation levels across different subcellular compartments (4–6). For example, although the Ca^{2+}-release channel ryanodine receptor type-2 (RyR2) is hyperphosphorylated, the L-type Ca^{2+} channel is hypophosphorylated in AF patients (4,7). By contrast, another study did not find any changes in PP1c expression levels in samples from patients with paroxysmal AF (PAF) (10). In experimental AF models, both unchanged PP1c levels with or without increased PP1c activity have been reported (6). These apparently contradictory findings may be due to the fact that PP1 is regulated at the level of its R-subunits, which underlie the heterogeneity in protein phosphorylation patterns within atrial myocytes.

The goal of our study was to assess the importance of PP1 R-subunits in PAF patients, because atrial remodeling is often still limited in such patients (10). We developed a novel proteomic method to quantify the levels of PP1c-bound R-subunits to characterize the full extent of the PP1-interactome in the human atria. This unbiased approach revealed extensive changes in the binding of various R-subunits to PP1c in PAF patients. This finding suggests that remodeling of the PP1 interactome could be one of the main causes of subcellular heterogeneity in protein phosphorylation associated with AF pathogenesis. A better understanding of these R-subunits may therefore lead to novel classes of drugs for treating AF.

METHODS

Animal studies were performed according to protocols approved by the institutional animal care and use committee at Baylor College of Medicine. Human right atrial appendages were collected with patients' written informed consent under protocols approved by the ethics committee of the Medical Faculty Mannheim, University Heidelberg (No.2011–216N-MA). Experimental details are provided in the Online Appendix. The mass spectrometry (MS) data are available online (11).

RESULTS

IDENTIFICATION AND LABEL-FREE QUANTIFICATION OF PP1C INTERACTORS

To study PP1 at the level of the R-subunits, we developed a method for profiling these R-subunits in an unbiased way in vivo on the basis of label-free quantification (LFQ) (Figure 1, Online Appendix). We optimized the protocol using lysates from mouse ventricles before using smaller atrial samples from 4 sinus rhythm patients (Online Table 1). We identified and quantified 483 and 145 proteins from mouse and human cardiac lysates, respectively. Of these, 133 and 78 proteins were at least 3-fold enriched with PP1c pull-down, indicative of specific binding to PP1c (Figure 2). To date, this is the largest number of putative PP1c interactors identified in cardiac tissue.

Of the 133 proteins differentially enriched from mouse hearts, 13 are known PP1 R-subunits with a "PPP1R" designation (i.e., PP1 regulatory subunit followed by a unique number) according to the HUGO Gene Nomenclature Committee (orange circles in Figure 2A) and 4 are R-subunits or interactors that do not yet have a PPP1R designation (blue circles in Figure 2A) (9). In addition, there are 2 other known interactors, Pfkm and Hspb6, that fell below the 3-fold cutoff (dotted line in Figure 2A), suggesting that these do not, or only partially, interact with PP1c in mouse hearts (Online Table 2). The remaining 116 are putative interactors that were not reported previously. Similarly, of the 78 proteins identified from human atria (Online Table 3), 7 are known PP1 R-subunits with a PPP1R designation, 2 are R-subunits or interactors that do not have a PPP1R designation, and the remaining 69 are putative interactors not previously reported (Figure 2B).

ANALYSES OF PP1C INTERACTORS

First, we analyzed the known R-subunits by determining their relative binding to PP1c. To do so, we normalized their LFQ intensities to their respective sequence lengths, because a

larger protein will generally be digested into more peptides analyzable by MS, resulting in a higher LFQ intensity (12). For the mouse sample, the top PP1c interactors or R-subunits which occupy the most number of PP1c molecules were Ppp1r7, Ppp1r2, and Ppp1r11, in descending order. For human atria, they were PPP1R7, PPP1R18, PPP1R2, and PPP1R8 (Figures 3A and 3B). Of these, PPP1R7 and PPP1R2 were consistently the most highly PP1c-bound R-subunits between mouse and human. Conversely, we did not detect PPP1R1A (or protein inhibitor-1 [I-1]) binding to PP1c, most likely because of its low abundance in the heart (13) and/or the fact that I-1 only interacts with PP1c when it is phosphorylated at T35 by PKA (8).

Next, we analyzed all the putative PP1c interactors in terms of 3 known PP1c docking motifs: RVxF, MyPhoNE, and SILK (14). Of the 135 PP1c interactors identified from mouse hearts, 105 have at least 1 of the motifs, with 53, 68, and 45 having the RVxF, MyPhoNE, and SILK motifs, respectively (Figure 3C, Online Table 2). Similarly, of the 78 PP1c interactors identified from human atria, 60 have at least 1 of the motifs, with 39, 42, and 27 having the RVxF, MyPhoNE, and SILK motifs, respectively (Figure 3C, Online Table 3). Of these, most have more than 1 motif, and some have all 3 motifs (Figures 3D and 3E).

In addition to bioinformatics, we further validated our MS method in vitro by cotransfecting 3 putative PP1c interactors, protein transport protein Sec31A (SEC31A), valosin-containing protein (VCP), and cold-shock domain protein A (CSDA), with PP1c in HEK293 cells. Using these samples, coimmunoprecipitation followed by Western blot (WB) showed a specific interaction between PP1c and these novel interactors, confirming our MS findings (Figure 4).

CHANGES IN PP1C INTERACTOME IN PAF PATIENTS

Because PP1c expression is altered (along with other extensive electrical and structural remodeling) in chronic AF, but not PAF (5), samples from PAF patients were used in this study to avoid potential confounders. Applying our new methodology, we discovered 3 specific alterations in the PP1c interactome: PPP1R7 (1.59-fold; $p = 0.044$), CSDA (5.67-fold; $p = 0.0010$), and phosphodiesterase type-5A (PDE5A) (1.75-fold; $p = 0.049$) all had significantly increased binding to PP1c in PAF (Figure 5, Online Table 4). Because PPP1R7 is the top interactor of PP1c in human atria as shown in Figure 2B with the highest LFQ signal and Figure 3B with the largest pie piece (43%), a 1.59-fold increase in this binding between PPP1R7 and PP1c translates to a large change in the PP1c interactome (e.g., 43% × 1.59 = 68%). Furthermore, we validated this increase in PP1c–PPP1R7 interaction using WB as shown in Figures 6A and 6B, even though WB detection is not nearly as sensitive or quantitative as MS.

Bioinformatic analyses showed that PDE5A harbored all 3 of the PP1c docking motifs (Online Tables 2 and 3), unlike CSDA, which may bind to PP1c through alternative means (Figure 4C) (9). Nevertheless, CSDA does have the highest increase in PP1c binding in PAF (Figures 5A and 5B), making it a potential key regulator of PP1 action in PAF.

To correlate these changes in PP1c binding to global protein levels, we performed WB and found that, relative to PP1c's global protein level, PPP1R7 and CSDA are unchanged, whereas PDE5A is up-regulated by 1.35-fold in PAF patients ($p < 0.05$) (Figures 6C and 6D). This suggests increases in binding of both PPP1R7 and CSDA to PP1c (Figure 5B) are independent of their global levels, which remained unchanged in PAF (Figures 6C and 6D). By contrast, the increase in binding between PDE5A and PP1c may be partially attributed to the increase in PDE5A's global level. When PDE5A's increase in binding (1.75-fold) is normalized to its increase in global level (1.35-fold), there is still a positive ratio of 1.3-fold. This suggests that some increase in binding between PDE5A and PP1c is independent of the increase in PDE5A's global level. Taken together, these findings may indicate that although PP1 is relatively unchanged in its global level in PAF (Figures 6C and 6D) (10), it is dysregulated at the level of at least 3 R-subunits/interactors.

TARGETING FUNCTION OF PP1C INTERACTORS

To further validate the 2 novel PP1c interactors CSDA and PDE5A, immunocytochemistry was performed using isolated mouse atrial myocytes with PPP1R7 as a positive control. We found that both CSDA and PDE5A localized to the periphery of the isolated myocytes, unlike PPP1R7, which stained mostly intracellularly (Figure 7). However, all 3 colocalized with Ppp1ca (PP1c). Of note, CSDA costained more strongly with PPP1CA at the periphery than PDE5A, whereas the latter costained more strongly with PPP1CA intracellularly (Figure 7). To assess whether these findings were atrial specific, we performed the same experiments in isolated mouse ventricular myocytes and found the same costaining patterns (Online Figure 1). Together, these results suggest that each interactor targets PP1c to distinct subcellular compartments where PP1c may dephosphorylate different target proteins.

To further test this hypothesis, we transfected HeLa cells with FLAG-tagged CSDA and performed immunocytochemistry to see how this may affect the subcellular localization of PP1c. Figure 8 shows that cells that were transfected with FLAG-CSDA demonstrated a distinct PP1c staining pattern compared with nontransfected cells or to cells transfected with the empty vector. Specifically, the white arrows in Figure 8 point to nuclear or perinuclear aggregations of PP1c, which are absent without CSDA and which colocalize perfectly with CSDA. This supports the hypothesis that these interactors target PP1c to distinct subcellular compartments, as summarized in the Central Illustration.

DISCUSSION

This study provides the first characterization of the extensive remodeling of the PP1c interactome in the atria of PAF patients. Using a novel proteomic method, we found that PP1c is dysregulated in PAF patients by altered binding to at least 3 interactors: PPP1R7, CSDA, and PDE5A. In addition, we identified 135 and 78 PP1c interactors in vivo from mouse and human cardiac tissues, respectively, and analyzed them according to the most common PP1c docking motifs, providing further insights into the mechanisms by which PP1 is regulated by these R-subunits/interactors. Taken together, our study suggests that alterations in the PP1c interactome via R-subunits may contribute to subcellular heterogeneities underlying AF pathogenesis.

COMPARISON TO PREVIOUS STUDIES ON PP1-REGULATORY SUBUNITS IN HEART

More than one-half of all human proteins undergo reversible phosphorylation, the dysregulation of which drives the pathogenesis of cardiac disease (6). To balance the action of a plethora of kinases, the limited number of PP catalytic subunits rely on their numerous R-subunits to convey localization and substrate specificity (8). Because R-subunits are at the crux of deciphering PP1 regulation, studies have increasingly recognized the need and importance of investigating individual R-subunits (15–19). However, these studies are few and far between, limited to specific R-subunits on the basis of prior (biased) knowledge.

The most-studied R-subunits in the heart are two PP1 inhibitors, PPP1R1A (I-1) and PPP1R2 (I-2), which were investigated in the setting of heart failure (HF) (6). To date, the functional roles of I-1 and I-2 with respect to global PP1c activity remain controversial. Some studies suggest that decreased I-1 or I-2 and the correspondingly increased PP1c activity drive HF progression (13,20–25). Others have found the opposite and suggest that decreased PP1c activity negatively affects the heart (26,27). All of these studies only measured or manipulated the global levels of I-1, I-2, or PP1c, all of which may have affected many cellular processes and thereby confounded the findings. A more precise way to tease apart the many roles of PP1 in HF would be to examine its entire interactome and identify hotspots of dysregulation, as we have done for PAF patients in this study and for cyclic adenosine monophosphate (cAMP) nodes in HF in another recent study (28). The methodology developed in this study may be easily adapted to study HF in both animal models and human patients.

POTENTIAL IMPLICATIONS FOR TARGETED DEPHOSPHORYLATION OF CARDIAC PROTEINS

In addition to I-1 and I-2, previous studies have identified a few other PP1 R-subunits that may play key roles in cardiac physiology and pathophysiology, such as PPP1R9B (spinophilin) and PPP1R3A (R_{GL}), which target PP1c to RyR2 and phosphoamban, respectively (6,19). To the best of our knowledge, only 6 such R-subunits had been studied in cardiomyocytes; our present study doubles this number with validated R-subunits as summarized in the Central Illustration. Furthermore, we identified another 67 putative R-subunits from human atria and more than 100 from mouse ventricles (Figures 2 and 3, Online Tables 2 and 3). It is highly likely that some of these novel R-subunits target PP1c to key cardiac proteins with currently unknown R-subunits, such as Na^+/Ca^{2+} exchanger and voltage-dependent Na^+ channel (Central Illustration) (6). Our study provides the relative binding levels of these R-subunits to PP1c (Figures 3A and 3B), which may be useful for future drug design considerations.

One of the previously unappreciated R-subunits in the heart is PPP1R7 (SDS22), which turned out to be the most highly bound R-subunit in both mouse ventricles and human atria (Figures 3A and 3B). PPP1R7 is required for cell-cycle progression or mitosis completion in yeast by targeting PP1c to the nucleus (8,29), which is consistent with our immunocytochemistry finding (Figure 7A, Online Figure 1A). However, PPP1R7 is highly expressed in the heart (Figure 6B) and is abundantly associated with PP1c (Figures 3A and 3B). Furthermore, the binding between PPP1R7 and PP1c is increased in PAF (Figures 5B,

6A, and 6B), yet its role in the heart is still virtually unknown. Future studies are needed to determine the precise role of PPP1R7 in both cardiac physiology and pathophysiology.

NOVEL FINDINGS AND POTENTIAL CLINICAL IMPLICATIONS

We discovered numerous novel PP1c interactors, 2 of which showed up-regulated binding to PP1c in PAF (Figure 5B). CSDA is a member of the protein family containing the highly conserved coldshock domain and are considered as transcriptional and translational regulators (30,31). As such, it is perceivable that the increase in CSDA–PP1c association may underlie some of the transcriptional changes observed in PAF and perhaps drive the eventual electrical and structural remodeling in chronic AF (32,33). However, other than one study showing by Northern blot that CSDA is highly expressed in cardiac muscles (34), its role in the heart is completely unknown. Here, we found that CSDA is not only a novel PP1c interactor but that it localizes specifically to the sarcolemma in isolated cardiomyocytes (Figure 7, Online Figure 1). This is different from its cytoplasmic and nuclear localization in HeLa cells (Figure 8) (34), suggesting an additional and previously unappreciated role for CSDA, particularly in the context of PAF.

PDE5A was the first cyclic guanosine mono phosphate (cGMP)-selective phosphodiesterase discovered, and it is expressed mainly in vascular smooth muscle, endothelium, and fibroblast. Its expression in cardiomyocytes is controversial (35). It is generally believed that PDE5A localizes to Z-disks. This is consistent with our findings in isolated myocytes, although we also detected a significant level at the sarcolemma (Figure 7C, Online Figure 1) (35). In cardiac hypertrophy and HF, PDE5A is up-regulated to reduce cGMP and PKG activity, which are associated with oxidative stress (35). In our study, we also detected both a global up-regulation as well as an increased binding to PP1c in PAF (Figures 5B, 6C, and 6D), similar to HF. On the other hand, another study suggested that PDE4's activity on cAMP is protective against atrial arrhythmia and showed that it decreases with age and in chronic AF patients (36). The fact that PDE5A is up-regulated both globally and in association with PP1c may point to a compensatory mechanism early in AF pathogenesis.

STUDY LIMITATIONS

This study is limited by the sample size of the atrial biopsies and by inherent variability among the patients. Consequently, we were not able to identify and quantify as many interactors as we did with mouse samples. The inherent variability in these samples may have masked the subtler, but potentially important, changes in some of the PP1c interactors. This study is also limited by the number of samples/patients used for each of the MS and biochemical experiments, and this diminished our overall ability to detect small changes and increased the probability of false positives. Future studies with larger sample sizes are needed to confirm our findings. Finally, the sinus rhythm and PAF patients are not perfectly matched in terms of age and sex although the differences are not statistically significant ($p > 0.05$) (Online Table 1).

CONCLUSIONS

In this study, we demonstrated that PP1 is dysregulated in PAF at the level of protein–protein interaction with R-subunits that modify PP1 enzymatic activity and/or subcellular targeting. We identified 3 major alterations in the PP1c interactome that may play a role in AF pathogenesis. In addition, we developed a novel, powerful technique for capturing and quantifying the PP1c interactome directly from cardiac tissues. This technique enabled us to identify 135 and 78 PP1c interactors in vivo from mouse ventricles and human atria, respectively. Overall, we present a rich dataset that may open up new avenues of research toward the understanding of both cardiac physiology and AF pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS AND ACRONYMS

AF	atrial fibrillation
CSDA	cold-shock domain protein A
HF	heart failure
I-1	protein inhibitor 1
I-2	protein inhibitor 2
LFQ	label-free quantification
MS	mass spectrometry
PAF	paroxysmal atrial fibrillation
PDE5A	phosphodiesterase type-5A
PP	protein phosphatase
PP1	serine/threonine protein phosphatase type-1

PP1c	PP1 catalytic subunit
R-subunit	regulatory subunit
WB	Western blot

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The interactome of PP1 is dysregulated in patients with paroxysmal atrial fibrillation at the level of protein–protein interaction between the PP1 catalytic subunit and its regulatory subunits. It is now possible to study the PP1 interactome directly from human cardiac tissues.

TRANSLATIONAL OUTLOOK: Clinical studies of PP1 in atrial fibrillation and other cardiac disorders might allow targeting of molecular sites of dysregulation for the development of novel therapeutic strategies.

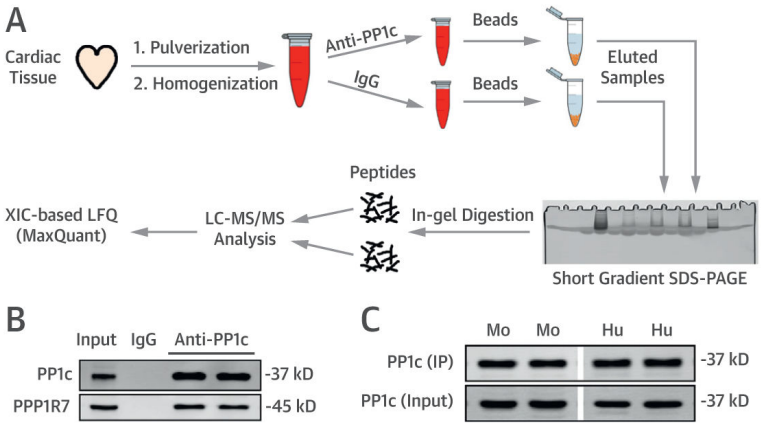


FIGURE 1. A Novel Unbiased Method for Profiling PP1c Interactors

(A) Schematic showing the major methodological steps. **(B)** Western blot validation of the coimmunoprecipitation of PP1c and 1 known R-subunit (PPP1R7) from mouse ventricular lysates. **(C)** Western blot demonstrating equal efficiency of anti-PP1c antibody in pulling down mouse (Mo) and human (Hu) PP1c. LC = liquid chromatography; LFQ = label-free quantification; MS/MS = tandem mass spectrometry; XIC = extracted-ion chromatogram.

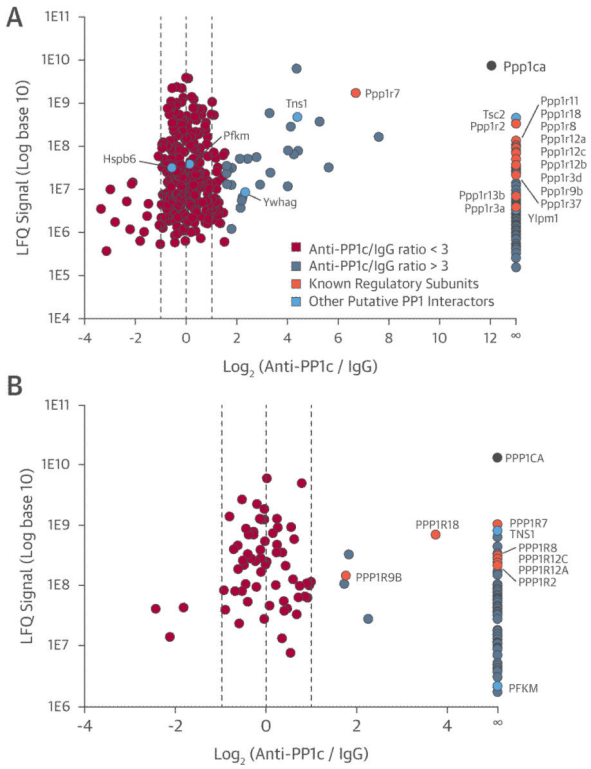


FIGURE 2. Identification and LFQ of PP1c Interactors

Cardiac lysates made from mouse ventricles **(A)** or human atria **(B)** were immunoprecipitated with anti-PP1c versus isotype-control IgGs and analyzed by mass spectrometry. The y-axis plots the label-free quantification (LFQ) signals of each protein from the anti-PP1c pull-down. The x-axis plots the ratios of the LFQ signals from the anti-PP1c pull-down over that from the control pull-down. Proteins with a ratio >3 are considered PP1c interactors. Proteins with an infinite ratio (∞) have no LFQ signals from the control pull-down. Known PP1c interactors are labelled with their gene names, whereas the baits Ppp1ca and PPP1CA are marked by a **black circle**.

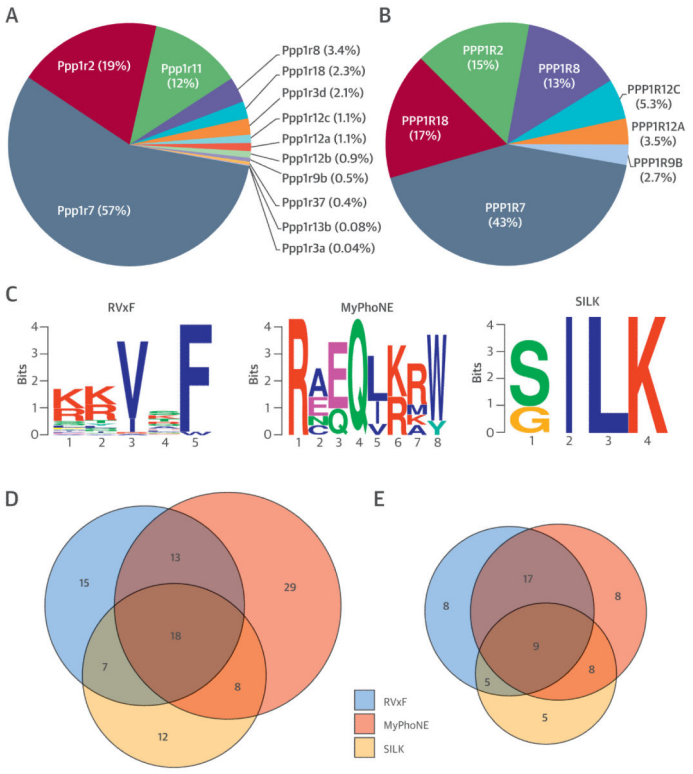


FIGURE 3. Bioinformatic Analyses of PP1c Interactors

Relative binding of known PP1 regulatory subunits to PP1c on the basis of LFQ signal intensities normalized to sequence length of each protein for mouse ventricles **(A)** and human atria **(B)**. **(C)** Sequence LOGOs of the 3 most common PP1c docking motifs (KVxF, MyPhoNE, and SILK) (9) generated from validated PP1c interactors using MEME. The E-values of the 3 motifs are 5.9E–4, 2.3E–6, and 3.8E3, respectively. PP1c interactors that have at least 1 of these motifs were plotted in Venn diagrams for mouse ventricles **(D)** and human atria **(E)**. LFQ = label-free quantification.

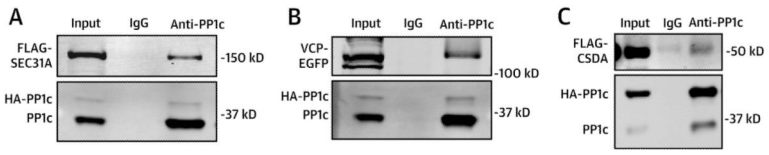


FIGURE 4. In Vitro Validation of MS Findings

Coimmunoprecipitation followed by Western blots were performed using lysates from HEK293 cells that were cotransfected with 1 of 3 putative PP1c interactors, SEC31A **(A)**, VCP **(B)**, and CSDA **(C)**, and HA-tagged PP1c. SEC31A and CSDA were FLAG tagged, whereas VCP was EGFP tagged. CSDA = cold-shock domain protein A; LFQ = label-free quantification; MS = mass spectrometry; SEC31A = protein transport protein Sec31A; VCP = valosin-containing protein.

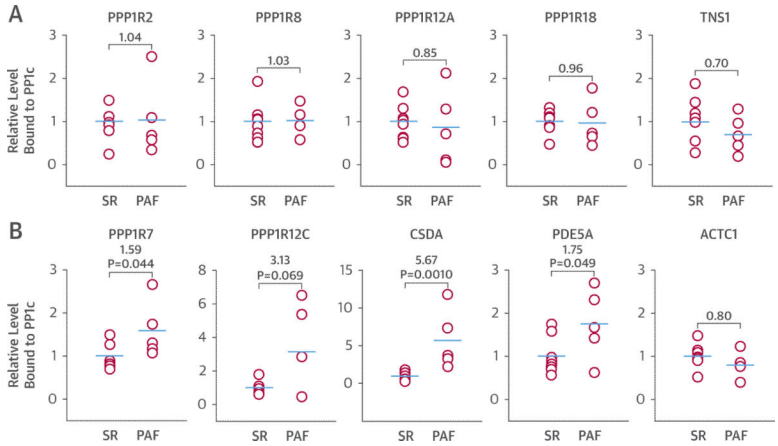


FIGURE 5. Comparison of Binding Between PP1c and Known or Novel Interactors in SR versus PAF Patients

(A) Known interactors that are unchanged in their binding to PP1c. **(B)** Interactors that are changed in their binding to PP1c in paroxysmal atrial fibrillation (PAF) patients (n = 5), with CSDA and PDE5A being novel PP1c interactors. Quantification is on the basis of LFQ signals, and ACTC1 is used as an internal control. For SR patients, n = 7 to 8. ACTC1 = actin-1; PDE5A = phosphodiesterase type-5A; SR = sinus rhythm; TNS1 = tensin-1; other abbreviations as in Figure 4.

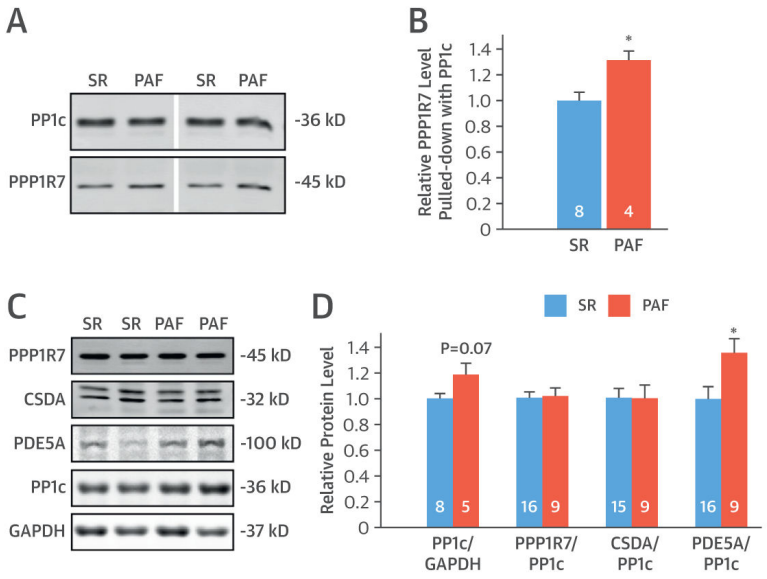


FIGURE 6. Global Levels of Select PP1c Interactors in SR Versus PAF Patients

(A and B) PP1c immunoprecipitation followed by Western blots confirming the increase in binding between PP1c and PPP1R7 in paroxysmal atrial fibrillation (PAF) patients. **(C and D)** Representative Western blots and quantification showing the global protein levels of the 3 interactors (PPP1R7, CSDA, and PDE5A) relative to that of PP1c. *$p < 0.05$ versus sinus rhythm (SR). Numbers in the bars indicate the numbers of patients per group. Abbreviations as in Figures 4 and 5.

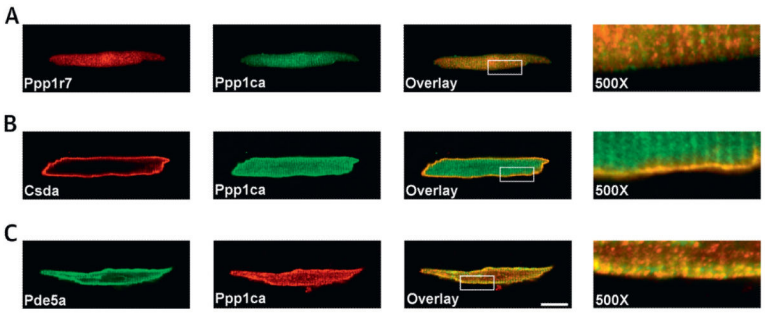


FIGURE 7. Colocalization of Ppp1ca (PP1c) and Its Interactors

Atrial myocytes isolated from adult mice were costained with different antibodies to show the colocalization between PPP1CA (PP1c) and 3 of its interactors: PPP1R7 **(A)**, CSDA **(B)**, and PDE5A **(C)**. Representative images were chosen from 10 to 12 cells from 2 to 3 mice. Scale bar = 20 mm. Abbreviations as in Figures 4 and 5.

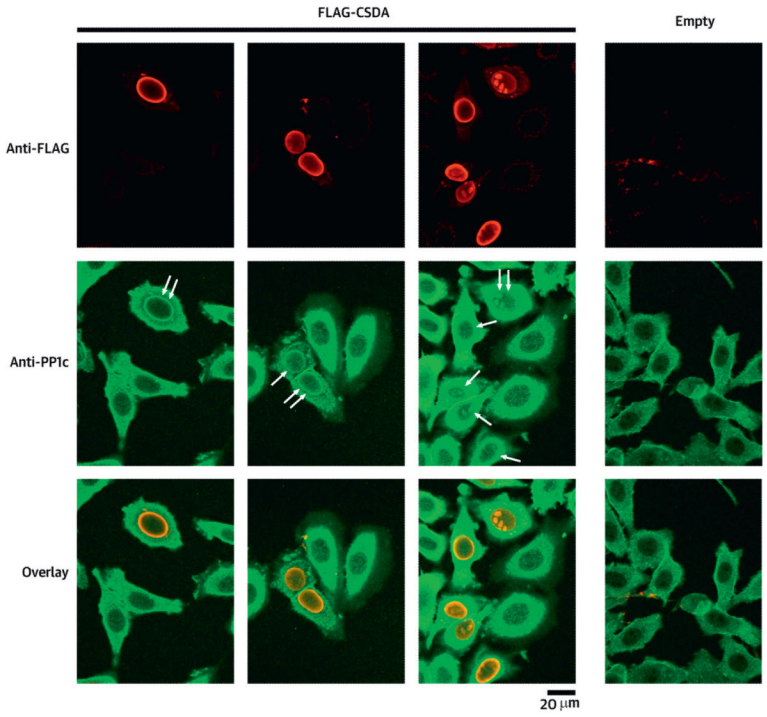
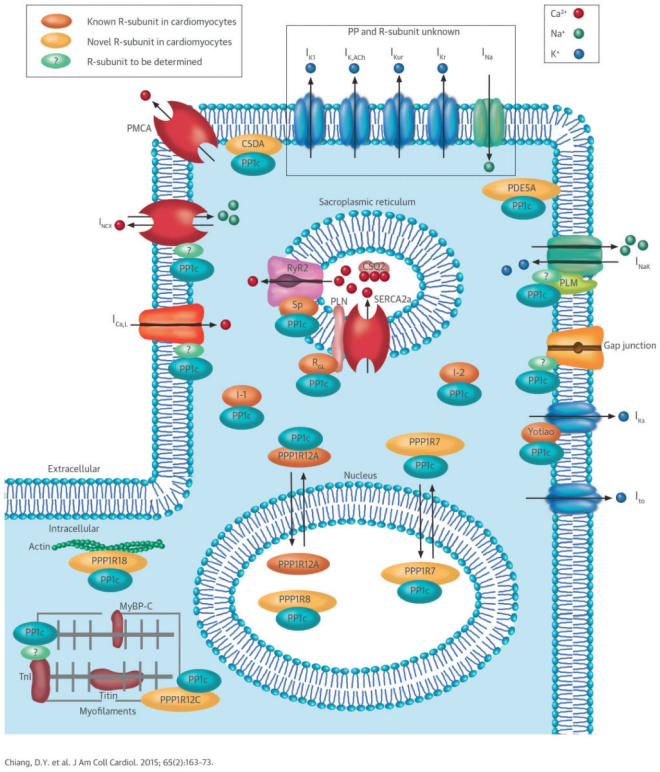


FIGURE 8. Subcellular Targeting of PP1c by CSDA

HeLa cells were transfected with either FLAG-CSDA or empty vector and costained with anti-FLAG and anti-PP1c antibodies. **White arrows in the middle panels** point to nuclear or perinuclear aggregations of PP1c, which are absent without CSDA and which colocalize perfectly with CSDA as shown in the **bottom panels.** Scale bar = 20 mm. CSDA = cold-shock domain protein A.



CENTRAL ILLUSTRATION. PP1c Interactome in Cardiomyocytes

Localization of novel PP1 regulatory (R)-subunits is on the basis of immunocytochemistry data (Figure 7, Online Figure 1) and previous studies that used noncardiac tissues. Online Table 5 contains the definitions of the abbreviations used in the Central Illustration.