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ORIGINAL RESEARCH - PRECLINICAL

Inhibition of Soluble Epoxide Hydrolase Reduces Inflammation and Myocardial Injury in Arrhythmogenic Cardiomyopathy

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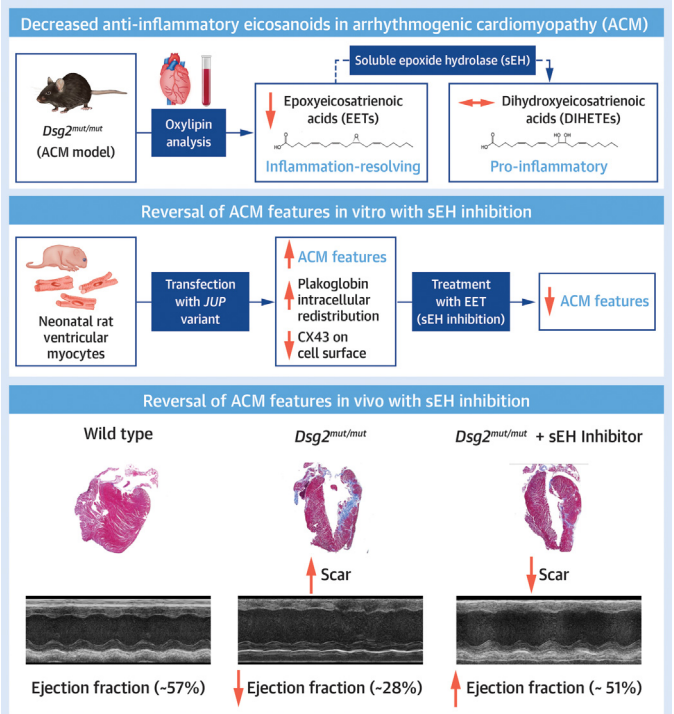
VISUAL ABSTRACT



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HIGHLIGHTS

- **No available therapies prevent or mitigate progression of myocardial injury in ACM.**
- **ACM is a chronic inflammatory disease in which innate immune signaling in cardiac myocytes drives injury and arrhythmias via the actions of CCR2-expressing macrophages.**
- **Here, we report an imbalance in pro- and anti-inflammatory eicsosonoids in a preclinical mouse model of ACM, and show that inhibition of sEH mitigates the disease phenotype and limits myocardial accumulation of CCR2+ cells.**
- **sEH inhibitors have completed phase 1a and 1b human safety trials with no adverse effects. They may be an effective therapy in patients with ACM.**

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ABBREVIATIONS AND ACRONYMS

AA = arachidonic acid

ACM = arrhythmogenic cardiomyopathy

CCR2 = C-C motif chemokine receptor 2

COX-2 = cyclooxygenase-2

CYP450 = cytochrome P450 monooxygenase enzyme

DHETs = dihydroxyeicosatrienoic acids

DiHOME = dihydroxy-octadecenoic acid

DMSO = dimethyl sulfoxide

EET = epoxyeicosatrienoic acid

EEZE = epoxyeicosa-5(Z)-enoic acid

EpFA = epoxy fatty acid

EpOME = epoxyoctadecenoic acid

ER = endoplasmic reticulum

iPSC = induced pluripotent stem cell

LA = linoleic acid

LTB4 = leukotriene B_4

HETE = hydroxyeicosatetraenoic acid

NFκB = nuclear factor κB

PET = positron emission tomography

PGE2 = prostaglandin E_2

PTUPB = 4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl)-benzene-sulfonamde

PUFA = polyunsaturated fatty acid

qPCR = quantitative polymerase chain reaction

sEH = soluble epoxide hydrolase

sn-RNAseq = single nucleus RNA sequencing

SPM = specialized pro-resolving mediator

TPPU = 1-trifluoro-methoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea

TXB_2 = thromboxane B_2

WT = wild-type

SUMMARY

We analyzed the role of pro- and anti-inflammatory eicosanoids in the pathogenesis of arrhythmogenic cardiomyopathy (ACM). Lipidomics revealed reduced levels of anti-inflammatory oxylipins in plasma and increased levels of pro-inflammatory eicosanoids in hearts of *Dsg2*$^{mut/mut}$ mice, a preclinical model of ACM. Disease features were reversed in vitro in rat ventricular myocytes expressing mutant *JUP* by the anti-inflammatory epoxyeicosatrienoic acid 14-15-EET, whereas 14,15-EEZE, which antagonizes the 14,15-EET receptor, intensified nuclear accumulation of the desmosomal protein plakoglobin. Inhibition of soluble epoxide hydrolase (sEH), an enzyme that converts anti-inflammatory EETs into polar, less active diols, prevented progression of myocardial injury in *Dsg2*$^{mut/mut}$ mice and promoted recovery of contractile function. This was associated with reduced myocardial expression of genes involved in innate immune signaling and fewer injurious macrophages expressing CCR2. These results suggest that pro-inflammatory eicosanoids contribute to the pathogenesis of ACM. Inhibition of sEH may be an effective, mechanism-based therapy for ACM patients.

Arrhythmogenic cardiomyopathy (ACM) is a familial nonischemic heart muscle disease characterized by arrhythmias and progressive myocardial injury, typically involving the right ventricle.[1,2] Most cases are caused by pathogenic variants in genes that encode desmosomal proteins.[1,2] We have previously reported that activation of nuclear factor κB (NFκB) signaling in cardiac myocytes drives myocardial injury and arrhythmias via actions of pro-inflammatory CCR2-expressing macrophages in a well-characterized preclinical mouse model of ACM involving homozygous knock-in of a variant in the gene for the desmosomal protein, desmoglein-2 (*Dsg2*$^{mut/mut}$ mice).[3] NFκB signaling is also activated in vitro in induced pluripotent stem cell (iPSC)-cardiac myocytes derived from ACM patients with disease-causing variants in *PKP2*[4] or *DSG2*,[5] and in rat ventricular myocytes expressing a disease-causing variant in *JUP* (all genes that encode desmosomal proteins).[4] ACM iPSC-cardiac myocytes and rat myocytes expressing mutant *JUP* produce and secrete large amounts of pro-inflammatory mediators under basal conditions without prior stimulation or provocation.[4-7] Taken together, these observations suggest that the pathogenesis of ACM is driven by a persistent, cardiac myocyte-autonomous innate immune response that fails to resolve.

A subset of cytochromes P450 can metabolize arachidonic acid to biologically active epoxides called epoxyeicosatrienoic acids (EETs), which are inflammation resolving, vasodilatory, angiogenic, and cardioprotective.[8,9] EETs are rapidly hydrolyzed to much less active diols by soluble epoxide hydrolase (sEH).[10,11] Inhibition or genetic disruption of sEH attenuates cardiac inflammation and improves recovery of heart function after ischemia.[9,12-14] 14,15-EET is the most abundant EET regioisomer produced by P450s and serves as a marker of P450 activity.[9,12,13] 14,15-EET is also the preferred substrate of sEH. Thus, a low ratio of 14,15-EET to its corresponding dihydroxyeicosatrienoic acid (14,15-EET:DHET) serves as a marker of increased sEH activity in vivo.[9,12,13] Cytochrome P450s also metabolize linoleic acid (LA), the most abundant unsaturated fatty acid in the human diet, to epoxyoctadecenoic acids (EpOMEs), which, in turn, are metabolized by sEH to produce pro-inflammatory dihydroxyoctadecenoic acids (DiHOMEs).[15-17]

Here, we show that levels of inflammation-resolving EETs are reduced in plasma and that levels of pro-inflammatory eicosanoids are increased in hearts of *Dsg2*$^{mut/mut}$ mice compared with wild-type (WT) mice. Hearts of *Dsg2*$^{mut/mut}$ mice also show increased expression of genes activated in endoplasmic reticulum (ER) stress, a hallmark of

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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unresolved inflammation.[18] In addition, ACM disease features are reversed in rat ventricular myocytes expressing mutant *JUP* by 14,15-EET, whereas 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE), a structural analog of 14,15-EET that antagonizes actions of the putative 14,15-EET receptor, intensifies nuclear accumulation of the desmosomal protein plakoglobin (aka, γ-catenin), which has been implicated in disease pathogenesis in ACM patients.[19] We also show that sEH is highly expressed in cardiac myocytes in hearts of WT and *Dsg2*$^{mut/mut}$ mice, confirming previous studies.[14] Finally, we show that inhibition of sEH prevents progression of the disease in *Dsg2*$^{mut/mut}$ mice and promotes significant recovery of contractile function associated with reduced myocardial injury, diminished expression of genes activated in the innate immune response, and fewer pro-inflammatory macrophages expressing CCR2. sEH inhibitors have completed phase 1a and phase 1b human safety trials with no adverse effects.[20] Thus, our results suggest that inhibition of sEH may be an effective mechanism-based therapy for patients with ACM.

METHODS

IN VIVO AND IN VITRO MODELS OF ACM. All animal studies were in full compliance with policies of Beth Israel Deaconess Medical Center and St. George's University of London, and conformed to the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health (NIH publication no. 85-23, revised 1996). Mice for in vivo studies were housed in a 12-hour light/dark cycle, climate-controlled facility with ad libitum access to water and standard rodent chow. Studies were performed in roughly equal numbers of male and female WT mice and mice with homozygous knock-in of a variant in *Dsg2*, the gene encoding the desmosomal cadherin desmoglein-2 (*Dsg2*$^{mut/mut}$ mice) as previously described.[3,4,6,7] This variant entails loss of exons 4 and 5, which causes a frameshift and premature termination of translation.

In vitro studies were performed in primary cultures of ventricular myocytes prepared from disaggregated ventricles of 1-day-old Wistar rat pups as previously described.[4,6,7] Cells were plated on collagen-coated plastic chamber slides at a density of 2.4×10^5 cells/cm^2. Two days after plating, monolayers were transfected in serum-free medium for 1 hour with a recombinant adenoviral construct (pAd/CMV/V5-DEST vector) containing *JUP2157del2*, after which the viral solution was replaced with complete medium. This protocol leads to expression of mutant *JUP* at a level roughly equivalent to that of the endogenous normal *JUP* gene product.[21] Then, 24 hours after transfection, cultures were incubated with 10 μM 14,15-EET or 10 μM 14,15-EEZE for an additional 24 hours. Other cultures were incubated for 24 hours with 1 μM TPPU (1-trifluoro-methoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea), a small-molecule inhibitor of sEH,[22] or 500 nM PTUPB (4-(5-phenyl-3-{3-[3-(4-trifluoromethyl-phenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide), a dual eicosanoid pathway (COX-2/sEH) inhibitor.[23] Transfected cultures treated with vehicle only and nontransfected cultures were used as controls. Thereafter, the cultures were rinsed in serum-free medium and fixed in 4% paraformaldehyde at 25 °C for 5 minutes in preparation for immunofluorescence microscopy as previously described.[4,6,7] Fixed cells were incubated with mouse monoclonal anti-plakoglobin (Sigma), anti-Cx43 (Millipore), and anti-RelA/p65 (LSBio) antibodies. Secondary antibodies included Cy3-conjugated goat anti-mouse or anti-rabbit IgGs (H+L; Jackson Immunoresearch). The cells were counterstained with DAPI and examined by laser scanning confocal microscopy (Nikon A1). The distribution of immunosignals was determined by inspection to be at or near the cell surface, within the cell or within the cell nucleus.

ANALYSIS OF PLASMA AND HEART EICOSANOIDS. Hearts and plasma were collected from 8- or 16-week-old WT or *Dsg2*$^{mut/mut}$ mice. Whole hearts were homogenized (100 mg tissue/mL) in Hanks' balanced salt solution (Sigma) containing 10 μM TPPU and 1 mm zirconia/silica beads (BioSpec Products) using a Tissuelyzer 2 (Qiagen) at 30 Hz for 10 minutes at 4 °C. Heart homogenate (100 μL containing 10 mg tissue) or 150 μL plasma were mixed with internal standards (PGE_2-d9, 3 ng; LTB_4-d4, 3 ng; 11,12-DHET-d11, 1.5 ng; 11,12-EET-d11, 3 ng; 15-HETE-d8, 3 ng; and AA-d11, 7.5 ng, all from Cayman Chemical), and lipids were extracted by liquid:liquid extraction with 600 μL ethyl acetate. The organic phase from plasma samples underwent an additional step by being passed through Maestro A phospholipid removal columns (Tecan) and washed with 1 mL acetonitrile. Plasma and heart extracts were dried under vacuum centrifugation and reconstituted in 50 μL 30% ethanol. Oxylipins were assayed on an Ultimate 3000 UHPLC equipped with an Xselect CSH C18, 2.1 × 50 mm, 3.5 μm particle column (Waters) and a TSQ Quantiva tandem mass spectrometer (Thermo Fisher Scientific) using multiple reaction monitoring as previously described.[24] Absolute oxylipin quantification was determined by calculating sample relative response ratios to curves generated with oxylipins standards purchased from Cayman Chemical.

INHIBITION OF sEH AND CHARACTERIZATION OF DISEASE PHENOTYPES IN *Dsg2*$^{mut/mut}$ MICE. The effects of the small-molecule sEH inhibitor TPPU were studied in age-matched male and female WT and *Dsg2*$^{mut/mut}$ mice. The 9-week-old animals underwent echocardiography before being implanted with intraperitoneal osmotic minipumps (Alzet, Model 1004) as previously described.[3,4,25] They received either vehicle or drug (TPPU dissolved in DMSO/polyethylene glycol). Drug-treated mice received 5 mg/kg/day TPPU via continuous infusion (1 μL/h for 28 days). Previous studies in rats have shown that at 1 mg/kg/day, TPPU achieves steady-state levels within blood and tissue by 8 days at a concentraton well above the IC_{50} required for inhibition of sEH.[26] Vehicle-treated mice received an equivalent volume of vehicle for 28 days. Final echocardiograms were obtained in both groups at 13 weeks of age. Thereafter, animals were sacrificed, and hearts and plasma were collected for additional studies, including tandem mass spectrometry to measure oxylipins (as detailed above), histology to measure the amount of ventricular fibrosis,[3,4] immunohistochemistry to measure the number of CCR2+ cells,[3] and qPCR to measure expression of genes related to the ER stress response and innate immune signaling as described in previous studies.[25,27]

STATISTICAL ANALYSIS. All data are presented using actual data points and the mean ± SEM; the sample size (n), and the statistical analyses performed for each experiment are indicated in the figure legends. Analysis of variance with Tukey post-hoc multiple comparisons was used as parametric statistical methods to compare the groups, as the Shapiro-Wilk significant departures from normality for the continuous variables of interest. $P < 0.05$ was considered statistically significant. All statistical analyses were analyzed using GraphPad Prism version 9.2, v10.0, or v10.4.1 for Mac, GraphPad Software).

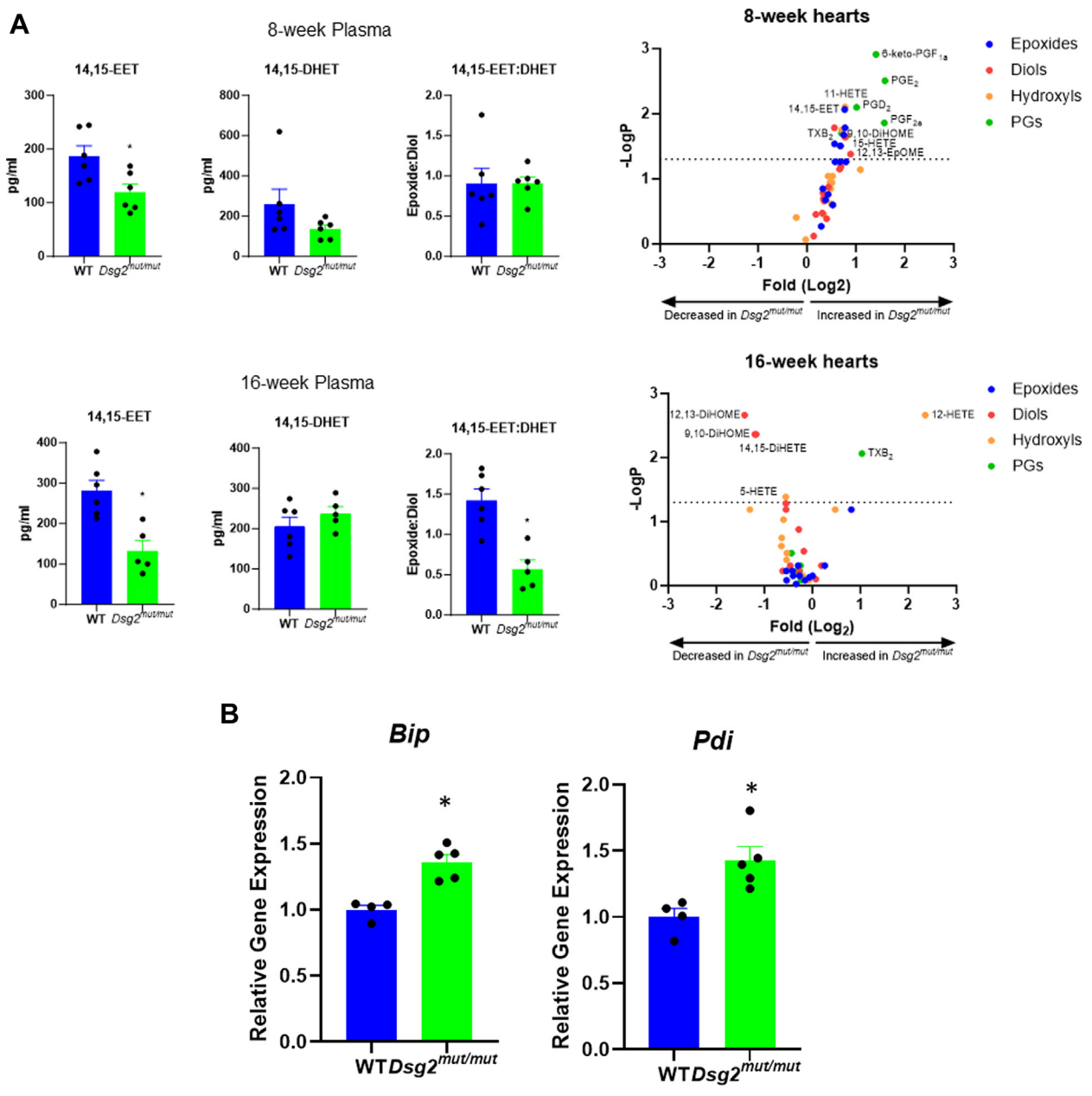
RESULTS

***Dsg2*$^{mut/mut}$ MICE HAVE REDUCED LEVELS OF EETs AND EXPRESS MARKERS OF ER STRESS.** Tandem mass spectrometry measurements of oxylipins in *Dsg2*$^{mut/mut}$ mice showed changes consistent with chronic progressive inflammation. Major findings are described here, and the full data set is included in Supplemental Tables 1 and 2. Plasma from 8-week *Dsg2*$^{mut/mut}$ mice, analyzed at a time when myocardial injury and arrhythmias were first becoming manifest,[3,4,6,7] showed reduced levels of inflammation-resolving 14,15-EET, which suggests systemic suppression of P450 activity (Figure 1A). Plasma from 16-week *Dsg2*$^{mut/mut}$ mice, analyzed at a time when these mice exhibited extensive myocardial injury, marked contractile dysfunction, and numerous arrhythmias,[3,4,6,7] also showed reduced 14,15-EET levels and significantly reduced 14,15-EET:DHET ratios, consistent with increased sEH-mediated hydrolysis of 14,15-EET to its less anti-inflammatory diol. Hearts of 8-week *Dsg2*$^{mut/mut}$ mice contained greatly increased levels of oxylipins derived from cyclooxygenase, lipoxygenase, and cytochrome P450 pathways compared with WT mice (Figure 1A). Such a broad, pathway-independent "eicosanoid storm" suggests inflammatory activation at the level of phospholipase A2.[24] Hearts from 16-week *Dsg2*$^{mut/mut}$ mice also showed increased markers of cyclooxygenase and lipoxygenase activity (eg, TXB_2 and 12-HETE) but decreased markers of P450-derived oxylipins (Figure 1A), consistent with effects of chronic persistent inflammation.[28,29] Taken together, these results suggest that persistent inflammation in *Dsg2*$^{mut/mut}$ mice reduces production of inflammation-resolving EETs and/or increases their inactivation by sEH.

ER stress is a hallmark of unresolved innate immune signaling.[18] As shown in Figure 1B, expression of the ER stress response genes for immunoglobulin binding protein (*BiP*), which acts as an ER chaperone, and protein disulfide isomerase (*Pdi*), involved in protein folding, was increased in hearts of *Dsg2*$^{mut/mut}$ mice compared with WT mice. These results provide additional independent evidence of ER stress and chronic progressive inflammation in ACM.

14,15-EET BLOCKS NFκB SIGNALING AND RESCUES ACM DISEASE FEATURES IN VITRO. We have previously reported that NFκB signaling is activated in a well-characterized in vitro model of ACM involving neonatal rat ventricular myocytes that express a variant in *JUP*, the gene for the desmosomal protein plakoglobin.[4] These cells exhibit characteristic features seen in ACM patients, including redistribution of junctional plakoglobin to intracellular and nuclear sites and loss of cell surface signal for Cx43, the major ventricular gap junction protein.[4,6,7] They also exhibit activation of NFκB signaling under basal conditions in vitro.[4] As shown in Figure 2, localization of plakoglobin and Cx43 signals at or near the cell surface was normalized when cells were incubated with the inflammation-resolving epoxy fatty acid (EpFA) 14,15-EET. In addition, NFκB signaling, indicated by the presence of nuclear signal for phospho-RelA/p65, was turned off in cells exposed to 14,15-EET. By contrast, nuclear accumulation of

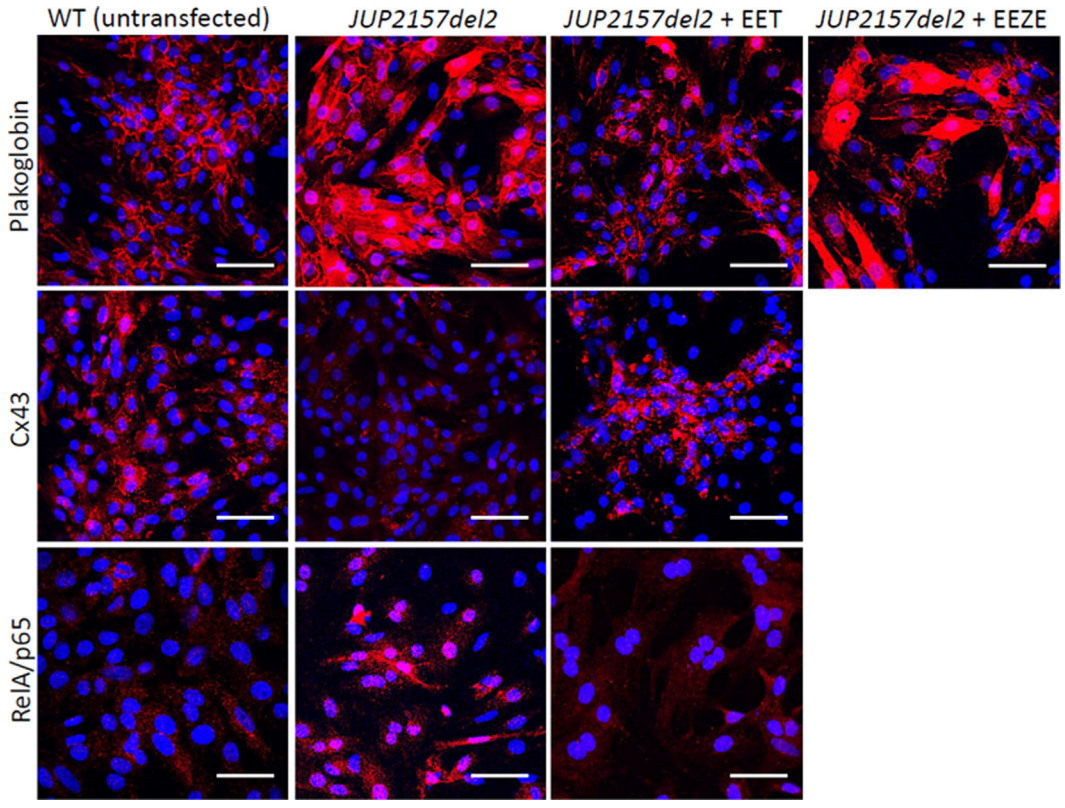
FIGURE 1 **Lipidomics Assays and Expression of Endoplasmic Reticulum Stress Genes in *Dsg2*$^{mut/mut}$ Mice**



(A) Free oxlipin levels in plasma and hearts from wild-type (WT) and *Dsg2*$^{mut/mut}$ mice at ages 8 or 16 weeks quantified by liquid chromatography/tandem mass spectrometry; n = 5-9, **P* < 0.05 vs WT. Heart oxylipins are displayed in a volcano plot of Log_2 fold relative concentration. Oxylipins above the dashed line represent significant differences between WT and *Dsg2*$^{mut/mut}$ mice. (B) quantitative polymerase chain reaction showing increased expression of the endoplasmic reticulum chaperone gene *BiP* and the protein folding protein disulfide isomerase gene *Pdi*, both markers of endoplasmic reticulum stress. Gene expression values in WT samples were normalized to 1; values in *Dsg2*$^{mut/mut}$ samples are shown as relative levels; **P* < 0.05 vs WT by *t*-test.

plakoglobin (aka γ-catenin) was greatly increased in cells incubated with 10μM 14,15-EEZE, which antagonizes actions of 14,15-EET (Figure 2). This is of particular interest, inasmuch as nuclear translocation of plakoglobin in cardiac myocytes is typically seen in patients with ACM[30] and has been implicated in altered Wnt signaling in the pathogenesis of ACM.[19]

FIGURE 2 Effects of 14,15-EET and its Antagonist on ACM Features In Vitro

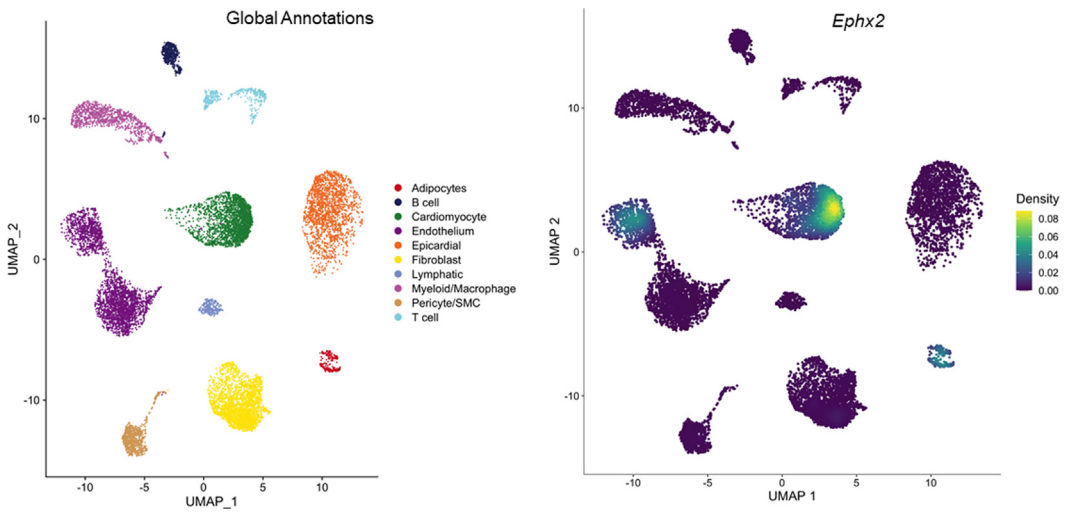


Effects of 14,15-eicosatrienoic acid (14,15-EET) and 14,15-eicosa-5(Z)-enoic acid (14,15-EEZE) on the distribution of immunoreactive signals for plakoglobin, connexin43 (Cx43), and RelA/p65 in primary cultures of wild-type (WT) neonatal rat ventricular myocytes and myocytes transfected to express the arrhythmogenic cardiomyopathy disease allele *JUP2157del2*.

sEH IS EXPRESSED MAINLY IN CARDIAC MYOCYTES IN HEARTS OF *Dsg2*$^{mut/mut}$ MICE. We previously performed single-nucleus RNA sequencing (sn-RNA-seq) in cells isolated from hearts of 16-week-old *Dsg2*$^{mut/mut}$ and WT mice.[3] Here, we analyzed the sn-RNAseq data to identify the specific cell types that expressed *Ephx2*, the major sEH gene in mice. In both WT and *Dsg2*$^{mut/mut}$ mouse hearts, sEH is expressed mainly in cardiac myocytes and, to a lesser extent, in endothelial and myeloid cells (**Figure 3**). These observations are in accordance with and confirm a recent report showing that sEH is expressed primarily by cardiac myocytes, less so in endothelial cells in mouse hearts and, furthermore, that selective disruption of sEH in cardiac myocytes (but not endothelial cells) improves recovery after ischemia/reperfusion injury in mouse hearts.[14] Of note, there was no apparent upregulation of sEH expression in cardiac myocytes of *Dsg2*$^{mut/mut}$ compared with WT mice. This suggests that metabolic changes in oxylipins (**Figure 1A**) are due to increased sEH activity rather than to increased expression in *Dsg2*$^{mut/mut}$ mice.

INHIBITION OF sEH NORMALIZES ACM DISEASE FEATURES IN VITRO AND PREVENTS MYOCARDIAL INJURY IN *Dsg2*$^{mut/mut}$ MICE IN VIVO. We have previously reported that inhibition of NFκB corrects ACM disease features in cultured rat myocytes expressing mutant *JUP*.[4] It also rescues the disease phenotype in *Dsg2*$^{mut/mut}$ mice.[3,4] To determine whether inhibition of sEH also mitigates the ACM disease phenotype and turns off NFκB signaling in vitro, we incubated cultures of rat myocytes expressing *JUP2157del2* with the sEH inhibitor TPPU or the dual COX-2/sEH inhibitor PTUPB. As shown in **Figure 4**, TPPU and PTUPB both restored the normal cell surface distribution of plakoglobin and Cx43 in rat myocytes expressing mutant *JUP* and eliminated nuclear signal for RelA/p65, indicating that NFκB signaling was reduced. Thus, inhibition of sEH in primary cultures of cardiac

FIGURE 3 **Uniform Manifold Approximation and Projection Plots Showing Populations of Cells Isolated From Hearts of 16-Week-Old *Dsg2*$^{mut/mut}$ Mice and Relative Density of *Ephx2* Expression in Each Population**



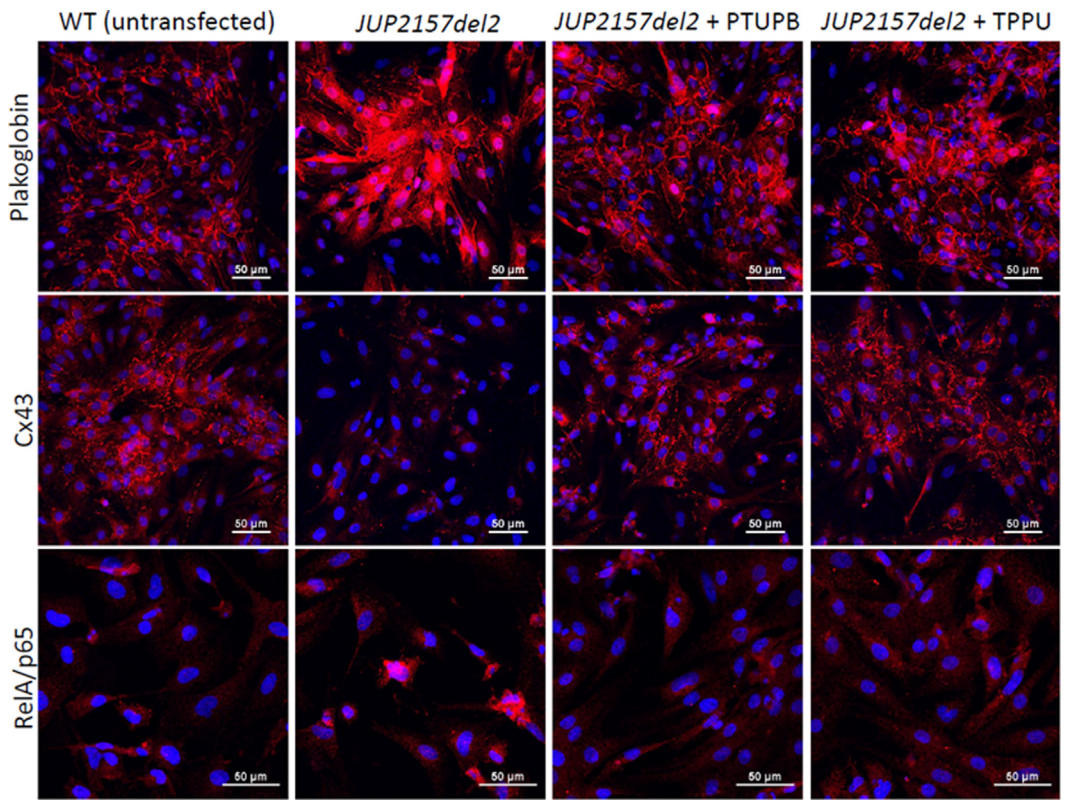
sEH (*Ephx2*) is expressed mainly in cardiac myocytes in the heart with lower levels of expression in endothelial cells.

myocytes in vitro reverses characteristic features of the ACM disease phenotype seen in patients. When considered in the context of the eicosanoid storm seen in hearts of 8-week old *Dsg2*$^{mut/mut}$ mice (**Figure 1A**), these data suggest that cyclooxygenase inhibitors might synergize with sEH inhibitors in reversing disease features in ACM. However, this conclusion cannot be definitively established solely from data in **Figure 4**. To determine whether inhibition of sEH can reduce inflammation and limit myocardial injury in vivo, we treated 9-week-old *Dsg2*$^{mut/mut}$ mice for 4 weeks with TPPU and monitored effects on left ventricular contractile function and immune signaling. As shown in **Figure 5**, disease progressed during the 4-week treatment interval in *Dsg2*$^{mut/mut}$ mice that received only vehicle. These mice exhibited significant deterioration of LV ejection fraction and reduced LV fractional shortening. By contrast, *Dsg2*$^{mut/mut}$ mice treated with TPPU showed significant improvement in left ventricular ejection fraction and fractional shortening to levels roughly equivalent to those seen in age-matched WT mice. Every treated *Dsg2*$^{mut/mut}$ mouse showed enhanced contractile performance, whereas untreated *Dsg2*$^{mut/mut}$ mice showed no improvement or exhibited functional deterioration (**Figure 5**). TPPU had no effect on left ventricular contractile function in WT mice. As previously reported, *Dsg2*$^{mut/mut}$ mice show little if any fibrosis at ∼8 weeks of age.[3,4,6,7] However, marked ventricular fibrosis was seen in 13-week vehicle-treated *Dsg2*$^{mut/mut}$ mice, whereas significantly less fibrosis was present in hearts of *Dsg2*$^{mut/mut}$ mice treated with TPPU (**Figure 6A**). Similarly, as previously reported,[3] the number of cells expressing CCR2 was increased by ∼5-fold in hearts of untreated *Dsg2*$^{mut/mut}$ mice, but they were significantly reduced in number in hearts of treated *Dsg2*$^{mut/mut}$ mice (**Figure 6B**). These observations are of particular interest because we have shown that pro-inflammatory myeloid cells expressing CCR2 are highly injurious to the heart in ACM.[3] Last, we have previously reported increased expression of key genes involved in the innate immune response in hearts of *Dsg2*$^{mut/mut}$ mice, including *Tnfα* (the gene for the pro-inflammatory cytokine tumor necrosis factor-α), *Tlr4* (which encodes Toll-like receptor 4, the major pattern recognition receptor on cardiac myocytes), and *Ccr2*, which is expressed by pro-inflammatory macrophages.[3,4] These findings reflect a state of persistent innate immune signaling in hearts of *Dsg2*$^{mut/mut}$ mice. As shown in **Figure 7**, we confirmed upregulation of these genes in *Dsg2*$^{mut/mut}$ mice and furthermore that inhibition of sEH reduced myocardial expression of these genes. Taken together with data in **Figure 6**, these results show that inhibition of sEH reduces inflammation, leading to significant functional recovery and cessation of ACM disease progression.

DISCUSSION

Inflammation is usually a self-limited process, designed to eliminate the cause of injury and restore

FIGURE 4 Effects of sEH Inhibition on ACM Features In Vitro

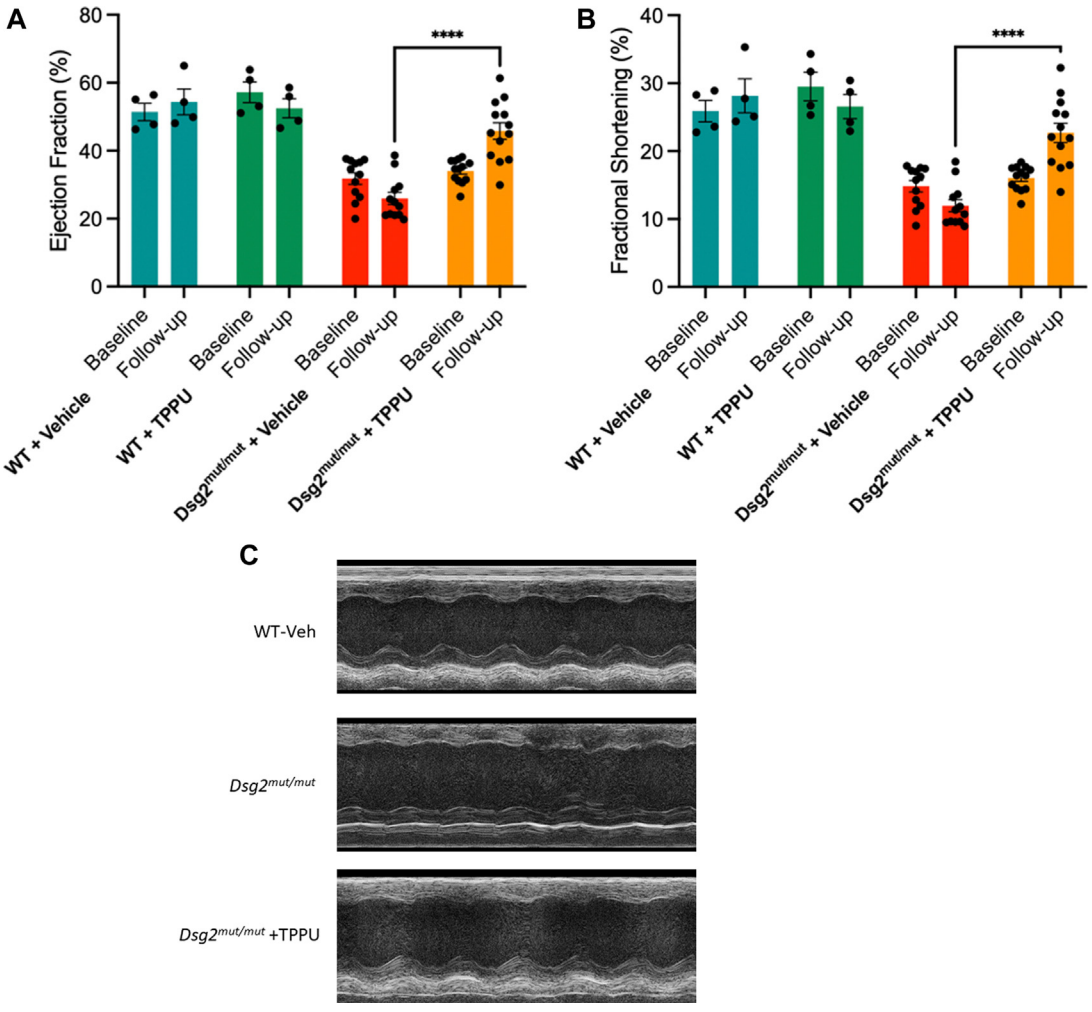


Effects of 1μM TPPU (1-trifluoro-methoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea) and 500 nM PTUPB (4-(5-phenyl-3-{3-[3-(4-trifluoromethylphenyl)-ureido]-propyl}-pyrazol-1-yl)-benzenesulfonamide) on the distribution of immunoreactive signals for plakoglobin, connexin43 (Cx43), and RelA/p65 in primary cultures of wild-type (WT) neonatal rat ventricular myocytes and myocytes transfected to express the arrhythmogenic cardiomyopathy disease allele *JUP2157del2*.

homeostasis.[31,32] If left uncontrolled, however, inflammation can cause progressive tissue damage. Indeed, diverse diseases such as sepsis, acute respiratory distress syndrome, cancer, and COVID-19 are driven by unresolved inflammation.[33,34] Resolution of inflammation is an active regulated process, orchestrated in part by various endogenous specialized proresolving mediators (SPMs) synthesized from n-3 polyunsaturated fatty acids (PUFAs) by lipoxygenase, cyclooxygenase and/or cytochrome P450 monooxygenase enzymes.[8] EpFAs not only increase SPM production but also are direct pro-resolving mediators.[24,35-37] However, most anti-inflammatory EpFAs are short lived. For example, EETs are rapidly metabolized by sEH to their corresponding, less active diols.[8,10,11] sEH also metabolizes linoleic acid-derived EpFAs to dihydroxyoctadecamonoenoic acids (DiHOMES), which may exert pro-inflammatory effects.[8,10,11] Small-molecule inhibitors of sEH stabilize levels of anti-inflammatory EpFAs and reduce tissue injury in diverse animal models or disease.[36-39] sEH inhibitors also shift arachidonic acid metabolic cascades from a pattern of initiation of inflammation to a pattern of resolution.[40] Importantly, both SPMs and EpFAs, synergized by inhibition of sEH, potently down-regulate NFκB pathways,[40,41] which are persistently activated in cardiac myocytes in a cell-autonomous fashion in ACM.[3-7]

Previous studies have shown that SPMs promote resolution of pathologic ER stress, a hallmark of unresolved inflammation.[18] Additional evidence implicates chronic ER stress in ACM. For example, prolonged ER stress promotes apoptosis,[42-44] a major type of myocyte death in ACM.[45,46] The unfolded protein response (UPR) is activated in human iPSC-cardiac myocytes expressing a variant in *PLN* (the gene for phospholamban), which has been linked to a complex overlap phenotype of DCM and ACM.[47] Expression of BiP (binding immunoglobulin protein), an essential regulator of the UPR, is increased in hearts

FIGURE 5 Effects of sEH Inhibition on Contractile Function in *Dsg2*$^{mut/mut}$ Mice

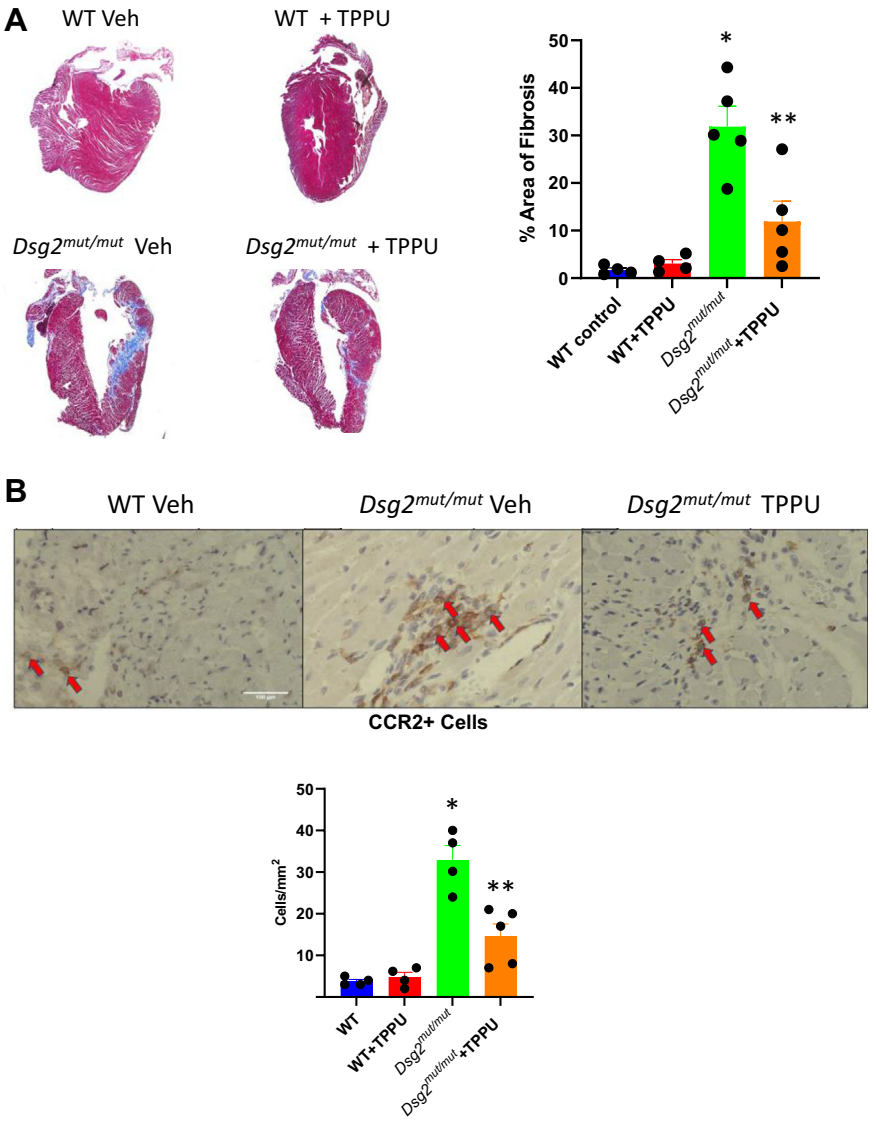


Effects of TPPU (1-trifluoro-methoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea) on left ventricular ejection fraction (A) and fractional shortening (B) in wild-type (WT) and *Dsg2*$^{mut/mut}$ mice. (C) Representative echocardiograms at the follow-up time point after treatment. Baseline echocardiography was performed in 9-week-old mice and then repeated after treatment for 4 weeks with TPPU or vehicle (Veh). Data are shown for each group (left) and each individual animal (right). The Shapiro-Wilk test was used to assess normality within each of the 4 groups. No significant deviations from normality were found, indicating an approximate normal distribution. Then, Tukey's 1-way analysis of variance was used. ****$P < 0.0001$ Dsg2$^{mut/mut}$ + Vehicle follow-up vs Dsg2$^{mut/mut}$ + TPPU follow-up. Data are shown as mean with SEM.

of ACM patients, reflecting a marked increase in unfolded proteins in the ER lumen.[47] The UPR has also been implicated in down-regulation of ion channel protein expression, leading to reduced I_{Na} and prolonged action potential duration,[48] thus further linking ER stress to key clinical features of ACM. Given the role of persistent ER stress in promoting cell injury and apoptosis and previous studies implicating the UPR in ACM, it is perhaps not surprising that ER stress markers are increased in hearts of *Dsg2*$^{mut/mut}$ mice and are normalized after sEH inhibition.

Increasing evidence suggests that chronic inflammation in ACM is the result of deficient resolution. The consistent presence of inflammatory infiltrates in the

FIGURE 6 Effects of sEH Inhibition on Fibrosis and CCR2+ Cells in Heart of *Dsg2*$^{mut/mut}$ Mice



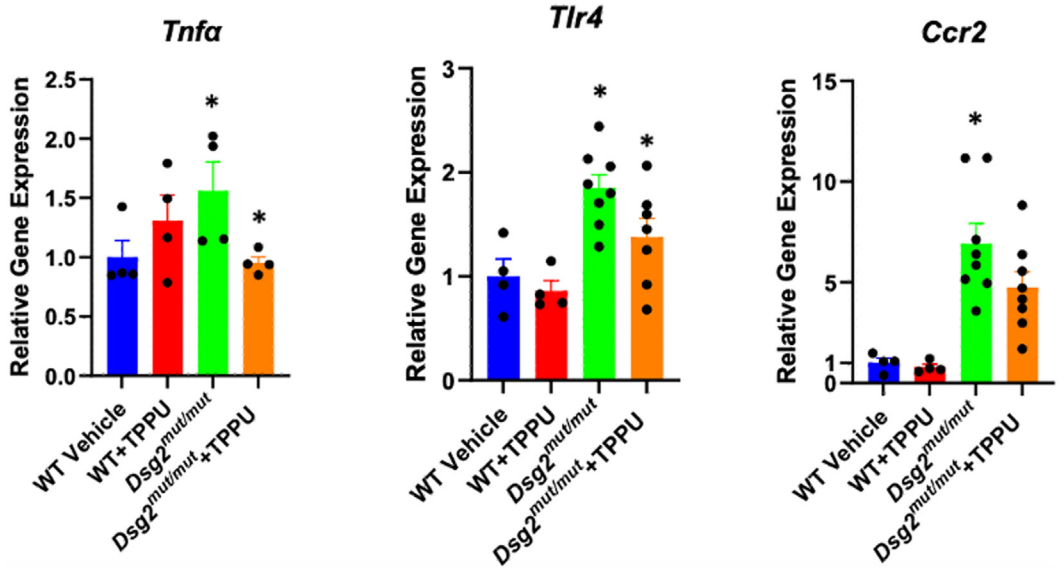
Effects of TPPU (1-trifluoro-methoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea) on the amount of myocardial fibrosis (A) and the number of cells expressing CCR2 (B) in wild-type (WT) and *Dsg2*$^{mut/mut}$ mice (positive cells identified by arrows in representative immunostained tissue sections). Hearts were excised from animals after treatment for 4 weeks with TPPU or vehicle (Veh) and analyzed by histology in trichrome stained sections or by immunohistochemistry in sections stained with an anti-CCR2 antibody. The Shapiro-Wilk test was used to assess normality within each of the 4 groups. No significant deviations from normality were found, indicating an approximate normal distribution. Then, Tukey's 1-way analysis of variance was used. *$P < 0.05$ WT vs vehicle-treated Dsg2$^{mut/mut}$; *$P < 0.05$ vehicle-treated *Dsg2*$^{mut/mut}$ vehicle-treated vs 1770-treated Dsg2$^{mut/mut}$ mice. Data are shown as mean with SEM.

hearts of ACM patients obviously implicates immune mechanisms.[1,2] Moreover, ACM patients have elevated circulating levels of cytokines, and their cardiac myocytes express multiple pro-inflammatory mediators.[49] Recurrent bouts of inflammation (rigorously defined as troponin elevation with normal coronary arteries, and typical ^{18}F-fluoro-deoxyglucose PET findings) occur in ACM patients with variants in *DSP*, the gene for the desmosomal protein desmoplakin.[50]

CONCLUSIONS

Here, we provide new evidence that 1) ACM is driven by a persistent innate immune response associated

FIGURE 7 Effects of sEH Inhibition on Expression of Innate Immune Signaling Genes



Effects of TPPU (1-trifluoro-methoxy-phenyl-3-(1-propionylpiperidin-4-yl) urea) on expression of *Tnfα*, *Tlr4*, and *Ccr2* in hearts of wildtype (WT) and $Dsg2^{mut/mut}$ mice treated with TPPU or vehicle (Veh). Gene expression values in wildtype samples treated with vehicle were normalized to 1; values in all other groups are shown as relative levels. The Shapiro-Wilk test was used to assess normality within each of the 4 groups. No significant deviations from normality were found, indicating an approximate normal distribution. All tests were 1-way analysis of variance. *$P < 0.05$ $Dsg2^{mut/mut}$ vs WT Veh; *$P < 0.05$ $Dsg2^{mut/mut}$ vs $Dsg2^{mut/mut}$ + TPPU. Data are shown as mean with SEM.

with increased sEH activity and reduced levels of anti-inflammatory bioactive lipid mediators, 2) the anti-inflammatory EpFA 14,15-EET can reverse disease features in an in vitro model of ACM, and 3) inhibition of the sEH enzyme in vivo mitigates the disease phenotype in a well-characterized animal model of ACM. sEH inhibitors are currently being evaluated in phase 1 clinical trials.[20] sEH inhibitors are in clinical development for hypertension,[51] chronic obstructive pulmonary disease,[52] chronic pain, and other conditions.[20,53] Dual COX-2/sEH inhibitors are also currently in clinical development for multiple inflammatory diseases.[54,55] Should these small-molecule drug candidates be found to be safe and free of toxic side effects, our observations indicate that they may be effective mechanism-based therapies in patients with ACM.

STUDY LIMITATIONS AND FUTURE DIRECTIONS. Expression of mutant *JUP* by neonatal rat ventricular myocytes results in highly reproducible and characteristic redistribution of plakoglobin and Cx43 from junctional sites to intracellular and intranuclear sites, similar to changes seen in the hearts of patients with ACM. These changes were normalized in transfected myocytes treated with the anti-inflammatory EpFA 14,15-EET. We acknowledge that the distribution of plakoglobin and Cx43 in the present study was assessed qualitatively (by inspection) rather than quantitatively. Nevertheless, we have reported a close correlation between the amount of Cx43 signal visualized at or near the cell surface and precise measurements of gap junctional conductance.[56] This provides reassurance that immunosignal for Cx43 seen at or near the cell surface resides in functional gap junctions and, similarly, that signal for plakoglobin at or near the cell surface is located within desmosomes. We have previously shown that CCR2+ cells, mobilized to the heart by signal from cardiac myocytes, mediate tissue damage in hearts of $Dsg2^{mut/mut}$ mice.[3] We also showed that virtually all cells expressing CCR2 in hearts of $Dsg2^{mut/mut}$ mice also expressed the pan-macrophage marker CD68.[3] Thus, although we did not repeat double labeling, it is likely that CCR2+ cells identified in the present study were macrophages. This conclusion was further supported by their characteristic size and shape and their interstitial and perivascular location. The main finding in the present study is that inhibition of sEH mitigates the disease phenotype in $Dsg2^{mut/mut}$ mice, a robust preclinical model of ACM. We also show a reduction in anti-inflammatory oxylipins in plasma and an increase in pro-inflammatory eicosanoids in

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hearts of *Dsg2*$^{mut/mut}$ mice. However, these observation do not prove that pro-inflammatory oxylipins play a causal role in the pathogenesis of ACM nor that inhibition of sEH reduces myocardial injury by limiting production of pro-inflammatory eicosanoids. Additional studies to define these mechanisms are required. For example, studies in *Dsg2*$^{mut/mut}$ mice crossed with lines with either germline or cardiac myocyte-specific deletion of sEH will determine whether actions of sEH in cardiac myocytes are the major driver of disease. Lipidomics assays in mice after pharmacologic or genetic blockade of sEH may provide insights into the pathogenic roles of specific pro-inflammatory oxylipins. The fact that hearts of 8-week *Dsg2*$^{mut/mut}$ mice contained greatly increased levels of oxylipins derived from cyclooxygenase, lipoxygenase, and cytochrome P450 pathways compared with WT mice suggests that combined inhibition of sEH and COX-2 with PTUPB may be more effective than inhibition of sEH alone in mitigating myocardial injury in ACM. Finally, diets rich in n-3 PUFAs increase production of anti-inflammatory lipids and synergize with sEH inhibitors to suppress inflammatory pathways in various experimental models, whereas diets high in n-6 PUFA increase production of pro-inflammatory eicosanoids via cyclooxygenase and lipoxygenase pathways.[57,58] Thus, dietary interventions that increase the availability of n-3 PUFA substrates may be beneficial in patients with ACM.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The only treatment proved to reduce risk of sudden death in ACM is an ICD. Although they are lifesaving, ICDs do not treat the underlying progressive muscle disease. Here, we report an imbalance in pro- and anti-inflammatory oxylipins in a preclinical mouse model of ACM and show that inhibition of sEH significantly mitigates the disease phenotype.

TRANSLATIONAL OUTLOOK: Small-molecule inhibitors of sEH are currently in phase 1b clinical trials. They could be a new mechanism-based therapy in patients with ACM.

REFERENCES

1. Corrado D, Link M, Calkins H. Arrhythmogenic right ventricular cardiomyopathy. *New Eng J Med*. 2017;376:61–72.

2. Corrado D, Basso C, Thiene G, et al. Spectrum of clinico-pathologic manifestations of arrhythmogenic right ventricular cardiomyopathy/dysplasia: a multicenter study. *J Am Coll Cardiol*. 1997;30:1512–1520.

3. Chelko SP, Penna V, Engle M, et al. NFκB signaling drives myocardial injury via CCR2+ macrophages in a preclinical model of arrhythmogenic cardiomyopathy. *J Clin Invest*. 2024;134(13):e183441. https://doi.org/10.1172/JCI183441

4. Chelko SP, Asimaki A, Lowenthal J, et al. Therapeutic modulation of the immune response in arrhythmogenic cardiomyopathy. *Circulation*. 2019;140:1491–1505.

5. Hawthorne RN, Blazeski A, Lowenthal J, et al. Altered electrical, biomolecular, and immunologic phenotypes in a novel patient-derived stem cell model of desmoglein-2 mutant ARVC. *J Clin Med*. 2021;10(14):3061. https://doi.org/10.3390/jcm10143061

6. Chelko SP, Asimaki A, Andersen P, et al. Central role for GSK3β in the pathogenesis of arrhythmogenic cardiomyopathy. *JCI Insight*. 2016;1(5):e85923. https://doi.org/10.1172/jci.insight.85923

7. Asimaki A, Kapoor S, Plovie E, et al. Identification of a new modulator of the intercalated disc in a zebrafish model of arrhythmogenic cardiomyopathy. *Sci Transl Med*. 2014;6:240ra74.

8. Node K, Huo Y, Ruan X, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science*. 1999;285:1276–1279. https://doi.org/10.1126/science.285.5431.1276

9. Spector AA, Norris AW. Action of epoxyeicosatrienoic acids on cellular function. *Am J Physiol Cell Physiol*. 2007;293(3):C996–C1012.

10. Morisseau C, Hammock BD. Impact of soluble expoxide hydrolase and epoxyeiconsanoids on human health. *Annu Res Pharmacol Toxicol*. 2013;53:37–58.

11. Ota K, Hammock BD. Cytosolic and microsomal epoxide hydrolases: differential properties in mammalian liver. *Science*. 1980;207:1479–1481.

12. Seubert JM, Sinal CJ, Graves J, et al. Role of soluble expoxide hydrolase in postischemic recovery of heart contractile function. *Circ Res*. 2006;99(4):442–450.

13. Sosnowski DK, Jamieson KL, Gruzdev A, et al. Cardiomyocyte-specific disruption of soluble epoxide hydrolase limits inflammation to preserve cardiac function. *Am J Physiol Heart Circ Physiol*. 2022;323(4):H670–H687.

14. Edin ML, Gruzdev A, Bradbury JA, et al. Disruption of Ephx2 in cardiomyocytes but not endothelial cells improves functional recovery after ischemia-reperfusion in isolated mouse hearts. *J Biol Chem*. 2023;299:103049. https://doi.org/10.1016/j.jbc.2023.103049

15. Hildreth K, Kodani SD, Hammock BD, Zhao L. Cytochrome P450-derived linoleic acid metabolites EpOMEs and DiHOMEs: a review of recent studies. *J Nutr Biochem*. 2020;86:108484. https://doi.org/10.1016/j.jnutbio.2020.108484

16. Frömel T, Hu J, Fleming I. Lipid mediators generated by the cytochrome P450-epoxide hydrolase pathway. *Adv Pharmacol*. 2023;97:327–373.

17. Moghaddam MF, Grant DF, Cheek JM, Williamson KC, Hammock BD. Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase. *Nat Med*. 1997;3:562–566. https://doi.org/10.1038/nm0597-562

18. Inceoglu B, Bettaieb A, Haj FG, Gomes AV, Hammock BD. Modulation of mitochondrial dysfunction and endoplasmic reticulum stress are key mechanisms for the wide-ranging actions of epoxy fatty acids and soluble epoxide hydrolase inhibitors. *Prostaglandins Other Lipid Mediat*. 2017;133:68–78.

19. Garcia-Gras E, Lombardi R, Giocondo MJ, et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythymogenic right ventricular cardiomyopathy. *J Clin Invest*. 2006;116:2012–2021.

20. Schmidt WK, Cortés-Puch I, McReynolds CB, et al. Randomized, double-blind, phase 1a single-ascending dose and food effect studies assessing safety and pharmacokinetics of EC5026 in health volunteers. *Clin Transl Sci*. 2024;17(9):e70033. https://doi.org/10.1111/cts.7033

21. Huang H, Asimaki A, Lo D, McKenna W, Saffitz J. Disparate effects of different mutations in plakoglobin on cell mechanical behavior. *Cell Motil Cytoskeleton*. 2008;65:964–978.

22. An G, Lee KSS, Yang J, Hammock BD. Target-mediated drug disposition: a class effect of soluble epoxide hydrolase inhibitors. *J Clin Pharmacol*. 2021;61:531–537.

23. Gartung A, Yang J, Sukhatme VP, et al. Suppression of chemotherapy-induced cytokine/lipid mediator surge and ovarian cancer by a dual COX-2/sEH inhibitor. *Proc Natl Acad Sci USA*. 2019;116:1698–1703.

24. Edin ML, Gruzdev A, Graves JP, et al. Effects of sEH inhibition on the eicosanoid and cytokine storms in SARS-CoV-2-infected mice. *FASEB J*. 2024;38(10):e23692. https://doi.org/10.1096/fj.202302202RR

25. Deng J, Yang H, Haak VM, et al. Eicosanoid regulation of debris-stimulated metastasis. *Proc Natl Acad Sci USA*. 2021;118(41):e2107771118. https://doi.org/10.1073/pnas.2107771118

26. Ostermann AI, Herbers J, Willenberg I, et al. Oral treatment of rodents with soluble epoxide hydrolase inhibitor 1-(1-propanoylpiperidin-4-yl)-3-[trifluoromethoxy)phenyl]urea (TPPU): resulting drug levels and modulation of oxylipid pattern. *Prostaglandins Other Lipid Mediat*. 2015;121:131–137.

27. Fishbein A, Wang W, Yang H, et al. Resolution of eicosanoid/cytokine storm prevents carcinogen and inflammation-initiated hepatocellular cancer progression. *Proc Natl Acad Sci USA*. 2020;117(35):21576–21587. https://doi.org/10.1073/pnas.2007412117

28. Graves JP, Bradbudy JA, Gruzdev A, et al. Expression of *Cyp2c/ Cyp2j* subfamily members and oxylipin levels during LPS-induced inflammation and resolution in mice. *FASEB J*. 2019;33(12):14784–14797. https://doi.org/10.1096/fj.201901872R

29. Theken KN, Deng Y, Kannon AM, Miller TM, Poloyac SM, Lee CR. Activation of the acute inflammatory response alters cytochrome P450 expression and eicosanoid metabolism. *Drug Metab Dispos*. 2011;39(1):22–29. https://doi.org/10.1124/dmd.110.035287

30. Asimaki A, Tandri J, Huang H, et al. A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. *N Eng J Med*. 2009;360:1075–1084.

31. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol*. 2005;6:1191–1197.

32. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature*. 2024;510:92–101.

33. Panigrahy D, Gilligan MM, Serhan CN, Kashfi K. Resolution of inflammation: an organizing principle in biology and medicine. *Pharmacol Ther*. 2021;227:10789. https://doi.org/10.1016/j/pharmthera.2021.107879

34. Chiang N, Serhan CN. Specialized pro-resolving mediator network: an update on production and actions. *Essays Biochem*. 2020;64:443–462.

35. Abdalla HB, Alvarez C, Wu YC, et al. Soluble epoxide hydrolase inhibition enhances production of specialized pro-resolving lipid mediator and promotes macrophage plasticity. *Br J Pharmacol*. 2023;180:1597–1615.

36. Abdalla HB, Puhl L, Rivas CA, et al. Modulating the sEH/EETs axis restrains specialized proresolving mediator impairment and regulates T cell imbalance in experimental periodontitis. *J Immunol*. 2024;212:433–445.

37. Gilroy DW, Edin ML, De Maeyer RPH, et al. CYP450-derived oxylipins mediate inflammatory resolution. *Proc Natl Acad Sci USA*. 2016;113(23):E3240–E3249. https://doi.org/10.1073/pnas.1521453113

38. Ono E, Dutile S, Kazani S, et al. Lipoxin generation is related to soluble epoxide hydrolase activity in severe asthma. *Am J Respir Crit Care Med*. 2014;190:886–897.

39. Flitter BA, Hvorecny KL, Ono E, et al. Pseudomonas aeruginosa sabotages the generation of host proresolving lipid mediators. *Proc Natl Acad Sci USA*. 2017;114:136–141.

40. Dong L, Zhou Y, Zhu ZQ, et al. Soluble epoxide hydrolase inhibitor suppresses the expression of triggering receptor expressed on myeloid cells by inhibiting NF-κB activation in murine macrophage. *Inflammation*. 2017;40:13–20.

41. Sham HP, Walker KH, Abdulmour RE, et al. 15-epi-lipoxin A4, resolving D2, and resolving D3

induce NF-κB in bacterial pneumonia. *J Immunol*. 2018;200:2757–2766.

42. Inceoglu B, Bettaieb A, Haj FG, Gomes AV, Hammock BD. Modulation of mitochondrial dysfunction and endoplasmic reticulum stress are key mechanisms for the wide-ranging actions of epoxy fatty acids and soluble epoxide hydrolase inhibitors. *Prostaglandins Other Lipid Mediat*. 2017;133:68–78.

43. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science*. 2011;334:1081–1086. https://doi.org/10.1126/science.1209038

44. Salminen A, Kauppinen A, Suuronen T, Kaarniranta K, Ojala J. ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. *J Neuroinflam*. 2009;6:41. https://doi.org/10.1186/1742-2094-6-41

45. Marcus FI, Fontaine GH, Guiraudon G, et al. Right ventricular dysplasia: a report of 24 adult cases. *Circulation*. 1982;65:384–398.

46. Austin KM, Trembley MA, Chandler SF, et al. Molecular mechanisms of arrhythmogenic cardiomyopathy. *Nat Rev Cardiol*. 2019;16:519–537.

47. Feyen DAM, Perea-Gill I, Maas RGC, et al. Unfolded protein response as a compensatory mechanism and potential therapeutic target in PLN R14dek cardiomyopathy. *Circulation*. 2021;144:382–392.

48. Lie M, Dudley SC. The role of the unfolded protein response in arrhythmias. *Channels*. 2018;12:335–345.

49. Asimaki A, Tandri H, Duffy ER, et al. Altered desmosomal proteins in granulomatous myocarditis and potential pathogenic links to arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhyth Electrophysiol*. 2011;4:743–752.

50. Smith ED, Lakdawala NK, Papoutsidakis N, et al. Desmoplakin cardiomyopathy, a fibrotic and inflammatory form of cardiomyopathy distinct from typical dilated or arrhythmogenic right ventricular cardiomyopathy. *Circulation*. 2020;141:1872–1884.

51. Ulu A, Harris TR, Morisseau C, et al. Anti-inflammatory effects of ω-3 polyunsaturated fatty acids and soluble epoxide hydrolase inhibitors in angiotensin-II-dependent hypertension. *J Cardiovasc Pharamacol*. 2013;62:285–297.

52. Lazaar AL, Yang L, Boardley RL, et al. Pharmacokinetics, pharmacodynamics and adverse event profile of GSK2256294, a novel soluble epoxide hydrolase inhibitor. *Br J Clin Pharmacol*. 2016;81:971–979.

53. Kodani SD, Hammock BD. The 2014 Bernard B. Brodie award lecture – epoxide hydrolases: drug metabolism to therapeutics for chronic pain. *Drug Metab Dispos*. 2015;43:788–802.

54. Dileepan M, Rastle-Simpson S, Greenberg Y, et al. Effect of dual sEH-COX-2 inhibition on allergen-induced airway inflammation. *Front Pharmacol*. 2019;10:1118.

55. Yang HH, Duan JX, Liu SK, et al. A COX-2/sEH dual inhibitor PTUPB alleviates lipopolysaccharide-induced acute lung injury in mice by inhibiting NLFP3 inflammasome activation. *Theranostics*. 2020;10:4749–4761.

56. Jin Q, Lee KY, Selimi Z, et al. Determinants of electrical propagation and propagation block in arrhythmogenic cardiomyopathy. *J Mol Cell Cardiol*. 2024;186:71–80.

57. Wall R, Ross RP, Fitzgerald GF, Stanton C. Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutrition Rev*. 2010;68:280–289.

58. MacIntosh BA, Ramsden CE, Faurot KR, Zamora D, Mangan M, Hibbeln JR, Mann JD. Low-*n*-6 and low-*n*-6 plus high-*n*-3 diets for use in clinical research. *Brit J Nutrition*. 2013;110:559–568.

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APPENDIX For supplemental tables, please see the online version of this paper.