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Wnt Signaling Interactor WTIP (Wilms Tumor Interacting Protein) Underlies Novel Mechanism for Cardiac Hypertrophy

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Abstract

BACKGROUND: The study of hypertrophic cardiomyopathy (HCM) can yield insight into the mechanisms underlying the complex trait of cardiac hypertrophy. To date, most genetic variants associated with HCM have been found in sarcomeric genes. Here, we describe a novel HCM-associated variant in the noncanonical Wnt signaling interactor *WTIP* (Wilms tumor interacting protein) and provide evidence of a role for WTIP in complex disease.

METHODS: In a family affected by HCM, we used exome sequencing and identity-by-descent analysis to identify a novel variant in WTIP (p.Y233F). We knocked down WTIP in isolated neonatal rat ventricular myocytes with lentivirally delivered short hairpin ribonucleic acids and in *Danio rerio* via morpholino injection. We performed weighted gene coexpression network analysis for WTIP in human cardiac tissue, as well as association analysis for WTIP variation and left ventricular hypertrophy. Finally, we generated induced pluripotent stem cell–derived cardiomyocytes from patient tissue, characterized size and calcium cycling, and determined the effect of verapamil treatment on calcium dynamics.

RESULTS: WTIP knockdown caused hypertrophy in neonatal rat ventricular myocytes and increased cardiac hypertrophy, peak calcium, and resting calcium in *D rerio*. Network analysis of human cardiac tissue indicated WTIP as a central coordinator of prohypertrophic networks, while common variation at the *WTIP* locus was associated with human left ventricular hypertrophy. Patient-derived *WTIP* p.Y233F-induced pluripotent stem cell–derived cardiomyocytes recapitulated cellular hypertrophy and increased resting calcium, which was ameliorated by verapamil.

CONCLUSIONS: We demonstrate that a novel genetic variant found in a family with HCM disrupts binding to a known Wnt signaling protein, misregulating cardiomyocyte calcium dynamics. Further, in orthogonal model systems, we show that expression of the gene *WTIP* is important in complex cardiac hypertrophy phenotypes. These findings, derived from the observation of a rare Mendelian disease variant, uncover a novel disease mechanism with implications across diverse forms of cardiac hypertrophy.

Keywords

calcium; cardiomyopathy, hypertrophic; humans; induced pluripotent stem cells; myocytes, cardiac; zebra fish

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Hypertrophic cardiomyopathy (HCM) shares critical cellular- and tissue-level phenotypes with more complex causes of ventricular hypertrophy, for example, hypertension and aortic stenosis, which are also associated with sudden cardiac death. Indeed, the study of Mendelian genetics has previously revealed therapeutic targets in complex forms

of disease ranging from hyperlipidemia and myocardial infarction to malignancies.[1,2] Therefore, illuminating new underlying causes of a Mendelian disease like HCM can also be informative for the treatment of related common diseases.

After decades of study, hundreds of different genetic variants have been associated with HCM, the vast majority located in sarcomeric genes. Despite this progress, half of HCM cases remain genetically unexplained. This missing heritability is most likely attributable to variation in other genes, which, if discovered, will significantly advance our understanding of the mechanisms behind the hallmark feature of HCM, cardiac hypertrophy.

Here, we describe a novel missense variant in a nonsarcomeric gene that is associated with HCM in a multigenerational family. This variant falls in an important functional domain of the noncanonical Wnt signaling–associated protein WTIP (Wilms tumor interacting protein), providing a putative mechanism of pathogenicity. Knockdown of *WTIP* expression in neonatal rat ventricular myocyte and zebra fish models indicate that this gene modulates cellular hypertrophy and calcium handling. Furthermore, population-level analysis demonstrates an association between variation in WTIP and left ventricular wall thickness. Therapeutic intervention targeted at this pathway in induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs) relieves disease-relevant phenotypes. These findings reveal an important nonsarcomeric genetic mechanism of cardiac hypertrophy that may represent a previously unreported targetable pathway in several forms of cardiomyopathy.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. The study was approved by the Stanford Institutional Review Board, and all subjects provided informed written consent (adults) or assent (minors). All methods are described in the Supplemental Material.

RESULTS

We performed exome sequencing of 6 individuals in a family with HCM (Table 1) without an identifiable genetic cause on clinical panel testing: 5 exhibiting HCM and 1 unaffected family member (Figure 1A and 1B; Table 2). Applying a hidden Markov model, we identified chromosomal segments shared identical by descent among affected individuals and found a region of chromosome 19 with identity by descent of 1 harboring 6 heterozygous novel variants cosegregating with HCM (Figure 1C). We then applied parametric linkage analysis to 5474 single nucleotide polymorphisms uniquely tagging 0.3-cm bins in targeted regions (average heterozygosity, 0.44). This yielded an logarithm of the odds score of 1.0 (maximum for this pedigree configuration) for this region (Figure 1C, inset). Sanger sequencing of the 6 candidate variants in all 6 individuals and 2 additional subsequently identified HCM-affected family members showed cosegregation of 3 variants with HCM (*WTIP* p.Y233F [NM_001080436.2], *SARS2* p.G502S [NM_017827.4], and *ZNF699* p.R603X [NM_198535.3]). All 3 of these genes are expressed in cardiac tissue. However, of these variants, only *WTIP* p.Y233F (Figure 1D) remained novel in gnomAD[3]

(*SARS2* p.G502S [p.G504S in the gnomAD reference transcript (CCDS54265)]: MAF, 0.00120%; *ZNF699* R603X MAF, 0.00278%). *WTIP* p.Y233F occurs in the first LIM domain of the protein (Figure 1E) in a predicted tyrosine kinase recognition site and disrupts an amino acid that is conserved from *Homo sapiens* through *Danio rerio* (Figure 1F). *WTIP* is reported to bind ROR2 (receptor tyrosine kinase-like orphan receptor 2)[4]—a Wnt receptor that regulates calcium homeostasis in noncardiac cell types.[5] We found that *WTIP* p.Y233F displays decreased binding affinity for ROR2 in coimmunoprecipitation assays (Figure 1G and 1H). We sequenced the coding exons of *WTIP* in 95 genomes of HCM patients without a known genetic diagnosis from the Mayo Clinic and did not identify this variant. In light of its population novelty, tissue-specific expression, and biological context, this variant was pursued as the primary candidate for disease causality.

To clarify the role of WTIP in functional cardiomyocyte phenotypes, we first assessed the effect of *WTIP* knockdown in neonatal rat ventricular myocytes. Lentiviral transduction of short hairpin ribonucleic acid targeting *Wtip* resulted in ≈50% knockdown of *Wtip* expression (Figure IA). This knockdown caused exaggerated phenylephrine-induced hypertrophy, as quantified by cell area and *NPPA* expression (Figure 2A; Figure IB).

As elevated cytoplasmic calcium activates prohypertrophic signaling in the concentric hypertrophy seen in HCM,[6] we further explored the role of WTIP on cardiac calcium dynamics. The WTIP interactor ROR2 (Figure 1G and 1H) modulates Wnt signaling to control cytoplasmic calcium content in neurons, and a ROR2 ligand is known to orchestrate calcium release during cardiac development in *D rerio*.[5,7] We, therefore, sought to understand the effect of modulating WTIP expression and ROR2 activation on calcium handling in the intact heart of *D rerio*, in which Wnt11 is the major activator of ROR2.[8] Morpholinos targeting WTIP and Wnt 11 translation were injected at the zygote stage of a *D rerio* line expressing a nppb::luciferase reporter.[9] Morpholinos to WTIP and concomitant knockdown with WTIP and Wnt11 both caused significantly increased luciferase expression, indicative of *nppb* expression and activated hypertrophic signaling (Figure 2B). Representative calcium fluorescence from the cardiac field is shown in Figure 2C. Peak calcium release was also increased after treatment with WTIP morpholinos or WTIP and Wnt11 morpholinos in combination (Figure 2D). The combination of Wnt11 and WTIP knockdown caused significantly increased resting calcium in addition to peak calcium release (Figure 2E), and this increase in mean cardiomyocyte calcium would be expected to have tonic effects on myosin activation. Both stroke volume and cardiac output were decreased without significant change in heart rate (Figure IC through 1E), potentially indicating reduced contractility or smaller, stiffer ventricles. This indicates that WTIP and ROR2 ligands act synergistically to maintain resting cardiac calcium and that WTIP loss of function and perturbation of its interaction with ROR2 increase resting calcium, calcium release, and hypertrophy in the *D rerio* heart.

To provide functional genomic context for WTIP within human cardiac tissue in adaptive hypertrophy, we performed network analysis[10,11] of 560 human myocardial tissue transcriptomic samples (whole genome microarray) from normal samples, samples with heart failure, and samples with cardiac hypertrophy. *WTIP* expression was significantly lower in heart failure and hypertrophy than in normal tissue and attained local and global

topological centrality in adaptive cardiac hypertrophy (as quantified by intramodular and global connectivity, respectively) when compared with normal tissue (Figure 3A). *WTIP* expression was significantly indirectly associated with module eigengene expression for all 3 of these networks (Pearson correlation coefficients, −0.59 to −0.69). Furthermore, WTIP was significantly coexpressed with 18 genes previously implicated in familial HCM (P=0.02; Figure 3B). Taken together, these results suggest a role for WTIP as a negative regulator of gene subnetworks involved in human adaptive cardiac hypertrophy and shared adaptive expression with specific gene members known to contribute to HCM pathogenesis.

We next investigated whether genetic variation near *WTIP* might explain some variance in left ventricular hypertrophy in the general population. We performed gene-wise meta-analysis of associations between common variants and echocardiographically defined left ventricular wall thickness (Figure 3C). Analysis of 6399 participants of the European ancestry in the EchoGen consortium showed a significant locus-wide association signal with left ventricular wall thickness (P=0.03). This signal was driven primarily by 3 single nucleotide polymorphisms, rs6510424 (meta-analysis P=0.0038 in single marker analysis), rs4805085 (P=0.019), and rs10416146 (P=0.02), all of which occur in 3′UTR or just downstream of *WTIP*. In a phenome-wide association scan of the UK Biobank, rs758217881 (≈50 kb downstream of WTIP) is associated with right bundle branch block—a common conduction system abnormality in cardiomyopathy—though it does not reach a threshold for phenome-wide significance (P=9.2×10^{-7}).[12]

Lastly, we applied the above findings to disease-relevant phenotypes in patient-derived iPSC-CMs. Based on elevated baseline and peak calcium fluorescence in *WTIP* knockdown in *D rerio*, we hypothesized that patient-derived *WTIP* p.Y233F iPSC-CMs would exhibit abnormal calcium cycling and associated hypertrophy. iPSC-CMs with the *WTIP* p.Y233F variant recapitulated the hypertrophy observed in the familial phenotype (Figure 4A and 4B). Calcium imaging with the ratiometric calcium fluorescence dye Fura-2 AM demonstrated higher resting calcium fluorescence in iPSC-CMs harboring the p.Y233F variant (P=0.004) but did not display significantly increased peak calcium fluorescence. Based on ROR2's known activation of voltage-gated calcium channels in neurons (Figure 4C), we hypothesized that verapamil, an L-type calcium channel antagonist, would ameliorate this phenotype. Indeed, verapamil treatment significantly decreased p.Y233F iPSC-CM resting calcium fluorescence (P<0.0001; Figure 4D and 4E), suggesting a specific avenue of therapy for this form of HCM.

DISCUSSION

Cardiac hypertrophy underlying both HCM and more common causes of left ventricular hypertrophy are associated with the activation of calcium-sensitive actors in the adult heart, including calcineurin and calcium calmodulin kinase, which have a major role in the cardiac hypertrophic response.[6] Although this calcium-dependent hypertrophic signaling program is well-described, upstream molecular pathways are incompletely understood: Most of the heritability of Mendelian cardiomyopathy is attributable to genes encoding the contractile machinery of cardiomyocytes, and the genetic underpinnings of more common causes of the maladaptive cardiac hypertrophic response[13] are even less well understood.

Our findings illuminate a novel avenue upstream of calcium-dependent hypertrophic signaling, through WTIP and the Wnt signaling pathway. Although the Wnt pathway has been implicated in other types of cardiomyopathy (eg, arrhythmogenic right ventricular cardiomyopathy)[14–16] and WTIP specifically is involved in cardiogenesis,[17–22] the role of WTIP in calcium overload and cardiomyocyte hypertrophy has not been reported. We show definitive evidence that WTIP is necessary for maintenance of cardiomyocyte and cardiac size and implicates disrupted calcium homeostasis as the underlying mechanism for this, a pathway known to be controlled by Wnt signaling in the developing heart and in other cell types.[5,7]

In functional genomics data, we demonstrate that WTIP is not only highly connected to a network of genes critical to human cardiac hypertrophy but also that regulatory variation around this locus is associated with ventricular wall thickness in EchoGen. Recent robust investigation of modifiers in HCM revealed hypertension, and specifically diastolic hypertension, to be a cause of increased hypertrophy in patients without an identified sarcomeric variant.[23] In this study, WTIP was not identified as a modifier of HCM, though this may be due to relatively small effect and rarity compared with variation in other large cardiomyopathy genes in the population. Of note, none of the family members studied here had hypertension at the time of diagnosis, and none who have been followed longitudinally have developed significant diastolic hypertension. It, therefore, remains possible and in fact highly likely that diastolic hypertension can modify wall thickness in the setting of a WTIP variant, certainly via pressure overload–related modulation of hypertrophy independent of high resting cytosolic calcium, and may also potentiate the effect of WTIP p.Y233F on resting calcium and downstream calcium-dependent hypertrophy signaling.

Further, we specifically find that the identified familial variant disrupts the binding of WTIP to ROR2. ROR2 is a known regulator of calcium dynamics in other cell types,[5] and its ligand Wnt 11 is implicated in calcium-based patterning of the developing heart.[7] Our findings, therefore, supply a putative mechanism by which WTIP controls hypertrophy and calcium homeostasis: it does so via Wnt signaling partners known to lie upstream of the well-described calcium-dependent hypertrophic signaling program common across etiologies of cardiac hypertrophy. Additionally, elevated resting calcium in iPSC-CMs harboring this variant is relieved by blocking voltage-gated calcium channels. This suggests a new, precision therapeutic for the family in question but also evidences the role of ROR2's activation of voltage-gated calcium channels in this mechanism, similar to that observed in neurons.[5] Voltage-gated calcium channels in the cardiomyocyte are responsible not only for the magnitude of calcium-induced calcium release but also for the maintenance of heart rhythm.[24,25] Therefore, WTIP and other Wnt pathway actors (eg, ROR2) may not only regulate calcium overload–induced hypertrophy but could be attractive targets to modulate calcium-dependent arrhythmogenesis in the adult heart.

The present study exemplifies the power of the natural experiment of rare human disease to inform our broader understanding of human disease mechanisms and cellular functions. The impact of our identification of WTIP as a new causative gene for a Mendelian disease (HCM) sheds light not only on the complex genetic architecture of HCM but on new key regulators of maladaptive cardiac hypertrophy across etiologies. At scale, the

study of constraint of loss-of-function variants in the population “human knockouts” can inform functional studies and even drug target selection.[26] However, these analyses are complicated by differential tissue expression and unknown evolutionary pressures. Here, because we were aware a priori of both the tissue and disease process associated with WTIP loss of function, we could circumvent these issues: this enabled the use of extant human cardiac tissue databases and precise disease modeling in vivo and in vitro to illuminate a new pathway controlling human cardiac hypertrophy. There are many other Mendelian-complex disease pairs in which rare human knockouts manifest severe forms of pathology or protection (eg, familial hypercholesterolemia, myocardial infarction risk, and ***PCSK9***[1,27–29]). As we show here with ***WTIP*** and maladaptive cardiac hypertrophy, studies of Mendelian disease will continue to inform our understanding of the underlying mechanisms on which rare and complex diseases converge.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Figure 4C was created using BioRender. A prior version of this article was incorporated into Dr De Jong’s doctoral dissertation.

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Disclosures

Dr Madhvani is currently an employee at Cytokinetics. Dr Chen is currently an employee at Personalis, Inc. Dr Ackerman is a consultant for Abbott, Audentes Therapeutics, Boston Scientific, Daiichi Sankyo, Invitae, LQT Therapeutics, Medtronic, MyoKardia, and UpToDate. Dr Ackerman and Mayo Clinic have an equity/royalty relationship with Alive Cor and Anumana. Dr MacRae has the following disclosures: Bayer, Merck, Affinia Tx, Design Tx, DiNAQor, Dewpoint, Myokardia, Atman Health, BioSymetrics, Foresite Labs, Dr Evidence, Apple, Verily, AstraZeneca, Bodyport, gWell, NeuCures, Clarify Health Solutions, Pfizer, and Quest Diagnostics. E.A. Ashley has the following financial disclosures: Personalis (founder and advisor), Deepcell (founder and advisor), Silicon Valley Exercise Analytics (founder and advisor), AstraZeneca (nonexecutive director), Gilead (advisor, clinical trial site PI), MyoKardia (academic grant, clinical trial site PI), Amgen (advisor, academic grant), Takeda (academic grant), Genome Medical (advisor), Avive (founding advisor), Samsung (scientific collaboration), Apple (advisor, scientific collaboration), Google (academic grants), Verily (scientific collaboration), Disney (advisor), Illumina (collaborative support in kind), Pacific Biosciences (collaborative support in kind), Oxford Nanopore (collaborative support in kind), Foresite Capital (advisor), and Sequence Bio (advisor).

Nonstandard Abbreviations and Acronyms

HCM	hypertrophic cardiomyopathy
iPSC-CM	induced pluripotent stem cell-derived cardiomyocyte
ROR2	receptor tyrosine kinase-like orphan receptor 2
WTIP	Wilms tumor interacting protein

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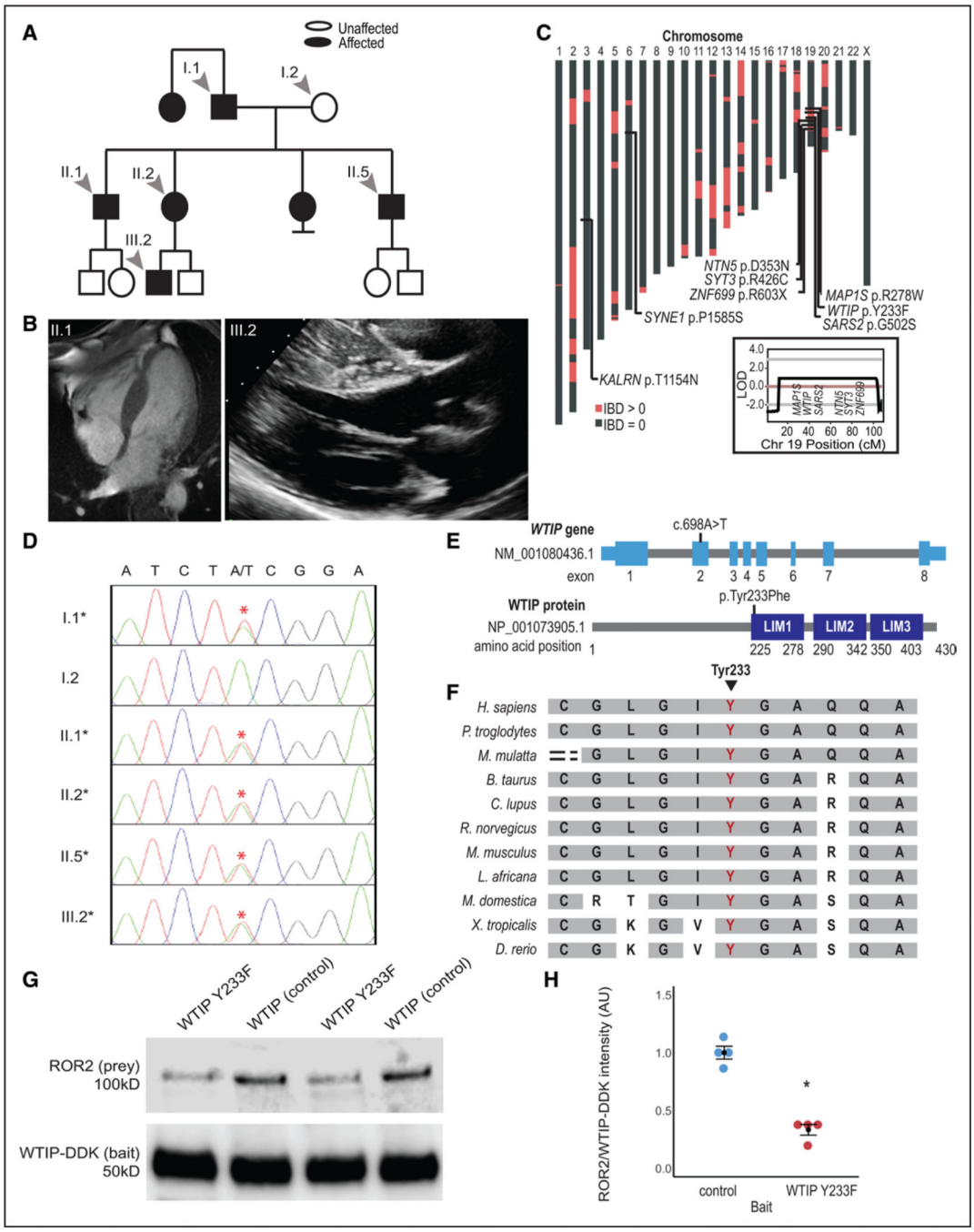

Figure 1. Exome sequencing identifies a region on chromosome 19 shared identical by descent among affected family members and a novel missense variant cosegregating with disease in the first LIM domain of Wilms tumor interacting protein (WTIP).

A, Partial family pedigree of study participants. Circles denote women, and squares denote men. Arrows indicate individuals who were sequenced for this study. **B**, Parasternal long-axis echocardiographic image of affected individual II.1 and cardiac magnetic resonance image of affected individual III.2. **C**, Identity by descent (IBD) sharing among affected family members according to 2 state (IBD, 0; IBD, >0) hidden Markov model. Six novel variants were identified in a region on chromosome 19 shared identical by descent among

all affected family members. Inset, parametric linkage analysis of the same region using MERLIN on exome data reduced to 1 single nucleotide polymorphism per 0.3 cm bin. **D**, Capillary sequence traces of *WTIP* p.Y233F. **E**, p.Y233F disrupts the first LIM domain of WTIP. **F**, Tyrosine 233 is fully conserved from *Homo sapiens* through *Danio rerio*. **G** and **H**, Compared with reference WTIP, WTIP p.Y233F shows decreased binding to ROR2 (receptor tyrosine kinase-like orphan receptor 2) as quantified by coimmunoprecipitation. Black circles indicate mean, and error bars indicate SE. **P*<0.05, Welch *t* test.

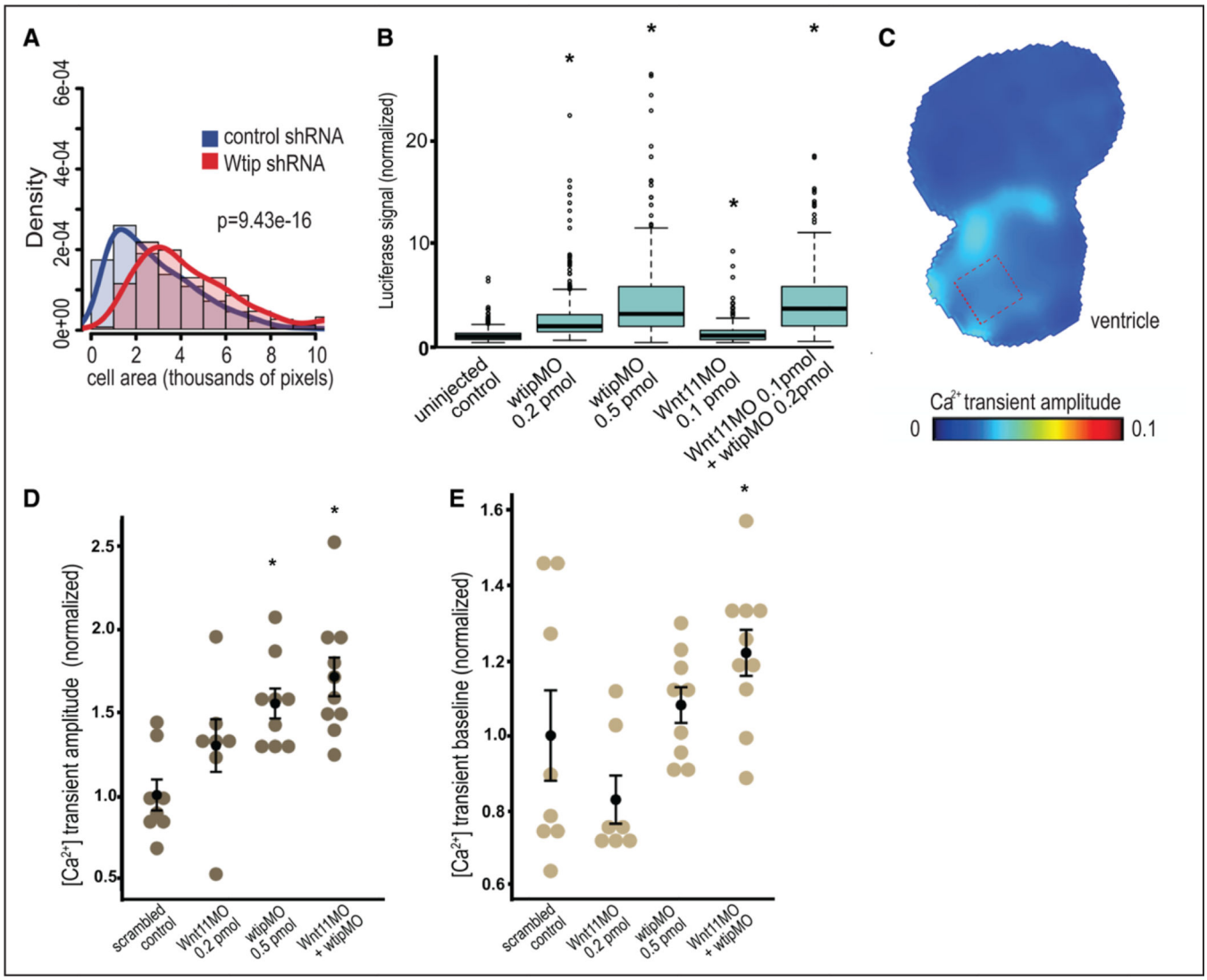

Figure 2. Knockdown of Wilms tumor interacting protein (WTIP) in independent model organisms recapitulates cardiac hypertrophy-associated phenotypes.

A, Lentiviral transduction of shRNA targeted to Wtip in neonatal rat ventricular myocytes exaggerates phenylephrine-induced cellular hypertrophy, 48 h after treatment. Histogram represents density of cell size in control shRNA (blue) and Wtip-targeted shRNA infected cultures (red). *P* denotes significance of difference in distributions as quantified by the Wilcoxon rank-sum test. **B**, Pathological natriuretic peptide (nppb) responses of live zebra fish to the wtipMO injected at 2 different morpholino (MO) concentrations, 0.2 pmol (n=252) and 0.5 pmol (n=192), the wnt11MO (n=204) at 0.1 pmol, with both MOs (n=180), and in uninjected control embryos (n=288) at 4 hours post fertilization. The nppb promoter firefly luciferase reporter line, nppb:F-Luc[9] and the wnt11MO[7] have been described and validated in detail elsewhere. *$P<0.05$ compared with uninjected control, Fisher LSD. **C**, Representative image of calcium fluorescence across *Danio rerio* heart field showing relative change in amplitude to background. **D**, Amplitude and (**E**) baseline levels of the intracellular Ca^{2+} transient in isolated hearts from scrambled MO control (n=8) embryos and after

injection with the wnt11MO (n=7), the wtipMO (n=9), and with both MOs (n=10) at the same concentrations. Black circles indicate mean, and error bars indicate SE. *P<0.05 compared with scrambled MO control, Fisher least significant difference shRNA, short hairpin ribonucleic acid.

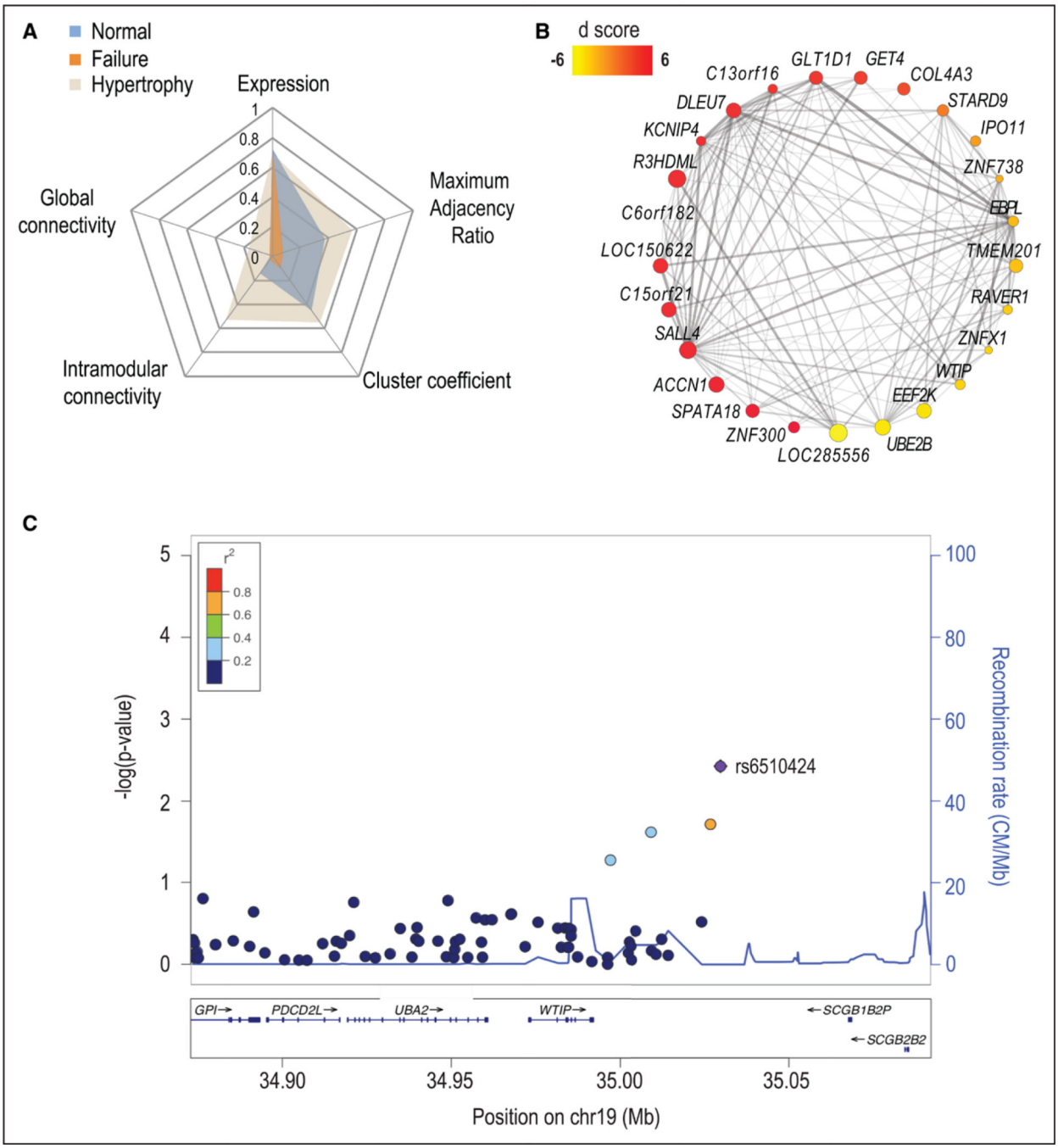

Figure 3. Wilms tumor interacting protein (WTIP) myocardial tissue coexpression patterns and local common variation are associated with adaptive cardiac hypertrophy.

A, *WTIP* is marginally differentially expressed but significantly more highly connected in global and local gene coexpression network topology in human cardiac hypertrophy than in normal tissue. **B**, Local modular network topology for *WTIP* in human cardiac hypertrophy. Line width corresponds to edge weight between genes as quantified by topological overlap, while node size is proportional to intramodular connectivity. Node color indicates significance of differential expression in cardiac hypertrophy compared with

normal tissue as quantified by the significance analysis of microarrays d score. Yellow indicates higher expression in normal tissue than in hypertrophy, while red indicates the converse. **C**, Common variation near *WTIP* is associated with the common phenotype of left ventricular wall thickness in 6399 White individuals from the EchoGen cohort. Subjects were genotyped using the Affymetrix 6.0 array, and 2.5 million genotypes represented in HapMap II were imputed using MACH. Association analysis was performed in each cohort using an additive genetic model and linear regression, and meta-analysis *P* values were generated using METAL. Locus zoom plot of meta-analysis *P* for associations between common genetic variants and left ventricular wall thickness, as quantified by sum of interventricular septal thickness and posterior wall thickness at end diastole, in the region 110 kb upstream to 40 kb downstream of *WTIP*.

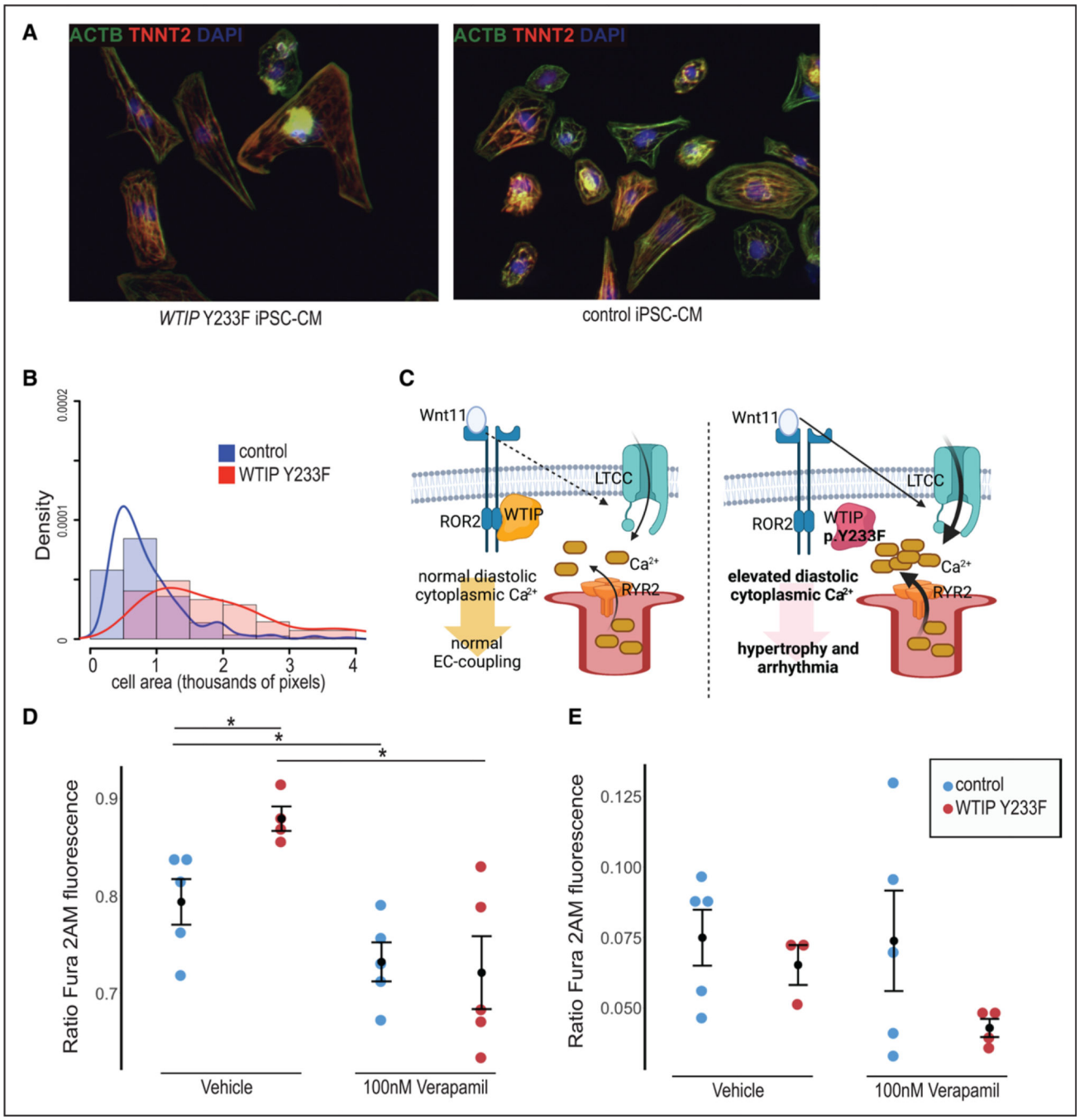

Figure 4. Patient-derived WTIP Wilms tumor interacting protein (WTIP) p.Y233F cardiomyocytes are hypertrophic and display elevated resting calcium that is alleviated by verapamil.

A, Representative images of induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from participants with (mutant WTIP) and without (control) mutations in the first LIM domain of WTIP. Cells were stained using antibodies against Actin beta (ACTB), Troponin T2, cardiac type (TNNT2), and with 4′,6-diamidino-2-phenylindole (DAPI). **B**, Cardiac cellular size is significantly greater in iPSC-CM from a carrier of mutant WTIP than in control cell line. Histogram represents density of cell size in control (blue) and

mutant WTIP iPSC-CM cultures (red). *P* denotes significance of difference in distributions as quantified by the Wilcoxon rank-sum test. **C**, Diagram of the regulation of cytoplasmic calcium content by WTIP (**left**) and hypothesized misregulation by WTIP pY233F (**right**). RYR2 denotes ryanodine receptor 2; LTCC denotes L-type calcium channel. **D**, Resting calcium fluorescence in WTIP p.Y233F iPSC-CM is significantly increased vs reference control iPSC-CMs. Verapamil administration decreases resting calcium fluorescence in both WTIP p.Y233F and reference lines. **E**, Peak calcium fluorescence amplitude does not significantly differ between WTIP p.Y233F and reference iPSC-CMs. Black circles indicate mean, and error bars indicate SE. ROR2 indicates receptor tyrosine kinase-like orphan receptor 2. *Bonferroni-adjusted $P<0.05$, ANOVA.

Table 1.

Clinical and Echocardiogram Characteristics of Study Participants

Participant	I.1*	I.2	II.1*	II.2*	II.5*	III.3*
Age, y	79	73	54	55	53	17
Height, cm	175	165	187	170	188	188
Body mass, kg	79	134	79	63	99	98
LVIDd, mm	41	48	35	35	44	43
IVSd, mm	15	11	19	14	17	31
PWTd, mm	15	10	11	9	11	11
LVEF, %	75	67	73	83	69	90
Lateral MV E′, cm/s	10.8	10.7	9.9	13.6	…	11.1
E/A	…†	1.2	1.1	1.6	2.0	1.7
LVOT gradient, rest, mm Hg	0	0	13	21	0	3
LVOT gradient, valsalva, mm Hg	0	0	32	28	0	5

IVSd indicates left ventricular septal wall thickness at end diastole; PWTd, posterior wall thickness; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal dimension at end diastole; LVOT, left ventricular outflow tract; and MV, mitral valve.

*Affected participant.

†Unable to calculate: subject in atrial fibrillation.

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Table 2.

Summary of Variants Discovered by Exome Sequencing

Participant	I.1*	I.2	II.1*	II.2*	II.5*	III.3*
All SNVs	34 810	35 403	35 264	35 821	35 741	35 397
NS/MS/SS SNVs	6006	6170	5995	6154	6078	6243
Rare NS/MS/SS SNVs	297	305	289	310	281	299
All indels	2659	2728	2723	2737	2732	2647
Coding indels	235	239	233	222	238	237
Frameshift coding indels	112	112	111	105	118	113
Rare frameshift coding indels	97	99	96	92	103	95

MS indicates missense; NS, nonsense; SNV, single-nucleotide variant; and SS, splice site.

*Affected subject.

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