HHS Public Access

Author manuscript

Circ Genom Precis Med. Author manuscript; available in PMC 2024 August 01.

Published in final edited form as:

Circ Genom Precis Med. 2023 August ; 16(4): 390–400. doi:10.1161/CIRCGEN.122.003912.

PRDM16 Deletion Is Associated with Sex-dependent Cardiomyopathy & Cardiac Mortality: A Translational, Multi-institutional Cohort Study

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Abstract

Background: 1p36 deletion syndrome can predispose to pediatric-onset cardiomyopathy. Deletion breakpoints are variable and may delete the transcription factor *PRDM16*. Early studies

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Disclosures: The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing performed at Baylor Genetics Laboratories.

suggest deletion of *PRDM16* may underlie cardiomyopathy in patients with 1p36 deletion; however, the prognostic impact of *PRDM16* loss is unknown.

Methods: This retrospective cohort included subjects with 1p36 deletion syndrome from four hospitals. Prevalence of cardiomyopathy and freedom from death, cardiac transplantation, or ventricular assist device (VAD) were analyzed. A systematic review cohort was derived for further analysis. A cardiac-specific *Prdm16* knockout mouse (*Prdm16* cKO) was generated. Echocardiography was performed at 4 and 6-7 months. Histology staining and qPCR were performed at 7 months to assess fibrosis.

Results: The retrospective cohort included 71 patients. Among individuals with *PRDM16* deleted, 34.5% developed cardiomyopathy versus 7.7% of individuals with *PRDM16* not deleted (p=0.1). In the combined retrospective and systematic review cohort (n=134), *PRDM16* deletion-associated cardiomyopathy risk was recapitulated and significant (29.1% versus 10.8%, p=0.03). *PRDM16* deletion was associated with increased risk of death, cardiac transplant, or VAD (p=0.04). Among those *PRDM16* deleted, 34.5% of females developed cardiomyopathy versus 16.7% of their male counterparts (p=0.2). We find sex-specific differences in the incidence and the severity of contractile dysfunction and fibrosis in female *Prdm16 cKO* mice. Further, female *Prdm16* cKO mice demonstrate significantly elevated risk of mortality (p=0.0003).

Conclusions: *PRDM16* deletion is associated with a significantly increased risk of cardiomyopathy and cardiac mortality. *Prdm16* cKO mice develop cardiomyopathy in a sex-biased way. Patients with *PRDM16* deletion should be assessed for cardiac disease.

Keywords

1p36 deletion syndrome; *PRDM16*; cardiomyopathy; mortality; sex differences

Background

1p36 deletion syndrome is the most common terminal deletion syndrome in humans, affecting approximately 1 in 5,000 newborns.[1] Children can present with a variety of deficits, including growth delay, intellectual disability, developmental delay, seizures, congenital heart defects, cardiomyopathy, and distinctive craniofacial abnormalities.[1-3] Cardiomyopathy is an important clinical feature of 1p36 deletion syndrome as symptomatic pediatric cardiomyopathy carries a 40% risk of death or cardiac transplant within 2 years.[4] Recently, one cohort of patients with 1p36 deletion syndrome found that 27% of patients had documented cardiomyopathy, particularly noncompaction cardiomyopathy (NCM) and dilated cardiomyopathy (DCM).[2] NCM is characterized by prominent ventricular trabeculations with deep intertrabecular recesses.[5,6] DCM is defined by a dilated left ventricle with systolic dysfunction, in the absence of an anatomic or hemodynamic cause.[7] Of note, NCM can also lead to decreased systolic function and has considerable overlap with DCM.[8] Within the 1p36 region, several candidate cardiomyopathic genes have been suggested, which has made the pathogenesis of 1p36 deletion syndrome-associated cardiomyopathy difficult to evaluate and prognosticate.[1,3,9]

PRDM16, which encodes a zinc-finger transcription factor and plays a role in negative TGF-β regulation, has been put forth as a candidate gene underlying the 1p36 deletion

syndrome-associated cardiomyopathy.[10,11] However, this remains controversial in humans as several cardiac genes are lost in 1p36 deletion syndrome and analysis of *PRDM16* variants demonstrates there are documented non-pathogenic *PRDM16* variants.[12] Recent associational analyses indicated truncating variants in *PRDM16* are associated with NCM,[13] further suggesting *PRDM16* loss is associated with or causes cardiomyopathy.

Multiple *Prdm16* knockout mouse models have been generated to further investigate the role of *PRDM16* deletion in cardiomyopathic pathogenesis. These models utilize cardiac-specific *PRDM16* knockouts, because germline knockouts are embryonically lethal. There is variation between models, with some demonstrating age-related hypertrophic cardiomyopathy while others demonstrate effects consistent with NCM due to failure of cardiac compaction and maturation.[14-18] All models demonstrate substantial cardiac morbidity and mortality caused by isolated *Prdm16* loss. Still, there is minimal evidence regarding the role of *PRDM16* in the human heart. *Prdm16*-knockout mice demonstrate cardiomyopathic effects similar to those observed clinically in patients with 1p36 deletion syndrome. Further, human data suggests loss of *PRDM16* is associated with NCM.[13] Still, it remains to be determined if *PRDM16* loss underlies cardiomyopathy observed in 1p36 deletion syndrome in humans and what the impact of this may be on outcomes.

To further elucidate the prognostic impact and clinical cardiovascular outcomes, such as cardiac diagnoses and medical management, of *PRDM16* deletion, this multi-center cohort study, investigates the outcomes and cardiac manifestations of *PRDM16* loss in 1p36 deletion syndrome. We hypothesize that within the spectrum of 1p36 deletion syndrome, deletion of *PRDM16* is associated with cardiomyopathy and confers an increased risk of mortality.

Methods

Full methods are available in the Supplemental Material. The study was approved by the Duke University Hospital IRB. Informed consent was waived. Due to the sensitive nature of the human data collected for this study, requests to access the dataset from qualified researchers trained in human subject confidentiality protocols may be sent to the corresponding author. Murine data are available from the corresponding author upon request.

Results

Cohort Characteristics

We identified a total of 71 patients with a deletion of 1p36 and at least one encounter which comprised the “retrospective cohort” (Table 1, Figure 1). The median age at diagnosis was 1.4 years (IQR 0.0-9.6). Thirty-seven subjects (52.1%) were female, and thirty-four subjects (47.9%) were male. Fifty-four individuals (76.1%) had a deletion of 1p36 and no other chromosomal deletions, while seventeen subjects (23.9%) had 1p36 deletion as well as another chromosomal abnormality. Thirty subjects (42.3%) lost *PRDM16* as part of their 1p36 deletion, henceforth referred to as the “*PRDM16* deleted” group. Twenty-seven (38.0%) did not lose *PRDM16* as part of their 1p36 deletion, referred to as the “*PRDM16* not deleted” group. Fourteen patients (19.7%) did not have chromosomal microarrays that

clearly identified *PRDM16* status and were eliminated from *PRDM16*-specific analyses. Those with *PRDM16* deleted were significantly more likely to have an isolated 1p36 deletion with no other chromosomal aberrations (p=0.003), to be diagnosed earlier (0.2 years versus 3.4 years, p=0.02), and to receive an echocardiogram (96.7% versus 48.1%, p<0.0001). There was a female predominance in those with *PRDM16* deleted (63.3% female, versus 37.0% female in those with *PRDM16* not deleted, p=0.06) compared to males.

To expand the number of individuals analyzed within this rare disease, this cohort was combined with the systematic review-acquired cases from the literature. This formed a "combined cohort" (Supplemental Table I). The combined cohort included a total of 134 subjects with defined *PRDM16* status; 81 subjects had a *PRDM16* deletion. As in the retrospective cohort, there was a female predominance in those with *PRDM16* deleted (69.1% female, p=0.002).

PRDM16 Deletion is Associated with Cardiomyopathy

To determine whether there is an association of *PRDM16* loss and cardiomyopathy, contingency tables were used to evaluate association between loss of *PRMD16* and diagnoses of cardiomyopathy. In the retrospective cohort, there were thirty-nine patients with at least one diagnostic echocardiogram available for review; this subset was used for analysis of cardiomyopathy development and assessment for structural defects (Figure 1). Interestingly, 93.3% (28/30) of patients with *PRDM16* deleted received an echocardiogram versus 40.7% (11/27) of those with *PRDM16* not deleted (p<0.0001, Table 1). Among those with *PRDM16* deleted, 35.7% (10/28) of patients were diagnosed with any cardiomyopathy versus 9.1% (1/11) of patients with *PRDM16* not deleted. For all cardiomyopathies, patients with *PRDM16* deleted were overrepresented when compared to their *PRDM16* not deleted peers (Figure 2A). In the retrospective cohort, the association between *PRDM16* deletion and diagnosis of any cardiomyopathy appeared noteworthy but underpowered, prompting the analysis of the combined cohort.

The combined cohort was comprised of 134 individuals of which 116 had diagnostic echocardiograms. The trends were similar and statistically significant. Of those with *PRDM16* deleted, 29.1% (23/79) carried a diagnosis of cardiomyopathy versus 10.8% of subjects with *PRDM16* not deleted (p=0.03, Figure 2B, Supplementary Table II). NCM was specifically associated with *PRDM16* deletion; 22.8% (18/79) of those with *PRDM16* deleted developed NCM versus 5.4% (2/37) of their *PRDM16* not deleted counterparts (p=0.03). Among the retrospective cohort, 15.4% (6/39) subjects had electrophysiologic abnormalities and 46.4% (26/56) had hemodynamically-significant congenital/structural heart defects; neither was associated with *PRDM16* deletion (Table 1). Taken together, these data demonstrate *PRDM16* deletion is associated with cardiomyopathy and, in particular, NCM.

PRDM16 Deletion is Associated with Death, Heart Transplant, and Ventricular Assist Devices

To assess the impact of *PRDM16* deletion on the cardiac mortality defined as death, heart transplant, or ventricular assist device (VAD) placement, a Kaplan-Meier survival estimate was generated. Among patients with 1p36 deletion syndrome in the retrospective cohort, loss of *PRDM16* significantly increases probability of death, heart transplant, and/or VAD placement (Figure 2C, p=0.04, log-rank test). Four subjects met the survival outcome: three subjects died, and one subjects received a left VAD followed by cardiac transplant. All four patients had *PRDM16* deleted, suggesting that *PRDM16* is a risk locus and is necessary for cardiac mortality in patients with 1p36 deletion syndrome. Of note, each patient who met this outcome did so prior to the age of 15, supporting the echocardiographic data suggesting *PRDM16* loss-mediated cardiac injury in 1p36 deletion syndrome is a primarily a pediatric disease. One additional subject was a fetus following intrauterine fetal demise and subsequent genetic testing discovered *PRDM16* deletion in 1p36 deletion syndrome; this subject was removed from this analysis.

Schoenfeld residual testing demonstrated proportionality was not violated, thus permitting covariate analysis for sex-specific effects. In this cohort, there were no significant sex-specific effects on freedom from death, VAD placement, or transplant (Supplemental Figure I). While the retrospective cohort was not powered to examine the role of sex in *PRDM16* deletion, of the 4 patients who met the composite survival outcome, 3 were female. In summary, we conclude that among those with 1p36 deletion, *PRDM16* deletion confers an increased risk of cardiac mortality.

Subjects with *PRDM16* Deleted Have High Risk of Systolic Dysfunction

Next, association between *PRDM16* loss and systolic dysfunction was analyzed using a Kaplan Meier estimator. This study analyzed freedom from systolic dysfunction in patients with 1p36 deletion, stratified by loss of *PRDM16* (Figure 2D). Notably, systolic dysfunction was common among individuals with *PRDM16* deleted, with a 64.4% probability of developing systolic dysfunction by age eighteen. These data suggest that patients who lose *PRDM16* as part of their 1p36 deletion are at substantial risk for pediatric-onset systolic dysfunction. Schoenfeld residual testing demonstrated proportionality was not violated, thus permitting covariate analysis for sex-specific effects. In this cohort, there were no significant sex-specific effects on freedom from systolic dysfunction (Supplemental Figure I). Altogether, this cohort found that patients with 1p36 deletion syndrome have elevated but variable risk of systolic dysfunction, and that nearly two-thirds of patients with *PRMD16* deleted develop pediatric-onset systolic dysfunction.

Medical Management of Cardiovascular Disease in 1p36 Deletion Syndrome

We next explored the medical management of these patients (Table 2). Of those with reported cardiac medications (41), 20 had *PRDM16* deleted and 21 had *PRDM16* not deleted. Patients with *PRDM16* deleted were significantly more likely to be receiving cardiac medications, with 50.0% (10/20) of patients with *PRDM16* deleted versus 9.5% (2/21) of patients with *PRDM16* not deleted (p=0.006). Patients with *PRDM16* deleted were significantly more likely to receive two classes of medications: beta blockers (p=0.04)

and ACE inhibitors (ACEI), angiotensin receptor blockers (ARBs), and/or angiotensin receptor-neprilysin inhibitors (ARNIs, p=0.02). The most common beta blockers were second generation, cardio-selective beta blockers (4 patients). The next most common class was third generation, vasodilatory beta blockers (3 patients). No patients received first generation, non-selective beta blockers. Nearly all patients with cardiomyopathy received cardio-active medications and few received cardiac medications without diagnosis of cardiomyopathy (Supplemental Figure II). Altogether, patients with *PRDM16* deleted were significantly more likely to receive cardiac medications of any kind. Patients with *PRDM16* deleted were specifically more likely to receive beta blockers and ACEI, ARBs, and/or ARNIs.

Females May be at Increased Risk of *PRDM16* Deletion-Associated Cardiomyopathy

We next explored the possibility that sex as a biological variable may influence phenotype in the setting of *PRDM16* deletion. Of those with *PRDM16* deletion, 63.3% are female versus 37.0% among those without a *PRDM16* deletion (p=0.06). This stark difference prompted analysis of sex effects in *PRDM16* deletion. As previously mentioned, among those with *PRDM16* deletion, females appear to have excess cardiac mortality and increased risk of systolic dysfunction, though this study was not powered to detect such differences (Supplementary Figure I, p=0.8 and p=0.6, respectively). The combined cohort includes a total of 81 individuals with *PRDM16* deletion and thus was targeted for analysis. Among those with *PRDM16* deletion in the combined cohort, females comprised 69.1% of the cohort and were significantly overrepresented (Figure 3A, p=0.0008). Among those with *PRDM16* deletion and cardiomyopathy, females comprised 82.6% of all subjects and were again overrepresented (p=0.003). Among those with *PRDM16* deleted, females were twice as likely to carry a diagnosis of cardiomyopathy: 34.6% of females (19/55) were diagnosed with cardiomyopathy versus 16.7% of males (4/24), though this relationship was not significant (Figure 3B, p=0.18). Altogether, these data suggest that females may be especially affected by *PRDM16* deletion given their overrepresentation in *PRDM16* deletion and potentially increased burden of cardiomyopathy.

Cardiac-Specific *Prdm16* Conditional Knockout Mice Develop Dilated Cardiomyopathy (DCM) and Fibrosis in a Sex-Biased Way

To further evaluate the effects of sex in *PRDM16* deletion, a *Prdm16* conditional knockout (cKO) murine model was generated. We monitored cardiac function through blinded echocardiography in *Prdm16* cKO mice at 4- and 6-7 months. At 4 months of age, female *Prdm16* cKO mice had ~38% reduction in ejection fraction (EF) and fractional shortening (FS) ($p<0.05$) when compared to age and sex-matched WT controls (Figures 4A-B). Female cKO mice also exhibited signs of left ventricular (LV) dilation as evidenced by a significant ($p<0.05$) increase in LV internal diameter at systole (Figure 4C). Interestingly, none of these parameters were affected in male cKO mice when compared to male WT mice. By 6-7 months of age, both female and male *Prdm16* cKO mice displayed a significant decrease in EF ($p<0.005$) and FS ($p < 0.005$) indicating impairment of systolic function (Figures 4G-H). Furthermore, *Prdm16* cKO mice demonstrate increased end-diastolic LV internal dimension ($p<0.05$) that were more severely affected in female cKO mice versus male cKO mice, when compared to their respective WT controls (Figures 4I-J). Finally, only female *Prdm16* cKO

mice had reduced LV posterior wall in systole, which indicates the development of DCM at 6-7 months (Figure 4K).

To examine if *Prdm16* ablation in cardiac cells induced fibrosis, we performed histological examination using Trichrome and Picrosirius Red staining on predominantly male WT and cKO mice, WT female mice and only one female cKO mouse that survived to 7 months. The results demonstrate a 3- ($p<0.05$) and 8-fold ($p<00005$) increase in % fibrosis area as assessed by trichrome and picrosirius red, respectively, in the hearts of *Prdm16* cKO mice at 7 months (Figures 5A-C). Consistent with the early cardiac dysfunction in female mice at 4 months of age, cardiac histology of the only female cKO mouse that survived to 7 months showed qualitatively more fibrosis when compared to age-matched make cKO mice.

As *Prdm16* loss was linked to myocardial fibrosis development[14,15] we next examined the expression of fibrotic genes by qPCR on male WT, male cKO mice, WT female mice, and one surviving female cKO mouse. In addition to observing the expected loss *Prdm16* mRNA expression in the hearts of *Prdm16* cKO mice (Figure 5D, $p<0.005$), we also observed elevated expression of the pro-fibrotic genes *Tgfb2*, *Tgfb3*, and *Cola1* (Figure 5D, $p<0.05$).[19,20] In addition, we detected a nearly tenfold increased expression of the natriuretic peptide gene *Nppb* though this was not significant (Figure 5D, $p=0.06$). Altogether the histological examination and qPCR analysis indicated a crucial role for Prdm16 in fibrosis in the murine heart.

Female *Prdm16* cKO Mice Have Unique Risk of Mortality

Given the increased mortality among 1p36 deletion patients who lost *PRDM16*, we next determined whether loss of *Prdm16* was associated with decreased survival. Indeed, *Prdm16* cKO mice demonstrate decreased survival (Figure 6A, $p=0.02$), consistent with the human cohort and suggestive that loss of *Prdm16* independently confers an increased risk of death. Because human cohort data suggested there was a potential sex-bias, we sought to evaluate the effects of sex; however, when introducing sex as a covariate, the proportional hazard assumption was violated (Supplemental Figure III, Schoenfeld residual $p < 0.05$) thus a stratification approach was used. Interestingly, *PRDM16* cKO demonstrated no effect on survival in male mice (Figure 6B, $p=0.8$). Female *Prdm16* cKO mice, however, demonstrate remarkably poor survival with 100% of the female mice dying by week 29 (Figure 6C, $p=0.0003$). These data demonstrate that *Prdm16* is critical for the maintenance of cardiac function and the prevention of fibrosis in the murine heart, with female mice demonstrating special vulnerability and especially deleterious outcomes.

Discussion

1p36 deletion syndrome is genetically and phenotypically heterogeneous. Genetically, patients present with a range of deletion sizes and some individuals may have additional chromosomal deletions or translocations. Phenotypically, patients display a spectrum of neurological, craniofacial, and cardiovascular defects.[1] In order to address the putative causative role of *PRDM16* loss in development of cardiomyopathy and adverse outcomes in 1p36 deletion syndrome, we assembled a multi-center cohort to date of patients with 1p36 deletion syndrome and stratified the cohort by *PRDM16* status. We then combined

this cohort with reported cases of *PRDM16* deletion in literature via a systematic review to validate *PRDM16*'s role in cardiomyopathy and investigate possible sex effects. In addition, we created a mouse model of *Prdm16* deletion in cardiomyocytes to examine the role of sex in *Prdm16* deletion-induced cardiomyopathy.

Our work builds on prior studies investigating the etiology of cardiac disease in 1p36 deletion syndrome. Arndt et al. performed multiallelic mapping in individuals with 1p36 deletion syndrome and cardiovascular disease and identified a common minimal region of loss containing *PRDM16*, which they supported with a zebrafish *prdm16* knockdown demonstrating bradycardia and reduced cardiac output.[10] They also identified five individuals with *PRDM16* variants in a cohort of patients with cardiomyopathy, associating *PRDM16* variants with NCM and DCM.[10] Important to note, however, is a letter of reply wherein De Leeuw et al. demonstrate the aforementioned region also contains other candidate cardio-active genes, such as *SKI*.[12] *SKI* is more distal on the 1p arm; thus, to lose *PRDM16*, patients with terminal deletions also lose *SKI*. Consequently, it is difficult to demonstrate from studies of 1p36 deletion syndrome alone that *PRDM16* underlies these cardiac effects.

Our cohort validates and builds upon these prior studies. 34.5% of patients with *PRDM16* loss were diagnosed with cardiomyopathy, primarily NCM and DCM. Losing *PRDM16* as part of 1p36 deletion was associated with significantly increased risk of death, heart transplant, and/or VAD placement; 20.0% of those with *PRDM16* deleted met this endpoint versus 0.0% among patients with two intact copies of *PRDM16*. Interestingly, these patients did not die of isolated progressive heart failure but rather complications from surgical correction of congenital heart disease or systemic infection. This suggests that *PRDM16* deletion generates a vulnerable myocardium susceptible to other stressors, which otherwise might be well-tolerated, that manifest this increased risk of death. This is consistent with literature that demonstrates there are genetic conditions that both predispose to cardiomyopathy and confer risk of heart failure and early mortality, such as truncating variants in *TTN*, encoding the sarcomeric protein titin, which both independently increase risk of DCM and have associated risk of heart failure/early mortality in the setting of peripartum cardiomyopathy or cardiomyopathy due to alcohol.[21-23] Regarding the role of *SKI*, we identified one patient who lost *SKI* but did not lose *PRDM16* as part of their deletion. This individual did not develop cardiomyopathy, consistent with the hypothesis that *PRDM16* loss is the disease susceptibility locus. This supports previous work that identified three patients with isolated individual would be needed to rule out *SKI* as a contributing factor for cardiomyopathy development.[24]

In addition to our main findings, we found that individuals with *PRDM16* loss were also significantly more likely to be managed on cardioactive medications (50.0% of patients), including beta blockers (30.0% of patients) and ACEI, ARB, and/or ARNIs (35.0% of patients). This is largely concordant with guidelines for pediatric heart failure, namely ACEI, ARBs, or ARNIs with possible escalation to beta blockers.[25,26] This work describes how patients with *PRDM16* deletion in 1p36 deletion syndrome-associated heart disease are presently managed; further work might build a guideline-informed, unified approach to this genetic subset of cardiomyopathy.

There were no differences between the *PRDM16* deleted and not deleted with regards to hemodynamically significant structural heart disease or arrhythmia, suggesting that *PRDM16* loss specifically increases risk of cardiomyopathy. This cohort did note female predominance in human *PRDM16* deletion (63% female in the retrospective cohort, 69% female in the combined cohort). Importantly, of those with *PRDM16* deleted, females appear to have a doubled risk of cardiomyopathy. In the combined cohort, 35% of females with *PRDM16* deletion were diagnosed with cardiomyopathy versus 17% of males, though this did not reach statistical significance. Such findings are especially relevant due to potential interface between PRDM16 and estrogen-receptor signaling[27] and previously demonstrated *Prdm16*-deletion associated hypotension in female mice.[18] Our cohort conclusions are limited, however, due to sample size, and thus attention was turned to a murine model.

Given difficulties in studying *PRDM16* loss in humans, several Prdm16-deficient animal models have been generated and have produced heterogeneous results. A zebrafish *prdm16* knockout model was generated that demonstrated bradycardia and reduced cardiac output.[10] Given embryonic lethality in germline murine *Prdm16* knockouts, several cardiac-specific conditional knockouts have been generated. Interestingly, the phenotypes have been varied: *α-MHC (Myh6)-Cre* driven knockout demonstrates hypertrophy in one model and LV dilation in another.[16,28] *Mesp1-Cre* driven knockout demonstrates age-related hypertrophy and heart failure,[15] and both *Xmlc2-Cre* and *cTnT-Cre* driven knockouts demonstrate noncompaction.[16] Possible explanations for these variations include efficiency of the *Cre* driver, the cell type(s) in which *Prdm16* is deleted, the timing of deletion, and background genetic effects.

Our murine model is similar to the one developed by Nam et al[14] using the *α-MHC (Myh6)-Cre* driven knockout. In contrast to the hypertrophic cardiomyopathy seen by Nam et al., our *Prdm16 cKO* mice develop a clear DCM phenotype characterized by increased LV dimensions, decreased ejection fraction, increased fibrosis, and increased mortality. The human cohort approached but did not reach statistical significance with regards to *PRDM16* loss and cardiomyopathy; the *Prdm16* cKO mice clearly demonstrate echocardiographic features consistent with dilated cardiomyopathy. Molecular characterization of these mice demonstrates increased expression of pro-fibrotic and heart failure-associated transcripts. Furthermore, for the first time, we demonstrate sex-specific effects of *Prdm16* loss on the incidence and the severity of contractile dysfunction as well as survival with female mice exhibiting unique poor outcomes with *Prdm16* cKO. The mechanism underlying this sex difference in survival in *Prdm16* cKO mice is not known and is currently being investigated in our lab. Altogether, because isolated *Prdm16* knockout resembles the cardiac features of 1p36 deletion syndrome observed in this cohort and confirm statistically significant cardiomyopathy risk in a sex-biased way, these data support the hypothesis that *PRDM16* loss underlies cardiomyopathy and adverse cardiac outcomes in patients with 1p36 deletion syndrome and females are more affected.

Overall, this study suggests that *PRDM16* loss is a risk allele for cardiomyopathy and adverse cardiac outcomes in 1p36 deletion syndrome. This is clinically relevant insofar as it supports cardiac monitoring of these children and informs further research on the role of *PRDM16* in cardiac development and maintenance in both sexes.

In summary, this cohort found patients with 1p36 deletion have a substantial burden of cardiac disease including cardiomyopathy and adverse cardiovascular outcomes, yet cardiology evaluation and follow-up was heterogeneous. Children with *PRDM16* loss demonstrate a significantly increased risk of death, transplant, or VAD placement, and nearly 75% were documented to have decreased systolic function. From these data, we propose that clinicians should screen children with 1p36 deletion syndrome, especially those with *PRDM16* deleted, for cardiovascular disease.

Study Limitations

There are several limitations to this study. Most notably, there are many genes in the 1p36 region that may influence cardiac development and function, and loss of genes other than *PRDM16* may underlie the variability. This is further complicated by varying deletion length in patients with 1p36 deletion syndrome. We address this by identifying a case of *SKI* loss without *PRDM16* loss who did not develop cardiomyopathy, and by generating a mouse model that recapitulated our clinical phenotypes with isolated *Prdm16* loss. While this study represents one of the largest cohorts today, our sample size was nonetheless small and retrospective in nature, and thus subject to selection bias. Future research regarding clinical features of *PRDM16* loss in 1p36 deletion syndrome, in particular the role of sex as a moderator on risk of cardiac disease, would include development of a validation cohort or prospective cohort analysis. Additionally, comparison between inducible *Cre* models of cardiac-specific *Prdm16* loss could allow for spatiotemporal characterization of Prdm16 expression throughout murine heart development and maintenance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Sources of Funding:

This work was supported by the National Heart, Lung and Blood Institute (NHLBI) grant R01HL149870-01A1 (S.B.) and the National Center for Advancing Translational Sciences (NCATS) grant UL1TR002538 (S.B.). APL is supported by National Institutes of Health (K08-HL136839, R01-HL149870, R01-EB032726), Doris Duke Charitable Foundation (CSDA-2020098), American Sudden Infant Death Syndrome Institute, John Taylor Babbitt Foundation, The Hartwell Foundation, Additional Ventures, Y.T. and Alice Chen Pediatric Genetics and Genomics Research Center. RJK is supported by the Sarnoff Cardiovascular Research Foundation. HRM is supported by the St. Jude Pediatric Research Recruitment Support Fund.

Nonstandard Abbreviations and Acronyms:

DCM	dilated cardiomyopathy
NCM	noncompaction cardiomyopathy
HCM	hypertrophic cardiomyopathy
VAD	ventricular assist device
cKO	conditional knockout

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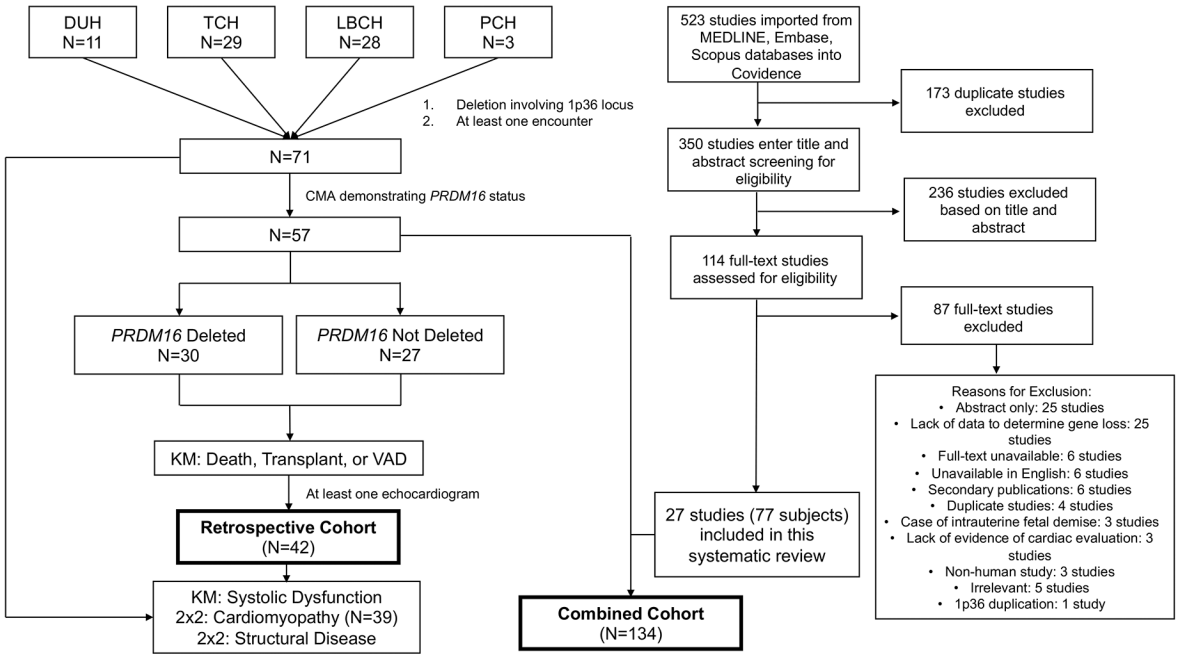

Figure 1:

Study overview for assembly and analysis of the retrospective cohort and the combined retrospective and systematic review cohort ("combined cohort"). (**A**) 71 subjects were acquired from 4 institutions. All 71 subjects were analyzed for structural disease. 57 subjects had chromosomal microarrays that delineated *PRDM16* deletion and a Kaplan Meier for the primary outcome of death, cardiac transplantation, or ventricular assist device placement was performed stratifying by genotype. Of the 57 patients with defined *PRDM16* status, 42 received echocardiograms and those subjects were analyzed using a Kaplan Meier for the secondary outcome of systolic dysfunction defined as ejection fraction outside of 55%-70% or reported qualitative read of abnormal. For cardiomyopathy analysis, 3 subjects were excluded due to nondiagnostic echocardiograms for a final N of 40 in genotype-cardiomyopathy analysis. (**B**) A systematic review was performed to acquire additional subjects with defined *PRDM16* status and cardiac workups. After filtering and excluding studies, a total of 27 studies remained providing 77 subjects. The combined cohort included a final N of 134.

Abbreviations: DUH = Duke University Hospital. TCH = Texas Children's Hospital. LBCH = Le Bonheur Children's Hospital. PCH = Primary Children's Hospital. CMA = chromosomal microarray, using single nucleotide polymorphisms (SNP). KM = Kaplan-Meier curve. 2x2 = 2 by 2 contingency table.

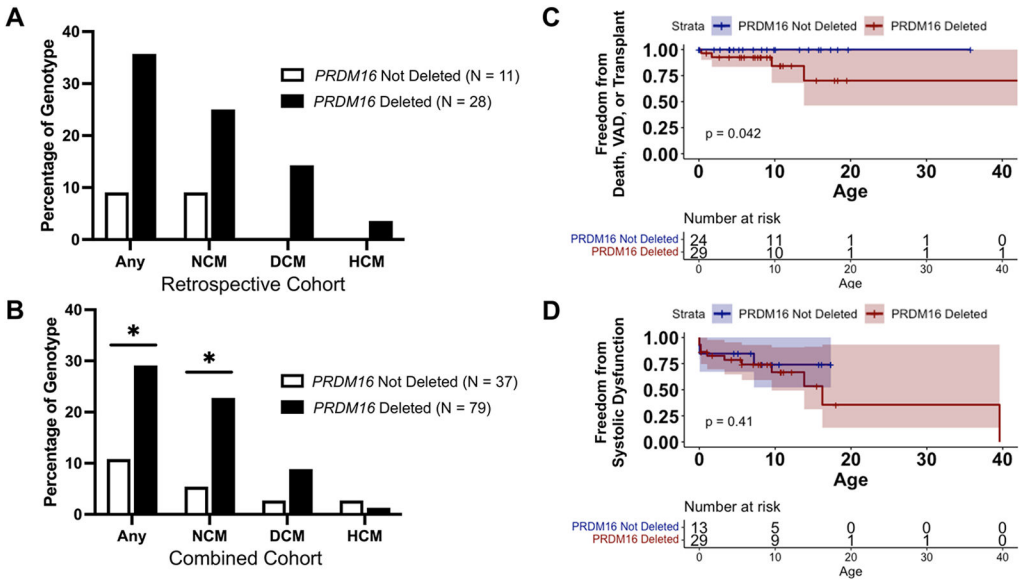

Figure 2:

Clinical outcomes in a retrospective cohort of patients with 1p36 deletion syndrome and in a combined cohort including patients with *PRDM16* deletion acquired via systematic review. (**A**) Prevalence of cardiomyopathy in the 1p36 deletion syndrome retrospective review cohort with and without *PRDM16* deleted. Any cardiomyopathy diagnosis, noncompaction cardiomyopathy, dilated cardiomyopathy, and hypertrophic cardiomyopathy were assessed. (**B**) Prevalence of cardiomyopathy in the combined cohort of retrospective review and systematic review with and without PRDM16 deleted ("Combined Cohort"). *PRDM16* deletion was associated with any cardiomyopathy diagnosis (p=0.03, Fisher's exact test) and noncompaction cardiomyopathy (p=0.03, Fisher's exact test). (**C**) A Kaplan Meier estimator was used to evaluate freedom from death, ventricular assist device placement, or cardiac transplantation among those with 1p36 deletion syndrome in the retrospective cohort (p=0.04, log-rank test). (**D**) A Kaplan Meier estimator was again used to evaluate freedom from systolic dysfunction defined as echocardiogram with ejection fraction outside 55%-70% or recorded qualitative read as abnormal among those with 1p36 deletion syndrome in the retrospective cohort (p=0.4, log-rank test).

Abbreviations: * = p<0.05. Abbreviations: NCM = noncompaction cardiomyopathy. DCM = dilated cardiomyopathy. HCM = hypertrophic cardiomyopathy.

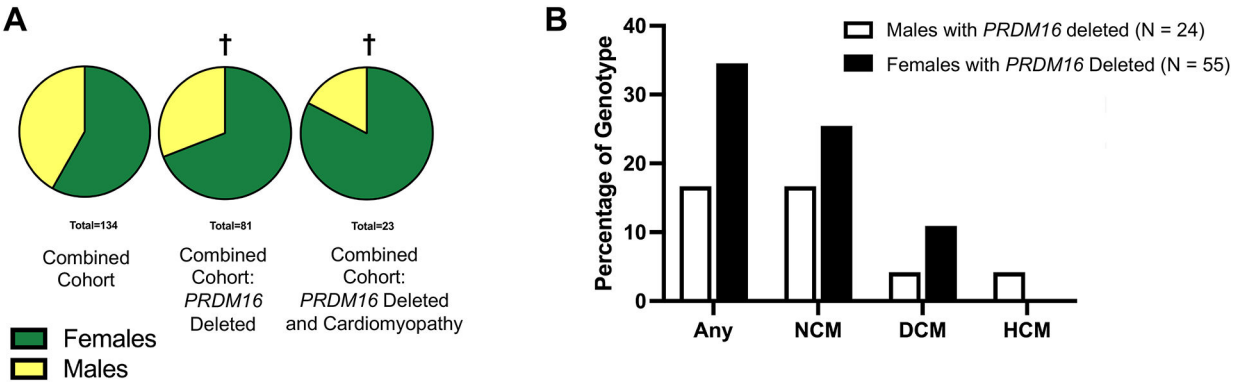

Figure 3:

Analysis of Sex Effects on Cardiomyopathy in the Combined Cohort. (**A**) Sex distribution was analyzed in the combined cohort. Subjects with *PRDM16* deletion were disproportionately female (p=0.0008, two-tailed Binomial test). Of subjects with *PRDM16* deleted who developed cardiomyopathy, females were again significantly overrepresented (p=0.003, two-tailed Binomial test). (**B**) Prevalence of cardiomyopathy was analyzed among those with *PRDM16* deleted in the combined cohort and stratified by sex. Approximately 35% of females with *PRDM16* deleted were diagnosed with cardiomyopathy versus 17% of their male peers (p=0.2, Fisher's exact test).

* = p<0.05, † = p<0.005. Abbreviations: NCM = noncompaction cardiomyopathy. DCM = dilated cardiomyopathy. HCM = hypertrophic cardiomyopathy.

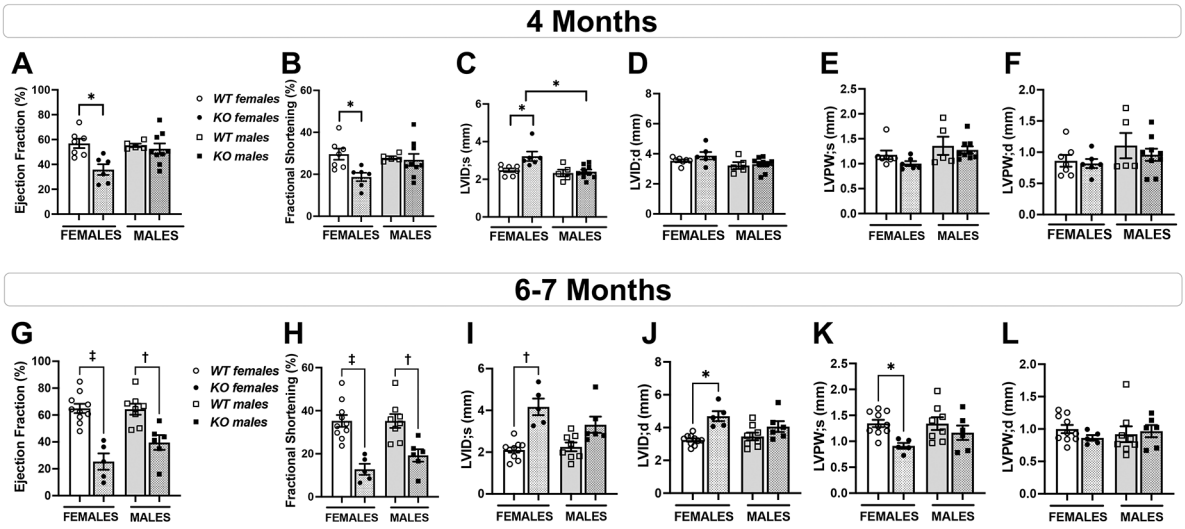

Figure 4:

Loss of Cardiac *Prdm16* Caused Dilated Cardiomyopathy in Mice in a sex-specific manner. (**A, G**) % Ejection fraction; (**B, H**) % Fractional shortening; (**C, I**) Left ventricular internal diameter in systole; (**D, J**) Left ventricular internal diameter in systole (LVIDs); (**E, K**) Left ventricular posterior wall in diastole (LVIDd) and (**F, L**) Left ventricular posterior wall in systole and diastole (LVPWs and LVPWd) in female and male wild-type and *Prdm16* cKO mice at 4 and 6-7 months respectively. Data are mean ± SEM. Number of mice per group: 4 months: WT female (n=7); cKO female (n=6); WT male (n=5) and cKO male (n=9) and 6-7 months: WT female (n=10); cKO female (n=5); WT male (n=8) and cKO male (n=6). Data was analyzed by a two-way ANOVA. * = $p<0.05$, † = $p<0.005$, ‡ = $p<0.0005$. Abbreviations: cKO = conditional knockout. WT = wild-type.

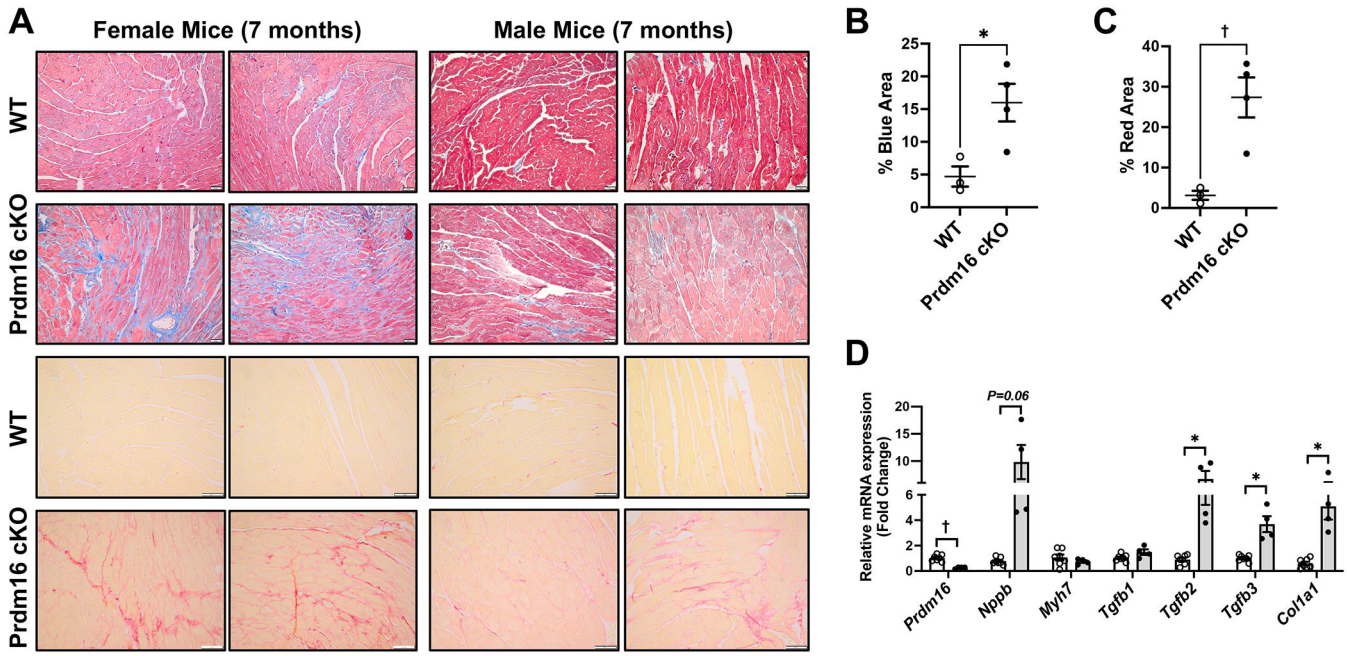

Figure 5:

Prdm16 cKO mice develop structural remodeling and fibrosis that is worse in female than male mice. (**A**) Representative images of heart sections stained with trichrome or picrosirius red from WT and *Prdm16* cKO female and male mice at 7 months. (**B, C**) Quantification of fibrosis as expressed by % blue area over total area for trichrome or % red area over total area for picrosirius red in hearts from 7 months male and female WT and Predm16 cKO mice combined. (**D**) Relative mRNA expression of *Prdm16*, *Nppb* (encoding natriuretic peptide b), *Myh7* (Myosin heavy chain 7), *Tgfb* (Transforming growth factor beta) and *Cola1* (Collagen type I alpha 1 chain) in hearts from 7 months male and female WT and *Prdm16* cKO mice combined. Data are mean ± SEM. n=3 WT and 4 cKO for **B** and **C** and n=7 WT and 4 cKO mice for **D**. Data was analyzed using a student *t* Test to compared WT versus cKO mice (male and female combined). * = p<0.05, † = p<0.005, ‡ = p<0.0005. Abbreviations: cKO = conditional knockout. WT = wild-type.

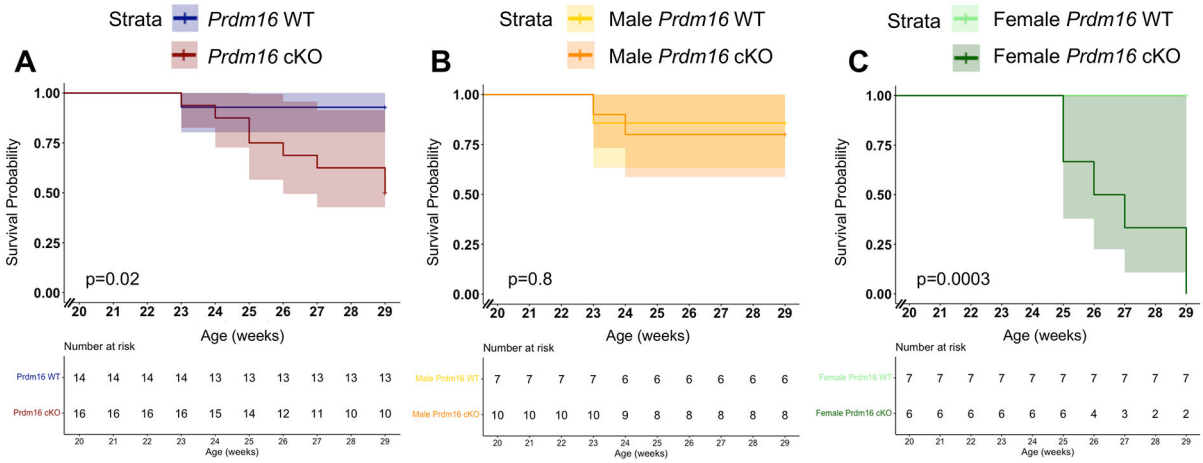

Figure 6:

Prdm16 deletion confers a significantly increased risk of death in female mice. (**A**) A Kaplan Meier estimator was used to evaluate freedom from death in mice with *Prdm16* cKO (n=16) versus WT mice (n=14) of all sexes. *Prdm16* cKO have worse survival (p=0.02, log-rank test). (**B**) A Kaplan Meier estimator was used to evaluate freedom from death in male *Prdm16* cKO mice (n=10) versus *Prdm16* WT mice (n=7). Among male mice, *Prdm16* cKO does not have an effect on survival (p=0.8, log-rank test) (**C**) A Kaplan Meier estimator was used to evaluate freedom from death in female *Prdm16* cKO mice, demonstrating female *Prdm16* cKO mice (n=6) have a uniquely poor survival (p=0.0003, long-rank test) compared to female *Prdm16* WT mice (n=7). Abbreviations: cKO = conditional knockout. WT = wild-type.

Table 1:

There were 71 subjects included in this cohort, of which 57 had defined PRDM16 status with 30 subjects harboring a PRDM16 deletion. Demographic, genetic, and clinical features of subjects were obtained. Prevalence was assayed, displayed as percentage with parentheses denoting number of subjects with that feature over total N available for that analysis. P value for count data were derived using a Fisher exact test. Continuous variables, displayed as the mean with the interquartile range and N in brackets, were compared using a Wilcoxon Rank-sum test with continuity correction. DCM indicates dilated cardiomyopathy; DUH, Duke University Hospital; HCM, hypertrophic cardiomyopathy; LBCH, Le Bonheur Children's Hospital; LVNC, left ventricular noncompaction; PCH, primary children's hospital; and TCH, Texas Children's Hospital.

Variable		**Total Cohort**	***PRDM16* Not Deleted**	***PRDM16* Deleted**	**Significance**
N	DUH	11	6	5	
	TCH	29	18	11	
	LBCH	28	3	11	
	PCH	3	0	3	
	Total	71	27	30	
PRDM16 Status	Deleted	42.3% (30/71)			
	Not Deleted	38.0% (27/71)			
	Data Unavailable	19.7% (14/71)			
Deletion Type	Isolated 1p36 Deletion	76.1% (54/71)	55.6% (15/27)	83.3% (25/30)	**p = 0.003**
	Multiple Deletions	23.9% (17/71)	44.4% (12/27)	16.7% (5/30)	
Demographic Information	Median Age at Diagnosis (Y)	1.4 [0.0-9.6, N=45]	3.4 [1.6-13.0, N=23]	0.2 [0.0-1.0, N=22]	**p = 0.02**
	Median Age at Last Follow Up (Y)	8.3 [4.3-14.2, N=64]	9.9 [5.0-16.1, N=23]	8.0 [4.0-11.4, N=28]	p = 0.32
Sex	Female	52.1% (37/71)	37.0% (10/27)	63.3% (19/30)	p = 0.06
	Male	47.9% (34/71)	63.0% (17/27)	36.7% (11/30)	
Severe Adverse Events	N	71	27	30	
	Death	5.6% (4/71)	0% (0/27)	13.3% (4/30)	p = 0.1

Variable		Total Cohort	*PRDM16* Not Deleted	*PRDM16* Deleted	Significance
	Transplant	1.4% (1/71) *	0% (0/27)	3.3% (1/30) *	p > 0.99
	Ventricular Assist Device	(1.4% (1/71) *	0% (0/27)	3.3% (1/30) *	p > 0.99
	Total	7.0% (5/71)	0% (0/27)	16.7% (5/30)	**p = 0.05**
Cardiac Phenotypes					
Echocardiogram	At Least 1 Present	78.9% (56/71)	48.1% (13/27)	96.7% (29/30)	**p < 0.0001**
Cardiomyopathy	N †	53	11	28	
	Noncompaction	28.3% (15/53)	9.1% (1/11)	25.0% (7/28)	p = 0.4
	Dilated	7.5% (4/53)	0% (0/11)	14.3% (4/28)	p = 0.3
	Hypertrophic	1.9% (1/53)	0% (0/11)	3.6% (1/28)	p > 0.99
	Total (Any)	34.0% (18/53)	9.1% (1/11)	35.7% (10/28)	p = 0.1
Arrhythmia	N	39	5	24	
	Baseline Abnormalities	15.4% (6/39)	40.0% (2/5)	16.7% (4/24)	p = 0.3
	Arrhythmia	0% (0/39)	0% (0/5)	0% (0/24)	p > 0.99
	Total	15.4% (6/39)	40.0% (2/5)	16.7% (4/24)	p = 0.3
Hemodynamically Significant Structural Heart Defects	N	55	13	29	
	Total	47.3% (26/55)	69.2% (9/13)	51.7% (15/29)	**p = 0.3**

* Occurred in the same patient

† Three individuals were removed from cardiomyopathy analysis after blinded review deemed their echocardiograms insufficient for evaluation of cardiomyopathy. Of these individuals, two had PRDM16 not deleted and one had PRDM16 deleted.

Table 2:

Medical management in patients with 1p36 deletion syndrome. Of those in the retrospective cohort, 20 subjects with *PRDM16* deleted and 21 subjects with *PRDM16* not deleted had medications eligible for review. Medications were queried and categorized into classes.

Variable	Total Cohort	*PRDM16* Not Deleted	*PRDM16* Deleted	Significance
N	41	21	20	
Beta Blocker *	17.0% (7/41)	4.8% (1/21)	30.0% (6/20)	**p = 0.04**
First Generation (Non-selective)	0% (0/41)	0% (0/21)	0% (0/20)	p > 0.99
Second Generation (Cardio-selective)	9.8% (4/41)	4.8% (1/21)	15.0% (3/20)	p = 0.3
Third Generation (Vasodilatory)	7.3% (3/41)	0% (0/21)	15.0% (3/20)	p = 0.1
ACEI/ARB/ARNI	19.5% (8/41)	4.8% (1/21)	35.0% (7/20)	**p = 0.02**
Diuretics	7.3% (3/41)	0% (0/21)	15.0% (3/20)	p = 0.1
CCB	4.9% (2/41)	0% (0/21)	10.0% (2/20)	p = 0.5
Inotrope	2.4% (1/41)	0% (0/21)	5.0% (1/20)	p > 0.99
Any Cardiac Medication	29.3% (12/41)	9.5% (2/21)	50.0% (10/20)	**p = 0.006**

* Beta blockers for non-cardiovascular indications were excluded.

ACEI = angiotensin converting enzyme inhibitor. ARB = angiotensin receptor blockers. ARNI = angiotensin receptor neprilysin inhibitor. CCB = calcium channel blocker.