Identification of Critical Regions and Candidate Genes for Cardiovascular Malformations and Cardiomyopathy Associated with Deletions of Chromosome 1p36

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Abstract

Cardiovascular malformations and cardiomyopathy are among the most common phenotypes caused by deletions of chromosome 1p36 which affect approximately 1 in 5000 newborns. Although these cardiac-related abnormalities are a significant source of morbidity and mortality associated with 1p36 deletions, most of the individual genes that contribute to these conditions have yet to be identified. In this paper, we use a combination of clinical and molecular cytogenetic data to define five critical regions for cardiovascular malformations and two critical regions for cardiomyopathy on chromosome 1p36. Positional candidate genes which may contribute to the development of cardiovascular malformations associated with 1p36 deletions include DVL1, SKI, RERE, PDLP1, SPEN, CLCNKA, ECE1, HSPG2, LUZP1, and WASF2. Similarly, haploinsufficiency of PRDM16—a gene which was recently shown to be sufficient to cause the left ventricular noncompaction–SKI, PRKCZ, RERE, UBE4B and MASP2 may contribute to the development of cardiomyopathy. When treating individuals with 1p36 deletions, or providing prognostic information to their families, physicians should take into account that 1p36 deletions which overlap these cardiac critical regions may portend to cardiovascular complications. Since several of these cardiac critical regions contain more than one positional candidate gene—and large terminal and interstitial 1p36 deletions often overlap more than one cardiac critical region—it is likely that haploinsufficiency of two or more genes contributes to the cardiac phenotypes associated with many 1p36 deletions.

Introduction

Approximately 1 in 5000 newborns has a terminal deletion affecting chromosome 1p36, making it the most common telomeric deletion in humans [1]. Individuals with terminal 1p36 deletions share a common set of phenotypes that constitute the 1p36 deletion syndrome [2,3]. These phenotypes include typical craniofacial features, cognitive impairment, behavioral problems, seizures, postnatal growth deficiency, eye/vision problems, hearing loss, cleft palate, cardiovascular malformations, cardiomyopathy and renal anomalies.

The distal critical regions for most 1p36 deletion syndrome phenotypes have been determined to reside within approximately 4 Mb from the 1p telomere [4]. However, non-overlapping interstitial deletions involving the proximal region of 1p36, starting approximately 8 Mb from the 1p telomere, have also been shown to cause many of the phenotypes associated with distal 1p36 deletions including cognitive impairment, seizures, postnatal growth deficiency, cardiovascular malformations and cardiomyopathy [5]. Some individuals have deletions of both the distal and proximal regions of 1p36 [6]. In such cases, the additive effects of haploinsufficiency of genes within both of these regions may account for the observation that individuals carrying larger 1p36 deletions are more severely affected and exhibit more of the features typically associated with 1p36 deletions [7,8].

Cardiovascular malformations and cardiomyopathy are among the most acutely life-threatening conditions associated with both distal and proximal deletions of 1p36. Heterozygous loss-of-function mutations in PRDM16—a gene in the distal portion of 1p36 (2,985,742–3,355,185; hg19)—were recently shown to be sufficient to cause left ventricular noncompaction [9]. Similarly, a heterozygous loss-of-function mutation in ECE1 (21,543,740–21,672,034; hg19) has been identified in an individual with patent ductus arteriosus, a small subaortic ventricular septal defect, and a small atrial septal defect, suggesting that haploinsufficiency of this proximal 1p36 gene may be sufficient to cause cardiovascular malformations in humans [10]. However, the other genomic
regions and dosage-sensitive genes that contribute to the cardiac phenotypes caused by 1p36 deletions have not been clearly defined. This paucity of information makes it difficult for physicians to create individualized medical plans and provide accurate prognostic information to patients and families affected by 1p36 deletions. This is true even though detailed information regarding the extent and location of an individual’s 1p36 deletion can be readily obtained on a clinical basis. Defining the cardiac-related regions and genes on 1p36 will not only allow physicians to provide improved medical care, but is also a prerequisite to understanding the molecular mechanisms that underlie the development of 1p36-related cardiovascular malformations and cardiomyopathy. Elucidating these molecular mechanisms may lead to the development of novel preventative and/or therapeutic interventions for cardiovascular disorders.

Using clinical and molecular cytogenetic data from individuals with isolated 1p36 deletions defined by array-based copy number analysis, we have identified five non-overlapping critical regions for cardiovascular malformations and two non-overlapping critical regions for cardiomyopathy on chromosome 1p36. A bioinformatic analysis of each of these cardiac critical regions revealed at least one positional candidate gene whose deletion may contribute to the development of cardiac phenotypes based on human studies and/or animal models. In some cases, haploinsufficiency of two or more such genes may contribute to the cardiac phenotypes associated with a 1p36 deletion. This is particularly likely in the case of large terminal and interstitial deletions that overlap more than one cardiac critical region.

**Materials and Methods**

**Ethics Statement**

These studies were performed under research protocols approved by the institutional review board of Baylor College of Medicine. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from parents or guardians on behalf of study participants all of whom were minors/children at the time of enrollment.

**Patient Identification and Accrual**

Molecular and clinical data from individuals with isolated 1p36 deletions associated with cardiovascular malformation and/or cardiomyopathy were collected from four sources. The first source consisted of data from 33,566 de-identified individuals referred to the Medical Genetics Laboratories at Baylor College of Medicine for array-based copy number analysis. Only data from individuals with isolated 1p36 deletions and an indication for testing that included a cardiovascular malformation or cardiomyopathy were included in this study.

The second source consisted of data from patients with isolated 1p36 deletions confirmed by array-based copy number analyses who were accrued from a group of individuals receiving care at Texas Children’s Hospital in Houston, TX, USA and by self-referral. In these cases, informed consent was obtained under a protocol approved by the institutional review board of Baylor College of Medicine after which clinical and molecular cytogenetic data was obtained from a review of the medical record and correspondence with the individual’s parents/family members.

The third source consisted of data from individuals with isolated 1p36 deletions associated with cardiac phenotypes who were listed in the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER; http://decipher.sanger.ac.uk/). In each case, representatives of the contributing institution–where consent for the submission of clinical and molecular cytogenetic data were obtained–were contacted and given an opportunity to provide further details of the phenotypes present in their patients.

**Table 1.** Summary of individuals with isolated 1p36 deletions and cardiovascular malformations referred to the Medical Genetics Laboratory (MGL) at Baylor College of Medicine.

| Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiovascular Malformations | Other | Critical Regions |
|------------|------------------|-----------|---------|-----------------------------|-------|------------------|
| Patient 1  | 1–2694017        | 2.7       | De novo | Hypoplastic right heart     |       | 1                |
| Patient 2  | 1–3581432        | 3.6       | De novo | Bicommissural aortic valve, mild aortic dilatation | Developmental delay, mild unilateral conductive hearing loss, concern for seizures | 1 |
| Patient 3  | 1–6551698        | 6.6       | De novo | Moderate secundum atrial septal defect, dilation of main pulmonary artery | Developmental delay, dysmorphic features, conductive hearing loss | 1 |
| Patient 4  | 1–6804034        | 6.8       | De novo | Moderate patent ductus arteriosus, multiple small muscular ventricular septal defects, small secundum atrial septal defect, heavily trabeculated left ventricle with normal function |       | 1 |
| Patient 5  | 1–6921434        | 6.9       | Unknown | Patent ductus arteriosus, multiple ventricular septal defects, secundum atrial septal defect, aberrant left subclavian artery | Developmental delay, cognitive impairment, dysmorphic features, intractable seizures, cortical blindness, contractures involving small joints of hand | 1 |
| Patient 6  | 8803013–11739523 | 2.9       | De novo | Ventricular septal defect | Microcephaly | 2 |
| Patient 7  | 12726755–20540759| 7.8       | Unknown | Tetralogy of Fallot, bicommissural aortic valve | Developmental delay, cognitive impairment, seizures, kyphosis of spine | 3* |
| Patient 8  | 27803719–31404471| 3.6       | De novo | Coarctation of the aorta | Moderate developmental delay, cognitive impairment, failure to thrive, gastrointestinal problems | 5* |

* = Deletion defines a cardiac critical region.
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Defining Cardiac Critical Regions

Individual cardiac critical regions on chromosome 1p36 were defined based on the smallest, non-overlapping deletion present within a single individual with a cardiovascular malformation and/or cardiomyopathy. In cases where both a minimal and a maximal deleted region were defined, the breakpoints of the maximal deleted region were used to define the critical region. This approach minimizes the risk that a cardiac-related gene located between the minimal and maximal deleted region will be erroneously excluded from the critical region.

Identifying Cardiac-related Genes within Critical Regions

Data regarding each gene located completely or partially within a cardiac critical region were downloaded into a searchable spreadsheet using GeneDistiller2 [http://www.genedistiller.org/]. This publicly-available online program allows the user to access information on genes within a defined interval from a variety of online sources. Information reviewed to identify cardiac-related candidate genes included: Online Mendelian Inheritance in Man (OMIM; [http://www.omim.org/]) reports, interaction data from the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; [http://string-db.org/]) and the Unified Human Interactome (UniHI; [http://www.unihi.org/]), and phenotype data from the Human Phenotype Ontology website ([http://www.human-phenotype-ontology.org/]), the Mouse Genome Database (MGD; [http://www.informatics.jax.org/phenotypes.shtml]) and the Gene Ontology Database ([http://www.geneontology.org/]). This information was augmented with manually curated data from recently published manuscripts cited in PubMed ([http://www.ncbi.nlm.nih.gov/pubmed]).

Results

To map and identify dosage-sensitive genes and genomic regions that may contribute to the development of cardiovascular malformations and/or cardiomyopathy, we identified individuals with these phenotypes who had isolated 1p36 deletions defined by array-based copy number detection techniques as described in the Materials and Methods. The location and extent of the 1p36

Table 2. Summary of individuals with isolated 1p36 deletions and cardiovascular malformations identified from the DECIPHER database.

| DECIPHER Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiovascular Malformations (Co-existing Cardiomyopathy) | Other | Critical Regions |
|---------------------|------------------|----------|----------|----------------------------------------------------------|-------|-----------------|
| 1229                | 928301–4708254   | 3.8      | De novo  | Ventricular septal defect (Cardiomyopathy)               | Intellectual disability, epileptic spasms, delayed cranial suture closure, stenosis of external auditory canal, dysmorphic features | 1     |
| 2483                | 4795388–17364849 | 12.6     | Not maternal, father unavailable | Secundum atrial septal defect | Intellectual disability, delayed speech and language development, feeding difficulties in infancy, microcephaly, submucous cleft hard palate, prenatal short stature, scoliosis, dysmorphic features | 2, 3 |
| 248448              | 7812397–13488491 | 5.7      | De novo  | Mild pulmonary valve stenosis | Intellectual disability, feeding difficulties in infancy, recurrent infections, sensorineural hearing impairment, proportionate short stature, cryptorchidism, short palhanges, broad thumbs, dysmorphic features | 2, 3 |
| 1803 Distal         | 18382579–19879460 | 1.5       | Unknown  | Atrial septal defect | Intellectual disability, feeding difficulties in infancy, high palate, dysmorphic features | 3     |
| 1803 Proximal       | 27358936–29807278 | 2.4      | De novo  | Atrial septal defect | See above | 5     |
| 1634                | 20555776–23438888 | 2.9      | De novo  | Patent ductus arteriosus, coarctation of the aorta, small ventricular septal defect | Intellectual disability, behavioral problems with aggression and mood swings, dysmorphic features, non-cleft velopharyngeal dysfunction, hypoplasia, bifid thumb, stiffness and progressive joint contractures with fixed kyphosis, fusion of 1st and 2nd cervical vertebrae | 4*    |

* = Deletion defines a cardiac critical region.
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Table 3. Summary of individuals with isolated 1p36 deletions and cardiovascular malformations identified from the literature.

| Reference and Patient Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiovascular Malformations (Co-existing Cardiomyopathy) | Other | Critical Regions |
|---------------------------------|-----------------|----------|----------|----------------------------------------------------------|-------|-----------------|
| El-Hattab et al. 2010 [11]      | 1–2418935       | 2.4      | De novo  | Ventricular septal defect, atrial septal defect, PDA, right-sided aortic arch | Developmental delay, renal malposition and malrotation, omphalocele, cloacal exstrophy, imperforate anus, multiple sacral segmentation defects, genital anomalies, diastasis of symphysis pubis, limb deformities | 1*   |
| Cremer et al. 2008 [15]         | 1–4888723       | 4.9      | De novo  | Ventricular septal defect (LVNC) | Cleft palate, unilateral choanal stenosis, hypothyroidism | 1    |
| Campeau et al. 2008, Patient 1 [16] | 1–10247416     | 10.2     | De novo  | Asymmetric ventricles, muscular ventricular septal defect, tortuous aortic arch, PDA | Hypotonia, single febrile seizure, bilateral colpocephaly, moderate to severe non-obstructive hydrocephalus, sensorineural hearing loss, short femurs, unilateral club foot, submucous cleft palate, velopharyngeal incompetence, dysmorphic features | 1,2  |
| Bursztejn et al. 2009 [17]      | 1–11809959      | 11.8     | De novo  | Atrial septal defect, ventricular septal defect | Infantile spasms, partial seizures, agenesis of the corpus callosum, ventricular dilation, dysmorphic features | 1,2  |
| Saito et al. 2008 [18]          | 1–11809959      | 11.8     | De novo  | Enlargement of right atrium, narrowing of right ventricle, ventricular septal defects, PDA, Ebstein anomaly (LVNC) | Bilateral perisyliyan polymicrogryria, periventricular nodular heterotopia, seizures, hypotonia, feeding difficulties, dysmorphic features | 1,2  |
| Nicoulaz et al. 2011 [6]        | 1–16177338      | 15.6     | De novo  | Tetralogy of Fallot | Joint contractures, ventriculomegaly, marked pachygyria, absent septum pellucidum, thinned corpus callosum, dysmorphic features | 1,2,3|
| Arndt et al. 2013, Patient 6 [9] | 564205–10821909 | 10.3     | De novo  | Atrial septal defect (LVNC) | Developmental delay, microcephaly, hypothyroidia, deep set eyes | 1,2  |
| Kang et al. 2007, Case 3 [5]    | 3768946–18563553| 14.8     | Unknown  | Cleft mitral valve, redundant tricuspid valve leaflets, ventricular septal defect, small atrial septal defect, mild pulmonary valve stenosis, PDA | Hypotonia, bilateral nasolacrimal duct obstruction, gastroesophageal reflux, severe biventricular hypertrophy, moderate sensorineural hearing loss, bilateral cleft lip and palate, posteriorly rotated ears, digital contractures | 2,3  |
| Arndt et al. 2013, Patient 16 [9]| 4089259–12054030| 8.0      | Unknown  | Ventricular septal defect (Cardiomyopathy) | Cognitive impairment, microcephaly, ptosis | 2    |
| Kang et al. 2007, Case 5 [5]    | 4317448–13867316| 9.5      | Unknown  | Partial anomalous pulmonary venous return with the left pulmonary veins draining into the innominate vein | Developmental delay, seizures, failure to thrive, hemivertebra, Wolff-Parkinson-White syndrome, ataxia, unsteady gait, appendicular hypertonia | 2,3  |
| Kang et al. 2007, Case 2 [5]    | 4843323–16397974| 11.6     | Unknown  | Two small right coronary artery fistulae terminating in the left atrium and right ventricle | Developmental delay, seizures, peripheral hypertension, gastroesophageal reflux, dysmorphic features | 2,3  |
| Kang et al. 2007, Case 4 [5]    | 8395179–11362893| 3.0      | Unknown  | Perimembranous ventricular septal defect, small septum secundum atrial septal defect | Developmental delay, failure to thrive, truncal hypotonia, dysmorphic features | 2*   |
| Kang et al. 2007, Case 1 [5]    | 9124551–21782714| 12.7     | Unknown  | High and mid muscular ventricular septal defect, bicuspid aortic valve, patent foramen ovale, PDA | Developmental delay, seizures, dysmorphic features | 2, 3,4|

* = Deletion defines a cardiac critical region, LVNC = left ventricular noncompaction, PDA = patent ductus arteriosus.
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Deletions identified in each individual are provided in Tables 1–6 along with a description of the individual’s cardiovascular malformations (Tables 1–3) or cardiomyopathy (Tables 4–6). These deletions are also represented graphically in Figures 1 and 2.

Using this data, we defined five non-overlapping critical regions for cardiovascular malformations (Table 7, Figure 1). For clarity, each critical region has been numbered sequentially starting with the most distal region on chromosome 1p36. The first critical region is defined by a de novo ~2.4 Mb terminal deletion reported.
by El-Hattab and colleagues in a patient with a ventricular septal defect, an atrial septal defect, patent ductus arteriosus and a rightsided aortic arch [11].

The second critical region is defined by an ~3.0 Mb interstitial deletion identified in Case 4 reported by Kang and colleagues in an individual with a perimembranous ventricular septal defect and a small septum secundum atrial septal defect [5]. In using Kang et al. Case 4 to define the second critical region, we note that the ~2.9 Mb interstitial deletion in Patient 6, who had a ventricular septal defect, begins slightly centromeric to the deletion in Kang et al. Case 4 and, therefore, does not involve the SCL4A5/1 gene. However, the deletion in Patient 6 extends farther into the proximal region of 1p36 than the deletion in Kang et al. Case 4 and includes UBE2V2P3, PTCCHD2, FBX02, FBX044, FBX06 and MAD2L2.

The third critical region is defined by an ~7.8 Mb interstitial deletion in Patient 7 who was referred to the Medical Genetics Laboratories at Baylor College of Medicine for copy number analysis with an indication for testing that included tetralogy of Fallot and a bicommissural aortic valve. The fourth critical region is defined by DECIPHER patient #1634 who had a patent ductus arteriosus, coarctation of the aorta and a small ventricular septal defect caused by a de novo ~2.9 Mb interstitial deletion. The fifth critical region is defined by a de novo ~3.6 Mb interstitial deletion in Patient 8 who was referred to the Medical Genetics Laboratories at Baylor College of Medicine for copy number analysis with an indication for testing that included coarctation of the aorta.

Using this same dataset, we defined two critical regions for cardiomyopathy (Table 8, Figure 2). The first critical region contains the PRDM16 gene and is defined by an ~1.5 Mb interstitial deletion in two siblings with left ventricular noncompaction described by Gajeka and colleagues. The second critical region is defined by an ~3.0 Mb interstitial deletion in an individual reported by Kang and colleagues (Case 4) who had dilated cardiomyopathy which coexisted with a perimembranous ventricular septal defect and a small septum secundum atrial septal defect [5]. Kang and colleagues indicated that both cardiac malformations spontaneously closed but the dilated cardiomyopathy remained and was still being treated with medications when the child was 5 years old. While this history suggests that he had a primary cardiomyopathy, we cannot rule out the possibility that his cardiovascular malformations contributed to his dilated cardiomyopathy. However, further evidence of the role of this region in the development of primary cardiomyopathy comes from Patient 16 described by Arndt and colleagues who had an overlapping ~8 Mb deletion associated with early-onset cardiomyopathy with transient heart failure whose only structural heart defect was a clinically insignificant ventricular septal defect [9]. Each of the cardiac-related critical regions we identified contains one or more positional candidate genes whose haploinsufficiency may contribute to the development of cardiovascular phenotypes. Genes which may contribute to the development of cardiovascular malformations associated with 1p36 deletions include DVL1, SKI, RERE, PDPS, SPEN, CLCNKA, ECE1, HSPG2, LUZP1, and WASP2. Genes which may contribute to the development of cardiomyopathy associated with 1p36 deletions include SKI, PRKCZ, PRDM16, RERE, UBE4B and MASP2. The cardiac-related phenotypes associated with each of these genes in humans, mice and zebrafish are summarized in Tables 7 and 8.

**Table 4.** Summary of individuals with isolated 1p36 deletions and cardiomyopathy referred to the Medical Genetics Laboratory (MGL) or recruited into a 1p36 research study at Baylor College of Medicine.

| Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiomyopathy | Other | Critical Regions |
|------------|------------------|-----------|----------|----------------|-------|-----------------|
| Patient 9  | 1−4470448        | 4.5       | Unknown  | Dilated cardiomyopathy |       | 1               |
| Patient 10 | 1−4330413        | 4.3       | De novo  | Dilated cardiomyopathy | Developmental delay, infantile spasms, hypotonia | 1 |
| Patient 11 | 1−4078518        | 4.1       | De novo  | Bilateral dilated cardiomyopathy | Seizures, cleft lip, intellectual disability, sensorineural hearing loss | 1 |

**Table 5.** Summary of individuals with isolated 1p36 deletions and cardiomyopathy identified from the DECIPHER database.

| DECIPHER Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiomyopathy (Co-existing Cardiovascular Malformations) | Other | Critical Regions |
|---------------------|------------------|-----------|----------|----------------------------------------------------------|-------|-----------------|
| 1229                | 1−4708254        | 4.7       | De novo  | Cardiomyopathy (Ventricular septal defect) | Intellectual disability, development delay, infantile spasms, narrow/atrietic auditory canal, dysmorphic features | 1 |
| 2353                | 3224674−12540397 | 9.3       | Unknown  | Dilated cardiomyopathy | Intellectual disability, development delay, seizures, myopia, sensorineural hearing loss, dysmorphic features | 1,2 |

**Table 4.** Summary of individuals with isolated 1p36 deletions and cardiomyopathy referred to the Medical Genetics Laboratory (MGL) or recruited into a 1p36 research study at Baylor College of Medicine.

| Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiomyopathy | Other | Critical Regions |
|------------|------------------|-----------|----------|----------------|-------|-----------------|
| Patient 9  | 1−4470448        | 4.5       | Unknown  | Dilated cardiomyopathy |       | 1               |
| Patient 10 | 1−4330413        | 4.3       | De novo  | Dilated cardiomyopathy | Developmental delay, infantile spasms, hypotonia | 1 |
| Patient 11 | 1−4078518        | 4.1       | De novo  | Bilateral dilated cardiomyopathy | Seizures, cleft lip, intellectual disability, sensorineural hearing loss | 1 |

**Table 5.** Summary of individuals with isolated 1p36 deletions and cardiomyopathy identified from the DECIPHER database.

| DECIPHER Identifier | Start-Stop (hg19) | Size (Mb) | Heredity | Cardiomyopathy (Co-existing Cardiovascular Malformations) | Other | Critical Regions |
|---------------------|------------------|-----------|----------|----------------------------------------------------------|-------|-----------------|
| 1229                | 1−4708254        | 4.7       | De novo  | Cardiomyopathy (Ventricular septal defect) | Intellectual disability, development delay, infantile spasms, narrow/atrietic auditory canal, dysmorphic features | 1 |
| 2353                | 3224674−12540397 | 9.3       | Unknown  | Dilated cardiomyopathy | Intellectual disability, development delay, seizures, myopia, sensorineural hearing loss, dysmorphic features | 1,2 |
remaining 15% had dilated cardiomyopathy. Similar patterns of cardiac anomalies were seen in the patients with terminal 1p36 deletion described in this report. Individuals with interstitial deletions of 1p36 also have high rates of cardiac abnormalities, although their exact incidences likely vary from one region to another and are more difficult to estimate based on the relatively low number of patients that have been identified [5].

Due to the high risk of cardiac-related problems, most children with 1p36 deletions are screened for cardiovascular anomalies at baseline and followed over time for the development of cardiomyopathy—often having several echocardiograms even if they are asymptomatic. Although this type of careful monitoring can help identify potentially treatable problems, it also places a burden on families and the health care system which could be avoided or reduced if an individual’s risk of having these problems could be estimated based on the location and extent of their individual 1p36 deletion. This type of risk stratification will require a better understanding of the 1p36 genes and genomic regions associated with the development of cardiovascular anomalies and cardiomyopathy.

In this report, we take the first step towards addressing this issue by defining five non-overlapping critical regions for cardiovascular malformations and two non-overlapping critical regions for cardiomyopathy. Each of these cardiac critical regions is defined by an isolated 1p36 deletion identified in a single individual. Hence, they represent genomic regions whose deletions are sufficient to cause cardiovascular malformation or cardiomyopathy. The size of these regions vary with the smallest being 1.5 Mb (28 genes) and the largest being 7.8 Mb (175 genes). As more 1p36 deletions are described, it is likely that these regions will be refined and some may be subdivided into more than one region. It is also possible that data from additional deletions will reveal novel critical regions on 1p36 that are associated with cardiac problems.

It is reasonable to assume that each of the cardiac critical regions we have delineated on chromosome 1p36 contains at least one dosage-sensitive, cardiac-related gene or regulatory region. Although one or more positional candidate genes from each interval can be identified based on their known function and/or their phenotypes in animal models, haploinsufficiency of only two genes have been shown to cause cardiac phenotypes in humans. Heterozygous loss-of-function mutations in PRDM16 have been identified in patients with 1p36 deletions.
shown to be sufficient to cause left ventricular noncompaction and a loss-of-function mutation in \textit{ECE1} was identified in an individual with patent ductus arteriosus, a small subaortic ventricular septal defect and a small atrial septal defect, Hirschsprung disease, and autonomic dysfunction [9,10].

Loss-of-function mutations in the remainder of the positional candidate genes identified in 1p36 cardiac critical regions–\textit{DVL1}, \textit{SKI}, \textit{RERE}, \textit{PDYN}, \textit{SPEN}, \textit{CLCNKA}, \textit{HSPG2}, \textit{LUZP1}, \textit{WASPF}, \textit{PRKCZ}, \textit{UBE4B} and \textit{MASP2}–have yet to be identified in individuals with cardiovascular phenotypes. The identification of \textit{de novo} loss-of-function mutations in these positional candidate genes, or dominantly inherited loss-of-function mutations segregating with a cardiac phenotype in multiple family members, would serve to confirm their pathogenic roles. Experience with other chromosomal regions suggests that these types of mutations are most likely to be identified in genes with particularly high impacts on cardiac development and function like \textit{GATA4} on 8p23.1, \textit{ZFPM2} on 8q23.1, and \textit{TBX1} on 22q11.2 [12–14].

\textit{De novo} loss-of-function mutations in cardiac genes whose haploinsufficiency makes a more modest contribution to cardiac
risk may be particularly difficult to identify. Loss-of-function mutations in such genes are more likely to be inherited from an asymptomatic parent and may combine with other genetic, environmental or stochastic factors to cause cardiac-related problems in sporadic cases following a multifactorial inheritance pattern. In such cases, animal models may provide the first evidence that a gene within one of the critical regions plays a role in cardiac development and/or function. Not only can animal models be used to effectively identify low penetrance phenotypes caused by haploinsufficiency of a candidate gene, but they can also be used to explore cardiac-related phenotypes—including defects that lead to embryonic lethality—that only become apparent when the expression of an individual gene is reduced by more than 50%.

Table 7. Cardiac-related genes within cardiovascular malformation critical regions.

| Cardiovascular Malformation Region 1: Chr1:1–2418935 (2.4 Mb), 111 genes |  |
|---|---|
| **Gene** | **Start (hg19)** | **Stop (hg19)** | **Related Cardiovascular Phenotypes** | **References** |
| **DVL1** | 1270658 | 1284492 | No cardiovascular phenotypes have been documented in Dvl-null mice. However, an extra copy of Dvl1 was able to rescue the lethal conotruncal heart defects seen in Dvl3-null mice suggesting that Dvl1 has redundant functions in cardiac development. | [20,21] |
| **SKI** | 2160134 | 2241652 | Mutations in SKI with putative dominant-negative potential have been shown to cause Shprintzen-Goldberg syndrome whose features include mitral valve prolapse, aortic root dilatation, vascular tortuosity and aortic aneurysms. Knockdown of the 2 paralogs of mammalian SKI in zebrafish (skia and skib) results in severe cardiac anomalies characterized by partial to complete failure in cardiac looping and malformations of the outflow tract. | [22] |

| Cardiovascular Malformation Region 2: Chr1:8395179–11362893 (3.0 Mb), 55 genes |  |
|---|---|
| **RERE** | 8412464 | 8877699 | Rere-null mouse embryos die of cardiac failure around E9.5 with unlooped hearts. RERE-deficient mouse embryos have aortic arch anomalies, double outlet right ventricle, transposition of the great arteries, and perimembranous ventricular septal defects. | [23,24] |

| Cardiovascular Malformation Region 3: Chr1:12726755–20540759 (7.8 Mb), 175 genes |  |
|---|---|
| **PDNP** | 13910252 | 13944452 | Pdpn-null mouse embryos have hypoplastic and perforated compact and septal myocardium, hypoplastic atrioventricular cushions resulting in atrioventricular valve abnormalities, and coronary artery abnormalities, hypoplasia of the sinoatrial node, and thin, perforated cardinal and pulmonary vein walls. | [25–27] |
| **SPEN** | 16174359 | 16266950 | Spen-null mouse embryos die in utero and have defects of the cardiac septum and muscles. | [28] |
| **CLCNKA** | 16348486 | 16360452 | A loss-of-function variant in the human CLCNKA gene is a risk factor for heart failure in Caucasians. | [29] |

| Cardiovascular Malformation Region 4: Chr1:20555776–23438888 (2.9 Mb), 50 genes |  |
|---|---|
| **ECE1** | 21543740 | 21672034 | A loss-of-function mutation in ECE1 was identified in an individual with patent ductus arteriosus, a small subaortic ventricular septal defect, and a small atrial-septal defect, Hirschsprung disease, and autonomic dysfunction. Ece1-null mice have interruptd aortic arch, absent right subclavian artery, poorly developed endocardial cushions, double outlet right ventricle, truncus arteriosus, double aortic arch, overriding aorta and ventricular septal defects. | [10,30,31] |
| **HSPG2** | 22148737 | 22263750 | HSPG2-deficient mouse embryos have hyperplastic conotruncal endocardial cushions, transposition of the great arteries, and malformations of the semilunar valves. However, recessive mutations in HSPG2 have been shown to cause Schwartz-Jampel syndrome, type 1 and dyssegmental dysplasia, Silverman-Handmaker type, neither of which are commonly associated with cardiac defects. | [32–34] |
| **LUZP1** | 23410516 | 23495351 | Luzp1-null mice have double outlet right ventricle, transposition of the great arteries, and ventricular septal defects. | [35] |

| Cardiovascular Malformation Region 5: Chr1:27803719–31404471 (3.6 Mb), 55 genes |  |
|---|---|
| **WASF2** | 27730734 | 27816678 | Wasf2-null mouse embryos die before E11.5 with abnormalities in vasculogenesis. These embryos also have small dorsal aortas and anterior cardinal veins at the 22 somite stage, and display incomplete cardiac looping and small hearts at E10.5. | [36] |

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Deleterious changes in genes that make a modest contribution to cardiac risk may also be identified in asymptomatic individuals from the general population. One source of information on potentially deleterious changes in various populations is the Database of Genomic Variants (http://dgv.tcag.ca/) which catalogues genomic variation among “normal controls” and population-based cohorts. It is interesting to note that exon-containing deletions in each of the positional candidate genes identified in this study have been reported in this database (Table S1). This suggests that the cardiac-related phenotypes caused by haploinsufficiency of each of these genes alone are likely to be incompletely penetrant or may go undetected in some individuals. This is consistent with the incomplete penetrance for cardiovascular malformations and cardiomyopathy seen among patients with overlapping terminal and interstitial deletions of 1p36 [2,5].

Several of the cardiac critical regions we have identified on chromosome 1p36 contain more than one positional candidate gene. We also note that large terminal and interstitial deletions often overlap more than one cardiac critical region. This suggests that haploinsufficiency of two or more genes may contribute to the cardiac phenotypes associated with many 1p36 deletions. Future studies aimed at understanding how adjacent 1p36 genes work together to impact cardiac development and function may provide information which can be used to design effective therapeutic and/or preventative measures which can minimize the impact of cardiovascular malformations and/or cardiomyopathy in such cases.

**Supporting Information**

Table S1 Exon-containing deletions in 1p36 cardiac-related genes found in various control and population-based cohorts catalogued in the Database of Genomic Variants.

**Author Contributions**

Conceived and designed the experiments: HPZ TFB DAS. Performed the experiments: HPZ TFB DAS. Analyzed the data: HPZ TFB KES SRL DAS. Contributed reagents/materials/analysis tools: AHG TM AVH BMA CP HG GH BEM SWC SRL DAS. Wrote the paper: HPZ KES DAS.
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