Mutations in DCHS1 cause mitral valve prolapse

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Mitral valve prolapse (MVP) is a common cardiac valve disease that affects nearly 1 in 40 individuals1–9. It can manifest as mitral regurgitation and is the leading indication for mitral valve surgery10. Despite a clear heritable component, the genetic etiology leading to non-syndromic MVP has remained elusive. Four affected individuals from a large multigenerational family segregating non-syndromic MVP underwent capture sequencing of the linked interval on chromosome 11. We report a missense mutation in the DCHS1 gene, the human homologue of the Drosophila cell polarity gene dachsous (do), that segregates with MVP in the family. Morpholino knockdown of the zebrafish homologue dachsous1b resulted in a cardiac atrioventricular canal defect that could be rescued by wild-type human DCHS1, but not by DCHS1 messenger RNA with the familial mutation. Further genetic studies identified two additional families in which a second deleterious DCHS1 mutation segregates with MVP. Both DCHS1 mutations reduce protein stability as demonstrated in zebrafish, cultured cells and, notably, in mitral valve interstitial cells (MVICs) obtained during mitral valve repair surgery of a proband. Dchst1+/− mice had prolapse of thickened mitral leaflets, which could be traced back to developmental errors in valve morphogenesis. DCHS1 deficiency in MVP patient MVICs, as well as in Dchst1+/− mouse MVICs, result in altered migration and cellular patterning, supporting these processes as aetiological underpinnings for the disease. Understanding the role of DCHS1 in mitral valve development and MVP pathogenesis holds potential for therapeutic insights for this very common disease.

In a previous study, based on specific diagnostic criteria9–11, MMVP2 (myxomatous mitral valve prolapse-2) was mapped to a 4.3 cM region of chromosome 11p15.4 in a family of Western European descent segregating non-syndromic mitral valve prolapse as an autosomal dominant trait with age-dependent penetrance (Fig. 1a, c). We performed tiled capture and high-throughput sequence analysis of genomic DNA from four affected individuals (Fig. 1a), identifying 4,891 single nucleotide variants (SNVs) and insertion/deletion polymorphisms in the targeted region (see Methods). After selecting rare protein-coding variants shared among all affected pedigree members, we identified three heterozygous protein-altering variants: two missense SNVs in DCHS1, a member of the cadherin superfamily10, resulting in p.P197L and p.R2513H (Fig. 1b), and a single missense SNV in APBB1, the amyloid beta (A4) precursor protein-binding family B member 1 gene resulting in p.R481H. Both DCHS1 mutations, p.P197L and p.R2513H, were rare in the population (the former observed twice in 4,300 European-American individuals from the NHLBI Exome Sequencing Project and the latter never observed), and both were predicted to be protein damaging by PolyPhen-2 (ref. 11) and LRT12. Additionally, Apbb1 is not expressed in murine cardiac valves (Extended Data Fig. 1a, b). Therefore, we were not confident that Apbb1 was contributing to MVP in this family.

The functional effect of the DCHS1 variants was evaluated in the zebrafish Danio rerio, as this model system lends itself to functional annotation of mutations implicated in human disease16–18. Zebrafish have two DCHS1 homologues, dachsous1a and dachsous1b. dachsous1b is located in a region of D. rerio chromosome 10 that is syntenic to the DCHS1 region of human chromosome 11. Knockdown of dachsous1a did not result in a cardiac phenotype despite reduction in mRNA levels.
Figure 1 | Pedigrees, mutation, and phenotype. Black symbols, MVP affected, grey, unknown, arrows, probands. If no genotype is shown, the individuals were unavailable for study. a, Pedigree linked to chromosome 11. #, Individuals under 15 years of age; *, individuals sequenced. Genotypes c.7538G>A (R2513H) of DCHS1 mutation are shown. b, DNA sequence of c.7538G>A (R2513H). c, Two-dimensional echocardiographic long-axis view of family 1 proband. Dashed line marks mitral annulus. d, e, Family 2 and 3 pedigrees. Genotype c.6988C>T (p.R2330C) shown. f, DNA sequence c.6988C>T (p.R2330C). g, Two-dimensional echocardiographic long-axis view of family 2 proband.

Figure 2 | Zebrafish Dchs1b is required for atrioventricular canal development. a, By 72 hpf, zebrafish hearts develop a constriction in the atrioventricular canal (AVC) that separates the atrium (a) from the ventricle (v). b, Knockdown of dchs1b plays a role in MVP beyond the linked family. By evaluating a cohort of MVP patients, we identified two additional families in which MVP segregated with the novel DCHS1 protein variant p.R2330C (Fig. 1d–g). The proband of family 2 underwent surgical mitral valve repair for severe MVP and mitral 

(Extended Data Fig. 1a, b); however, knockdown of dachsous1b (dchs1b) led to significant changes in cardiac morphology (Fig. 2a; Extended Data Fig. 1a). Control zebrafish hearts undergo looping and develop an atrioventricular constriction by 48 h post-fertilization (hpf), whereas dchs1b knockdown disrupts this process, resulting in impaired formation of the atrioventricular constriction (Fig. 2a, b). While control embryos have unidirectional blood flow between the atrium and ventricle at 72 hpf (Supplementary Video 4), dchs1b knockdown causes regurgitation of blood from the ventricle into the atrium (Supplementary Video 5). An atrioventricular canal defect was defined as failure of cardiac looping combined with any atrioventricular regurgitation at 72 hpf. Using a high morpholino dose (1.5 ng) to establish AVC defects (Fig. 2c). Injection of the mutant DCHS1 mRNA alone did not cause atrioventricular canal defects, supporting a loss-of-function mechanism for the DCHS1 mutation. Having demonstrated segregation of a loss-of-function DCHS1 mutation with MVP in our large pedigree, we sought to determine if genetic variation in DCHS1 plays a role in MVP beyond the linked family. By evaluating a cohort of MVP patients, we identified two additional families in which MVP segregated with the novel DCHS1 protein variant p.R2330C (Fig. 1d–g). The proband of family 2 underwent surgical mitral valve repair for severe MVP and mitral
regurgitation at age 21. Valve tissue was resected to repair the posterior leaflet and examination of the tissue showed classic myxomatous degeneration (Extended Data Fig. 5). Mitral valve interstitial cells were isolated from a portion of the posterior leaflet resected during surgery, providing a unique resource for the functional studies described below. The proband’s sister, evaluated at age 27 and also heterozygous for the p.R2330C mutation, demonstrated classical MVP and did not carry the mutation. The mother (age 49) and maternal grandfather (age 76) are both affected with MVP and both carry p.R2330C. In family 3, the mother (age 49) and maternal grandfather (age 76) are both heterozygous for the p.R2330C mutation, demonstrated classical MVP and exhibited mitral regurgitation with posterior leaflet prolapse requiring surgery at age 72. The proband’s sister, evaluated at age 27 and also heterozygous for the p.R2330C mutation, also carries p.R2330C, however his MVP status is indeterminate due to mild left ventricular inferior wall hypokinesis that tethers the leaflets down into the left ventricular cavity, masking regurgitation due to mild leaflet prolapse anteriorly (Fig. 4) as in the proband from family 1 (Fig. 1c, Supplementary Videos 1–3, 6, 7). Micro-MRI analyses and 3D reconstructions of adult Dchs1−/− mice reveal prominent posterior leaflet prolapse compared to wild-type controls (Fig. 4). Cycloheximide treatment revealed that wild-type DCHS1 protein in transfected cells had a half-life of 5.8 h, while the mutant DCHS1 protein (p.P197L/p.R2513H) had a half-life of 1.6 h (Fig. 3d, e). DCHS1 constructs harbouring either p.P197L or p.R2513H were evaluated, showing that p.R2513H markedly reduced protein levels, implicating this variant as pathogenic in the family (Fig. 1, Extended Data Fig. 6).

A similar analysis of DCHS1 protein half-life was conducted using DCHS1 wild-type (WT) and mutant (p.P197L/p.R2513H) transfectants or MVICs from the proband of family 2. Consistent with the data obtained from the p.R2513H transfectants, these studies showed significant reduction in protein half-life compared to control MVICs (t1/2 = 0.46 h versus 1.73 h, Fig. 3d, e). Together, our studies show that p.R2513H markedly reduced protein levels, implicating this variant as pathogenic in humans.

Homozygous knockout of Dchs1 in mice results in neonatal lethality and multi-organ impairment; however, the relevant genetic model for human MVP is the heterozygous Dchs1 mouse. Dchs1−/− mice exhibit mitral valve prolapse with pronounced involvement of the posterior leaflet, which is elongated and shifts the leaflet coaptation anteriorly (Fig. 4) as in the proband from family 1 (Fig. 1c, Supplementary Videos 1–3, 6, 7). Micro-MRI analyses and 3D reconstructions of adult Dchs1−/− mice reveal prominent posterior leaflet prolapse compared to wild-type controls (Fig. 4). Cycloheximide treatment revealed that wild-type DCHS1 protein in transfected cells had a half-life of 5.8 h, while the mutant DCHS1 protein (p.P197L/p.R2513H) had a half-life of 1.6 h (Fig. 3d, e). DCHS1 constructs harbouring either p.P197L or p.R2513H were evaluated, showing that p.R2513H markedly reduced protein levels, implicating this variant as pathogenic in the family (Fig. 1, Extended Data Fig. 6).

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thickening with quantitative increases in valve volume (Fig. 4 and Supplementary Video 8). All other echocardiographic measurements were unchanged (Extended Data Fig. 7). Histological and molecular characterization of Dchs1+/− mice confirmed leaflet thickening and showed myxomatous degeneration with increased proteoglycan accumulation in both mitral leaflets (Fig. 4). These results clearly show that Dchs1 heterozygosity results in mitral valve prolapse in mice.

To determine if MVP has a developmental origin, we performed expression and functional analyses at embryonic and fetal time points. RNA in situ hybridization and immunohistochemistry showed expression of Dchs1 in endocardial and mesenchymal cells of atrioventricular valve leaflets at all time points examined (Extended Data Fig. 8). While no morphological defects were observed in the Dchs1+/− mice during early embryonic development (E11.5–E13.5), at later time points (E15.5–E17.5) Dchs1+/− mice displayed changes in mitral-valve shape (Fig. 5a–c), which were more severe in Dchs1−/− animals. Histology and three-dimensional reconstructions of anterior and posterior mitral leaflets at E17.5 of Dchs1+/+, Dchs1+/− and Dchs1−/− mice showed comparable leaflet volumes. However, Dchs1+/− and Dchs1−/− animals exhibited statistically significant changes in valvular length and width (Supplementary Video 9 and Fig. 5a–c). In most leaflet regions measured, Dchs1−/− animals displayed an intermediate phenotype, demonstrating a gene dosage effect. These shape changes implicate Dchs1 as critical for proper anatomical patterning of the valve, consistent with previous reports of dachsous function in the Drosophila wing. Thus, in vivo lineage-tracing studies were performed on Dchs1+/+ and Dchs1−/− mice. Crossing the WT1-Cre/ROSA-eGFP line onto both Dchs1+/+ and Dchs1−/− backgrounds allowed visualization of patterning defects of epicardial-derived cells (EPDCs) during migration into the posterior leaflet. This EPDC population initially migrates into the posterior leaflet as a sheet of cells. However, in the Dchs1−/− mice this sheet-like appearance is disrupted and an increase in EPDCs infiltrating diffusely throughout the valve tissue is observed (Extended Data Fig. 9a–c, Supplementary Video 10), concomitant with altered cellular patterning and alignment (Extended Data Fig. 9d, e). In vitro studies of MVICs from Dchs1−/− and from the family 2 MVP proband (p.R2330C) also show this increased migration phenotype (Extended Data Fig. 10a, b). Taken together, the mouse and human studies support a developmental aetiology for MVP, and invoke a model for MVP in which cell migration and patterning defects mediated by DCHS1 contribute to disease pathogenesis.

MVP is one of the most common cardiovascular diseases, affecting nearly 1 in 40 people worldwide. Although its heritability and variable expression in large pedigrees has been known for decades, its genetic underpinnings have remained elusive. We report the discovery of two loss-of-function mutations in DCHS1 that segregate with MVP in three families. Our mouse models exhibit classical MVP, a phenotype that was traced back to developmental errors during valve morphogenesis. These findings provide a model for understanding inherited non-syndromic MVP as a developmentally based disease that progresses over the lifespan of affected individuals, consistent with previous reports on the natural history of MVP. A robust estimate of the total contribution of rare DCHS1 genetic variation to sporadic MVP has yet to be determined and will require sequencing of large cohorts of MVP patients. Nonetheless, discovery of this novel mechanistic pathway elucidated by intensively studying rare familial mutations will facilitate the identification of additional MVP genes and reveal pathogenic mechanisms that hold the potential for pre-surgical therapy for this very common cardiac disease.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**Figure 5 | Developmental aetiology for MVP. a, b, Haematoxylin and eosin and 3D reconstructions of E17.5 Dchs1+/+, Dchs1−/− and Dchs1−/− mouse hearts showing thickening of anterior and posterior leaflets (AL, PL) in Dchs1−/− mice compared to Dchs1+/+ mice. Dchs1+/+ valves display an intermediate phenotype. c, Quantification of valve dimensions showing Dchs1+/+ (green bars) and Dchs1−/− (red bars) anterior and posterior lengths were significantly reduced compared to Dchs1+/+ (blue bars) leaflets. Dchs1−/− and Dchs1+/− valves displayed increased thickness throughout the leaflets compared to Dchs1+/+. Scale bars, 100 μm. n = 5 per genotype and two-tailed Student’s t-test was used to calculate P values; *P < 0.01.**
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Supplementary Information is available in the online version of the paper.

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Author Contributions R.D., M.L., C.S., C.J., M.P., X.J., J.-J.S., D.T., S.O., X.E., F.C., and S.A.S. participated in genetic analysis, sequencing and mutation cloning. R.D., F.N.D., L.A.F., and R.D. wrote the manuscript. R.A.L., A.H., S.A.S. and J.J.S. coordinated the Leducq Mitral Network. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.A.S. (slaugenhaupt@chgr.mgh.harvard.edu) or R.A.N. (norrisra@musc.edu).
METHODS

Study participants. Family 1 was originally recruited through the Echocardiography Laboratory at Massachusetts General Hospital as part of a phenotype-driven genetic study of MVP. MVP was diagnosed by specific criteria (>3 mm atrial leaflet displacement in a parasternal long-axis view)2. The study was approved by the Institutional Review Board of Partners Healthcare, Boston, Massachusetts, and all participants provided written informed consent. Complete details of the linkage analysis on this large, multigenerational family have been previously published1. In brief, the family contains 41 individuals in five generations. Echocardiograms and DNA were obtained on 28 subjects, of whom 12 were diagnosed with MVP, three were confirmed to have normal mitral leaflet displacement and were considered unaffected for the original linkage analysis. The proband had prominent MVP with thickened leaflets, severe mitral regurgitation, and heart failure ultimately requiring surgical valve repair (Fig. 1c and Supplementary Videos 1–3). Other affected members also showed diffusate leaflet thickening, prolapse, and mitral regurgitation of varying severity; one required surgical repair. No extracardiac manifestations of connective-tissue abnormalities or Marfan syndrome were present in any family members. Following a complete genome scan, parametric and non-parametric analyses confirmed linkage of this family to a 4.3 Mb region of chromosome 11p15.4. Consistent with the model of sex- and age-dependent penetrance, several of the unaffected members who carried the MVP allele were less than 15 years old at the time of evaluation (Fig. 1a). Importantly, an analysis using only affected individuals confirmed the linkage result.

DNA sequencing and variant calling in family 1. In order to identify the mutation, four affected individuals who shared the disease haplotype were chosen for sequencing (Fig. 1a). To reduce the likelihood of random haplotype sharing, we selected individuals with four distinct haplotypes on the non-MVP allele. A 2.1 Mb region of human chromosome 11 (5094774–7420926; NCBI36 coordinates) was targeted and screened for repetitive regions using the SureSelect system (Agilent). DNA extraction was performed using the AutoGenFlex STAR automated system (Autogen) and FlexGen DNA purification reagents (Qiagen) according to the manufacturer’s instructions. Bait oligonucleotides were designed to the non-repetitive regions of the targeted linkage peak, resulting in 1.03 Mb of target sequence using the SureSelect in-solution long read DNA baits (Agilent). Captured DNA was amplified and quantified using the Agilent High Sensitivity DNA Kit for the Agilent 2100 Bioanalyzer, and sequenced using Illumina sequencing chemistry (paired-end, 100 cycles) at the Venter Institute supported by the NHLBI Resequencing and Genotyping Program. One hundred bases were sequenced from each end of the captured DNA fragments. Image analysis and base calling were performed using Illumina’s GA Pipeline version 1.6.0. Sequence reads were mapped to the human genome (.ncbi36) and variants identified using clc-ngs-cell-2.0.5-linux_64 (clc_ref_assemble_long -q -p fb ss 180 360 –I –r, and find_ mapped to the human genome (ncbi36) and variants identified using clc-ngs-cell-

Exome sequencing, genotyping, and variant evaluation. Exome-enriched genomes were multiplexed by flow cell for 101-bp paired-end read sequencing according to the protocol for the Hiseq 2000 sequencer (vers. 1.7.0; Illumina) to allow a minimum coverage of 30x. Reads were aligned to the human reference genome (UCSC NCBI36/hg19) using the Burrows-Wheeler Aligner (version 0.5.9). Evaluation of the DCHS1 gene yielded 4 novel coding sequence variants that confirmed following repeat Sanger sequencing: 6646587 G/A (p.R2330C); 6646709 G/A (p.A2289V); 6648584 C/T (p.A1896T); 6648929 A/G (p.V1817A), based upon positions in the 1000 Genomes database. These four variants, in addition to the variants identified in family 1, were genotyped using Sequenom technology in the sporadic cohort. The major steps included primer and multiplex assay design using Sequenom’s MassARRAY Designer software, DNA amplification by PCR, post-PCR nucleotide deactivation using shrimp alkaline phosphatase (SAP) to remove phosphate groups from unincorporated dNTPs, single-base extension reaction for allele differentiation, salt removal using ion-exchange resin, and mass correlated genotype calling using SpectroCHIP array and MALDI-TOF mass spectrometry. Quality control to determine sample and genotyping quality and to potentially remove poor SNPs and/or samples was performed in PLINK, a whole genome association analysis toolset. We predicted the impact on gene function using PolyPhen218, Mutation Taster19 and LRT20.

Identification of family 2 and 3. In order to identify other mutations in DCHS1, we first evaluated DCHS1 in the exome sequence data described above, reasoning that early onset forms may be more likely to have strong genetic aetiologies. Rare variants causing amino acid substitutions in DCHS1 were identified in four individuals (p.V1817A, p.A1896T, p.A2289V, and p.R2330C) and genotyped in a cohort of 1,864 sporadic MVP patients that included the 21 individuals with exome data; two of these variants, both localized to exon 19, were observed in the MVP cohort (p.A2289V in two cases and p.R2330C in three cases). The proband in family 2 carried the p.R2330C variant and underwent surgery for MVP in Paris. We were able to collect DNA and exonic sequences on first-degree relatives at that time. Additional clinical characteristics of the proband in family 2 included congestive heart failure (NYHA II/III) with left ventricular dilatation (70/50 mm end-diastolic/end-systolic dimensions), impaired left ventricular systolic function (ejection fraction 53%, low for this volume overload), recurrent symptomatic atrial fibrillation, sustained ventricular tachycardia, and exercise-induced pulmonary hypertension (70 mm Hg systolic). The proband in family 3 was originally collected in Amiens, France. All echocardiograms were read in both Boston and Paris and readers were blind to genotype data. D. rerio studies. Husbandry, knockdown and expression analyses were performed in the wild-type D. rerio (zebrafish) strain Tubingen AB. Morpholinos were injected at a dose of 1.5 ng (after dose optimization) into single-cell embryos to achieve gene knockdown, and phenotypes were examined at 48 and 72 h post-fertilization in three separate experiments of 50–75 embryos and compared to controls using Fisher’s exact test. Morpholino GeneTools LLC (Philomath, OR) sequences were as follows: apbbl AACAAAGGAGTACCTACGATATTAGGC, dchs1a TAAAGAAGATGCAGCTCTACCTGCA, and dchs1b CATAACTGTGAAGTTTCGCGCTAGA. Knockdown was confirmed by quantitative polymerase chain reaction. qPCR was performed as previously described19. In brief, 20–30 ng of the captured end was detected at 73 h post-injection in liquid nitrogen. TRIZol (Sigma) was added, RNA was purified according to the manufacturer’s instructions, and cDNA was prepared using a Superscript III Kit (Invitrogen). Primer sets were as follows: apbbl, 5'-GGTGGAGGTTAAGCAAAGAC, 5'-CCAGCAGGAGATCCGAGTC; dchs1a, 5'-GTCTCTAGGGAGATCCGAG, 5'-CTTATTACACCCACCTTAC; dchs1b, 5'-GCTTCTTTTGAGGAAAGCCG, 5'-GGCCACCCCTACGAGG. qPCR was performed using SYBR Green (Applied Biosystems) in triplicate on an Applied Biosystems 7500 Fast Real-Time PCR instrument and normalized against β-actin. All zebrafish experiments were performed under protocols approved by the Institutional Animal Care and Use Committee at Massachusetts General Hospital.

D. rerio in situ hybridizations. In situ hybridizations were performed as previously described48 using a partial clone of dchs1b (Open Biosystems, Clone ID 7136458) amplified with primers containing a T7 RNA polymerase site engineered onto the 3’ end of the reverse primer (Forward 5’-GGCACTGATCGTTGTTGGT. Reverse: TAAATAGACATTATATACAGGGTTTAAACTCTAACAGGCACT, T7 site underlined). The dchs1b probe was produced using a T7 RNA polymerase (Ambion) and digoxigenin-labelled dNTPs (Roche). Other riboprobe usages in the study have been previously described48.

Generation of DCHS1 expression constructs. Human DCHS1 and the mutant c.590C>T (p.A1896T) were excised from pCRII and cloned into the pGREENII vector. Both plasmid containing the c.590C>T and c.5383C>G, A–sequence changes were synthesized by Integrated DNA Technologies. A unique EcoRI site, a T7 promoter and a Kozak sequence were added to the 5’ end of each gene, while a V5 tag and unique XhoI site were added to the 3’ end. Each gene was then subcloned into the expression vector pcDNA3.1. Additional expression constructs were generated.

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that contained only the c.590G>T (p.P197L) mutation or only the c.7538G>A (p.R2513H) mutation. These constructs were made using the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) as per the manufacturer’s instructions. The P197L construct was generated from the double mutant construct by changing the R2513H (c.7538G>A) mutation back into the wild type sequence using the following primers: 5’-gtctggagacgcagcagctccg, 3’-agcggacttgccgcttcgtgctcc (The underlined bold base indicates the base pair changed.). The R2513H construct was generated by introducing the (c.7538G>A) mutation into the wild type DCHS1 construct using the following primers: 5’-gtctggagacgcagcagctccg, 3’-agcggacttgccgcttcgtgctcc. Preparation and injection of DCHS1 mRNA. mRNA was prepared from the wild-type DCHS1 and DCHS1 mutant expression vectors using a T7 mMessage mMachine Kit (Ambion) according to the manufacturer’s instructions. Injection mixtures containing 0.75 ng dcdhs1 MO alone, or 0.75 ng dcdhs1 MO plus 7 fg µg⁻¹ of human DCHS1 mRNA (either wild type or mutant) were injected into one-cell embryos, and fish were scored for atrioventricular canal defects (failure to loop, and presence of regurgitation) 72 h later. Data were collected from three independent experiments performed with 20–30 embryos each and comparisons made using Fisher’s Exact test.

Isolation of DCHS1 mRNA. PCR and control patient mitral valve tissue and valvular interstitial cells. Resected posterior mitral valve tissue was used for culture and histology. For culture, valve pieces were minced in phosphate buffered saline (PBS) and washed in DMEM with antibiotics (penicillin/streptomycin (P/S) and fungizone) and incubated in DMEM with collagenase type II (Worthington) (1 mg ml⁻¹) at 37 °C for 12 h. Following mechanical dissociation in DMEM, the cell suspension was filtered through a 40-µm cell strainer and cells were cultured in DMEM with 15% fetal calf serum and antibiotics (P/S, fungizone). Although rare valve endothelial cells were present at P0, only cells with a fibroblastic phenotype (VFs) remained following passage 2. For all experiments, these valvular interstitial cells (VICs) were cultured for 10 days. Cells were used before passage 5. For histology: valves were fixed in formalin, embedded in paraffin and sectioned at 5 µm. Movat’s Pentachrome histological stain was performed using standard procedures.

Cell culture studies. Wild type, p.P197L, p.R2513H and p.P197L/R2513H DCHS1 constructs were either synthesized by Integrated DNA Technologies or generated by site-directed mutagenesis (as described above), with an amino-terminal V5 epitope tag. Except where indicated, “mutant DCHS1” indicates the double mutant p.P197L/p.R2513H haplotype in family 1. These constructs were expressed in mycoplasma-free HEK293 cells (ATCC, not independently authenticated) using cationic lipid-mediated transient transfection (Lipofectamine LTX, Invitrogen). Protein expression of transfected HEK cells was measured by quantifying western blots using an antibody to the V5 epitope tag (Invitrogen). Patient cells: for patient cells, control and p.R2330C valvar interstitial fibroblasts from posterior leaflets were plated at 2.5 × 10⁵ cells in a 24-well dish. 24 h later, protein stability experiments were performed. Protein stability experiments involved addition of cycloheximide 24 h after transfection (WT and p.R2513H transfectants) or stability experiments were performed. Protein stability experiments involved addition of cycloheximide 24 h after transfection (WT and p.R2513H transfectants) or plating (control or p.R2330C patient cells). For the cycloheximide experiments, media containing 100 ng ml⁻¹ of cycloheximide was added at 24 h post-transfection and each well was harvested as above at the indicated time points. Western blots were performed after a mousered and their hearts were perfused fixation and immersed 1:10 (12.5 mmol) Gadolinium (ProHance) in 10% formalin overnight before imaging. MRI was undertaken at 7T using a Bruker Biospin console (Pavasion 5.1) with a volume transmitter coil and a phased array surface coil. Gradient echo FLASH 3D images were collected with repetition time/echo time = 50 ms/5.4 ms, flip angle = 30°, number of excitations = 3, matrix = 256 × 256 × 256 and pixel resolution = 55 × 55 × 59 µm. Images in DICOM format were imported into AMIRA 3D reconstruction software and volume quantification were performed as described previously. Pairwise comparison of phenotypes were performed and statistical significance was determined by unpaired t-tests (two-tailed) using GraphPad Prism 7. All data were estimated under protocols approved by the Institutional Animal Care and Use Committee, Medical University of South Carolina. Prior to cardiac resection, mice were euthanized in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

RNA expression analyses of Dchs1 and Apbb1. Section in situ hybridization was performed as previously described on 4 embryos at each time point to localize Dchs1 expressing cells throughout cardiac development. A Dchs1 digoxigenin-labelled riboprobe (Roche) was generated against region 9222–10180 of accession number NM_001262943 and used for in situ hybridization at E11.5, E13.5, and E15.5. Dchs1 mRNA in situ hybridization at E14.5 was performed through GenePaint®. Two separate riboprobes were used to analyse Apbb1 mRNA expression at E14.5. These probes were generated against regions 1676–2506 and 370–1697 of accession number NM_001262943. These probes span all known isoforms for Apbb1 and provide similar spatial RNA expression patterns.
Protein expression. Dchs1 antibodies were generated by immunizing rabbits with a synthetic peptide corresponding to rat Dchs1 protein sequence: CSTYMVES PDLVEADSAA (region 1308–1324 of accession number NP_001101014)\(^a\). Immunohistochemistry was performed as described previously\(^a\) using a 1:100 dilution of primary antibody.

In vivo lineage trace. To trace the fate of epicardially derived cells in Dchs1\(^+/−\) and Dchs1\(^+/+\) mitral leaflets, the Wt1/IREs/GFP-Cre mouse\(^b\) was bred with the Dchs1\(^−/−\) and Dchs1\(^−/−\) mice (n = 4 per genotype). Mice were euthanized at neonatal day 0 (P0), hearts were isolated and fixed overnight at 4°C in 4% paraformaldehyde dissolved in PBS. Hearts were processed through a series of graded ethanol, cleared in toluene, and embedded in Paraplast Plus (Fisherbrand, 23-021-400). Hearts were sectioned at 5 μm and slides were treated with 15 ml of antigen unmasking solution (Vector Biolabs, H-3300) in 1,600 ml of distilled water for 10 min in a pressure cooker (Cuisinart) followed by incubation for 1 h at room temperature with 1% BSA (Sigma, B4287) in PBS. Expression of EGFP after Cre recombination was detected by immunofluorescence using antibodies against GFP (Abcam, 13970) and myosin heavy chain (MF20; DSHB). 5 μm sections throughout the entire valve were used for 3D reconstructions using Amira software. The volume of GFP positive cells and the volume of each mitral leaflet were measured using this software. Cell counting was done on GFP positive and GFP negative cells every 15 μm throughout the entire valve. Pairwise comparison of littermates were performed and statistical significance was determined (Student’s t-test) with P = 0.04 for posterior leaflet and P = 0.86 for anterior leaflet.

In vitro migration. Human mitral valve interstitial cells were isolated from a control and the patient with the DCHS1 mutation (p.R2230C) (proband family 2) and seeded into the Radius 24-well Cell Migration Assay plate containing hydrogels (Cell Biolabs, CBA-125). Cells were allowed to adhere overnight and then gels were dissolved. Wells were imaged over a period of 24 h and area of the cell free region was measured in Photoshop v.10.0.01 and subtracted from the initial area of the hydrogel to generate area migrated over time. Migration in the Dchs1\(^+/−\) and Dchs1\(^−/−\) mice was assessed by explanting P0 neonatal posterior mitral leaflets onto plastic. Images of the explants and migrating cells were captured at multiple time points. Distance migrated was measured as the distance from the explant to the farthest migrating cell. Measurements were taken at 5 points around the explant and averaged to calculate distance migrated. After 24 h, cells were fixed in ice-cold 100% methanol for 10 min and immunofluorescence was performed using an antibody against N-cadherin (1:1,000 dilution, BD Transduction Labs, 610920). Pairwise comparisons were performed and statistical significance was determined (Student’s t-test) with P < 0.05.

Statistical considerations. In all experiments sample sizes were chosen to provide power of 0.8 to detect biologically significant differences between test groups with two-sided α = 0.05. Specific statistical tests are listed in the methods for each individual experiment. Assumptions of normal distributions were made for quantitative biological measurements and comparison groups were assumed to have similar variances. For zebrafish experiments, fertilized oocytes were randomly selected within each clutch for injection with active compound versus controls. Mouse experiments were interpreted blinded to genotype.

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Extended Data Figure 1 | Measurement of endogenous and exogenous gene expression in *D. rerio*. a, Corresponding representative embryos of each morpholino knockdown on the left, with close-up of heart on the right. b, To assess efficiency of morpholino knockdown, 20 embryos were collected 72 h after injection, mRNA was collected, and quantitative PCR was performed with three technical replicates. We demonstrate that morpholino (MO) knockdown of each indicated gene results in reduced mRNA expression, after normalization to beta-actin expression, compared to mock-injected controls (two-sided Student’s *t*-test). *P* values are noted on graphs. c, Western blotting of 20 pooled embryos injected with *DCHS1* mRNA demonstrates the production of protein. Mutant mRNA refers to the compound mutant P197L/R2513H.
Extended Data Figure 2 | *Apbb1* is not expressed during cardiac morphogenesis. *Apbb1* RNA expression was analysed at E14.5 in sagittal sections using 2 separate antisense probes. Whereas strong cranial and neural expression is observed for *Apbb1*, no detectable cardiac expression or valve expression (arrow) is evident.
Extended Data Figure 3 | Dachsous1b expression at the atrioventricular junction. *In situ* hybridization reveals the presence of *dchs1b* in the atrioventricular canal (avc) at 54 hpf (a, b) and 72 hpf (c). The *dchs1b* expression is purple while a counterstain for cardiac tissue is brown (a). White arrows highlight the *dchs1b* signal in the atrioventricular canal.
Extended Data Figure 4 | Dachsous1b knockdown alters atrioventricular ring markers. *In situ* hybridization at 48 hpf and 72 hpf, as indicated, was performed for known atrioventricular ring markers. In contrast to WT (a) *bmp4* expression is expanded into the ventricle at 48 hpf in *dchs1* knockdown embryos at 48 hpf (b), *spp1* and *notch1b* expression was largely unperturbed (c–f), and *has2* expression was not detected at 48 hpf, and is faint at 72 hpf in *dchs1* knockdown, compared to identically handled and stained controls (i–l).
Extended Data Figure 5 | Histopathology mitral valves. Human posterior leaflets of control, Barlow’s with MVP, and DCHS1 p.R2330C were isolated, fixed and stained with Movat’s pentachrome. Leaflet thickening, elongation and myxomatous degeneration is observed in the Barlow’s and DCHS1 p.R2330C leaflets compared to controls. Expansion of the proteoglycan layer (blue) and disruption of the normal stratification of matrix boundaries is observed in the Barlow’s and DCHS1 p.R2330C leaflets. Blue, proteoglycan; yellow, collagen; black, elastin; red, fibrin or cardiac muscle. Scale bars, 0.5 cm.
Extended Data Figure 6 | Protein expression of uncoupled mutations. In order to determine which family 1 DCHS1 mutation is leading to the observed decrease in protein expression, constructs were generated that harboured only the p.P197L or the p.R2513H variant. Mutant refers to the double mutant P197L/R2513H construct. Western blot analyses from transfected HEK293 cells, three independent biological replicates, demonstrate that the p.R2513H mutation causes a significant decrease in DCHS1 protein expression, similar to that of the construct with both variants (mutant), suggesting pathogenicity. Percent difference in protein levels is depicted. Normalization of data was accomplished by qPCR specific to the transfected constructs. P values from the Student’s t-test are indicated in graphs.
Cardiac Function (Echocardiography)

| Measurement      | Mode       | Parameter | Units | Dchs1 +/- (N=6) | STD     | Dchs1 +/- (N=6) | STD     | p-value |
|------------------|------------|-----------|-------|-----------------|---------|-----------------|---------|---------|
| IVS;d            | M-Mode     | Depth     | mm    | 0.820±0.935     | 0.187755| 0.906±0.155     | 0.140688| 0.170463|
| IVS;s            | M-Mode     | Depth     | mm    | 1.290±1.867     | 0.243251| 1.369±0.117     | 0.200513| 0.393879|
| LVID;d           | M-Mode     | Depth     | mm    | 4.282±7.233     | 0.515571| 4.045±3.183     | 0.217379| 0.28511 |
| LVID;s           | M-Mode     | Depth     | mm    | 2.887±3.983     | 0.33268 | 2.735±7.167     | 0.362674| 0.544435|
| LVPW;d           | M-Mode     | Depth     | mm    | 0.914±7.866     | 0.210393| 0.961±2.98      | 0.215238| 0.691322|
| LVPW;s           | M-Mode     | Depth     | mm    | 1.291±7.033     | 0.237193| 1.385±0.96      | 0.239801| 0.252632|

Extended Data Figure 7 | Cardiac function is not altered in Dchs1^{+/−} mice.
M-mode analyses were performed to determine whether cardiac structure and/or function were perturbed in the Dchs1^{+/−} mice. No statistically significant differences were observed in either cardiac structure or calculated cardiac function (n = 6 for each genotype). IVS, interventricular septum; d, diastole; s, systole; LVID, left ventricular internal dimension; LVPW, left ventricular posterior wall; EF, ejection fraction; FS, fractional shortening; LV, left ventricle.
Extended Data Figure 8 | Dchs1 expression during cardiac development.

Top, RNA expression of Dchs1 was analysed during embryonic gestation (E11.5, E13.5, and E15.5) by section in situ hybridization. At E11.5 Dchs1 RNA (blue staining) expression is observed in the endocardium and mesenchyme of the superior and inferior cushions (sAVC and iAVC, respectively). A gradient pattern of expression is observed at this time point with more intense expression near the endocardium. At E13.5 and E15.5, a similar pattern is observed in the forming anterior and posterior mitral leaflets (AL and PL, respectively). Bottom, Dchs1 protein expression (red) is observed throughout cardiac development in the endothelial cells and interstitial cells of the developing valves. Dchs1 shows asymmetric expression in the valvular interstitial cell bodies around E15.5 (arrowheads). Dchs1 protein is also observed in the epicardium and atrioventricular sulcus (arrows). (Red Dchs1; green MF20; blue Hoescht).
Extended Data Figure 9 | Dchs1 deficiency causes altered valvular interstitial cell patterning in vivo. a, IHC for eGFP of postnatal day 0 (P0) lineage traced Wt1-Cre/Rosa-eGFP/Dchs1<sup>+/+</sup> neonatal mice show epicardial-derived cells (EPDCs) migrating into the posterior leaflet as a sheet of cells directly under the endothelium of the atrialis. This normal patterning is perturbed in the Wt1-Cre/Rose-eGFP/Dchs1<sup>+/−</sup> mice. 3D reconstructions were used to examine all EPDCs in the posterior leaflet of both genotypes to obtain a complete fate map of these cells. b, c, Total volume of the leaflet is unchanged at this time point. However, the total volume of EPDCs as well as total EPDC cell number is significantly increased. There is a significant decrease in the number of non-EPDCs in the posterior leaflet with no overall change in total cell number. These data demonstrate that a minimum threshold of Dchs1 expression is required for normal migration of EPDCs into the posterior leaflet, normal patterning of this cell population, and cross-talk between EPDC and non-EPDC cell types in the valve. **P < 0.01. d, Isolated anterior mitral leaflet from fetal (E17.5) Dchs1<sup>+/+</sup>, Dchs1<sup>+/−</sup>, and Dchs1<sup>−/−</sup> mice were used to quantify cellular alignment of valvular interstitial cells. Vector maps were generated from histological (haematoyxin and cosin) stains to show orientation and alignment of cells in relationship to each other. Boxes in each vector map panel are represented as zoomed images of regions within each of the valves to show cell orientation. e, Cell alignment and polarity were quantified as the number of cells that deviate >10 degrees from the proximal-distal (P–D) axis of the leaflet. 90% of the cells in Dchs1<sup>+/+</sup> show proper alignment with each other and along this P–D axis. Haploinsufficiency (Dchs1<sup>+/−</sup>) results in a 50% reduction in cell alignment, which is further reduced in Dchs1<sup>−/−</sup> (**P values < 0.01).
Extended Data Figure 10 | Mice and MVP patients with Dchs1 deficiency exhibit migratory defects in vitro. a, Posterior leaflets of P0 neonatal Dchs1+/+ and Dchs1+/- mice were explanted and interstitial cells were allowed to migrate out for 24 h. Dchs1+/- mice exhibit increased migration (black lines drawn from explants) coincident with loss of cell–cell contacts and N-cadherin expression at focal adhesions. Whereas N-cadherin expression (red) is found at the membrane at points of cell–cell contract in Dchs1+/+ valvular interstitial cells (arrows), this membrane expression is lost in the Dchs1+/- cells and is prominently expressed in the cytoplasm (arrows). Nuclei, blue. b, Migration assays using control and MVP patient (p.R2330C) valvular interstitial cells exhibit a similar affect as observed in the mouse cells whereby the p.R2330C cells exhibit an increase in migration. P values are indicated in graphs.