Knockout mice represent an important tool for the multisystemic study of human monogenic heart disease

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Abstract

Mouse models are relevant to study the functionality of genes involved in human diseases, however, translation of phenotypes can be challenging due to differences in physiology. Herein, we systematically investigated genes related to monogenic forms of cardiovascular disease based on the Genomics England PanelApp and aligned them to the International Mouse Phenotyping Consortium (IMPC) data. Our analyses found a total of 153 genes with a strong link to cardiomyopathy, cardiac arrhythmias or congenital heart disease in humans. Among those, 151 had a corresponding one2one mouse ortholog. For 37.7% (57/151) viability and heart data captured by electrocardiography, transthoracic echocardiography, heart morphology and pathology from embryos and young adult mice was available. In single gene knockout mice, 75.4% (43/57) of these genes are associated with non-viable phenotypes, whereas records of prenatal, neonatal or infant death for the associated disorders in humans were found for 35.1% (20/57). When viable, multisystem phenotypes are common, with 58.8% (20/34) of heterozygous (homozygous lethal) and 78.6% (11/14) of homozygous (viable) mice showing cardiovascular, metabolic/homeostasis, musculoskeletal,

hematopoietic, nervous system and/or growth abnormalities mimicking the clinical manifestations observed in patients. This IMPC data is critical beyond cardiac diagnostics given its multisystemic nature that allows detecting abnormalities in various physiological systems, providing a valuable resource to understand pleiotropic effects.

Introduction

Mouse models provide an invaluable resource to investigate the functionality of genes as well as contribute to the discovery of new genes involved in human disease[1]. Genetically modified mice have markedly facilitated the understanding and characterisation of Mendelian disorders, providing insights into the disease mechanisms and the development of therapeutic options[2,3]. Reproducibility is vital in mouse disease modeling. Inappropriate breeding, animal husbandry and quality control can lead to irreproducible results. Having validated models, appropriate use of controls, rigorous experimentation and statistical standards for carrying out and reporting mouse phenotypic procedures are essential to strengthen the translational impact of model organisms[4,5]. However, translation of phenotypes across different species presents some challenges. It is not always straightforward to assess whether a statistically significant abnormal phenotype, i.e. some deviation from normal morphology, physiology, or behaviour[6] detected in knockout mice will be observed when loss-of-function mutations for the ortholog gene occur in humans[7]. Conversely, knockout mice do not necessarily replicate the phenotypes observed in humans due to potential differences in biology, genetic background, or alternate pathways; some of these limitations in mouse models have previously been discussed[8,9]. Phenotypes of adult animals are defined during development, so understanding the molecular evolution of phenotypic traits in mammals, including mice, is important to understand trait discrepancies (e.g., non-detectability) between species[10].

The International Mouse Phenotyping Consortium (IMPC) is a collaborative, international program that aims to generate and phenotype null mutants for every protein-coding mouse gene[11]. It involves the creation of a repository and the implementation of standardized phenotypic assays to systemically characterize mouse strains across all organ systems. The standardised screens, that involve an ever increasing number of wild-type mice, controlled for gender and other factors, are key for reproducibility. How well mouse models perform at mimicking disease phenotypes can be explored as a whole[12] or focusing on specific types of disorders[13,14]. The IMPC is an evolving and frequently updated resource, with an increasing number of mouse knockouts and corresponding phenotype data points caputured by data releases that provide stable and versioned reference sets of analysed data. At the time of the present analysis (DR 16.0), it contained 8,093 phenotyped mouse genes and 93,235 phenotype calls[15].

Here we systematically investigated the phenotypic abnormalities observed in the knockout mouse orthologs to human genes with evidence of cardiovascular function in humans. For that we used a set of Mendelian disease associated genes from expert-curated gene panels used in clinical diagnostics from

Genomics England PanelApp[16]. We focused our analyses to a set of genes involved in monogenic forms of cardiomyopathy, cardiac arrhythmia and congenital heart disease showing clinical manifestations in humans that could be potentially captured by targeted IMPC assays, including electrocardiogram, transthoracic echocardiogram, organ morphology, gross pathology, embryo gross morphology and primary viability assessment.

Results

According to gene panels related to human Mendelian disorders, a total of 153 genes in the Genomics England PanelApp[16] have a high level of evidence for association with human cardiomyopathy, cardiac arrhythmia or congenital heart disease. For 151, we found the corresponding one2one mouse ortholog and out of these, 57 had phenotypic data from the IMPC. All 57 genes were shown to have one or more abnormal phenotypes when knocked out in mice. These included pre-weaning lethality and/or effects on various physiological systems in the young adult (9-16 weeks) mice. For many genes both were observed. We found 59.6% (34/57) of the homozygous knockouts to be lethal (absence of live null homozygous pups pass the weaning stage) and 15.8% (9/57) subviable (<12.5% live homozygous pups) in a primary viability assessment. For the non-viable (lethal and subviable) lines (43/57, 75.4%), the heterozygous knockouts entered the early adult phenotypic pipeline where 69.8% (30/43) were found to be phenodeviants. All the homozygous viable lines (14/14) showed abnormalities in a broad range of physiological systems.

Lethality in cardiac genes

The percentage of lethal (and subviable) lines among the set of cardiovascular genes (43/57, 75.4%) compared to 35.03% for the total number of lines with viability data according to DR17 (July 2022) reflects how essential these genes are for organism development (**Fig 1a**). Cardiovascular disease associated genes have a 5.6-fold increase in the odds of being lethal/subviable in the mouse compared to 4-fold for neurodevelopmental disorder genes (**Fig 1b**). When investigating lethality in humans, 46.5% (20/43) of the lethal and subviable cardio genes in the mouse have also been associated with prenatal, neonatal or infant death up to 1 year of age according to OMIM clinical records. If we include childhood mortality (1-10 years of age) this percentage increases to 55.8% (24/43). Conversely, 28.5% (4/14) of the viable genes in mouse show records of neonatal/infant death in humans. When we investigated potential sources of discrepancy in lethality between mouse and human, we found a higher percentage of (total) missense pathogenic variants reported in ClinVar[17] among the set of lethal/subviable genes in the mouse compared to the viable categories (p-value = 5.56e-31) (**Fig 1c**). When investigating the presence of pathogenic LoF and nonLoF variants at the gene level, we also observed a lower percentage of genes with only nonLoF (mainly

missense) variants reported, although the differences between viability categories are not statistically significant (**Fig 1d**). In the studied set of knockout mice the phenotype is invariably the result of a null - loss of gene function (LoF) -mutation.

Out of the 153 diagnostic grade genes associated with cardiomyopathy, cardiac arrhythmias and congenital heart disease in humans, 54 (35%) are biallelic (autosomal recessive) , 68 (44%) monoallelic (autosomal dominant), 21 (14%) both biallelic and monoallelic and 10 (7%) X-linked. For the subset of these genes investigated in the previous genes with IMPC mouse data available, we find a shift in the percentage of biallelic forms, 24 (42%), compared to 23 (40%) monoallelic, 8 (14%) both and 2 (4%) X-linked (**Fig 1e**). Interestingly, we do not observe differences in viability when we compare AR and AD disease associated genes: for 19/24 AR disease genes and 19/23 AD genes, the mouse is homozygous lethal or subviable, 79% and 83% respectively (p-value = 1). A curation of OMIM clinical records indicates that 15/19 (79%) AR and homozygous mouse lethal genes have records of prenatal to infant lethality in humans while this is true for only 1/19 (5%) AD and homozygous knockouts lethal genes (**Fig 1f**).

Phenotype abnormalities

A full description of the significant phenotypes other than lethality, i.e. phenotypes for the heterozygous knockout in homozygous knockout lethal lines, and the homozygous knockouts for the viable lines are shown in **Fig 2a** and **Fig 2b** respectively. The majority of lethal lines (26/34, 76.4%) show an abnormal phenotype in heterozygous viable adult mice. The phenotypes observed cover the whole range of physiological systems, and multisystem phenotypes are common (19/23, 82.6%) (**Fig 2a**). Despite only 6 mouse models displaying cardiac abnormalities in the homozygous embryo or heterozygous early adult MPC mice, abnormalities in other physiological systems mimicking some of the clinical phenotypes observed in patients were captured. This is reflected by the PhenoDigm scores[18] (**See Methods**) used to compute the phenotypic similarity between the mouse model (mammalian phenotype ontology (MP) terms) and the human disease phenotypes (HPO terms). A PhenoDigm percentage score greater than 0 implies at least one HPO–MP match. Based on these scores, 69.2% (18/26) of the homozygous lethal lines showing phenotyping abnormalities in the heterozgous model are able to partially recapitulate the phenotypic manifestations observed in patients. After manual curation of prenatal to infant lethal phenotypes observed in humans, this percentage increases to 92.3% (24/26). All of the viable lines show at least one physiological system affected, with all but three genes (11/14, 78.6%) showing abnormal phenotypes in different organ systems (**Fig 2b**). If we compare the phenotypes observed in patients according to Human Phenotype Ontology (HPO) annotations, with the abnormal phenotypes identified in the knockout mice, some of the organ systems more frequently affected are shared between the two organisms: cardiovascular, metabolism/homeostasis, muskuloskeletal and nervous system abnormalities. Multisystemic phenotypes are predominant both in mouse and humans (**Fig 2d**). Based on HPO annotations for these

genes, only 4 genes have associated abnormal phenotypes restricted to the cardiovasuclar system (*JPH2, TNNI3K*, *COA5* and *PKP2),* being the median number for this set of genes of 12 physiological systems, which illustrates the multisystemic nature of the associated disorders (**Fig 2d**).

Viable lines

For the viable lines, 5 out of 14 genes showed some variation of cardiovascular phenotypic abnormality. Abnormalities in other physiological systems, e.g. homeostasis and/or hematopoietic system reported for the associated disorders were also recorded in the mutant mice. Most of the viable lines (11/14, 78.6%) mimic at least partially the phenotypes described in patients. This includes genes for which some of the phenotyping procedures aimed at identifying abnormal cardiovascular phenotypes have not yet been performed, e.g. *IDH2,* and *MYPN* with no ECG or TTE data. For three genes (*MYPN, NRAP* and *PRKAG2)* no overlapping abnormal phenotypes have been found between mouse and human (**Fig 2b**).

Phenotyping coverage

The IMPC *in-vivo* electrocardiography (ECG) and *in-vivo* transthoracic echocardiography (TTE) constitute the main phenotyping procedures aimed at capturing structural and functional heart anomalies of the heart in the mice. ECG peak and interval detection are used to capture electrical conduction problems whereas TTE recordings were performed to capture morphological and functional phenotypes of the left ventricle at end-systole and end-diastole. *Ex-situ* iodine-contrast high spatial resolution microcomputed tomography imaging of embryo hearts are applied at different developmental stages to identify morphological cardiac abnormalities or developmental delays. Gross Pathology and Tissue collection allows to detect and record abnormal external findings and macroscopic alterations in the heart, including heart weight. The IMPC phenotyping pipeline is performed across different centers with slightly variable coverage. At stage, nine IMPC centers measure ECG and six centers perform TTE recordings at 12 weeks of age in mice. *Ex-situ* imaging of the embryo heart is solely applied to homozygous lethal or homozygous subviable single-gene knockouts to identify structural heart defects. No embryo imaging was performed on viable knockout lines so embryo data was available for only a small subset of the total knockout genes used in this study. This results in a situation where the current phenotyping may have not yet covered the relevant physiological systems affected in patients, consequently, cardiac phenotype coverage is gaping, or the specific test can not be included anymore herein not all lines can be evaluated for the complete set of cardiovascular parameters. **Fig 3a** shows the coverage of the main procedures aimed at capturing cardiovascular phenotypes in the set of selected genes.

Bootstrap analysis

For each phenotyping procedure, the IMPC data is processed by different statistical methods to identify genotype-phenotype associations by comparing knockout mice to wild type mice of the same background strain. Given the number of tests performed, a pre-set and conservative significance threshold (0.0001) for Type I error has been established. This cutoff is used to identify phenodeviants and result in an MP term being associated with a knockout line. Given the observed depletion of cardiovascular phenotypes, we further investigated the raw statistical results to check the p-values for the parameters evaluated through the two main TTE and ECG procedures. Bootstrap was applied to assess whether the proportion of nominally significant p-values (<0.05) among the selected genes was higher than expected (**See Methods**). The proportion of genes with at least one significant parameter (p-value <0.05) in the set of cardiovascular gene pairs with TTE procedures is 0.89 (17/19) compared to 0.66 for the entire set of genes with TTE procedures (empirical bootstrap p-value = 0.0214). Applying the same procedure to genes with ECG parameters, the proportion of p-values <0.05 in the set of genes of interest is 0.53 (20/38) compared to 0.49 for the entire set of genes with ECG procedures (empirical bootstrap p-value = 0.5356) (**Fig 3a, b**). This suggests there is an enrichment for nominally significant p-values for the TTE procedure.

Extended analysis of genes with moderate evidence

The previous analysis was expanded to include genes for which the level of evidence for their association with the phenotype targeted in the gene panel from panelApp is moderate or low (‘Amber’ and ‘Red’). This added 90 additional genes labelled as ‘Amber’ and 104 genes classified as ‘Red’. It is worth mentioning that a single gene may have a different status in several panels, i.e. *SLC25A4* is rated as ‘Green’, in the *Cardiomyopathies - including childhood onset* panel and as ‘Red’ in the *Hypertrophic cardiomyopathy - teen and adult.* Out of 194 extra genes, 78 have entered the IMPC phenotyping pipeline. A summary of viability information and phenotypic abnormalities observed in the knockout mouse for these genes is shown in **Fig 4**. Interestingly, even a higher percentage of amber and red genes show cardiovascular abnormalities in the mouse compared to green genes, although the differences between genes baed on the level of evidence for the disease associaton are not significant (viability, p-value = 0.239; cardiovascula phenotype p-value = 0.726. The complete set of genes and annotations is available in **Sup Table 1.**

Discussion

Lethal phenotypes

Genes having a preweaning lethality phenotype in the mouse are known to be enriched in human disease genes, however the percentage of lethal/subviable lines found among the set of cardiovascular genes investigated (75.4%) is exceptionally high compared to that of the entire set of Mendelian disease-associated genes (55.6%) or neurodevelopmental disease genes (64.7%)[19,20]. By identifying early lethal phenotypes through curation of OMIM records, 50.9% of the associated disorders also had clinical reports

of prenatal, neonatal, infantile or childhood lethality. The discrepancy between the genes showing homozygous LoF lethality in the mouse but not in human disease may be explained by different factors such as i) 97.5% of the IMPC mutant lines are derived from the C57BL/6 substrain genetic background, compared to a higher polygenic background found in humans that may affect the penetrance and / or severity of some cardiac phenotypes and impact the phenotypic associations observed in more genetically diverse patients[21], ii) genes with monoallelic (heterozygous) variants account for 40.3% (23/57) of the genes included in this study and, biallelic (homozygous) variants could lead to embryonic lethality in humans. Reports of prenatal lethality may be underestimated in current disease annotation databases with the homozygous variant not documented in humans; iii) even for biallelic forms of disease, a wide range of phenotypic outcomes in terms of severity could be expected, with the difficulty to establish a molecular diagnosis when prenatal lethality occurs, iv) particularly among the genes showing mouse lethality, there is a non negligible percentage of genes (22% overall, 27% among lethal genes) where only non LoF variants have been classified as pathogenic which could be mildly deleterious and thus associated with less severe phenotypes[22]. This higher percentage of genes with only missense variants observed in patients in genes that are lethal in the mouse compared to viable genes may again indicate that LoF variants are not seen in the population because they lead to prenatal death in humans. Conversely, for the set of viable genes, LoF variants may not lead to early death but less severe phenotypes while missense variants may not impact the protein function..

Detecting abnormal phenotypes through ECG and TTE

Despite considerable differences in heart size and conduction rate, the development of the four-chambered heart and cardiac anatomy is remarkably similar in mice and humans[23]. Hence, single gene knockout mice lines with abnormal electrocardiography and/or echocardiography are well-established model systems for evaluating genotype-to-phenotype associations that may cause or contribute to human heart disease. *In-vivo* ECG and TTE, two state-of-the-art diagnostic procedures[24] have here enabled us to recapitulate phenotypes in mice known to be manifested in congenital monogenic forms of cardiac arrhythmias and cardiomyopathies in neonates and children. There is a variable coverage for certain phenotypes assays, including ECG and TTE (a challenge of the high-throughput and multicentre approach) and therefore not all mouse lines undertake the same number of tests. This gap can partially justify the rather marginal abnormal cardiovascular phenotype detection rate of IMPC for the known cardiac genes described in PanelApp (**Fig 2a,b**). This incompleteness is certainly a reason for lack of phenotypic recapitulation in the mouse. For example, for the 14 viable lines, 9 did not show any cardiovascular phenotype, but for 4 lines neither ECG or TTE were performed and for 11 TTE was not performed which is required to detect the types of morphological and functional heart phenotypes described in human patients (e.g. cardiomyopathy). Furthermore, in high-throughput phenotyping as pursued by the IMPC, the detectability of differences in the

interval lengths of ECG parameters and thus initial indications of abnormal gene function in the innervation of the heart were recorded, but not arrhythmias.

Another issue raised during our analyses, is whether the stringent thresholds to detect abnormal phenotypes through the ECG and TTE are obscuring some true effects. Multiple testing correction is required to avoid inflated false positive rates in the detection of the phenotype associations, but the enrichment of unadjusted p-values for the TTE parameters among the set of cardiovascular genes suggests that this particular procedure could be reviewed to investigate alternative cutoffs (e.g. 8/17, 47% genes with TTE data would have a significant abnormal heart phenotype with an alpha significance level of 0.005). The focus of the IMPC is to unlock, through systematic genotyping in mice, a landscape of previously unknown or poorly described genes that play a role in congenital monogenic heart disease and have great potential for translation.

Capturing multisystemic phenotypes

Cross-species phenotypic similarity algorithms allow for automated analysing of phenotype information and evaluation of mouse models for known disease-gene associations. PhenoDigm[18] relies on the entire set of mouse and disease annotations using all the pairwise mouse and human phenotype comparisons and thus identifying partial matches, i.e. a shared abnormal phenotype in one particular physiological system.

Neuromuscular disorders are commonly associated with cardiomyopathy, e.g. Emery–Dreifuss muscular dystrophy, Duchenne muscular dystrophy, with skeletal muscle phenotypes showing an earlier age of onset[25]. Similarly, specific mitochondrial and storage diseases and inborn errors of the metabolism are also associated with cardiomyopathy[25,26]. Congenital heart disease is also associated with a higher risk of neurodevelopmental delay[27]. Unsurprisingly, these are the physiological systems more commonly affected along with cardiovascular abnormalities both in patients and knockout mice, indicating that the unbiased nature of the IMPC pipeline is able to capture this wide range of co-occurrent phenotypic outcomes, even when specific cardiac abnormalities were not detected. It is important to note the variable spectrum of clinical features reported in patients that are summarised by one set of HPO associated terms. Given this and other factors involved, e.g. LoF vs non LoF variants or the complex nature of the genotype-phenotype relationship, it is not unexpected to find that only a subset of all the clinical manifestations observed in humans is mimicked in the mouse.

For the genes in the selected panels with IMPC data, only 3 are not included as ‘Green’ genes in any other panel (*ALPK3*, *COA5*, *NRAP*), *RYR2*, *TNNC1*, *CALM2*, *CASQ2*, *FHOD3*, *RBM20*, *TMEM43, DSG2*, *MYL2* are also included in the sudden cardiac death panel. The remaining 44 genes are considered as diagnostic genes in several other panels, predominantly neurology and neurodevelopmental disorders (23), Metabolic disorders (22), Dysmorphic and congenial abnormal syndromes (6) and Endocrine disorders (5). Similarly,

based on HPO annoations, only a small percentage of the studied genes (7%, 4/57) have associated abnormal phenotypes restricted to the cardiovasuclar system.

The IMPC resource and the data presented here are not without limitations. Due to its high-throughput nature, the current pipeline focuses on phenotyping a minimal number of animals required to detect strong phenotypic effects[28]. The phenotypic screens have been selected to facilitate modeling of human disease, but some key biological functions are not fully covered or studied in the heart (e.g. right ventricular cardiac diagnostics, blood pressure measurements), or in some cases only in center-specific pipelines or pilot studies, thus incomplete phenotype coverage across mouse lines for certain procedures is experienced. Further, only protein-coding LoF effects of single genes are characterised and importantly, the practical totality of IMPC lines are derived from one substrain genetic background, C57BL/6 and differences in the phenotypic outcomes of mutations from different genetic backgrounds could be observed given potential effects on penetrance and expresivity[29,30].

In summary, the analysis of the outcomes of a systematic phenotyping pipeline performed on knockout mice for a subset of genes involved in monogenic forms of cardiovascular disease uncovered an important finding of gene-essentiality. A significantly higher number of lethal lines compared to other disease categories was identified, reflecting how this set of genes have an indispensable role in organism development. Specific cardiovascular phenotypes were, however, not identified in the majority of heterozygous knockout or the homozygous knockout viable lines and different hypotheses were provided for this lack of recapitulation. The approach followed in the present study highlights the benefits of the multisystem nature of the phenotyping protocol, allowing to detect abnormalities in several other physiological systems important to mimicking the clinical manifestations observed in patients, and is thus providing a useful transformative resource to clinicians.

Here we focused on a set of diagnostic-grade genes, however for some of the genes, there is not evidence from unrelated families and/or enough functional evidence to validate the association with the observed phenotypes. The highly standardized high-throughput screening pipelines such as the IMPC contribute to generating a high volume of data and provide mouse models that can assist the prioritisation and validation of variants found in those genes. The identification of genes, mutations, and their mechanisms of action that cause and/or contribute to congenital heart disease in humans is far from complete and alternative approaches for novel disease-gene discovery can benefit from this genotype-phenotype knowledge base [7].

Methods

Monogenic cardiovascular disease gene selection

Genomics England PanelApp, a publicly available resource that contains expert curated gene panels for human Mendelian disorders, was used to identify genes of interest. Genes are categorised accoring to the level of evidence to support the gene-disease association. We retrieved ‘Green’ genes, i.e. genes with high confidence to being associated with disease, included in any of these three categories: Cardiomyopathy, Congenital heart disease, Cardiac arrhythmia. [https://panelapp.genomicsengland.co.uk/; Data accessed 23/11/2022][16]. A singe gene can belong to multiple gene panels.

Gene panel	Number of genes
Arrhythmogenic cardiomyopathy	10
Brugada syndrome	1
Cardiac arrhythmias	14
Cardiac arrhythmias - additional genes	1
Cardiomyopathies - including childhood onset	107
Catecholaminergic polymorphic VT	6
Dilated cardiomyopathy - adult and teen	32
Dilated Cardiomyopathy and conduction defects	35
Familial non syndromic congenital heart disease	25
Hypertrophic cardiomyopathy - teen and adult	22
Idiopathic ventricular fibrillation	1
Left Ventricular Noncompaction Cardiomyopathy	6
Long QT syndrome	9
Sudden death in young people	4
Total number of unique genes	153

Subsequent analysis was limited to the subset of genes with mouse orthologs that have undergone the IMPC phenotypic pipeline (n = 57).

An additional analysis was performed to include genes in the same aformentioned panels labelled as ‘Amber’ (moderate evidence for the gene-disease association, it should not be used for genome interpretation] and ‘Red’ (not enough evidence for the gene-disease association, it should not be used for genome interpretation). This added 90 ‘Amber’ and 104 ‘Red’ genes, 41 and 37 respectively with a one2one mouse orthologue that entered the IMPC phenotypic pipeline.

IMPC Mouse Phenotyping and Procedures

Data was obtained from knockout mice phenotyped under the viabiliy and adult and embryonic phenotype pipelines. All IMPC phenotyping data is publicly available through the IMPC portal. Detailed information on

the experimental procedures and standardised phenotyping protocols is available through the Mouse Phenotyping Resource of Standardised Screens (IMPReSS) (https://www.mousephenotype.org/impress).

Phenotyping procedures to capture the phenotypic manifestations potentially present in these disorders include: Primary viability, TTE (IMPC_ECH_001), ECG (IMPC_ECG_001, IMPC_ECG_002) to assess the functional and morphological abnormalities of the heart., Gross Pathology and Tissue Collection and Gross Morphology Embryo at different developmental stages (E9.5, E12.5, E14.5-E15.5). The complete list of viability outcomes and significant phenotypes corresponding to the IMPC Data Release 16.0 are publicly available to download and use (https://www.ebi.ac.uk/about/terms-of-use)[19,31]

. For each phenotyping screen, the IMPC data is processed by applying SoftWindowing[32] implemented in the SmoothWin R package accompanied by the OpenStats, an R package that provides a set of statistical methods to identify genotype-phenotype (MP term) associations by comparing knockout to wild type mice[33]. The genotype effect is assessed using Linear Mixed Models and under the setting of 0.0001 for the Type I error. All measurements from knckout mouse are accompanied by corresponding data from matched wildtype controls that have been assessed at the same time point and facility. All this data is further annotated with metadata. The SoftWindowing procedure metioned aboved assigns more weight to the set of control mice that were assessed proximally to the knockout mice. Batch effects are also considered in the modelling approach (https://www.mousephenotype.org/help/data-analysis/mutants-and-controls/ ; https://www.mousephenotype.org/help/data-analysis/statistical-analysis/).

The IMPC strategy for the upcoming years is to prioritise mouse orthologues of human genes that are not well understood, with very limited knowledge about their function, i.e. the dark genome[34] (https://www.mousephenotype.org/about-impc/)

Human phenotypes

Human phenotypes for the selected genes were retrieved using the HPO annotation files (https://hpo.jax.org/app/download/annotation)[17,35], and through OMIM (https://www.omim.org/) curations of the associated clinical records for evidence of early lethality[36].

PhenoDigm scores

The PhenoDigm algorithm[18] computes individual scores for each HPO–MP phenotypic match, based on the proximity of the two terms in the overall cross-species ontology (Jaccard index; simJ) and the observed frequency of the phenotype in common from the entire set of disease and mouse annotations (Information Content; IC). The geometric mean of the IC and simJ was used to generate the HPO–MP pairwise score. The overall score, which is a percentage-based score, is the result of comparing the best and mean scores for all the pairwise HPO–MP comparisons relative to the maximum possible scores for a mouse model

perfectly mimicking the disease phenotypes. A PhenoDigm percentage score greater than 0 implies at least one HPO–MP match.

Variant functional annotations

Molecular consequences for the pathogenic variants reported for each gene were obtained from ClinVar (https://www.ncbi.nlm.nih.gov/clinvar). Only pathogenic variants according to clinical significance were considered for the analysis. Molecular consequence categories were classified as LoF (frameshift, nonsense, splice site) and non LoF (missense, ncRNA, near gene, UTR). Each gene was labeled as LoF if at least one LoF pathogenic variant was reported or nonLoF if only nonLoF pathogenic variants were reported[17]

Bootstrap approach

For the bootstrap approach we retrieved the raw p-values for each gene-parameter pair from the ECG and TTE procedures (https://www.mousephenotype.org/understand/accessing-the-data/)[37]. The empirical bootstrap aims to estimate parameters by iteratively sampling from the data[38]. We employed bootstrap to check whether the proportion of nominally significant p-values (<0.05) among the selected genes for the procedures of interest was higher than expected. To this end, The empirical bootstrap distribution of the proportion of pairs with nominally significant p-values was computed. A total of 10,000 iterations (equally sized without replacement, with a sample size equal to the number of genes with data for each procedure) are drawn from the pool of genes with raw p-values for the TTE and ECG procedures, and the proportion of genes in each iteration with at least one significant raw p-values is estimated. Based on the bootstrapped proportions, the empirical p-values are obtained by computing the number of iterations with a proportion of genes greater or equal to the observed proportion in the gene group of interest.

Lethal lines enrichment and comparison across viability categories

For each disease category, Odds Ratio (OR) were computed from a contingency table with the number of disease and non-disease associated genes for each of two viability categories (lethal/subviable and viable) for all those genes with IMPC data on viability according to IMPC DR16. ORs were calculated by unconditional maximum likelihood estimation and normal approximation (Wald) confidence intervals as implemented in the epitools R package[39]. Two-sided p-values for the test of independence were computed using Fisher's exact test. The p-values that are represented in the text and figures are adjusted using Benjamini-Hochberg (BH) correction method.

All the data analysis was performed using R[40].

Ethical approval

All the IMPC international member institutes that breed mice and collect phenotyping data are guided by their own and individual ethical review panels, licensing and accrediting bodies, reflecting the national and/or geo-political legislation under which they operate. Cardiovascular mouse phenotyping was carried out under the auspice of the following animal protocols: the German Mouse Clinic Helmholtz Zentrum München - German Animal Welfare Act; Medical Research Council Harwell - Animal Welfare and Ethical Review Body (AWERB), RIKEN Tsukuba Institute - The Animal Experiments Committee; The Centre for Phenogenomics - Animal Care Committee (ACC), Baylor College of Medicine - Institutional Animal Care and Usage Committee, the Jackson Laboratory - Institutional Animal Careand Use Committee (IACUC); the University of California Davis - Institutional Animal Care and Use Committee (IACUC).

Detailed information on mouse preoduction including breeding, housing and husbandy, animal care and monitoring as well as study design, experimental procedures and sample size can be found here: https://www.mousephenotype.org/about-impc/animal-welfare/ ; https://www.mousephenotype.org/about-impc/animal-welfare/arrive-guidelines/. Animal welfare was routinely assessed.

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Contributions

P.C. contributed to conceptualization, data analysis, data/results interpretation and presentation and writing the manuscript. H.H.M., V.G.-D., M.H.d.A., H.F. and D.S. contributed to reviewing and editing the manuscript. N.S. contributed to conceptualization, data/results interpretation and writing the manuscript. M.H.d.A., H.F. and D.S are PIs of the key programs who contributed to the management and execution of the work.

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Figure legends

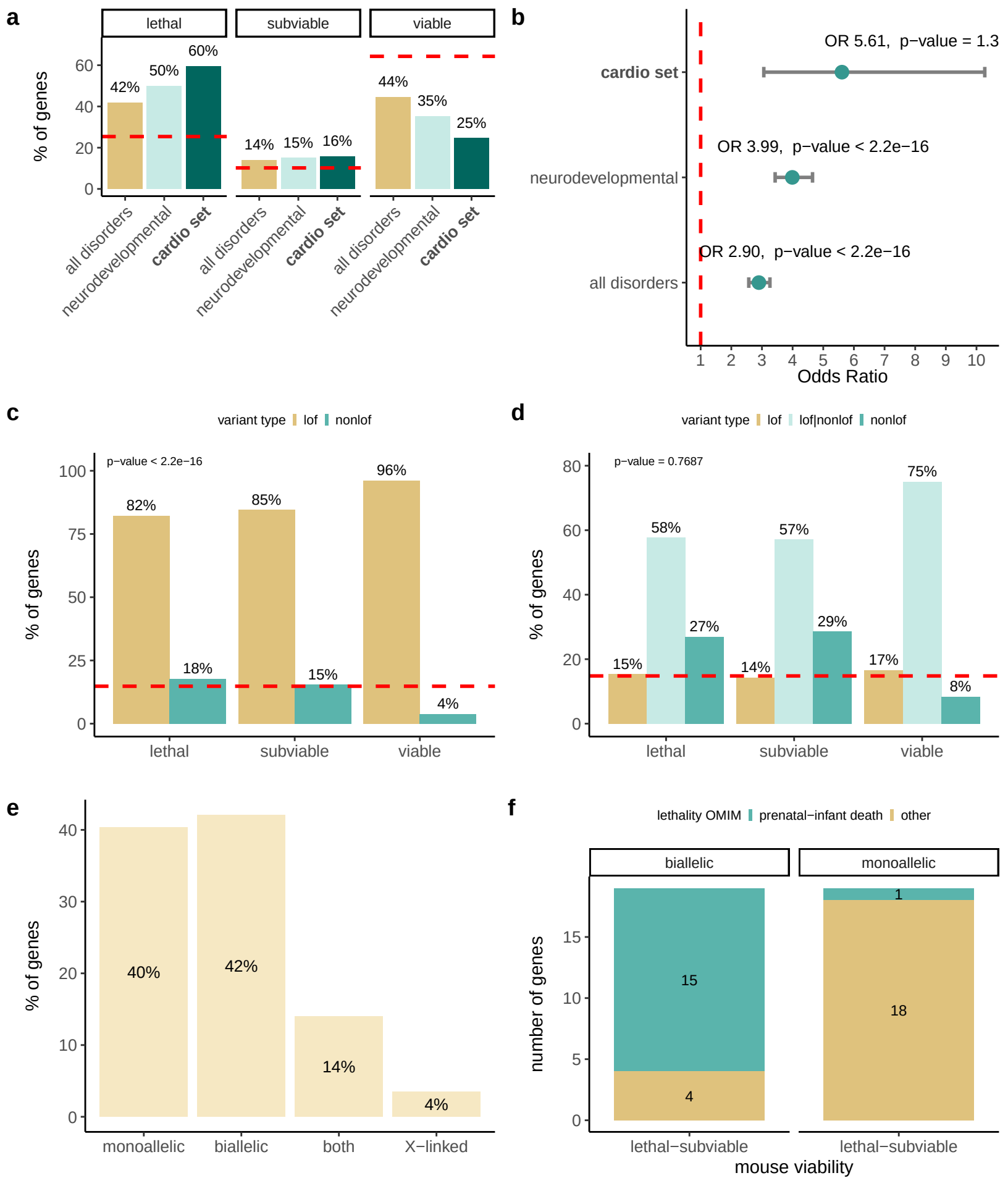
Fig 1 **Viability in the mouse orthologues of Mendelian cardiovascular disease genes**; **a)** Percentage of lethal, subviable and viable lines for different sets of disease associated genes. In the investigated cardiovascular set of genes, 60% of the lines were homozygous lethal or subviable with respect to 25% for the entire set of genes that have undergone viability assessment (dashed red line); **b)** The association between Mendelian disease genes and lethality in mice is statistically significant. Cardiovascular disease associated genes have 5.6-fold higher odds of being lethal in the mouse primary viability assessment; **c**) LoF variants are predominant in ClinVar, with the total number of pathogenic variants reported for the cardiovascular genes investigated being significantly higher among genes viable in the IMPC primary viability screen; **d**) 27% of the cardiovascular genes showing lethality in mice have non-LoF variants (mainly missense) reported as pathogenic in ClinVar. This percentage decreases to 8% for those showing viability in mice; **e**) Distribution of the set of genes investigated based on associated allelic requirement / mode of inheritance, showing a similar proportion of monoallelic and biallelic genes; **f**) Most of the cardiovascular biallelic genes that are lethal/subviable in the knockout mouse show records of prenatal to infant lethality in humans. This is not observed in the set of monoallelic genes. OR: Odds Ratio; LoF: loss-of-function.

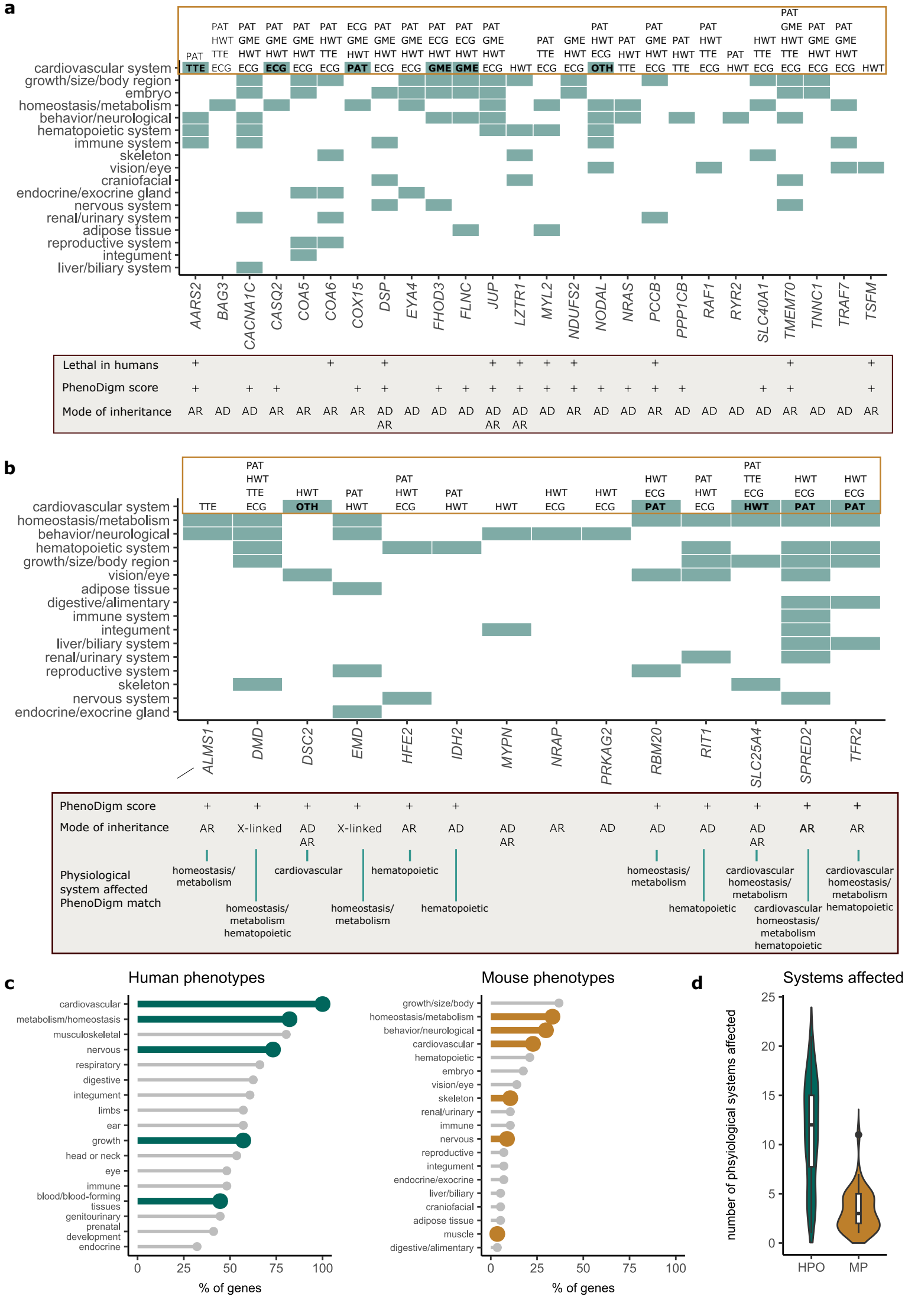
Fig 2 **Phenotypes observed in the mouse orthologues of Mendelian cardiovascular disease genes**; **a)** IMPC heterozygous knockout mouse phenotypes for lethal lines; **b**) IMPC homozygous knockout mouse

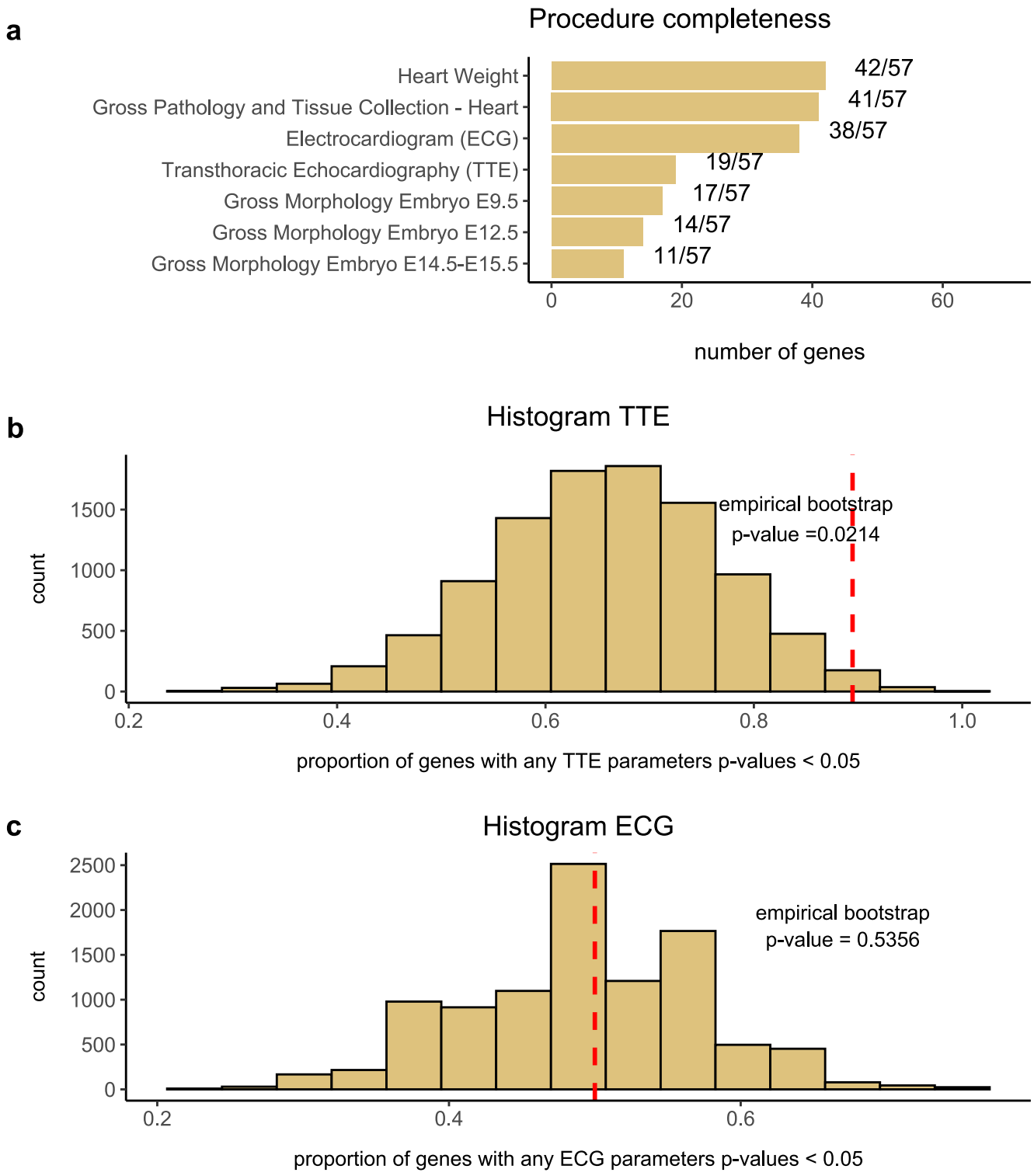
phenotypes for viable lines. For genes in figures **a**) and **b**), the physiological systems showing abnormal phenotypes are shown. For the cardiovascular system phenotypes, the phenotyping procedures that have been successfully performed are indicated at the top of the figure, those where a significant phenotype association was found are highlighted in bold. For the lethal lines, those genes for which early lethal phenotypes in humans have been reported are indicated, as well as those genes with positive PhenoDigm scores. For the viable lines, the physiological systems affected that are shared among patients and the knokout mice as captured by the PhenoDigm algorithm are highlighted. Additionally, two subviable genes, *ALPK3* and *DSG2* also showed cardiac abnormalities in the knockout mouse; **c**) Human and mouse physiological systems associated to the set of cardiovascular genes according to HPO and IMPC MP annotations. Cardiovascular phenotypes are reported for 100% of the genes, while only 23% show a cardiac abnormality in the mouse. **d**) The distribution of the number of physiological systems affected per gene illustrates the multisystemic nature of the associated disorders. TTE: transthoracic echocardiography; ECG: electrocardiography; GEM: gross embryo morphology; PAT: gross pathology and tissue collection; HWT: heart weight; Other : corneal vascularization; AD: autosomal dominant; AR: autosomal recessive.

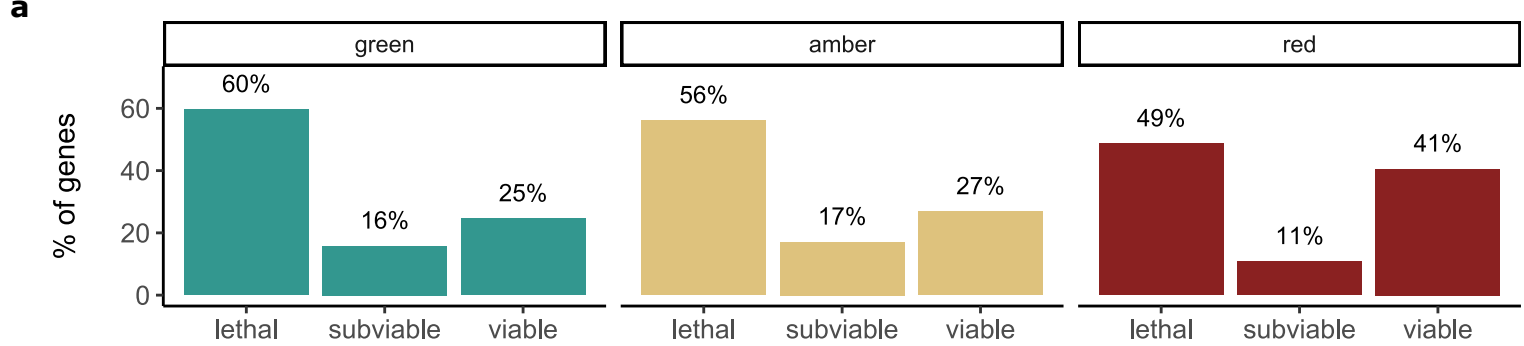
Fig 3 Phenotyping procedures aimed at capturing cardiac abnormalities a) Percentage of the IMPC phenotyping lines by procedure (or trait); **b)** Empirical bootstrap distribution of the proportion of genes with significant nominal p-values for any of the parameters evaluated through the TTE and ECG procedures.

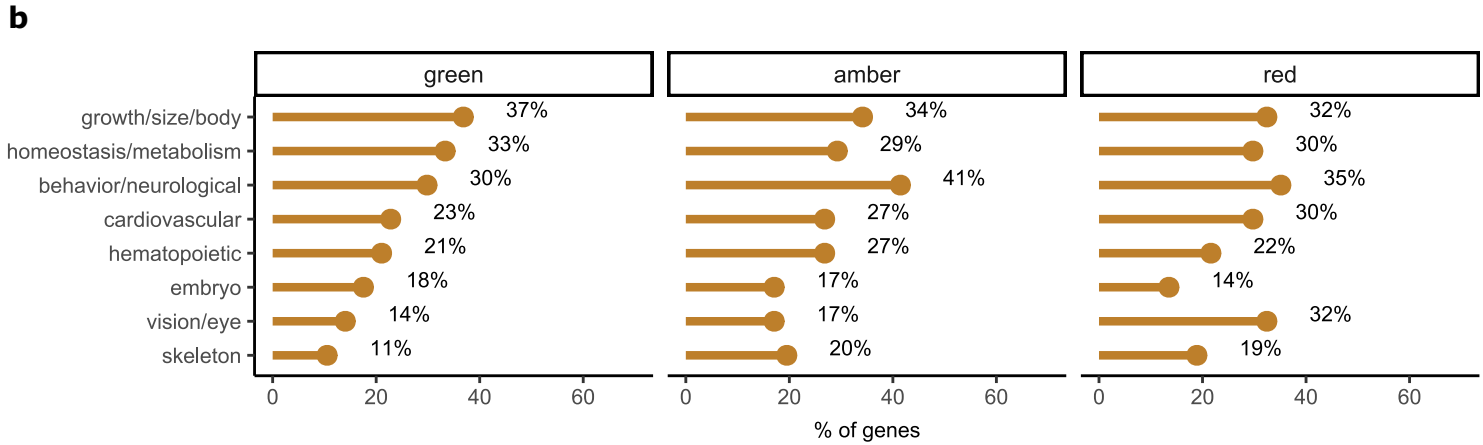
Fig 4 Additional panelApp genes based on the level of evidence for the gene-phenotype association a) Percentage of lethal, subviable and viable lines for different sets of cardiovascular genes based on the panelApp rating that is used for genome interpretation. **b)** Percentage of genes in each category showing abnormal phenotypes for the main phyisological systems affeccted; **c**) Most of the genes labelled as ‘green’ are also considered diagnositic grade geens in other gene panels (95%), compared to 62% of genes classified as ‘red’.


a
lethal
subviable
viable
% of genes
42%
50%
60%
14%
15%
16%
44%
35%
25%
all disorders
neurodevelopmental
cardio set
b
OR 5.61, p−value = 1.3
cardio set
OR 3.99, p−value < 2.2e−16
neurodevelopmental
OR 2.90, p−value < 2.2e−16
all disorders
Odds Ratio
c
variant type lof nonlof
p−value < 2.2e−16
% of genes
82%
18%
85%
15%
96%
4%
lethal
subviable
viable
d
variant type lof lof|nonlof nonlof
p−value = 0.7687
% of genes
15%
58%
27%
14%
57%
29%
17%
75%
8%
lethal
subviable
viable
e
% of genes
40%
42%
14%
4%
monoallelic
biallelic
both
X−linked
f
lethality OMIM prenatal−infant death other
biallelic
monoallelic
number of genes
15
4
1
18
lethal−subviable
lethal−subviable
mouse viability


a
cardiovascular system
growth/size/body region
embryo
homeostasis/metabolism
behavior/neurological
hematopoietic system
immune system
skeleton
vision/eye
craniofacial
endocrine/exocrine gland
nervous system
renal/urinary system
adipose tissue
reproductive system
integument
liver/biliary system
AARS2 BAG3 CACNA1C CASQ2 COA5 COA6 COX15 DSP EYA4 FHOD3 FLNC JUP LZTR1 MYL2 NDUFS2 NODAL NRAS PCCB PPP1CB RAF1 RYR2 SLC40A1 TMEM70 TNNC1 TRAF7 TSFM
Lethal in humans
PhenoDigm score
Mode of inheritance
b
cardiovascular system
homeostasis/metabolism
behavior/neurological
hematopoietic system
growth/size/body region
vision/eye
adipose tissue
digestive/alimentary
immune system
integument
liver/biliary system
renal/urinary system
reproductive system
skeleton
nervous system
endocrine/exocrine gland
ALMS1 DMD DSC2 EMD HFE2 IDH2 MYPN NRAP PRKAG2 RBM20 RIT1 SLC25A4 SPRED2 TFR2
PhenoDigm score
Mode of inheritance
Physiological system affected PhenoDigm match
homeostasis/ metabolism
homeostasis/ metabolism hematopoietic
cardiovascular
homeostasis/ metabolism
hematopoietic
hematopoietic
homeostasis/ metabolism
hematopoietic
cardiovascular homeostasis/ metabolism
cardiovascular homeostasis/ metabolism hematopoietic
cardiovascular homeostasis/ metabolism hematopoietic
c
Human phenotypes
cardiovascular
metabolism/homeostasis
musculoskeletal
nervous
respiratory
digestive
integument
limbs
ear
growth
head or neck
eye
immune
blood/blood-forming tissues
genitourinary
prenatal development
endocrine
0 25 50 75 100
% of genes
Mouse phenotypes
growth/size/body
homeostasis/metabolism
behavior/neurological
cardiovascular
hematopoietic
embryo
vision/eye
skeleton
renal/urinary
immune
nervous
reproductive
integument
endocrine/exocrine
liver/biliary
craniofacial
adipose tissue
muscle
digestive/alimentary
0 25 50 75 100
% of genes
d
Systems affected
number of phsyiological systems affected
0 5 10 15 20 25
HPO
MP


a
Procedure completeness
Heart Weight
Gross Pathology and Tissue Collection - Heart
Electrocardiogram (ECG)
Transthoracic Echocardiography (TTE)
Gross Morphology Embryo E9.5
Gross Morphology Embryo E12.5
Gross Morphology Embryo E14.5-E15.5
42/57
41/57
38/57
19/57
17/57
14/57
11/57
0
20
40
60
number of genes
b
Histogram TTE
count
1500
1000
500
0
empirical bootstrap
p-value =0.0214
0.2
0.4
0.6
0.8
1.0
proportion of genes with any TTE parameters p-values < 0.05
c
Histogram ECG
count
2500
2000
1500
1000
500
0
empirical bootstrap
p-value = 0.5356
0.2
0.4
0.6
proportion of genes with any ECG parameters p-values < 0.05





c

- 13/57 show cardiovascular abnormalities in the mouse
- 3/57 are not diagnostic grade genes 'green' in any other gene panel: *ALPK3*, *COA5A*, *NRAP*

- 11/41 show cardiovascular abnormalities in the mouse
- 7/41 are not diagnostic grade genes 'green' in any other gene panel: *ANKRD1*, *CACNA2D1*, *CACNB2*, *FLII*, *GATA5*, *RBHDF1*, *TECRL*

- 11/37 show cardiovascular abnormalities in the mouse
- 14/37 are not diagnostic grade genes 'green' in any other gene panel: *AKAP9*, *ATP5E*, *GPD1L*, *ILK*, *KIF20A*, *LAMA4*, *MYOM2*, *NEBL*, *NOS1AP*, *SCN2B*, *SNC3B*, *SCN4B*, *SEMA3D*, *SLMAP*