Article:
Bowley, G., Kugler, E. orcid.org/0000-0003-2536-6140, Wilkinson, R. et al. (6 more authors) (2022) Zebrafish as a tractable model of human cardiovascular disease. British Journal of Pharmacology, 179 (5). pp. 900-917. ISSN 0007-1188

https://doi.org/10.1111/bph.15473

Received: 15 December 2020 | Revised: 18 March 2021 | Accepted: 24 March 2021

DOI: 10.1111/bph.15473

REVIEW ARTICLE

Zebrafish as a tractable model of human cardiovascular disease

George Bowley[1,2] | Elizabeth Kugler[1,2,3] | Rob Wilkinson[4] | Allan Lawrie[1] | Freek van Eeden[2,5] | Tim J. A. Chico[1,2] | Paul C. Evans[1,2] | Emily S. Noël[2,5] | Jovana Serbanovic-Canic[1,2]

[1]Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, UK

[2]Bateson Centre, University of Sheffield, Sheffield, UK

[3]Institute of Ophthalmology, Faculty of Brain Sciences, University College London, London, UK

[4]School of Life Sciences, University of Nottingham, Nottingham, UK

[5]Department of Biomedical Science, University of Sheffield, Sheffield, UK

Correspondence
Paul C. Evans and Jovana Serbanovic-Canic, Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, UK.
Email: paul.evans@sheffield.ac.uk; j.serbanovic-canic@sheffield.ac.uk

Emily S. Noël, Bateson Centre, University of Sheffield, Sheffield, UK.
Email: e.s.noel@sheffield.ac.uk

Funding information
Medical Research Council; Biotechnology and Biological Sciences Research Council; National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs); British Heart Foundation

Mammalian models including non-human primates, pigs and rodents have been used extensively to study the mechanisms of cardiovascular disease. However, there is an increasing desire for alternative model systems that provide excellent scientific value while replacing or reducing the use of mammals. Here, we review the use of zebrafish, *Danio rerio*, to study cardiovascular development and disease. The anatomy and physiology of zebrafish and mammalian cardiovascular systems are compared, and we describe the use of zebrafish models in studying the mechanisms of cardiac (e.g. congenital heart defects, cardiomyopathy, conduction disorders and regeneration) and vascular (endothelial dysfunction and atherosclerosis, lipid metabolism, vascular ageing, neurovascular physiology and stroke) pathologies. We also review the use of zebrafish for studying pharmacological responses to cardiovascular drugs and describe several features of zebrafish that make them a compelling model for in vivo screening of compounds for the treatment cardiovascular disease.

KEYWORDS
cardiovascular, disease model, endothelial, vascular, zebrafish

1 | INTRODUCTION

The purpose of this review is to provide an overview on the use of zebrafish models of cardiovascular physiology and disease. We compare the anatomy and physiology of zebrafish and mammalian systems and briefly review the advantages of zebrafish models in terms of gene modification and unique imaging approaches. The manuscript then summarizes zebrafish models of cardiac pathology with emphasis on congenital heart defects, cardiomyopathy, conduction disorders and regeneration. We then summarize zebrafish models of vascular pathology with focus on endothelial dysfunction and atherosclerosis, lipid metabolism, vascular ageing, neurovascular

Abbreviations: AP, action potential; CFD, computational fluid dynamics; dpf, days post fertilization; dpi, days post injury; DPIV, digital particle image velocimetry; DTA, diphtheria toxin; ECs, endothelial cells; GWAS, genome-wide association study; hpf, hours post fertilization; hpi, hours post injury; MI, myocardial infarction; NVC, neurovascular coupling; WSS, wall shear stress.

George Bowley, Elizabeth Kugler Paul C. Evans, Emily S. Noël and Jovana Serbanovic-Canic have equal contributions.

physiology and stroke. It should be noted that we have not have not referenced every zebrafish study within these subject areas for the sake of brevity. We also highlight many examples where cardiovascular research has combined pharmacological and genetic approaches, summarize recent technological advances and how they can drive future research in cardiovascular disease.

2 | ZEBRAFISH AND MAMMALS: COMPARATIVE PHYSIOLOGY

2.1 | The heart

As they have a single circulatory system, zebrafish have a 2-chambered heart comprised of a single atrium and a single ventricle, connected to the circulatory system by the bulbus arteriosus which regulates BP to the gill vasculature. In addition to the lack of chamber septation and the presence of the teleost-specific bulbus arteriosus, adult zebrafish hearts are also hyper-trabeculated compared with endothermic vertebrate hearts, with comparatively little compact myocardium in the ventricular wall (Jensen et al., 2016). Despite the relative simplicity of the zebrafish heart when compared with mammals, most of the specialized cell types and structures (e.g. pacemaker, atrioventricular valve, aortic valve, trabeculae and coronary vasculature) and contributing cell types (myocardium, endocardium, epicardium, cardiac neural crest, second heart field and fibroblasts) are conserved. Similarly, the signalling pathways and morphogenetic processes driving early heart development are also well conserved (Staudt & Stainier, 2012), and zebrafish are now widely used as a model to investigate cardiac development and morphogenesis.

Zebrafish are increasingly employed to analyse disorders of cardiac function. Heart rate in zebrafish is more similar to human than those of other model organisms, and ECG analysis of adult zebrafish identifies clear P, QRS and T waves, with a QT duration that indicates a comparable repolarization time (Milan et al., 2006), demonstrating that electrophysiology of the adult zebrafish heart is highly similar to human (Lin et al., 2018; Liu et al., 2016; Nemtsas et al., 2010). Despite the small size of embryonic zebrafish hearts, techniques for measuring ECG profile in embryos have been developed (Dhillon et al., 2013), identifying similar ECG features from 3 days post fertilization (dpf) onwards as those observed in adult zebrafish. Because zebrafish are ectotherms, these electrophysiological dynamics are temperature dependent, and thus, it is important that comparative analyses of electrical activity in the zebrafish heart are temperature-controlled. The similarities in ECG activity between zebrafish and human hearts, in particular the distinct QT interval, are due to a highly comparable ventricular action potential (AP), with both zebrafish and human exhibiting a long plateau phase at positive voltage during repolarization. Many of the key ion channels that govern AP dynamics in human have orthologues in zebrafish, for example, the Na^+ channel encoded by ***SCN5A***, the inward rectifier $I_{K,ACh}$ K^+ channels encoded by ***KCNJ3*** and ***KCNJ5***, and the outward rectifying I_{Kr} K^+ channel encoded by ***KCNH2***. However, there are also key differences between human and zebrafish AP dynamics and channel composition or function, with atrial AP less similar between species, different composition of the inward rectifier I_{K1} channel, and absence of delayed rectifying current I_{Ks}-encoding genes (see Ravens, 2018). Although these similarities make the zebrafish a highly suitable model to understand human cardiac electrophysiology, the variation in ion channel composition and slight differences in AP dynamics should be kept in mind during pharmacological analyses.

2.2 | Vascular system

The basic structure of vascular anatomy is highly conserved between zebrafish and other mammalian models, including humans (Isogai et al., 2001). The unique advantage of the zebrafish model is that due to the optical transparency of the embryos and larvae, the morphological and functional changes of blood vessels can be observed non-invasively in living animals. Developmental processes involved in vascular development, including vasculogenesis, angiogenesis and vascular remodelling, are comparable between zebrafish and mammals, requiring tight regulation of the same key molecular pathways (Gore et al., 2012). The main axial vessels, the dorsal aorta and posterior cardinal vein form by vasculogenesis starting from around 10 somite stage (14 h post fertilization [hpf]) from the progenitors in the lateral plate mesoderm. The intersomitic vessels of the trunk which are among the first angiogenic vessels to form in vertebrates, start sprouting from the dorsal aorta from around 22 hpf. Circulating blood cells can be observed in the main axial vessels shortly after the onset of heart contractions at 24 hpf. Even though the cardiovascular system is one of the first to form during development, the zebrafish embryo obtains oxygen by passive diffusion from water for the first several days. This feature enables studies of experimental manipulations and cardiovascular mutant phenotypes, which would be lethal in humans and other mammals (Stainier et al., 1996).

Important differences to be noted between fish and mammals include the relative sparsity of mural cells associated with the endothelium as well as low arterial BP (0.3–0.4 mmHg in larvae and 1.5–2.15 mmHg in adult zebrafish) (Michel, 2020). Nevertheless, there seems to be a conserved response of the vasculature to the classical vasodilators and vasoconstrictors, as well as to a number of cardiovascular drugs (Fritsche et al., 2000; Margiotta-Casaluci et al., 2019). Treatment of zebrafish larvae with the **NO** donor **sodium nitroprusside** resulted in a significant increase in both arterial and venous vessel diameters, whereas application of NOS inhibitor **N^G-nitro-L-arginine methyl ester (L-NAME)** led to a significant decrease in vessel diameters, which corresponds to responses in mammals. Similarly, in larvae pretreated with L-NAME to inhibit endogenously produced NO, addition of **adrenaline** resulted in vasoconstriction (Fritsche et al., 2000). A comparative analysis of the in vivo cardiovascular responses of zebrafish, rat, dog and human to three cardiovascular drugs, which modulate β-adrenergic and renin-angiotensin systems (**propranolol**, **losartan** and **captopril**) showed that

zebrafish and human responses were largely comparable in >80% of drug/endpoint combinations, demonstrating the translational power of zebrafish (Margiotta-Casaluci et al., 2019).

3 | ZEBRAFISH MODELS: UNIQUE GENETIC AND IMAGING SYSTEMS

The use of zebrafish as a model to study cardiovascular function or disease has accelerated in recent years, with a literature search retrieving in excess of 5000 publications since 2010. This rise in popularity can be attributed to several advantages the zebrafish has over mammalian systems, primarily, the potential for examination of the formation and function of the cardiovascular system, non-invasively, in a live organism. Its small size, optical translucency, high fertility, rapid development and affordability make the zebrafish an attractive model to study human disease. Moreover, analysis of early zebrafish embryos can in many instances replace studies of rodents and other mammals, thereby reducing the use of more sentient animals in research. Approximately 70% of human genes share an orthologue with zebrafish, and 82% of known human disease genes are also present in the zebrafish genome (Howe et al., 2013). This high degree of conservation coupled with its amenity for genetic manipulation has positioned the zebrafish at the forefront of biomedical research.

3.1 | The zebrafish genetic toolbox

One of the major advantages of using zebrafish in biomedical research is the ability to perform genetic modification with relative ease. This technology has facilitated temporal and spatial control of gene expression allowing both gain-of- and loss-of-function genetic studies. It has been applied extensively to create transgenic lines with fluorescently labelled cells allowing cell behaviour to be studied in unparalleled detail. A wide range of methods are available for disrupting gene function in zebrafish for reverse genetic studies. However, these methods have now largely been replaced by CRISPR-Cas9 mediated gene editing, which is cheaper and simpler to implement. In addition, gene expression can be transiently inhibited throughout the embryo at the message or protein level using morpholino oligonucleotides (MOs) or in specific tissues using CRISPR-Interference (CRISPRi) (Savage et al., 2019). Genetically, zebrafish have undergone a partial genome duplication, which can make studying the role of certain genes more challenging due to functional redundancy of paralogous genes. Alternatively, this redundancy can also be advantageous in some contexts because later functions of some genes that are embryonically lethal in mammals can be uncovered in zebrafish.

3.2 | The imaging toolbox

The zebrafish cardiovascular system can be visualized using a multitude of approaches, which range from traditional in situ hybridization in fixed tissues to real-time analysis of transgenic reporter lines, which express fluorescent proteins in cardiac and vascular-specific cell types. In recent years, fluorescence microscopy techniques have shifted towards higher resolution, increased tissue penetration depth and a reduction of image acquisition artefacts. Although confocal and AiryScan microscopy are often used to achieve increased in vivo resolution, light sheet fluorescence microscopy (LSFM) has become a crucial image acquisition method, allowing data acquisition at a greater anatomical depth and imaging duration from hours-to-days (Huisken et al., 2004). Of particular relevance, visualization of cardiac dynamics has received increasing attention, allowing for 3D (+time) data acquisition and analysis, using gating approaches in imaging and post-processing (Mickoleit et al., 2014). Additionally, the ability to control cardiac function optogenetically (Arrenberg et al., 2010) and analyse endothelial cell calcium dynamics (Savage et al., 2019) has opened up new avenues for cardiovascular studies.

4 | CARDIAC DISEASE MODELS

Zebrafish have been used to model many aspects of cardiac development, function and disease. Here, we highlight specific examples where zebrafish have made substantial contributions to our understanding of cardiac defects, along with the genetic and experimental techniques used to generate and interrogate these models.

Congenital heart defects (CHD) are the most common birth defect, affecting approximately 1% of live births worldwide and comprise a spectrum of structural malformations, including septal defects, inflow and outflow tract malformation, chamber hypoplasia and valve dysgenesis. Despite the comparative simplicity of the zebrafish two-chambered heart when compared with the four-chambered heart of mammals with dual circulatory systems, the molecular regulation and morphogenetic processes underlying zebrafish heart development are highly conserved. Here, we highlight specific examples where zebrafish have provided novel insights into CHD aetiology.

Heterotaxia syndrome is caused by perturbations in left–right patterning of the body during embryogenesis, resulting in loss of concordance of organ lateralization and disruption to internal asymmetries within organ systems. The heart is a highly asymmetric organ and correct asymmetric morphogenesis is vital for correct cardiac function. Thus, individuals with heterotaxia often present with CHD. Key players in the pathways regulating early establishment of embryonic asymmetry are well established, with zebrafish mutants exhibiting heterotaxia phenotypes similar to those present in patients with mutations in the homologous genes (Figure 1). Recent studies identified novel variants in candidate genes potentially causative for heterotaxia (Liang et al., 1866; Liu et al., 2018), with functional validation through CRISPR-Cas9-mediated embryonic mutagenesis confirming *dnah10*, *rnf115*, *flna*, *kif7* and *kmt2d* to regulate cardiac asymmetry (Liang et al., 1866; Liu et al., 2018). Zebrafish mutant models have also shed light on the mechanisms underlying CHDs in individuals carrying mutations in *CFAP53*, encoding a ciliary protein

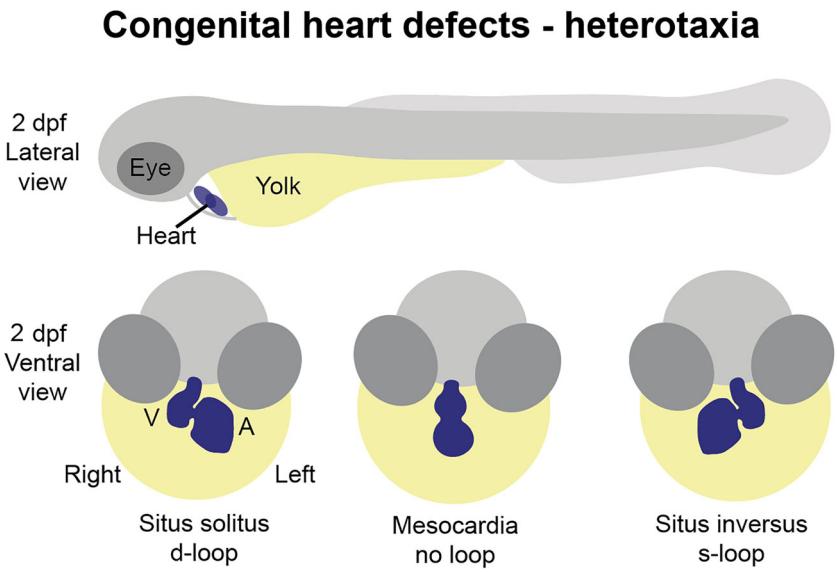

FIGURE 1 Zebrafish models of congenital heart defects. Congenital heart defects in zebrafish are often analysed at 2 dpf, when the heart has undergone initial looping morphogenesis and is positioned over the yolk, ventrally and posterior to the head. Morphology of the atrium (A) and ventricle (V) can be distinguished, and the atrioventricular canal and outflow tract can be visualized. Heterotaxia phenotypes can be assessed by the directionality of looping morphology of the heart, which usually undergoes a d-loop under normal left–right patterning (situs solitus) but can be reversed (s-loop) or remain at the midline (no-loop) if embryonic laterality is disrupted

(Liang et al., 1866; Liu et al., 2018; Noel et al., 2016). The genetics underlying CHD phenotypes are complex, and this highlights the value of zebrafish in dissecting the pathways and interactions that underlie cardiac morphogenesis.

Zebrafish represent an excellent model to investigate the effects of environmental factors on cardiac development. PM2.5 is fine particulate matter, high levels of which are found in urban areas with poor air quality or high levels of pollution. PM2.5 has been associated with increased incidence of CHD and exposing zebrafish embryos to PM2.5 resulted in developmental heart defects, including heart malformations and bradycardia (Duan et al., 2017; Jiang et al., 2019; Ren et al., 2020; Yue et al., 2017; Zhang et al., 2016). This model has been exploited to identify compounds, which ameliorate the impact of PM2.5 on the heart, such as **folic acid**. Protective effects of folic acid on developmental heart defects have also been described in a zebrafish model of fetal alcohol spectrum disorder (Sarmah & Marrs, 2013), which demonstrated the negative impact of continuous alcohol exposure during early development on cardiac morphology and growth. Zebrafish have also been used to model the effects of diabetes/hyperglycaemia during pregnancy on cardiac development (Liang et al., 2010). Together, zebrafish can model the mechanisms underlying CHDs with either genetic or environmental origins.

Cardiomyopathy represents a spectrum of heart diseases encompassing structural changes of the myocardium, comprised of three main classes. Dilated cardiomyopathy (DCM) is a progressive disease characterized by an enlarged, often left, ventricle, thinning of the myocardial wall, and reduced cardiac output. Hypertrophic cardiomyopathy (HCM) is associated with a thickening of the ventricular wall. Arrhythmogenic cardiomyopathy (ACM) involves degeneration of cardiomyocytes, and gradual replacement with fibrofatty scar tissue, affecting heart function and leading to structural heart remodelling. Typically, cardiac function is progressively impaired, often leading to heart failure. Hallmarks of cardiomyopathy and heart failure are conserved in zebrafish, including the up-regulation of genes associated with cardiomyopathy and heart failure, such as ***nppa*/*nppb*** (Becker et al., 2012). Thus, studies in zebrafish have contributed significantly to our understanding of the mechanisms underlying cardiomyopathies through functional validation of novel cardiomyopathy candidate genes (Figure 2). For example, a 2015 study describing transcriptomic analysis of embryonic and adult zebrafish hearts identified zebrafish homologues for 49 out of 51 DCM-associated genes (Shih et al., 2015).

TITIN (*TTN*) encodes a giant sarcomeric protein containing binding sites for a number of proteins along its length, which associate with the Z-disc, I- and A-bands, and M-line regions of the sarcomere. Mutations in *TTN* result in both DCM and skeletal myopathy and may account for as many as 25% of DCM cases (Herman et al., 2012). However, the location of mutations within the *TTN* gene appears to influence disease severity, with mutations in the C-terminal more likely to be associated with profound DCM (Herman et al., 2012; Roberts et al., 2015). Targeted CRISPR-Cas9-mediated mutagenesis in zebrafish has shed light on why the position of truncating mutations within *TTN* may influence disease severity (Zou et al., 2015). In the latter study, the authors generated six different truncating mutations in the zebrafish *ttnb* gene (which most resembles human *TTN*), resulting in truncations either in the z-disc, proximal/mid I-band or distal A-band domains. Although all mutations exhibit reduced cardiac function, mutants with C-terminal truncations also displayed disrupted skeletal muscle sarcomeres and paralysis. An additional promoter was identified within an intron of *ttnb*, which produces a short *ttnb* transcript encoding only the C-terminal of the protein and which is expressed at higher levels in skeletal muscle than cardiac muscle. Because this shorter C-terminal transcript is still expressed in embryos with N-terminal truncations of full-length *ttnb*, this could explain why N-terminal *ttnb* mutations predominantly result in cardiac defects, whereas C-terminal mutations, which affect both transcripts, result in cardiac and skeletal defects. This internal promoter is conserved in mouse, and moreover, the position of this putative promoter region lies at the border between alleles causing different phenotypic severity in the human *TTN* gene (Zou et al., 2015), providing insights into

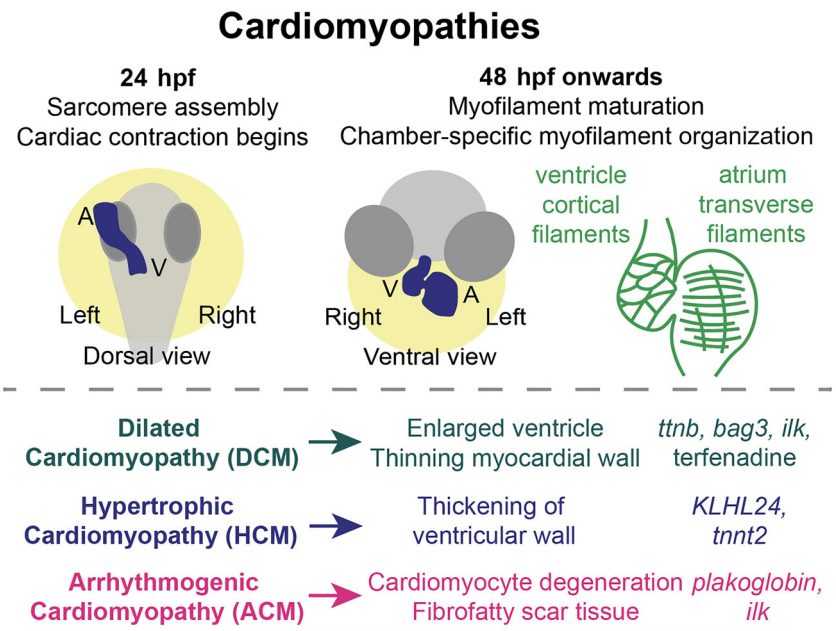

FIGURE 2 Zebrafish models of cardiomyopathy. Cardiac contractility in zebrafish begins around 24 hpf, when the heart tube has first formed and sarcomeres are assembled. As the heart develops the myofilaments mature, and by 48 hpf atrial and ventricular cardiomyocytes display different myofilament organisation. Ventricular wall cardiomyocytes displaying cortical basal actin while atrial cardiomyocytes forming long myofilaments spanning the cell running perpendicular to the direction of blood flow. Disruptions in sarcomere assembly and function result in diverse cardiomyopathies, represented by zebrafish mutants or mis-expression models, including those listed

why the position of genetic lesions in *TTN* may contribute to DCM severity.

BLC2-associated athanogene 3 (*BAG3*) encodes a heat shock protein co-chaperone implicated in DCM. Initially, morpholino-mediated knockdown of zebrafish *bag3* was performed after *BAG3* CNV identification in a DCM (Norton et al., 2011), resulting in pericardial oedema and reduced fractional shortening in embryos, confirming a requirement for Bag3 in heart function. However, generation of a stable zebrafish *bag3* mutant revealed that although dispensable for embryonic heart function, *bag3* is required in adulthood. This is exemplified with adult *bag3* zebrafish mutants exhibiting a decline in cardiac function and reduced exercise tolerance at 6 months (Ding et al., 2019), consistent with DCM onset age in patients with pathogenic *BAG3* variants. Moreover, functional analyses of single myofibrils, isolated from dissected *bag3* mutant zebrafish hearts, revealed defects suggestive of hypocontractility, a phenotype associated with DCM rather than HCM, allowing classification of the cardiomyopathy in *bag3* mutants similar to that employed in patients. Comparative analysis of combinatorial *bag3*/*mTor* zebrafish mutants revealed that reduction of **mTor** signalling can partially rescue the hallmarks of DCM observed in *bag3* mutants (Ding et al., 2019). This suggests that mTor represents a candidate therapeutic target for *bag3*-associated DCM and that the *bag3* mutant may represent a suitable model to screen novel compounds to alleviate DCM associated with *BAG3* mutations.

The laminin/integrin/integrin linked kinase (**ILK**) axis links the extracellular matrix to the cytoskeleton. The identification of a zebrafish *ilk* mutant, exhibiting hallmarks of DCM, provided the first evidence that disruptions to laminin/integrin signalling may result in cardiomyopathy (Knoll et al., 2007). Targeted sequencing of *LAMA4* and *ILK* in DCM cohorts subsequently revealed a missense mutation in *ILK* and 10 variants in *LAMA4* that were linked with DCM, representing the first identification of laminin/integrin mutations associated with DCM (Knoll et al., 2007).

Heterozygous mutations in *ILK* have also recently been linked with ACM (Brodehl et al., 2019). In this context, generation of transgenic zebrafish expressing either wild type or disease variant human ILK-GFP fusions demonstrated that these ILK variants cause fractional shortening at 3 dpf, and specific variants result in death at juvenile stages (Brodehl et al., 2019). Another zebrafish *ilk* mutant allele, *main squeeze* (*msq*), carries a mutation in the kinase domain of ILK, and although DCM has not been described in *msq* mutants, they do have defects in cardiac contractility (Bendig et al., 2006). **Protein kinase B (PKB)** phosphorylation is down-regulated in *msq* mutants. However, injection of constitutively active PKB can restore heart contractility, suggesting that PKB phosphorylation is required for heart development. A subsequent study took advantage of this by using the *msq* mutants as a tool to screen for compounds that could restore contractile function (Pott et al., 2018), identifying calyculin A and **okadaic acid** as agents that could restore ventricular fractional shortening in *msq* mutants, as well as partly restoring PKB phosphorylation.

The ability to perform targeted knockdown, create disease-specific mutations or overexpress disease variants has made zebrafish an invaluable model to validate novel candidate genes implicated in DCM, HCM and ACM. In addition to the better-characterized genes or pathways described above, functional studies in zebrafish have also identified novel roles for a diverse array of genes in cardiomyopathy, for example, *Raf1*, *Taf1a* and *Asn* (Dhandapany et al., 2014; Long et al., 2017; Verhagen et al., 2019). Zebrafish DCM models have also been generated through pharmacological induction by administering the K^+ channel blocker **terfenadine** to embryos (Gu et al., 2017), whereas a zebrafish model of ACM was developed using cardiomyocyte-driven expression of a plakoglobin disease variant, resulting in cardiomegaly and thinning of atrial and ventricular walls in early-adult fish (Asimaki et al., 2014). By combining this ACM model with an *nppa*:luciferase transgenic line (up-regulated in ACM zebrafish hearts), a chemical library was screened for compounds that would

modify the up-regulation of *nppa* in the ACM model, along with potential rescue of cardiac function. This approach identified that the compound **SB216763**, a GSK-3 inhibitor, could normalize defects in action potential observed in the ACM model (Asimaki et al., 2014), highlighting the tractability of the zebrafish model for performing functional screens for potential therapeutic agents which can alleviate DCM-type phenotypes.

Conduction disorders encompass a variety of pathologies, the precise nature of which depends on how generation or propagation of electrical impulses throughout the heart is disrupted. Despite the relative structural simplicity of the zebrafish heart compared with that of mammals, ECG analysis of adult zebrafish reveals that action potential dynamics of adult zebrafish cardiomyocytes is highly similar to human (Figure 3) (Lin et al., 2018; Liu et al., 2016; Nemtsas et al., 2010). Recent improvements to ECG in zebrafish include refinements in temperature considerations and the anaesthesia protocol to minimize impacts of environmental factors on action potential analysis (Lin et al., 2018; Yan et al., 2020), optimization of probe positioning and opening of the pericardial sac to improve signal-to-noise ratio (Yan et al., 2020). These efforts to standardize measurements, and establish baseline variation in ECG parameters, allow more detailed analysis of how arrhythmia develops in the aging animal, in genetic models of arrhythmia, or upon inhibition of the parasympathetic nervous system (Yan et al., 2020). Cardiac contractility in embryos and adults can be captured and quantified via echocardiography (Denvir et al., 2008; Fang et al., 2020; Wang et al., 2017), whereas cardiac performance is also indirectly measured through exercise tolerance using swim tunnels, where adult zebrafish undergo timed swims against a current with increasing flow rate, and time to exhaustion is recorded.

In addition to the similarities between zebrafish and human cardiomyocyte action potentials, conduction dynamics in the zebrafish heart are also sensitive to channel blockers and activators used in humans, along with stimulants such as **isoprenaline** and **noradrenaline** (Denvir et al., 2008; Langheinrich et al., 2003; Lin et al., 2018; Mittelstadt et al., 2008; Nemtsas et al., 2010; Tsai et al., 2011; Yan et al., 2020). Many of these compounds have effects on heart function or conduction similar to those observed in humans. Administration of compounds that stimulate sympathetic regulation of cardiac function, such as noradrenaline or isoprenaline, causes tachycardia in zebrafish (Denvir et al., 2008; Lin et al., 2018; Tsai et al., 2011), whereas **atropine**, which inhibits parasympathetic input, also increases heart rate (Yan et al., 2020). Ion channel modulators have effects on cardiac conduction in zebrafish, similar to those seen in humans. ECG

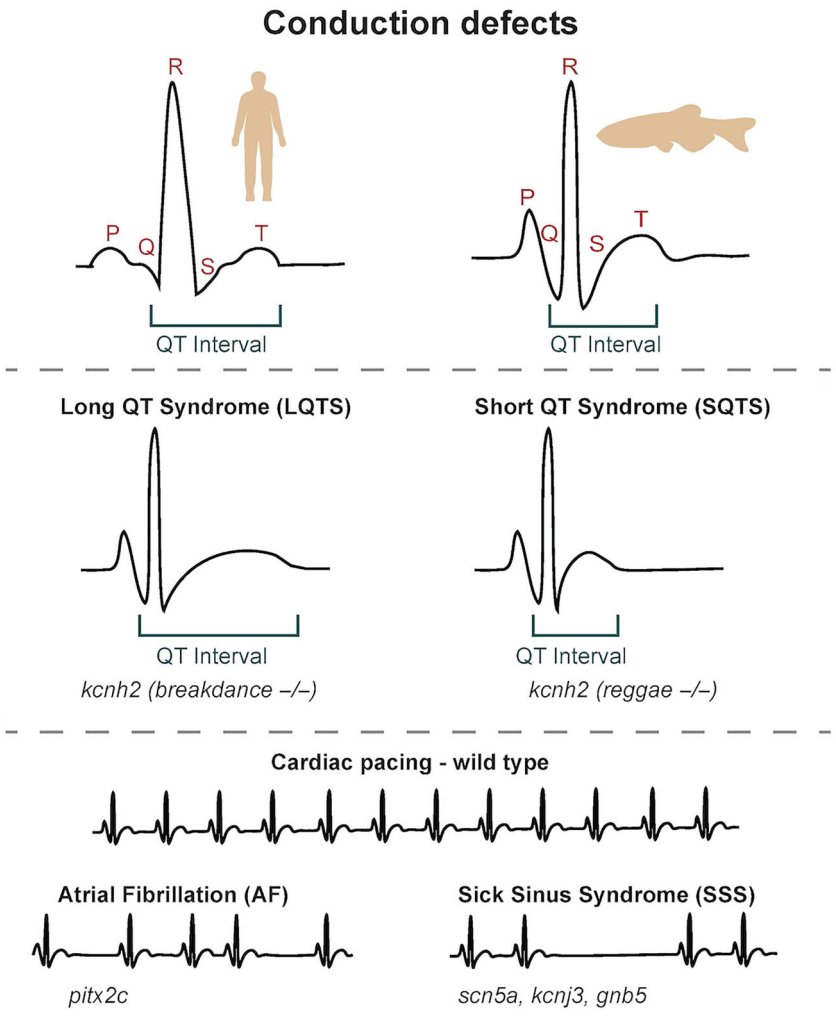

FIGURE 3 Zebrafish models of cardiac conduction defects. ECG recordings of embryonic and adult zebrafish hearts closely resemble those obtained from humans, with distinguishable P wave, QRS complex and T wave, allowing quantification of comparative parameters such as QT interval. Both Long and short QT syndrome (LQTS and SQTS) can result from mutations in the potassium channel *kcnh2*, and are modelled by the zebrafish *breakdance* and *reggae* mutants respectively. Cardiac pacing defects such as atrial fibrillation (AF, loss of regular sinus rhythm) and sick sinus syndrome (SSS, defective sinus pacing, including sinus pause) have also been modelled using zebrafish loss of function and mis-expression models

analysis of embryos exposed to compounds affecting potassium channels reveals a conserved atrial-specific response to **acetylcholine** and **carbachol** (Nemtsas et al., 2010), whereas administration of E4031, stemizole, terfenadine, **haloperidol**, **diphenhydramine** and **orphenadrine** (***hERG/KCNH2*** K^+ channel blockers) prolongs action potential duration (APD) and QT interval (Dhillon et al., 2013; Nemtsas et al., 2010; Tsai et al., 2011). Analysis of action potential dynamics reveals that the sodium channel blocker **tetrodotoxin** reduces AP upstroke (Nemtsas et al., 2010), whereas the L-type Ca channel blocker **nifedipine** shortened plateau phase, shaping AP duration (Nemtsas et al., 2010). Both nifedipine and the calcium channel antagonist **verapamil** induce bradycardia (Langheinrich et al., 2003; Lin et al., 2018), whereas **BayK8644** (an L-type calcium channel activator) prolongs QT interval (Langheinrich et al., 2003; Tsai et al., 2011). In some cases, these compounds also induce additional cardiac defects in a concentration-dependent manner, including arrythmias and AV block (Dhillon et al., 2013). Conserved effects of anti-arrhythmic drugs also been demonstrated in zebrafish, with both **amiodarone** and **quinidine** (which can result bradycardia and prolong QT interval) similarly prolonging QT interval in zebrafish (Lin et al., 2018; Tsai et al., 2011; Yu et al., 2010). However, not all compounds used in patients elicit the same response. **HMR1556** (an I_{KS} blocker) shortened APD instead of prolonging it (Nemtsas et al., 2010), whereas the QT-prolonging drugs sotalol, **erythromycin**, quinidine and **amitriptyline** did not have similar effect in zebrafish (Langheinrich et al., 2003). These discrepancies could result from some differences in ion channel composition or function or poor uptake of the compounds. The effects of these pharmacological compounds on heart function can reveal information about the molecular pathways underlying cardiac electrophysiology in health and disease. This is enhanced by additional imaging tools, such as genetically encoded calcium sensors driven by myocardial promoters, which enable live in vivo imaging of transient calcium dynamics in the embryonic and larval heart (Chi et al., 2008; Salgado-Almario et al., 2020; van Opbergen et al., 2018; Weber et al., 2017). Optical voltage mapping also provides high-resolution electrophysiological analysis of the heart. This can be achieved either through staining with a dye (Milan et al., 2009; Panakova et al., 2010), genetically encoded voltage sensors (van Opbergen et al., 2018), or a recently developed myocardial mitochondrial ATP sensor (Kioka et al., 2020).

Long QT syndrome (LQTS), atrial fibrillation (AF) and sick sinus syndrome (SSS) are conduction defects that were modelled in zebrafish. LQTS is characterized by prolonged myocardial repolarization time, diagnosed by increased QT interval duration. Mutations in the ion channel **K_v11.1**, encoded by ***KCNH2***, are causative for LQTS, and the zebrafish *breakdance* mutant, harbouring a *kcnh2* mutation, exhibits an atrioventricular (AV) block recapitulating that observed in paediatric LQTS (Langheinrich et al., 2003). The *breakdance* mutant was used as the background for a chemical screen for compounds, which could suppress the AV block (Peal et al., 2011). Administration of either 2-MMB or the steroid **flurandrenolide** shortened action potential duration (APD) and rescued AV block in the *breakdance* mutant, identifying two potential therapeutics which can shorten myocardial repolarization. Patch clamp analysis, following the application of the I_{Kr} potassium channel blocker terfenadine to explanted hearts, reveals similarly increased AP duration to *kcnh2* mutants, providing further functional evidence that I_{Kr} is critical for repolarization in the zebrafish ventricle (Arnaout et al., 2007).

Despite the fact that mutations in *KCNH2* are associated with LQTS, a heterozygous variant of *KCNH2* was associated with short QT syndrome (SQTS). Expression of this *KCNH2* variant in zebrafish revealed a gain-of-function phenotype (Brugada et al., 2004), providing explanation for the opposing effect of the mutation on QT interval when compared with conventional LQTS-associated *KCNH2* mutations. The zebrafish *reggae* mutant provides further evidence that specific *KCNH2* variants can have opposing effects on QT interval. *Reggae* mutants harbour a lesion in the voltage-sensing domain of *kcnh2* (Hassel et al., 2008). However, unlike the *breakdance* mutants, *reggae* mutants exhibit sinus block instead of AV block (Hassel et al., 2008; Langheinrich et al., 2003). Administration of terfenadine reduces potassium currents and rescues the phenotype of *reggae* mutants, suggesting the lesion results in gain-of-function. Supporting this, action potential duration is significantly reduced in *reggae* mutants, with a shorter QT interval (Hassel et al., 2008). Thus, *breakdance* and *reggae* mutants represent zebrafish models of LQTS and SQTS, with the *reggae* mutant representing the first described animal model for SQTS (Figure 3).

AF is characterized by irregular atrial pacing and often increased heart rate. Enhancer analyses in zebrafish identified putative *PITX2c* regulatory sequences which overlap genetic regions containing common AF-associated single nucleotide polymorphisms (SNPs) in humans suggesting dysregulation of PITX2 could be causative of AF(Ye et al., 2016). ECG analysis of adult *pitx2c* zebrafish mutants reveals impaired cardiac function, accompanied by enlarged atria and increased atrial fibrosis (Collins et al., 2019). This suggests *PITX2c* variants may be a causal factor in AF.

Sick Sinus Syndrome (SSS, also termed sinus node dysfunction) arises when the sinus node, the pacemaker of the heart, fails to generate a regular physiological heart rate resulting in arrhythmia. Mutations in *GNB5*, a guanine nucleotide-binding protein subunit involved in recruiting proteins to inward rectifier potassium channels, have been identified in individuals with early-onset sinus node dysfunction (Lodder et al., 2016). Analysis of the effect of carbachol and isoprenaline on a zebrafish *gnb5* mutant suggests that *GNB5* is required for parasympathetic control of heart rate, but not for sympathetic control. This indicates that loss of *GNB5* would be associated with extreme bradycardia at rest - a finding in line with abnormally low resting heart rates in patients with *GNB5* mutations (Lodder et al., 2016). Zebrafish studies validated gain–of-function activity of a *KCNJ3* variant identified in a family with autosomal dominant bradyarrhythmia (Yamada et al., 2019), and demonstrated that administration of the I_{KACh} channel blocker NIP-151 improves bradyarrhythmia phenotypes in the zebrafish model.

Functional measurements, conduction sensors and genetic mutants all provide suitable backgrounds that can be used as the foundation for pharmacological screens, identifying novel genes

implicated in cardiac conduction, or compounds which modify conduction dynamics. A screen of almost 300 insertional mutants was carried out to identify new genes and pathways that regulate cardiac conduction. Cross-matching the positive hits with LQTS-associated genome-wide association study (GWAS) data revealed a novel role for mitotic regulator *Gins3* in action potential dynamics (Milan et al., 2009). In another study, screening of synthetic compounds that restore heart function in *tremblor* mutants (a **sodium-calcium exchanger Ncx1** mutant with erratic heart contractility) revealed that treatment with **efsevin** restored steady calcium transients and cell coupling, rescuing heart function (Shimizu et al., 2015).

4.1 | Regeneration

The inability of human to replenish lost cardiomyocytes, following a myocardial infarction (MI) results in persistent scarring, impaired heart function, cardiac remodelling, and eventually heart failure. Although the adult mouse heart has limited regenerative potential, neonatal mouse hearts are able to mount a regenerative response (Porrello et al., 2011). However, this capacity is lost by 7 days post-partum, and long term follow-up of neonatal mice that have undergone ventricular resection suggests long term scarring as well as dilated cardiomyopathy (Andersen et al., 2016). Conversely, zebrafish are able to fully regenerate their hearts after injury, providing an invaluable model to understand the molecular mechanisms to improve regenerative potential in humans. Zebrafish models of heart regeneration have provided significant insights into the temporal processes underlying regeneration, along with the molecular mechanisms that underpin these responses. The early response from 3 h post injury (hpi) includes expression of proinflammatory molecules and recruitment of immune (Bevan et al., 2020; de Preux Charles et al., 2016; Huang et al., 2013; Simoes et al., 2020) cells, which are important for scar deposition and the subsequent regenerative response. Simultaneously, endocardial cells in the uninjured tissue undergo morphological changes and re-express developmental genes (Kikuchi et al., 2011). After inflammation and endocardial activation have occurred, the endocardium and epicardium are the first cell layers to undergo large-scale regeneration. Between 3 to 5 dpi, the endocardial cells around the injury site proliferate (Munch et al., 2017), before migrating to cover the internal face of the wound area. Onset of coronary revascularization is initiated rapidly post-injury, and the vascular network is required to promote regeneration (Marin-Juez et al., 2016, 2019). ECG analysis of regenerating hearts shows that despite prolongation of QT interval during regeneration, action potential dynamics return to normal, after regeneration (Chablais et al., 2011; Yu et al., 2010).

Several zebrafish cardiac injury models have been established (Figure 4), with ventricular amputation capturing the ability of the heart to undergo regeneration, forming a fibrin clot which is gradually replaced with muscle in 30–60 dpi (Poss et al., 2002; Raya et al., 2003). Myocardial infarction induces death of multiple cell types alongside inflammation and fibrosis. Thus, regeneration requires not

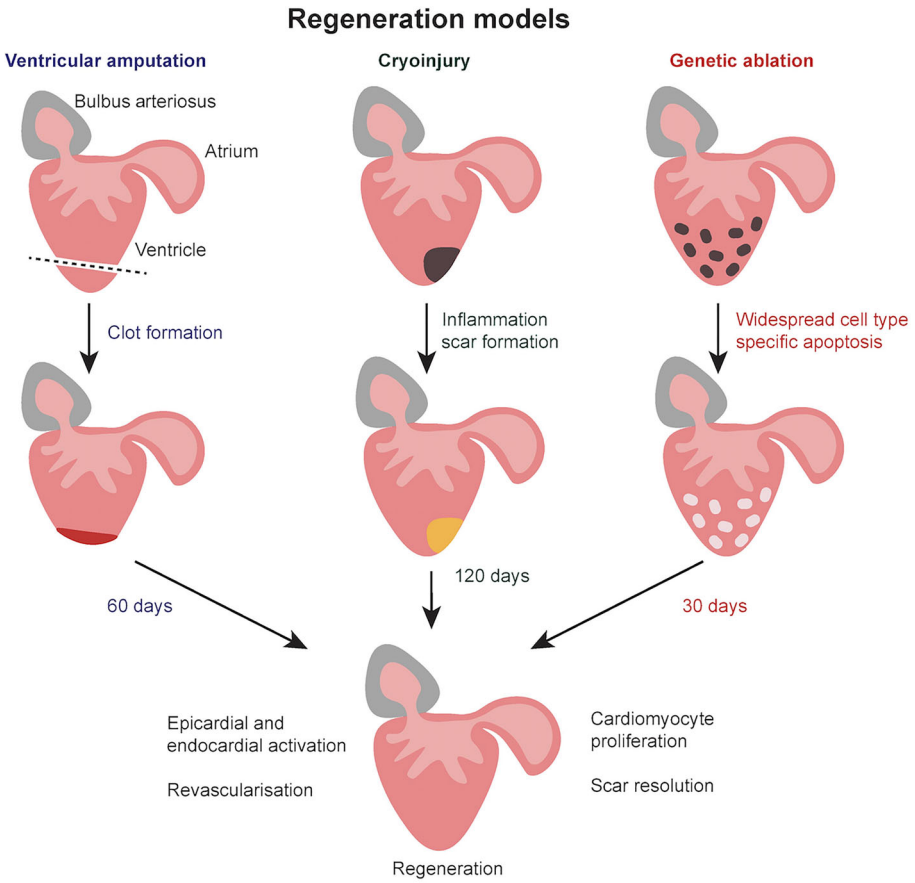

FIGURE 4 Zebrafish models of regeneration. The zebrafish heart is capable of fully regenerating after injury. In the resection model the ventricular apex is amputated resulting in formation of a fibrin clot, and new heart tissue grows within 60 days. In the cryoinjury model, a cryoprobe is applied to the ventricle causing localized cell death. Inflammation and clearance of cell debris subsequently occur, and a scar is formed at the injury site. This scar is resolved after around 120 days, and the heart is regenerated

only the replacement of lost cardiomyocytes, but also the removal of dead cells, matrix remodelling, revascularization, and re-establishment of electromechanical coupling in the heart (Frangogiannis, 2006). This can be modelled in zebrafish by cryoinjury which results in substantial localized cell death in the injured portion of the ventricle, resulting in apoptosis of all cardiac cell types and replicating the cardiac necrosis that occurs post-MI (Gonzalez-Rosa et al., 2011). Inducible genetic ablation models, where cell type-specific promoters drive expression of either diptheria toxin (DTA), or nitroreductase (an enzyme which converts **metronidazole** into a cytotoxic agent) provides further mechanistic insights into how different cell types contribute to cardiac regeneration (Curado et al., 2007; Kefalos et al., 2019; Sánchez-Iranzo et al., 2018; Wang et al., 2011; Zhang et al., 2013).

Interestingly, although the regenerative response to cryoinjury is robust, scar resorption diminishes with repeated injuries and after six cryoinjuries, hearts fail to resolve fibrotic tissue (Bise et al., 2020). Although this demonstrates that the heart can regenerate after multiple insults (the ability to regenerate cardiomyocytes themselves does not appear to be affected after multiple injuries), it suggests there is a limit to the ability to replace the fibrotic tissue with new cardiomyocytes. The multiple-injury model provides opportunities to separate the pro-regenerative programme from that of fibrosis and scar resolution, which may have implications for improving cardiac function in MI despite the presence of scarring to the heart.

The combination of zebrafish genetic and regeneration models can also provide insights into how cardiac dysfunction alters regenerative capacity, and how therapeutic agents may be developed to improve this capacity. Cardiac regeneration is impaired in the zebrafish *breakdance* mutant LQTS model, associated with increased extracellular matrix (ECM) deposition and excessive inflammation (Xu et al., 2019). Although administration of inflammatory compounds such as **dexamethasone** or MMP inhibitors promotes scar resolution and regeneration in this model (Xu et al., 2019), timing and evolution of the immune response during regeneration is likely to be crucial in mediating regeneration (Lai et al., 2019), and zebrafish represent an excellent model to directly investigate how the immune response could be manipulated to promote regeneration in humans. Similarly, targeting ECM-remodelling represents a promising therapeutic avenue. A 2016 study demonstrated that ECM from regenerating zebrafish hearts has pro-regenerative effects in mammalian non-regenerative models (Chen et al., 2016) suggesting that specific ECM composition may be key in promoting specific aspects of regeneration. In line with this, recent studies have reported that administration of the ECM component **agrin** to post-MI mouse and pig hearts improves cardiac regeneration (Baehr et al., 2020; Bassat et al., 2017), demonstrating that insights from zebrafish can lay the foundations for developing therapeutic strategies.

Although significant insights into cardiac regeneration have been made using zebrafish, one limitation lies in the ability to monitor morphological and functional recovery live, relying on fixed tissue analyses (although light-sheet imaging facilitates visualization of regeneration within whole-tissue context). Advances in MRI imaging of the regenerating heart provides new opportunities to assess regeneration in the same animal over time (Koth et al., 2017), whereas the development of a fluidic device to culture explanted injured hearts allows live imaging of processes such as revascularization, providing more detailed insights into specific cellular interactions during regeneration (Yip et al., 2020).

Overall, the ability to perform live, in vivo analyses of cardiac development, function, and regeneration in zebrafish provides a unique opportunity to define the relationships between morphological and functional abnormalities during development, and cardiac dysfunction and structural remodelling over the life course.

5 | VASCULAR DISEASE MODELS

5.1 | Endothelial dysfunction and atherosclerosis

Cardiovascular development is a complex and tightly regulated process, highly conserved among vertebrates. Many of the conserved developmental pathways regulating cardiovascular development, including **BMP**, **TGFβ**, **Notch** and **Wnt**, are re-activated in arteries in adults and play critical roles in the development of cardiovascular diseases, including atherosclerosis (Souilhol et al., 2020). Thus, using zebrafish embryos and larvae not only as a vertebrate developmental model, but as a model for processes involved in human diseases, may provide important insights into molecular mechanisms regulating vascular disease (Figure 5).

Atherosclerosis is a chronic inflammatory disease that can lead to myocardial infarction and stroke. Despite the systemic nature of many of the associated risk factors, atherosclerosis is a focal disease which develops in the regions of arterial trees where wall shear stress (WSS) magnitude is low and flow patterns are disturbed, this is particularly the case at vessel bends, branches and bifurcations. Disturbed flow and low WSS activate endothelial cells (ECs) leading to a pro-inflammatory, proliferative, and pro-apoptotic phenotype, contributing to endothelial injury and leading to development of atherosclerotic lesions. In contrast, laminar, unidirectional flow and physiologically high WSS confer a protective, quiescent phenotype to ECs. Transparency of the zebrafish embryos combined with availability of transgenic lines allow imaging and measurement of blood flow parameters and haemodynamic forces in the developing zebrafish vasculature using digital particle image velocimetry (DPIV) and computational fluid dynamics (CFD). These studies indicate that WSS magnitudes in the zebrafish vasculature are comparable to those in humans, with Lee et al. reporting the mean WSS in the dorsal aorta between 4 and 8 dyne cm^{-2} and in the caudal vein between 1 and 2 dyne cm^{-2} at 15 dpf (Lee et al., 2015). In contrast, WSS magnitudes in mice are approximately 10 times higher than in humans, primarily due to differences in size and heart rate. (Suo et al., 2007)

Although zebrafish have been used to study the roles of flow and WSS in vascular development and remodelling (Baeyens et al., 2015; Franco et al., 2015; Geudens et al., 2019; Goetz et al., 2014; Gray et al., 2013; Lenard et al., 2015; Weijts et al., 2018) endothelial

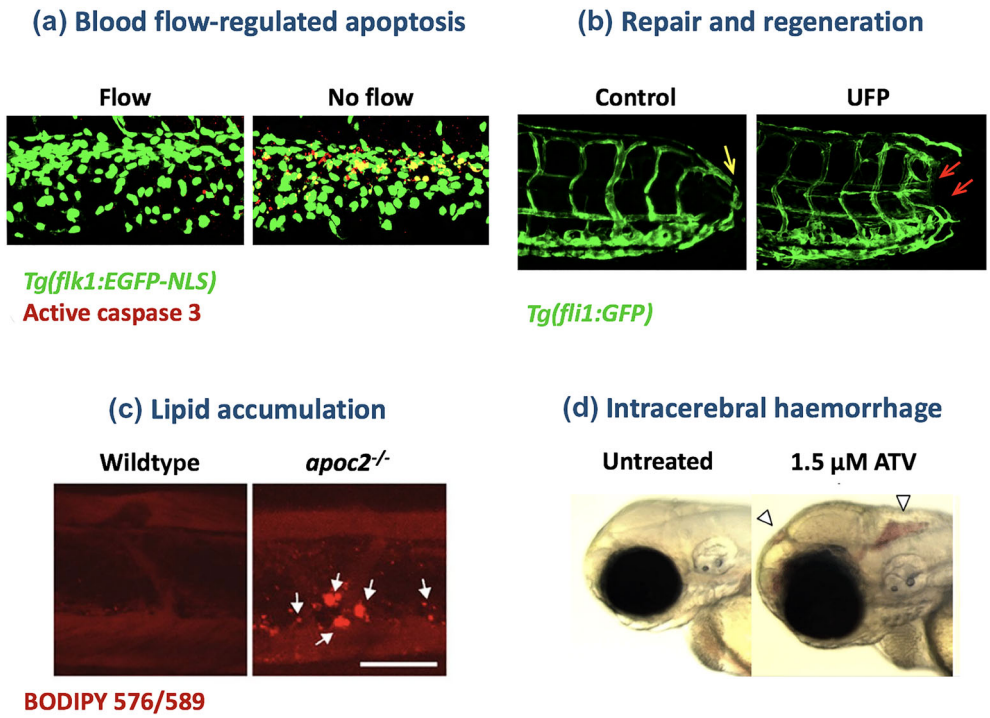

FIGURE 5 Zebrafish embryos and larvae can be used to study vascular responses to flow, high fat feeding and as models of intracerebral haemorrhage. (a) Blocking blood flow leads to increased endothelial cell apoptosis in the aorta and caudal vein plexus of zebrafish embryos at 30 h post fertilisation (hpf). Whole-mount active caspase-3 (red) staining of 30 hpf *flk1:EGFP-NLS* zebrafish embryos (green EC nuclei) in the presence or absence of flow (*sih* morpholino oligonucleotide). Apoptotic ECs are shown in yellow. (b) Vascular injury by tail amputation at 3 days post fertilization (dpf) results in shear stress-dependent vessel repair and regeneration by 3 days post amputation (dpa), which is impaired upon exposure to ultrafine particles (UFP). Control fish developed vascular regeneration connecting the dorsal aorta with the dorsal longitudinal anastomotic vessel at 3 dpa (yellow arrow). Fish exposed to UFP developed impaired vascular repair and a disrupted vascular network (red arrows) at 3 dpa. (c) Vascular lipid deposits in 14 dpf wildtype (WT) and *apoc2* mutant larvae (*apoc2*$^{-/-}$) fed a normal diet. (d) Exposure of zebrafish embryos to atorvastatin (ATV) results in intracranial haemorrhage. Bleeds are formed in both forebrain and mid-hindbrain regions (arrows denote haemorrhages). (b) Images kindly provided by Prof. Tzung Hsiai. Images adapted from Liu et al. (2015) (c) and Crilly et al. (2018) (d) under Creative Commons Licence

responses to flow, relevant for development of atherosclerosis, require further examination. In a recently established zebrafish model of flow-mediated EC apoptosis, preventing blood circulation using *tnnt2a* morpholino (*silent heart* embryos) or the anaesthetic tricaine, resulted in increased EC apoptosis in the vascular plexus of embryos (Serbanovic-Canic et al., 2017). The model was subsequently used for functional validation of a number of putative apoptotic regulators found to be enriched at a disease-prone site. It will be of interest to determine whether haemodynamic forces have an effect on other features of EC dysfunction in zebrafish, such as proliferation and inflammatory responses.

Reduction of viscosity-dependent shear stress in zebrafish embryos decreased induction of the glycolytic metabolite, dihydroxyacetone by flow-sensitive **VEGFR-PKCε-PFKFB3** signalling. This led to impaired vascular regeneration following zebrafish tail amputation, whereas injecting erythropoietin mRNA to increase shear stress promoted vascular repair (Baek et al., 2018). In conclusion, zebrafish embryos can model several features that are important in the pathogenesis of atherosclerosis including EC apoptosis, impaired vascular repair and regeneration.

5.2 | Lipid metabolism

In addition to haemodynamic factors, lipid metabolism plays an important role in atherosclerosis development. Accumulation of LDL, the main carrier of **cholesterol**, in the subendothelial layer and its subsequent oxidation into oxidized LDL triggers inflammatory and immune responses, which initiate plaque formation. Interestingly, feeding zebrafish a high cholesterol diet (HCD) can replicate some of the processes involved in the early stages of atherosclerosis, such as hypercholesterolaemia, lipoprotein oxidation, vascular lipid accumulation and myeloid cell recruitment to the vasculature (Stoletov et al., 2009). Feeding of HCD to zebrafish for up to 10 days starting from 5 dpf resulted in increased expression of inflammatory markers **TNF-α** and **IL-1β**, as well as decreased expression of the gene for the anti-inflammatory protein **PPARγ** in the endothelium, prior to myeloid cell accumulation and lipid deposition (Luo et al., 2019).

So far, two zebrafish genetic models of hyperlipidaemia have been reported: apolipoprotein C-II (*apoc2*) and LDL receptor (*ldlr*) mutants, which have been generated using TALEN- and CRISPR/Cas9-mediated genome editing, respectively (Liu et al., 2015, 2018).

In accordance with the phenotype observed in human patients with APOC2 deficiency, Apoc2 zebrafish mutants fed a normal diet displayed hypertriglyceridaemia, which was rescued by injection of plasma from wild-type zebrafish or by injection of a human APOC2 mimetic peptide. Apoc2-deficient zebrafish larvae exhibited accumulation of lipid and lipid-laden macrophages in the vasculature, in resemblance to early human and mouse atherosclerotic lesions (Liu et al., 2015). *dlr* mutants, on the other hand, developed moderate hypercholesterolaemia when fed a normal diet, which was exacerbated following a short-term, 5 day HCD feeding starting at 4.5 dpf (Liu, Kim, et al., 2018). Therefore, hyperlipidaemia can be modelled in zebrafish using either dietary and genetic approaches, and such models can be useful for screening of novel lipid-lowering compounds.

Vascular ageing is a significant and independent risk factor for a range of degenerative diseases, including cardiovascular diseases. Vascular ageing is characterized by increased endothelial dysfunction and medial wall calcification, contributing to arterial stiffness (North & Sinclair, 2012). Zebrafish are an attractive model for studying age-related diseases, due to their short life span, which is similar to that of mice, and conservation of mechanisms involved in DNA damage repair and regulation of telomere length. Interestingly, telomere shortening in vivo has been observed in the regions susceptible to atherosclerosis and telomere lengths are shorter in atherosclerotic plaques compared with healthy vessels (Salpea & Humphries, 2010), indicating a role for telomere homeostasis in the pathogenesis of atherosclerosis.

5.3 | Neurovascular physiology and stroke

Due to the combination of larval transparency, experimental accessibility of embryos and experimental tools available, zebrafish have become a crucial model to study neurovascular development. This is exemplified by the ability to study blood–brain-barrier (BBB) formation in vivo and over time (Eliceiri et al., 2011; Fleming et al., 2013; Quinonez-Silvero et al., 2020; van Leeuwen et al., 2018; Xie et al., 2010), showing that BBB formation and cerebrovascular angiogenesis occur in parallel (Umans et al., 2017) and the identification of functional and genetic regulators for BBB formation (Bostaille et al., 2016; O'Brown et al., 2019; Vanhollebeke et al., 2015). Recent work showed that a functional interaction between the brain vasculature and neurons (i.e. neurovascular coupling, NVC) develops in zebrafish between 6 and 8 dpf (Chhabria et al., 2020), establishing the first non-rodent model to study NVC. Similarly, careful observations revealed the existence of perivascular cells (Venero Galanternik et al., 2017), shed light on vascular mural cell coverage (Ando et al., 2016; Whitesell et al., 2019), established the dynamics of cerebrovascular spinal fluid movements (Olstad et al., 2019), and discovered a new endothelial cell membrane behaviour, termed *kugeln* (Kugler et al., 2019). Increasing understanding in vascular diseases and the ability to model those in zebrafish, such as stroke (Crilly et al., 2019) or thrombosis (Freire et al., 2020), sheds light on vascular bed-specific disease progression, such as vascular dementia. Additionally, we are beginning to understand how vascular regulation develops (Bahrami & Childs, 2020). This is complemented by studies examining the role of blood flow on cerebrovascular patterning and cell death, as well as novel computational analysis approaches, which bridge the gap between data acquisition and extraction of meaningful results for clinical translation using quantitative objective image analysis (Daetwyler et al., 2019).

Ischaemic stroke, which results from occlusion of cerebral blood vessels, accounts for ~85% of all acute strokes. On the other hand, haemorrhagic stroke, caused by intracerebral haemorrhage, is less common (~15%), but it is associated with higher morbidity and mortality rates. There have been a limited number of studies attempting to establish a zebrafish model for studying ischaemic stroke (Walcott & Peterson, 2014). In contrast, zebrafish have more extensively been used to study intracerebral haemorrhage. Thus, bleeding in the brain of zebrafish larvae induced quantifiable pathological and inflammatory phenotypes which mimicked key features of human intracerebral haemorrhage (Crilly et al., 2018). One of the advantages of zebrafish over mammalian models is the ability of zebrafish larvae to exhibit spontaneous brain-specific bleeding which can be observed noninvasively. Intracerebral haemorrhage can result from genetic mutations (*arhgef7*, *pak2a* and *notch3*) or can be induced pharmacologically (with **atorvastatin**) (Eisa-Beygi et al., 2013; Withers et al., 2020). However, there are limitations to using the zebrafish model which include the lack of fully developed cranium and rapid recovery rates from brain injury (Crilly et al., 2018).

6 | CARDIOVASCULAR DRUG DISCOVERY AND FUTURE DIRECTIONS

Despite considerable advances in therapeutic approaches, cardiovascular diseases are still the leading cause of death worldwide. The zebrafish model offers exciting possibilities for drug discovery by combining advantages of both in vitro systems (including cost, size, ease of use and amenability to automation) and mammalian systems, as a holistic and physiologically relevant model with high level of genetic and functional conservation between zebrafish and humans (Novodvorsky et al., 2013).

However, limitations of the zebrafish model must also be considered. Firstly, high throughput screening is limited to water-soluble compounds that can be added to the water in which the fish develop. Additionally, due to solubility issues, especially at higher compound concentrations, it may be challenging to predict the relationship between administered compound dose and actual exposure. Although methods for robotic injection into the zebrafish yolk are available and suitable for injection of DNA, microbes or cells (Spaink et al., 2013), microinjection into the blood circulation is less amenable for high throughput applications. Secondly, lack of standardized protocols, including zebrafish strains, husbandry practices and experimental design, can lead to experimental variability and lack of reproducibility (Hamm et al., 2019). Despite these limitations, there are currently eight compounds discovered in zebrafish that have advanced into

clinical trials which demonstrates the translational value of the zebrafish model (Cassar et al., 2020). The combination of genetic and pharmacological models mimicking human cardiac dysfunction (including advances in genome-editing which allow the creation of human disease-specific variants in zebrafish), high resolution live in vivo imaging, electrophysiological analysis of cardiac function, and high-throughput screening capacity, position the zebrafish as an excellent translational model. This is exemplified by screens identifying compounds which improve cardiac function in specific zebrafish cardiomyopathy models (Asimaki et al., 2014; Peal et al., 2011; Pott et al., 2018; Shimizu et al., 2015; Yamada et al., 2019), providing the foundation for further investigation.

Recent technological advancements allow for fully automated high throughput screening assays, with robotics-mediated handling steps and automated image acquisition and analysis. Burns et al established a 96-well-based assay for measuring heart rate using automated microscopy of *Tg* (*cmlc2:GFP*) embryos expressing GFP specifically in the myocardium (Burns et al., 2005). Some of the challenges, such as the requirement for anaesthesia or restraint, fluorescently labelled animals, as well as the level of throughput, have been addressed in subsequent studies (Gierten et al., 2020; Martin et al., 2019). A wide range of cardiovascular parameters, including heart rate, rhythm, contractility, vessel diameter and blood circulation, can now be assessed automatically or semi-automatically using 96-well or 384-well format (Kithcart & MacRae, 2017).

In addition to drug discovery, the zebrafish is also a valuable model for cardiotoxicity studies. Cardiotoxicity, manifested by conduction abnormalities, arrhythmias and depression of myocardial contractility is a serious side effect of many drugs which can limit their clinical application. One small molecule screen in zebrafish found that 22 out of 23 tested compounds which cause QT interval prolongation in humans, caused bradycardia and atrioventricular block in the zebrafish, demonstrating suitability of the zebrafish model (Milan et al., 2003). An example of a cardiotoxic agent is **doxorubicin**, a highly effective chemotherapy drug, the use of which is limited by cumulative, dose-related myocardial damage which can lead to heart failure. Using a zebrafish model of doxorubicin-induced cardiomyopathy for small-molecule screening, the cytochrome P450 family, **CYP1**, has been recently identified as a candidate therapeutic target for clinical cardioprotection (Asnani et al., 2018; Lam et al., 2020).

Zebrafish, first introduced as a developmental model in 1970s, have become an increasingly attractive and valuable model for studying human diseases. With its unique advantages, the zebrafish model has proven its utility in studying disease mechanisms, drug screenings and toxicology studies, complementing murine models and in vitro systems. With the emergence of large amounts of data generated by human–omics studies, the identification of new, robust and reliable zebrafish models for functional screening of genes with putative roles in cardiovascular health and disease is required. Zebrafish are emerging as a valuable model for functional validation of GWAS data from patients with diverse cardiovascular disease, providing additional disease-specific models which can inform therapeutic development (von der Heyde et al., 2020).

Despite its amenability to high throughput screening, automated imaging and phenotypic scoring still remain a challenge. Continued technological advances and application and development of automated zebrafish screening platforms will increase the clinical significance of the zebrafish model, leading to a better understanding of disease mechanisms and new therapeutic targets for human diseases, including cardiovascular disease.

6.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY (http://www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Cidlowski, et al., 2019; Alexander, Fabbro, et al., 2019a; 2019b; Alexander, Kelly, et al., 2019; Alexander, Mathie, et al., 2019).

ACKNOWLEDGEMENTS

The authors are funded by the British Heart Foundation, National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), Biotechnology and Biological Sciences Research Council and Medical Research Council.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article because no new data were created or analysed in this study.

ORCID

Elizabeth Kugler https://orcid.org/0000-0003-2536-6140

REFERENCES

Alexander, S. P. H., Cidlowski, J. A., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Nuclear hormone receptors. *British Journal of Pharmacology*, *176*, S229–S246. https://doi.org/10.1111/bph.14750

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019a). The Concise Guide to PHARMACOLOGY 2019/20: Catalytic receptors. *British Journal of Pharmacology*, *176*, S247–S296. https://doi.org/10.1111/bph.14751

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L. Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019b). The Concise Guide to PHARMACOLOGY 2019/20: Enzymes. *British Journal of Pharmacology*, *176*, S297–S396. https://doi.org/10.1111/bph.14752

Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F. Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, CL., Buneman, O. P., Cidlowski, J. A.,

Christopoulos, A., Davenport, A. P., Fabbro, D., Spedding, M., Striessnig, J., Davies, J. A., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Other Protein Targets. *British Journal of Pharmacology*, *176*, S1–S20. https://doi.org/10.1111/bph.14747

Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., Southan, C., Davies, J. A., & CGTP Collaborators. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Ion channels. *British Journal of Pharmacology*, *176*, S142–S228. https://doi.org/10.1111/bph.14749

Andersen, D. C., Jensen, C. H., Baun, C., Hvidsten, S., Zebrowski, D. C., Engel, F. B., & Sheikh, S. P. (2016). Persistent scarring and dilated cardiomyopathy suggest incomplete regeneration of the apex resected neonatal mouse myocardium--a 180 days follow up study. *Journal of Molecular and Cellular Cardiology*, *90*, 47–52. https://doi.org/10.1016/j.yjmcc.2015.11.031

Ando, K., Fukuhara, S., Izumi, N., Nakajima, H., Fukui, H., Kelsh, R. N., & Mochizuki, N. (2016). Clarification of mural cell coverage of vascular endothelial cells by live imaging of zebrafish. *Development*, *143*, 1328–1339. https://doi.org/10.1242/dev.132654

Arnaout, R., Ferrer, T., Huisken, J., Spitzer, K., Stainier, D. Y., Tristani-Firouzi, M., & Chi, N. C. (2007). Zebrafish model for human long QT syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 11316–11321. https://doi.org/10.1073/pnas.0702724104

Arrenberg, A. B., Stainier, D. Y., Baier, H., & Huisken, J. (2010). Optogenetic control of cardiac function. *Science*, *330*, 971–974. https://doi.org/10.1126/science.1195929

Asimaki, A., Kapoor, S., Plovie, E., Karin Arndt, A., Adams, E., Liu, Z., James, C. A., Judge, D. P., Calkins, H., Churko, J., Wu, J. C., MacRae, C. A., Kléber, A. G., & Saffitz, J. E. (2014). Identification of a new modulator of the intercalated disc in a zebrafish model of arrhythmogenic cardiomyopathy. *Science Translational Medicine*, *6*, 240ra74. https://doi.org/10.1016/j.tcm.2020.07.007

Asnani, A., Zheng, B., Liu, Y., Wang, Y., Chen, H. H., Vohra, A., Chi, A., Cornella-Taracido, I., Wang, H., Johns, D. G., Sosnovik, D. E., & Peterson, R. T. (2018). Highly potent visnagin derivatives inhibit Cyp1 and prevent doxorubicin cardiotoxicity. *JCI Insight*, *3*, e96753. https://doi.org/10.1172/jci.insight.96753

Baehr, A., Umansky, K. B., Bassat, E., Jurisch, V., Klett, K., Bozoglu, T., Hornaschewitz, N., Solyanik, O., Kain, D., Ferraro, B., Cohen-Rabi, R., Krane, M., Cyran, C., Soehnlein, O., Laugwitz, K. L., Hinkel, R., Kupatt, C., & Tzahor, E. (2020). Agrin promotes coordinated therapeutic processes leading to improved cardiac repair in pigs. *Circulation*, *142*, 868–881. https://doi.org/10.1161/CIRCULATIONAHA.119.045116

Baek, K. I., Li, R., Jen, N., Choi, H., Kaboodrangi, A., Ping, P., Liem, D., Beebe, T., & Hsiai, T. K. (2018). Flow-responsive vascular endothelial growth factor receptor-protein kinase C isoform epsilon signaling mediates glycolytic metabolites for vascular repair. *Antioxidants & Redox Signaling*, *28*, 31–43. https://doi.org/10.1089/ars.2017.7044

Baeyens, N., Nicoli, S., Coon, B. G., Ross, T. D., Van den Dries, K., Han, J., Lauridsen, H. M., Mejean, C. O., Eichmann, A., Thomas, J. L., Humphrey, J. D., & Schwartz, M. A. (2015). Vascular remodeling is governed by a VEGFR3-dependent fluid shear stress set point. *eLife*, *4*, e04645. https://doi.org/10.7554/eLife.04645

Bahrami, N., & Childs, S. J. (2020). Development of vascular regulation in the zebrafish embryo. *Development (Cambridge, England)*, *147*, dev183061.

Bassat, E., Mutlak, Y. E., Genzelinakh, A., Shadrin, I. Y., Baruch Umansky, K., Yifa, O., Kain, D., Rajchman, D., Leach, J., Riabov Bassat, D., Udi, Y., Sarig, R., Sagi, I., Martin, J. F., Bursac, N., Cohen, S., & Tzahor, E. (2017). The extracellular matrix protein agrin promotes heart regeneration in mice. *Nature*, *547*, 179–184. https://doi.org/10.1038/nature22978

Becker, J. R., Robinson, T. Y., Sachidanandan, C., Kelly, A. E., Coy, S., Peterson, R. T., & MacRae, C. A. (2012). In vivo natriuretic peptide reporter assay identifies chemical modifiers of hypertrophic cardiomyopathy signalling. *Cardiovascular Research*, *93*, 463–470. https://doi.org/10.1093/cvr/cvr350

Bendig, G., Grimmler, M., Huttner, I. G., Wessels, G., Dahme, T., Just, S., Trano, N., Katus, H. A., Fishman, M. C., & Rottbauer, W. (2006). Integrin-linked kinase, a novel component of the cardiac mechanical stretch sensor, controls contractility in the zebrafish heart. *Genes & Development*, *20*, 2361–2372. https://doi.org/10.1101/gad.1448306

Bevan, L., Lim, Z. W., Venkatesh, B., Riley, P. R., Martin, P., & Richardson, R. J. (2020). Specific macrophage populations promote both cardiac scar deposition and subsequent resolution in adult zebrafish. *Cardiovascular Research*, *116*, 1357–1371. https://doi.org/10.1093/cvr/cvz221

Bise, T., Sallin, P., Pfefferli, C., & Jazwinska, A. (2020). Multiple cryoinjuries modulate the efficiency of zebrafish heart regeneration. *Scientific Reports*, *10*, 11551.

Bostaille, N., Gauquier, A., Twyffels, L., & Vanhollebeke, B. (2016). Molecular insights into Adgra2/Gpr124 and Reck intracellular trafficking. *Biology Open*, *5*, 1874–1881. https://doi.org/10.1242/bio.021287

Brodehl, A., Rezazadeh, S., Williams, T., Munsie, N. M., Liedtke, D., Oh, T., Ferrier, R., Shen, Y., Jones, S. J. M., Stiegler, A. L., Boggon, T. J., Duff, H. J., Friedman, J. M., Gibson, W. T., Consortium, F. C., Childs, S. J., & Gerull, B. (2019). Mutations in ILK, encoding integrin-linked kinase, are associated with arrhythmogenic cardiomyopathy. *Translational Research*, *208*, 15–29. https://doi.org/10.1016/j.trsl.2019.02.004

Brugada, R., Hong, K., Dumaine, R., Cordeiro, J., Gaita, F., Borggrefe, M., Menendez, T. M., Brugada, J., Pollevick, G. D., Wolpert, C., Burashnikov, E., Matsuo, K., Wu, Y. S., Guerchicoff, A., Bianchi, F., Giustetto, C., Schimpf, R., Brugada, P., & Antzelevitch, C. (2004). Sudden death associated with short-QT syndrome linked to mutations in HERG. *Circulation*, *109*, 30–35. https://doi.org/10.1161/01.CIR.0000109482.92774.3A

Burns, C. G., Milan, D. J., Grande, E. J., Rottbauer, W., MacRae, C. A., & Fishman, M. C. (2005). High-throughput assay for small molecules that modulate zebrafish embryonic heart rate. *Nature Chemical Biology*, *1*, 263–264. https://doi.org/10.1038/nchembio732

Cassar, S., Adatto, I., Freeman, J. L., Gamse, J. T., Iturria, I., Lawrence, C., Muriana, A., Peterson, R. T., Van Cruchten, S., & Zon, L. I. (2020). Use of zebrafish in drug discovery toxicology. *Chemical Research in Toxicology*, *33*, 95–118. https://doi.org/10.1021/acs.chemrestox.9b00335

Chablais, F., Veit, J., Rainer, G., & Jazwinska, A. (2011). The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. *BMC Developmental Biology*, *11*, 21. https://doi.org/10.1186/1471-213X-11-21

Chen, W. C., Wang, Z., Missinato, M. A., Park, D. W., Long, D. W., Liu, H. J., Zeng, X., Yates, N. A., Kim, K., & Wang, Y. (2016). Decellularized zebrafish cardiac extracellular matrix induces mammalian heart regeneration. *Science Advances*, *2*, e1600844. https://doi.org/10.1126/sciadv.1600844

Chhabria, K., Plant, K., Bandmann, O., Wilkinson, R. N., Martin, C., Kugler, E., Armitage, P. A., Santoscoy, P. L., Cunliffe, V. T., Huisken, J., McGown, A., Ramesh, T., Chico, T. J., & Howarth, C. (2020). The effect of hyperglycemia on neurovascular coupling and cerebrovascular patterning in zebrafish. *Journal of Cerebral Blood Flow and Metabolism*, *40*, 298–313. https://doi.org/10.1177/0271678X18810615

Chi, N. C., Shaw, R. M., Jungblut, B., Huisken, J., Ferrer, T., Arnaout, R., Scott, I., Beis, D., Xiao, T., Baier, H., Jan, L. Y., Tristani-Firouzi, M., & Stainier, D. Y. (2008). Genetic and physiologic dissection of the vertebrate cardiac conduction system. *PLoS Biology*, *6*, e109. https://doi.org/10.1371/journal.pbio.0060109

Collins, M. M., Ahlberg, G., Hansen, C. V., Guenther, S., Marin-Juez, R., Sokol, A. M., El-Sammak, H., Piesker, J., Hellsten, Y., Olesen, M. S., Stainier, D. Y. R., & Lundegaard, P. R. (2019). Early sarcomere and metabolic defects in a zebrafish pitx2c cardiac arrhythmia model. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 24115–24121. https://doi.org/10.1073/pnas.1913905116

Crilly, S., Njegic, A., Laurie, S. E., Fotiou, E., Hudson, G., Barrington, J., Webb, K., Young, H. L., Badrock, A. P., Hurlstone, A., Rivers-Auty, J., Parry-Jones, A. R., Allan, S. M., & Kasher, P. R. (2018). Using zebrafish larval models to study brain injury, locomotor and neuroinflammatory outcomes following intracerebral haemorrhage. *F1000Res*, *7*, 1617. https://doi.org/10.3389/fimmu.2020.623119

Crilly, S., Njegic, A., Parry-Jones, A. R., Allan, S. M., & Kasher, P. R. (2019). Using zebrafish larvae to study the pathological consequences of hemorrhagic stroke. *Journal of Visualized Experiments: JoVE*, (148). https://doi.org/10.3791/59716

Curado, S., Anderson, R. M., Jungblut, B., Mumm, J., Schroeter, E., & Stainier, D. Y. (2007). Conditional targeted cell ablation in zebrafish: A new tool for regeneration studies. *Developmental Dynamics: An Official Publication of the American Association of the Anatomists*, *236*, 1025–1035. https://doi.org/10.1002/dvdy.21100

Daetwyler, S., Günther, U., Modes, C. D., Harrington, K., & Huisken, J. (2019). Multi-sample SPIM image acquisition, processing and analysis of vascular growth in zebrafish. *Development (Cambridge, England)*, *146*, bio056200. https://doi.org/10.1242/bio.056200

de Preux Charles, A. S., Bise, T., Baier, F., Marro, J., & Jazwinska, A. (2016). Distinct effects of inflammation on preconditioning and regeneration of the adult zebrafish heart. *Open Biology*, *6*, 160102. https://doi.org/10.1098/rsob.160102

Denvir, M. A., Tucker, C. S., & Mullins, J. J. (2008). Systolic and diastolic ventricular function in zebrafish embryos: Influence of norepenephrine, MS-222 and temperature. *BMC Biotechnology*, *8*, 21. https://doi.org/10.1186/1472-6750-8-21

Dhandapany, P. S., Razzaque, M. A., Muthusami, U., Kunnoth, S., Edwards, J. J., Mulero-Navarro, S., Riess, I., Pardo, S., Sheng, J., Rani, D. S., Rani, B., Govindaraj, P., Flex, E., Yokota, T., Furutani, M., Nishizawa, T., Nakanishi, T., Robbins, J., Limongelli, G., … Gelb, B. D. (2014). RAF1 mutations in childhood-onset dilated cardiomyopathy. *Nature Genetics*, *46*, 635–639. https://doi.org/10.1038/ng.2963

Dhillon, S. S., Dóró, E., Magyary, I., Egginton, S., Sík, A., & Müller, F. (2013). Optimisation of embryonic and larval ECG measurement in zebrafish for quantifying the effect of QT prolonging drugs. *PLoS One*, *8*, e60552. https://doi.org/10.1371/journal.pone.0060552

Ding, Y., Dvornikov, A. V., Ma, X., Zhang, H., Wang, Y., Lowerison, M., Packard, R. R., Wang, L., Chen, J., Zhang, Y., Hsiai, T., Lin, X., & Xu, X. (2019). Haploinsufficiency of mechanistic target of rapamycin ameliorates bag3 cardiomyopathy in adult zebrafish. *Disease Models & Mechanisms*, *12*, dmm040154.

Duan, J., Hu, H., Zhang, Y., Feng, L., Shi, Y., Miller, M. R., & Sun, Z. (2017). Multi-organ toxicity induced by fine particulate matter PM2.5 in zebrafish (*Danio rerio*) model. *Chemosphere*, *180*, 24–32. https://doi.org/10.18632/aging.202777

Eisa-Beygi, S., Hatch, G., Noble, S., Ekker, M., & Moon, T. W. (2013). The 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) pathway regulates developmental cerebral-vascular stability via prenylation-dependent signalling pathway. *Developmental Biology*, *373*, 258–266. https://doi.org/10.1016/j.ydbio.2012.11.024

Eliceiri, B. P., Gonzalez, A. M., & Baird, A. (2011). Zebrafish model of the blood-brain barrier: Morphological and permeability studies. *Methods in Molecular Biology*, *686*, 371–378. https://doi.org/10.1007/978-1-60761-938-3_18

Fang, Y., Sun, Y., Luo, C., Gu, J., Shi, Z., Lu, G., Silvestre, J. S., & Chen, Z. (2020). Evaluation of cardiac dysfunction in adult zebrafish using high frequency echocardiography. *Life Sciences*, *253*, 117732. https://doi.org/10.1016/j.lfs.2020.117732

Fleming, A., Diekmann, H., & Goldsmith, P. (2013). Functional characterisation of the maturation of the blood-brain barrier in larval zebrafish. *PLoS One*, *8*, e77548. https://doi.org/10.1371/journal.pone.0077548

Franco, C. A., Jones, M. L., Bernabeu, M. O., Geudens, I., Mathivet, T., Rosa, A., Lopes, F. M., Lima, A. P., Ragab, A., Collins, R. T., Phng, L. K., Coveney, P. V., & Gerhardt, H. (2015). Dynamic endothelial cell rearrangements drive developmental vessel regression. *PLoS Biology*, *13*, e1002125. https://doi.org/10.1371/journal.pbio.1002163

Frangogiannis, N. G. (2006). The mechanistic basis of infarct healing. *Antioxidants & Redox Signaling*, *8*, 1907–1939. https://doi.org/10.1089/ars.2006.8.1907

Freire, C., Fish, R. J., Vilar, R., Di Sanza, C., Grzegorski, S. J., Richter, C. E., Shavit, J. A., & Neerman-Arbez, M. (2020). A genetic modifier of venous thrombosis in zebrafish reveals a functional role for fibrinogen AαE in early hemostasis. *Blood Advances*, *4*, 5480–5491. https://doi.org/10.1182/bloodadvances.2020001472

Fritsche, R., Schwerte, T., & Pelster, B. (2000). Nitric oxide and vascular reactivity in developing zebrafish, Danio rerio. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *279*, R2200–R2207. https://doi.org/10.1152/ajpregu.2000.279.6.R2200

Geudens, I., Coxam, B., Alt, S., Gebala, V., Vion, A. C., Meier, K., Rosa, A., & Gerhardt, H. (2019). Artery-vein specification in the zebrafish trunk is pre-patterned by heterogeneous notch activity and balanced by flow-mediated fine-tuning. *Development*, *146*, dev-181024. https://doi.org/10.1242/dev.181024

Gierten, J., Pylatiuk, C., Hammouda, O. T., Schock, C., Stegmaier, J., Wittbrodt, J., Gehrig, J., & Loosli, F. (2020). Automated high-throughput heartbeat quantification in medaka and zebrafish embryos under physiological conditions. *Scientific Reports*, *10*, 2046.

Goetz, J. G., Steed, E., Ferreira, R. R., Roth, S., Ramspacher, C., Boselli, F., Charvin, G., Liebling, M., Wyart, C., Schwab, Y., & Vermot, J. (2014). Endothelial cilia mediate low flow sensing during zebrafish vascular development. *Cell Reports*, *6*, 799–808. https://doi.org/10.1016/j.celrep.2014.01.032

Gonzalez-Rosa, J. M., Martin, V., Peralta, M., Torres, M., & Mercader, N. (2011). Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish. *Development*, *138*, 1663–1674. https://doi.org/10.1242/dev.060897

Gore, A. V., Monzo, K., Cha, Y. R., Pan, W., & Weinstein, B. M. (2012). Vascular development in the zebrafish. *Cold Spring Harbor Perspectives in Medicine*, *2*, a006684. https://doi.org/10.1101/cshperspect.a006684

Gray, C., Bratt, D., Lees, J., daCosta, M., Plant, K., Watson, O. J., Solaymani-Kohal, S., Tazzyman, S., Serbanovic-Canic, J., Crossman, D. C., Keavney, B. D., Haase, A., McMahon, K., Gering, M., Roehl, H., Evans, P. C., & Chico, T. J. (2013). Loss of function of parathyroid hormone receptor 1 induces notch-dependent aortic defects during zebrafish vascular development. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *33*, 1257–1263. https://doi.org/10.3390/ijerph18052469

Gu, G., Na, Y., Chung, H., Seok, S. H., & Lee, H. Y. (2017). Zebrafish larvae model of dilated cardiomyopathy induced by Terfenadine. *Korean Circulation Journal*, *47*, 960–969. https://doi.org/10.4070/kcj.2017.0080

Hamm, J. T., Ceger, P., Allen, D., Stout, M., Maull, E. A., Baker, G., Zmarowski, A., Padilla, S., Perkins, E., Planchart, A., Stedman, D., Tal, T., Tanguay, R. L., Volz, D. C., Wilbanks, M. S., & Walker, N. J. (2019). Characterizing sources of variability in zebrafish embryo screening protocols. *ALTEX*, *36*, 103–120. https://doi.org/10.14573/altex.1804162

Hassel, D., Scholz, E. P., Trano, N., Friedrich, O., Just, S., Meder, B., Weiss, D. L., Zitron, E., Marquart, S., Vogel, B., Karle, C. A., Seemann, G., Fishman, M. C., Katus, H. A., & Rottbauer, W. (2008). Deficient zebrafish ether-a-go-go-related gene channel gating causes

short-QT syndrome in zebrafish reggae mutants. *Circulation*, *117*, 866–875. https://doi.org/10.1161/CIRCULATIONAHA.107.752220

Herman, D. S., Lam, L., Taylor, M. R., Wang, L., Teekakirikul, P., Christodoulou, D., Conner, L., DePalma, S. R., McDonough, B., Sparks, E., Teodorescu, D. L., Cirino, A. L., Banner, N. R., Pennell, D. J., Graw, S., Merlo, M., Di Lenarda, A., Sinagra, G., Bos, J. M., … Seidman, C. E. (2012). Truncations of titin causing dilated cardiomyopathy. *The New England Journal of Medicine*, *366*, 619–628. https://doi.org/10.1056/NEJMoa1110186

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., McLaren, S., Sealy, I., Caccamo, M., Churcher, C., Scott, C., Barrett, J. C., Koch, R., Rauch, G. J., White, S., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*, 498–503.

Huang, W. C., Yang, C. C., Chen, I. H., Liu, Y. M., Chang, S. J., & Chuang, Y. J. (2013). Treatment of glucocorticoids inhibited early immune responses and impaired cardiac repair in adult zebrafish. *PLoS One*, *8*, e66613. https://doi.org/10.1371/journal.pone.0083627

Huisken, J., Swoger, J., Del Bene, F., Wittbrodt, J., & Stelzer, E. H. (2004). Optical sectioning deep inside live embryos by selective plane illumination microscopy. *Science*, *305*, 1007–1009. https://doi.org/10.1126/science.1100035

Isogai, S., Horiguchi, M., & Weinstein, B. M. (2001). The vascular anatomy of the developing zebrafish: An atlas of embryonic and early larval development. *Developmental Biology*, *230*, 278–301. https://doi.org/10.1006/dbio.2000.9995

Jensen, B., Agger, P., de Boer, B. A., Oostra, R. J., Pedersen, M., van der Wal, A. C., Nils Planken, R., & Moorman, A. F. (2016). The hypertrabeculated (noncompacted) left ventricle is different from the ventricle of embryos and ectothermic vertebrates. *Biochimica et Biophysica Acta*, *1863*, 1696–1706. https://doi.org/10.1016/j.bbamcr.2015.10.018

Jiang, Y., Li, J., Ren, F., Ji, C., Aniagu, S., & Chen, T. (2019). PM2.5-induced extensive DNA methylation changes in the heart of zebrafish embryos and the protective effect of folic acid. *Environmental Pollution*, *255*, 113331. https://doi.org/10.1093/jac/dkab106

Kefalos, P., Agalou, A., Kawakami, K., & Beis, D. (2019). Reactivation of notch signaling is required for cardiac valve regeneration. *Scientific Reports*, *9*, 16059.

Kikuchi, K., Holdway, J. E., Major, R. J., Blum, N., Dahn, R. D., Begemann, G., & Poss, K. D. (2011). Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration. *Developmental Cell*, *20*, 397–404. https://doi.org/10.1016/j.devcel.2011.01.010

Kioka, H., Kato, H., Fujita, T., Asano, Y., Shintani, Y., Yamazaki, S., Tsukamoto, O., Imamura, H., Kogo, M., Kitakaze, M., Sakata, Y., & Takashima, S. (2020). In vivo real-time ATP imaging in zebrafish hearts reveals G0s2 induces ischemic tolerance. *The FASEB Journal*, *34*, 2041–2054. https://doi.org/10.1096/fj.201901686R

Kithcart, A., & MacRae, C. A. (2017). Using zebrafish for high-throughput screening of novel cardiovascular drugs. *JACC: Basic to Translational Science*, *2*, 1–12. https://doi.org/10.1016/j.jacbts.2017.01.004

Knoll, R., Postel, R., Wang, J., Kratzner, R., Hennecke, G., Vacaru, A. M., Vakeel, P., Schubert, C., Murthy, K., Rana, B. K., Kube, D., Knoll, G., Schafer, K., Hayashi, T., Holm, T., Kimura, A., Schork, N., Toliat, M. R., Nurnberg, P., … Bakkers, J. (2007). Laminin-alpha4 and integrin-linked kinase mutations cause human cardiomyopathy via simultaneous defects in cardiomyocytes and endothelial cells. *Circulation*, *116*, 515–525. https://doi.org/10.1161/CIRCULATIONAHA.107.689984

Koth, J., Maguire, M. L., McClymont, D., Diffley, L., Thornton, V. L., Beech, J., Patient, R. K., Riley, P. R., & Schneider, J. E. (2017). High-resolution magnetic resonance imaging of the regenerating adult zebrafish heart. *Scientific Reports*, *7*, 2917.

Kugler, E. C., van Lessen, M., Daetwyler, S., Chhabria, K., Savage, A. M., Silva, V., Plant, K., MacDonald, R. B., Huisken, J., Wilkinson, R. N., Schulte-Merker, S., Armitage, P., & Chico, T. J. (2019). Cerebrovascular endothelial cells form transient notch-dependent cystic structures in zebrafish. *EMBO Reports*, *20*, e47047. https://doi.org/10.15252/embr.201847047

Lai, S. L., Marín-Juez, R., & Stainier, D. Y. R. (2019). Immune responses in cardiac repair and regeneration: A comparative point of view. *Cellular and Molecular Life Sciences: CMLS*, *76*, 1365–1380. https://doi.org/10.1007/s00018-018-2995-5

Lam, P. Y., Kutchukian, P., Anand, R., Imbriglio, J., Andrews, C., Padilla, H., Vohra, A., Lane, S., Parker, D. L. Jr., Cornella Taracido, I., Johns, D. G., Beerens, M., MacRae, C. A., Caldwell, J. P., Sorota, S., Asnani, A., & Peterson, R. T. (2020). Cyp1 inhibition prevents doxorubicin-induced cardiomyopathy in a zebrafish heart-failure model. *Chembiochem*, *21*, 1905–1910. https://doi.org/10.1002/cbic.201900741

Langheinrich, U., Vacun, G., & Wagner, T. (2003). Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe arrhythmia. *Toxicology and Applied Pharmacology*, *193*, 370–382. https://doi.org/10.1016/j.taap.2003.07.012

Lee, S. J., Choi, W., Seo, E., & Yeom, E. (2015). Association of Early Atherosclerosis with Vascular Wall shear stress in Hypercholesterolemic zebrafish. *PLoS One*, *10*, e0142945. https://doi.org/10.1371/journal.pone.0142945

Lenard, A., Daetwyler, S., Betz, C., Ellertsdottir, E., Belting, H. G., Huisken, J., & Affolter, M. (2015). Endothelial cell self-fusion during vascular pruning. *PLoS Biology*, *13*, e1002126. https://doi.org/10.1371/journal.pbio.1002126

Liang, J., Gui, Y., Wang, W., Gao, S., Li, J., & Song, H. (2010). Elevated glucose induces congenital heart defects by altering the expression of tbx5, tbx20, and has2 in developing zebrafish embryos. *Birth Defects Research. Part a, Clinical and Molecular Teratology*, *88*, 480–486. https://doi.org/10.1002/bdra.20654

Liang, S., Shi, X., Yu, C., Shao, X., Zhou, H., Li, X., Chang, C., Lai, K. S., Ma, J., & Zhang, R. (1866). Identification of novel candidate genes in heterotaxy syndrome patients with congenital heart diseases by whole exome sequencing. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *2020*, 165906.

Lin, M. H., Chou, H. C., Chen, Y. F., Liu, W., Lee, C. C., Liu, L. Y., & Chuang, Y. J. (2018). Development of a rapid and economic in vivo electrocardiogram platform for cardiovascular drug assay and electrophysiology research in adult zebrafish. *Scientific Reports*, *8*, 15986. https://doi.org/10.1038/s41598-018-33577-7

Liu, C., Cao, R., Xu, Y., Li, T., Li, F., Chen, S., Xu, R., & Sun, K. (2018). Rare copy number variants analysis identifies novel candidate genes in heterotaxy syndrome patients with congenital heart defects. *Genome Medicine*, *10*, 40.

Liu, C., Gates, K. P., Fang, L., Amar, M. J., Schneider, D. A., Geng, H., Huang, W., Kim, J., Pattison, J., Zhang, J., Witztum, J. L., Remaley, A. T., Dong, P. D., & Miller, Y. I. (2015). Apoc2 loss-of-function zebrafish mutant as a genetic model of hyperlipidemia. *Disease Models & Mechanisms*, *8*, 989–998. https://doi.org/10.1242/dmm.019836

Liu, C., Kim, Y. S., Kim, J., Pattison, J., Kamaid, A., & Miller, Y. I. (2018). Modeling hypercholesterolemia and vascular lipid accumulation in LDL receptor mutant zebrafish. *Journal of Lipid Research*, *59*, 391–399. https://doi.org/10.1194/jlr.D081521

Liu, C. C., Li, L., Lam, Y. W., Siu, C. W., & Cheng, S. H. (2016). Improvement of surface ECG recording in adult zebrafish reveals that the value of this model exceeds our expectation. *Scientific Reports*, *6*, 25073.

Lodder, E. M., De Nittis, P., Koopman, C. D., Wiszniewski, W., Moura de Souza, C. F., Lahrouchi, N., Guex, N., Napolioni, V., Tessadori, F., Beekman, L., Nannenberg, E. A., Boualla, L., Blom, N. A., de Graaff, W., Kamermans, M., Cocciadiferro, D., Malerba, N., Mandriani, B., Coban

Akdemir, Z. H., … Merla, G. (2016). GNB5 mutations cause an autosomal-recessive multisystem syndrome with sinus bradycardia and cognitive disability. *American Journal of Human Genetics*, *99*, 786.

Long, P. A., Theis, J. L., Shih, Y. H., Maleszewski, J. J., Abell Aleff, P. C., Evans, J. M., Xu, X., & Olson, T. M. (2017). Recessive TAF1A mutations reveal ribosomopathy in siblings with end-stage pediatric dilated cardiomyopathy. *Human Molecular Genetics*, *26*, 2874–2881. https://doi.org/10.1093/hmg/ddx169

Luo, H., Li, Q. Q., Wu, N., Shen, Y. G., Liao, W. T., Yang, Y., Dong, E., Zhang, G. M., Liu, B. R., Yue, X. Z., Tang, X. Q., & Yang, H. S. (2019). Chronological in vivo imaging reveals endothelial inflammation prior to neutrophils accumulation and lipid deposition in HCD-fed zebrafish. *Atherosclerosis*, *290*, 125–135. https://doi.org/10.1016/j.atherosclerosis.2019.09.017

Margiotta-Casaluci, L., Owen, S. F., Rand-Weaver, M., & Winter, M. J. (2019). Testing the translational power of the zebrafish: An interspecies analysis of responses to cardiovascular drugs. *Frontiers in Pharmacology*, *10*, 893. https://doi.org/10.3389/fphar.2019.00893

Marin-Juez, R., El-Sammak, H., Helker, C. S. M., Kamezaki, A., Mullapuli, S. T., Bibli, S. I., Foglia, M. J., Fleming, I., Poss, K. D., & Stainier, D. Y. R. (2019). Coronary revascularization during heart regeneration is regulated by Epicardial and endocardial cues and forms a scaffold for Cardiomyocyte repopulation. *Developmental Cell*, *51*, 503–515e4. https://doi.org/10.1016/j.devcel.2019.10.019

Marin-Juez, R., Marass, M., Gauvrit, S., Rossi, A., Lai, S. L., Materna, S. C., Black, B. L., & Stainier, D. Y. (2016). Fast revascularization of the injured area is essential to support zebrafish heart regeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 11237–11242. https://doi.org/10.1073/pnas.1605431113

Martin, W. K., Tennant, A. H., Conolly, R. B., Prince, K., Stevens, J. S., DeMarini, D. M., Martin, B. L., Thompson, L. C., Gilmour, M. I., Cascio, W. E., Hays, M. D., Hazari, M. S., Padilla, S., & Farraj, A. K. (2019). High-throughput video processing of heart rate responses in multiple wild-type embryonic zebrafish per imaging field. *Scientific Reports*, *9*, 145.

Michel, J. B. (2020). Phylogenic determinants of cardiovascular frailty, focus on hemodynamics and arterial smooth muscle cells. *Physiological Reviews*, *100*, 1779–1837. https://doi.org/10.1152/physrev.00022.2019

Mickoleit, M., Schmid, B., Weber, M., Fahrbach, F. O., Hombach, S., Reischauer, S., & Huisken, J. (2014). High-resolution reconstruction of the beating zebrafish heart. *Nature Methods*, *11*, 919–922. https://doi.org/10.1038/nmeth.3037

Milan, D. J., Jones, I. L., Ellinor, P. T., & MacRae, C. A. (2006). In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation. *American Journal of Physiology. Heart and Circulatory Physiology*, *291*, H269–H273. https://doi.org/10.1152/ajpheart.00960.2005

Milan, D. J., Kim, A. M., Winterfield, J. R., Jones, I. L., Pfeufer, A., Sanna, S., Arking, D. E., Amsterdam, A. H., Sabeh, K. M., Mably, J. D., Rosenbaum, D. S., Peterson, R. T., Chakravarti, A., Kaab, S., Roden, D. M., & MacRae, C. A. (2009). Drug-sensitized zebrafish screen identifies multiple genes, including GINS3, as regulators of myocardial repolarization. *Circulation*, *120*, 553–559. https://doi.org/10.1161/CIRCULATIONAHA.108.821082

Milan, D. J., Peterson, T. A., Ruskin, J. N., Peterson, R. T., & MacRae, C. A. (2003). Drugs that induce repolarization abnormalities cause bradycardia in zebrafish. *Circulation*, *107*, 1355–1358. https://doi.org/10.1161/01.CIR.0000061912.88753.87

Mittelstadt, S. W., Hemenway, C. L., Craig, M. P., & Hove, J. R. (2008). Evaluation of zebrafish embryos as a model for assessing inhibition of hERG. *Journal of Pharmacological and Toxicological Methods*, *57*, 100–105. https://doi.org/10.1016/j.vascn.2007.10.004

Munch, J., Grivas, D., Gonzalez-Rajal, A., Torregrosa-Carrion, R., & de la Pompa, J. L. (2017). Notch signalling restricts inflammation and serpine1 expression in the dynamic endocardium of the regenerating zebrafish heart. *Development*, *144*, 1425–1440. https://doi.org/10.1242/dev.143362

Nemtsas, P., Wettwer, E., Christ, T., Weidinger, G., & Ravens, U. (2010). Adult zebrafish heart as a model for human heart? An electrophysiological study. *Journal of Molecular and Cellular Cardiology*, *48*, 161–171. https://doi.org/10.1016/j.yjmcc.2009.08.034

Noel, E. S., Momenah, T. S., Al-Dagriri, K., Al-Suwaid, A., Al-Shahrani, S., Jiang, H., Willekers, S., Oostveen, Y. Y., Chocron, S., Postma, A. V., Bhuiyan, Z. A., & Bakkers, J. (2016). A zebrafish loss-of-function model for human CFAP53 mutations reveals its specific role in laterality organ function. *Human Mutation*, *37*, 194–200. https://doi.org/10.1002/humu.22928

North, B. J., & Sinclair, D. A. (2012). The intersection between aging and cardiovascular disease. *Circulation Research*, *110*, 1097–1108. https://doi.org/10.1161/CIRCRESAHA.111.246876

Norton, N., Li, D., Rieder, M. J., Siegfried, J. D., Rampersaud, E., Zuchner, S., Mangos, S., Gonzalez-Quintana, J., Wang, L., McGee, S., Reiser, J., Martin, E., Nickerson, D. A., & Hershberger, R. E. (2011). Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. *American Journal of Human Genetics*, *88*, 273–282. https://doi.org/10.1016/j.ajhg.2011.01.016

Novodvorsky, P., Da Costa, M. M., & Chico, T. J. (2013). Zebrafish-based small molecule screens for novel cardiovascular drugs. *Drug Discovery Today: Technologies*, *10*, e109–e114. https://doi.org/10.1016/j.ddtec.2012.01.005

O'Brown, N. M., Megason, S. G., & Gu, C. (2019). Suppression of transcytosis regulates zebrafish blood-brain barrier function. *eLife*, *8*, e47326. https://doi.org/10.7554/eLife.47326

Olstad, E. W., Ringers, C., Hansen, J. N., Wens, A., Brandt, C., Wachten, D., Yaksi, E., & Jurisch-Yaksi, N. (2019). Ciliary beating compartmentalizes cerebrospinal fluid flow in the brain and regulates ventricular development. *Current Biology*, *29*, 229–241e6. https://doi.org/10.1016/j.cub.2018.11.059

Panakova, D., Werdich, A. A., & Macrae, C. A. (2010). Wnt11 patterns a myocardial electrical gradient through regulation of the L-type ca(2+) channel. *Nature*, *466*, 874–878. https://doi.org/10.1038/nature09249

Peal, D. S., Mills, R. W., Lynch, S. N., Mosley, J. M., Lim, E., Ellinor, P. T., January, C. T., Peterson, R. T., & Milan, D. J. (2011). Novel chemical suppressors of long QT syndrome identified by an in vivo functional screen. *Circulation*, *123*, 23–30. https://doi.org/10.1161/CIRCULATIONAHA.110.003731

Porrello, E. R., Mahmoud, A. I., Simpson, E., Hill, J. A., Richardson, J. A., Olson, E. N., & Sadek, H. A. (2011). Transient regenerative potential of the neonatal mouse heart. *Science*, *331*, 1078–1080. https://doi.org/10.1126/science.1200708

Poss, K. D., Wilson, L. G., & Keating, M. T. (2002). Heart regeneration in zebrafish. *Science*, *298*, 2188–2190. https://doi.org/10.1126/science.1077857

Pott, A., Shahid, M., Kohler, D., Pylatiuk, C., Weinmann, K., Just, S., & Rottbauer, W. (2018). Therapeutic chemical screen identifies phosphatase inhibitors to reconstitute PKB phosphorylation and cardiac contractility in ILK-deficient zebrafish. *Biomolecules*, *8*, 153. https://doi.org/10.3390/biom8040153

Quinonez-Silvero, C., Hubner, K., & Herzog, W. (2020). Development of the brain vasculature and the blood-brain barrier in zebrafish. *Developmental Biology*, *457*, 181–190. https://doi.org/10.1016/j.ydbio.2019.03.005

Ravens, U. (2018). Ionic basis of cardiac electrophysiology in zebrafish compared to human hearts. *Progress in Biophysics and Molecular Biology*, *138*, 38–44. https://doi.org/10.1016/j.pbiomolbio.2018.06.008

Raya, A., Koth, C. M., Buscher, D., Kawakami, Y., Itoh, T., Raya, R. M., Sternik, G., Tsai, H. J., Rodriguez-Esteban, C., & Izpisua-Belmonte, J. C. (2003). Activation of notch signaling pathway precedes heart regeneration in zebrafish. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(Suppl 1), 11889–11895.

Ren, F., Ji, C., Huang, Y., Aniagu, S., Jiang, Y., & Chen, T. (2020). AHR-mediated ROS production contributes to the cardiac developmental toxicity of PM2.5 in zebrafish embryos. *Science of the Total Environment*, *719*, 135097. https://doi.org/10.1021/acsami.1c01720

Roberts, A. M., Ware, J. S., Herman, D. S., Schafer, S., Baksi, J., Bick, A. G., Buchan, R. J., Walsh, R., John, S., Wilkinson, S., Mazzarotto, F., Felkin, L. E., Gong, S., MacArthur, J. A., Cunningham, F., Flannick, J., Gabriel, S. B., Altshuler, D. M., Macdonald, P. S., … Cook, S. A. (2015). Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titin truncations in health and disease. *Science Translational Medicine*, *7*, 270ra6.

Salgado-Almario, J., Vicente, M., Vincent, P., Domingo, B., & Llopis, J. (2020). Mapping calcium dynamics in the heart of zebrafish embryos with Ratiometric genetically encoded calcium indicators. *International Journal of Molecular Sciences*, *21*, 6610. https://doi.org/10.3390/ijms21186610

Salpea, K. D., & Humphries, S. E. (2010). Telomere length in atherosclerosis and diabetes. *Atherosclerosis*, *209*, 35–38. https://doi.org/10.1016/j.atherosclerosis.2009.12.021

Sánchez-Iranzo, H., Galardi-Castilla, M., Sanz-Morejón, A., González-Rosa, J. M., Costa, R., Ernst, A., Sainz de Aja, J., Langa, X., & Mercader, N. (2018). Transient fibrosis resolves via fibroblast inactivation in the regenerating zebrafish heart. *Proceedings of the National Academy of Sciences of the United States of America*, *115*, 4188–4193. https://doi.org/10.1073/pnas.1716713115

Sarmah, S., & Marrs, J. A. (2013). Complex cardiac defects after ethanol exposure during discrete cardiogenic events in zebrafish: Prevention with folic acid. *Developmental Dynamics*, *242*, 1184–1201. https://doi.org/10.1002/dvdy.24015

Savage, A. M., Kurusamy, S., Chen, Y., Jiang, Z., Chhabria, K., MacDonald, R. B., Kim, H. R., Wilson, H. L., van Eeden, F. J. M., Armesilla, A. L., Chico, T. J. A., & Wilkinson, R. N. (2019). tmem33 is essential for VEGF-mediated endothelial calcium oscillations and angiogenesis. *Nature Communications*, *10*, 732. https://doi.org/10.1096/fba.2020-00133

Serbanovic-Canic, J., de Luca, A., Warboys, C., Ferreira, P. F., Luong, L. A., Hsiao, S., Gauci, I., Mahmoud, M., Feng, S., Souilhol, C., Bowden, N., Ashton, J. P., Walczak, H., Firmin, D., Krams, R., Mason, J. C., Haskard, D. O., Sherwin, S., Ridger, V., … Evans, P. C. (2017). Zebrafish model for functional screening of flow-responsive genes. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *37*, 130–143. https://doi.org/10.1161/ATVBAHA.116.308502

Shih, Y. H., Zhang, Y., Ding, Y., Ross, C. A., Li, H., Olson, T. M., & Xu, X. (2015). Cardiac transcriptome and dilated cardiomyopathy genes in zebrafish. *Circulation. Cardiovascular Genetics*, *8*, 261–269. https://doi.org/10.1161/CIRCGENETICS.114.000702

Shimizu, H., Schredelseker, J., Huang, J., Lu, K., Naghdi, S., Lu, F., Franklin, S., Fiji, H. D., Wang, K., Zhu, H., Tian, C., Lin, B., Nakano, H., Ehrlich, A., Nakai, J., Stieg, A. Z., Gimzewski, J. K., Nakano, A., Goldhaber, J. I., … Chen, J. N. (2015). Mitochondrial ca(2+) uptake by the voltage-dependent anion channel 2 regulates cardiac rhythmicity. *eLife*, *4*, e04801. https://doi.org/10.7554/eLife.04801

Simoes, F. C., Cahill, T. J., Kenyon, A., Gavriouchkina, D., Vieira, J. M., Sun, X., Pezzolla, D., Ravaud, C., Masmanian, E., Weinberger, M., Mayes, S., Lemieux, M. E., Barnette, D. N., Gunadasa-Rohling, M., Williams, R. M., Greaves, D. R., Trinh, L. A., Fraser, S. E., Dallas, S. L., … Riley, P. R. (2020). Macrophages directly contribute collagen to scar formation during zebrafish heart regeneration and mouse heart repair. *Nature Communications*, *11*, 600.

Souilhol, C., Serbanovic-Canic, J., Fragiadaki, M., Chico, T. J., Ridger, V., Roddie, H., & Evans, P. C. (2020). Endothelial responses to shear stress in atherosclerosis: A novel role for developmental genes. *Nature Reviews. Cardiology*, *17*, 52–63. https://doi.org/10.1038/s41569-019-0239-5

Spaink, H. P., Cui, C., Wiweger, M. I., Jansen, H. J., Veneman, W. J., Marin-Juez, R., de Sonneville, J., Ordas, A., Torraca, V., van der Ent, W., Leenders, W. P., Meijer, A. H., Snaar-Jagalska, B. E., & Dirks, R. P. (2013). Robotic injection of zebrafish embryos for high-throughput screening in disease models. *Methods*, *62*, 246–254. https://doi.org/10.1016/j.ymeth.2013.06.002

Stainier, D. Y., Fouquet, B., Chen, J. N., Warren, K. S., Weinstein, B. M., Meiler, S. E., Mohideen, M. A., Neuhauss, S. C., Solnica-Krezel, L., Schier, A. F., Zwartkruis, F., Stemple, D. L., Malicki, J., Driever, W., & Fishman, M. C. (1996). Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. *Development*, *123*, 285–292.

Staudt, D., & Stainier, D. (2012). Uncovering the molecular and cellular mechanisms of heart development using the zebrafish. *Annual Review of Genetics*, *46*, 397–418. https://doi.org/10.1146/annurev-genet-110711-155646

Stoletov, K., Fang, L., Choi, S. H., Hartvigsen, K., Hansen, L. F., Hall, C., Pattison, J., Juliano, J., Miller, E. R., Almazan, F., Crosier, P., Witztum, J. L., Klemke, R. L., & Miller, Y. I. (2009). Vascular lipid accumulation, lipoprotein oxidation, and macrophage lipid uptake in hypercholesterolemic zebrafish. *Circulation Research*, *104*, 952–960. https://doi.org/10.1161/CIRCRESAHA.108.189803

Suo, J., Ferrara, D. E., Sorescu, D., Guldberg, R. E., Taylor, W. R., & Giddens, D. P. (2007). Hemodynamic shear stresses in mouse aortas: Implications for atherogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *27*, 346–351. https://doi.org/10.1161/01.ATV.0000253492.45717.46

Tsai, C. T., Wu, C. K., Chiang, F. T., Tseng, C. D., Lee, J. K., Yu, C. C., Wang, Y. C., Lai, L. P., Lin, J. L., & Hwang, J. J. (2011). In-vitro recording of adult zebrafish heart electrocardiogram - a platform for pharmacological testing. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *412*, 1963–1967. https://doi.org/10.1093/jalm/jfab034

Umans, R. A., Henson, H. E., Mu, F., Parupalli, C., Ju, B., Peters, J. L., Lanham, K. A., Plavicki, J. S., & Taylor, M. R. (2017). CNS angiogenesis and barriergenesis occur simultaneously. *Developmental Biology*, *425*, 101–108. https://doi.org/10.1016/j.ydbio.2017.03.017

van Leeuwen, L. M., Evans, R. J., Jim, K. K., Verboom, T., Fang, X., Bojarczuk, A., Malicki, J., Johnston, S. A., & van der Sar, A. M. (2018). A transgenic zebrafish model for the in vivo study of the blood and choroid plexus brain barriers using claudin 5. *Biology Open*, *7*, bio030494. https://doi.org/10.1242/bio.030494

van Opbergen, C. J. M., Koopman, C. D., Kok, B. J. M., Knopfel, T., Renninger, S. L., Orger, M. B., Vos, M. A., van Veen, T. A. B., Bakkers, J., & de Boer, T. P. (2018). Optogenetic sensors in the zebrafish heart: A novel in vivo electrophysiological tool to study cardiac arrhythmogenesis. *Theranostics*, *8*, 4750–4764. https://doi.org/10.7150/thno.26108

Vanhollebeke, B., Stone, O. A., Bostaille, N., Cho, C., Zhou, Y., Maquet, E., Gauquier, A., Cabochette, P., Fukuhara, S., Mochizuki, N., Nathans, J., & Stainier, D. Y. (2015). Tip cell-specific requirement for an atypical Gpr124- and Reck-dependent Wnt/beta-catenin pathway during brain angiogenesis. *eLife*, *4*, e06489. https://doi.org/10.7554/eLife.06489

Venero Galanternik, M., Castranova, D., Gore, A. V., Blewett, N. H., Jung, H. M., Stratman, A. N., Kirby, M. R., Iben, J., Miller, M. F., Kawakami, K., Maraia, R. J., & Weinstein, B. M. (2017). A novel perivascular cell population in the zebrafish brain. *eLife*, *6*, e24369. https://doi.org/10.7554/eLife.24369

Verhagen, J. M. A., van den Born, M., van der Linde, H. C., Nikkels, P. G. J., Verdijk, R. M., Kivlen, M. H., van Unen, L. M. A., Baas, A. F., Ter

Heide, H., van Osch-Gevers, L., Hoogeveen-Westerveld, M., Herkert, J. C., Bertoli-Avella, A. M., van Slegtenhorst, M. A., Wessels, M. W., Verheijen, F. W., Hassel, D., Hofstra, R. M. W., Hegde, R. S., … van de Laar, I. (2019). Biallelic variants in ASNA1, encoding a cytosolic targeting factor of tail-anchored proteins, cause rapidly progressive pediatric cardiomyopathy. *Circ Genom Precis Med.*, *12*, 397–406. https://doi.org/10.1111/petr.14002

von der Heyde, B., Emmanouilidou, A., Mazzaferro, E., Vicenzi, S., Höijer, I., Klingström, T., Jumaa, S., Dethlefsen, O., Snieder, H., de Geus, E., Ameur, A., Ingelsson, E., Allalou, A., Brooke, H. L., & den Hoed, M. (2020). Translating GWAS-identified loci for cardiac rhythm and rate using an in vivo image- and CRISPR/Cas9-based approach. *Scientific Reports*, *10*, 11831. https://doi.org/10.1038/s41598-020-68567-1

Walcott, B. P., & Peterson, R. T. (2014). Zebrafish models of cerebrovascular disease. *Journal of Cerebral Blood Flow and Metabolism*, *34*, 571–577. https://doi.org/10.1038/jcbfm.2014.27

Wang, J., Panáková, D., Kikuchi, K., Holdway, J. E., Gemberling, M., Burris, J. S., Singh, S. P., Dickson, A. L., Lin, Y. F., Sabeh, M. K., Werdich, A. A., Yelon, D., Macrae, C. A., & Poss, K. D. (2011). The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. *Development (Cambridge, England)*, *138*, 3421–3430. https://doi.org/10.1093/nar/gkab218

Wang, L. W., Huttner, I. G., Santiago, C. F., Kesteven, S. H., Yu, Z. Y., Feneley, M. P., & Fatkin, D. (2017). Standardized echocardiographic assessment of cardiac function in normal adult zebrafish and heart disease models. *Disease Models & Mechanisms*, *10*, 63–76. https://doi.org/10.1242/dmm.026989

Weber, M., Scherf, N., Meyer, A. M., Panakova, D., Kohl, P., & Huisken, J. (2017). Cell-accurate optical mapping across the entire developing heart. *eLife*, *6*, e28307. https://doi.org/10.7554/eLife.28307

Weijts, B., Gutierrez, E., Saikin, S. K., Ablooglu, A. J., Traver, D., Groisman, A., & Tkachenko, E. (2018). Blood flow-induced notch activation and endothelial migration enable vascular remodeling in zebrafish embryos. *Nature Communications*, *9*, 5314.

Whitesell, T. R., Chrystal, P. W., Ryu, J. R., Munsie, N., Grosse, A., French, C. R., Workentine, M. L., Li, R., Zhu, L. J., Waskiewicz, A., Lehmann, O. J., Lawson, N. D., & Childs, S. J. (2019). foxc1 is required for embryonic head vascular smooth muscle differentiation in zebrafish. *Developmental Biology*, *453*, 34–47. https://doi.org/10.1016/j.neuron.2020.11.011

Withers, S. E., Parry-Jones, A. R., Allan, S. M., & Kasher, P. R. (2020). A multi-model pipeline for translational intracerebral Haemorrhage research. *Translational Stroke Research*, *11*, 1229–1242. https://doi.org/10.1007/s12975-020-00830-z

Xie, J., Farage, E., Sugimoto, M., & Anand-Apte, B. (2010). A novel transgenic zebrafish model for blood-brain and blood-retinal barrier development. *BMC Developmental Biology*, *10*, 76. https://doi.org/10.1186/1471-213X-10-76

Xu, S., Liu, C., Xie, F., Tian, L., Manno, S. H., Manno, F. A. M. 3rd, Fallah, S., Pelster, B., Tse, G., & Cheng, S. H. (2019). Excessive inflammation impairs heart regeneration in zebrafish breakdance mutant after cryoinjury. *Fish & Shellfish Immunology*, *89*, 117–126. https://doi.org/10.1016/j.fsi.2019.03.058

Yamada, N., Asano, Y., Fujita, M., Yamazaki, S., Inanobe, A., Matsuura, N., Kobayashi, H., Ohno, S., Ebana, Y., Tsukamoto, O., Ishino, S., Takuwa, A., Kioka, H., Yamashita, T., Hashimoto, N., Zankov, D. P., Shimizu, A., Asakura, M., Asanuma, H., … Takashima, S. (2019). Mutant KCNJ3 and KCNJ5 potassium channels as novel molecular targets in Bradyarrhythmias and atrial fibrillation. *Circulation*, *139*, 2157–2169.

Yan, J., Li, H., Bu, H., Jiao, K., Zhang, A. X., Le, T., Cao, H., Li, Y., Ding, Y., & Xu, X. (2020). Aging-associated sinus arrest and sick sinus syndrome in adult zebrafish. *PLoS One*, *15*, e0232457. https://doi.org/10.1371/journal.pone.0232457

Ye, J., Tucker, N. R., Weng, L. C., Clauss, S., Lubitz, S. A., & Ellinor, P. T. (2016). A functional variant associated with atrial fibrillation regulates PITX2c expression through TFAP2a. *American Journal of Human Genetics*, *99*, 1281–1291. https://doi.org/10.1016/j.ajhg.2016.10.001

Yip, J. K., Harrison, M., Villafuerte, J., Fernandez, G. E., Petersen, A. P., Lien, C. L., & McCain, M. L. (2020). Extended culture and imaging of normal and regenerating adult zebrafish hearts in a fluidic device. *Lab on a Chip*, *20*, 274–284. https://doi.org/10.1039/C9LC01044K

Yu, F., Li, R., Parks, E., Takabe, W., & Hsiai, T. K. (2010). Electrocardiogram signals to assess zebrafish heart regeneration: Implication of long QT intervals. *Annals of Biomedical Engineering*, *38*, 2346–2357. https://doi.org/10.1007/s10439-010-9993-6

Yue, C., Ji, C., Zhang, H., Zhang, L. W., Tong, J., Jiang, Y., & Chen, T. (2017). Protective effects of folic acid on PM2.5-induced cardiac developmental toxicity in zebrafish embryos by targeting AhR and Wnt/beta-catenin signal pathways. *Environmental Toxicology*, *32*, 2316–2322. https://doi.org/10.1002/casp.2507

Zhang, H., Yao, Y., Chen, Y., Yue, C., Chen, J., Tong, J., Jiang, Y., & Chen, T. (2016). Crosstalk between AhR and wnt/beta-catenin signal pathways in the cardiac developmental toxicity of PM2.5 in zebrafish embryos. *Toxicology*, *355–356*, 31–38.

Zhang, R., Han, P., Yang, H., Ouyang, K., Lee, D., Lin, Y. F., Ocorr, K., Kang, G., Chen, J., Stainier, D. Y., Yelon, D., & Chi, N. C. (2013). In vivo cardiac reprogramming contributes to zebrafish heart regeneration. *Nature*, *498*, 497–501. https://doi.org/10.1038/nature12322

Zou, J., Tran, D., Baalbaki, M., Tang, L. F., Poon, A., Pelonero, A., Titus, E. W., Yuan, C., Shi, C., Patchava, S., Halper, E., Garg, J., Movsesyan, I., Yin, C., Wu, R., Wilsbacher, L. D., Liu, J., Hager, R. L., Coughlin, S. R., … Deo, R. C. (2015). An internal promoter underlies the difference in disease severity between N- and C-terminal truncation mutations of Titin in zebrafish. *eLife*, *4*, e09406. https://doi.org/10.7554/eLife.09406

How to cite this article: Bowley, G., Kugler, E., Wilkinson, R., Lawrie, A., van Eeden, F., Chico, T. J. A., Evans, P. C., Noël, E. S., & Serbanovic-Canic, J. (2021). Zebrafish as a tractable model of human cardiovascular disease. *British Journal of Pharmacology*, 1–18. https://doi.org/10.1111/bph.15473