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# THE ZEBRAFISH AS A MODEL SYSTEM FOR HUMAN DISEASE

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## 1. ABSTRACT

The zebrafish (*Danio rerio*) has been widely utilised for the study of developmental biology, which has lead to the evolution of sophisticated cellular and molecular approaches. More recently, the rapid progress of various zebrafish genomic infrastructure initiatives is facilitating the development of zebrafish models of human disease. This review aims to describe several representative examples of how the zebrafish can be successfully used to identify novel genes and assign gene function, providing invaluable clues to human pathophysiology.

## 2. INTRODUCTION

The zebrafish (*Danio rerio*) provides a number of advantages as a model organism for the study of vertebrate development and its perturbation in disease (1). Their reasonably short reproductive cycle, the large number of progeny that can be produced, and the relatively small space and expense required to maintain large numbers of offspring, make zebrafish an efficient system for genetic analysis. In addition, their embryos are transparent and develop externally, which makes them accessible from fertilisation onwards, while various techniques facilitating mutant screening are established, such as ethyl nitrosourea (ENU) germline mutagenesis. Ploidy can be manipulated to unmask recessive phenotypes and to permit highly efficient mapping strategies to be successfully employed (2). Furthermore, there is strong conservation to humans both anatomically and genetically, which allows modelling of complex developmental processes, including those required to generate kidney, multi-chambered heart, multi-lineage hematopoietic cells, and the notochord.

Several large-scale genetic screens have exploited these characteristics with great success, resulting in the identification of over 500 mutant phenotypes relevant to various aspects of vertebrate development (3,4), including several highly reminiscent of human disease states (Table 1). In this way, it has been possible to investigate complex biological processes without prior knowledge of the genes involved, providing new insights into the pathophysiology of disease. Moreover, the positional cloning of the genes identified by such mutations is feasible and the resources for mapping are rapidly expanding, with an international commitment to facilitate zebrafish gene cloning (5-8). This is allowing for the identification of the molecular basis of diseases – sometimes for the first time.

This review presents several examples of zebrafish mutant phenotypes with clinical relevance and the progress being made to characterize them and identify the underlying genetic mutations.

## 3. DISEASE MODELS

### 3.1. Hematopoietic models

The zebrafish is especially suitable for the study of hematopoiesis. The anatomical and morphological features of both primitive and definitive hematopoiesis are comparable to those in mammals (9,10). Zebrafish possess both erythroid and myeloid compartments, the latter including macrophage and two distinct granulocytic lineages (9,11,12). In addition, there is strong conservation of hematopoietic gene expression and function at the

**Table 1.** Defined zebrafish mutants with molecularly analogous human diseases

| Disease group | Zebrafish mutant | Mutated gene | Analagous human disease | Reference |
|---|---|---|---|---|
| Blood-heme synthesis | *sauternes* | *d-aminolevinate synthetase* | Sideroblastic anemia | 21 |
| | *yquem* | *uroporphyrinogen decarboxylase* | Porphyria cutanea tarda & Hepatoerythropoietic porphyria | 19 |
| | *dracula* | *ferrochelatase* | Erythropoietic protoporphyria | 20 |
| Blood-iron transport | *weissherbst* | *ferroportin1* | Hemochromatosis | 22 |
| Blood-erythrocyte structure | *riesling* | *ß-spectrin* | Hereditary spherocytosis | 31 |
| | *retsina* | *band3* | Congenital dyserythropoietic anemia type II (HEMPAS) | 32 |
| Blood-other | *zinfandel* | *globin locus* | Resembles thalassemia | 24 |
| Pigmentation pattern | *sparse* | *kit* | Piebaldism | 35 |
| Other | *panther* | *fms* | No known congenital disease association; (somatic loss associated with myelodysplasia/leukemia in 5q- syndrome) | 36 |

molecular level, which extends to transcription factors and signalling components (13-15). The ease of identifying red hemoglobin pigment in the transparent zebrafish embryo has facilitated direct visual screens for mutants with defects in erythroid development since anemia is immediately obvious (16,17), with more than 50 mutants identified which fall into 26 complementation groups, representing all stages of hematopoietic development (18) Several mutants obtained from these screens present almost identical phenotypes to those of congenital human hematopoietic diseases, and serve to effectively illustrate the power of zebrafish genetics to unravel specific molecular events in hematopoiesis.

For example, phenotypes suggestive of a group of disorders known as porphyrias in humans – which are caused by mutations in enzymes of the heme biosynthesis pathway – were identified by observing the concurrence of anemia, photosensitivity of erythrocytes, and in some cases, fluorescence from porphyrin pigments. The *yquem* mutant is characterized by photosensitive, auto-fluorescent blood and deficiency in uroporphyrinogen decarboxylase (UROD) activity, an enzyme in the heme biosynthetic pathway. The human diseases porphyria cutanea tarda and the rarer hepatoerythropoietic porphyria result from mutations in the gene encoding UROD. Hence *UROD* was studied as a candidate gene and the mutant phenotype shown to be the result of a homozygous mutation in the zebrafish *urod* gene (19). The *dracula* mutant shows light-dependent hemolysis and liver disease, with a build-up of protoporphyrin IX, similar to erythropoietic protopophyria. This is due to a mutation in a splice donor site of the *ferrochelatase* gene, the terminal enzyme in the heme biosynthetic pathway (20). While these mutations did not uncover new genes involved in human porphyria, they have provided animal models for studying the pathogenesis of this class of disease. Furthermore, the rescue of zebrafish mutant phenotypes by transient replacement of the wild-type gene provides examples of genetic therapeutic approaches.

Another zebrafish mutant affects the first step in the heme synthesis pathway although, like the analogous human disease, it does not result in porphyria. The *sauternes* mutant is characterized by delayed erythroid maturation and abnormal globin expression, resulting in a microcytic, hypochromic anemia. Positional cloning identified the mutant gene as encoding δ-aminolevulinate synthase (ALAS2), an erythroid specific enzyme required for the first step in heme biosynthesis (21). Mutations in human ALAS2 result in congenital sideroblastic anemia.

Zebrafish mutants have also been identified which exhibit hypochromic anemia, such as *weißherbst* and *zinfandel*. Recently, it has been revealed that the gene *ferroportin1* is responsible for the *weißherbst* phenotype and encodes a novel transmembrane domain iron exporter, which has orthologues in mice and humans homologues and suggest functional conservation (22). An autosomal dominant form of human hemochromatosis is also due to a defective *ferroportin* gene (23). In this case, applying zebrafish genetics has yielded totally new information, including a novel gene. In contrast, the *zinfandel* mutant maps to the globin locus and thus probably represents a thalassemia disorder (9,24). Additional hypochromic zebrafish mutants include *chardonnay*, *chianti*, and *clear blood*. The further characterisation of these mutants has the potential to yield new insights into other aspects of red cell biology, including heme synthesis, iron metabolism, and globin expression.

Other zebrafish hematopoietic mutants identified include those that impact on stem cell generation or maintenance, such as *cloche*, *spadetail*, and *moonshine* (14,25,26). These mutants show a severe decrease or a complete absence of blood cells at the onset of circulation. In *cloche* and *spadetail*, both primitive and definitive hematopoeitic cells are affected, while in *moonshine* only embryonic erythrocytes are affected. The *cloche* mutant also affects endothelial cell development, and may represent a defect in a gene important for the generation or survival of the hemangioblast, a putative bipotential precursor cell for both blood and endothelial progenitors (25). The mutated gene in *spadetail* is a novel T-box transcription factor gene (27), although this does not correlate with a known human disease. The phenotype of

*cloche* is that which might be expected of a lesion in either the *scl* or *hhex* gene from our understanding of the role of these genes in mammalian hematopoiesis, but both these candidates have been dismissed (28-30).

A range of other mutants show defects in either progenitor proliferation or differentiation including *frascati*, *thunderbird*, *grenache*, *retsina*, *merlot*, *reisling*, *chablis*, and *cabernet*. These mutants show normal expression of early hematopoietic gene markers and normal levels of circulating cells at 1 days post fertilization (dpf), but display defects after this time. The genetic lesion in *riesling* is in the *ß-spectrin* gene (31); in humans, ß-spectrin lesions result in the disease hereditary spherocytosis, although unlike the autosomal recessive zebrafish model, most human pedigrees of this disease show autosomal dominant inheritance. *Retsina* is recently reported to be a lesion in the *erythroid anion exchanger 1/band3* gene, making it a model of the rare human congenital disease dyserythropoietic anaemia type II (HEMPAS) (32). Interestingly, both the zebrafish and human diseases are characterized by bilobed nuclei in red cell precursors, and cell biological studies in zebrafish have provided insight into the role of this protein in cytokinesis. In *frascati*, *thunderbird*, and *grenache* mutants, most erythroid cells die by 2 dpf, whereas in the other mutants the number of circulating blood cells decreases at various stages between 2–5 dpf (10).

All of these mutants were detected due to their alteration of the red cell compartment, which is easily scorable by direct microscopic analysis. Other screens currently underway in several laboratories instead utilise specific gene markers to focus on other hematopoietic lineages, including stem, myeloid and lymphoid cells (33). It is anticipated that the mutants recovered will increase our understanding of normal hematopoietic processes as well as the pathophysiology of hematopoietic disorders, including leukemia.

However, not all zebrafish mutants are close recapitulations of the mammalian disease phenotype – this is illustrated by the phenotypes of the zebrafish mutants *sparse* and *panther*, with lesions in the zebrafish orthologues of c-kit and c-fms respectively. In mice, inactivating mutations of the c-kit tyrosine kinase receptor results in anemia, pigmentation and germ cell defects, and in humans are associated with the autosomal dominant pigmentation disorder of piebaldism (34). The recessive zebrafish *c-kit* mutant *sparse* displays neural crest migration defects resulting in an altered pigment pattern, but not hematopoietic or germ cell defects. All four zebrafish alleles lack the transmembrane domain and are hence likely null alleles. There are several possible explanations for this phenotypic discordance. It may represent divergence of c-kit functions between mammals and zebrafish (indeed, *c-kit* expression could not be detected in the zebrafish genital ridge by in situ hybridization), or subtleties of the specific molecular lesions in the different species, or the presence of other c-kit-related genes in zebrafish (35). Another example of imperfect concordance between the zebrafish and mammalian disease phenotypes is the consequence of lesions in the c-*fms* gene, the receptor for macrophage colony stimulating factor (M-CSF). The zebrafish *fms* mutant *panther* has pigmentation defects as its most striking abnormality, indicating a unique role for *fms* in these neural crest-derived cells in zebrafish. The c-*fms* gene is also expressed in both zebrafish macrophages and osteoclasts, and a quantitative defect in osteoclast numbers demonstrated in *panther* (36); this initial report did not quantitate macrophages by an independent macrophage-lineage marker. c-*Fms*-deficient mice do not exist, but M-CSF ligand-deficient mice have macrophage deficiencies and are osteopetrotic (37). Human osteopetrosis is not yet associated with *FMS* mutations (38), although it remains theoretically possible that this is an infrequent cause of this already rare disease, and an example has just not yet been identified. In the absence of a human pedigree and a murine gene knockout strain, a role for human Fms in mammalian pigmentation cannot be excluded, although it would not be expected from the expression profile of the gene.

### 3.2. Renal models

The pronephric kidney of zebrafish embryos is a rather simple organ, possessing two nephrons with fused glomeruli and paired, bilateral pronephric ducts (39), compared with the metanephric kidney of humans. However, many details of renal function and development are comparable to that of other vertebrates (40), and so zebrafish provides a simple and accessible system for study.

Large-scale mutagenesis screens have identified 18 mutations affecting development of the zebrafish pronephros, the majority of which display glomerular or tubular cystic formation (39). Such mutants cover all aspects of nephrogenesis, including specification from mesoderm, nephron patterning, development of epithelial polarity, and glomerulus vascularization. Several of them again show similarities to human disease, including autosomal dominant polycystic kidney disorders (ADPKD). Zebrafish homologues of several genes important in renal development in mammals are cloned (e.g. *wt1*, *pax2*, *gdnf*, *lim-1*) although they have not yet been linked with any particular zebrafish mutant. In the mutant *double bubble*, glomerular cysts form as blood filtration commences. At later time points, changes are seen in the glomerular basement membrane and targeting of the membrane-bound $Na^+/K^+$ ATPase, which is required to establish ion gradients across the pronephric epithelia. Since mislocation of the $Na^+/K^+$ ATPase has been associated with ADPKD (41), the *double bubble* mutant provides a useful system for further study of this disease. Similarly, two other mutants, *elipsa* and *fleer*, exhibit combined renal-retinal dysplasia (39,42) comparable to that observed in the human disease Senior-Loken syndrome (43). In this case, complementation grouping suggests that the mutants represent distinct genes involved in the same pathway, and will likely provide new insight into the possible molecular basis of this disease.

### 3.3. Cardiac models

The embryonic heart of zebrafish closely resembles that of a human embryo at approximately three weeks gestation, being divided into atrial and ventricular chambers and lined by endocardium, with cardiac valves forming at the chamber boundaries (44). Beating of the

heart commences as a peristaltic wave at around 22 hpf, giving way to coordinated contractions of the atrium and ventricle by 36 hpf. Zebrafish are not dependent on circulation for survival at early stages of development. Therefore, perturbations in heart development do not result in the immediate death of the embryo. Coupled with the ease of visualisation of the heart, this makes the zebrafish an especially attractive cardiac model.

A range of zebrafish mutants have been identified with defects in cardiac development and/or function (44,45). In the mutant *pandora*, the heart valve is absent, while in *jekyll* the entire ventricle is absent. In *heart and soul*, which has a lesion in a PKC lambda gene (46), heart size is greatly reduced, while in *santa* it is four times larger than normal. In *miles apart* there is no fusion of the two primitive heart tubes, leading to the formation of two cardiac structures on either side of the midline. In contrast, the mutants *passive aggressive*, *pickwick* and *hal* affect contractility, while *slow mo*, *trembler* and *reggae* exhibit defects in rhythmicity. The *slow mo* mutant has a bradycardia, with a heart rate slower than wildtype fish (47), with the mutation decreasing pacemaker current (48). Another mutant, *gridlock*, resembles the human congenital disorder coarctation of the aorta (49) – there is a lack of blood circulation to the tail, caused by a localized vascular defect in which the paired lateral dorsal aorta fail to fuse and instead generate a single dorsal aorta in the anterior trunk. This leads to the development of a collateral circulation, which permits survival (50). Recently, Zhong et al. have shown that the gene responsible for *gridlock* encodes a basic helix-loop-helix protein of the Hairy/Enhancer of split related family genes (51), a family whose function is unknown. These examples again highlight the usefulness of zebrafish for gene discovery.

A more recent mutagenesis screen has identified greater than 20 mutants with perturbations in cardiac induction and/or patterning using specific cardiac gene markers (52). The identification of the underlying genetic lesions responsible for these mutants will likely provide further molecular details of these important developmental processes.

### 3.4. Other developmental diseases

In addition to the examples discussed above, a number of other vertebrate-specific and clinically-relevant developmental processes are being investigated using the zebrafish and zebrafish mutants. For example, *one eyed pinhead* displays cyclopia and lacks ventral neuroectoderm, a phenotype resembling the human congenital disorder, holoprosencephaly (53,54), while the *no optokinetic response* mutant represents a possible model for human retinal disease (55). Other investigators are aiming to use behavioural/neurological testing to investigate functional disorders such as drug addiction (56).

Several unique aspects of zebrafish biology can make it attractive to use the alternate reverse genetic approach of first isolating zebrafish gene implicated in a human disease, and then exploiting its biology in the fish. For example, isolation of zebrafish *dystrophin* – orthologous to the human gene implicated in the fatal muscle wasting disease Duchene Muscular Dystrophy (57) – provides further opportunities for the creation of a zebrafish model of this disease.

The function of novel genes isolated from zebrafish themselves can also be directly evaluated in zebrafish – an example of this is the creation of a form of polycythemia rubra vera by the transgenic expression of a dominant-negative form of a novel cell death receptor in the erythroid compartment of zebrafish (58).

### 3.5. Diseases resulting from exposure to environmental agents

The potential of using zebrafish for the study of malignancy is becoming evident, with reports of successful tumor induction in zebrafish (59-61). The range of tumors induced varies with the chemical agent used. Ethyl nitrosourea treatment resulted in benign papillomas; no malignant skin lesions were seen (59). Other agents (N-methyl-N'-nitro-N-nitrosoguanidine [MNNG] and 7,12-dimethylbenz[a]anthracene [DMBA]) have different spectra of target organ sensitivities. MNNG induced hepatomas and mesenchymal tumours including various forms of sarcoma (61). Fry exposure to DMBA induced various tumours in liver, gill and blood vessels, and treatment of juveniles resulted in intestinal epithelial neoplasia (61). These observations collectively demonstrate that zebrafish are susceptible to benign and malignant tumour development in tissues derivative of all three germ layers.

An extension of this approach has been the application of "chemical genetics" to zebrafish. The feasibility of screening panels of small molecules for specific effects on early developmental processes has been shown (62). The primary intent of these screens has been to identify molecules that, by virtue of the specific phenotype they induce, suggest a specific molecular action worthy of further biochemical study. However, a corollary of this approach is that candidate teratogens are identified that may model a range of environmentally-dependent congenital diseases.

There are as yet no genetic zebrafish models of tumour propensity, and the evaluation of oncogenic proteins by ubiquitous over-expression very early in development may result in unexpected developmental defects (63). It may be necessary to confine the cancer-predisposing genetic lesion to a particular compartment using a transgenic or conditional approach.

## 4. PERSPECTIVE

The zebrafish is proving itself as a useful animal model system for studying human disease, especially in elucidating the molecular basis of congenital disease. As a versatile tool for in vivo functional genomics approaches, the zebrafish is well-positioned to contribute to the next phase of understanding the now-sequenced vertebrate genome. Whole genome sequencing has generated unprecedented quantities of sequence data, including the

sequence of many genes of unknown function. Genetic studies in zebrafish, particularly the unbiased phenotype-first surveying of the genome for lesions in genes that is possible with mutagenesis and screening approaches, can be expected to play a significant role in assigning functions to genes. In the process of doing so, many interesting zebrafish models of human disease will be generated.

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## 6. REFERENCES

1. Driever, W., and M. C. Fishman: The zebrafish: heritable disorders in transparent embryos. *J Clin Invest* 97, 1788-1794 (1996)
2. Postlethwait, J. H., and W. S. Talbot: Zebrafish genomics: from mutants to genes. *TIG* 13, 183-189 (1997)
3. Haffter, P., M. Granato, M. Brand, M. C. Mullins, M. Hammerschmidt, D. A. Kane, J. Odenthal, F. J. van Eeden, Y. J. Jiang, C. P. Heisenberg, R. N. Kelsh, M. Furutani-Seiki, E. Vogelsang, D. Beuchle, U. Schach, C. Fabian, and C. Nusslein-Volhard: The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*. *Development* 123, 1-36 (1996)
4. Driever, W., L. Solnica-Krezel, A. F. Schier, S. C. Neuhauss, J. Malicki, D. L. Stemple, D. Y. Stainier, F. Zwartkruis, S. Abdelilah, Z. Rangini, J. Belak, and C. Boggs: A genetic screen for mutations affecting embryogenesis in zebrafish. *Development* 123, 37-46 (1996)
5. Postlethwait, J. H., Y.-L. Yan, M. A. Gates, S. Horne, A. Amores, A. Brownlie, A. Donovan, E. S. Egan, A. Force, Z. Gong, C. Goutel, A. Fritz, R. Kelsch, E. Knapik, E. Liao, B. Paw, D. Ransom, A. Singer, M. Thomson, T. S. Abduljabbar, P. Yelick, D. Beier, J.-S. Joly, D. Larhammar, F. Rosa, M. Westerfield, L. I. Zon, S. L. Johnson, and W. S. Talbot: Vertebrate genome evolution and the zebrafish gene map. *Nature Genet* 18, 345-349 (1998)
6. Talbot, W. S., and N. Hopkins: Zebrafish mutations and functional analysis of the vertebrate genome. *Genes Dev* 14, 755-762 (2000)
7. Shimoda, N., E. W. Knapik, J. Ziniti, C. Sim, E. Yamada, S. Kaplan, D. Jackson, F. de Sauvage, H. Jacob, and M. C. Fishman: Zebrafish genetic map with 2000 microsatellite markers. *Genomics* 58, 219-232 (1999)
8. Geisler, R., G. J. Rauch, H. Baier, F. van Bebber, L. Brobeta, M. P. Dekens, K. Finger, C. Fricke, M. A. Gates, H. Geiger, S. Geiger-Rudolph, D. Gilmour, S. Glaser, L. Gnugge, H. Habeck, K. Hingst, S. Holley, J. Keenan, A. Kirn, H. Knaut, D. Lashkari, F. Maderspacher, U. Martyn, S. Neuhauss, P. Haffter, and et al.: A radiation hybrid map of the zebrafish genome. *Nat Genet* 23, 86-89 (1999)
9. Chan, F.-Y., J. Robinson, A. Brownlie, R. A. Shivdasani, A. Donovan, C. Brugnara, J. Kim, B.-C. Lau, H. E. Witkowska, and L. I. Zon: Characterization of Adult a- and b-globin genes in the zebrafish. *Blood* 89, 688-700 (1997)
10. Amatruda, J. F., and L. I. Zon: Dissecting hematopoiesis and disease using the zebrafish. *Dev Biol* 216, 1-15 (1999)
11. Lieschke, G. J., A. C. Oates, M. O. Crowhurst, A. C. Ward, and J. E. Layton: Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. *Blood* 98, 3087-3096 (2001)
12. Bennett, C. M., J. P. Kanki, J. Rhodes, T. X. Liu, B. H. Paw, M. W. Kieran, D. M. Langenau, A. Delahaye-Brown, L. I. Zon, M. D. Fleming, and A. T. Look: Myelopoiesis in the zebrafish, Danio rerio. *Blood* 98, 643-651 (2001)
13. Conway, G., A. Margoliath, S. Wong-Madden, R. J. Roberts, and W. Gilbert: Jak1 kinase is required for cell migrations and anterior specification of zebrafish embryos. *Proc Natl Acad Sci USA* 94, 3082-3087 (1997)
14. Liao, E. C., B. H. Paw, A. C. Oates, S. J. Pratt, J. H. Postlethwait, and L. I. Zon: SCL/Tal-1 transcription factor acts downstream of cloche to specify hematopoietic and vascular progenitors in zebrafish. *Genes Dev* 12, 621-626 (1998)
15. Oates, A. C., A. Brownlie, S. J. Pratt, D. V. Irvine, E. C. Liao, B. H. Paw, K. J. Dorian, S. L. Johnson, J. H. Postlethwait, L. I. Zon, and A. F. Wilks: Gene duplication of zebrafish JAK2 homologs is accompanied by divergent embryonic expression patterns: only jak2a is expressed during erythropoiesis. *Blood* 94, 2622-2636 (1999)
16. Ransom, D. G., P. Haffter, J. Odenthal, A. Brownlie, E. Vogelsang, R. N. Kelsh, M. Brand, F. J. van Eeden, M. Furutani-Seiki, M. Granato, M. Hammerschmidt, C. P. Heisenberg, Y. J. Jiang, D. A. Kane, M. C. Mullins, and C. Nusslein-Volhard: Characterization of zebrafish mutants with defects in embryonic hematopoiesis. *Development* 123, 311-319 (1996)
17. Weinstein, B. M., A. F. Schier, S. Abdelilah, J. Malicki, L. Solnica-Krezel, D. L. Stemple, D. Y. Stainier, F. Zwartkruis, W. Driever, and M. C. Fishman: Hematopoietic mutations in the zebrafish. *Development* 123, 303-309 (1996)
18. Paw, B. H., and L. I. Zon: Zebrafish: a genetic approach in studying hematopoiesis. *Curr Opin Hematol* 7, 79-84 (2000)
19. Wang, H., Q. Long, S. D. Marty, S. Sassa, and S. Lin: A zebrafish model for hepatoerythropoietic porphyria. *Nature Genet* 20, 239-243 (1998)
20. Childs, S., B. M. Weinstein, M. A. Mohideen, S. Donohue, H. Bonkovsky, and M. C. Fishman: Zebrafish dracula encodes ferrochelatase and its mutation provides a model for erythropoietic protoporphyria. *Curr Biol* 10, 1001-1004 (2000)
21. Brownlie, A., A. Donovan, S. J. Pratt, B. H. Paw, A. C. Oates, C. Brugnara, H. E. Witkowska, S. Sassa, and L. I. Zon: Postional cloning of the zebrafish *sauternes* gene: a model for congenital sideroblastic anaemia. *Nature Genet* 20, 244-250 (1998)
22. Donovan, A., A. Brownlie, Y. Zhou, J. Shepard, S. J. Pratt, J. Moynihan, B. H. Paw, A. Drejer, B. Barut, A. Zapata, T. C. Law, C. Brugnara, S. E. Lux, G. S. Pinkus, J. L. Pinkus, P. D. Kingsley, J. Palis, M. D. Fleming, N. C. Andrews, and L. I. Zon: Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature* 403, 776-781 (2000)
23. Montosi, G., A. Donovan, A. Totaro, C. Garuti, E. Pignatti, S. Cassanelli, C. C. Trenor, P. Gasparini, N. C. Andrews, and A. Pietrangelo: Autosomal-dominant

hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. *J Clin Invest* 108, 619-623 (2001)
24. Dooley, K., and L. I. Zon: Zebrafish: a model system for the study of human disease. *Curr Opin Genet Dev* 10, 252-256 (2000)
25. Stainier, D. Y., B. M. Weinstein, H. W. Detrich, 3rd, L. I. Zon, and M. C. Fishman: Cloche, an early acting zebrafish gene, is required by both the endothelial and hematopoietic lineages. *Development* 121, 3141-3150 (1995)
26. Thompson, M. A., D. G. Ransom, S. J. Pratt, H. MacLennan, M. W. Kieran, H. W. I. Detrich, B. Vail, T. L. Huber, B. Paw, B. A.J., A. C. Oates, A. Fritz, M. A. Gates, A. Amores, N. Bahary, W. S. Talbot, H. Her, D. R. Beier, J. H. Postlethwait, and L. I. Zon: The cloche and spadetail genes differentially affect hematopoiesis and vasculogenesis. *Dev Biol* 197, 248-269 (1998)
27. Griffin, K. J., S. L. Amacher, C. B. Kimmel, and D. Kimelman: Molecular identification of spadetail: regulation of zebrafish trunk and tail mesoderm formation by T-box genes. *Development* 125, 3379-3388 (1998)
28. Liao, W., B. W. Bisgrove, H. Sawyer, B. Hug, B. Bell, K. Peters, D. J. Grunwald, and D. Y. Stainier: The zebrafish gene cloche acts upstream of a flk-1 homologue to regulate endothelial cell differentiation. *Development* 124, 381-389 (1997)
29. Parker, L., and D. Y. Stainier: Cell-autonomous and non-autonomous requirements for the zebrafish gene cloche in hematopoiesis. *Development* 126, 2643-2651 (1999)
30. Liao, W., C. Y. Ho, Y. L. Yan, J. Postlethwait, and D. Y. Stainier: Hhex and scl function in parallel to regulate early endothelial and blood differentiation in zebrafish. *Development* 127, 4303-4313 (2000)
31. Liao, E. C., B. H. Paw, L. L. Peters, A. Zapata, S. J. Pratt, C. P. Do, G. Lieschke, and L. I. Zon: Hereditary spherocytosis in zebrafish riesling illustrates evolution of erythroid beta-spectrin structure, and function in red cell morphogenesis and membrane stability. *Development* 127, 5123-5132 (2000)
32. Paw, B. H.: Cloning of the zebrafish retsina blood mutation: a genetic model for dyserythropoiesis and erythroid cytokinesis. *Blood Cells Mol Dis* 27, 62-64 (2001)
33. Trede, N. S., A. Zapata, and L. I. Zon: Fishing for lymphoid genes. *Trends Immunol* 22, 302-307 (2001)
34. Giebel, L. B., and R. A. Spritz: Mutation of the KIT (mast/stem cell growth factor receptor) protooncogene in human piebaldism. *Proc Natl Acad Sci U S A* 88, 8696-8699 (1991)
35. Parichy, D. M., J. F. Rawls, S. J. Pratt, T. T. Whitfield, and S. L. Johnson: Zebrafish sparse corresponds to an orthologue of c-kit and is required for the morphogenesis of a subpopulation of melanocytes, but is not essential for hematopoiesis or primordial germ cell development. *Development* 126, 3425-3436 (1999)
36. Parichy, D. M., D. G. Ransom, B. Paw, L. I. Zon, and S. L. Johnson: An orthologue of the kit-related gene fms is required for development of neural crest-derived xanthophores and a subpopulation of adult melanocytes in the zebrafish, Danio rerio. *Development* 127, 3031-3044 (2000)
37. Yoshida, H., S. Hayashi, T. Kunisada, M. Ogawa, S. Nishikawa, H. Okamura, T. Sudo, and L. D. Shultz: The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature* 345, 442-444 (1990)
38. de Vernejoul, M. C., and O. Benichou: Human osteopetrosis and other sclerosing disorders: recent genetic developments. *Calcif Tissue Int* 69, 1-6 (2001)
39. Drummond, I. A., A. Majumdar, H. Hentschel, M. Elger, L. Solnica-Krezel, A. F. Schier, S. C. Neuhauss, D. L. Stemple, F. Zwartkruis, Z. Rangini, W. Driever, and M. C. Fishman: Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. *Development* 125, 4655-4667 (1998)
40. Dressler, G. R.: Kidney development branches out. *Dev Genet* 24, 189-193 (1999)
41. Wilson, P. D.: Epithelial cell polarity and disease. *Am J Physiol* 272, F434-442 (1997)
42. Malicki, J., S. C. Neuhauss, A. F. Schier, L. Solnica-Krezel, D. L. Stemple, D. Y. Stainier, S. Abdelilah, F. Zwartkruis, Z. Rangini, and W. Driever: Mutations affecting development of the zebrafish retina. *Development* 123, 263-273 (1996)
43. Gusmano, R., G. M. Ghiggeri, and G. Caridi: Nephronophthisis-medullary cystic disease: clinical and genetic aspects. *J Nephrol* 11, 224-228 (1998)
44. Warren, K. S., and M. C. Fishman: "Physiological genomics": mutant screens in zebrafish. *Am J Physiol* 275, H1-7 (1998)
45. Fishman, M. C., D. Y. Stainier, R. E. Breitbart, and M. Westerfield: Zebrafish: genetic and embryological methods in a transparent vertebrate embryo. *Methods Cell Biol* 52, 67-82 (1997)
46. Horne-Badovinac, S., D. Lin, S. Waldron, M. Schwarz, G. Mbamalu, T. Pawson, Y. Jan, D. Y. Stainier, and S. Abdelilah-Seyfried: Positional cloning of heart and soul reveals multiple roles for PKClambda in zebrafish organogenesis. *Curr Biol* 11, 1492-1502 (2001)
47. Baker, K., K. S. Warren, G. Yellen, and M. C. Fishman: Defective "pacemaker" current (Ih) in a zebrafish mutant with a slow heart rate. *Proc Natl Acad Sci U S A* 94, 4554-4559 (1997)
48. Warren, K. S., K. Baker, and M. C. Fishman: The slow mo mutation reduces pacemaker current and heart rate in adult zebrafish. *Am J Physiol Heart Circ Physiol* 281, H1711-1719 (2001)
49. Towbin, J. A., and T. C. McQuinn: Gridlock: a model for coarctation of the aorta? *Nat Med* 1, 1141-1142 (1995)
50. Weinstein, B. M., D. L. Stemple, W. Driever, and M. C. Fishman: Gridlock, a localized heritable vascular patterning defect in the zebrafish. *Nat Med* 1, 1143-1147 (1995)
51. Zhong, T. P., M. Rosenberg, M. A. Mohideen, B. Weinstein, and M. C. Fishman: gridlock, an HLH gene required for assembly of the aorta in zebrafish. *Science* 287, 1820-1824 (2000)
52. Alexander, J., D. Y. Stanier, and D. Yelon: Screening mosaic F1 females for mutations affecting zebrafish heart induction and patterning. *Dev Genet* 22, 288-299 (1998)
53. Hammerschmidt, M., G. N. Serbedzija, and A. P. McMahon: Genetic analysis of dorsoventral pattern formation in the zebrafish: requirement of a BMP-like ventralizing

activity and its dorsal repressor. *Genes Dev* 10, 2452-2461 (1996)
54. Wallis, D. E., and M. Muenke: Molecular mechanisms of holoprosencephaly. *Mol Genet Metab* 68, 126-138 (1999)
55. Van Epps, H. A., C. M. Yim, J. B. Hurley, and S. E. Brockerhoff: Investigations of photoreceptor synaptic transmission and light adaptation in the zebrafish visual mutant nrc. *Invest Ophthalmol Vis Sci* 42, 868-874 (2001)
56. Gerlai, R., M. Lahav, S. Guo, and A. Rosenthal: Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. *Pharmacol Biochem Behav* 67, 773-782 (2000)
57. Chambers, S. P., A. Dodd, R. Overall, T. Sirey, L. T. Lam, G. E. Morris, and D. R. Love: Dystrophin in adult zebrafish muscle. *Biochem Biophys Res Commun* 286, 478-483 (2001)
58. Long, Q., H. Huang, E. Shafizadeh, N. Liu, and S. Lin: Stimulation of erythropoiesis by inhibiting a new hematopoietic death receptor in transgenic zebrafish. *Nat Cell Biol* 2, 549-552 (2000)
59. Beckwith, L. G., J. L. Moore, G. S. Tsao-Wu, J. C. Harshbarger, and K. C. Cheng: Ethylnitrosourea induces neoplasia in zebrafish (Danio rerio). *Lab Invest* 80, 379-385 (2000)
60. Spitsbergen, J. M., H. W. Tsai, A. Reddy, T. Miller, D. Arbogast, J. D. Hendricks, and G. S. Bailey: Neoplasia in zebrafish (Danio rerio) treated with 7,12-dimethylbenz[a]anthracene by two exposure routes at different developmental stages. *Toxicol Pathol* 28, 705-715 (2000)
61. Spitsbergen, J. M., H. W. Tsai, A. Reddy, T. Miller, D. Arbogast, J. D. Hendricks, and G. S. Bailey: Neoplasia in zebrafish (Danio rerio) treated with N-methyl-N'-nitro-N-nitrosoguanidine by three exposure routes at different developmental stages. *Toxicol Pathol* 28, 716-725 (2000)
62. Peterson, R. T., B. A. Link, J. E. Dowling, and S. L. Schreiber: Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc Natl Acad Sci U S A* 97, 12965-12969 (2000)
63. Thisse, C., H. Neel, B. Thisse, S. Daujat, and J. Piette: The Mdm2 gene of zebrafish (Danio rerio): preferential expression during development of neural and muscular tissues, and absence of tumor formation after overexpression of its cDNA during early embryogenesis. *Differentiation* 66, 61-70 (2000)

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