Advancing human disease research with fish evolutionary mutant models

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Abstract

Model organism research is essential to understand disease mechanisms, but laboratory-induced forward and reverse genetic models usually lack genetic variation and often fail to mimic the full spectrum of disease severity. Evolutionary mutant models (EMMs) of human diseases are species with evolved phenotypes that mimic maladaptive human diseases. EMMs complement traditional laboratory models by providing unique avenues to study gene-by-environment interactions, genetic variants and modular mutations in non-coding regions that reflect the spectrum of disease severity and their evolved compensations. EMMs have improved our understanding of complex diseases including cancer, diabetes, and aging, and illuminated disease mechanisms in many organ systems. Rapid advancements of sequencing technologies and genome editing tools have catapulted the utility of EMMs and led to a boom in the discovery, description, and use of EMMs, particularly in fish. The deep radiation of fish, the most diverse group of vertebrates, generated a kaleidoscope of specialized phenotypes, many that would be pathogenic in humans but are adaptive in the species' specialized habitat. Importantly, evolved compensations can suggest avenues for novel disease therapies. This review summarizes current research using fish EMMs to advance our understanding of human diseases.

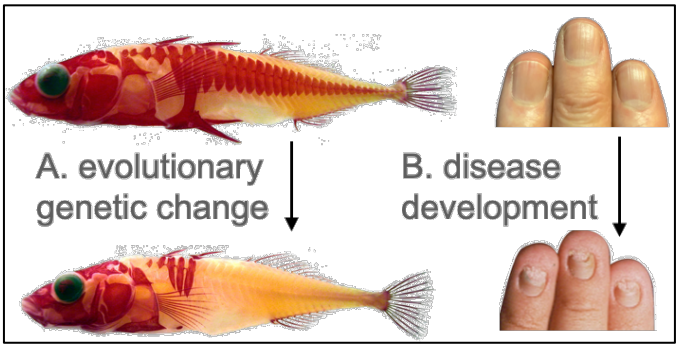
Evolutionary mutant models in precision medicine

Humans differ in the probability of developing disease, the severity of disease phenotypes, and in their responses to disease prevention efforts and treatments, in part due to standing genetic variation among individuals [1–3]. Understanding the influence of each individual's genetics on disease etiology led to advances in the field of "precision" medicine through the development of therapies tailored to genotypes [4]. Research on human patients is critical for revealing connections between genetic variation and differences in health outcomes but is difficult to perform, often involves isolated material such as cell lines rather than intact organisms, and uses Genome Wide Association Studies (GWAS) in which variation is often limited to specific populations.

Model organism research facilitates diagnosis, mechanistic understanding, and searches for therapies for human diseases and relies on forward and reverse genetic screens [5,6]. Forward genetic screens identify desired phenotypes in a randomly mutagenized population followed by characterizing the mutant genotype. Traditionally, this is accomplished using laboratory models such as worms, flies, zebrafish, and medaka and provides enormously successful models to identify genetic variants underlying an observed phenotype [7]. Human medical genetics is fundamentally a forward genetic screen where the patient displays a phenotype that geneticists link to a specific mutated human gene. Forward genetic screens in model organisms, however, often identify only variants exhibiting extreme phenotypes arising from single gene defects. In contrast, many human diseases, especially chronic pathologies, are rarely caused by full loss-of-function alleles, are often polygenic, occur in our highly outbred population, and can involve altered regulatory regions. Conversely, reverse genetic experiments generate desired genotypes by targeted mutagenesis, made possible with the advent of recombinant DNA, followed by identification of the mutant phenotype [5,8]. Nevertheless, reverse genetics still struggles with phenotypes caused by multiple loci, involve pleiotropy, or appear only with age.

An important addition to forward genetics and reverse genetics comes from evolutionary genetics: evolutionary mutant models (EMM) are animals with evolved phenotypes that mimic maladaptive human diseases. Evolutionary genetic screens take advantage of natural phenotypic traits and provide an alternative for discovering underlying genetic variants without the need for laboratory mutagenesis. Instead, EMMs arise when natural selection or **genetic drift** (see Glossary) shifts the frequencies of alleles underlying phenotypes within populations. While such phenotypes mimic diseases in humans, these traits may be adapted to the particular environment the EMMs inhabit [9]. In some cases, changes involve variants in **orthologs** of known disease genes and result in phenotypes directly mimicking human diseases. These genetic variants and subsequent EMMs arise gradually through similar natural processes as human disease variants, and not from chemical mutagenesis as in forward genetics or recombinant DNA technology as in reverse genetics. Furthermore, EMM organisms can be amenable to reverse genetics, such as CRISPR-Cas9 genome editing. The utility of EMMs for forward and reverse genetics make them ideal bridges between human and laboratory animal model research (Box 1).

Box 1 Evolutionary Mutant Model Strategy



Evolutionary mutant models (EMMs) of human disease show evolved phenotypes that mimic human disease but nevertheless suit the species in its environment. For example, anadromous stickleback fish have heavy bony plates in their skin. Thousands of times in Asia, North America, and Europe, anadromous stickleback became landlocked in freshwater lakes. Over occasionally remarkably short periods of time, freshwater stickleback evolved into forms with greatly reduced ectodermal plates (Figure A) [10,11]. Some human diseases, like ectodermal dysplasia, also disrupt ectodermal modifications including small nails, hair loss, reduced sweat glands and missing teeth (Figure B: Photo Credit Bottom: National Foundation for Ectodermal Dysplasias [i]).

Understanding evolved molecular genetic mechanisms that cause a disease-like phenotype to develop in the EMM can lead to novel insights into the etiologies of human disease. In the case of stickleback plates, crosses of anadromous individuals to freshwater morphs led to the mapping of this feature to the stickleback *ectodysplasin* gene [12,13]. Likewise, mutations in the human *ectodysplasin* gene give rise to ectodermal dysplasia disease in people, including reduced and malformed nails (Figure B) [14]. Investigating the molecular genetic mechanisms that integrate the EMM's disease-like phenotype into a functioning whole might provide insights into managing human disease.

EMMs can model the spectrum of genetic complexity in human disease

The spectrum of human disease phenotypes is based on genetic complexity and environmental interactions. Some human diseases, like cystic fibrosis [15], are genetically simple, resulting from mutation(s) in a single gene, and are well suited for study in classical reverse genetic models. More commonly, however, a disease can occur due to mutations in and of several different genes (e.g., Fanconi Anemia [16]) or when variants in several genes aberrantly interact (e.g., in Alzheimer's disease [17]). Sometimes, different phenotypes can arise from different alleles of the same gene (complete loss-of-function alleles (amorphs), gain-of-function alleles (neomorphs), or over- or under-expression alleles (hypermorphs or hypomorphs, respectively)) [18,19]. Additional nuanced genetic changes, including suboptimal gene interactions or incompatibility between a mutant gene product and other substances (e.g., proteins, RNAs, or metabolites) can cause a spectrum of disease phenotypes emerging from variants at one or more loci with altered, but not entirely null, function [20–23]. In addition, some pathogenic phenotypes may only appear when certain genotypes experience special environmental conditions such as variations in regulatory elements gradually diminishing the expression of lactase in adults, resulting in lactose intolerance [24]. EMMs provide a wide range of genetic models from genetically simple diseases with low environmental influence, to polygenic diseases with high environmental influence [9].

EMMs can identify background-specific disease variants

Studying diseases in a small number of genetic backgrounds is problematic because disease-related genetic variants may only become apparent on a specific genetic background. In humans, **epistatic variation** can lead to ranges of disease severity in different populations. For example, variants of alpha- and beta-globins, including variation up-regulating gamma-globin, interact in complex ways influencing the severity of malaria in a population-dependent manner across sub-Saharan Africa [25]. Additionally, environmental conditions impact some genetic backgrounds more than others, in gene-by-environment (GxE) interactions. For example, diabetes in Pima people living in Arizona is higher than in non-Native American populations and is much higher than genetically related Pima people living in Mexico (38% vs. 7%) [26].

EMMs harbor genetic variation absent in inbred laboratory models, and can be used in manipulative, controlled studies to understand GxE interactions. Specifically, EMMs can help identify population-specific disease variants by studying genetically diverse populations in a common environment and are amenable to powerful techniques to identify population-specific variants such as **Quantitative Trait Locus (QTL)** Mapping or GWAS [27,28]. Chances of encountering reduced **linkage disequilibrium**, increased recombination rates, and often quite advanced backcrosses in natural populations increase the power and resolution of these techniques to detect relevant loci underlying phenotypes [28]. These techniques are commonly used to study disease resistance by crossing resistant and non-resistant individuals and comparing the genetics and resistance levels of offspring [29]. Also, because natural populations can be locally adapted to differing environments, new avenues for studying the impact of the environment and genetic background on phenotypic variation can be explored under natural conditions by crossing individuals adapted to different areas either in a

laboratory setting or in naturally existing hybrid zones where individuals from different environments interbreed [30].

EMMs can model non-coding genetic variants

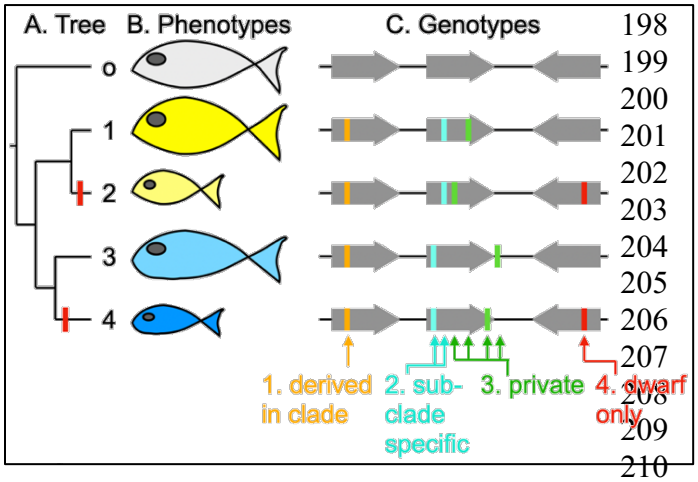
The study of non-coding genetic variants is essential to understand complex human diseases because 88% of loci linked to diseases in GWAS are in non-coding regions [31]. Human disease research, however, focuses mostly on coding regions even when associated variants are identified in non-coding regions. For example, multiple GWAS identified the first intron of the dioxygenase gene *FTO* to be associated with obesity, leading to extensive study of *FTO* in obesity research [32]. No direct links, however, were found between *FTO* expression and obesity. Instead, a long-range enhancer in this region that regulates *IRX3* several megabases away was identified that has direct links to obesity [32].

Some conserved regulatory functions lie in **conserved non-coding elements (CNEs)** that become apparent in whole-genome alignments performed across multiple species [33]. Comparing genetically diverse species with phenotypic characters of interest can reveal CNEs with specific trait associations. These types of associations have been used to identify CNEs altered in human diseases, such as the disruption of an *IRF6* enhancer binding site in cleft lip and palate [34] and a mutation in a *RET* enhancer in Hirschsprung Disease [35].

Due to a high level of sequence and synteny conservation within mammals, DNA sequence alignments with fishes are particularly valuable for identifying and assigning CNEs to their target gene [36]. Missing or highly derived CNEs in EMMs may indicate regulatory elements involved in the development of their unique features; therefore, these CNEs are candidate targets to explore for the diseases they model. For example, syngnathid fishes are potential models for craniofacial disorders (they are toothless and possess uniquely shaped craniofacial bones adapted to allow for rapid suction feeding) [37]. Researchers identified a deeply conserved CNE in an intron of *dlx1a* that was lost in the Gulf pipefish, a syngnathid [38]. Based on the role of *dlx1* in craniofacial development, this CNE is a potential target to explore in the context of human disease [39].

Variants of CNE or other regulatory mutations can also be identified in EMMs by crossing EMMs from different populations and comparing gene expression patterns to phenotypes via **expression QTL mapping (eQTL)** or phylomapping (Box 2) [27]. Non-coding mutations also can cause nuanced changes in gene expression that may contribute to the spectrum of disease severity [9]. For example, people with hypodontia develop many fewer than the normal number of teeth and those with hyperdontia develop many more teeth than normal [40]. Stickleback fish model these conditions. Benthic freshwater stickleback populations have twice as many pharyngeal teeth as marine populations [41]. Crosses of hypodontic by hyperdontic stickleback and gene expression analyses showed that responsible changes were in regulatory elements for *bmp6* (bone morphogenetic protein-6) leading to increased *bmp6* expression in the population with more teeth [41,42].

Box 2 Phylomapping for Fish Evolutionary Mutant Models



Phylomapping is an important methodology to identify candidate loci that underlie naturally occurring phenotypes mimicking human disease [43]. Phylomapping involves a clade with an outgroup (o) and inferred phylogenetic relationships to orient evolutionary change (Fig. A). The clade contains multiple lineages that independently evolved disease-like phenotypes, for example, skeletal development that mimics dwarfism (Fig. B, species 2 and 4). Comparing genome sequences within the clade compared to the outgroup can identify: 1) variants derived and shared by the queried clade, 2) variants unique to various subclades, 3) variants private to each species, and 4) lesioned genes or regulatory motifs that are shared only by lineages showing the disease-like phenotype (Fig. C). If the shared, lesioned, phenotype-associated genes have the same genomic changes in different species showing the phenotype, then they might be derived by lineage sorting from standing genetic variation, as has occurred in stickleback [10,12,44]. Alternatively, lesioned genes shared by independently derived evolutionary mutant model phenotypes might have different molecular genetic changes in the same genes, indicating independently derived mutations leading to similar phenotypes.

Mutated candidate genes in laboratory models, like zebrafish or medaka, that result in phenotypes similar to the EMM support the hypothesis that the candidate gene causes the observed 'pathogenic' phenotype. This approach led to the identification of fibroblast growth factor receptor variants as candidates for the reduction of bony scales in fishes of the genus *Phoxinellus* [43]. Future phylomapping studies of phenotypes that vary within a specific clade, such as longevity, pigment, craniofacial features, behavioral traits, and many other characters, promise to identify not only protein-coding genes, but also potential regulatory elements and non-coding genes associated with the evolution of disease-like traits.

The Utility of Fish EMMs

EMMs for human diseases exist in a diverse array of animal groups including anoles as models for congenital heart disease [45] and giraffes and pikas as models for hypertension [46,47]. Recently, EMMs with superior antiviral defenses including pangolins, bats, and naked mole rats have proven particularly useful in the study of highly transmissible viruses like SARS-CoV-2 [48]. EMMs, however, are especially prevalent in fishes for a variety of reasons. The clade of ray-finned fished (*Actinopterygii*), to which the models in this review belong, contains roughly half of all extant vertebrate species [49–54]. The ancient origin of this clade, in combination with the often-large population sizes and extensive geographic ranges of species in this group has likely contributed to the clade being very speciose. Phenotypic diversity arises from the wide range of habitats in which fish have evolved. Adaptations to extreme temperatures, salinities, light levels, water pressures, and primary producer abundances resulted in wide-ranging phenotypes. Genome duplication events, which are numerous among actinopterygian lineages [50–53,55–58], also likely provided a substrate for diversification by increasing gene numbers and relaxing molecular evolutionary constraints [53,55]. Gene duplicates have greatly expanded avenues for evolution providing multiple copies of genes that can evolve new functions (neofunctionalization) or partition pre-existing functions (subfunctionalization) [56,57]. In some cases, these duplicates contribute to highly derived morphologies and unique adaptations that can be used to study orthologous disease phenotypes in humans. Advances in molecular and genomic tools have greatly expanded EMM utility; many of these organisms now have high-quality reference genome sequences and can be manipulated using laboratory techniques such as CRISPR-Cas9 genome editing. Below, we describe seven fish EMMs used to tackle research on nine groups of human diseases (Box 3; Table 1; Fig 1; Fig 2).

Box 3 Validating Evolutionary Mutant Models for Human Disease.

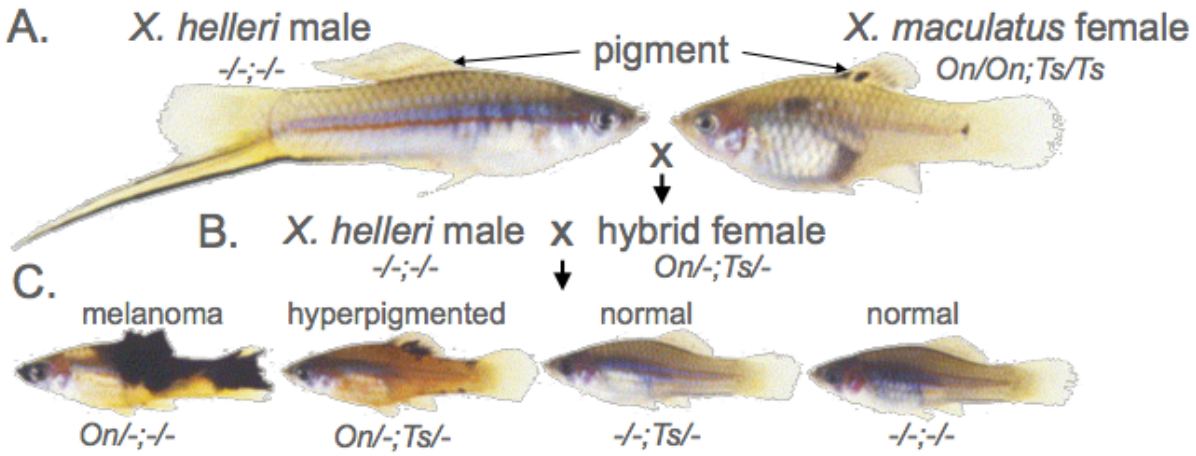
From November 2020 to January 2021, the National Institutes of Health (ORIP, NHLBI, NIA, NIDDK, NIGMS, NINDS) held a workshop entitled: *Validation of Animal Models and Tools for Biomedical Research* [ii]. Principles from those workshops apply to models of human disease in general and include three types of validation.

Face Validity*:* Does the model *replicate* human disease clinical findings? Are the pathological phenotypes similar? Do they involve corresponding organs and cell types? Face validity can be achieved in fish EMMs because fish and humans share vertebrate organs and organ systems.

Construct Validity*:* Does the model *reflect the mechanisms* of the human disease? The model should involve orthologous genes with similar gene expression patterns, utilize similarly acting non-coding elements, and display comparable epigenetic marks; the model's proteins should share intracellular locations with the corresponding human protein; and the model should react to pharmacological agents as people do. Genomic studies showed that fish have orthologs of at least 82% of human genes with morbidity descriptions [59].

Predictive validity: Can the EMM *foretell* currently unknown aspects of human disease? The true value of EMMs is that they can lead to a deeper understanding of disease mechanisms and thus to previously unknown disease features researchers can verify in human patients, potentially leading to novel disease therapies.

A case study: EMM validation and hybrid Xiphophorus malignant melanoma.



Melanoma is one of the deadliest forms of cancer, with post-diagnosis survival averaging only about a year [60,61]. The earliest fish EMM, a small Mexican fish in the genus *Xiphophorus* described in 1927, has provided a number of important insights into malignant melanoma (MM) [62–65]. The southern platyfish (*X. maculatus*) has a dark pigment spot on the dorsal fin but the green swordtail (*X. helleri*) lacks this pigmentation state (Fig. A; photo credit Manfred Schartl). A quarter of hybrid backcrossed individuals of these *Xiphophorus* species develop MM; a quarter show hyperpigmentation; and half are normal [64,66–68], as expected from Mendelian segregation of two unlinked epistatic loci (Fig. B, C; photo credit Manfred Schartl).

***Xiphophorus* MM shows Face Validity** because it replicates clinical findings in human MM. Fish have melanocytes in their epidermis like humans, whereas mice have melanocytes in hair bulbs but not their skin [69]. Furthermore, *Xiphophorus* MM proceeds from pigmented nevi through radial growth, then into a vertical invasive growth phase, as do human melanomas.

***Xiphophorus* MM shows Construct Validity** by reflecting the mechanisms of human MM. The platyfish melanoma driver oncogene is a constitutively activated EGF-receptor (EGFR) homolog called *xmrk* [66]. This discovery reflects the importance of the mutation of *EGFR* in human melanoma, malignant glioma, lung cancers, and more. In addition, the transcriptional disease signature of *Xiphophorus* MM matches human melanoma transcriptomes [70] suggesting that orthologous genes and pathways lead to the same pathologies by the same genetic and cellular mechanisms in fish and humans. Additionally, drugs that are effective in fighting human melanoma, like Trametinib and Cisplatin, also act on *Xiphophorus* MM [70], confirming shared pathogenic mechanisms of MM in fish and humans.

***Xiphophorus* MM has Predictive Validity** because results from *Xiphophorus* studies forecast findings shown later for human MM. *Xiphophorus* MM genetics first introduced the concept of tumor promotor genes (now called oncogenes) and tumor suppressor genes [71]. *Xiphophorus* results predicted that the RTK/RAS/RAF/MAPK pathway is the critical driver for human melanoma a decade before it was detected in humans [72]. *Xiphophorus* studies predicted a mechanism of interferon resistance in first-line melanoma treatments [73]. Osteopontin was first identified in *Xiphophorus* MM as a key factor in the transition from radial to vertical growth, and this finding led to its use in diagnostics and as a prognostic marker in the clinic [73–75]. Recent results showed that *rab3d* is the *Xiphophorus* tumor suppressor gene [76]. Human RAB3D is involved in exocytosis [77] and its elevated expression promotes cancer progression and metastasis [78], but its role in MM in humans has not yet been shown. Studies of *rab3d* mechanisms in *Xiphophorus* may suggest new avenues for developing therapies for human melanomas.

Molecular identification of the *xmrk*; *rab3d* pair in *Xiphophorus* is particularly promising for studying novel melanoma therapies because small aquarium fish enable high-throughput screens for drug-like molecules with potential therapeutic use [79]. The demonstration that the *Xiphophorus* model for human malignant melanoma meets criteria for face, construct, and predictive validation verifies its use as an important EMM for human disease.

African Turquoise Killifish, a Model of Aging

Aging is the primary risk factor for many pathologies, with age-related diseases affecting most aspects of human health. Unfortunately, modeling age-related diseases, particularly in vertebrates, is difficult due to long lifespans [80]. While invertebrate models of aging, including flies and nematodes, have been useful in studying highly conserved age-related genetic pathways, invertebrates lack (or have ancestral versions of) many critical human organ systems (e.g., cartilage and bone, spleen, gall bladder) and cannot be used to study the effects of aging in those organs relevant to age-related diseases [81]. Organisms with exceptionally long lifespans are used to study aging pathways and age-related diseases [82–84], but genetic analysis of aging can also be performed in an expedited fashion using short-lived species. Importantly, employing phylomapping or other genome wide comparisons between short- and long-lived species could help identify relevant genetic changes in age-related pathways (Box 2).

The African Turquoise Killifish *Nothobranchius furzeri* is a unique short-lived model for aging because it has the shortest lifespan (4-9 months) of any vertebrate that can be kept in captivity [85,86], shares vertebrate organs and genes with humans, and exhibits typical age-dependent pathologies (e.g., cognitive decline, loss of fertility, and cancer) [81]. Their telomere length is also more similar to that in humans than mammalian models such as mice [81], making them useful to study mechanisms of aging. While similar telomere size is observed in other fish species, including zebrafish (*Danio rerio*), studies of genetically manipulated African Turquoise Killifish can be more rapidly studied due to their shortened lifespan and early onset of age-related pathologies, including telomere shortening, making them useful to tackle high-throughput screening of age-related genes. Importantly, variation of lifespan varies across populations providing unique avenues for comparative genomics between populations to identify genetic changes associated with changes in lifespan [87].

The recent assembly and analysis of the African Turquoise Killifish genome helped elucidate the complex genetics of aging [85]. Molecular clock studies identified 497 genes evolving adaptively, 22 of which were previously associated with roles in truncated lifespan in mouse and human [85]. Some of these genes were known human disease genes. Specifically, *rtel1* showed evidence of adaptive evolution in African Turquoise Killifish and functions as a helicase involved in telomere elongation [85]. In humans, loss of *RTEL1* function is tied to dyskeratosis congenita, a disease characterized by shortened telomeres and premature aging [88]. The same molecular clock study also revealed adaptive evolution in *lmna(3of3)* [85]; mutations of the human ortholog can cause Hutchinson-Gilford Progeria Syndrome associated with decreased longevity [89]. Rapid evolution of these aging-related genes in African Turquoise Killifish provides evidence of conserved genetic mechanisms of aging and illustrated the value of studying aging in this system. In 12 other cases, comparisons with exceptionally long-lived species including the naked mole rat, Brandt's bat, and the bowhead whale revealed similar signals of positive selection although on different residues including positive selection on the insulin/IGF signaling gene *igf1r* in Brandt's bat and the marmoset, the shortest-lived primate [85]. Interestingly, this study also identified genes that may play roles in aging but have not yet been linked to age-related diseases in other animal models or humans and included Gene Ontology enrichment of genes involved in signal transduction pathways, metabolism, development, proteostasis, and

immunity [85]. Future functional analysis of these genes is needed to discern their roles in aging.

African Turquoise Killifish provides an example of how study of EMMs can lead to novel disease therapies [90]. In humans and in African Turquoise Killifish, aging is associated with a decrease in the number of species in the gut microbiome. This finding raised the possibility of a novel treatment – microbiome transfer – to slow the process of aging [90]. The transfer of the gut microbiome of young fish to middle-aged fish resulted in increased longevity of the recipient [90]. Importantly, weeks after transfer, the diversity of the recipient microbiota in the recipient was much greater than in the control group, meaning the recipient's microbiome continued to resemble that of a younger fish. RNA-seq on host tissue also demonstrated microbial transfer impacts on gene expression of pathways typically associated with younger fish including proliferation as well as ribosomal genes *rps* and *rpl* associated with aging [90]. These experiments suggest the African Turquoise Killifish could provide insight into alternative mechanisms to slow the process of aging and suggest new therapies for aging via diet, fecal transfer, probiotics, or whole microbiome transfer.

Notothenioids, Models of Skeletal Dysplasias

Skeletal dysplasias are a heritable group of more than 450 disorders that primarily affect bone and cartilage formation and occur in approximately one in 5,000 births [91]. Among skeletal dysplasias, Osteogenesis Imperfecta (OI, "brittle bone disease") presents at birth and results in bones that can break easily, often from little or no apparent trauma. The severity of this disorder varies among affected people and ranges from mild cases increasing the risk of bone fractures to severe forms causing death before or shortly after birth [92,93].

Notothenioids (*Notothenioidei*) have unique adaptations, making them an excellent model to explore the genetic bases of skeletal dysplasias and OI. Notothenioids are endemic to Antarctic and sub-Antarctic waters and derived from a common ancestor confined to the sea floor due to a highly mineralized skeleton and the absence of a swim bladder, the organ that allows fish to achieve neutral buoyancy. As species diversified, some lineages independently colonized the food-rich water column left vacant by species unable to survive the freezing waters of Antarctica [94]. The transition into the water-column was accompanied by increased buoyancy, which in most cases resulted from reducing "heavy" body parts like bones, and accruing "lighter" substances, such as lipids [95,96].

Notothenioids had previously been proposed as a model for osteopenia and osteoporosis [9,97], however, the developmental and morphological characteristics of the notothenioid skeleton do not solidly align with the progressive, degenerative nature of osteoporosis. Indeed, morphological and molecular skeletal analyses of notothenioids revealed developmental heterochronies (alteration in the timing and rates of bone development) generally reflecting paedomorphic characters. The most buoyant notothenioids show delayed mineralization of bony elements, slow rates of osteological development, smaller bones with persistent cartilage between bone veneers, or even bone loss (Fig 3) [98–100]. Thus, notothenioids do not lose bone as they age, but instead never form well-developed bones, hinting at developmental alterations similar to human skeletal dysplasias, including OI. Supporting this hypothesis, a recent phylogenomic study found that the genes *col1a1a* and *col1a2*, the human orthologs of

which account for up to 90% of OI cases in human [92,93], experienced positive selection in the evolutionary branch leading to the subgroup of notothenioids with reduced skeletons [101]. Furthermore, *col1a* gene mutations in zebrafish show phenotypes that mimic human OI [102], and during larval development of notothenioids, the chondrogenic genetic program, revealed by *col2a1* expression, persists, while an osteogenic program, revealed by *col1a1* expression, is delayed and reduced [103]. In addition, genes such as *sparc* and *notch2*, associated with OI Type XVII [93] and Hadju Cheney Syndrome, respectively, which display phenotypic features overlapping OI [92,104], are both required for craniofacial skeleton development in zebrafish [105,106] and both genes also experienced positive selection in the ancestor of notothenioids displaying skeletal reduction [101].

While previous genetic studies on notothenioid skeletal evolution queried only genes already known to be associated with bone development, future research should explore the role of candidate genes with as yet unknown roles in skeletogenesis to illuminate ways by which notothenioids evolved an adaptive reduced skeleton and potentially identify novel regulators of bone formation and maintenance in human. Furthermore, different lineages of notothenioids show varying degrees of skeletal reduction and different combinations of gene variants, thus displaying the potential to associate specific genetic variants with phenotypic variations, as seen in human patients.

Antarctic Icefishes, Models of Anemias

Anemias affect the abundance and morphology of red blood cells or the function of hemoglobin, which can result in decreased capacity to deliver oxygen to tissues and to return carbon dioxide for elimination. Anemias affect up to 27% of the world's population, mostly in developing countries, with young children and women of reproductive age being particularly affected [107]. The 16 species of white-blooded Antarctic icefishes (*Notothenioidei*, *Channichthyidae*) are unique among vertebrates because they lack functional hemoglobin genes, and in six species, also fail to express cardiac myoglobin [108]. Icefish blood, therefore, is opalescent white, and transports only about 10% as much oxygen as blood in a red-blooded relative, making them particularly sensitive to hypoxia [109] (Fig 4). Antarctic icefishes also do not form mature erythrocytes, with the most advanced erythropoietic cells displaying abnormal morphologies and fragility [110–112]. This extreme phenotype makes icefishes an excellent model for several human blood disorders, such as anemias, hemoglobinopathies, and thalassemias.

Genomic studies revealed that, of alpha- and beta-globin genes possessed by related red-blooded species, icefishes lost all except a single exon of one alpha-globin gene [113–115]. In addition to studies that aim to understand the genetic mechanisms associated with the loss of hemoglobin and the failure of erythropoiesis in icefishes [116–121], research efforts also focus on the physiological adaptations that enabled Antarctic icefishes to survive and diversify despite their maladaptive blood condition. Specifically, recent studies revealed evolutionary compensations in the vasculature supplying oxygen to the oxygen-thirsty retina [122,123], in the physiological capacities of hearts lacking hemoglobin and myoglobin [124–126], in the mitochondria [114,127], and in the innovation of a plasma-accessible carbonic anhydrase that catalyzes CO_2 excretion in the gills [128]. These adaptations show that the loss of hemoglobin in icefish is a harmful trait that required compensation. Understanding the genetic

mechanisms involved in these physiological compensations and tissue remodeling that permit Antarctic icefishes to survive has the potential to uncover alternative strategies to treat human anemias.

Electric Fishes, Models of Channelopathies

Channelopathies are diseases caused by aberrant function of ion channels that commonly impact the nervous, cardiovascular, respiratory, and immune systems [129]. Generally, channelopathies are caused by mutations that alter channel gating, causing improper fluctuations of ion concentrations. These mutations are tied to many common diseases, including epilepsy, cystic fibrosis, and cardiomyopathy, and are responsible for at least one in five cases of Sudden Infant Death Syndrome [129,130]. In electric fishes, electrocytes that compose electric organs contain highly modified ion channels. Ions move synchronously through these channels to produce electric organ discharges. Fine tuning of channel functions gives rise to a vast diversity of electric signals in these fish [131], performing many roles, including self-defense, navigation, and communication [132].

Mutations that alter channel function (such as altered channel inactivation time) allow electric fish to produce electric signals, but mutations in homologous genes in humans lead to channelopathies [133]. Electric organs in mormyrids (elephantfishes) and most gymnotiforms (knifefishes) are useful for modeling channelopathies afflicting skeletal muscle [134]. In these two fish lineages, the ion channel gene *scn4aa* experienced numerous amino acid substitutions at sites that cause disease phenotypes in the human homologue *SCN4A* [135]. One such mutation alters the inactivation gate that closes the channel leading to membrane hyperexcitability and likely aids in signal production for the electric organ but results in pathological muscle stiffness in humans [135,136]. Mutations induced in *scn4aa* via CRISPR/cas9 in both a mormyrid and gymnotiform resulted in decreased signal amplitude, demonstrating that the electric signal is modulated by changes in ion channel sequence, providing a novel and quantifiable method for testing genetic modifications on electric signals [137]. Recent work also showed interesting evolutionary histories of the mormyrid voltage-gated potassium ion channel gene *kcna7a.* In humans, KCNA7 is has been linked with two human cardiac disorders via mapping studies: progressive familial heart block type 1 (PFHB1) and isolated cardiac conduction (ICC) though no causative mutations have been found [138]. The earliest diverging member of the electric fish clade, *Gymnarchus niloticus,* retained a Kcna7a protein sequence similar to non-electric sister taxa, while other members of the clade accumulated changes indicating positive selection [139]. These genetic modifications cause differences in channel activation sensitivity by modifying the S3-S4 linker domain [139], a previously underexplored region of this protein. These studies show that electric fish are an excellent system to discover and explore mutations in ion channels that may underlie human channelopathies.

Mexican Cavefish, a Model of Diabetes and Metabolic Diseases

Metabolic diseases, particularly those related to the regulation of blood glucose, are pervasive threats to the wellness and longevity of hundreds of millions of people worldwide [140]. These concerns are increasing due to modern western diets that are especially high in sugars and fats [141], with obesity and diabetes recently reaching epidemic proportions [142]. Species adapted to extreme dietary conditions should be

useful for understanding metabolic diseases such as diabetes mellitus, hepatic steatosis, metabolic syndrome, and obesity.

Cave-dwelling populations of the Mexican tetra *Astyanax mexicanus* repeatedly evolved from ancestral riverine populations [143]. Brief periods of abundant food in caves are followed by long periods of nutritional deficit, so life in caves requires the ability to survive through extended stretches of starvation [143]. Cavefish show frenzied "binge eating" and an adaptive metabolism that favors long-term usage of stored energy reserves [144]. Interestingly, cave-adapted *A. mexicanus* exhibit many traits similar to metabolic disease states in humans [144–146]. For example, cave-dwelling Mexican tetra populations are insulin-resistant [145], a trait commonly observed in type-2 diabetes, but in Mexican tetras, this trait provides an advantageous metabolism without the negative effects of diabetes, including inflammation.

Insulin-resistance in cavefish is caused by reduced binding of insulin to its receptor due to a point mutation in the receptor. CRISPR-Cas9 genome editing to induce the same single-base-pair mutation in zebrafish resulted in an observed increase both in body mass and in insulin resistance [145]. Cave-dwelling and surface fish experience similar lifespans and similar advanced glycation end-product (AGE) levels in their blood, making *A. mexicanus* a potentially valuable model for understanding compensatory mechanisms to extreme insulin resistance [145]. Precise knowledge of the mechanisms by which cavefish limit accumulation of AGEs amid extreme blood glucose level fluctuations could ultimately lead to therapeutics. For instance, because excessive AGEs lead to vascular damage and ultimately cardiovascular diseases [147] and retinopathy [148], preventing the accumulation of AGEs could mitigate these severe symptoms of diabetes. Such treatments, perhaps through gene therapy approaches (e.g., mimicking AAV delivery of *FGF21* in diabetic mice [149]), could be revealed by future insights from the genetics of cavefish-derived metabolic pathways.

Mexican Cavefish, also a Model of Cardiac Regeneration

Globally, myocardial infarctions or heart attacks, are the most prevalent cause of human mortality. These deadly episodes result in the death of billions of cardiomyocytes and the deposition of a permanent fibrotic scar [150] caused by the inability of heart tissue to heal completely and regenerate. Several fish species are capable of varying degrees of regeneration including zebrafish which can regenerate cardiac tissue and have been useful in studying mechanisms of regeneration [150]. Interestingly, while the uniform way in which zebrafish are able to regenerate tissue has been useful in identifying important candidate genes and genetic pathways, comparisons between closely related individuals with differing morphologies is not possible in these species [150]. Instead, Mexican cavefish are being developed as a model for cardiac regeneration not only for their regenerative abilities, but for the population-specific manner in which they regenerate cardiac tissue. Surface-dwelling Mexican cavefish have pronounced differences in heart development, morphology, and ability to regenerate cardiac tissue compared to nearby cave-dwelling conspecifics including the ability to regenerate cardiac tissue providing an emerging system to study mechanisms underlying this potentially life-saving adaptation [151].

Morphologically, Pachón cavefish have smaller ventricles and a lower heart rate than surface-dwellers [152]. Surface-dwellers, however, are capable of cardiac regeneration, while Pachón cavefish form fibrotic scars as do humans after heart attacks [151].

Because Pachón cavefish and surface fish can interbreed, QTL analyses are feasible and identified genes associated with cardiac regeneration [150], including three extracellular matrix related genes, *si:dkeyp-69c1.7, ncam1a,* and *itgam,* that were not previously associated with heart regeneration [151]. Transcriptomic analysis of heart ventricle tissue of the two morphs revealed numerous genes associated with immune and scarring responses, including *lrrc10*, the human ortholog of which is associated with dilated cardiomyopathy and was upregulated in regenerating hearts of surface fish [151]. Follow-up reverse-genetic studies in zebrafish showed that *llrc10* knockout reduced regeneration, but not cardiomyocyte proliferation, therefore no scar was formed [151]. Important work comparing immunopathological responses between surface-dwelling and cave-dwelling cavefish provides a strong hypothesis for the origins of the differences in fibrotic scarring. Researchers identified differences in immune cell composition using single-cell approaches and demonstrated that while surface-dwellers maintain balanced use of the innate and adaptive immune systems, cave-dwellers rely heavily on the adaptive immune system, likely due to long-term adaptation to low parasite load [153]. This result means that cave-dweller immune systems are challenged less frequently, leading to altered robustness of certain immune responses, including more sensitive and prolonged immune response of proinflammatory cytokines [153]. These early experiments demonstrated the strength of *A. mexicanus* as an EMM for cardiac regeneration. Interbreeding cave and surface fish populations provide a unique opportunity to identify new loci and functionally test candidate genes including those identified in existing QTL datasets.

Mummichog, a Model of Mitochondrial Diseases

Unsuccessful interactions between mitochondrial and nuclear genomes can result in mitochondrial dysfunctions (mt-dys) that causes mitochondrial diseases [154–156]. Unfortunately, diagnosis of mitochondrial diseases has been historically challenging because many genetic pathways impacting mitochondrial activity remain undescribed. Primary Mitochondrial Diseases (PMD) are characterized by perturbed Oxidative Phosphorylation (OXPHOS) due to mutations in an OXPHOS protein or a protein that directly interacts with them. Mutations in either the mitochondrial genome or the nuclear genome can perturb OXPHOS function and causes diseases like Leigh syndrome (mitochondrial-encoded *ATP6*), Barth syndrome (nuclear-encoded *TAZ*), and Pearson syndrome (various mitochondrial gene deletions) [157]. PMDs present a wide range of symptoms but are commonly characterized by neuromuscular degeneration. As studies of mitochondrial activity have increased, however, common disorders previously unassociated with mt-dys have been re-characterized as secondary mitochondrial dysfunctions (SMD), including Alzheimer's disease, Parkinson's disease, Muscular Dystrophy, and Autism Spectrum Disorder [157]. Study of SMDs is complicated by the strong role of genetic variation in nuclear and mitochondrial genomes and the influence of the environment in mt-dys [157,158].

The mummichog *Fundulus heteroclitus* provides a unique system to understand the roles of mitochondrial and nuclear genetic variation on the progression of mt-dys. This species includes two morphologically distinct and interbreeding subspecies (*F. heteroclitus heteroclitus* and *F. heteroclitus macrolepidotus*) along the east coast of the United States with different mitochondrial haplotypes. These subspecies are largely separated at the Hudson River in New Jersey due to previous glaciation. The northern

population, *F. h. macrolepidotus*, lives in conditions up to 12 °C colder than the southern *F. h. heteroclitus.* In a hybrid zone between these interbreeding populations, **admixture** of mitotypes and nuclear alleles results in many mito-nuclear combinations that can be used to study the complex polygenic nature of mt-dys in the broader context of environmental and genotypic variations [159–161]. This hybridization population is particularly useful for studying the effects of two common environmental stressors on mt-dys: temperature and oxygen availability, which both influence mitochondrial physiology, with low temperatures and low oxygen both suppressing mitochondrial function and reducing metabolic rates [159,161]. This natural hybrid population of mummichogs has been utilized to study not only classical primary mitochondrial disease genetics, but nuclear genetic variants that might not have previously been associated with mt-dys or perhaps only result in mt-dys under certain environmental stressors [159]. More work is needed to fully characterize this system, though its evolutionary history has the potential to provide insight into the complex GxE interactions underlying mt-dys.

Threespine Stickleback, a Model of Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBD), which include ulcerative colitis and Crohn's disease, affect nearly seven million people worldwide [140] and cause chronic inflammation of the gastrointestinal tract that can lead to debilitating pain, anemia, and an increased risk for colorectal cancer [162,163]. The pathologic chronic inflammation of IBD can be identified using markers of inflammation including presence of excessive numbers of neutrophil cells. Several interacting factors are associated with IBD, including genetic variation, gut microbiome composition, and diet. Understanding the complex nature of these diseases at mechanistic and systems levels has proven to be a major challenge [164]. Experimentally tractable animal models with high levels of standing genetic variation, like threespine stickleback *Gasterosteus aculeatus*, have the potential to be especially useful for understanding IBD.

Countless natural stickleback populations were founded through repeated colonization of freshwater habitats by oceanic ancestors over hundreds of thousands of years, resulting in substantial inter-population differentiation in genotypes and phenotypes [12,165]. Recent experiments showed that the stickleback genotype influences the makeup of its gut microbiome [166,167], with some of the same host genotype-driven bacterial lineages comprising stickleback and mammalian guts [166,168]. Further investigation of oceanic and freshwater populations revealed important inflammation-related differences in the abundance of intestinal neutrophils [169]. The adaptation of gnotobiotic experimental procedures to stickleback allowed testing of how neutrophil abundance depends on the genetic background of the host in response to a microbial stimulus [169]. Controlled microbial exposure using gnotobiotic animals revealed a more robust microbial stimulation of neutrophil activity, indicating a more robust immune response to resident microbes, in an oceanic population compared to a fresh-water population thus mimicking humans with IBD [169]. A subsequent study of the same populations showed an especially large effect of genetic background on gene expression in the gut, with subtle indications of microbial and "host genotype-by-microbial" interactive effects on genes such as complement cascade components and *nlrc3s*, which are orthologs of known inflammatory genes in humans [170].

Leveraging differences in intestinal health evaluated by the number of neutrophils between these same oceanic and freshwater populations, an F2-intercross QTL mapping study identified candidate regions of the stickleback genome associated with increased neutrophil counts [171]. Several associated regions contained orthologs of human genes tied to neurological/neuromuscular syndromes that include secondary gut inflammation symptoms, such as Amyotrophic Lateral Sclerosis, Autism Spectrum Disorder, and Muscular Dystrophy [172–179]. This finding is significant because some neurological and neuromuscular diseases can be successfully slowed using microbial interventions [180,181]. Together, these studies suggest potential genetic links between neurological disorders and intestinal inflammation and support further exploration of dietary interventions for neurological diseases. Threespine stickleback therefore provide a needed model to test the impact of genetic variation and intestinal inflammation on systemic health.

Challenges and Future Directions

The discovery, description, and use of fish EMMs have greatly increased in the last ten years. Further exploration of fish EMMs is needed to advance our understanding of human disease. High-quality genome assemblies offer new avenues of research to elucidate complex genetic underpinnings of disease particularly though phylomapping. Importantly, molecular advances, including CRISPR-Cas9 genome editing, have made functional testing of candidate genes easier in outbred evolutionary mutant models. Attention to areas of fundamental science and searches for new EMMs will inform new directions for the study of human disease in the context of genetic variation, enhance our understanding of complex genetic diseases, and may lead to novel treatments not apparent in current inbred animal models. Still, there are several resources that need developed to advance fish EMM research (Box 4).

Additional challenges lay ahead in advancing the utility of fish EMMs. First, while fish EMMs are used to study multiple organ systems, differences between fish and human anatomy must be considered when applying findings in EMMs to human disease treatments. The ability to compare across species is further complicated when researchers do not have complete descriptions of molecular processes and systems in an EMM. For example, while fish immunology is a growing field, fundamental questions remain unanswered. First, the timing of onset of adaptive immunity across fish species varies, with different fish species developing essential components of adaptive immunity at vastly different developmental stages [182]. Fish and mammalian immune systems also differ some in composition, including a lack of fully formed germinal centers (GCs) in fish, which are important for B cell humoral immune responses. Instead, some fish, including Teleosts, rely on melanomacrophage centers (MMCs) which are not found in mammals [183]. Additionally, some fish EMMs are more amenable to large-scale studies than others depending on whether the species can survive and breed in captivity and the size of the fish (Table 1). In some cases, this limits high-throughput studies to larval or juvenile fish, or to adults from natural populations. Unfortunately, working with smaller organisms can also limit the amount of biological material that can be sampled from individuals, though these are not problems unique to fish EMMs. The lack of biological material can be particularly challenging when working with difficult to obtain samples such as blood, which can be drawn from fish, though this is not a trivial process [184].

Another growing area of significant benefit using fish EMMs is chemical and drug screening, particularly given the high throughput manner in which thousands of molecules can be screened across thousands of embryos [185–188]. Testing toxicity of compounds on large numbers of vertebrate animals can be useful in early screens. Zebrafish have been used in high-throughput screens of ototoxicity, epilepsy, and cancer, and have led to the development of FDA-approved drugs such as leflunomide used to treat arthritis [189], but there are some important considerations that must be accounted for when performing these trials. First, the method of delivery in fish is to submerge fish in the compounds being screened. Full submersion in chemicals is mechanistically very different than how drugs are commonly delivered in mammalian systems, can prevent tissue-specific treatment, and can require large quantities of compound which can be expensive. Estimating compound solubility is also an important part of drug development -- oral ingestion, the most common drug delivery method, may result in different availability to target cells compared to dissolving the same drug in the fish water [190]. Unfortunately, both pharmacokinetic-pharmacodynamic (PK/PD) modelling and compound solubility can be difficult in fish due to varying water temperature and salinity conditions impacting drug activity across species [190–192].

Finally, teleost fish researchers must always be aware of the teleost genome duplication event and the phenomenon of **ohnologs** gone missing, that paralogs from genome duplication events [53,193–196] that occurred before the divergence of tetrapod and teleost lineages were sometimes reciprocally shared between the two lineages, so that for a few genes, neither lineage has an ortholog of the most closely related gene in the other lineage, although their functions are likely to be quite similar.

These potential challenges of using and drawing inferences from models are more than offset by the benefits of the evolutionary diversity of EMMs. Together with traditional model organisms, and powered by the rapid advances in tools, the future is very bright. Expansion of work in fish EMMs will support continued scientific breakthroughs using the vast diversity of models created by nature's grand laboratory.

Box 4 Resources for aquatic evolutionary mutant models of human diseases.

To optimize contributions that fish EMMs can make to understand human health, a number of resources are needed that encompass methodological, informatic, phenotyping, and community needs; several such needs surfaced in a workshop entitled "Validation of Non-Zebrafish Aquatic Models for Preclinical Research" organized by the Division of Comparative Medicine at NIH [iii]

Methodological resource needs:

• *Protein localization tools*: antibodies that work across a broad range of fish species would help validate, or fail to validate, that proteins in an EMM occupy the same cells and sub-cellular compartments as they do in humans.

• *Precise genome editing tools:* Methods to replace the sequence in an EMM genome with the orthologous human sequence, and CRIMIC-like vectors [197] that work in fish to simultaneously knock-in reporter constructs while knocking out and replacing genes, would provide critical tests of sequences responsible for phenotypes.

• *Single cell transcriptome sequencing*: Widespread and inexpensive access to scRNA-seq methodologies, especially methods that retain spatial information [198] and scRNA-seq atlases of fish development [199] would provide cellular-level resolution of mechanisms underlying EMM phenotypes.

Informatic resource needs:

• *Gene nomenclature uniformity*: It is essential that true orthologs are identified among EMMs, other model organisms, and humans, a task that is not always straight forward due to the teleost genome duplication and ohnologs-gone-missing [53,193–196].

• *A pan-fish informatics database*: A database like ZFIN [iv] that curates orthologs, gene expression information, and literature across many fish EMM species that is linked to the Alliance of Genome Resources [v] would greatly improve the connectivity of EMMs with each other and to human genomic medicine.

Phenotyping resource needs:

• *A phenotype ontology*: The widespread use of a standard ontology for anatomical terms and descriptors for deviation from 'normal', like the one in ZFIN [vi] would foster accurate interspecies comparisons.

• *Fuller phenotype descriptions*: Researchers often focus solely on the single EMM phenotype that interests them rather than describing full EMM phenotypes. More complete descriptions would call attention of other researchers to phenotypes that may mimic human diseases that the original researcher hadn't recognized or can't study.

Community needs:

• *Conferences*: We encourage broad participation of EMM researchers at meetings such as the semi-annual 'Aquatic Models of Human Disease Conference' sponsored by the NIH Division of Comparative Medicine [vii].

• *Exploration*: EMMs often occupy extreme and rare habitats, many of which may disappear as climates change and humans encroach on wild spaces. The community must explore existing biodiversity for EMMs before these remarkable, beautiful, and useful animals become extinct.

Glossary

admixture: the presence of DNA from individuals of distantly related, formerly genetically isolated populations resulting from interbreeding.

Conserved Noncoding Element (CNE): a noncoding genomic region identified by sequence conservation among multiple species.

epistatic variation: genetic polymorphisms that interact to produce joint, non-additive effects on phenotypes.

expression QTL (eQTL): genomic loci that are correlated with variation of expression levels of transcripts.

genetic drift: changes in the relative frequency of alleles in a population due to chance

linkage disequilibrium: non-random association of alleles from different loci.

ohnolog: gene duplicates created by a whole genome duplication event.

ortholog: genes in different species that evolved from a single gene in the last common ancestor.

Quantitative Trait Locus (QTL): genomic loci that correlate with an observed quantitative trait.

syngnathid: a family of teleost fishes including seahorses, pipefishes, and seadragons characterized by an elongated snout and male pregnancy.

teleost: ray-finned fish that have a dorso-ventrally symmetrical tail fin, in contrast to basally diverging ray-finned fish like their sister group, the Holostei (bowfins and garfish), and more basally diverging fish, like Chondrostei (sturgeons and redfish), that have asymmetrical tail fins.

Competing Interests

The authors declare no competing interests.

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Figure Legends

Figure 1. Summary of fish evolutionary mutant models and affected organ systems.

Each circle includes a line drawing of a ray-finned evolutionary mutant model with common name and the diseased organ system primarily modeled in that species. (Mexican cavefish/heart and pancreas; Antarctic Icefish/bones and blood; Turquoise Killifish/ whole body; Platyfish/skin; Electric fish/whole body; threespine stickleback/intestines; Mummichog/brain). Lines connect these groups to their human counterpoints.

Figure 2. Summary of fish evolutionary mutant models and affected organ systems.

Phylogenetic relationships among the fish EMMs discussed in this review (in blue), in addition to the traditional model zebrafish (*Danio rerio*) and the non-**teleost** outgroup spotted gar (*Lepisosteus oculatus*). These relationships, published by Rabosky et al. 2018, are time-calibrated, with branch lengths in units of millions of years (MY) and scaled according to the bar below the tree. Also noted is the timing of the teleost genome duplication (TGD) event.

Figure 3. Antarctic Icefishes, Models of Anemias.

Compared to their red-blooded relative (e.g., A. Bullhead notothen), the 16 species of white-blooded icefishes lack functional hemoglobin genes (e.g., B. South Georgia icefish), and in six species, also fail to express cardiac myoglobin (e.g., C. Blackfin icefish). Compared to dark-red hearts of red-blooded species (A1), hearts of icefish species expressing myoglobin are pink (B1) and beige in icefish species failing to express myoglobin (C1). The blood of red-blooded species is dark-red and contains a large fraction of erythrocytes (A2; left, fresh blood; right, blood after centrifugation) that are ovoid (A3, Wright-Giemsa stain). In comparison icefish blood appears opalescent white and contains only limited amount of blood cells, majorly white-blood cells and thrombocytes (B2, C2). A few erythropoietic cells are present in icefish blood, however, they display abnormal morphologies and fragility (B3, C3). a, atrium; c, blood cells; e, erythrocytes; o, outflow tract; p, plasma; v, ventricle.

Figure 4. Notothenioids, Models of skeletal Dysplasias.

Morphological and molecular analyses of notothenioids revealed alterations in the timing and rates of bone development in several lineages. Compared to bottom dwellers (e.g., Humphead notothen), more buoyant notothenioids (e.g., Ocellated icefish) show delayed mineralization of bony elements, slow rates of osteological development, smaller bones, or even bone loss as seen in CT-scans of craniofacial regions of juveniles of comparable sizes.

Figure 5. Threespine Stickleback, A models of Inflammatory Bowel Diseases.

Threespine stickleback from various populations exhibit pronounced differences in neutrophil activity in the gut. Pictured here are two threespine stickleback gut sections stained for neutrophils (black dots). (A) Healthy stickleback gut with neutrophils primarily located outside intestinal villi (arrow). (B) Inflamed stickleback gut with neutrophils congregating inside the villi (box).

Table 1. Summary of fish evolutionary mutant models and their benefits as medical models.

Common Name	Scientific Name(s)	Disease	Breed in Captivity	Genome sequence Available
Swordtail Platyfish	*Xiphophorus helleri* *Xiphophorus maculatus*	Melanoma	Y	Y[68]
African Turquoise Killifish	*Nothobranchius furzeri*	Aging	Y	Y[85]
Notothenioid	*Notothenioidei*	Skeletal Dysplasia	Y/N	Y[113]
Antarctic Icefish	*Notothenioidei: Channichthyidae*	Anemias	N	Y[114]
Electric Fish	*Mormyridae* and *Gymnotiformes*	Channelopathies	Y	Y[134,200]
Mexican Cavefish	*Astyanax mexicanus*	Cardiac Regeneration Metabolic Disease	Y	Y[201]
Mummichog	*F. h. heterocolitis* *F. h. macrolepidotus*	Mitochondrial Disease	Y	Y[202]
Threespine stickleback	*Gasterosteus aculeatus*	Inflammatory Bowel Disease	Y	Y[203]

Resources:

i) https://www.nfed.org/
ii) https://orip.nih.gov/about-orip/workshop-reports
iii) https://orip.nih.gov/sites/default/files/Validation_Session_VII_Meeting_Report_Final_508.pdf
iv) http://zfin.org/
v) https://www.alliancegenome.org/
vi) https://zfin.org/action/ontology/search
vii) https://www.mbl.edu/aqmhd/

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