Genes & Diseases (2023) 10, 468–479

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

REVIEW ARTICLE

The recent advances and future perspectives of genetic compensation studies in the zebrafish model

Muhammad Abdul Rouf [a,1], Lin Wen [a,1], Yoga Mahendra [a], Jinxuan Wang [a], Kun Zhang [a], Shuang Liang [a], Yuming Wang [a], Zhenggong Li [b], Yeqi Wang [a,], Guixue Wang [a,*]**

[a] *Key Laboratory for Biorheological Science and Technology of Ministry of Education State and Local Joint Engineering Laboratory for Vascular Implants Bioengineering College of Chongqing University, Chongqing 400030, China*
[b] *Center of Cardiology, Chongqing General Hospital, University of Chinese Academy of Sciences, Chongqing 400013, China*

Received 27 June 2021; received in revised form 8 November 2021; accepted 1 December 2021
Available online 5 January 2022

KEYWORDS
Epigenetic machinery;
Genetic compensation;
Genetic robustness;
Knockdown-mediated;
Knockout-mediated;
NMD;
PTC;
Zebrafish

Abstract Genetic compensation is a remarkable biological concept to explain the genetic robustness in an organism to maintain its fitness and viability if there is a disruption occurred in the genetic variation by mutation. However, the underlying mechanism in genetic compensation remain unsolvable. The initial concept of genetic compensation has been studied in model organisms when there was a discrepancy between knockout-mediated and knockdown-mediated phenotypes. In the zebrafish model, several studies have reported that zebrafish mutants did not exhibit severe phenotype as shown in zebrafish morphants for the same genes. This phenomenon in zebrafish mutants but not morphants is due to the response of genetic compensation. In 2019, two amazing works partially uncovered genetic compensation could be triggered by the upregulation of compensating genes through regulating NMD and/or PTC-bearing mRNA in collaboration with epigenetic machinery in mutant zebrafish. In this review, we would like to update the recent advances and future perspectives of genetic compensation studies, which including the hypothesis of time-dependent involvement and addressing the discrepancy between knockout-mediated and knockdown-mediated to study gene

* Corresponding author.
** Corresponding author.
E-mail addresses: yeqi.wang@cqu.edu.cn (Y. Wang), wanggx@cqu.edu.cn (G. Wang).
Peer review under responsibility of Chongqing Medical University.
[1] These authors contributed equally.

https://doi.org/10.1016/j.gendis.2021.12.003

function in the zebrafish model. At last, the study of genetic compensation could be a potential therapeutic strategy to treat human genetic disorder related diseases.

Introduction

Henry David Thoreau, a well-known American philosopher, once said that, "If we will be quiet and ready enough, we shall find compensation in every disappointment." In the biological world, we might hear about *Dosage Compensation* and *Genetic Compensation* which are quite similar, but have slightly different meanings and mechanisms. *Dosage Compensation* is an epigenetic regulatory mechanism by which organisms increase the transcription of genes located on the single X chromosome in males to equalize the expression of genes on the both X chromosomes in females.[1] While *Genetic Compensation* is a biological concept to explain the genetic robustness, which ensures an organism to maintain its fitness and viability when something disrupts its genetic variation such as by mutation or the genetic perturbations occurred.[2,3] Both dosage compensation and genetic compensation likely share the pattern similarity for organisms in terms of maintaining their genetic variation by means of compensation if biological disappointment happened.

Genetic compensation has been further studied and reported before by several studies using different model organisms such as in *Arabidopsis*,[4] mice,[5] and zebrafish (*Danio rerio*),[6] where the lack of phenotype was found in their mutants. Genetic compensation may result from different mechanisms including the key point—the upregulation of gene(s), or from a more complex response within metabolic signaling pathways.[7] The upregulating genes which could be triggered in genetic compensation (genetic robustness) might be gained from the redundancy of gene function, when a gene has one or more homologous gene(s) which play the same or similar biochemical function in the genome. For those respective organisms, genetic robustness has a beneficial role to help organisms survive when one copy of the homologous genes becomes non/malfunction due to mutation.[2]

In the most recent study, the phenomenon of genetic compensation response has been widely studied and observed in the zebrafish model especially for the concept in which several mutant zebrafish for different genes performed normal phenotype.[6,8–14] The selection of zebrafish model for this genetic compensation study is due to the versatile convenient vertebrate model system with remarkable advantages.[15,16] Kok et al observed that there was a discrepancy between knockdown-mediated and knockout-mediated in the zebrafish model, in which not all mutant zebrafish (by knockout-mediated) showed the same phenotype as in gene knocked-down zebrafish.[6] They reported that several zebrafish mutants for different genes failed to mimic the previously published morpholino-induced phenotypes.[6] To support this finding, Rossi et al reported their observation and revealed the reason behind the discrepancy, where deleterious mutation by knockout rather than gene knockdown triggered genetic compensation in the zebrafish model.[17]

Naturally, in conventional wisdom, we kept in mind that when we modify or disrupt a gene to inactivate the encoded protein by 'knockout', the organisms should have more severe effects than merely reducing the gene expression level by performing 'knockdown'. However, there are many cases in which the opposite occurs. In fact, the knockout of a gene sometimes has no discernible impact, whereas the reduction of expression 'knocking down' of the same gene causes major defects.[18] Off-target or toxic effects of the reagent (morpholino) used for gene knockdown have sometimes been found to be the things responsible,[6] but not always,[17] making severe defects in organisms. Another factor is that the effect of genetic compensation response which has been reported in the deleterious mutation of an organism could be the possible reason why mutant organisms did not exhibit severe phenotypic defects.[17] Here, in this review we try to update the recent advances and future perspectives of genetic compensation studies in the zebrafish model.

Knockdown-mediated versus knockout-mediated in zebrafish

Zebrafish has been more powerful and widely accepted as model organisms to study vertebrate biology both in developmental and genetic analysis studies. Large-scale genetic screens have been developed and successfully identified hundreds to thousands of mutant phenotypes, many of them resemble and mimic human clinical disorders such as human genetic disorder. The creation of critical genetic reagents, coupled with the rapid progress of the zebrafish genome initiative directed by the National Institutes of Health (NIH), up lifted this model system to its full beneficial for the study of vertebrate biology, physiology, development, and even human diseases.[19] These studies have been taking the advantages of many of which zebrafish provided, including the rapid external development of the transparent zebrafish embryos and the hardy nature of zebrafish adults and their ability to produce large clutch sizes facilitated subsequent mapping of causative mutant genes.[20]

By studying and observing the embryonic morphology of zebrafish, researchers discovered various genes required for distinct stages in embryogenesis, including gastrulation,[21] hematopoiesis,[22] cardiovascular morphogenesis,[23] and many others. Subsequent forward genetic screens have been applied to determine the genetic basis, however, the

screening to saturation in zebrafish have been challenging.[20] At the same time, the overwhelm of sequence data over the past decades has revealed a wealth of genes, however, the mutated genes data in zebrafish are still limited.[6] Therefore, reverse genetic approaches to study the functions of a specific gene, by focusing the two mechanism procedures namely gene knockout and gene knockdown methods are needed.

Morpholino induced knockdown-mediated phenotype

The introduction of morpholino oligonucleotides (MOs) was warmly welcomed with excitement in the zebrafish community as a prominent tool to study gene function. The MOs etiquette differs from standard nucleic acid oligonucleotides in which it possesses a six-ring heterocycle backbone and non-ionic phosphorodiamidate linkages.[24] These big modifications discovered MOs with more highly stable *in vivo*, allowed it to have a high affinity for RNA, and supposedly reduced their off-target binding to macromolecules.[25] MOs is designed to block translation or splicing process and it could recapitulate the known mutant phenotypes by injecting it into one cell stage of zebrafish embryos and could directly analyze the gene function at desired indicated stages.[26,27] MOs is also able to block microRNA maturation and their binding to target 3' untranslated regions (UTRs).[28,29] Given its ease, MOs is an accessible and straightforward method to study gene function by knockdown-mediated in zebrafish embryos and analyzing the given phenotypes. By utilizing this method, hundreds or even more reports studying various gene functions in the zebrafish model have been published.[6]

However, researchers found that the injection of a gene specific MOs to disrupt the translation process, often resulted in an observable phenotype in the injected embryos or *morphants* embryos.[27] The side effects in which the use of MOs should be considered are those, MOs could induce p53-dependent apoptosis and the off-target cell-type-specific effect might change in gene expression that made researchers confused during observing the morphant phenotypes.[8,30–32] Moreover, the efficiency of MOs must be checked and confirmed by using protein assay targeting the translation start codon ATG as well as transcript analysis for MOs targeting splicing sites of the target genes. To minimize the effect of DNA damage response by MOs, co-injection with p53 MOs is needed and co-injection with wild-type mRNA to rescue the morphant phenotypes is used to verify the specification of MOs.[8] And recently, knockout-mediated based technology to generate a stable mutant in zebrafish have been successfully developed and widely applied to replace MOs. Therefore, with the various deficiencies that MOs has, the use of this method as a knockdown based technology needs to be reevaluated to study the specific gene function.

Knockout-mediated mutant phenotypes

The rapid development of some techniques in genome editing, especially the generation of mutants by using ZFN, TALENs and the most recent one, CRISPR/Cas9, have been widely used as the sophisticated tool to study gene function.[33–36] Researchers have used these techniques to generate mutants of their target gene in animal models such as mice and zebrafish. These technologies, generally performed similar procedures in which they genetically modified cells or even direct animal models to stop their gene expression, which means the specific gene of theirs would completely be silenced.[36]

In zebrafish, the induced mutagenesis of interested genes could be achieved with relatively straightforward experimental setup. The CRISPR/Cas9 technology performing the highest mutagenesis efficiency is based on microinjection technique application of an *in vitro* preassembled complex of guide RNA (gRNA) and Cas9 protein in one-cell stage of zebrafish embryos. There could be two possible strategies using CRISPR/Cas9 based genetic editing technology to study gene function in zebrafish (Fig. 1). First, the phenotypes of the injected F_0 embryos or *CRISPRant*, carrying mosaic loss of function (LOF) mutations, could directly be observed and used to study the respective gene function. This strategy by using CRISPRant embryos is known as *the transient knockout approach*. The other strategy, the CRISPRant zebrafish obtained from the injected F_0 embryos could be grown into sexual maturity and crossed with wildtype to generate F_1 heterozygous carriers and followed by the inter-crossed between F_1 heterozygous to obtain F_2 homozygous mutant embryos (25% of the total progeny based on Mendelian ratio). This strategy is called *the isogenic stable knockout*. The generation of isogenic stable knockout in zebrafish takes at least 4–6 months, it is much longer compared to the transient knockout approach where the CRISPRant embryos could be analyzed directly. However, the isogenic stable knockout allows to obtain hundreds of F_2 embryos with different genotypes including homozygous or wildtype (25%), heterozygous (50%) and homozygous mutants (25%) which could be used to evaluate the parallel gene functions among the three genotypes.[35,37]

Together, this knockout-mediated mutant phenotype has been suggested by researchers and zebrafish community to become standard metric to study gene function in zebrafish, while the MOs based knockdown-mediated could be a supplementary technique to achieve the trusted gene function study in the zebrafish model.[6]

The discrepancy between morphant and mutant in zebrafish

In 2015, Kok and colleagues reported that there was a huge gap between morphants and mutants in the zebrafish model. Some mutant genes of zebrafish that have been generated with stable conditions failed to perform the expected phenotypes, while morphants had severe defects in their phenotypes. By using ZFN, TALEN, CRISPR/Cas9 technology, the authors found that among 24 selected gene mutants recorded in the Sanger Zebrafish Mutation Project for comparison, the phenotypes of 80% genes in morphants were not observed in the mutant embryos.[6]

To support this finding, subsequently several researches have been developed and published to give more insights to study the discrepancy between morphants and mutants in

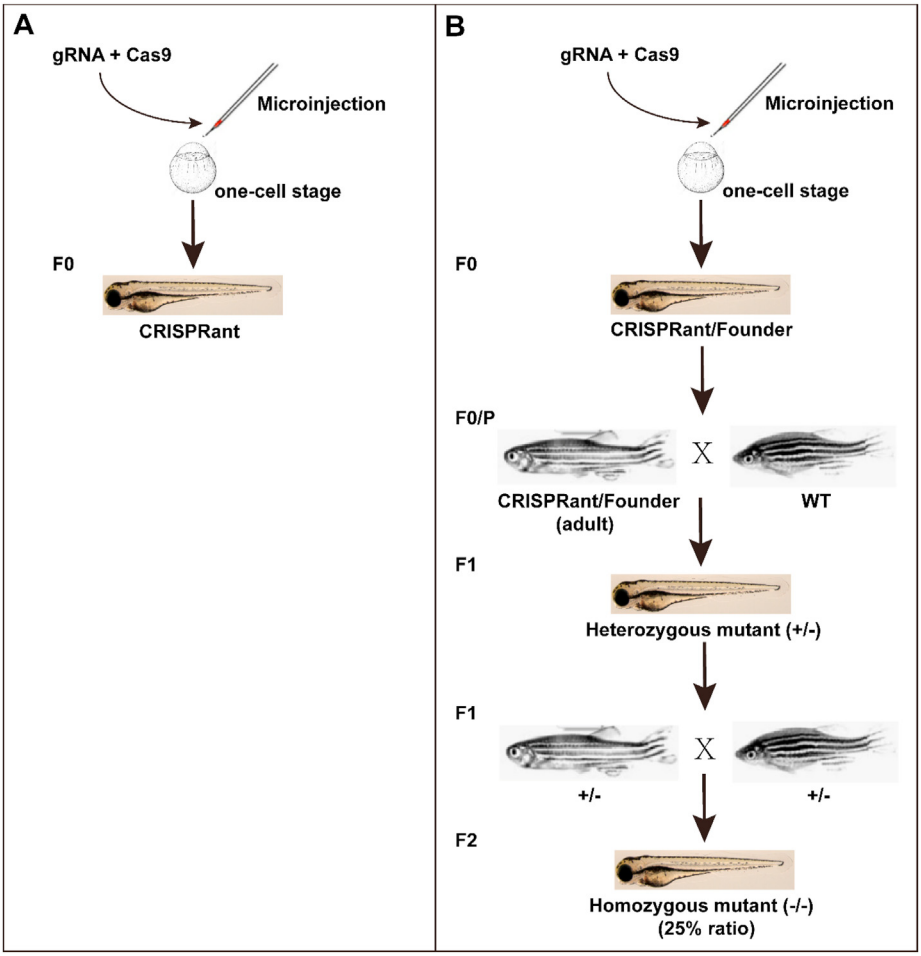

Figure 1 Diagram representative for two strategies using CRISPR/Cas9 technology-based knockout-mediated to study gene function in zebrafish. **(A)** The transient knockout method. **(B)** The isogenic stable knockout method.

zebrafish. For example, mutation of the *EGF-like-domain, multiple 7* (*egfl7*) gene, an endothelial extracellular matrix gene of therapeutic interest in zebrafish has been generated by TALENs based-knockout method. Phenotypic analysis revealed that *egfl7* zebrafish mutants did not perform any obvious phenotypes, while in contrast *egfl7* zebrafish morphants showed severe vascular defects. Further observation revealed that, *egfl7* mutants instead of *egfl7* morphants could upregulate a set of proteins and genes such as *emilin3a* as their compensating gene.[17] Similar case, a work done by Moreno et al also reported the same phenomenon in the zebrafish model. Zebrafish primary motor neurons have been studied to differ from one to another with respect to morphology, muscle targets and electrophysiological properties.[12] The authors then checked the transcription factor (TF), *Islet2a*, whether or not this TF is expressed in CaP, in which CaP plays a role in specifying the stereotypic electrophysiological properties in zebrafish. By using both TALENs and morpholino induction, the authors observed the phenotypic analysis by performing the electrophysiological experiments, and found that CaPs in morphants exhibited truncated axons, while mutants showed normal axons. Further analysis, by testing the gene expression profiles of whole embryos in morphant, control, and mutant embryos showed that morphants had 174 genes, while mutants and controls had 201 genes respectively which were differentially expressed.[12]

Another example, the study of genetic compensation in pronephros development using *epoa* zebrafish mutants done by She et al.[38] Erythropoietin (Epo) is a glycoprotein hormone traditionally considerd to have an essential function for erythropoiesis or red blood cell formation.[39] A recent morpholino-mediated knockdown of *epoa* expression in zebrafish could induce the alteration of pronephros development which is driven by blocking apoptosis in developing kidneys. In contrast, by generating stable mutant of *epoa* in zebrafish using CRISPR/Cas9 technology, these mutant embryos did not perform any defect in pronephros development, however the *epob* gene was identified and upregulated to be a compensating gene in *epoa* zebrafish mutants but not in morphants.[38]

These reported discrepancies raised a big concern either about the credibility of using MOs to study gene function in zebrafish or there is another effect (genetic compensation response) lies in mutant zebrafish but not in morphants. For more reliable and trusted results of a gene function study in zebrafish and any animal models, morpholino-induced morphants could reliably be applied for ancillary analyses along with the standard metric, which is the use of knockout-mediated for mutant phenotypes (Table 1).

Table 1 The summary of knockout-mediated *vs.* knockdown-mediated in zebrafish.

Comparison	Knockout	Knockdown
Definition	A genetic perturbation by inactivating or deleting the gene of an organism.[40]	A genetic perturbation by reducing the amount of functional RNA or protein level in an organism.[24,25]
Reverse genetic approaching method	TALENs,[33,36] ZFN,[33,36] CRISPR/Cas9.[34,35]	Morpholino oligonucleotides (MOs).[24,25]
Organism Calling	Mutant	Morphant
Example discrepancy between mutant and morphant in zebrafish model		
a. *egfl7*[17]	No vascular defect/normal phenotype. Upregulated *emilin3a* as the compensating gene.	Severe vascular defects. No upregulation occurred.
b. *Islet2a*[12]	Showed normal axons. Had 201 gene expression profiles.	Showed truncated axons. Had 174 gene expression profiles.
c. *Epoa*[38]	No pronephros development defect/normal phenotype. Upregulated *apob* as the compensating gene.	Alteration of pronephros development. No upregulation occurred.
Deficiency	Some mutants showed normal phenotypes due to genetic compensation response.[17,18]	Toxicity and off-target effects.[6,30–32]

CRISPRant versus mutant in zebrafish

The CRISPR/Cas9 technology is the most advanced technique and has been widely used in gene editing method to study gene function especially in the zebrafish model.[34,35,37] This technique could induce stable mutation of interested genes and analyze the gene function by two ways, using *CRISPRant* and *mutant* (Fig. 1). However, in contrast, a recent study reported that between CRISPRant and mutant zebrafish have significant differences in their phenotypes. The gene function study of *epoa* in zebrafish by applying CRISPR/Cas9 technology was reported that *epoa* zebrafish CRISPRant at 48 hpf showed pronephros development defect by increased glomerulus length and decreased neck length in pronephric structures. While in the same stage of *epoa* zebrafish homozygous mutants (from F_2 generation) performed normal pronephros development.[38]

In the latest example, the study of genetic compensation in a stable *sl25a46* mutant gene of zebrafish done by Buglo and colleagues.[14] Loss function of *SLC25A46* in human has been studied to associate with the spectrums of disorders, ranging from optic atrophy to Charcot-Marie-Tooth type 2, Leigh syndrome, progressive myoclonic ataxia and lethal congenital pontocerebellar hypoplasia.[41–46] Another study observed in the mice model where mutation in *slc25a46* caused cerebellar ataxia, optic atrophy, peripheral neuropathy, and neuromuscular junction defects.[47,48] Here, Buglo et al observed that *sl25a46* zebrafish CRISPRants had several phenotypes similar to zebrafish morphants with defective phenotypes in motor neuron, smaller eyes, heart edema and shorter trunk size, while *slc25a46* zebrafish mutants showed none of those phenotypes aka normal phenotype.[14] This phenomenon was speculated due to the genetic compensation response which was observed in a stable *slc25a46* zebrafish mutant but neither in CRISPRants nor in morphants.[14] This finding indicated that the discrepancy phenotypes between CRISPRant and mutant in some specific genes could be observed within the knockout-mediated itself.

By mimicking the morphant phenotypes, the use of direct analysis of F_0 generation of CRISPRant zebrafish could be an attractive and potential alternative approach to study gene function *in vivo*. This method serves as a shortcut method avoiding the burden of months and even years of animal husbandry in generating zebrafish mutants and time required for genotyping and confirming a stable mutant line. Importantly, direct analysis using F_0/CRISPRant method has no or minimal cytotoxicity and off-target effect compared to in knockdown morpholino models (morphants) and does not perform genetic compensation response as found in mutant models (Table 2).

The concept and mechanism in genetic compensation

Genetic compensation (re)gained more attention nowadays and its concept becoming crystal clear, when the amazing works done by El-Brolosy and colleagues,[49] as well as Ma and colleagues provided a captivating mechanism on how genetic compensation occurs in the zebrafish model.[50] By studying the effects of a variety of mutations in zebrafish embryos, both El-Brolosy et al and Ma et al found that the upregulation of compensating genes is specifically triggered by mutations with the involvement of mRNA degradation and the generated short nucleotide sequences known as premature termination codons (PTCs).[49,50] This concept and mechanism of genetic compensation discovered in the zebrafish model could open up a new and high prospect to treat human genetic disorders.

Deleterious mutation induced genetic compensation by upregulating the compensating gene

Before the two concepts and mechanisms provided by El-Brolosy et al and Ma et al,[49,50] a concept of genetic compensation has been studied a few years earlier in the

Table 2 The summary of CRISPRant *vs.* mutant in zebrafish.

Comparison	CRISPRant	Mutant
Definition	The injected F_0 embryos carrying mosaic loss of function (LOF) mutations.[35,37]	The injected F_0 embryos which grow into sexual maturity and crossed with wildtype to generate F_1 heterozygous carriers and followed by the inter-crossed between F_1 heterozygous to obtain F_2 homozygous mutant embryos.[35,37]
Reverse genetic approaching method	CRISPR/Cas9 technology.[34,35,37]	CRISPR/Cas9 technology.[34,35,37]
Method name	The transient knockout approach.[35,37]	The isogenic stable knockout.[35,37]
Example discrepancy between CRISPRant and mutant in zebrafish model		
a. *epoa*[38]	Showed pronephros development defect by increased glomerulus length and decreased neck length in pronephric structures.	Normal pronephros development.
b. *sl25a46*[14]	Showed defective phenotypes in motor neurons, had smaller eyes, heart edema and shorter trunk size.	Showed normal phenotype.
Advantage	Serves as a shortcut method to study gene function, has no/minimal cytotoxicity and off-target effect.[35,37]	Has ability to evaluate the parallel gene functions among the three genotypes (wildtype, heterozygous and homozygous mutant).[35,37]
Deficiency	The effectiveness and efficiency need to be further validated.	Time and cost consuming,[35,37] some mutants showed normal phenotypes due to the response of genetic compensation.[17,18]

zebrafish model. In August 2015, Rossi and colleagues reported their finding that deleterious mutation but not gene knockdown could induce genetic compensation by upregulating the expression of its gene family member harboring homologous sequence.[17] As mentioned earlier, the authors studied this phenomenon by using *egfl7* gene in zebrafish, where *egfl7* zebrafish morphants exhibited severe vascular defect, while *egfl7* zebrafish mutants performed normal phenotype with activating the *emilin3a* which served as the compensating gene.[17] This finding could be the initial discovery about the concept and mechanism in genetic compensation, in which deleterious mutation but not gene knockdown could induce genetic compensation by activating the compensating gene.

NMD mechanism is required for genetic compensation

One of the wonders in the biological process, especially in terms of central dogma pathway in eukaryotes is the presence of nonsense-mediated mRNA decay (NMD) which is also participated in dosage compensation.[51] NMD is a surveillance machine or pathway that exists in all eukaryotes. The main function of this machine is to check whether there is something inappropriate in the transcriptional mechanism pathway. NMD will reduce the errors in gene expression by removing the mRNA transcripts that contain PTC.[52] NMD was initially discovered when the mRNA levels with low concentration in the cells were transcribed from alleles carrying nonsense mutations.[53] The nonsense mutation could be detected by the presence of PTC which leads to the shortening and even truncation of protein which may or may not be functional, depending on the severity of what is not translated.[54] In some cases, translation of these aberrant mRNA (mRNA decay) could lead to deleterious gain-of-function or dominant—negative activity of the resulting proteins.[52] NMD has been reported to have the possible roles to not only limit the translation of abnormal proteins, but it could occasionally also cause detrimental effects in specific genetic mutations.[54]

The involvement of NMD mechanism in genetic compensation was further studied by El-Brolosy and colleagues.[49] In that study, the authors initially analyzed zebrafish mutants and cells from knockout mice that either have a PTC or have their last exon deleted. Mutation of six genes including *hbegfa*, *vcla*, *hif1ab*, *vegfaa*, *egfl7* and *alcama* in zebrafish could activate their respective mRNA expression level of a gene paralogue namely *hbegfb*, *vclb*, *epas1a* and *epas1b*, *vegfab*, *emilin3a* and *alcamb* as their compensating genes. While, in heterozygous zebrafish mutants of *hbegfa*, *hif1ab*, *vegfaa* and *alcama* also upregulated the wild type transcripts, indicating that the mutant transcript was necessary to induce genetic compensation.[49] Further analyses that the cells derived from *Fermt2*, *Rela*, *Actg1* and *Actb* knockout mice could also upregulate mRNA level of their family member genes namely *Fermt1*, *Rel*, *Actg2* and *Actg1*, respectively. The interesting phenomenon in that study, when the authors injected and transfected the wild type mRNA into corresponding zebrafish and mouse cells mutants, the authors did not observe the upregulation of homologous genes of their respective mutants.[49] These clearly indicated that transcriptional adaptation is a dominant phenomenon in deleterious mutation by upregulating their corresponding homologous genes.

As reported, those observed mutant genes carrying genetic compensation had a PTC and reduced mRNA level by mRNA decay. The authors speculated that NMD pathway

might be involved in genetic compensation response.[49] Then, the authors disrupted the expression of *Upf1* by mutation in respective *hbegfa*, *vegfaa* and *vcla* zebrafish mutants. As *Upf1* is the key component of NMD, the authors found that loss function by mutation of *Upf1* reduced mutant mRNA decay and resulted in the loss of transcriptional adaptation, hence the genetic compensation response was not observed. Concomitant with these results, knockdown of *Upf1* in the respective *Rela* and *Actb* mouse mutant cells exhibited loss of transcriptional adaptation. Further analysis, the authors also observed a strong genetic compensation when they injected respective un-capped *hif1ab* and *vegfaa* mRNA into wildtype zebrafish embryos as well as transfected un-capped *Actb* RNA into wild type mESCs. This is because the inducing mRNA degradation in wildtype both zebrafish and mouse cells by using un-capped RNA could be quickly degraded by 5′ to 3' exonucleases and triggered transcriptional adaptation.[49] Those suggested that mRNA degradation is a key factor to induce genetic compensation.

Consistent with those mechanisms, the authors also found that injecting un-capped transcripts containing similar sequences in some cases resulted in upregulation of its homologous genes. One of their findings, by using *hif1ab* zebrafish mutant, the authors analyzed the transcripts containing sequences similar to *epas1a* could increase the *epas1a* mRNA expression levels. It indicated that sequence similarity is also required for upregulating the compensating gene to induce genetic compensation response. In addition to that, the authors also reported that mutant genes which were unable to produce a transcript failed to gain genetic compensation.[49]

It has been reported that mRNA decay factor could translocate to the nucleus and interact with histone modifiers or chromatin remodelers to induce gene expression.[55–58] In order to further elucidate the role of NMD in genetic compensation, El Brolosy et al speculated that through an epigenetic machinery, the NMD factor could mediate genetic compensation.[49] Here, the authors performed a targeted siRNA screen in *Rela* knockout mouse cells to analyze the epigenetic modulators involved in genetic compensation. Knocking down of the histone lysine demethylases *KDM4* or *KDM6*, which removed the inhibitory H3K9me3 or H3K27me3 histone marks respectively as well as *WDR5*, a key component of the COMPASS complex, reduced the transcriptional adaptation response with knocking down of *WDR5* had the strongest effect. Further confirmation by performing chromatin immunoprecipitation (ChIP), *WDR5* in the help of H3K4me3 could enhance the promoters of *Fermt1*, *Rel* and *Actg2* in respective *Fermt2*, *Rela* and *Actg* knockout mouse cells.[49] Those indicated that, degradation of mutant mRNA and translocation of NMD factors to nucleus with the recruitment of either histone modifier and/or chromatin remodelers or COMPASS complex onto the promoters of the homologous genes together could upregulate their transcription to perform the genetic compensation response (Fig. 2).

PTC-bearing mRNA is involved in genetic compensation

In 2019, Ma and colleagues also proposed a detailed mechanism for genetic compensation. They believed that genetic compensation could be triggered by mRNA bearing PTC. To confirm their hypothesis, firstly, the authors used both *capn3a* zebrafish morphants and mutants and observed their phenotypes. In zebrafish, *capn3a* or *calpain3a*, a member of the calpain family is highly expressed in the brain and endodermic region at 1–4 dpf and in liver buds from 2.5 dpf.[50] Phenotypic analysis found that loss function of mutation harboring 14bp deletion in exon 1 carrying PTC

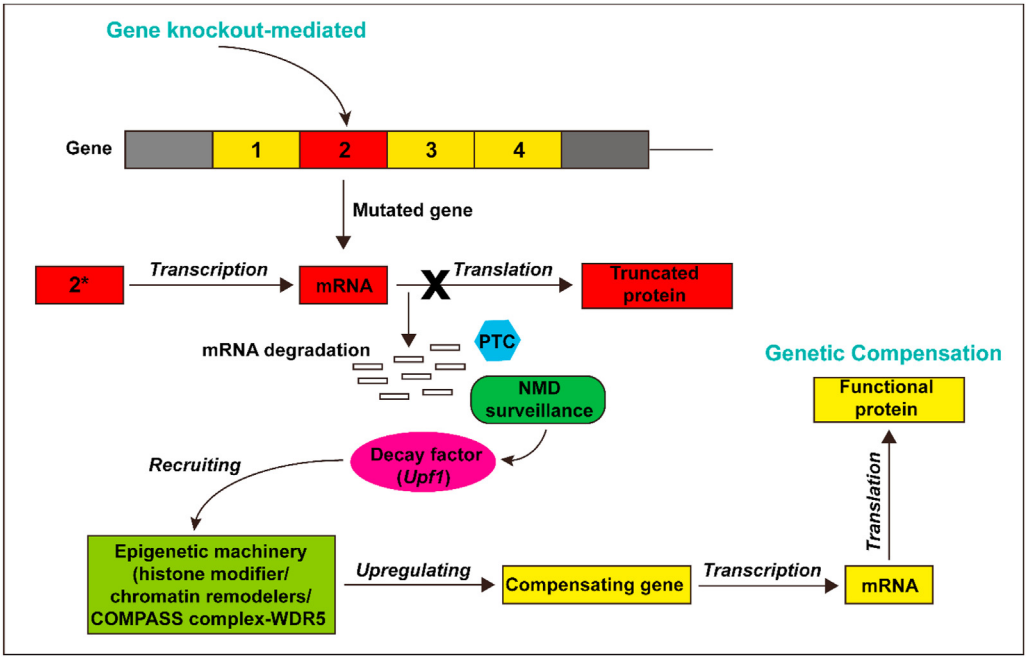

Figure 2 The requirement of NMD mechanisms in response to genetic compensation.

in *capn3a* showed normal phenotypes. However, in contrast, *capn3a* zebrafish morphants exhibited small liver phenotype, indicating that there was a genetic compensation response.[50] By qRT-PCR analysis, the authors also reported that at 1.5 dpf *capn3a* zebrafish mutants instead of morphants performed the upregulation of 10 out of 19 *capn3a* family members, in which two of them, *capn8* and *capn12* showing the highest increase. For further analysis, the authors then generated five additional *capn3a* mutant alleles with different exon locations but not in the last and found that most of those mutations gained a PTC bearing mRNA and elicited genetic compensation. It confirmed that genetic compensation could be triggered only when the PTC is not located in the last exon.[50]

It has been observed that the degree of genetic compensation response of different genes often varied in different mutants. To validate it, Ma et al analyzed two zebrafish mutants of *capn3* and *nid1a* and observed their genetic compensation response.[50] The authors found that there was a different response between *capn3* and *nid1a* genes in terms of upregulating genes. It was reported that in *capn3* heterozygous mutants, the wild type *capn3* transcripts were upregulated, but instead of increasing, the wildtype *nid1a* transcripts were reduced in *nid1a* heterozygous mutants.[50] It indicated that, not all mutant genes could perform the same level of response to genetic compensation.

El-Brolosy et al reported that the induction un-capped mRNA into wildtype could trigger mRNA degradation and required for genetic compensation.[49] Ma et al observed the contrast result.[50] By injecting un-capped *capn3* mutant mRNA while having PTC bearing into wildtype zebrafish embryos failed to upregulate *capn8* and *capn12*.[50] The transcription of PTC bearing mRNA but not mRNA degradation is crucial for genetic compensation response. To confirm this concept, the authors generated transgenic zebrafish containing six different transgene designs harboring exons and introns derived from *capn3* with or without PTC created in different artificial exons. The authors found that the endogenous *capn3* expression was upregulated only in transgenic fish harboring transgene containing a *capn3* homologous sequence and a functional PTC which was located not in the last exon.[50]

NMD pathway has been associated to the presence of PTC in eukaryotic system.[52] Similar to El-Brolosy et al.'s work,[49] Ma and colleagues also investigated the role of NMD factors involved in genetic compensation.[50] Here, the authors introduced the key important factors of NMD pathway namely *Upf1*, *Upf2* and *Upf3* (with two homologous genes *Upf3a* and *Upf3b*) in vertebrates, where the recruitment of those NMD factors to the exon—junction complex (EJC) is required for mutant mRNA degradation by NMD pathway itself.[50,59,60] To analyze the role of those NMD factors in genetic compensation, the authors then knocked down the expression of respective *Upf1*, *Upf2*, *Upf3a* and *Upf3b* with gene-specific morpholinos both in wildtype and *capn3a* zebrafish mutants. Interestingly, the authors found that only knockdown of *Upf3a* in *capn3a* zebrafish mutants successfully mimicked the *capn3a* zebrafish morphants by exhibiting small liver phenotype and significantly reduced the *capn8* and *capn12* transcripts as their compensating genes. Those indicated the involvement of NMD factors-*Upf3a* in response to genetic compensation.[50]

Further analysis, Ma et al conducted experiments to observe the interaction between NMD factor-Upf3a and histone modifier and/or chromatin remodelers or COMPASS complex.[50] Similar to previous report, the authors found that Wdr5 and other components including Setd1a, Ash2 and Rbpp5 from COMPASS complex were also associated to mediate genetic compensation response.[49,50] Important result from the observation is that *Upf3a* could achieve its duty likely through direct interaction with Wdr5, then bridged Rbbp5 to form a functional genetic compensation mechanism.[50] In summary, the authors successfully uncovered a mechanism by which PTC-bearing mRNA elicits genetic compensation response (Fig. 3).

Is time-dependent involved in genetic compensation?

It has been our curiosity to study the involvement of time-dependent in the mechanism of genetic compensation. Our questions, is time-dependent really involved in genetic compensation response? and how the mechanism works? Due to the lack of knowledge and very limited literature sources reported so far, the involvement of time-dependent is still under hypothesis. In late 2019, an answer which could at least be the initial step to confirm this hypothesis came to light. A work done by Anne and colleagues reported that age-dependent could change in glucose homeostasis in male deiodinase type 2 (Dio2) zebrafish mutants.[61] Thyroid hormones (THs) have been studied to have essential determinants of vertebrate glucose metabolism and insulin sensitivity.[62,63] Further studies reported that an activity-reducing mutation in *Dio2*, the major TH-activating enzyme, has been linked to the type 2 diabetes mellitus both in humans and mice model.[64,65] Here, by using *Dio2KO* zebrafish lines, Anne et al reported that at a young stage of *Dio2* zebrafish mutants exhibited hyperglycemia both in a fasted (6- and 9-month age) and a fed state (9-month age).[61] At that age, *Dio2* zebrafish mutants could not produce a sufficient amount of insulin to restore the blood sugar level into normal. Meanwhile, in contrast, older mutant zebrafish of *Dio2* (18- to 24-month age) showed normoglycemia with increased insulin and glucagon expression with accompanied by the increase in pancreatic islet size, α and β cell numbers to normalize the blood sugar level.[61] Those indicated that there was a compensatory response in older *Dio2KO* zebrafish but not in younger mutants. Magdalena group found 75% maternal zygotic *(MZ)cep290* mutation embryos showed a mild axis curvature at 2—3 dpf, and recovered a straight body axis at 7—8 dpf because the upregulation of *arl3*, *arl13b*, *uncll119b*. But the phenotype is transient. The adult *MZcep290* mutants showed various degrees of spinal scoliosis.[66] Zhu et al provides another example of deleterious mutations in zebrafish basement membrane glycoprotein Nidogen 1 (*nid1*) leading to genetic compensation by a Nidogen family member *nid2*.[67] The *nid1a* mutations showed a short body at 1—3 dpf, and recovering a straight body at 5

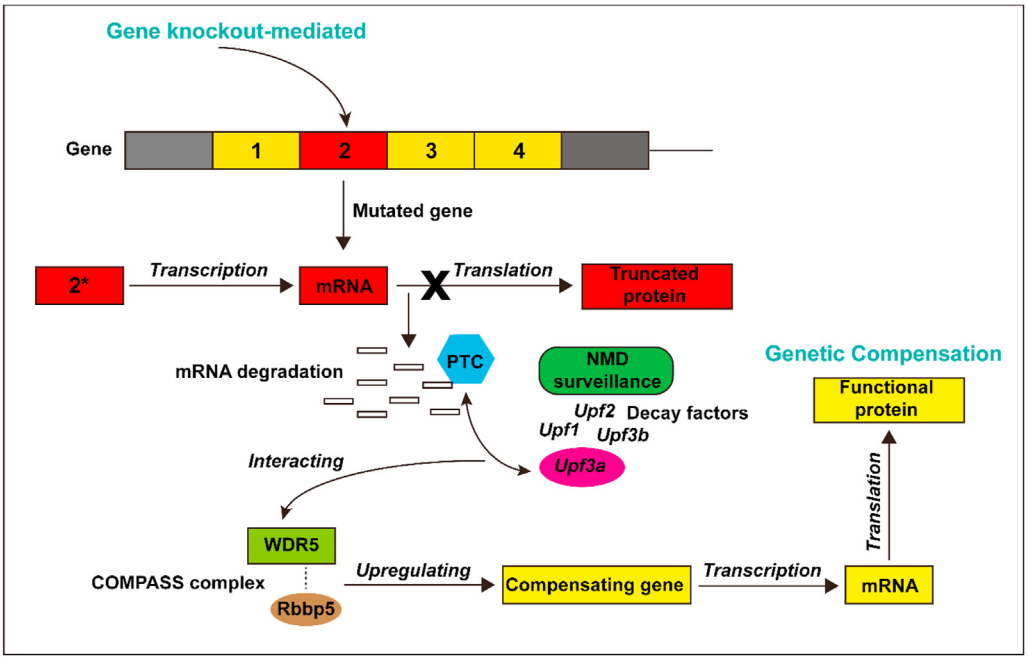

Figure 3 The involvement of PTC-bearing mRNA in response to genetic compensation via *Upf3a* and COMPASS complex.

dpf, but not morphant. The loss of function in *nid1a* gene was compensated by the up-regulation of *nid1b* and *nid2a*.[67]

Although there are some reports about time-dependent compensation, the evidences for it are less robust. To explore the time-dependent compensation, first step is to classifying the severity of the mutant phenotype, than explore whether there are genetic compensatory differences among mutants with different phenotypes. Next, observe and compare whether there are compensatory differences between the offspring of the same parents at different periods and between mutants of different generations. Based on the above observations, epigenetic modification changes at the level of RNA or DNA can be detected and further examine whether these epigenetic modification changes are involved in genetic compensation.

Human disease and genetic compensation

Nemaline myopathies are a heterogenous group of congenital myopathies often resulting in early death, which commonest caused by the genes encoding skeletal α-actin (*ACTA1*) and nebulin (*NEB*) genetic mutation.[68] About 10% genetic compensation of *ACTA1* gene complete loss, leading to a milder phenotype by upregulating of its paralogues gene cardiac muscle α-actin (*ACTC1*).[68] This phenotype also mimics the phenotype in the zebrafish. Zebrafish *actc1b* (the *ACTA1* orthologue) mutants display a mild myopathy phenotype compared with that observed in morphants, due to the upregulation of *actc1a* (the *ACTC1* orthologue).[7]

Spinal muscular atrophy (SMA) is an autosomal recessive hereditary disease mutation in *SMN1*, result in degeneration of motor neurons in the anterior horn of the spinal cord leading to muscle weakness and atrophy. The clinical manifestations of SMA vary greatly. The *SMN1* gene mutations result in a nonfunctional protein, however, its homologous gene *SMN2* can be transcriptional activation to producing small amounts of full-length and fully functional SMN-protein.[69,70] But the efficiency of genetic compensation response is affected by the copy number of the *SMN2*, which is contributed to SMA's highly variable phenotypic spectrum.[70,71] Over the past decade, gene therapy is applied to gene disease. Splicing modification of *SMN2* is a new treatment strategy to replacing the *SMN1* gene. Nusinersen is an antisense-oligonucleotide (ASO) that the first drug to apply to SMA treatment.[72,73] It binds to an intronic splice-silencing-site in intron 7 of *SMN2* to suppressing the binding of other splice-factors, which increased the proportion of *SMN2*-mRNA with included exon 7 and produced more functional full-length SMN2 protein.[74] Another strategy to increase the full length MSN2 protein are small molecules such as RG7916 (risdiplam) and LMI070 (branaplam) by taking orally.[75]

Magdalena et al demonstrated that the cilia-related disease ventral axis curvature in *cep290* CRISPR/Cas9 zebrafish mutants are more mild than *cep290* morphant.[66] However, nonsense-associated altered splicing or exon skipping is not account for the genetic compensation of MZ*cep290* mutant.[66] RNAseq analysis of the *cep290* morphants, mutants and WT embryos showed that upregulation of the delivery of cilia membrane proteins arl3, arl13b or unc119b compensate for the *MZcep290* deficiency.[66] A 4-fold induction of UNC119B from urine-derived renal epithelial cells (URECs) from Joubert Syndromes (JBTS) patients compared with control patients URECs samples.[66] Injection of *arl13b* and *unc119b* RNA in *cep290* morphant embryos completely rescued Kupffer's vesicle cilia. Expression of *arl3* RNA also rescued the length of the cilia but only partially. Injection of *arl3*, *arl13b* and *unc119b* mRNAs alone or in combination with cep119ex25 morpholino induced a partial rescue of photoreceptor outer

segment length defects in *MZcep290* morphant embryos. Based on this result, upregulating the *arl3*, *arl13b*, *unc119b* RNA expression may be the strategy to cure the cilia-associated syndromes caused by *cep290* mutation.[66]

Perspective and future prospect

The study of genetic e*gfl7* compensation has recently become a hot topic in biology and genetics fields. The concept and mechanism behind genetic compensation have begun slowly to unfold. Here, we reviewed the morphants, CRISPRants and mutants in zebrafish gene study and the recent studies in gene compensation mechanisms. The phenotype discrepancies between knockdown-mediated and knockout-mediated as the initial concept of genetic compensation especially in the zebrafish model has been slowly solved. The use of morpholino induced knockdown-mediated is debatable due to the side effects such as toxicity and off-target effect.[6,30–32] Nevertheless, using morpholino for preliminary screening of gene function would be a great time saver and much more efficient. While in knockout-mediated by using mutant genes, some mutant zebrafish do not exhibit any desired phenotypes due to the response of genetic compensation. Here, the use of direct F_0 or CRISPRant in zebrafish which provided several advantages including less time and cost consuming compared to mutant zebrafish could be the prominent and potential strategy to study gene function in the future. Yet, the use of CRISPRant zebrafish needs to be further validated.

Genetic compensation is beneficial for natural harmful mutations and organism survival, but it is a huge obstacle to the study of gene function. More than 80% of zebrafish genes are knocked out without phenotypes, so it is difficult to study the function of these genes, most of which is due to genetic compensation. To solve this problem, we can block the abnormal compensation according to the mechanism found in the studies by El Brolosy et al and Ma et al.[49,50] At the same time, we should avoid constructing mutants that cause nonsense mutations as far as possible, such as by knocking out gene promoter to reduce the occurrence of gene compensation reaction.

The results of human genome sequencing show that there are a large number of homozygous nonsense mutations in the genomes of normal people, some of which may cause serious human genetic diseases due to missense mutations. Genetic compensation effect may be an important reason for this phenomenon. Recently, the works done by El Brolosy et al and Ma et al have become an oasis in the middle of desert for giving hope to identify and develop new therapeutic targets for genetic or hereditary diseases.[49,50] Ma et al showed that a PTC is required to trigger the GC, E1-Brolosy demonstrated that mRNA degradation products are sufficient and the PTC-bearing mRNA may only serve as a substrate of the NMD pathway to prudence the degradation products.[50]

In conclusion, the discovery of genetic compensation could have a remarkable implication to cure human genetic diseases. Based on the human genome sequences, many diseases have been identified as homozygous nonsense mutation. The concept and mechanism of genetic compensation probably could explain that individuals with homozygous nonsense mutation diseases showed apparent well and healthy. Another type of mutation such as gain of function mutations, the introduction of PTC in the mutant gene could be the potential therapeutic approach to activate their compensating genes to respond to the genetic compensation and treat those related diseases. For loss-of-function genetic diseases, the introduction of nonsense mutations may be a clinical approach for the treatment of genetic diseases with compensating genes. For human genetic diseases caused by missense mutations, we can activate the genetic compensation in human body by knocking out these genes or transferring into homologous DNA with missense mutations. The involvement of time-dependence in genetic compensation is also could be a prominent concept treating genetic diseases in older patients. Somehow, the curing of human genetic diseases is no longer a mere dream. Because we shall find compensation in every disappointment.

The open questions include: Q1. Which proteins are required for mutant mRNA degradation and transcriptional adaptation? Q2. In some cases, gene mutations trigger genetic compensation resulting variable phenotypes by regulating homologous genes or genes with common domains at different levels. What are the mechanisms of this phenomenon? Q3. Is PTC-bearing mutation involved in the severity of human genetic disease? And is it possibility that genetic compensation can benefit the serious human disease caused by missense mutation? Therefore, the study of genetic compensation has a long way to go. There are many more concepts and mechanisms behind in genetic compensation that need to be further elucidated and reinforced by the involvement of future research from *in vivo* mammalian models.

Author contributions

G.W. and Y.W. designed the study. M.A.R and L.W. drafted the manuscript. Y.M., J.W., K.Z, Y,W, Z.L and S.L gave input to discussion. G.W, Y.W and S,L provided funding supporting. G.W., Y.W. and L.W. made critical revisions of the manuscript and G.W. approved the final version of the manuscript.

Conflict of interests

Authors declare no conflict of interests.

Funding

This work was supported by the National Natural Science Foundation of China (No. 31971242, 12032007, and 31771599), Key grants from Chongqing Science and Technology Bureau, China (No. cstc2019jcyj-zdxmX0028) and the State Key Project Specialized for Infectious Diseases, China (No. 2017ZX10201201-001-005, and 2017ZX10201201-002-005) as well as Visiting Scholar Foundation of Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, China (No. CQKLBST-2020-007).

References

1. Lucchesi JC, Kuroda MI. Dosage compensation in Drosophila. *Cold Spring Harbor Perspect Biol*. 2015;7(5):a019398.
2. Peng J. Gene redundancy and gene compensation: an updated view. *J Genet Genomics*. 2019;46(7):329—333.
3. Mather K. Genetical control of stability in development. *Heredity*. 1953;7(3):297—336.
4. Bouché N, Bouchez D. Arabidopsis gene knockout: phenotypes wanted. *Curr Opin Plant Biol*. 2001;4(2):111—117.
5. White JK, Gerdin AK, Karp NA, et al. Genome-wide generation and systematic phenotyping of knockout mice reveals new roles for many genes. *Cell*. 2013;154(2):452—464.
6. Kok FO, Shin M, Ni CW, et al. Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. *Dev Cell*. 2015;32(1):97—108.
7. Sztal TE, McKaige EA, Williams C, et al. Genetic compensation triggered by actin mutation prevents the muscle damage caused by loss of actin protein. *PLoS Genet*. 2018;14(2):e1007212.
8. Robu ME, Larson JD, Nasevicius A, et al. p53 activation by knockdown technologies. *PLoS Genet*. 2007;3(5):e78.
9. Karakas B, Weeraratna AT, Abukhdeir AM, et al. P21 gene knock down does not identify genetic effectors seen with gene knock out. *Cancer Biol Ther*. 2007;6(7):1025—1030.
10. Law SH, Sargent TD. The serine-threonine protein kinase PAK4 is dispensable in zebrafish: identification of a morpholino-generated pseudophenotype. *PLoS One*. 2014;9(6):e100268.
11. Novodvorsky P, Watson O, Gray C, et al. klf2ash317 mutant zebrafish do not recapitulate morpholino-induced vascular and haematopoietic phenotypes. *PLoS One*. 2015;10(10):e0141611.
12. Moreno RL, Williams K, Jones KL, et al. Investigation of Islet2a function in zebrafish embryos: mutants and morphants differ in morphologic phenotypes and gene expression. *PLoS One*. 2018;13(6):e0199233.
13. San B, Rougeot J, Voeltzke K, et al. The ezh2(sa1199) mutant zebrafish display no distinct phenotype. *PLoS One*. 2019;14(1):e0210217.
14. Buglo E, Sarmiento E, Martuscelli NB, et al. Genetic compensation in a stable slc25a46 mutant zebrafish: a case for using F0 CRISPR mutagenesis to study phenotypes caused by inherited disease. *PLoS One*. 2020;15(3):e0230566.
15. Arunachalam M, Raja M, Vijayakumar C, et al. Natural history of zebrafish (Danio rerio) in India. *Zebrafish*. 2013;10(1):1—14.
16. Kozol RA, Abrams AJ, James DM, et al. Function over form: modeling groups of inherited neurological conditions in zebrafish. *Front Mol Neurosci*. 2016;9:55.
17. Rossi A, Kontarakis Z, Gerri C, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. *Nature*. 2015;524(7564):230—233.
18. El-brolosy MA, Stainier DYR. Genetic compensation : a phenomenon in search of mechanisms. *PLoS Genet*. 2017;13(7):e1006780.
19. Dooley K, Zon LI. Zebrafish: a model system for the study of human disease. *Curr Opin Genet Dev*. 2000;10(3):252—256.
20. Lawson ND, Wolfe SA. Forward and reverse genetic approaches for the analysis of vertebrate development in the zebrafish. *Dev Cell*. 2011;21(1):48—64.
21. Hammerschmidt M, Pelegri F, Mullins MC, et al. Mutations affecting morphogenesis during gastrulation and tail formation in the zebrafish, Danio rerio. *Development*. 1996;123:143—151.
22. Ransom DG, Haffter P, Odenthal J, et al. Characterization of zebrafish mutants with defects in embryonic hematopoiesis. *Development*. 1996;123:311—319.
23. Stainier DY, Fouquet B, Chen JN, et al. Mutations affecting the formation and function of the cardiovascular system in the zebrafish embryo. *Development*. 1996;123:285—292.
24. Summerton J, Weller D. Morpholino antisense oligomers: design, preparation, and properties. *Antisense Nucleic Acid Drug Dev*. 1997;7(3):187—195.
25. Summerton JE. Morpholino, siRNA, and S-DNA compared: impact of structure and mechanism of action on off-target effects and sequence specificity. *Curr Top Med Chem*. 2007;7(7):651—660.
26. Draper BW, Morcos PA, Kimmel CB. Inhibition of zebrafish fgf8 pre-mRNA splicing with morpholino oligos: a quantifiable method for gene knockdown. *Genesis*. 2001;30(3):154—156.
27. Nasevicius A, Ekker SC. Effective targeted gene 'knockdown' in zebrafish. *Nat Genet*. 2000;26(2):216—220.
28. Choi WY, Giraldez AJ, Schier AF. Target protectors reveal dampening and balancing of Nodal agonist and antagonist by miR-430. *Science*. 2007;318(5848):271—274.
29. Kloosterman WP, Lagendijk AK, Ketting RF, et al. Targeted inhibition of miRNA maturation with morpholinos reveals a role for miR-375 in pancreatic islet development. *PLoS Biol*. 2007;5(8):e203.
30. Ekker SC, Larson JD. Morphant technology in model developmental systems. *Genesis*. 2001;30(3):89—93.
31. Pickart MA, Klee EW, Nielsen AL, et al. Genome-wide reverse genetics framework to identify novel functions of the vertebrate secretome. *PLoS One*. 2006;1(1):e104.
32. Amoyel M, Cheng YC, Jiang YJ, et al. Wnt1 regulates neurogenesis and mediates lateral inhibition of boundary cell specification in the zebrafish hindbrain. *Development*. 2005;132(4):775—785.
33. Huang P, Zhu Z, Lin S, et al. Reverse genetic approaches in zebrafish. *J Genet Genomics*. 2012;39(9):421—433.
34. Chang N, Sun C, Gao L, et al. Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos. *Cell Res*. 2013;23(4):465—472.
35. Hwang WY, Fu Y, Reyon D, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nat Biotechnol*. 2013;31(3):227—229.
36. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *J Clin Invest*. 2014;124(10):4154—4161.
37. Cornet C, Di Donato V, Terriente J. Combining zebrafish and CRISPR/Cas9: toward a more efficient drug discovery pipeline. *Front Pharmacol*. 2018;9:703.
38. She J, Wu Y, Lou B, et al. Genetic compensation by epob in pronephros development in epoa mutant zebrafish. *Cell Cycle*. 2019;18(20):2683—2696.
39. Jelkmann W. Erythropoietin: back to basics. *Blood*. 2010;115(21):4151—4152.
40. Housden BE, Muhar M, Gemberling M, et al. Loss-of-function genetic tools for animal models: cross-species and cross-platform differences. *Nat Rev Genet*. 2017;18(1):24—40.
41. Abrams AJ, Hufnagel RB, Rebelo A, et al. Mutations in SLC25A46, encoding a UGO1-like protein, cause an optic atrophy spectrum disorder. *Nat Genet*. 2015;47(8):926—932.
42. Charlesworth G, Balint B, Mencacci NE, et al. SLC25A46 mutations underlie progressive myoclonic ataxia with optic atrophy and neuropathy. *Mov Disord*. 2016;31(8):1249—1251.
43. Wan J, Steffen J, Yourshaw M, et al. Loss of function of SLC25A46 causes lethal congenital pontocerebellar hypoplasia. *Brain*. 2016;139(11):2877—2890.
44. Hammer MB, Ding J, Mochel F, et al. SLC25A46 Mutations associated with autosomal recessive cerebellar ataxia in north African families. *Neurodegener Dis*. 2017;17(4—5):208—212.
45. Nguyen M, Boesten I, Hellebrekers DM, et al. Novel pathogenic SLC25A46 splice-site mutation causes an optic atrophy spectrum disorder. *Clin Genet*. 2017;91(1):121—125.
46. Sulaiman RA, Patel N, Alsharif H, et al. A novel mutation in SLC25A46 causes optic atrophy and progressive limb spasticity,

with no cerebellar atrophy or axonal neuropathy. *Clin Genet*. 2017;92(2):230—231.
47. Li Z, Peng Y, Hufnagel RB, et al. Loss of SLC25A46 causes neurodegeneration by affecting mitochondrial dynamics and energy production in mice. *Hum Mol Genet*. 2017;26(19):3776—3791.
48. Terzenidou ME, Segklia A, Kano T, et al. Novel insights into SLC25A46-related pathologies in a genetic mouse model. *PLoS Genet*. 2017;13(4):e1006656.
49. El-Brolosy MA, Kontarakis Z, Rossi A, et al. Genetic compensation triggered by mutant mRNA degradation. *Nature*. 2019;568(7751):193—197.
50. Ma Z, Zhu P, Shi H, et al. PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components. *Nature*. 2019;568(7751):259—263.
51. Yin S, Deng W, Zheng H, et al. Evidence that the nonsense-mediated mRNA decay pathway participates in X chromosome dosage compensation in mammals. *Biochem Biophys Res Commun*. 2009;383(3):378—382.
52. Baker KE, Parker R. Nonsense-mediated mRNA decay: terminating erroneous gene expression. *Curr Opin Cell Biol*. 2004;16(3):293—299.
53. Sharma J, Keeling KM, Rowe SM. Pharmacological approaches for targeting cystic fibrosis nonsense mutations. *Eur J Med Chem*. 2020;200:112436.
54. Holbrook JA, Neu-Yilik G, Hentze MW, et al. Nonsense-mediated decay approaches the clinic. *Nat Genet*. 2004;36(8):801—808.
55. Collins SR, Miller KM, Maas NL, et al. Functional dissection of protein complexes involved in yeast chromosome biology using a genetic interaction map. *Nature*. 2007;446(7137):806—810.
56. Berretta J, Pinskaya M, Morillon A. A cryptic unstable transcript mediates transcriptional trans-silencing of the Ty1 retrotransposon in S. cerevisiae. *Genes Dev*. 2008;22(5):615—626.
57. Pinskaya M, Gourvennec S, Morillon A. H3 lysine 4 di- and trimethylation deposited by cryptic transcription attenuates promoter activation. *EMBO J*. 2009;28(12):1697—1707.
58. Haimovich G, Medina DA, Causse SZ, et al. Gene expression is circular: factors for mRNA degradation also foster mRNA synthesis. *Cell*. 2013;153(5):1000—1011.
59. Lykke-Andersen J, Shu MD, Steitz JA. Human Upf proteins target an mRNA for nonsense-mediated decay when bound downstream of a termination codon. *Cell*. 2000;103(7):1121—1131.
60. Shum EY, Jones SH, Shao A, et al. The antagonistic gene paralogs Upf3a and Upf3b govern nonsense-mediated RNA decay. *Cell*. 2016;165(2):382—395.
61. Houbrechts AM, Beckers A, Vancamp P, et al. Age-dependent changes in glucose homeostasis in male deiodinase type 2 knockout zebrafish. *Endocrinology*. 2019;160(11):2759—2772.
62. Saghatelian A, Cravatt B. Glucagon and thyroid hormone: a championship team. *Cell*. 2016;167(3):604—605.
63. Kim SR, Tull ES, Talbott EO, et al. A hypothesis of synergism: the interrelationship of T3 and insulin to disturbances in metabolic homeostasis. *Med Hypotheses*. 2002;59(6):660—666.
64. Canani LH, Capp C, Dora JM, et al. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2005;90(6):3472—3478.
65. Dora JM, Machado WE, Rheinheimer J, et al. Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. *Eur J Endocrinol*. 2010;163(3):427—434.
66. Cardenas-Rodriguez M, Austin-Tse C, Bergboer JGM, et al. Genetic compensation for cilia defects in cep290 mutants by upregulation of cilia-associated small GTPases. *J Cell Sci*. 2021;134(14):jcs258568.
67. Zhu P, Ma Z, Guo L, et al. Short body length phenotype is compensated by the upregulation of nidogen family members in a deleterious nid1a mutation of zebrafish. *J Genet Genomics*. 2017;44(11):553—556.
68. Nowak KJ, Sewry CA, Navarro C, et al. Nemaline myopathy caused by absence of alpha-skeletal muscle actin. *Ann Neurol*. 2007;61(2):175—184.
69. Feldkötter M, Schwarzer V, Wirth R, et al. Quantitative analyses of SMN1 and SMN2 based on real-time lightCycler PCR: fast and highly reliable carrier testing and prediction of severity of spinal muscular atrophy. *Am J Hum Genet*. 2002;70(2):358—368.
70. Butchbach ME. Copy number variations in the survival motor neuron genes: implications for spinal muscular atrophy and other neurodegenerative diseases. *Front Mol Biosci*. 2016;3:7.
71. Calucho M, Bernal S, Alías L, et al. Correlation between SMA type and SMN2 copy number revisited: an analysis of 625 unrelated Spanish patients and a compilation of 2834 reported cases. *Neuromuscul Disord*. 2018;28(3):208—215.
72. Rigo F, Hua Y, Krainer AR, et al. Antisense-based therapy for the treatment of spinal muscular atrophy. *J Cell Biol*. 2012;199(1):21—25.
73. Prior TW, Swoboda KJ, Scott HD, et al. Homozygous SMN1 deletions in unaffected family members and modification of the phenotype by SMN2. *Am J Med Genet*. 2004;130A(3):307—310.
74. Passini MA, Bu J, Richards AM, et al. Antisense oligonucleotides delivered to the mouse CNS ameliorate symptoms of severe spinal muscular atrophy. *Sci Transl Med*. 2011;3(72):72ra18.
75. Poirier A, Weetall M, Heinig K, et al. Risdiplam distributes and increases SMN protein in both the central nervous system and peripheral organs. *Pharmacol Res Perspect*. 2018;6(6):e00447.