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Metabolic changes in response to food intake in *somatostatin 1.1* deficient zebrafish

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ABSTRACT

Somatostatin is a multifunctional hormone with several genes in teleost fishes. A zebrafish CRISPR/Cas9 knockout of the *somatostatin 1.1* (*sst1.1*) with persistent hyperglycaemia and hyperlipidaemia displayed reduced fecundity when fed brine shrimp ad libitum. Here, we investigated the effect of feeding brine shrimp one to three times a day on fecundity and liver transcriptomics of the *sst1.1* mutant compared to their wild-type siblings to unravel molecular pathways associated with the phenotype. We find that the *sst1.1* deficient zebrafish had high mortality when fed at the highest rate and that in both genotypes, growth and fecundity were proportional to food intake. Although glucose and cholesterol decreased substantially at the lowest level of feeding, they were still higher in the mutant than in the wild-type zebrafish. Furthermore, *sst1.1* deficiency had a small but significant effect on the hepatic expression of protein, carbohydrate, and fatty acid biosynthesis genes, contributing to the mutant's diabetic phenotype.

1. Introduction

Somatostatins (SST) are cyclic peptides with multiple functions produced in several tissues. The product of the mammalian *SST1* gene, preprosomatostatin, yields three bioactive peptides: neuronostatin, an N-terminus amidated peptide of 13 amino acid residues, and shorter (SST-14) and longer (SST-28) forms of SST from the C-terminus. Neuronostatin is produced in the spleen, pancreas, cerebrum and hypothalamus; SST-14 is produced in the central nervous system, including the hypothalamus, peripheral nerves, and pancreatic δ-cells; SST-28 is produced in the gastrointestinal δ-cells (Ampofo et al., 2020). SST-14, among other functions, plays a vital role in glucose regulation through paracrine inhibition of both insulin and glucagon secretion from islet α-cells and β-cells (Briant et al., 2018; Klein and Sheridan, 2008; Malaisse, 2014; Rorsman and Huising, 2018). Exogenous SST-14 and SST-28 treatments inhibit insulin and glucagon secretion (Strowski et al., 2000), while glucose supplementation can partly reverse neonatal mortality in δ-cell-deficient mice (Li et al., 2018). Both insulin and glucagon release are inhibited through the tonic influence of SST from δ-cells, although other factors produced by δ-cells also contribute (Hauge-Evans et al., 2009; Li et al., 2018). The SST actions are mediated mainly through SST receptors 2 (SSTR2) and 5 (SSTR5), which are expressed in both pancreatic α- and β-cells (Hauge-Evans et al., 2009; Strowski et al., 2000). In turn, the δ-cells are a target of multiple factors that regulate SST secretion, including glucose, ghrelin and urocortin 3 (DiGruccio et al., 2016; van der Meulen et al., 2015; Wang et al., 2014).

SST peptides from teleost fishes, in common with those from mammals, are produced in several tissues and are essential modulators of carbohydrate and lipid metabolism (Eilertson and Sheridan, 1993). However, unlike mammals, which have only two SST family genes, *SST1* and *SST2* (*CORT* or *cortistatin*) (Spier and de Lecea, 2000), teleost fishes have six SST family genes, five of which are already present in the ancestral gnathostome, some being lost in the mammalian lineage (Liu et al., 2010; Tostivint et al., 2019). SST-14 and SST-25 isoforms have been identified to be produced in the pancreas of salmonids and have both glycolytic and lipolytic effects (Eilertson and Sheridan, 1993, 1995; Sheridan and Hagemeister, 2010; Sheridan et al., 1987). Their pancreatic release is regulated by insulin, glucagon, and SST-14 and modulated by glucose (Eilertson et al., 1995). The Brockman bodies in teleost fishes contain islets of endocrine pancreas surrounded by exocrine tissue,

Abbreviations: sst1.1, *somatostatin 1.1*; sstr, SST receptor; BL, body length; BW, body weight; TG, triglyceride; TC, total cholesterol; PCA, principal component analysis; DEGs, differential expressed genes; GO, Gene ontology; KEGG, Kyoto encyclopedia of genes andgenomes.

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maintaining a structure and cellular architecture conserved in the mammalian pancreas (Argenton et al., 1999; Biemar et al., 2001). They are scattered around the spleen and large intestine and vary in number (Biemar et al., 2001; Tehrani and Lin, 2011). Ablation studies are therefore challenging, and immune suppression has only occasionally been used to study the role of SST on metabolism (Plisetskaya et al., 1989). Instead, we have used the CRISPR/Cas9 system to establish a zebrafish *sst1.1* knockout. This mutant line showed the characteristics of a diabetic phenotype, including hyperglycaemia in larvae and adults, glucose intolerance, and increased lipid levels due to increased α-cell mass. Compared to wild-type siblings, these fish also had reduced fecundity when fed ad libitum but not under normal feeding (Chen et al., 2024). *SST1* deficient mice also had impaired metabolism, were more sensitive to a high-fat than a low-fat diet, exhibiting altered growth and body composition (fat/lean percentage) and impaired glucose/insulin metabolism, especially in males (Luque et al., 2016). Here, we investigated the effect of feeding level on the metabolic phenotype and fecundity of *sst1.1* deficient zebrafish. We analysed their blood chemistry and compared the liver transcriptome at high and low feeding levels. We found feeding level and genotype to influence mortality, plasma glucose and cholesterol levels with no effect on fecundity, while changes in liver gene expression in the mutant included carbohydrate, protein and fatty acid synthesis.

2. Materials and methods

2.1. Zebrafish maintenance and spawning

The Shanghai Ocean University Animal Ethics Committee approved the zebrafish experimental protocols (SHOU-DW-2-2020-027). The zebrafish were kept in an Aquaneering (San Diego, USA) facility with recirculating water (10 % replacement per hour), mechanical and charcoal filtration, and UV treatment at 26–28 °C, pH 7.6–8.0 and conductivity 300–600 μS, oxygen 6–8 mg/l under a 14:10-h light:dark circadian cycle.

Heterozygous zebrafish carrying an allele with the *sst1* mutation (Chen et al., 2024) were incrossed to produce wild-type (WT, *sst1.1*$^{+/+}$) and homozygous mutants (MT, *sst1.1*$^{-/-}$) and maintained in a mixed population to avoid tank effects. They were fed *Paramecium* two to three times a day until day 28 post-fertilisation. From day 14 onwards, they were fed brine shrimp (*Artemia* spp.). At 3.5 months, fish were genotyped, and WT and MT zebrafish were divided into three groups in triplicate tanks, each receiving 3 male/female pairs. The three groups of WT and MT mutants were fed brine shrimp nauplii at different feeding frequencies: group “high” was fed three times a day, group “medium” was fed once a day, and group “low” was fed once every two days for the 16 weeks duration of the experiment. The brine shrimp were continuously added to the experimental tanks in small volumes for 15 min at each feeding.

The brine shrimp used in the feeding experiments were freshly hatched from freeze-dried cysts (50 g; Qicaiyu, China) in artificial seawater. After 36 h, they were harvested with a mesh, washed, and placed in a 3 L tank with aerated water until used within 8–10 h. The brine shrimp suspension was diluted 10 times with zebrafish water, and brine shrimp density was determined by counting the larvae under a stereoscope.

To estimate breeding efficiency (% pairs that successfully mated), breeding pairs were established in the afternoon and maintained separated through a partition in the middle of the breeding tank. The partition was removed the following day, and one hour later, the number of eggs at the bottom of the tank was counted to determine the fecundity (number of eggs spawned).

2.2. Morphometry and liver collection

The fish were anesthetised in 40 μg/ml tricaine, pH 7, for body weight and body length measurements. The fish were blotted on no-dust tissue, measured to the nearest mm and weighed to the nearest 0.01 g at the beginning of the feeding experiments, followed by every two weeks in the first two months, and then monthly. Each measurement took no >2 min. At the end of the experiment, fish were euthanised with an overdose of anaesthetic, and blood (1 μl) was taken in a heparinised microcapillary from the severed tail, centrifuged at 5000 rpm 4 °C for 10 min. Livers were dissected and stored at −80 °C until RNA extraction.

2.3. Blood biochemistry

Biochemical measurements were made using specific kits after diluting the plasma 100 times in the corresponding assay buffers following the manufacturer's instructions: Amplex™ Red Glucose/glucose oxidase Assay kit (Invitrogen, #A22189); Triglyceride Assay Kit (Abcam, #ab65336); Cholesteryl Ester Assay Kit (TC; Abcam, #ab65359).

2.4. Liver transcriptomics

Total RNA from the liver of WT and MT from the high-frequency feeding and low-frequency feeding groups ($n = 5$) was isolated by Eastep® Super total RNA isolation Kit (Promega). Sequencing libraries were prepared and quantified following the manufacturer's protocol (VAHTS Universal V6 RNA-seq Library Prep Kit). They were sequenced on an Illumina Novaseq 6000 platform, and 150 bp paired-end reads were generated as raw reads. Raw reads were first processed using fastq, and low-quality reads were removed to obtain clean reads for subsequent analyses. Clean Reads were aligned to the zebrafish genome (GRCz11) using HISAT2 (Kim et al., 2015; Liao et al., 2019). HTSeq was used to calculate the read counts FPKM of each gene(Anders et al., 2014). Differentially expressed genes (DEGs) were determined using DESeq2 based on Q value <0.05 and |log2(fold change) ≥1. GO and KEGG pathway enrichment analysis of DEGs were performed using ClusterProfiler (Yu et al., 2012). Transcriptome data visualisation (Vulcano plot, Principal Components and Cluster Analysis) was done with SRplot (Tang et al., 2023). Sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA947539.

2.5. Statistics

A three-way analysis of variance (ANOVA) was used to test the effect of genotype, feeding level and time on length and log-transformed weight. A two-way ANOVA was used to compare the amount of food given and biochemical parameters according to feeding level and genotype. Tuckey's post hoc test was used to determine the significance levels in each factor. Person correlations with Dunn-Sidak corrections between biochemical parameters were calculated. Mortality was compared using Kaplan-Meier plots and the log-rank (Mantel-Cox) test. SYSTAT v13.2 (SYSTAT Inc., USA) was used to compute ANOVA statistics and GraphPad Prism v. 9.4.0 (Domatics, USA) to compute Kaplan-Meier mortality plots and statistics. Values are shown as mean ± standard error of the mean (SEM). The statistical significance level was 5 %.

3. Results

3.1. Growth and reproduction and mortality

Fish ingested significantly different amounts of feeding ($F_{(2,420)} = 144.8$, $P < 0.001$) according to treatment with no significant differences between genotypes (Table 1).

A high dietary intake (three times a day) caused higher mortality in the *sst1.1* mutant starting ca. day 20 after the experiment began, reaching 20 % at ca 70 days and 50 % at 120 days. In fish fed once a day or every other day, mortality did not differ between MT and WT, i.e. ca. 5–10 % mortality (Fig. 1).

Table 1
Number of daily *Artemia* nauplii ingested per individual in each feeding group and genotype (mean ± standard deviation).

Genotype	High	Medium	Low
Wild-type	2631 ± 714	1053 ± 273	600 ± 592
Sst1.1 mutant	2273 ± 612	917 ± 240	506 ± 501

For length (Fig. 2a) there was a significant two-way interaction between treatment and time ($F_{(10,574)} = 4.613$, P<001) and no significant effect of genotype. Fish that received the higher dietary intake grew significantly faster in length, followed by the medium and the low intake, with statistically significant differences between the three groups ($P < 0.05$) already two weeks into the diet. Similarly, for weight (Fig. 2b), there was a significant two-way interaction between treatment and time ($F_{(10,574)} = 4.707$, $P < 0.001$), but there were also small but significant main effects of genotype ($F_{(1,574)} = 42.140$, $P < 0.001$) with WT mean weight 9 % higher than MT. At the low feeding level, there was a significant increase in length only after week 12 compared to week 2 ($P < 0.05$), but there was no change in weight.

Fecundity strongly depended on feeding ($F_{(2,188)} = 73.416$, $P < 0.001$), with differences between genotypes only at the high feeding level ($F_{(1,188)} = 5.676$, $P < 0.02$) (Fig. 3a). There were no differences in breeding efficiency except in the low-fed group ($F_{(1,36)} = 6.997$, $P = 0.012$) (Fig. 3b).

3.2. Blood biochemistry

There was a significant two-way interaction for glucose between feeding level and genotype ($F_{(2,64)} = 6.629$, $P = 0.002$). The low-feeding groups had a significant reduction in plasma glucose compared to the high-feeding group, and only in the low and medium-feeding groups was glucose significantly higher in the mutant compared to WT ($P < 0.05$) (Fig. 4a).

The main effects of genotype ($F_{(2,64)} = 43.952$, $P < 0.001$) and feeding level ($F_{(1,64)} = 28.889$, $P < 001$) on total blood cholesterol (TC) were statistically significant with no interaction. Mutants in all groups had a higher cholesterol level than WT, and the three feeding groups differed significantly in cholesterol levels. There was a strong positive correlation between glucose and cholesterol ($R = 0.645$, $n = 33$, $P < 0.0001$), albeit slightly reduced in the MT ($R = 0.484$, $n = 29$, $P = 0.023$) (Fig. 4b).

There was a significant main effect of feeding frequency on triglyceride (TG) levels ($F_{(2,56)} = 4.123$, $P = 0.02$) due to the difference between the WT high-feeding group compared to WT medium-feeding with no significant difference between genotypes (Fig. 4c).

3.3. Transcriptomic response

Each library produced 44–50 million clean reads with Q30 base pairs between 93.1 % and 94.4 % and an average GC content of 47.3 %. The reads' alignment rate to the zebrafish genome is 90.8 % to 94.9 %, indicating good quality of the sequencing data. One low-feeding individual was excluded because of contamination with cardiac muscle transcripts.

The two first components of the principal component analysis (PCA) explained 48.4 % of the variation (Fig. 5a). The feeding level was well separated along PC1, but the genotype was poorly separated. Although MT and WT seemed better separated in the high-feeding group, this group was also more variable. However, in the cluster analysis, MT separated clearly from WT in both feeding levels (Fig. 5b and c).

In the high-feeding group (three times a day), there were 190 DEGs between WT and MT, of which 106 were up-regulated and 84 were down-regulated (Fig. 6a, Supplemental Table 1). Among the most upregulated were annotated genes associated with cytoskeleton (*saxo2*, *cfap45*, *iqcg*), angiogenesis (*angptl1b*), calcium homeostasis (*pvalb6*), immune response (*serpinb1l2*), cell survival and cell proliferation (*pimr188*), bicellular tight junction assembly and cell adhesion associated (*cldn11a*), odorant receptors (*or109–13*) and thyroid hormone transporter (*slco1c1*) (Supplemental Table 1). Among the most downregulated genes were iron metabolism (*hamp*), cytoskeleton (*spata5*), protein modifications (*tmtc1*, *spcs2*), translocation of phospholipids (*abca1b*), extracellular matrix (*pcolce2b*) amino acid, nucleoside and nucleobase transporters (*slc3a2b*, *slc29a1a*) and signal transduction regulator (*rgs2*, *cc2d1a*) (Supplemental Table 1).

In the low-feeding group (every other day), there were 1339 DEGs between WT and MT, of which 738 were up-regulated and 601 down-regulated (Fig. 6a, Supplemental Table 1). Among the most upregulated annotated genes in this group were transmembrane transport (*aqp8a.2*, *slc6a19a.2*), protein glycosylation (*gant8a.1*), extracellular components (*col10a1a*), immune response (*ccl25a*), neuropeptide signalling (*penka, glra2*), metal ion binding (*btr05*) and metabolic process (*acsl5*, *chia.3*). Among the most downregulated annotated genes in this group were associated with cartilage development (*ostn*), extracellular region (*nbl1*), nucleotide binding (*hsc70*), membrane component (*duox2, mhc1uba, mhc1lda*), zinc ion binding (*gch2*), granule secretion (*scg2a*), nucleic acid binding (*elavl4*) and purine metabolism (*pde9al*) (Supplemental Table 1).

The main enriched biological processes associated with high feeding in MT compared to WT were protein synthesis (downregulated) and ciliary movement (upregulated) (Fig. 7a, Supplemental Table 2). In contrast, the main low-feeding biological processes were cholesterol biosynthetic process (downregulated), transmembrane transport (upregulated), fatty acid biosynthetic process (upregulated), rRNA processing (downregulated) and regulation of intracellular pH (upregulated) (Fig. 7b, Supplemental Table 2). The significant KEGG pathways

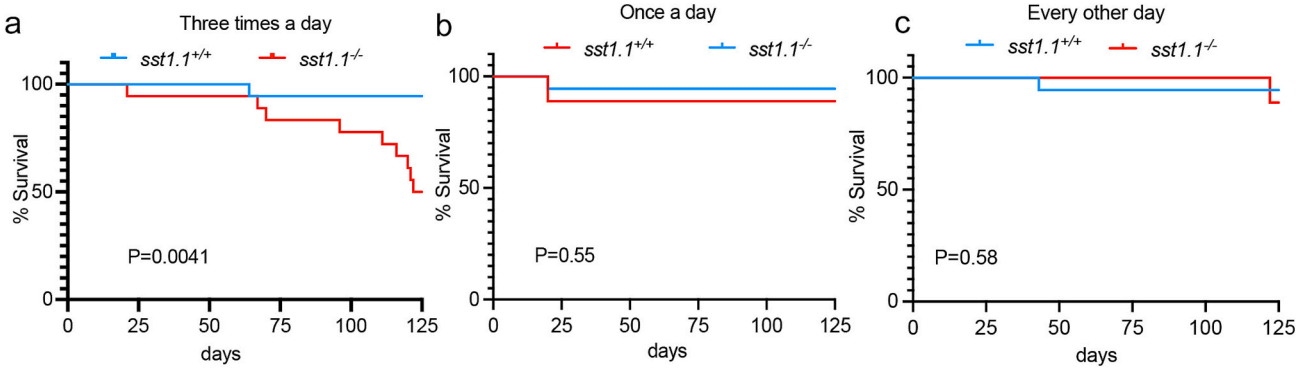

Fig. 1. Kaplan-Meier mortality plots of WT (*sst1.1*$^{+/+}$) and MT (*sst1.1*$^{-/-}$) according to the three feeding regimes. P indicates the significance level of the comparison of survival between WT and MT.

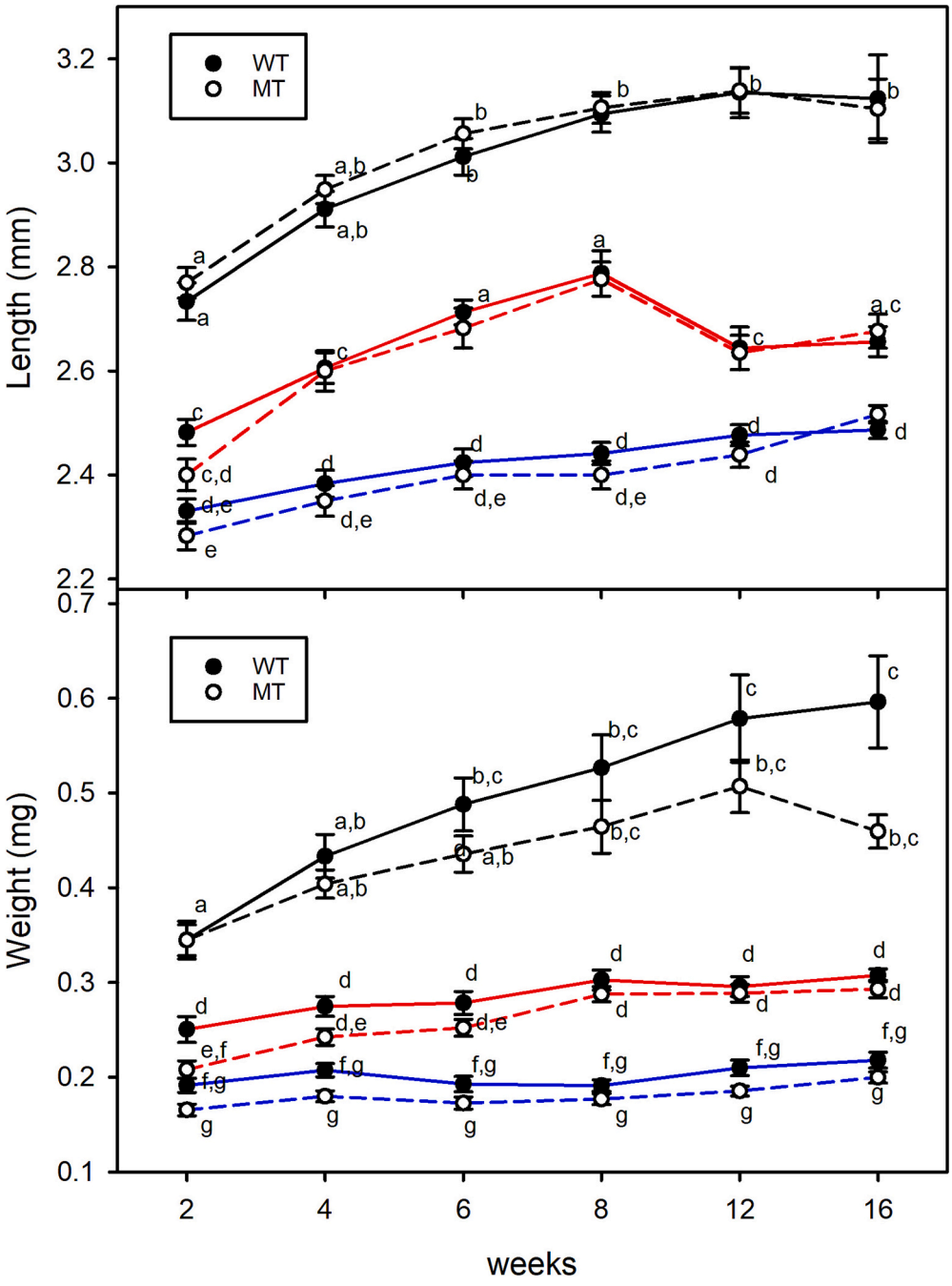

Fig. 2. Growth curves of WT (*sst1.1*$^{+/+}$) and MT (*sst1.1*$^{-/-}$). Body length (top) and body weight (bottom) are shown for each genotype and the three feeding regimes: three times a day (black), once a day (red) and every other day (blue). Error bars indicate the standard error of the mean; three-way ANOVA followed by the Holm-Sidak post hoc test; different letters indicate statistically significant differences. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated with high feeding were protein synthesis (downregulated) and carbohydrate metabolism (upregulated) (Fig. 7c, Supplemental Table 2). For the low feeding, they were fatty acid biosynthesis, PPAR signalling and ribosome biogenesis in eukaryotes (downregulated) (Fig. 7c, Supplemental Table 2).

4. Discussion

Feeding levels significantly affected growth and fecundity in both genotypes, with high feeding causing lower growth and high mortality in the MT genotype and low feeding causing lower growth and fecundity in the two genotypes. Blood glucose and TC were significantly decreased only in the low-feeding group of both genotypes, with higher levels in the MT.

Mortality was high in the MT high-feeding group and at normal levels (similar to the WT) in both the medium and low-feeding groups. Since glucose, TC, and TG did not vary significantly between the high and medium feeding groups, the cause of mortality may be related to an imbalance in another unknown physiological factor that we did not measure or to a mixture of factors.

As expected, the reduction of feeding caused a significant decrease in growth with the low feeding group just above maintenance, as indicated by the slight increase in length and no increase in weight. Other feeding experiments in which zebrafish were fed 1, 3 and 5 times daily to

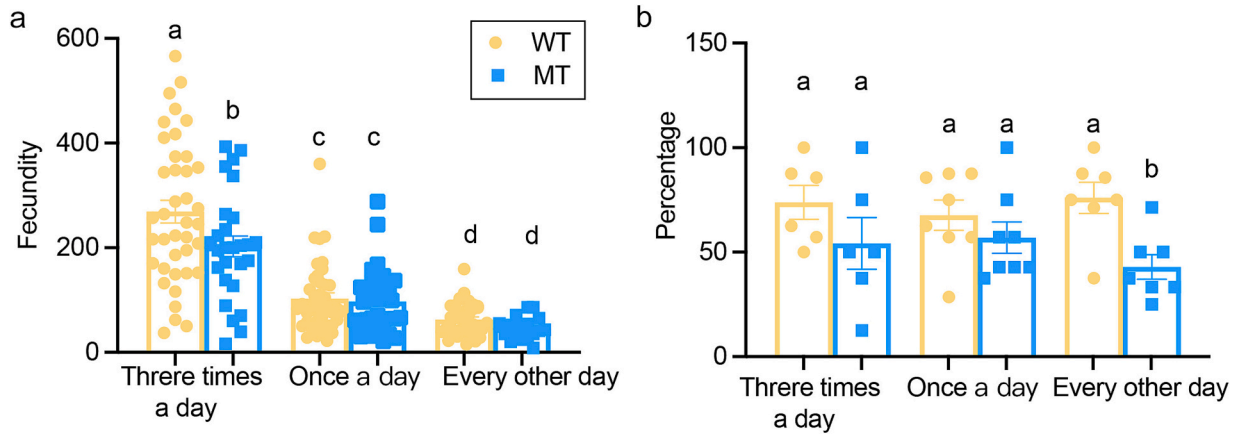

Fig. 3. Fecundity and feeding efficiency of *sst1.1* deficient zebrafish and their wild-type siblings according to three feeding regimes: three times a day, once a day and every other day. (a) Number of eggs per clutch spawned every 3–5 days; (b) Breeding efficiency of mating pairs (% that bred). Error bars indicate the standard error of the mean; two-way ANOVA followed by the Holm-Sidak post hoc test; different letters indicate statistically significant differences.

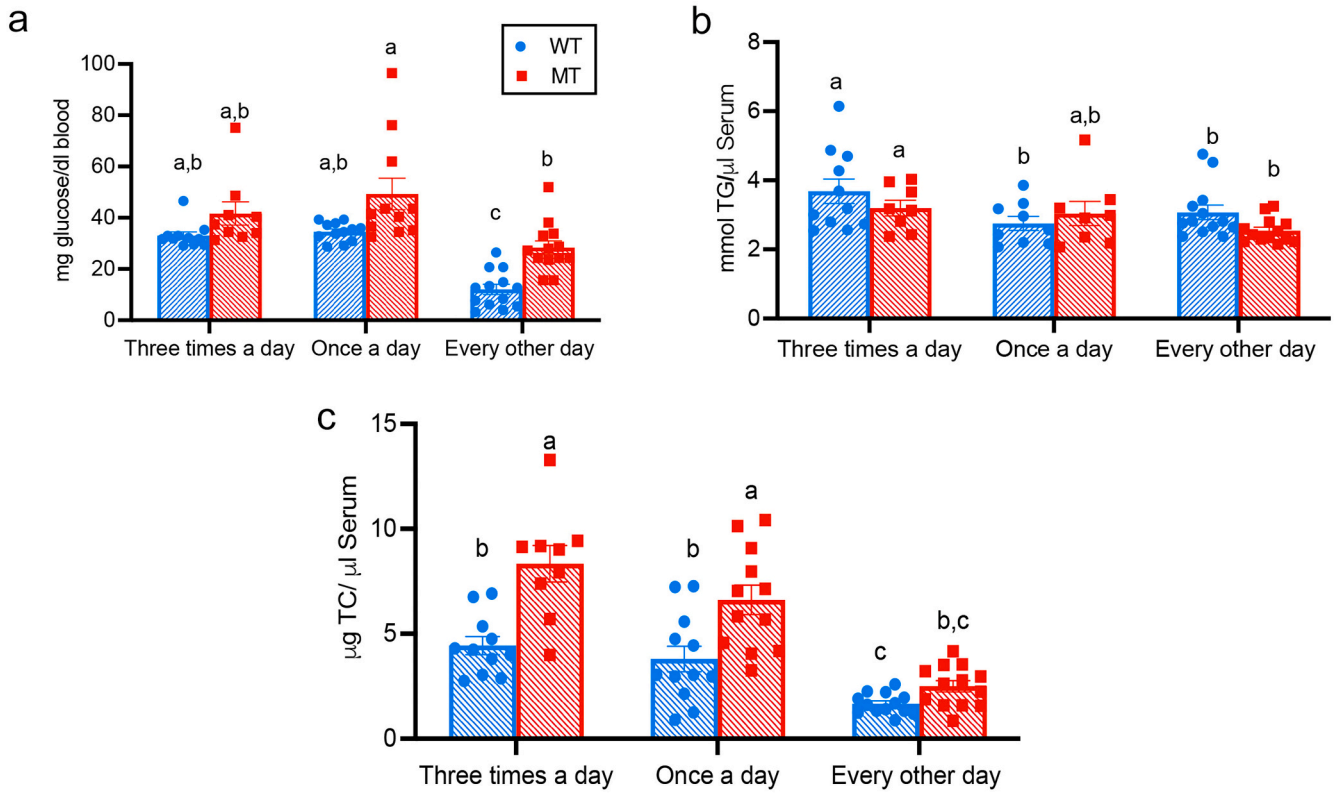

Fig. 4. Blood glucose, total cholesterol and triglyceride levels of WT (*sst1*$^{+/+}$) and MT (*sst1*$^{-/-}$) according to three feeding regimes: three times a day, once a day and every other day. (a) Blood glucose; (b) total cholesterol, TC, (c) triglyceride, TG. Error bars indicate the standard error of the mean; two-way ANOVA followed by the Holm-Sidak post hoc test; different letters indicate statistically significant differences.

satiation found a similar relationship between growth and feeding level (Lawrence et al., 2012). They also found that the higher feeding caused reduced breeding success despite no differences in fecundity (Lawrence et al., 2012). Our *sst1.1* deficient fish also showed a drastic reduction in breeding success and fecundity when fed ad libitum, possibly related to the diabetic phenotype (Chen et al., 2024). We found that fecundity was proportional to the feeding amount, although WT laid more eggs than MT only at high feeding levels. This suggests that at high feeding, possibly the same factors contributing to mortality also impair egg production. Since there are no significant differences in glucose and TC between the high and medium feeding levels, these may not contribute to mortality or fecundity.

In rainbow trout, SST-14 and SST-25 injections elevated plasma glucose and increased hepatic glycogenolysis (Eilertson and Sheridan, 1993). SS-14 and SST-25 treatment of cultured liver and adipose tissue caused stimulation of hepatic fatty acid and glycerol release, and simultaneously, liver triacylglycerol lipase activity was elevated (Eilertson and Sheridan, 1994). Similar lipolytic and hyperglycaemic effects also occurred in other teleost fish and lamprey via indirect and direct actions (Kao et al., 1998; Sheridan and Kao, 1998; Sheridan and Kittilson, 2004). Those observations are unlike our *sst1.1* deficient zebrafish, which generally had significantly elevated glucose and TC

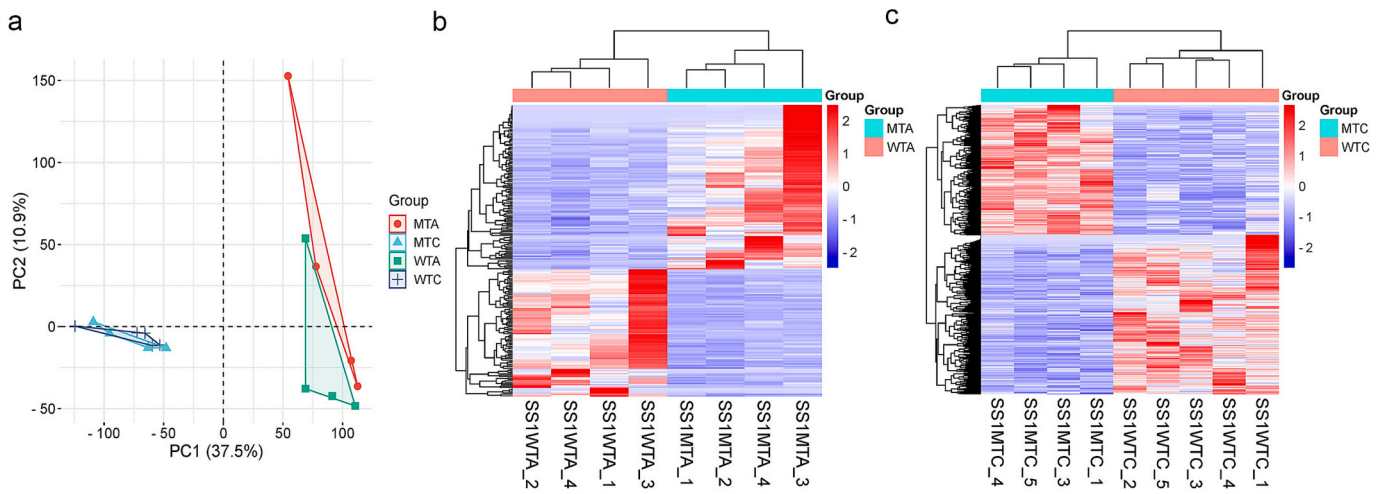

Fig. 5. Clustering of DEGs in the high (MTA and WTA) and low-feeding (MTC and WTC) groups liver transcriptomes of WT (sst1$^{+/+}$) and MT (sst1$^{-/-}$) zebrafish. (a) Principal component analysis. (b) high feeding and (c) low feeding heatmaps.

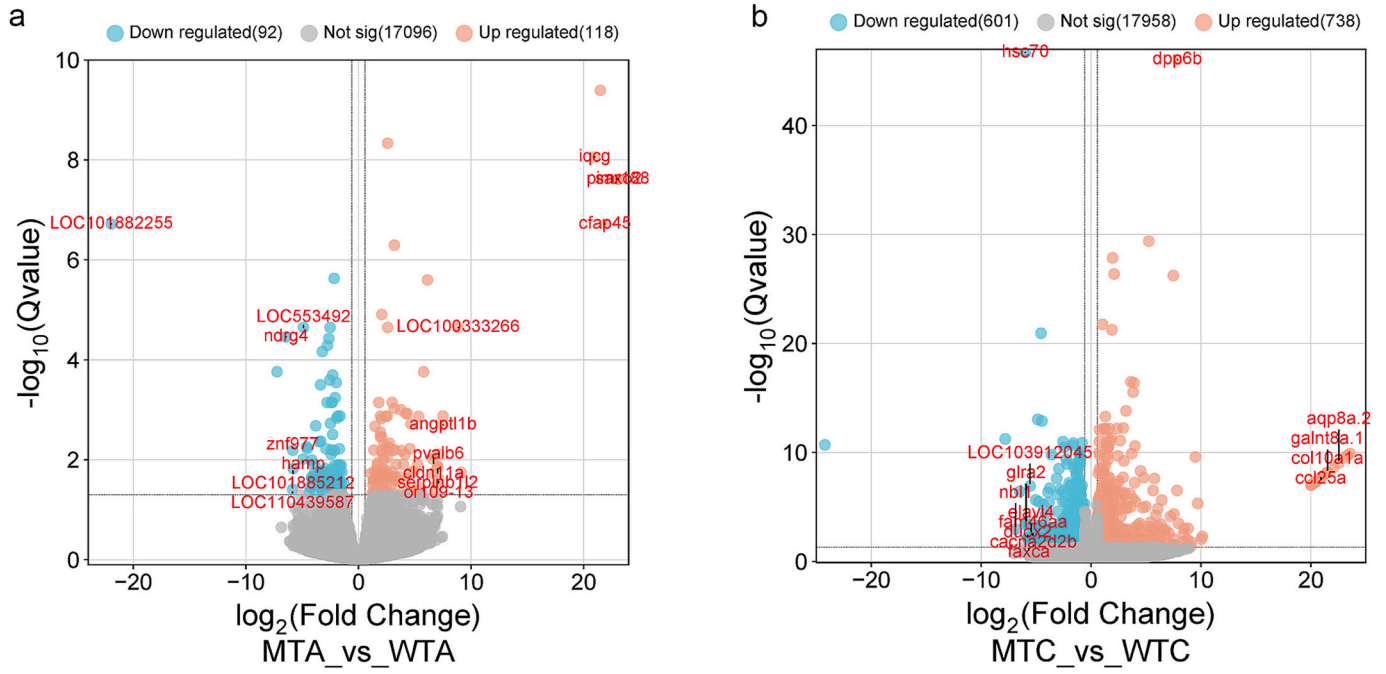

Fig. 6. Volcano plots of differentially expressed genes in WT (sst1$^{+/+}$) and MT (sst1$^{-/-}$) zebrafish liver transcriptomes of in response to feeding. (a) High feeding and (b) low feeding. Blue: downregulated, red: upregulated, threshold fold change ≥2 and Q value ≤0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

levels. Since both glucose and insulin promote cholesterol biosynthesis and uptake, and somatostatin inhibits insulin (Xiao et al., 2022), SST-14 deficiency (a product of *sst1.1*) is expected to lead to high cholesterol, considering the observed blood hyperglycaemia. This is supported by the high correlation between glucose and cholesterol levels in the WT and, to a lesser extent, in the MT. The difference between genotypes could be partly related to cholesterol biosynthesis genes being down-regulated in the MT low-feeding group despite plasma TC being elevated compared to the WT.

In contrast to glucose and TC, TG levels were only affected by feeding level, not genotype. The differences in glucose response between our sst1.1. deficient zebrafish and the trout treated with SST-14 and SST-25 could be related to the fact that in injection experiments with intact fish, endogenous hormone levels may contribute to the response, and the ratio of endogenous SST peptides also altered in the zebrafish mutant. The *sst1.1* transcript only produces SST-14 (Tostivint et al., 2013), whose production was eliminated in the mutant. However, the zebrafish mutant still produces SST-14 isoforms differing from 1 to 4 amino acids from the remaining 5 SST family genes (Tostivint et al., 2013), and the balance of peptides from different SST genes may result in specific effects, such as in the *sst1.1* mutant. Furthermore, the *sst1.1* mutant produces an excess of pancreatic α-cells, which could lead to a high glucagon-to-insulin ratio and elevated levels of plasma glucose (Chen et al., 2024; Lee et al., 2016; Parrilla et al., 1974), although confirmation requires measurement of the plasma hormone. Notably, the *glucagon receptor betta* (*gcgrb*) was significantly upregulated in the low-feeding group, suggesting increased sensitivity to glucagon. Glucagon action in the liver is a central response to fasting and type 2 diabetes (Tavares et al., 2020) and could also play an essential role in the expression of our MT phenotype.

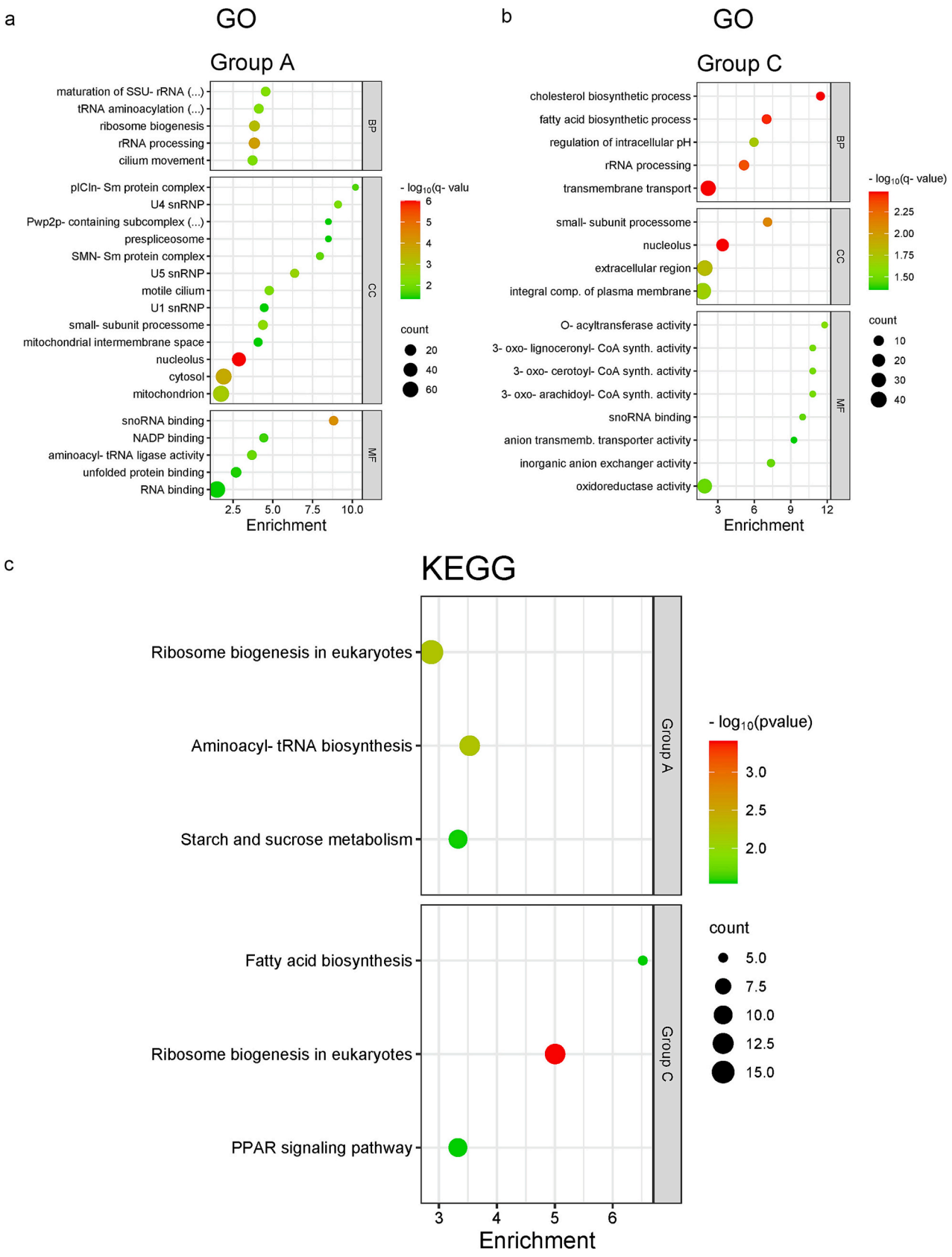

Fig. 7. Dot plot of GO enrichment and KEGG pathways for DEGs of WT (sst1$^{+/+}$) and MT (sst1$^{-/-}$) zebrafish liver transcriptomes in response to feeding. (a) GO enrichment of high feeding group, (b) GO enrichment of low feeding group, (c). KEGG enrichment analysis of high-feeding (Group A) and low-feeding groups (Group C).

While feeding induced significant changes in the liver transcriptome, reflecting large metabolic changes between high and low feeding levels, changes between genotypes, albeit significant, were much smaller, with higher variation in the high-feeding group, as revealed by the PCA.

Interestingly, the MT protein synthesis machinery was enriched in downregulated genes, particularly rRNA processing, maturation and biogenesis in both the high and low-feeding groups. We do not have an explanation for this observation. However, it has been reported that a high-fat diet represses rRNA transcription in mice, reducing intracellular energy consumption mediated by the nucleolar factor nucleomethylin (Nlm), essential for energy storage (Oie et al., 2014). Although, we did not find a differentially expressed *nlm* transcript, *NOC2-like nucleolar associated transcriptional repressor (noc2l)*, involved in the biogenesis and processing of rRNA (Lu et al., 2023), was downregulated in the high and low-feeding groups. This could indicate a slowdown of metabolic processes, although further studies are required for confirmation. Excess feeding (*Artemia* 6 times per day) can induce a type 2 diabetic phenotype (Meng et al., 2017; Zang et al., 2017) and in overfed zebrafish (*Artemia* 4 times per day), hepatic fatty acid biosynthesis was upregulated, and glucose metabolism was downregulated (Ge et al., 2023), indicating storage processes. This contrasts with our results in which the liver of the high-feeding MT was enriched with upregulated carbohydrate metabolism genes compared to WT. However, this was not reflected in significantly higher blood glucose levels (possibly because of glucose-related differential mortality). In contrast, in the low-feeding group, processes and pathways related to lipid biosynthesis (fatty acid and cholesterol) were also enriched (mainly downregulated) and in agreement with the higher levels of plasma metabolites.

In conclusion, our study demonstrated that food intake influenced growth and fecundity in zebrafish and that *sst1.1* deficiency had a small but significant effect on the hepatic expression of protein, carbohydrate, and fatty acid biosynthesis genes. This was reflected in higher plasma glucose and cholesterol levels, contributing to the mutant's diabetic phenotype.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.genrep.2024.102062.

CRediT authorship contribution statement

Jie Chen: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Huiming Yuan:** Methodology, Investigation, Data curation. **Jing Gao:** Methodology, Data curation. **Lu Liu:** Data curation. **Adelino V.M. Canario:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

Ethics statement

The Animal Welfare Committee of Shanghai Ocean University reviewed and approved the study.

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Declaration of competing interest

Adelino V. M. Canario reports financial support was provided by Foundation for Science and Technology (Portugal). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

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