

RESEARCH ARTICLE

Genetic analysis of body weight in wild populations of medaka fish from different latitudes

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Abstract

The genetic bases of growth and body weight are of economic and scientific interest, and teleost fish models have proven useful in such investigations. The *Oryzias latipes* species complex (medaka) is an abundant freshwater fish in Japan and suitable for genetic studies. We compared two wild medaka stocks originating from different latitudes. The Maizuru population from higher latitudes weighed more than the Ginoza population. We investigated the genetic basis of body weight, using quantitative trait locus (QTL) analysis of the F₂ offspring of these populations. We detected one statistically significant QTL for body weight on medaka chromosome 4 and identified 12 candidate genes that might be associated with body weight or growth. Nine of these 12 genes had at least one single nucleotide polymorphism that caused amino acid substitutions in protein-coding regions, and we estimated the effects of these substitutions. The present findings might contribute to the marker-assisted selection of economically important aquaculture species.

Introduction

Growth and body weight are economically important traits in the livestock industry and in aquaculture. Such traits involve complex physiological processes that are controlled by various environmental and genetic factors. Quantitative trait locus (QTL) mapping and marker-assisted selection for economic traits, including growth and body weight in aquaculture, have recently been conducted in several studies using molecular markers such as microsatellites and single nucleotide polymorphisms (SNP) [1–7].

Body weight depends not only on growth traits but also on body composition and metabolism. Genome-wide association studies (GWAS) of body mass index (BMI) over the past decade have associated several hundred SNPs with body weight and obesity [8,9].

[index.html](#)) under the accession numbers DRR226810-DRR226964.

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Animal models play essential roles in most aspects of medicine. Diet-induced obesity in zebrafish and mammalian obesity are pathophysiologically similar; thus, the roles of genes associated with visceral adiposity have been examined in zebrafish models of human obesity [10,11]. Understanding the genetic basis of body weight in teleost fish models could help deepen the medical understanding of obesity.

Ecological profiles of body size are distributed within species according to the Bergmann's rule, which states that animals living at high latitudes are generally larger than those living in low latitudes [12]. Some exceptions exist, but the findings of several studies are in line with the Bergmann's rule and its applicability in many types of mammals [13], birds [14,15], and ectothermic vertebrate and invertebrate taxa [16]. However, the underlying genetic mechanisms that result in a body size cline remain obscure.

Medaka are small freshwater fish that are native to Japan, Korea, and China. Japanese wild populations of the *Oryzias latipes* species complex are widely distributed from high to low latitudes throughout the Japanese archipelago. Previous phylogeographic studies using allozymes, mitochondrial DNA (mtDNA) sequences, and genome-wide SNP analysis revealed that Japanese wild medaka comprise Northern and Southern Japanese populations [17–20]. The average rate of SNPs between two inbred strains derived from the two populations is 3.4% [21]. Inbred strains and wild stocks of medaka originating from Japanese wild populations are currently available through the National BioResource Project (NBRP) (<https://shigen.nig.ac.jp/medaka/top/top.jsp>). Phenotypic variations between these two populations have been described for several traits, including brain [22] and craniofacial morphology [23], body color and sexual dimorphism [24], vertebral regionalization and number [25], aggressiveness [26], startle behavior [27], and male-specific ossified processes and sex steroid levels [28,29]. Several QTL have been detected by focusing on these phenotypic variations using genetic analyses based on draft [21] and updated medaka genome sequences (http://utgenome.org/medaka_v2/#!/Top.md). We investigated two medaka wild stocks originating from different latitudes. Body weight was higher in the Maizuru population than in the Ginoza population from high and low latitudes, respectively. We investigated the genetic basis of body weight via QTL analysis of the F₂ offspring of these medaka populations. We detected one statistically significant QTL for body weight on chromosome 4 and assessed candidate genes located within that QTL region.

Materials and methods

Ethics statement

The Animal Experiment Committee at the National Institutes of Natural Sciences, Japan approved the study protocol (14A108, 15A047). The medaka used in these experiments were treated according to the animal experiment guidelines of the National Institutes of Natural Sciences, Japan.

Animals

The NBRP Medaka (<https://shigen.nig.ac.jp/medaka/>) supplied adult medaka (G₀ generations) from stocks originating from wild North and South Japanese populations at Maizuru City (Maizuru stock; strain ID, WS215) located at 35° 28' N 135° 23' E, Kyoto Prefecture) and Ginoza Village (Ginoza stock; strain ID, WS255) located at 26° 28' N 127° 58' E, Kunigamigun, Okinawa Prefecture), respectively. We then raised several generations of these fish in the laboratory. The Maizuru and Ginoza stocks were crossed to collect the G₁ generations, respectively. Then, Maizuru females were mass-mated with Ginoza males and vice versa to obtain the F₁ offspring, which was used to analyze body weight. Five Maizuru × Ginoza pairs were mated to

generate the F₂ offspring for QTL mapping. Three of these pairs comprised Ginoza females and Maizuru males and two comprised Maizuru females and Ginoza males.

Two to four weeks after hatching, all the generations were transferred outdoors between June and July, 2014, and maintained for 1 year under natural temperature and photoperiod at the outdoor experimental field of the National Institute of Basic Biology (34° 57' N 137° 9' E) in Okazaki, Aichi, Japan. Between June and July 2015, the fish were transferred to experimental aquariums and maintained in water circulation systems at 26°C ± 1°C for 1 month. The fish were fed with artificial dry food twice daily. Body weight was analyzed in 10 Ginoza G₁, 26 Maizuru G₁, 15 F₁ hybrid, and 126 F₂ fish euthanized with 0.05% 3-aminobenzoic acid ethyl ester methanesulfonate salt (MS222). Water was removed from the fish by blotting them with paper towels; then, each fish was weighed. Thereafter, the fish were frozen in liquid nitrogen and stored in a deep freezer (-80°C).

Restriction site-associated DNA sequencing (RAD-Seq) and SNP markers

Genomic DNA was extracted from muscle tissue using DNeasy Blood & Tissue Kits (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The concentration of DNA was determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific Waltham, MA, USA). Genomic DNA (40 ng) from each sample was digested using two restriction enzymes, BglII and EcoRI, ligated with a Y-shaped adaptor, and amplified by polymerase chain reaction (PCR) using KAPA HiFi HS ReadyMix (Kapa Biosystems Inc., Wilmington, MA, USA). Fragments (~300–360 bp) were selected using E-Gel Size Select (Life Technologies, Carlsbad, CA, USA). Details of the library preparation method are described elsewhere [30]. The fragments were sequenced on a HiSeq 2500 platform (Illumina Inc., San Diego, CA, USA) in 50-bp single-end mode. We conducted RAD-Seq in 10 parent fish from the 5 Maizuru × Ginoza pairs, 3–4 from the F₁ generation of each Maizuru × Ginoza pair, and 126 of the F₂ generation. The reads were quality filtered using Trimmomatic [31] under the following parameters: trimmomatic-0.32.jar SE -threads 8 -phred33 ILLUMINACLIP TruSeq3-PE-2.fa:2:30:10 LEADING:19 TRAILING:19 SLIDINGWINDOW:30:20 AVGQUAL:20 MINLEN:51. The trimmed reads were mapped to the draft genome of the Hd-rR inbred medaka strain (v. 2.2.4, http://utgenome.org/medaka_v2/#!/Assembly.md), and SNPs were called using the Stacks pipeline [32]. We identified RAD tags with a homozygous genotype in all Maizuru and all Ginoza and those that had different alleles between all Maizuru and all Ginoza parents for SNP marker selection. Among these markers, we selected those that were heterozygous in all the F₁ individuals and genotyped in > 80% of the 126 F₂ fish. Finally, we selected 371 RAD markers for QTL analysis (S1 Table). Genetic distances (cM) involving each chromosome were calculated using the Kosambi map function [33].

Raw sequence data were deposited in the DDBJ Sequence Read Archive (DRA) (<https://www.ddbj.nig.ac.jp/dra/index.html>) under the accession numbers DRR226810–DRR226964.

Analysis of QTL

Quantitative trait loci associated with body weight were mapped in the 126 F₂ fish, and simple interval mapping [34] proceeded using R/qtl software [35,36]. Genome-wide significant (5%) and suggestive (10%) thresholds of a single QTL were determined by 1000 permutation tests. Bayesian credible intervals (95% CI) were computed using the R/qtl function. Physical lengths of credible intervals (Mb) were predicted by extending the physical position of the nearest flanking markers.

Analyses of SNPs in the candidate genes

We selected genes within the 95% CI that were positioned according to the NCBI ASM223467v1 (GCF_002234675.1) assembly and associated with growth, body weight, and obesity on the basis of a literature search. We sequenced the selected candidate genes for the Maizuru and Ginoza fish and cataloged the SNPs located on their coding regions (S2 Table). We then analyzed these polymorphisms using GENETYX software (version 13, GENETICS Inc., Tokyo, Japan) to detect variants that could cause amino acid substitutions. We only considered polymorphisms in which all eight sequenced Ginoza and Maizuru individuals ($n = 4$ each) were homozygous for the allele. We then analyzed the amino acid substitutions using Protein Variation Effect Analyzer (PROVEAN) v1.1 to estimate their functional effects on the encoded protein [37]. A non-synonymous amino acid substitution with a potential functional effect was found on one of the genes (*sned1*), and its protein sequence (accession number XP_011471983.1) was compared with those of two teleost fishes, *Gasterosteus aculeatus* (stickleback; ENSGACG0000003698) and *Danio rerio* (zebrafish; XP_017212114.1), *Mus musculus* (mice; XP_006529380.1), and *Homo sapiens* (humans; XP_011509233) using the sequence alignment tool, ClustalW (version 2.1, DNA Data Bank of Japan).

Results

Body weight variation

Fig 1 shows that body weight was significantly higher in the Maizuru than in the Ginoza ($p < 0.01$, Student *t*-test), and Fig 2 shows a broader range of body weight distribution in the F_2 generation than in the parental populations.

Identification of body weight QTL

A genetic map was constructed using 371 SNP markers obtained by RAD-seq (Fig 3). The total length of the genetic map of the 24 chromosomes was 1496.32 cM, and the average calculated interval between each marker was 4.51 cM (Table 1). Physical lengths were determined based on reference medaka genome data. The total physical length was 676.74 Mb, and the average interval between each marker was 1.75 Mb (Table 1).

A QTL analysis of the 126 F_2 fish identified a statistically significant QTL region on the distal arm of chromosome 4, with a maximum LOD of 4.14 (Figs 3 and 4). The closest SNP marker was 56900. We calculated the mean body weight of the F_2 individuals that were homozygous for the Maizuru and Ginoza alleles of the closest marker and the heterozygous fish. The mean body weight was higher among individuals that carried a homozygous or heterozygous Maizuru allele than among those that carried a homozygous Ginoza allele (Fig 4C). We confirmed a linkage between an amplified fragment length polymorphism marker located on the distal position of chromosome 4 (0.68 Mb, S3 Table) and the SNP marker 56900 (4.7 cM) by genotyping using PCR.

Differences in the amino acid sequences of the candidate genes between Maizuru and Ginoza

The 95% Bayesian CI of the QTL was 0–7 cM (Fig 4B), and the physical location of the CI estimated from the genetic and physical positions of the markers 56900 (0 cM, 2.38 Mb) and 55681 (17.7 cM, 11.01 Mb) that were the closest to the QTL (Fig 3B) was 0–5.74 Mb. Among the 141 genes encoding proteins within the CI, we identified 12 that are reportedly associated with body weight or growth. Nine of these genes had at least one SNP that caused substitutions of amino acids in the coding regions (Table 2). We estimated, using PROVEAN, that most of

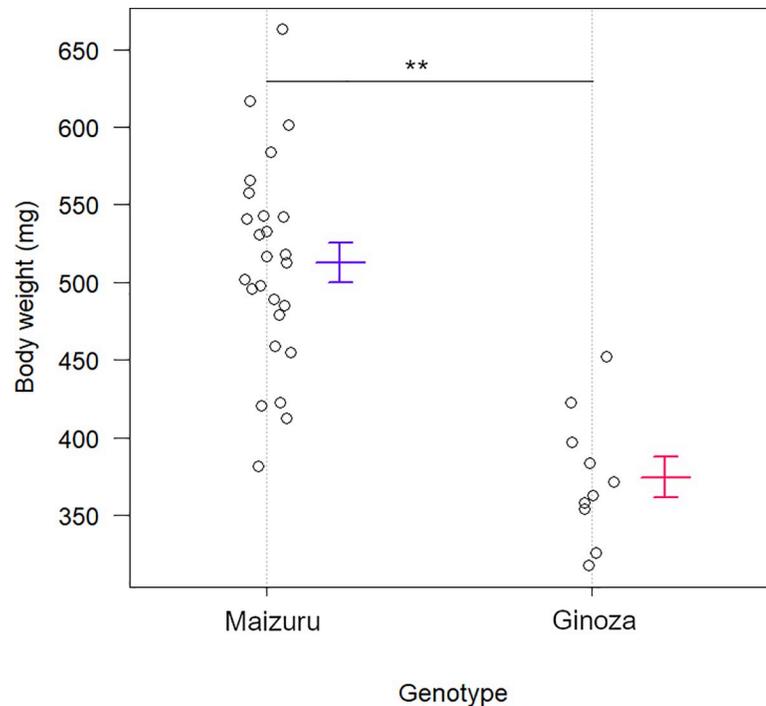


Fig 1. Mean body weight of Maizuru (n = 26) and Ginoza (n = 10). Data are shown as the means and standard errors (SE). Body weight was significantly higher in Maizuru than in Ginoza stock (512.6 vs. 374.7 mg; $**p < 0.01$; Student *t*-tests).

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these amino acid substitutions exerted neutral protein functional effects. However, one substitution in the protein SNED1 encoded by the gene *sned1* at G1013S appeared to have a deleterious (significantly different function) effect with a PROVEAN score of -2.648 (cutoff, -2.5). The amino acid glycine at 1013 in Ginoza SNED1 is conserved across other vertebrates, such as teleost, stickleback and zebrafish, as well mice and humans (Fig 5). In contrast, serine, which substituted for glycine in Maizuru SNED1, was not found in any other analyzed species.

Discussion

Medaka fish found at various latitudes provide an excellent model for investigating the genetic basis of body weight. Therefore, we compared two wild medaka stocks from Maizuru and Ginoza at different latitudes. Fish from the parental populations and the F₁ and F₂ generations were reared under the same environmental conditions to avoid the effects of plastic responses to temperature and other variables such as food availability during growth. Mean body weight differed significantly between the Maizuru and Ginoza individuals (Fig 1), reflecting the involvement of genetic components in the determination of body size. The Bergmann's rule is currently defined as a within-species tendency for body size to increase as latitude increases [12]. Our results conformed to Bergmann's rule, as body weight was greater among the Northern Maizuru population than the Southern Ginoza population.

The identification of genes that regulate complex multigenic traits such as growth and body weight has proven challenging. Over 6000 genes are considered to influence body weight in mice [38]. Multiple QTL regions are associated with body weight in Atlantic salmon (*Salmo salar*) [2]. Our QTL analysis identified a significant QTL on chromosome 4 (peak LOD, 4.14) (Fig 3), and the effects of the allele of the marker with the highest score supported the

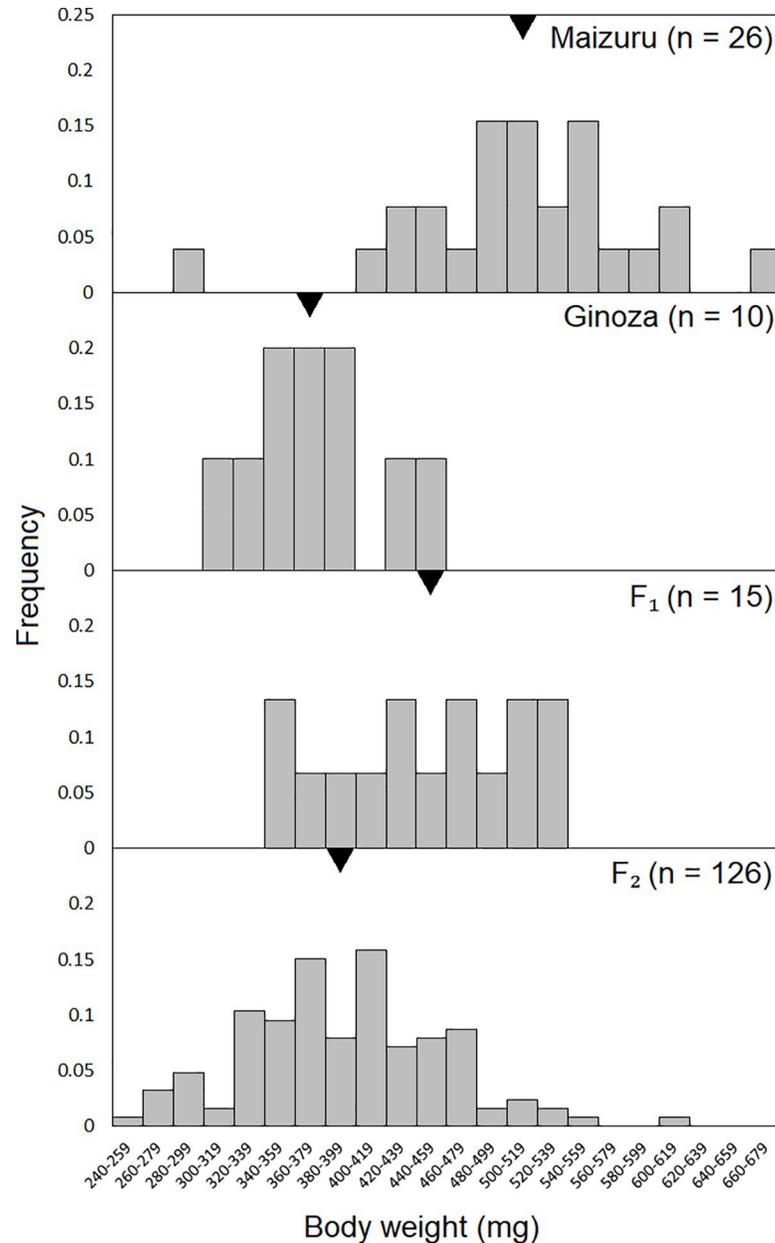


Fig 2. Distribution of body weight of the Ginoza, Maizuru, F₁, and F₂ fish. The Y axis indicates the frequency; the X axis indicates the body weight categories at 20-mg intervals. Black triangles indicate the means.

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phenotype found in the parental strains (the body weight was higher for the Maizuru allele than the Ginoza allele in the F₂ medaka). This explains 14% of the variance.

We identified 12 candidate genes involved in body weight and growth regulation within the significant QTL region. The genes *cilp2*, expressing cartilage intermediate layer protein 2, and *sned1*, expressing sushi, nidogen and EGF-like domain-containing protein 1, are associated with BMI in humans [39]; *mef2b* (myocyte-specific enhancer factor 2B), *rfxank* (regulatory factor X-associated ankyrin-containing protein), and *rab6b* (Ras-related protein Rab-6B) are associated with body weight and growth traits in sheep [40–42]; *sgcb* (sarcoglycan beta) is

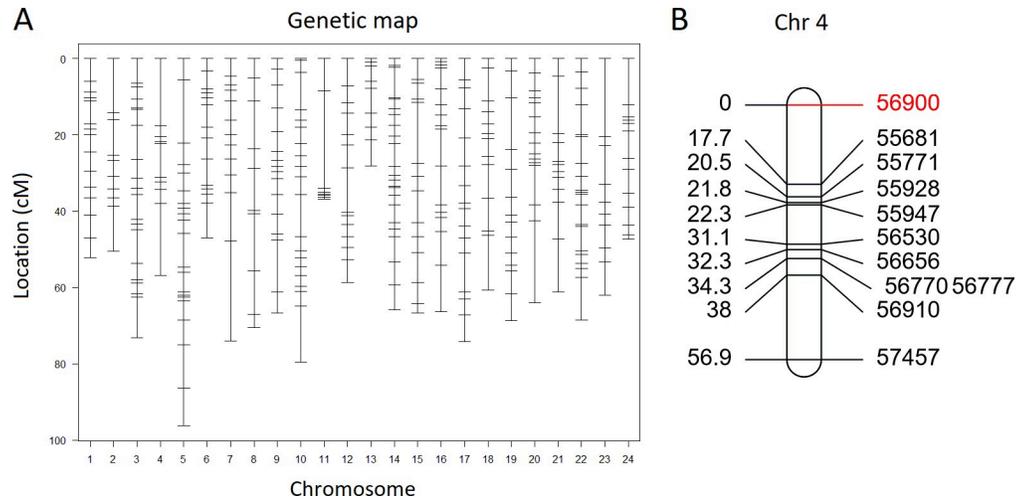


Fig 3. Genetic map of the single nucleotide polymorphism (SNP) markers. (A) Marker distribution across the 24 medaka chromosomes. (B) Genetic map of chromosome 4 show the marker names and locations (cM). Markers with the highest logarithm of odds (LOD) scores in the QTL analysis are shown in red.

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Table 1. Summary of markers used in the present study.

Chromosome no.	No. of markers	Genetic length (cM)	Average marker interval (cM)	Physical length (Mb)	Average marker interval (Mb)
1	16	52.23	3.48	34.38	2.05
2	10	50.5	5.61	22.52	2.1
3	22	73.18	3.48	37.51	1.68
4	11	56.87	5.69	29.86	2.75
5	21	96.32	4.82	32.35	1.61
6	14	47.04	3.62	28.5	1.32
7	15	74.12	5.29	33.17	1.92
8	10	70.61	7.84	26.12	2.79
9	16	66.73	4.45	33.05	1.72
10	21	79.51	3.98	31.09	1.52
11	15	36.82	2.63	23.49	1.65
12	16	58.75	3.92	24.39	1.25
13	10	28.19	3.13	26.89	1.46
14	24	65.83	2.86	28.27	1.01
15	14	66.65	5.13	29.59	1.9
16	19	66.33	3.68	29.84	1.25
17	16	74.15	4.94	31.74	1.92
18	13	60.63	5.05	28.49	1.57
19	14	68.67	5.28	25.31	1.75
20	16	64.06	4.27	24.71	1.63
21	13	61.22	5.1	29.82	2.35
22	22	68.5	3.26	24.06	1.1
23	10	62.08	6.9	21.47	2.38
24	13	47.29	3.94	20.12	1.43
Total	371	1496.28	4.51	676.74	1.75

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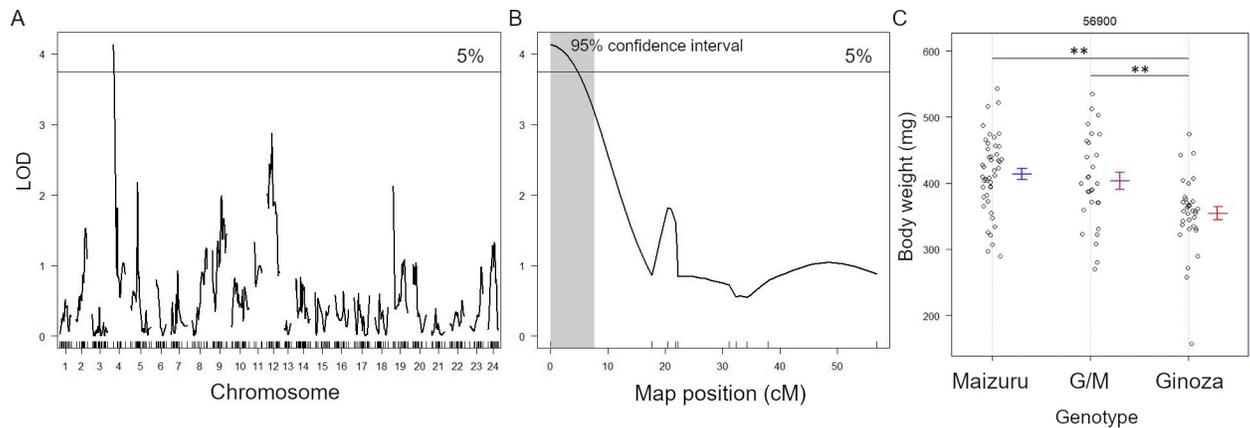


Fig 4. QTL analysis of the F₂ generation. (A) Results for all chromosomes. The significant QTL on chromosome 4 (B) has a peak LOD of 4.14. Gray areas indicate 95% Bayesian CI. (C) Body weight for genotypes of SNP marker 56900, which had the highest LOD score among the markers. Points represent the body weight of the F₂ individuals homozygous for the Maizuru alleles (n = 29), heterozygous for the Ginoza and Maizuru alleles (G/M, n = 46), and homozygous for the Ginoza alleles (n = 34). The means and standard errors (SE) are presented for each genotype. Differences between the mean body weight of the Ginoza and G/M genotypes and of the Ginoza and Maizuru fish were statistically significant (*p < 0.01, ANOVA and Tukey's honestly significance difference (HSD) test) but those between the G/M and Maizuru fish were not.

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associated with body weight in broilers [43]; *scl*y (selenocysteine lyase), *cep19* (19-kDa centrosomal protein), *dhcr24* (24-dehydrocholesterol reductase), and *lmo4* (LIM domain transcription factor) are associated with body weight and obesity traits in mammals [44–47]; and *mrpl55* (mitochondrial ribosomal protein L55) has a critical role in development and body size in *Drosophila* [48]. Furthermore, *sgta* (small glutamine-rich tetratricopeptide repeat-containing protein alpha) is a regulator of growth hormone receptors, which consequently influence body weight, because *sgta* knockout mice are smaller than wild-type mice [49].

Among the identified candidate genes, nine had amino acid substitutions that distinguished Maizuru from Ginoza (Table 2). The SNED1 amino acid substitution G1013S was predicted to be deleterious (functionally different) according to the PROVEAN score. In addition, the Ginoza amino acid variant, glycine, was prevalent among other vertebrate groups, whereas the serine residue in Maizuru was not found in other investigated species (Fig 5). SNED1 in humans is known as insulin-responsive sequence DNA-binding protein 1 (IRE-BP1); it is a transcription factor involved in the determination of BMI [37] and the activation of insulin-responsive genes and obesity [50]. *SNED1* is located at the terminal region of chromosome 2 in humans. Patients with a deletion in that region, with break-points at or within cytogenetic band 2q37, have a short stature among other features [51]. *Sned1* might be involved in mouse skeletal development, as *Sned1* knockout mice have craniofacial malformations and growth defects [52]. Therefore, we speculate that a functional difference in SNED1 protein activity caused by the amino substitution that distinguished Maizuru from Ginoza induced the difference in body weight. However, unidentified genes in the QTL region might also influence body weight through mechanisms other than differences in protein function, such as changes in their expression levels and/or profiles. These speculations await further investigation.

Future studies are necessary to identify the genetic variation(s) responsible for the differences in body weight between the medaka populations studied here. Nevertheless, the present results will contribute to the marker-assisted selection of economically important aquaculture species and provide a better understanding of the genetic mechanisms underlying ecological differences in body weight among populations at different latitudes.

Table 2. SNPs with non-synonymous substitutions in the candidate genes in Maizuru and Ginoza.

Gene symbol (Accession no.) ¹	Description	Position of mRNA (bp) ¹	SNP			Number of amino acids	Non-synonymous substitutions		
			Position (bp) ¹	Maizuru	Ginoza		Position (aa) ¹	Maizuru	Ginoza
<i>sgcb</i> (XP_020558516.1)	sarcoglycan beta	1356188– 1364186	1360124	G	C	296	98	Leu	Val
<i>cilp2</i> (XP_011471792.1)	cartilage intermediate layer protein 2	2139202– 2161322	2161297	T	C	1302	3	Lys	Arg
			2161159	G	A		49	Ser	Leu
			2149453	T	A		146	Thr	Ser
			2149400	A	C		163	Asp	Glu
			2149359	C	T		177	Ser	Asn
			2147355	T	C		326	Asp	Gly
			2147167	C	T		389	Val	Ile
			2146872	C	T		420	Gly	Asp
			2141205	T	A		961	Tyr	Phe
			2141068	T	A		1007	Met	Leu
			2140229	G	C		1286	Ile	Met
<i>mef2b</i> (XP_011471858.1)	myocyte-specific enhancer factor 2B	2306973– 2324496	2314502	G	A	421	218	Pro	Leu
			2314431	A	C		242	Ser	Ala
			2313743	C	T		266	Gly	Ser
			2308851	A	C		298	Val	Gly
			2308220	G	C		360	Ser	Thr
			2308128	T	C		391	Ile	Val
<i>rfxank</i> (XP_011471878.1)	DNA-binding protein RFXANK	2358970– 2362447	–	–	–	208	–	–	–
<i>rab6b</i> (XP_004067647.1)	ras-related protein Rab-6B	2693698– 2739982	–	–	–	215	–	–	–
<i>sned1</i> (XP_011471983.1)	sushi, nidogen and EGF-like domain- containing protein 1	2824005– 2850954	2850480	T	C	1349	39	Lys	Glu
			2843533	T	G		207	Gln	Pro
			2842008	T	A		372	Thr	Ser
			2841981	A	G		381	Tyr	His
			2837261	A	G		559	Leu	Pro
			2837260	C	T				
			2837250	C	T		563	Ala	Thr
			2835940	T	G		660	Asn	Thr
			2835939	G	T				
			2835923	C	G		666	Val	Leu
			2835883	G	C		679	Thr	Ser
			2835439	G	C		772	Gln	Glu
			2835283	A	G		792	Tyr	His
			2833621	T	G		912	Lys	Gln
			2833038	T	C		1013	Ser	Gly
			2830096	T	G		1070	Thr	Pro
			2829960	A	G		1115	Val	Ala
			2829735	C	A		1146	Ala	Ser
			2829731	C	T		1147	Arg	Lys
			2829460	C	G		1213	Ser	Thr
2825995	T	A	1315	Gln	Leu				
2825713	T	A	1346	Asn	Ile				

(Continued)

Table 2. (Continued)

Gene symbol (Accession no.) ¹	Description	Position of mRNA (bp) ¹	SNP			Number of amino acids	Non-synonymous substitutions		
			Position (bp) ¹	Maizuru	Ginoza		Position (aa) ¹	Maizuru	Ginoza
<i>scl</i> (XP_023809718.1)	selenocysteine lyase	2908002–2933175	2908316	A	C	445	411	Ser	Ala
<i>cep19</i> (XP_023809746.1)	centrosomal protein 19 kDa	3244247–3245808	3244607	C	T	157	128	Asp	Asn
<i>dhcr24</i> (XP_004067663.1)	delta(24)-sterol reductase	3287533–3295716	3288635	A	C	516	396	Ser	Ala
			3288401	G	A		452	Ala	Val
<i>lmo4</i> (XP_023809796.1)	LIM domain transcription factor LMO4	4586958–4596199	–	–	–	165	–	–	–
<i>sgta</i> (XP_023809802.1)	small glutamine-rich tetratricopeptide repeat-containing protein alpha	5201953–5215418	–	–	–	342	–	–	–
<i>mrpl55</i> (XP_023809802.1)	39S ribosomal protein L55, mitochondrial	5446241–5450258	5446451	T	C	138	16	Thr	Met
			5446578	C	A		21	Pro	Thr
			5446708	T	A		64	Leu	Gln
			5450099	T	A		118	Ser	Thr

¹Genes and positions are according to the NCBI ASM223467v1 (GCF_002234675.1) assembly.

The G1013S substitution in SNED1 with a deleterious effect (significantly different function) estimated by PROVEAN is shaded in gray.

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Maizuru_medaka   HVLSPLDGSEARTAVLNISTTQYTFRSLGPGQMYTVDVLTQSGIRPDEFPPSTSHSAGPL 1041
Ginoza_medaka   HVLSPLDGSEARTAVLNISTTQYTFRSLGPGQMYTVDVLTQSGIRPDEFPPSTSHSAGPL 1041
stickleback     HVSLTPADGGSEARTAVLNASATEHTFSSLVPGQMYTVDVMTQSGIRPDEFPPSTSHSAGPL 1069
zebrafish       RVSLVPADGGSGRTAVLNSVTDYTFSTLLPGQMYTVDVVTQSGLRPEDLPSTSKSAGPL 1070
mouse           RVSILYPEASAVQSTEVDRSVDRLTFGDLLPGRRYTVRLTTLSPGGAEYPTESLASAPL 1040
human           RVSIRHPEALRDQATVDVDRSDFRFRALLPGKRYTIQLTTLGLRGEHPTESLATAPT 1122
***           :.  :.  :.  :.  *.  **  *  *.  **:  :  ***  :  *:  *  :  :  *
    
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Fig 5. SNED1 protein sequence comparison. Glycine at position 1013 in Ginoza SNED1 (red square) is conserved among other vertebrate species. Serine (blue square) was found only in Maizuru SNED1.

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Supporting information

S1 Table. Single nucleotide polymorphism (SNP) markers used for quantitative trait locus (QTL) mapping and genotyping for each SNP in the parental, F₁, and F₂ generations.

Number of markers used for QTL mapping: 371.

(XLSX)

S2 Table. Single nucleotide polymorphisms (SNPs) on the coding regions in 12 candidate genes from the Maizuru and Ginoza individuals. Number of Maizuru and Ginoza fish: n = 4 each.

(XLSX)

S3 Table. Genotyping amplified fragment length polymorphism marker ch4_0.68M and SNP marker 56900 in the parental and F₂ generations.

The forward and reverse primer sequences of ch4_0.68M for genotyping using PCR are 5'-caattgctgtttgtcagttacac-3' and 5'-cgcctaagcactccagcac-3', respectively. Their locations are 685055–685078 bp and 685136–685156 bp on chromosome 4, respectively. The sizes of the amplified fragments separated using Microchip Electrophoresis System MCE®-202 MultiNA microchip electrophoresis (Shimadzu Corporation, Kyoto, Japan).

(XLSX)

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References

1. Feng X, Yu X, Fu B, Wang X, Liu H, Pang M, et al. A high-resolution genetic linkage map and QTL fine mapping for growth-related traits and sex in the Yangtze River common carp (*Cyprinus carpio haematopterus*). *BMC Genomics*. 2018; 19(1): 230. <https://doi.org/10.1186/s12864-018-4613-1> PMID: 29609551
2. Gutierrez AP, Lubieniecki KP, Davidson EA, Lien S, Kent MP, Fukui S, et al. Genetic mapping of quantitative trait loci (QTL) for body-weight in Atlantic salmon (*Salmo salar*) using a 6.5K SNP array. *Aquaculture*. 2012;358–359: 61–70.
3. Hosoya S, Kai W, Fujita M, Miyaki K, Suetake H, Suzuki Y, et al. The genetic architecture of growth rate in juvenile takifugu species. *Evolution*. 2013; 67(2): 590–598. <https://doi.org/10.1111/j.1558-5646.2012.01781.x> PMID: 23356630
4. Liu F, Sun F, Xia JH, Li J, Fu GH, Lin G, et al. A genome scan revealed significant associations of growth traits with a major QTL and GHR2 in tilapia. *Sci Rep*. 2014; 4: 1–9.
5. Pang M, Fu B, Yu X, Liu H, Wang X, Yin Z, et al. Quantitative trait loci mapping for feed conversion efficiency in crucian carp (*Carassius auratus*). *Sci Rep*. 2017; 7(1): 1–11. <https://doi.org/10.1038/s41598-016-0028-x>
6. Wenne R. Single nucleotide polymorphism markers with applications in aquaculture and assessment of its impact on natural populations. *Aquat Living Resour*. 2018; 31: 2.
7. Yue GH. Recent advances of genome mapping and marker-assisted selection in aquaculture. *Fish*. 2014; 15(3): 376–396.
8. Fall T, Ingelsson E. Genome-wide association studies of obesity and metabolic syndrome. *Mol Cell Endocrinol*. 2014; 382(1): 740–757. <https://doi.org/10.1016/j.mce.2012.08.018> PMID: 22963884
9. Loos RJ. The genetics of adiposity. *Curr Opin Genet Dev*. 2018; 50(1): 86–95.
10. Oka T, Nishimura Y, Zang L, Hirano M, Shimada Y, Wang Z, et al. Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. *BMC Physiol*. 2010; 10(1): 21.
11. Shimada Y, Kuroyanagi J, Zhang B, Ariyoshi M, Umemoto N, Nishimura Y, et al. Downregulation of Max dimerization protein 3 is involved in decreased visceral adipose tissue by inhibiting adipocyte differentiation in zebrafish and mice. *Int J Obes*. 2014; 38(8): 1053–1060.
12. Blackburn TM, Gaston KJ, Loder N. Geographic gradients in body size: A clarification of Bergmann's rule. *Divers Distrib*. 1999; 5(4): 165–174.
13. Ashton KG, Tracy MC, de Queiroz A. Is Bergmann's rule valid for mammals? *The American Naturalist*. 2000; 156(4): 390–415. <https://doi.org/10.1086/303400> PMID: 29592141
14. Ashton KG. Meta-analysis patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology & Biogeography*. 2002; 11: 505–523.
15. Meiri S, Dayan T. On the validity of Bergmann's rule. *J Biogeogr*. 2003; 30(3): 331–351.
16. Ashton KG, Feldman CR. Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution*. 2003; 57(5): 1151–1163. <https://doi.org/10.1111/j.0014-3820.2003.tb00324.x> PMID: 12836831
17. Katsumura T, Oda S, Mitani H, Oota H. Medaka population genome structure and demographic history described via genotyping-by-sequencing. 2019; 9: 217–228.

18. Matsuda AM, Yonekawa H, Hamaguchi S, Sakaizumi M. Geographic variation and diversity in the mitochondrial DNA of the medaka, *Oryzias latipes*, as determined by restriction endonuclease analysis. *Zoological Science*. 1997; 14(3): 517–526.
19. Sakaizumi M, Moriwaki K, Egami N. Allozymic variation and regional differentiation in wild populations of the fish *Oryzias latipes*. *Copeia*. 1983; 2: 311–318.
20. Takehana Y, Nagai N, Matsuda M, Tsuchiya K, Sakaizumi M. Geographic variation and diversity of the cytochrome b gene in Japanese wild populations of medaka, *Oryzias latipes*. *Zoolog Sci*. 2003; 20: 1279–1291. <https://doi.org/10.2108/zsj.20.1279> PMID: 14569151
21. Kasahara M, Naruse K, Sasaki S, Nakatani Y, Qu W, Ahsan B, et al. The medaka draft genome and insights into vertebrate genome evolution. *Nature*. 2007; 447(7145): 714–719. <https://doi.org/10.1038/nature05846> PMID: 17554307
22. Ishikawa Y, Yoshimoto M, Yamamoto N, Ito H. Different brain morphologies from different genotypes in a single teleost species, the medaka (*Oryzias latipes*). *Brain Behav Evol*. 1999; 53(1): 2–9. <https://doi.org/10.1159/000006577> PMID: 9858800
23. Kimura T, Naruse K, Takeda H, Shinya M. Genetic analysis of craniofacial traits in the medaka. *Genetics*. 2007; 177(4): 2379–2388. <https://doi.org/10.1534/genetics.106.068460> PMID: 18073435
24. Asai T, Senou H, Hosoya K. *Oryzias sakaizumii*, a new ricefish from northern Japan (Teleostei: *Adrianiichthyidae*). *Ichthyol Explor Freshwaters*. 2011; 22(4): 289–299.
25. Kimura T, Shinya M, Naruse K. Genetic analysis of vertebral regionalization and number in medaka (*Oryzias latipes*) inbred lines. *G3: Genes, Genomes, Genetics*. 2012; 2: 1317–1323.
26. Kagawa N. Comparison of aggressive behaviors between two wild populations of Japanese medaka, *Oryzias latipes* and *O. sakaizumii*. *Zoolog Sci*. 2014; 31(3): 116–121. <https://doi.org/10.2108/zsj.31.116> PMID: 24601772
27. Tsuboko S, Kimura T, Shinya M, Suehiro Y, Okuyama T. Genetic control of startle behavior in medaka fish. *PLoS One*. 2014; 9(11): e112527. <https://doi.org/10.1371/journal.pone.0112527> PMID: 25393539
28. Kawajiri M, Fujimoto S, Yoshida K, Yamahira K, Kitano J. Genetic architecture of the variation in male-specific ossified processes on the anal fins of Japanese medaka. *G3: Genes, Genomes, Genetics*. 2015; 5: 2875–2884.
29. Kawajiri M, Uchida K, Chiba H, Moriyama S, Yamahira K. Variation in the ontogeny of sex steroid levels between latitudinal populations of the medaka. *Zool Lett*. 2015; 1: 31.
30. Sakaguchi S, Sugino T, Tsumura Y, Ito M, Crisp MD et al. High-throughput linkage mapping of Australian white cypress pine (*Callitris glaucophylla*) and map transferability to related species. *Tree Genetics & Genomes*. 2015; 11: 21.
31. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014; 30(15): 2115–2120.
32. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. Stacks: An analysis tool set for population genomics. *Mol Ecol*. 2013; 22(11): 3124–3140. <https://doi.org/10.1111/mec.12354> PMID: 23701397
33. Kosambi DD. The estimation of map distances from recombination values. *Ann Eugen*. 1944; 12: 172–175.
34. Haley CS, Knott SA. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. 1992; 69: 315–324. <https://doi.org/10.1038/hdy.1992.131> PMID: 16718932
35. Broman KW, Wu H, Sen S, Churchill GA. R/qtl: QTL mapping in experimental crosses. *Bioinformatics*. 2003; 19(7): 889–890. <https://doi.org/10.1093/bioinformatics/btg112> PMID: 12724300
36. Broman KW, Sen S. A guide to QTL mapping with R/qtl. New York, NY: Springer New York; 2009.
37. Choi Y, Chan AP. PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics*. 2015; 31(16): 2745–2747. <https://doi.org/10.1093/bioinformatics/btv195> PMID: 25851949
38. Reed DR, Lawler MP, Tordoff MG. Reduced body weight is a common effect of gene knockout in mice. *BMC Genet*. 2008; 9: 4. <https://doi.org/10.1186/1471-2156-9-4> PMID: 18182103
39. Rzehak P, Covic M, Saffery R, Reischl E, Wahl S, Grote V, et al. DNA-methylation and body composition in preschool children: epigenome-wide-analysis in the European Childhood Obesity Project (CHOP)-Study. *Sci Rep*. 2017; 7(1): 1–13. <https://doi.org/10.1038/s41598-016-0028-x>
40. Zhang L, Ma X, Xuan J, Wang H, Yuan Z, Wu M, et al. Identification of *MEF2B* and *TRHDE* gene polymorphisms related to growth traits in a new Ujumqin sheep population. *PLoS One*. 2016; 11(7): e0159504. <https://doi.org/10.1371/journal.pone.0159504> PMID: 27472808

41. Zhang L, Liu J, Zhao F, Ren H, Xu L, Lu J, et al. Genome-wide association studies for growth and meat production traits in sheep. *PLoS One*. 2013; 8(6): e66569. <https://doi.org/10.1371/journal.pone.0066569> PMID: 23825544
42. Ghasemi M, Zamani P, Vatankhah M, Abdoli R. Genome-wide association study of birth weight in sheep. *Animal*. 2018; 13(9): 1797–1803.
43. Tarsani E, Kranis A, Maniatis G, Avendano S, Hager-Theodorides AL, Kominakis A. Discovery and characterization of functional modules associated with body weight in broilers. *Sci Rep*. 2019; 9(1): 1–12. <https://doi.org/10.1038/s41598-018-37186-2>
44. Seale LA, Gilman CL, Hashimoto AC, Ogawa-Wong AN, Berry MJ. Diet-induced obesity in the seleno-cysteine lyase knockout mouse. *Antioxidants Redox Signal*. 2015; 23(10): 761–74.
45. Shalata A, Ramirez MC, Desnick RJ, Priedigkeit N, Buettner C, Lindtner C, et al. Morbid obesity resulting from inactivation of the ciliary protein CEP19 in humans and mice. *Am J Hum Genet*. 2013; 93(6): 1061–1071. <https://doi.org/10.1016/j.ajhg.2013.10.025> PMID: 24268657
46. Dhana K, Braun KVE, Nano J, Voortman T, Demerath EW, Guan W, et al. An epigenome-wide association study of obesity-related traits. *Am J Epidemiol*. 2018; 187(8): 1662–1669. <https://doi.org/10.1093/aje/kwy025> PMID: 29762635
47. Zhou X, Gomez-Smith M, Qin Z, Duquette PM, Cardenas-Blanco A, Rai PS, et al. Ablation of *LMO4* in glutamatergic neurons impairs leptin control of fat metabolism. *Cell Mol Life Sci*. 2012; 69(5): 819–828. <https://doi.org/10.1007/s00018-011-0794-3> PMID: 21874351
48. Tselykh TV, Roos C, Heino TI. The mitochondrial ribosome-specific MrpL55 protein is essential in *Drosophila* and dynamically required during development. *Exp Cell Res*. 2005; 307(2): 354–366. <https://doi.org/10.1016/j.yexcr.2005.03.037> PMID: 15894314
49. Philp LK, Day TK, Butler MS, Laven-Law G, Jindal S, Hickey TE, et al. Small glutamine-rich tetratricopeptide repeat-containing protein alpha (*SGTA*) ablation limits offspring viability and growth in mice. *Sci Rep*. 2016; 6: 1–14. <https://doi.org/10.1038/s41598-016-0001-8>
50. Chahal J, Chen CC, Rane MJ, Moore JP, Barati MT, Song Y, et al. Regulation of insulin-response element binding protein-1 in obesity and diabetes: Potential role in impaired insulin-induced gene transcription. *Endocrinology*. 2008; 149(10): 4829–4836. <https://doi.org/10.1210/en.2007-1693> PMID: 18566119
51. Falk RE, Casas KA. Chromosome 2q37 deletion: Clinical and molecular aspects. *Am J Med Genet Part C Semin Med Genet*. 2007; 145C(4): 357–371. <https://doi.org/10.1002/ajmg.c.30153> PMID: 17910077
52. Naba A, Jan K, Barqué A, Nicholas CL, Hynes RO. Knockout of the gene encoding the extracellular matrix protein Sned1 results in craniofacial malformations and early neonatal lethality. *bioRxiv* [Preprint]. 2018;440081. Available from: <https://www.biorxiv.org/content/10.1101/440081v1>