Supplemental Data

Congenital Joint Dislocations Caused by Carbohydrate

Sulfotransferase 3 Deficiency in Recessive

Larsen Syndrome and Humero-Spinal Dysostosis

Pia Hermanns, Sheila Unger, Antonio Rossi, Antonio Perez-Aytes, Hector Cortina, Luisa Bonafé, Loredana Boccone, Valeria Setzu, Michel Dutoit, Luca Sangiorgi, Fabio Pecora, Kerstin Reicherter, Gen Nishimura, Jürgen Spranger, Bernhard Zabel, and Andrea Superti-Furga

Material and Methods

Genomic DNA was extracted from the subjects' peripheral leucocytes with the Gentra genomic DNA blood isolation kit (Gentra, Minneapolis, MN, USA) according to the manufacturer's protocol. One hundred nanograms of genomic DNA was used as template in the PCR reaction. Exons 2 and 3 of the CHST3 gene are protein coding. A 391 bp product encompassing the whole of exon 2 (247 bp) and containing a 140 bp of coding sequence was generated. Exon 3 of the CHST3 gene consists of 6393 bp, of which 1.3 kb are coding. Given the size of the coding region, exon 3 was amplified in three PCR reactions with overlapping sequences of 86 bp and 93 bp, respectively. Primer information is available upon request. With this approach 66 bp of the noncoding 3′UTR and 85 bp of intron 2 were sequenced. The PCR reaction was carried out with the PCR Master Mix (Promega) in a 25 μl reaction at an annealing temperature of 58°C for exon 2 and 60°C for exon 3 (all three parts). In addition, the PCR reactions of exon 3 were supplemented with 5% glycerol. The PCR products were purified with the PCR purification Kit from Promega (Madison, WI, USA) and directly sequenced with Dye Terminator v3.1 on a 3130 Genetic Analyzer (Applied Biosystems) according to the manufacturer's recommendations. DTDST mutations had been excluded in patients 1, 4, and 5, and FLNB mutations had been excluded in patient 2 by routine molecular analysis.[1,2]

For separation of the alleles of patient 5, the PCR products were subcloned into the pGEM T vector (Promega) and transformed into DH5α cells. Ten single clones were picked and the plasmid DNA was isolated (Eppendorf) and sequenced with the vector-specific primers T7 and SP6, with Dye Terminator kit v3.1 (Applied Biosystems, Forster City, CA, USA).

Skin biopsies were obtained from patients 1 and 2 with informed consent. Skin fibroblasts from patients and controls were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS) and antibiotics at 37°C in a humidified atmosphere containing 5% CO_2.

Disaccharide analysis of glycosaminoglycan (GAG) chains synthesized in fibroblast cultures was performed as described previously.[3] In brief, confluent cells in 10 cm^2 dishes were washed with PBS to eliminate any sulfotransferase activity that might be present in the FCS and then preincubated for 4 hr in the presence or absence of 1 mM p-nitrophenyl β-D-xylopiranoside (Sigma) in DMEM without FCS in 5% CO_2 at 37°C. Labeling with [6-^{3}H]glucosamine (GE Healthcare) was performed in the same medium at a final concentration of 50 μCi/ml. After 24 hr labeling, an equal volume of 100 mM sodium acetate buffer, pH 5.8, containing 8 M urea, 4% Triton X-100, 20 mM EDTA, 20 mM NEM, and 1 mM PMSF was added to the Petri dishes, and samples were loaded on 1 ml DEAE Sephacel columns. After column washing with 50 mM sodium acetate, pH 6.0, 8 M urea, 0.15 M NaCl, 0.5% Triton X-100, and proteinase inhibitors, proteoglycans and hyaluronic acid were eluted with 1 M NaCl in the same buffer, recovered by precipitation with 9 volumes of ethanol and desalted by ultrafiltration with Centricon-10 (Millipore). PGs were then separated from hyaluronic acid by digestion with *Streptomyces* hyaluronidase (Seikagaku Corporation, Tokio, Japan) followed by ultrafiltration with Biomax Ultrafree-0.5 devices with 10kDa cut-off membrane (Millipore). PGs in the retentate were then digested with 30 mU of both chondroitinase ABC and ACII (Seikagaku Corporation) in 30 mM sodium acetate, 30 mM Tris-acetate, pH 7.35, at 37°C overnight. Undigested products were removed by precipitation with 4 volumes of ethanol;[4] the disaccharide constituents of chondroitin sulfate (dimers of glucuronic acid and N-acetyl-D-galactosamine, either unsulfated or sulfated on the positions 4 or 6 of the GalNAc residue; ΔDi-0S, ΔDi-4S, ΔDi-6S) in the supernatants were fractionated with a Supelcosil SAX1 (Supelco, Bellefonte, USA) HPLC column (4.6 × 250 mm) with a gradient of 5–400 mM KH_2PO_4, pH 4.2–4.5, at

room temperature, and the elution profile was measured at 232 nm.[5] Standard unlabeled disaccharides (ΔDi-0S, ΔDi-4S, ΔDi-6S) were added as internal reference to radiolabeled samples, and the corresponding peaks at 232 nm were collected and the concentration of radioactive labeled disaccharides determined by liquid scintillation counting.

Supplemental References

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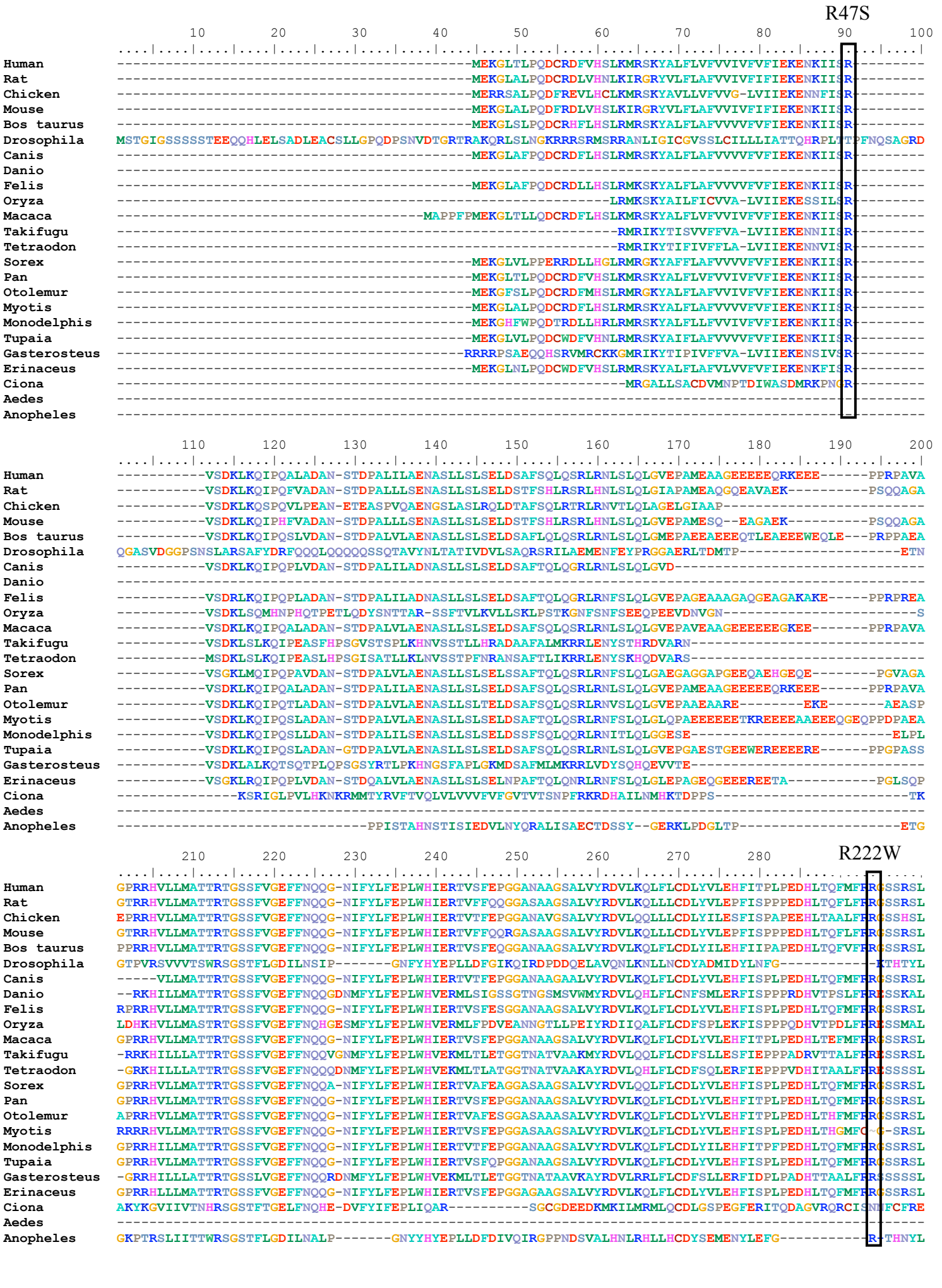
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Canis
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Myotis
Monodelphis
Tupaia
Gasterosteus
Erinaceus
Ciona
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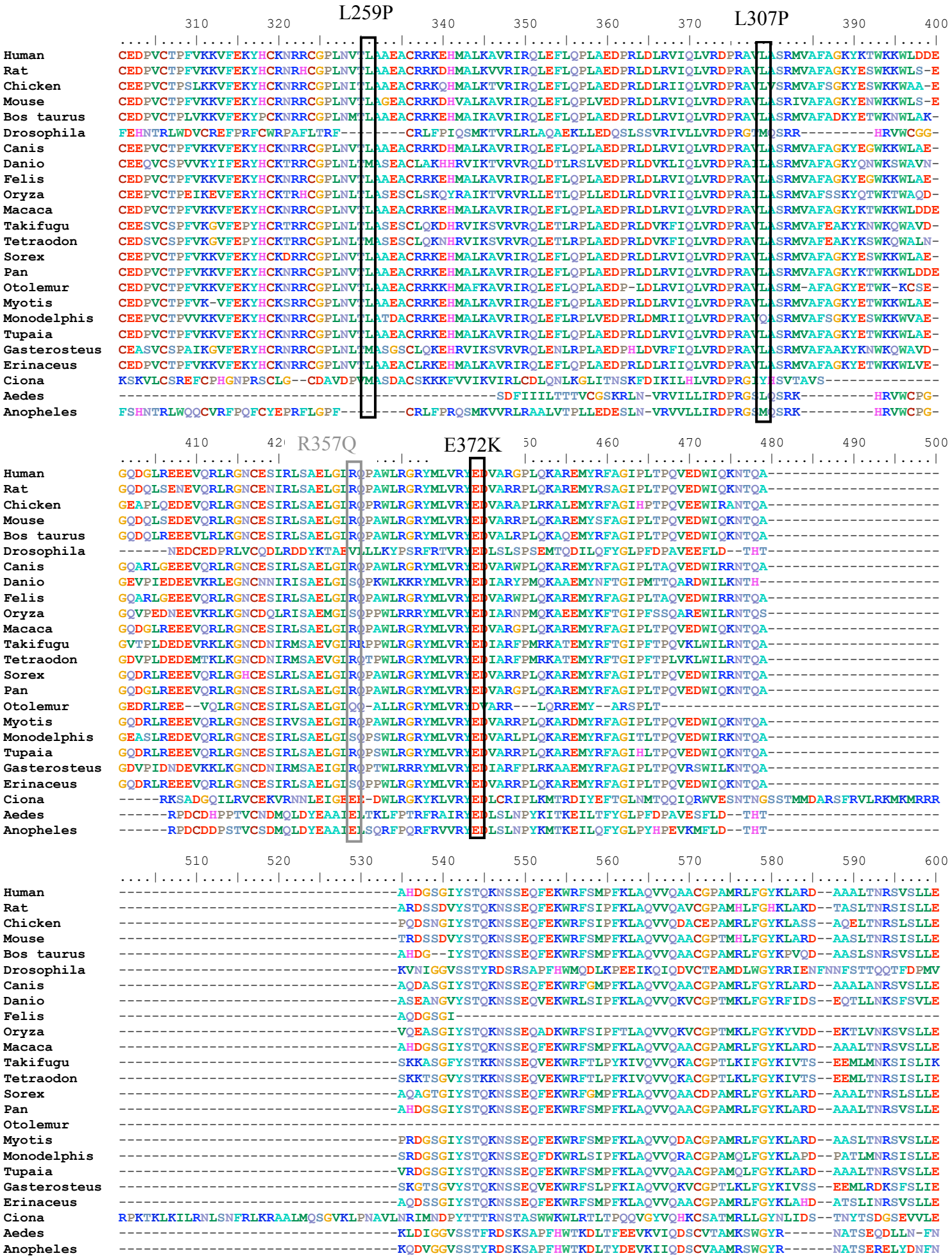
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L307P
R357Q
E372K
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Chicken
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Bos taurus
Drosophila
Canis
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Takifugu
Tetraodon
Sorex
Pan
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Monodelphis
Tupaia
Gasterosteus
Erinaceus
Ciona
Aedes
Anopheles

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Human           ERGTFWVT---
Rat             ERGTFWVT---
Chicken         EGPPTRIT---
Mouse           ERGTFWVT---
Bos taurus      ERGTFWVT---
Drosophila      MPPPFT-----
Canis           ERGTFWVT---
Danio           EK---------
Felis           ----------
Oryza           EKQF-------
Macaca          ERGTFWVT---
Takifugu        DK---------
Tetraodon       ----------
Sorex           ERGTFWVT---
Pan             ERGTFWVT---
Otolemur        ----------
Myotis          ERGTFWVT---
Monodelphis     DRGIFWVT---
Tupaia          ERGTFWVT---
Gasterosteus    D----------
Erinaceus       EGGTFWVT---
Ciona           PLCNDVTIGDC
Aedes           PLLPYSVS---
Anopheles       PLLPYSVS---
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