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SUBCHAPTER 29D

Angiotensin Converting Enzymes

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Additional names/abbreviations: Angiotensin converting enzyme, dipeptidyl carboxypeptidase I, peptidase P, kininase II, angiotensin I-converting enzyme/ACE; angiotensin converting enzyme 2, peptidyl-dipeptidase A, peptidyl-dipeptidase A/ACE2

ACE possesses dual actions to convert Ang I to Ang II and degrade bradykinin. The development of ACE inhibitor was the first effective drug for hypertension caused by high renin activity. ACE2 was identified as the receptor for SARS (severe acute respiratory syndrome) coronavirus, which caused the outbreak of an epidemic in 2002–2003.

Discovery

ACE was discovered in the mid-1950s by the observation that dialysis of plasma and kidney extract with water and saline before incubation had produced two separate pressor substances, Ang I and Ang II respectively [1]. It was discovered for a second time in 1966 during the characterization of bradykinin (BK) degrading enzyme from kidney and this enzyme was named kininase II; it later was found to be the same enzyme as ACE. ACE2 was discovered in 2000 when two independent research groups cloned homologous ACE that could convert Ang I to Ang(1–9) and yet also is captopril-insensitive [2,3].

Structure

Structural Features

Two isozymes of ACE are present in mammals: somatic ACE and testis ACE. Somatic ACE possesses two catalytic domains (N- and C-domains) and a C-terminal transmembrane segment (stalk) (Figure 29D.1). Somatic and testis ACEs in humans contain 1,306 and 665 aa residues, respectively. Testis ACE only possesses one catalytic domain. Both catalytic domains are zinc-metallopeptidase with the active motif HEMGH where the two histidine residues coordinate the zinc ion. The stalk anchors the enzyme on the membrane and is susceptible to be cleaved by shedding enzymes, resulting in plasma ACE activity (Figure 29D.1). ACE2 is a chimaera protein with a single catalytic domain of ACE, and a C-terminal highly resembling collectrin, which may act as a chaperone protein to deliver other proteins to the brush border membrane.

Synthesis and Release

Gene and mRNA

ACE and *ACE2* genes are located at chromosome 17q23 and Xp22 in humans, respectively. Testis *ACE* is transcribed from the same gene with an alternative transcription starting site on the 13th intron of the *ACE* gene, resulting in only C-domain and stalk segment with a unique additional 67 aa N-terminal sequence in humans. The two catalytic domains are the result of gene/domain duplication and the duplication occurred multiple times in evolution as the cnidarians, crustaceans, insects, and vertebrates possess ACE-like enzymes with one or two catalytic domains. No expression studies so far have been performed for non-mammalian *ACE* and *ACE2*.

Distribution of mRNA

Somatic *ACE* is expressed in various tissues including blood vessels, kidney, intestine, adrenal gland, liver, and uterus, and is especially abundant in highly vascular organs such as retina and lung. Testis *ACE* is expressed by postmeiotic male germ cells and high-level expression is found in round and elongated spermatids. *ACE2* is expressed in lung, liver, intestine, brain, testis, heart, and kidney.

Tissue and Plasma Concentrations

Lung possesses the highest amount of ACE and contributes to 0.1% of total protein. Serum ACE levels in humans ranged from 299.3 ± 49 μg/l (DD) to 494.1 ± 88.3 μg/l (II) with heterozygous individuals 392.6 ± 66.8 μg/l [4]. (ID: see the section "Pathophysiological Implications" for the genotype definition.) Several enzymatic assays have been developed for the measurement of ACE activity in plasma and tissues and usually involve artificial substrates such as hippuryl-His-Leu or N-[3-(2-furyl)acryloyl]L-phenylalanyl-glycyl-glycine (FAPGG), in combination with captopril inhibition. These methods were developed in mammals but were also extended to other vertebrates including birds, amphibians, and fishes [5]. However, these enzymatic methods may be erroneous because the enzyme specificity on the artificial substrates could be different. Lamprey ACE activities in different tissues were measured but captopril failed to decrease the ACE activities, indicating a possible nonspecific enzyme measurement. In amphibian, high captopril-sensitive ACE activities were found in gonad, intestine, kidney, and lung, moderate activities were presented in liver, heart, and skin, and low or negligible activities were observed in plasma, muscle, and erythrocytes.

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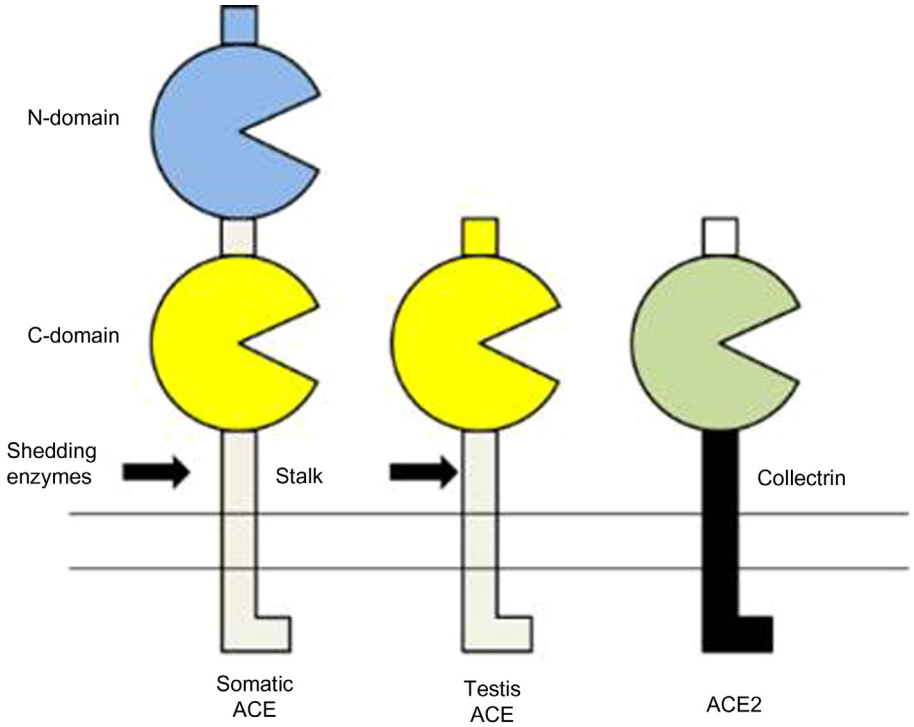

Figure 29D.1 Schematic diagram showing the functional domains of ACE and ACE2. Two extracellular enzymatic domains are present in ACE. An alternative transcript of ACE form testicular ACE which only possess a single enzymatic domain. The stalk anchors the enzyme on the membrane and is suspectible to be cleaved by shedding enzymes, resulting in plasma ACE activity. ACE2 is a chimaera protein with a single catalytic domain of ACE, and a C-terminal highly resemble collectrin, which may act as a chaperone protein to deliver other proteins to the brush border membrane.

Regulation of Synthesis and Release

Expression of *ACE* is affected by steroids and thyroid hormone, but the details of the regulation are not clear. *ACE* is under promoter regulation by hypoxia-inducing factor 1α (HIF-1α), which upregulates the *ACE* expression under hypoxic conditions, resulting in an increase in Ang II concentration. Under hypoxia, *ACE2* will be downregulated but it was shown that it is indirectly controlled by Ang II, but not HIF-1α [6]. Testis *ACE* expression control is highly specific and regulated by a tissue-specific promoter located immediately −59 bp of the transcription start site, which is frequently used in testis-specific overexpression studies. Hypoxia induced by high temperature decreased gill ACE activity but had no effect on kidney in carp. Promoters of *ACE2* from mammals, amphibians, and teleosts drive specific expression in the heart. *Cis*-element search results discovered WGATAR motifs in all putative *ACE2* promoters from different vertebrates, suggesting a possible role of GATA family transcriptional factors in *ACE2* expression regulation.

Receptors

None.

Inhibitors

The first ACE inhibitor was a peptide antagonist called SQ 20,881 (GWPRPEIPP) discovered from snake venom but it was not orally active. The snake venom peptides were further studied to produce the first orally active form, captopril, that lowers the blood pressure of essential hypertensive patients [7]. The most common side effects of captopril are cough, skin rash, and loss of taste, and therefore derivatives such as enalapril, lisinopril, and ramipril were developed with fewer side effects. After the discovery of N- and C-domains of ACE, specific domain inhibitors were developed to increase specificity. Ang I is mainly hydrolyzed by the C-domain *in vivo* but BK is hydrolyzed by both domains. By developing a C-domain selective inhibitor (RXPA380) some degradation of BK by the N-domain would be permitted and this degradation could be enough to prevent accumulation of excess BK causing angioedema [8].

Biological Functions

Target Cells/Tissues and Functions

The well-known function of ACE is the conversion of Ang I to Ang II and degradation of BK, which all play an important role in controlling blood pressure. ACE also acts on other natural substrates including encephalin, neurotensin, and substance P. Besides being involved in blood pressure control, ACE possesses widespread functions including renal development, male fertility, hematopoiesis, erythropoiesis, myelopoiesis, and immune responses [1]. ACE2 can convert Ang II to Ang(1−7), thereby reducing the concentration of Ang II and increasing that of Ang(1−7). ACE2 can also convert Ang I to Ang(1−9), which is subsequently converted into Ang(1−7) by ACE. The high expression of ACE2 favors the balance of Ang(1−7) over Ang II, which accounts for the cardioprotective role of ACE2 via the Ang(1−7)/Mas signaling pathway [9].

Phenotype in Gene-Modified Animals

ACE-knockout mice display normal blood pressure under normal conditions, but are sensitive to changes in blood pressure such as exercise. ACE-knockout also affects renal function, renal development, serum and urine electrolyte composition, haematocrit, and male reproductive capacity

[10]. Deficiency in testis ACE affects male fertility but its exact role is still not clear. Although mice with testis ACE deficiency mate normally and their sperm quantity and motility are no different from those of wild-type mice, the survival of sperm in the oviduct and fertilization rate are highly reduced [1]. Overexpression of *ACE2* in hypertensive models, but not in normotensive animals, reduced blood pressure. ACE2-knockout mice displayed progressive cardiac dysfunction resembling that of long-term hypoxia after coronary artery disease or bypass surgery in human, which could be reversed by concurrent ACE-knockout. It was suggested that the cardioprotective function of ACE2 is to counterbalance the effects of ACE.

Pathophysiological Implications

Clinical Implications

Inclusion (II) or deletion (DD) of 287 bp Alu repeats in the 16th intron affects the human plasma ACE levels and the DD genotype was more frequently found in patients with myocardial infarction but no convincing evidence was available on the association of the DD genotype with hypertension [4]. ACE2 was identified as the receptor for SARS (severe acute respiratory syndrome) coronavirus. SARS virus binding down-regulates the cellular expression of *ACE2*, and the binding induces clathrin-dependent internalization of virus/receptor (SARS/ACE2) complex. Not only has ACE2 facilitated the invasion of SARS virus for rapid replication, but also ACE2 is depleted from the cell membrane and therefore the damaging effects of Ang II are enhanced, resulting in acute deterioration of lung tissues.

Use for Diagnosis and Treatment

ACE has been the target of hypertension control since the 1970s. ACE inhibitors are prescribed as the sole or combinational treatment of high blood pressure, for the dual effects of lowering Ang II and slowing down BK degradation. In human hypertensive patients, ACE2 levels are lower in both kidney and heart compared to normotensive volunteers.

References

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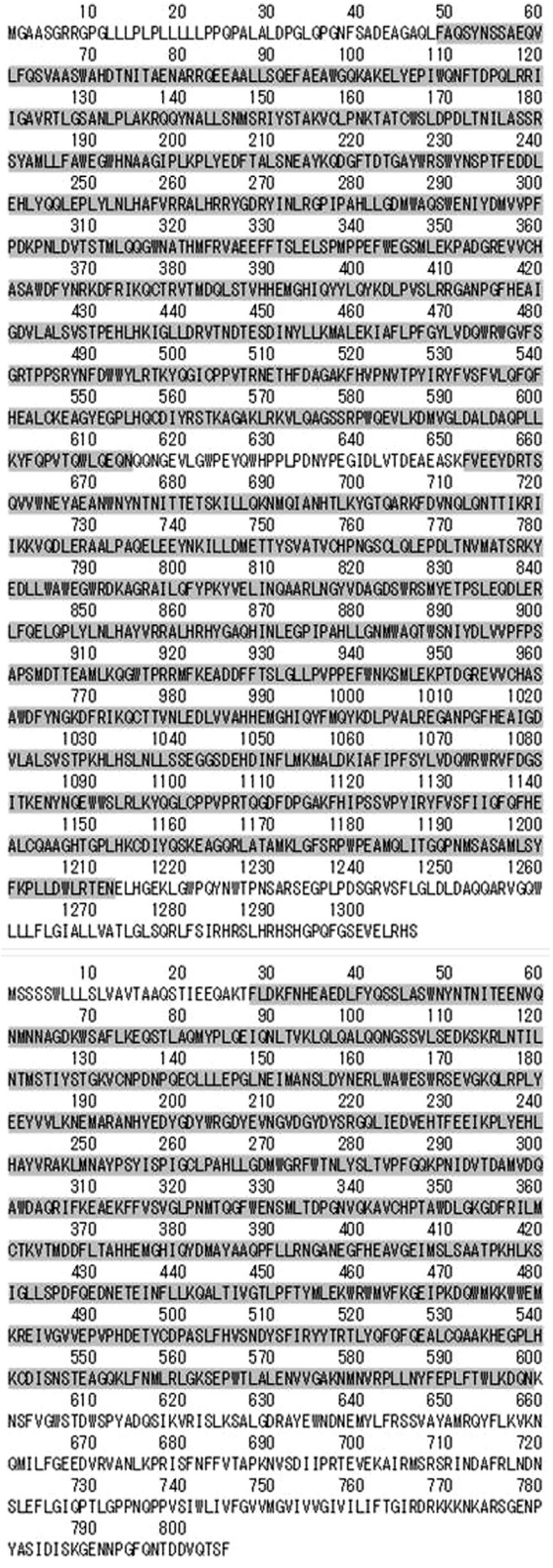
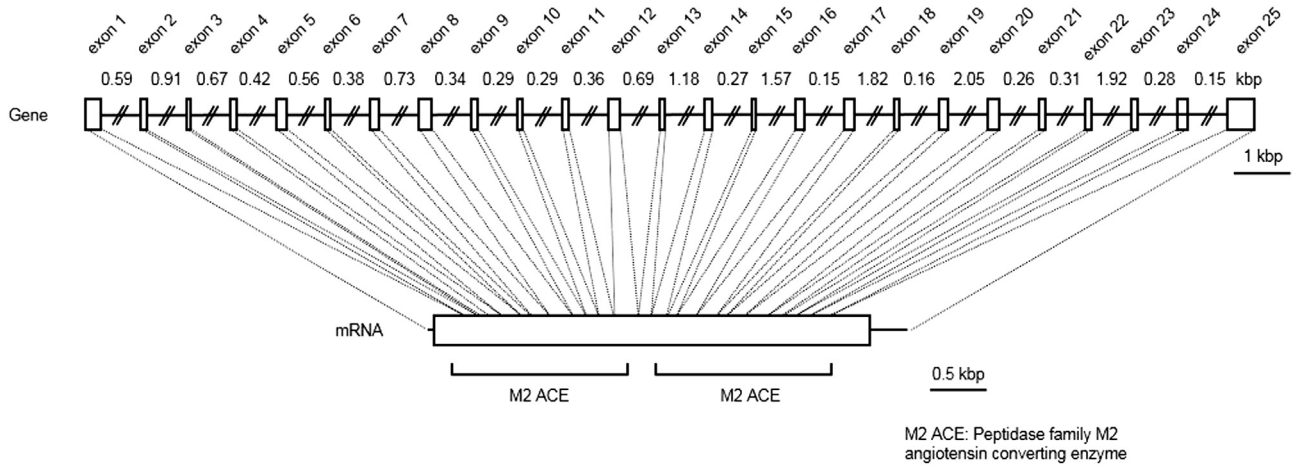
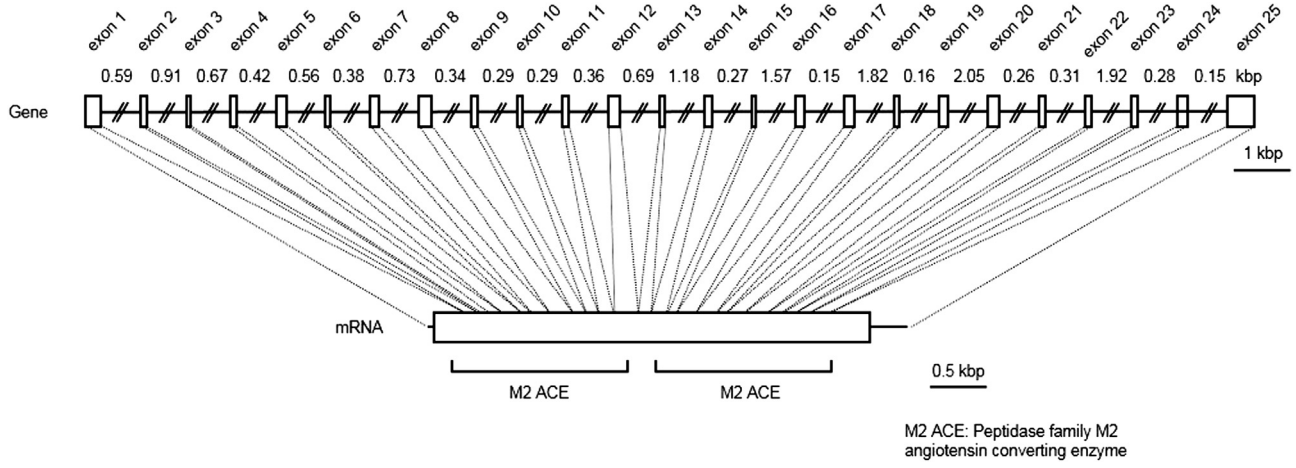
Supplemental Information



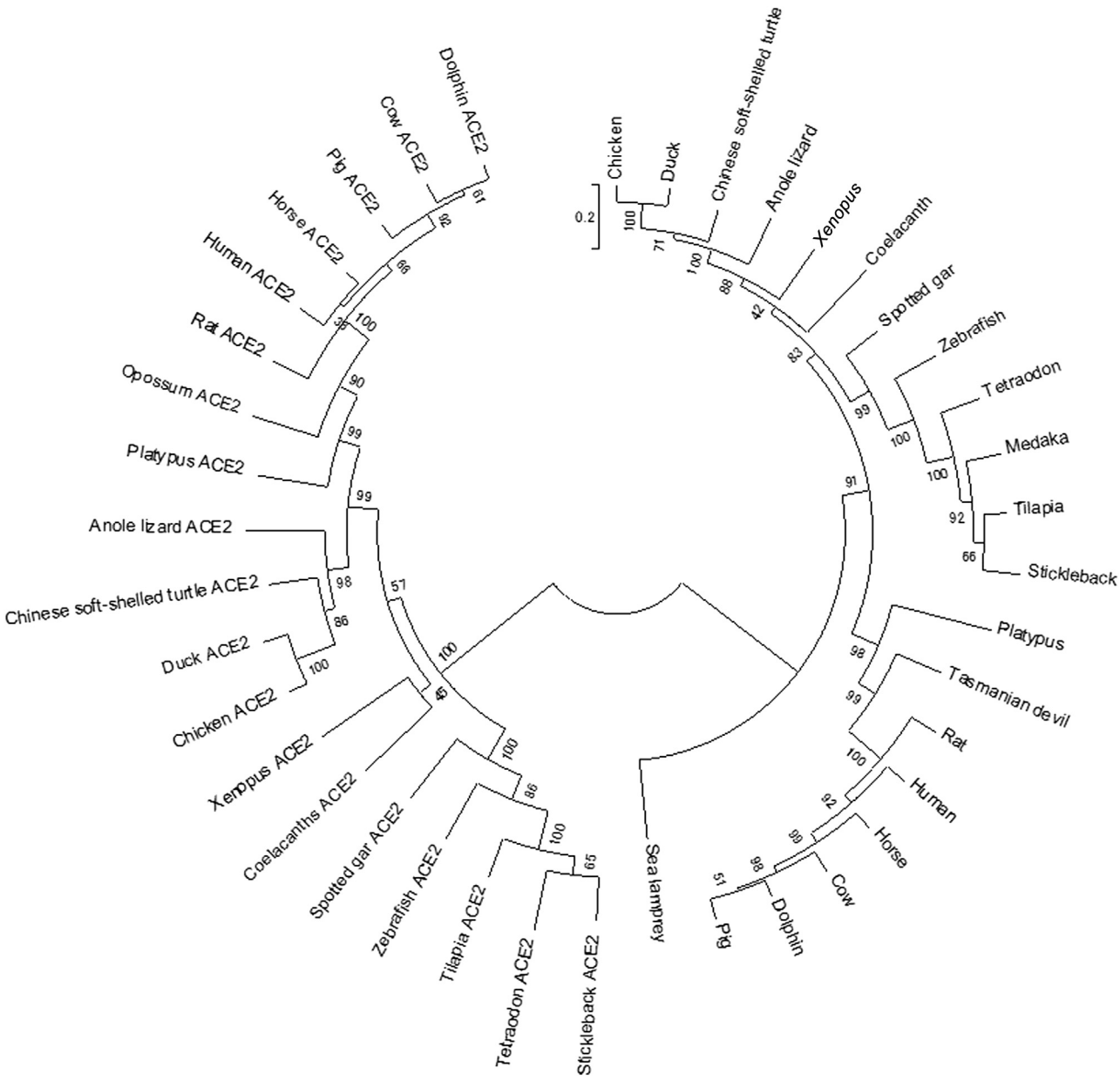
E-Figure 29D.1 Protein sequences and structural features of ACE and ACE2 of human. M2 gluzincin family domains are shaded. ACE possesses two M2 gluzincin family domains while ACE2 possesses only one catalytic domain.



E-Figure 29D.2 Gene, mRNA, and domain structure of the human angiotensin converting enzyme. Human angiotensin converting enzyme: *ACE*, location 17q23.3.



E-Figure 29D.3 Gene, mRNA, and domain structure of the human angiotensin converting enzyme 2. Human angiotensin converting enzyme 2: *ace2*, location Xp22.



E-Figure 29D.4 Phylogenetic tree of the angiotensin converting enzymes in vertebrates. The unrooted phylogenetic tree of the angiotensin converting enzyme (ACE) and angiotensin converting enzyme 2 (ACE2) was constructed with the maximum likelihood method using full-length sequences from representative vertebrate species. The numbers on the branches indicate the bootstrap values from 1,000 replicates. ACE and ACE2 form separate sub-clades.

E-Table 29D.1 Accession Numbers of Vertebrate Angiotensin Converting Enzymes (*ACE* and *ACE2*)

	Accession Number	
Species	**ACE**	**ACE2**
Anole lizard	ENSACAG00000013067	ENSACAG00000016963
Chicken	ENSGALG00000000498	ENSGALG00000016554
Chinese soft-shelled turtle	ENSPSIG00000004735	ENSPSIG00000006623
Coelacanth	ENSLACG00000012780	ENSLACG00000014299
Cow	ENSBTAG00000024950	ENSBTAG00000034402
Dolphin	ENSTTRG00000001667	ENSTTRG00000003589
Duck	ENSAPLG00000014288	ENSAPLG00000014477
Horse	ENSECAG00000012910	ENSECAG00000000430
Human	NM_000789	AB046569
Medaka	ENSORLG00000004282	
Opossum		ENSMODG00000017222
Pig	ENSSSCG00000017296	ENSSSCG00000012138
Platypus	ENSOANG00000003975	ENSOANG00000002574
Rat	AF201332	NM_001012006
Sea Lamprey	ENSPMAG00000007309	
Spotted gar	ENSLOCG00000012317	ENSLOCG00000007694
Stickleback	ENSGACG00000009898	ENSGACG00000015100
Tasmanian devil	ENSSHAG00000010621	
Tetraodon	ENSTNIG00000012854	ENSTNIG00000009505
Tilapia	ENSONIG00000019905	ENSONIG00000003407
Xenopus	ENSXETG00000005315	ENSXETG00000022452
Zebrafish	ENSDARG00000079166	ENSDARG00000016918