Gene 507 (2012) 36–43

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Phylogenetic and molecular evolution of the ADAM (A Disintegrin And Metalloprotease) gene family from *Xenopus tropicalis*, to *Mus musculus*, *Rattus norvegicus*, and *Homo sapiens*

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ARTICLE INFO

Article history:
Accepted 12 July 2012
Available online 25 July 2012

Keywords:
ADAM
Phylogeny
Gene duplication
Gene synteny
Positive selection

ABSTRACT

ADAM (a disintegrin and metalloprotease) genes have been identified in various tissues and species, and recently associated with several important human diseases such as tumor and asthma. Although various biological processes have been known for the ADAM family in different species including fertilization, neurogenesis, infection and inflammation, little is known about its detailed phylogenetic and molecular evolutionary history. In this study, the ADAMs of *Xenopus (Silurana) tropicalis*, *Mus musculus*, *Rattus norvegicus*, and *Homo sapiens* were collected and analyzed by using the Bayesian analysis and gene synteny analysis to establish a comprehensive phylogenetic relationship and evolutionary drive of this gene family. It was found that there were more ADAMs in the two rodents than in the amphibian, suggesting an expansion of the ADAM gene family during the early evolution of mammals. All ADAMs from this expansion were retained in both the rodents, but other duplication events occurred subsequently in the two rodents, respectively, leading to the classification of rodent ADAMs as classes I, II and III. Moreover, these duplicated ADAM genes in the rodents were found to be driven by positive selection, which might be the major force to retain them in the genome. Importantly, it was also found that orthologs of ADAM3 and 5 have been lost in humans. These results not only provide valuable information of the evolution of ADAM genes, but may also help in understanding the role of ADAM genes in the pathobiology of relevant diseases.

1. Introduction

ADAM (a disintegrin and metalloprotease) genes are members of the zinc metalloproteinase gene superfamily, which have recently been reported to be associated with various diseases (Duffy et al., 2003; Gandy and Petanceska, 2000; Lammich et al., 1999; Mochizuki and Okada, 2007; Moss and Bartsch, 2004; Van Eerdewegh et al., 2002). ADAMs share homologies in amino acid sequences with snake venom metalloproteinases (Stone et al., 1999; Weskamp and Blobel, 1994; Wolfsberg et al., 1995a, 1995b), and are characterized by the presence of multiple functional domains, including an N-terminal secretion and pro-domain, a metalloprotease domain, a disintegrin domain, a cysteine-rich domain and an EGF-like domain, as well as a cytoplasmic tail (Seals and Courtneidge, 2003). The pro-domain could keep the metalloprotease site of the ADAMs inactive through a cysteine switch (Van Wart and Birkedal-Hansen, 1990); the metalloprotease domain may regulate the proteolytic activity of the ADAMs (Maskos et al., 1998); the disintegrin domain works together with the cysteine-rich domain to mediate cell–cell and cell–matrix interaction by interacting with integrins (Becherer and Blobel, 2003; Seals and Courtneidge, 2003; White et al., 2005); the EGF-like domain may stimulate membrane fusion (Primakoff and Myles, 2000); and the cytoplasmic tail may regulate the catalytic activity and transporting protein to its correct location in the cell (Cao et al., 2002).

By now, multiple ADAMs have been identified and their functions have been determined. Among them, about half, namely ADAMs 2, 7, 18, 20, 21, 29, and 30, have been found to be expressed either exclusively or predominately in the testis of mammals (Cerretti et al., 1999; Choi et al., 2003; Hooft van Huijsduijnen, 1998; Wei et al.,

Abbreviations: ADMA, A Disintegrin And Metalloprotease; ADAMDEC, ADAM-like, decysin; BLAST, Basic Local Alignment Search Tool; EGF, Epidermal Growth Factor; LRT, Likelihood Ratio Test; MEGA, Molecular Evolutionary Genetics; NCBI, National Center of Biotechnology Information; PAML, Phylogenetic Analysis by Maximum Likelihood.

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http://dx.doi.org/10.1016/j.gene.2012.07.016

2010). These testis-specific/predominate ADAMs are reported to be involved in spermatogenesis and sperm functions such as sperm–egg binding (ADAM 2) and sperm–egg fusion (ADAM20 and 21) (Hooft van Huijsduijnen, 1998) (Cho et al., 2000; Edwards et al., 2008; Han et al., 2009; Kim et al., 2006b; Nishimura et al., 2001; Stein et al., 2005). Other ADAMs have been found to be expressed predominantly in either nervous systems (ADAM11, 22 and 23) or somatic tissues (ADAM9, 10, 12, 15, 17 and 19) (Edwards et al., 2008).

ADAMs have also been found in different species at different levels of evolution from sea quirt (*Ciona intestinalis*) and sea urchin, to mouse and rat (*Mus musculus* and *Rattus norvegicus*), as well as human (*Homo sapiens*). While most ADAMs with testis-specific/predominant gene expression could be phylogenetically divided into two groups (Choi et al., 2003), and some ADAMs are commonly found in different species, e.g. ADAM22 and 34 have been found in both *M. musculus* and *H. sapiens*, respectively (Brocker et al., 2009; Choi et al., 2004). However, it has been found that some mouse ADAMs do not have orthologs or their orthologs are pseudogenes in the human genome (Choi et al., 2004), suggesting that some of the orthologs of mammalian ADAMs might have been lost during the evolution of invertebrate (Edwards et al., 2008). Unfortunately, there were only limited sequences and species used in these previous studies so that the origin and evolutionary history, and detailed framework of the rodent and/or mammalian ADAMs were not understood. Therefore, it remains largely unknown as regards how some of the ADAMs were kept in mouse but lost in human.

To address this question, we investigated the phylogenetic and molecular evolution of the ADAMs in a clawed frog (*Xenopus (Silurana) tropicalis*), two rodents (*M. musculus* and *R. norvegicus*), and humans (*H. sapiens*). We found that there were more ADAMs in the rodents than in the amphibian (the frog), implying an expansion of the ADAM family in the early evolution of the mammals (rodents). The rodents retained all the duplicated ADAMs from this duplication. Afterwards, other duplication events occurred during the evolution of the ADAMs in *M. musculus* and *R. norvegicus*, respectively, which eventually evolved into three different classes (I, II and III). The origin of all the ADAMs present in the genome of modern rodents could be traced to these duplication events, and positive selection has been found to drive the evolution of these duplicated ADAM genes in rodents. Perhaps more importantly, it was found that in *H. sapiens* the ADAM3 and 5 genes became pseudogenes, respectively. From an evolutionary point of view, the loss or gain of ADAM genes in a certain species is generally thought to be attributable to the requirement of fecundity for the survival of the species (Puente et al., 2005). However, it would not be inconceivable that the evolution of ADAM genes may also contribute to disease pathobiology in humans, which remains to be investigated.

2. Material and methods

2.1. Data source and retrieval of ADAMs gene sequences

The ADAM genes reported by Brocker et al. (2009) were retrieved from Genbank (http://www.ncbi.nlm.nih.gov). With these genes as queries, the BLAST program was used to search in NCBI against *X. (Silurana) tropicalis*, *M. musculus*, *R. norvegicus*, and *H. sapiens* genome assemblies. To gain a full list of ADAM homologs, BLAST searches were performed for several rounds until no new hits were obtained. During these processes, the matching sequences identified in previous searches were used as new queries in subsequent searches.

2.2. Alignment and phylogenetic analysis

The ADAM genes were aligned by webPRANK (http://www.ebi.ac.uk/goldman-srv/webPRANK/), and the Bayesian analysis was conducted with MrBayes version 3.1.2 to generate the phylogenetic tree of the ADAM genes (Huelsenbeck and Ronquist, 2001). Each analysis was run for 20,000 generations with four chains to achieve a mean standard deviation less than 0.01; one tree was saved every 100 generations, and 1750 trees were summarized to produce the final cladogram. The final tree was visualized with MEGA5 (Tamura et al., 2011).

2.3. Evolutionary analysis

The ADAM codon alignments were generated by webPRANK (http://www.ebi.ac.uk/goldman-srv/webPRANK/). The selective pressures acting on the ADAM genes were estimated by the CODEML program implemented in the PAML4 package (Yang, 2007). In site-specific models, M0, M1a and M7 are null models which assume the same ω for all sites, and M2a, M3, and M8 are alternative models which allow $\omega > 1$. The comparison of M3 with M0 was used to test for variable ω among sites, and the comparison of either M2a with M1a, or of M8 with M7 was used to test for positive selection. The difference between each comparison was evaluated by the likelihood ratio test (LRT), of which statistical significance was estimated by comparing twice the log likelihood difference to a chi-square statistic with the degrees of freedom generated by the difference in the number of parameters between two given models.

Because the site-specific models allow variable selective pressures among sites with a fixed average ω over lineages, a few branches with different rates in an alignment may be undetectable. Therefore, the branch-site method was adopted to test for positive selection in specific branches. These branches of interest were marked as a foreground branch. To construct a likelihood ratio test (LRT), a model which allows for positive selection in the foreground branch (Model A) was used along with one that does not (Model A null).

3. Results

3.1. Phylogenetic analysis

To better understand the evolution of the ADAM family, we identified the number of ADAM genes in *X. (Silurana) tropicalis, M. musculus*, *R. norvegicus*, and *H. sapiens*. The results indicate that there were 14, 37, 30, and 21 ADAM genes in these four species, respectively as shown in Table 1. Interestingly, it shows that there were more ADAM genes in the two rodents than in either *X. (Silurana) tropicalis* or *H. sapiens*, which seems to be consistent with the expansion of the ADAM genes in the mouse genome (Choi et al., 2004). Using these ADAM genes, a Bayesian phylogenetic tree was reconstructed as shown in Fig. 1.

This phylogenetic tree displayed clearly resolved relationships of all the ADAM genes and that most of the bootstrap supports were high (Fig. 1). Furthermore, the ADAM genes could be classified into 6 clades on the tree topology. According to the nomenclature established by Brocker et al. (2009), the 6 clades of the ADAMs were named as clade A to clade F with clades A, B, D, E and F belonging to one large lineage and clade C belonging to another lineage on the tree topology. In the first lineage (clades A, B, D, E and F), members of ADAMs existed in all the four species, suggesting that these ADAMs have an ancient common ancestor before the emergence of the amphibian. In contrast, orthologs of clade C were present only in the mammalian species. Taken together, these results suggest that the ADAMs found in the amphibian and the mammalian species are likely decedents of two different ancestors.

Moreover, in clades A, E and F each ADAM subfamily appeared to be completely conserved across the species, suggesting that these clades are the most conserved groups in the ADAM family (Fig. 1). The only difference of the ADAM subfamilies in these clades was their positions on the phylogenetic tree, as they located either in the root position for *X. (Silurana) tropicalis*, or in the middle position for the rodents, or in the interior position for *H. sapiens*, respectively. Such phylogenetic relationships of these ADAMs are consistent with those known for species from amphibians to primates. The phylogenetic tree also shows that in clade E, the bootstrap values for the branches of ADAM12, 15, 19 and 33 were all equal to 100, suggesting that these four subfamilies

Table 1
The ADAM genes used in this study.

Gene name	Genbank ID	Gene name	Genbank ID	Gene name	Genbank ID
HsaADAM10	NP_001101.1	MumuADAM24	NP_034216.3	RnoADAM21like	XP_002726808.1
HsaADAM11	NP_002381.2	MumuADAM25	NP_035911.2	RnoADAM22	XP_002729322.1
HsaADAM12	NP_067673.2	MumuADAM25b	NP_001009548.1	RnoADAM23	NP_001025070.1
HsaADAM15	NP_997078.1	MumuADAM26A	NP_034215.2	RnoADAM24	XP_224892.1
HsaADAM17	NP_003174.3	MumuADAM26b	NP_001009547.1	RnoADAM26A	NP_001162590.1
HsaADAM18	NP_055052.1	MumuADAM28	NP_001041640.1	RnoADAM26B	NP_001128562.1
HsaADAM19	NP_075525.2	MumuADAM29	NP_787953.2	RnoADAM28	NP_859044.1
HsaADAM2	NP_001455.3	MumuADAM3	NP_033749.2	RnoADAM30	NP_001103070.1
HsaADAM20	NP_003805.3	MumuADAM30	NP_081941.1	RnoADAM32	NP_001164053.1
HsaADAM21	NP_003804.2	MumuADAM32	NP_700446.2	RnoADAM33	XP_002729280.1
HsaADAM22	NP_068369.1	MumuADAM33	NP_001157001.1	RnoADAM34like	XP_344524.4
HsaADAM23	NP_003803.1	MumuADAM34	NP_665688.2	RnoADAM3A	NP_064698.1
HsaADAM28	NP_055080.2	MumuADAM34b	NP_001073400.1	RnoADAM4	NP_064701.1
HsaADAM29	NP_001124177.1	MumuADAM34c	NP_001020411.1	RnoADAM4b	NP_001102747.1
HsaADAM30	NP_068566.2	MumuADAM39	NP_001020551.3	RnoADAM5	NP_064699.1
HsaADAM32	NP_659441.3	MumuADAM4	NP_033750.1	RnoADAM6	NP_620261.1
HsaADAM33	NP_079496.1	MumuADAM4b	NP_001034084.2	RnoADAM7	NP_064697.1
HsaADAM7	NP_003808.2	MumuADAM5	NP_031427.2	RnoADAM8	XP_001056204.1
HsaADAM8	NP_001100.3	MumuADAM6A	NP_777479.2	RnoADAM9	NP_001014772.1
HsaADAM9	NP_003807.1	MumuADAM6b	NP_001009545.1	RnoADAMdec	NP_001099516.1
HsaADAMDEC1	NP_055294.1	MumuADAM7	NP_031428.2	XtrADAM10	NP_001037869.1
MumuADAM10	NP_031425.2	MumuADAM8	NP_031429.1	XtrADAM11	ADK56764.1
MumuADAM11	NP_001104248.1	MumuADAM9	NP_031430.2	XtrADAM12	ABD52382.1
MumuADAM12	NP_031426.2	MumuADAMDEC	NP_067450.1	XtrADAM15	XP_002943163.1
MumuADAM15	NP_001032811.2	RnoADAM1	NP_064463.1	XtrADAM17	NP_001182159.1
MumuADAM17	NP_033745.4	RnoADAM10	ENSRNOP00000021066[a]	XtrADAM19	ABD52384.1
MumuADAM18	NP_034214.2	RnoADAM11	NP_001101770.1	XtrADAM22	NP_001123768.1
MumuADAM19	NP_033746.1	RnoADAM12	XP_001054670.2	XtrADAM23	XP_002936436.1
MumuADAM1a	NP_742124.2	RnoADAM15	NP_064704.1	XtrADAM28a	ADK56770.1
MumuADAM1b	NP_742123.2	RnoADAM17	NP_064702.1	XtrADAM28b	NP_001120446.1
MumuADAM2	NP_033748.2	RnoADAM18	XP_001072121.2	XtrADAM33	NP_001035102.1
MumuADAM21	NP_065063.1	RnoADAM19	NP_001153700.1	XtrADAM9a	XP_002932764.1
MumuADAM22	NP_001007221.1	RnoADAM2	NP_064462.1	XtrADAM9b	XP_002933016.1
MumuADAM23	NP_001171071.1	RnoADAM20	XP_001056657.2	XtrADAM9b1	XP_002937848.1

[a] This sequence was retrieved from Ensemble database.

should have arisen from one common ancestor whereas the three subfamilies in clade A and the two subfamilies in clade F should have arisen from another common ancestor.

In clade D, two ADAMs (ADAM28a, 28b) were present in *X. (Silurana) tropicalis* and clustered into a monophyletic group (Fig. 1), suggesting gene duplications in the amphibian species. Orthologs of ADAM7, 8, 28 and ADAMDEC subfamilies were present in *M. musculus*, *R. norvegicus and H. sapiens*, but not in *X. (Silurana) tropicalis*. The ADAM7, 28 and ADAMDEC clustered into a group with the two ADAMs in *X. (Silurana) tropicalis* being placed at its root (Fig. 1), suggesting that ADAM7, 28 and ADAMDEC might be produced by gene duplications occurring after the mammalian divergence from amphibians. Hence, the ADAMs in clade D should have originated from one common ancestor.

In clade B, all the ADAM subfamilies appeared to be present only in the mammals except for the ADAM9 subfamily that appeared to exist in all the species (Fig. 1). The ADAM2, 18 and 32 clustered into three different monophyletic groups, suggesting that they have arisen from duplication events after the divergence of mammalians from the amphibians. As regards the ADAM9 present in all the species, the two amphibian subfamilies found in *X. (Silurana) tropicalis* showed far distant relationships with the mammalian ADAM9 subfamilies and had low bootstrap values on the tree topology (data not shown). Therefore, they were excluded from this analysis. In addition, the pseudogenes of ADAM3 and 5 were found in humans (data not shown), which suggests that ADAM3 and 5 are specific to mammals but have been lost in humans during evolution.

The whole clade C was absent in *X. (Silurana) tropicalis* (Fig. 1). It might have been either lost in the amphibian or particularly gained in the mammalian species. Orthologs of ADAM21 and 30 were present in both the rodents and humans, indicating that they were conserved during mammalian evolution. However, ADAM21 was clustered to an additional ADAM20 in *H. sapiens*, whereas it was linked to a distinct ADAM20 *M. musculus*. ADAM29 existed in both *H. sapiens* and *R. norvegicus* but not in *M. musculus*. Although ADAM4 existed in both *M. musculus* and *R. norvegicus*, they formed two different monophyletic groups on the phylogeny. And these two groups further clustered together with high support. Similar clusters were also found for the ADAM6 subfamilies. This demonstrates that a common ancestor had firstly given rise to the rodent ADAM4 and 6 that then expanded in *M. musculus* and *R. norvegicus*, respectively, after the species split. It has been reported that ADAM25s in *M. musculus* cluster as a monophyletic group (39). Here, it was also found that ADAM4, 26, and 34 in *M. musculus*, and ADAM4 and 26 in *R. norvegicus* cluster as similar monophyletic subgroups, respectively. Besides, ADAM1, 4, 6, 24, 25, 26, 34 and 39 did not appear in *H. sapiens*.

3.2. Gene synteny

Analyses of genomic location of the ADAM loci resulted in the following findings. For ADAM1a and 1b, there was no hit found for either of them in the genome of *X. (Silurana) tropicalis*, but the unit of both ADAMs was conserved in *M. musculus* and *R. norvegicus*, though the ADAM1b became a pseudogene in *R. norvegicus*. The MumuADAM1a had a closer relationship to the RnoADAM1a than to the MumuADAM1b on the phylogenetic tree, suggesting that the unit of ADAM1a–1b should have appeared before the split between *M. musculus* and *R. norvegicus*, while the pseudogenization occurred to the RnoADAM1b after the split. Both of the two ADAMs became pseudogenes in the human genome (Fig. 2), indicating the ADAM1a and 1b cluster may be lineage-specifically gained in mammals.

For ADAM2, the HsaADAM2, MumuADAM2 and RnoADAM2 clustered together on the phylogeny with high bootstrap values (Fig. 2). However, except for rodent ADAM2, ADAMs in clade B were all located in tandem on one chromosome. These results suggest that genomic rearrangements might have occurred in this region. The genomic

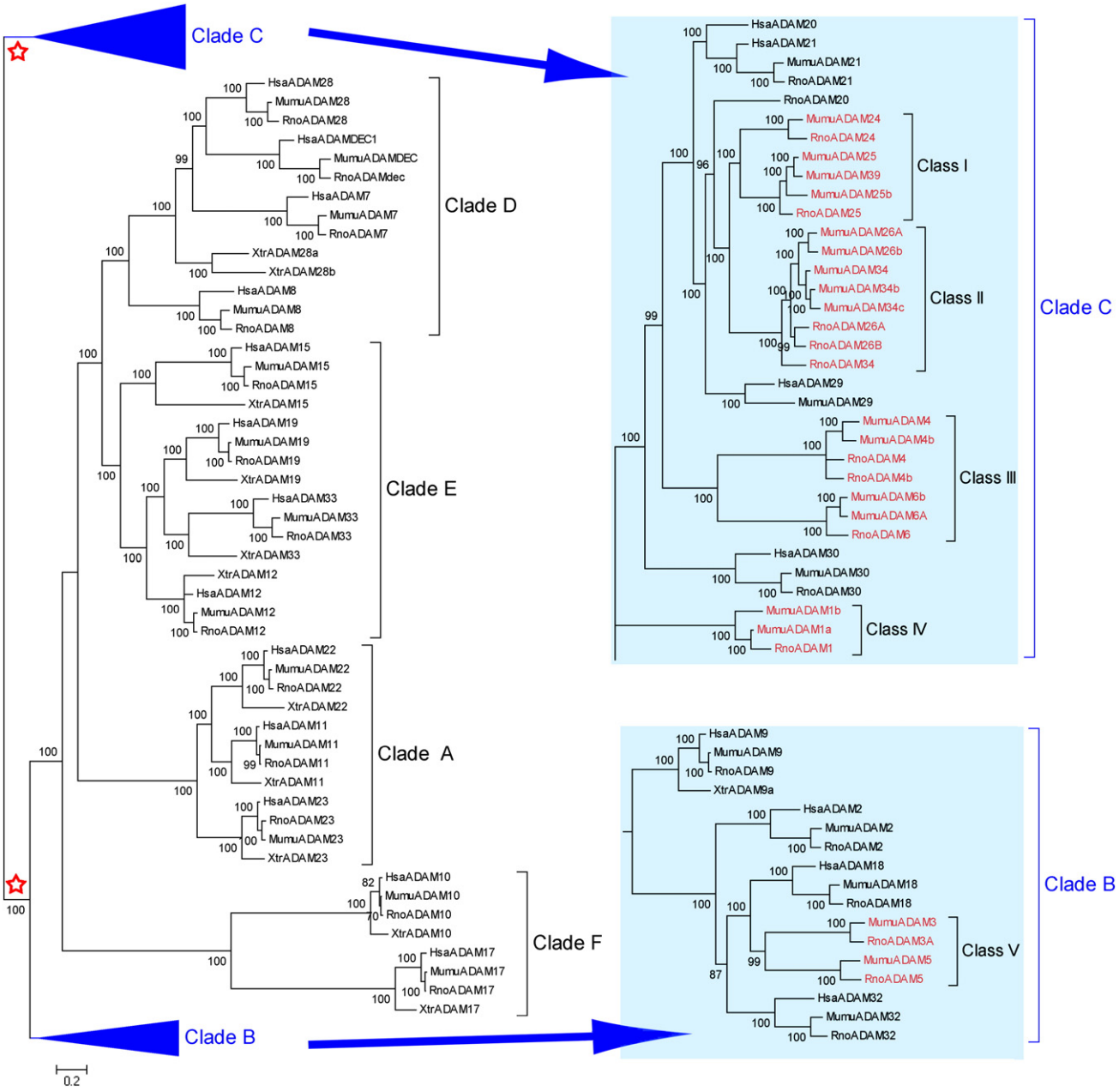

Fig. 1. Bayesian phylogenetic tree of the ADAM protein sequences from *Xenopus (Silurana) tropicalis, Mus musculus, Rattus norvegicus*, and *Homo sapiens.* The red stars indicate 2 earlier progenitors of the ADAM family. The tree is unrooted and Bayesian posterior probability values (>70%) are shown at interior nodes. The scale bar corresponds to 0.1 substitutions per site. The duplicated ADAMs in the five classes are marked in red. The species and accession numbers are listed in Table 1. The corresponding amino acid sequence alignment is provided in Fig. S1. The abbreviations used are as follows: Hsa, *Homo sapiens*: Mumu, *M. musculus*; Rno, *Rattus norvegicus*; Xtr, *Xenopus (Silurana) tropicalis.*

structure of clade B was conserved from rodents to humans, and the gene unit of Tm2D2-ADAM9 was even conserved from *X. (Silurana) tropicalis* to humans (Fig. 2). ADAM3 and 5 in clade B were identified as pseudogenes in the human genome (Fig. 2).

For ADAM4, the unit of ADAM4-Cox16-Synj2bp-ADAM21 was well conserved in rodents, and the ADAM4 was clustered with ADAM21 on the phylogeny (Fig. 2), indicating that this gene unit should have been generated by tandem duplications before the divergence of *M. musculus* and *R. norvegicus*. Furthermore, given that MumuADAM4 and MumuADAM4b are closer to each other than to RnoADAM4s, it seems that ADAM4 might have been duplicated separately in *M. musculus* and *R. norvegicus* after their split. In *H. sapiens* orthologs of ADAM4s were absent; instead at the orthologous region where ADAM4s were supposed to be there were pseudogenes of ADAM21 and 25. And ADAM20 and its pseudogene also appeared at the right flank of ADAM21, which was not seen in rodent genome (Fig. 2).

For ADAM6, there was a single member in *R. norvegicus*, as compared to two ADAM6s neighboring to each other on the chromosome in *M. musculus*, indicating lineage specific expansion in *M. musculus* (Fig. 2). On the other hand, the rodent ADAM4 and 6 clustered together with high bootstrap values on the tree topology, but located on different chromosomes, indicating that the ancestors of the ADAM4 and 6 should have arisen from segmental duplication.

For ADAM24 and 25, the rodent ADAM24s clustered nearly together on the phylogeny, and both ADAM24 and 25 located on one chromosome (Figs. 1, 2), indicating that this gene unit should have been produced by tandem duplication before the divergence of *M. musculus* and *R. norvegicus.* In this region, however, there were additional ADAM25b

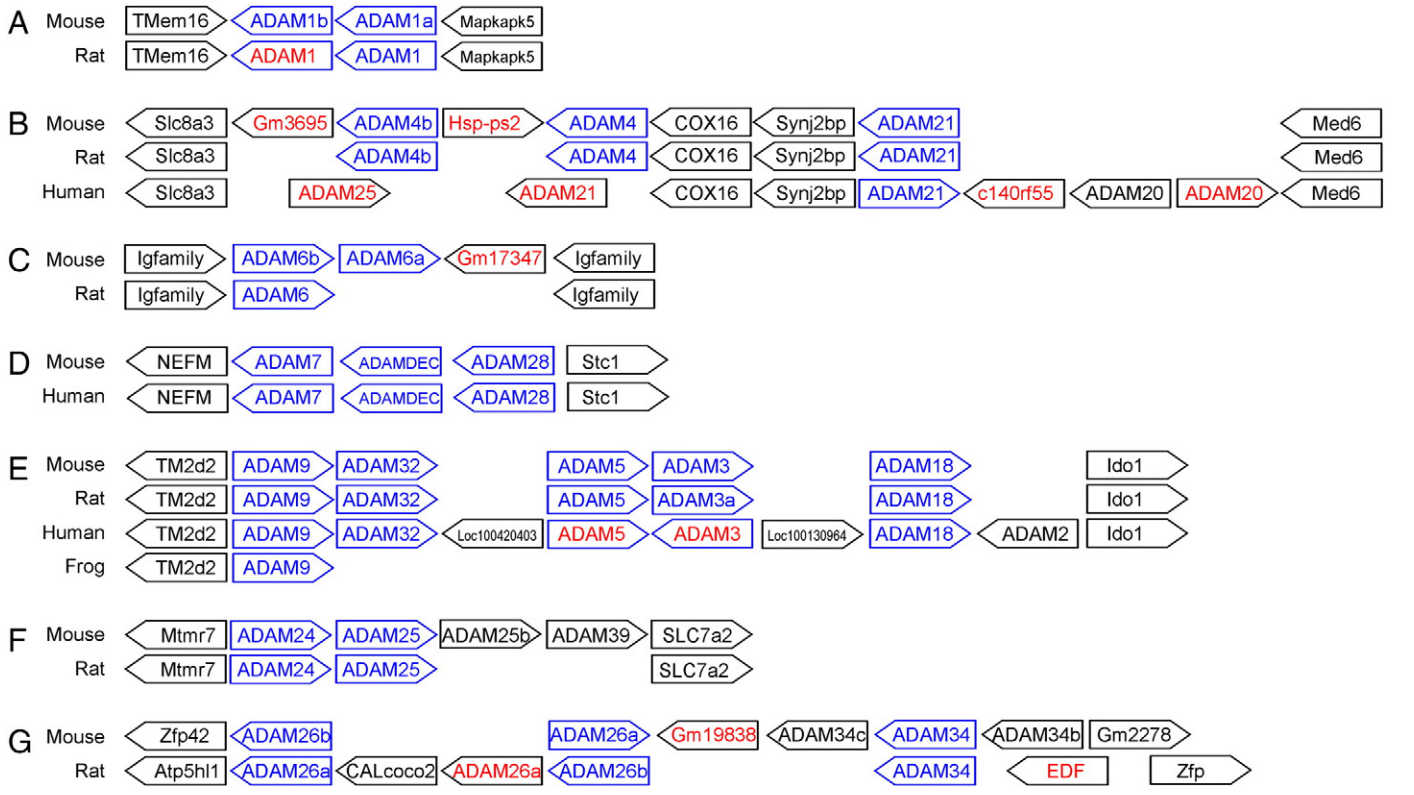

Fig. 2. Order and orientation of genes syntenic to (A) ADAM1a and ADAM1b; (B) ADAM4, ADAM4b and ADAM21; (C) ADAM6a and ADAM6b; (D) ADAM7, ADAMDEC and ADAM28; (E) ADAMs in clade B; (F) ADAMs in class I; (G) ADAM in class II. Genes are intentionally aligned in columns to facilitate visualization of synteny. For other details, please see the text.

and 39 in *M. musculus* but not in *R. norvegicus*, suggesting individual gene duplications in the former. Besides, the ADAM26s and 34s in *M. musculus* were found to be on the same chromosome and cluster as a monophyletic group on the phylogenetic tree (Fig. 2). These suggest that ADAM26s and 34s in *M. musculus* have originated from lineage specific tandem duplications. Similarly, in the orthologous region of ADAM26 in *R. norvegicus*, ADAM26s and 34 locate next to each other, and a pseudogene of ADAM26 was also found (Fig. 2), suggesting that at least two rounds of gene duplications have occurred in *R. norvegicus*. In the two rodents the gene units ADAM24–ADAM25(s) (39), ADAM26s–ADAM34s, and ADAM18–ADAM3–ADAM5–ADAM32–ADAM9 were factually on the same chromosome (chromosome 8, and 16 for *M. musculus*, and *R. norvegicus*, respectively) (Fig. 2). This suggests that the ancestors of these three gene units might have been derived from tandem duplications before species divergence.

3.3. Selection analysis

The evolutionary analyses by the CODEML program implemented in PAML4 package indicate that on the phylogenetic tree, all the duplicated ADAM genes in the two rodents belong to clades B and C, and can be further classified into classes I, II, III, IV, and IV (Fig. 1). Results of the site-specific model tests for clades B and C are shown in Tables 2, and 3, respectively. For clade B, M3 was preferred to M0 ($p<0.01$), but the comparison of neither M2a with M1a, nor M8 with M7 was significant ($p>0.05$), indicating no positive selection detectable (Table 2). For clade C, M3 was also a preferred model to fit the data ($p<0.01$), and the comparison of either M2a with M1a, or M8 with M7 was found statistically significant ($p<0.01$) (Table 3), indicating positive selection. It was also found that the significant positive selection occurred with $\omega=2.9$ for 3% sites, and 1.7 for 4% sites in M2a, and M8, respectively.

For the five ADAM gene classes, classes I and II were combined in the test against site-specific models because they clustered together on the tree topology, and classes IV and V were excluded for the test due to insufficient sequences. The results for the three classes are shown in Tables 4 and 5, respectively. For classes I and II. M3, and M8 fitted the data better than the null models M0, M1a and M7 ($p<0.01$), respectively (Table 4). In M2a and M8, about 5% and 6% sites were under positive selection ($\omega2=3.85$, 3.16 for M2a, M8, respectively) (Table 4). These results suggest that strong positive selection has acted on the ADAM genes in classes I and II after the duplication events. For class III, M3 was also preferred over M0 ($p<0.01$), and the comparison of M2a with

Table 2
Site model test for all ADAM genes in Clade B.

Model code	lnL	Estimates of parameters	2Δl	PSS
M0 (one-ratio)	−20,967.43	$\omega=0.25341$	988.74	N/A
M3 (discrete)	−20,473.06	p0 = 0.17230 p1 = 0.49220 p2 = 0.33550 ω0 = 0.01297 ω1 = 0.19124 ω2 = 0.61536	p<0.01	Not shown
M1a (nearly neutral)	−20,639.18	p0 = 0.66816 p1 = 0.33184 ω0 = 0.17445 ω1 = 1.00000	0	N/A
M2a (positive selection)	−20,639.18	p0 = 0.66816 p1 = 0.28877 p2 = 0.04306 ω0 = 0.17445 ω1 = 1.00000 ω2 = 1.00000	p>0.05	None
M7 (beta)	−20,476.79	p = 0.76474 q = 1.73552	0.06	N/A
M8 (beta&w>1)	−20,476.76	p0 = 0.99302 p = 0.77377 q = 1.78950 (p1 = 0.00698) ω = 1.00000	p>0.05	None

lnL: the log-likelihood difference between the two models; 2Δl: twice the log-likelihood difference between the two models.
Positively selected sites (PSS) were identified with posterior probability $p\geq0.90$.

Table 3
Site model test for all ADAM genes in Clade C.

Model code	lnL	Estimates of parameters	2Δl	PSS
M0 (one-ratio)	−18,185.94	ω = 0.30	1783.49 P<0.01	N/A
M3 (discrete)	−17,294.19	p0 = 0.33729 p1 = 0.36680 p2 = 0.29591 ω0 = 0.02661 ω1 = 0.29864 ω2 = 0.92318		Not shown
M1a (nearly neutral)	−17,438.54	p0 = 0.52830 p1 = 0.47170 ω0 = 0.09861 ω1 = 1.00000	26.71 P<0.01	N/A
M2a (positive selection)	−17,425.18	p0 = 0.51823 p1 = 0.44778 p2 = 0.03399 ω0 = 0.09925 ω1 = 1.00000 ω2 = 2.28576		104S** 149S** 298L 318Y*
M7 (beta)	−17,279.68	p = 0.38768 q = 0.64732	15.63 P<0.01	N/A
M8 (beta&w > 1)	−17,271.86	p0 = 0.96324 p = 0.40845 q = 0.74883 (p1 = 0.03676) ω = 1.65396		104S* 149S*

lnL: the log-likelihood difference between the two models; 2Δl: twice the log-likelihood difference between the two models.
Positively selected sites (PSS) were identified with posterior probability p ≥ 0.90.
One asterisk indicates posterior probability p ≥ 0.95 and two asterisks p ≥ 0.99.

M1a, or M8 with M7 was statistically significant (p<0.05, or 0.01), respectively (Table 5). These results demonstrate that positive selection has driven the evolution of the ADAM genes in class III, after the duplication events, though only at two sites.

The results of the branch-site specific model tests on the five ADAM gene classes are shown in Table 6. For classes I and II, Model A was preferable to Model A null (p<0.01) and about 8% of sites showed positive selection (ω = 2.52). For class III, the model A was also preferable (p<0.01) and strong positive selection was found (ω = 999.00 for 5% of sites) (Table 6). Similarly, for classes IV and V, Model A was preferable (p<0.01). There were 8% and 5% of sites in classes IV (ω = 146.67), and V (ω = 48.73) that have experienced positive selection, respectively.

4. Discussion

ADAM genes have been reported to be involved in a wide variety of biological processes, such as spermatogenesis and some diseases. Though ADAM genes are conserved in the evolution of the vertebrate, several ADAM members cannot be found in some mammals, and the evolutionary history of these members remains largely unclear. In this study, we investigated the phylogenetic positions of ADAM genes in species from *X. (Silurana) tropicalis, M. musculus*, *R. norvegicus* to *H. sapiens*, which is important in understanding the evolutionary history of the ADAM genes family in vertebrates. We show that the phylogeny of the ADAM genes was well supported. ADAM genes in clades A, E and F were highly conserved during evolution from *X. (Silurana) tropicalis* to *H. sapiens* (Fig. 1). After the diversification from the amphibian *X. (Silurana) tropicalis*, the mammalian rodents gained additional ADAM genes in clades B, C, D resulting from either duplication of existing genes or the birth of new genes. For example, the rodents seemed to have ADAM2, 3, 5, 18 and 32 in clade B as a result of several rounds of duplications after diversification from the amphibian. Clade C appeared only in the mammals used in this study. Further evolutionary events may have occurred, however, in the ADAM genes family as rodents diversified to primates.

According to the phylogeny of the clade C in mammals, ADAM1 was first established in evolution, and then orthologs of this subfamily became pseudogenes in *H. sapiens*. Other subfamilies should have arisen from an ADAM1 like gene. ADAM1a and ADAM1b located adjacent to each other on the same chromosome (Fig. 2), and this ADAM1 region was shown to be produced by tandem duplications predating the divergence between *M. musculus* and *R. norvegicus*.

In clade B, all the ADAMs except for ADAM2, were found to locate on the same genomic region (Fig. 2). This orthologous region was conserved in both rodents and humans despite the pseudogenization of ADAM3 and 5 in humans. Nevertheless, the ortholog of ADAM9 in this cluster was found to be present in *X. (Silurana) tropicalis*. This seems to indicate that, in the evolution of from rodent to human, by tandem duplication, an ADAM9 like gene had given birth to the ancestor of other ADAMs of clade B, which then produced ADAM2 by segmental duplication, and ADAM3, 5, 18 and 32 by tandem duplications, respectively. ADAM4, 4b, and 21 located on the same chromosome, and shared a common ancestor on the phylogeny, implying that ADAM21 had produced ADAM4 which then further produced ADAM4b by tandem duplications after the diversification of the rodents. In *H. sapiens*, ADAM20 and 21 were tandem duplicated but their copies had become pseudogenes during evolution (Fig. 2). In addition, the ADAMs in class I and II located in the same genomic region, and shared a similar structure, as well as were closely related on the phylogeny with ADAM29 (20) on the root position (Fig. 1). In *M. musculus* and *R. norvegicus*, ADAMs in classes I and II and ADAM29 (20) were on the same chromosome (Fig. 2). All these suggest that rounds of tandem duplications with ADAM29 (20) as the template had given birth to ADAMs in classes I and II, and a duplication event occurred at the early stage of diversification of the rodents from the amphibian *X. tropicalis*.

The phylogenetic tree also showed two large lineages in the ADAM genes family, with one including clades A, B, D, E and F, and the other comprising clade C alone. This implies that the ADAMs in either amphibians or mammals have originated from two different ancestors. It also showed prevalent gene duplications in rodent, resulting in more ADAM genes in rodents especially in the *M. musculus* as

Table 4
Site model test for duplicated ADAM genes in classes I and II (*Mus musculus* and *Rattus norvegicus*).

Model code	lnL	Estimates of parameters	2Δl	PSS
M0 (one-ratio)	−11,823.50	ω = 0. 47630	287.84	N/A
M3 (discrete)	−11,535.66	p0 = 0. 43789 p1 = 0. 49765 p2 = 0. 06446 ω0 = 0.08810 ω1 = 0.78163 ω2 = 3.12446	p<0.01	Not shown
M1a (nearly neutral)	−11,570.03	p0 = 0.54294 p1 = 0.45706 ω0 = 0.12614 ω1 = 1.00000	31.02	N/A
M2a (positive selection)	−11,539.01	p0 = 0. 50523 p1 = 0. 44909 p2 = 0. 04569 ω0 = 0.12508 ω1 = 1.00000 ω2 = 3.85280	p<0.01	256Y** 257A* 260P** 264T 267G** 286T** 301D 333S* 384S* 612V*
M7 (beta)	−11,571.58	p = 0.37585 q = 0.41674	37.33	N/A
M8 (beta&w > 1)	−11,534.25	p0 = 0.94071 p = 0.42551 q = 0.50146 (p1 = 0.05929) ω = 3.16678	p<0.01	256Y** 257A* 260P** 264T* 267G** 286T** 301D* 333S* 384S* 395P 588V 612V**

lnL: the log-likelihood difference between the two models; 2Δl: twice the log-likelihood difference between the two models.
Positively selected sites (PSS) were identified with posterior probability p ≥ 0.90.
One asterisk indicates posterior probability p ≥ 0.95 and two asterisks p ≥ 0.99.

Table 5
Site model test for ADAM 4 and 6 genes (class III, *Mus musculus* and *Rattus norvegicus*).

Model code	lnL	Estimates of parameters	2Δl	PSS
M0 (one-ratio)	−7074.33	ω = 0. 42256	134.91	N/A
M3 (discrete)	−6939.42	p0 = 0. 38646 p1 = 0. 50016 p2 = 0.11338 ω0 = 0. 04857 ω1 = 0.66093 ω2 = 2.46798	p<0.01	Not shown
M1a (nearly neutral)	−6947.71	p0 = 0.49173 p1 = 0.50827 ω0 = 0.08522 ω1 = 1.00000	6.16	N/A
M2a (positive selection)	−6941.55	p0 = 0. 47844 p1 = 0. 48380 p2 = 0.03776 ω0 = 0.08554 ω1 = 1.00000 ω2 = 4.03070	p<0.05	343T 499E
M7 (beta)	−6949.07	p = 0.29181 q = 0.29526	9.37	N/A
M8 (beta&w > 1)	−6939.69	p0 = 0.91706 p = 0.36190 q = 0.46185 (p1 = 0.08294) ω = 2.73889	p<0.01	343T 499E

lnL: the log-likelihood difference between the two models; 2Δl: twice the log-likelihood difference between the two models.
Positively selected sites (PSS) were identified with posterior probability p ≥ 0.90.

compared to either the amphibian (*X. (Silurana) tropicalis*) or human. This, however, may not be a result of lineage-specific expansions in rodents because pseudogenes of these rodent-specific ADAMs have been identified in the human genome. The clades also showed that ADAM10 and ADAM17 genes were separated from all other ADAM genes. This major diversification fits in the assumption that ADAM10 and ADAM17 are mostly responsible for the shedding of membrane proteins and that under pathological conditions, other ADAM proteases become functionally important. This might be the reason why ADAM10 and ADAM17 are so well conserved in all the species.

Site model analyses further demonstrated a positive selection of ADAM genes in clade C but not in clade B (Tables 2–5), suggesting that different ADAM subfamilies might have been subjected to different levels of evolutionary force during the evolution of vertebrates, even though ADAM genes in both clades B and C have been reported to undergo intensified selection in general (Dorus et al., 2010). Since the ADAM genes in clade C are known to be testis-specific or primarily expressed in reproductive tissues and the genes with a role in reproduction are likely to evolve rapidly (Cerretti et al., 1999; Choi et al., 2004; Clark et al., 2007; Glassey and Civetta, 2004; Haerty et al., 2007; Han et al., 2009; Kim et al., 2006a; Nishimura et al., 2004; Singh and Kulathinal, 2000; Swanson and Vacquier, 2002; Wyckoff et al., 2000; Zhu et al., 1999, 2001, 2009), it is not surprising to find the strong adaptive selection in clade C. Moreover, because positive selection occurs during a short period, it might be difficult to detect it in large groups (Jimenez et al., 2009). Thus, site model tests were performed on the five classes separately, which observed adaptive selection in classes I–III according to both site and branch-site model analyses and accelerated selection in class IV according to branch-site model analysis (Tables 3–5).

The duplicated genes may lead to several different long-term fates such as nonfunctionalization, subfunctionalization or neofunctionalization. The finding of pseudogenes of ADAM1 and 26 in *R. norvegicus* indicates that the duplicated genes in the rodent should have gone through nonfunctionalizations. It is reported that most ADAM genes with a potential role in sperm maturation or fertilization have significantly higher nonsynonymous rates than other ADAM genes (Civetta, 2003). Interestingly, positive selection was found along the rodent ADAM3 and 5 branches by branch-site model (Table 6), indicating that duplicated ADAM3/5 had been retained in rodent by adaptive selection but had been eliminated in *H. sapiens* as a result of functional redundancy.

Taken together, these results suggest that positive selection contributes to the retaining of those ADAM copies after gene duplication, possibly due to the fact that the ADAM genes are partly related to reproduction. The identification that the mammalian ADAM genes had experienced a wide variety of duplication events which were followed by pseudogenization and/or positive selection would help to understand not only the evolutionary history of the ADAM genes and their functional divergence, but also their roles in the emergence and development of human diseases that deserve further investigations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2012.07.016.

Acknowledgment

This research was supported by the National Natural Science Foundation of China (Grant nos. 11172340 and 31101926), the Chongqing University Postgraduates' Science and Innovation Funds (No. CDJXS11231180), Training Program for Hundreds of Distinguished Leading Scientists of Chongqing, Chongqing Natural Science Foundation (Project no. CSTC, 2010BA5001), and the Sharing Fund of Chongqing University's Large-Scale Equipment (nos. 2010063057, 2011063048, and 2011063049), and was also supported by the Visiting Scholar Foundation of Key Laboratory of Biorheological Science and Technology, Ministry of Education in Chongqing University and the Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Table 6
Branch site model tests for duplicated ADAM genes from *Mus musculus* and *Rattus norvegicus*.

Class	Model code	lnL	Estimates of parameters	2Δl	PSS
I and II	Model A null	−17,426.31	p0 = 0.49219 p1 = 0.42643 (p2 + p3 = 0.08139) ω0 = 0.09159 ω1 = 1.00000 ω2 = 1.00000	8.36	N/A
	Model A	−17,422.13	p0 = 0.49347 p1 = 0.42959 (p2 + p3 = 0.07694) ω0 = 0.09631 ω1 = 1.00000 ω2 = 2.52405	p<0.01	14H* 104S* 124R 172P 318Y*
III	Model A null	−17,434.49	p0 = 0.46771 p1 = 0.41968 (p2 + p3 = 0.11261) ω0 = 0.09537 ω1 = 1.00000 ω2 = 1.00000	11.57	N/A
	Model A	−17,428.70	p0 = 0.50113 p1 = 0.45229 (p2 + p3 = 0.04657) ω0 = 0.09510 ω1 = 1.00000 ω2 = 998.99621	p<0.01	119S* 157A*
IV	Model A null	−17,435.38	p0 = 0.48950 p1 = 0.42952 (p2 + p3 = 0.08098) ω0 = 0.09693 ω1 = 1.00000 ω2 = 1.00000	8.04	N/A
	Model A	−17,431.36	p0 = 0.49152 p1 = 0.42561 (p2 + p3 = 0.08287) ω0 = 0.09857 ω1 = 1.00000 ω2 = 146.66598	p<0.01	62G
V	Model A null	−20,637.87	p0 = 0.53867 p1 = 0.26862 (p2 + p3 = 0.19271) ω0 = 0.17237 ω1 = 1.00000 ω2 = 1.00000	8.14	N/A
	Model A	−20,633.81	p0 = 0.63273 p1 = 0.31622 (p2 + p3 = 0.05105) ω0 = 0.17167 ω1 = 1.00000 ω2 = 48.72643	p<0.01	167S* 477N 549R

The phylogenetic trees with the foreground and background branches marked out could refer to Fig. S2 and Fig. S3.
lnL: the log-likelihood difference between the two models; 2Δl: twice the log-likelihood difference between the two models.
Positively selected sites (PSS) were identified with posterior probability p ≥ 0.90.
One asterisk indicates posterior probability p ≥ 0.95.

References

Becherer, J.D., Blobel, C.P., 2003. Biochemical properties and functions of membrane-anchored metalloprotease-disintegrin proteins (ADAMs). Curr. Top. Dev. Biol. 54, 101–123.

Brocker, C.N., Vasiliou, V., Nebert, D.W., 2009. Evolutionary divergence and functions of the *ADAM* and *ADAMTs* gene families. Hum. Genom. 4, 43–55.

Cao, Y., Kang, Q., Zhao, Z., Zolkiewska, A., 2002. Intracellular processing of metalloprotease disintegrin ADAM12. J. Biol. Chem. 277, 26403–26411.

Cerretti, D.P., DuBose, R.F., Black, R.A., Nelson, N., 1999. Isolation of two novel metalloproteinase-disintegrin (ADAM) cDNAs that show testis-specific gene expression. Biochem. Biophys. Res. Commun. 263, 810–815.

Cho, C., Ge, H., Branciforte, D., Primakoff, P., Myles, D.G., 2000. Analysis of mouse fertilin in wild-type and fertilin beta(−/−) sperm: evidence for C-terminal modification, alpha/beta dimerization, and lack of essential role of fertilin alpha in sperm-egg fusion. Dev. Biol. 222, 289–295.

Choi, I., Woo, J.M., Hong, S., Jung, Y.K., Kim, D.H., Cho, C., 2003. Identification and characterization of *ADAM32* with testis-predominant gene expression. Gene 304, 151–162.

Choi, I., et al., 2004. Characterization and comparative genomic analysis of intronless Adams with testicular gene expression. Genomics 83, 636–646.

Civetta, A., 2003. Positive selection within sperm-egg adhesion domains of fertilin: an ADAM gene with a potential role in fertilization. Mol. Biol. Evol. 20, 21–29.

Clark, A.G., et al., 2007. Evolution of genes and genomes on the Drosophila phylogeny. Nature 450, 203–218.

Dorus, S., Wasbrough, E.R., Busby, J., Wilkin, E.C., Karr, T.L., 2010. Sperm proteomics reveals intensified selection on mouse sperm membrane and acrosome genes. Mol. Biol. Evol. 27, 1235–1246.

Duffy, M.J., Lynn, D.J., Lloyd, A.T., O'Shea, C.M., 2003. The ADAMs family of proteins: from basic studies to potential clinical applications. Thromb. Haemost. 89, 622–631.

Edwards, D.R., Handsley, M.M., Pennington, C.J., 2008. The ADAM metalloproteinases. Mol. Aspects Med. 29, 258–289.

Gandy, S., Petanceska, S., 2000. Regulation of Alzheimer beta-amyloid precursor trafficking and metabolism. Biochim. Biophys. Acta 1502, 44–52.

Glassey, B., Civetta, A., 2004. Positive selection at reproductive *ADAM* genes with potential intercellular binding activity. Mol. Biol. Evol. 21, 851–859.

Haerty, W., et al., 2007. Evolution in the fast lane: rapidly evolving sex-related genes in drosophila. Genetics 177, 1321–1335.

Han, C., et al., 2009. Comprehensive analysis of reproductive ADAMs: relationship of ADAM4 and ADAM6 with an ADAM complex required for fertilization in mice. Biol. Reprod. 80, 1001–1008.

Hooft van Huijsduijnen, R., 1998. ADAM 20 and 21; two novel human testis-specific membrane metalloproteases with similarity to fertilin-alpha. Gene 206, 273–282.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Jimenez, S., Lawton-Rauh, A.L., Reighard, G.L., Abbott, A.G., Bielenberg, D.G., 2009. Phylogenetic analysis and molecular evolution of the dormancy associated MADS-box genes from peach. BMC Plant Biol. 9, 81.

Kim, E., et al., 2006a. Mouse sperm lacking ADAM1b/ADAM2 fertilin can fuse with the egg plasma membrane and effect fertilization. J. Biol. Chem. 281, 5634–5639.

Kim, T., et al., 2006b. Expression and relationship of male reproductive *ADAMs* in mouse. Biol. Reprod. 74, 744–750.

Lammich, S., et al., 1999. Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. Proc. Natl. Acad. Sci. U.S.A. 96, 3922–3927.

Maskos, K., et al., 1998. Crystal structure of the catalytic domain of human tumor necrosis factor-alpha-converting enzyme. Proc. Natl. Acad. Sci. U.S.A. 95, 3408–3412.

Mochizuki, S., Okada, Y., 2007. ADAMs in cancer cell proliferation and progression. Cancer Sci. 98, 621–628.

Moss, M.L., Bartsch, J.W., 2004. Therapeutic benefits from targeting of ADAM family members. Biochemistry 43, 7227–7235.

Nishimura, H., Cho, C., Branciforte, D.R., Myles, D.G., Primakoff, P., 2001. Analysis of loss of adhesive function in sperm lacking cyritestin or fertilin beta. Dev. Biol. 233, 204–213.

Nishimura, H., Kim, E., Nakanishi, T., Baba, T., 2004. Possible function of the ADAM1a/ADAM2 fertilin complex in the appearance of ADAM3 on the sperm surface. J. Biol. Chem. 279, 34957–34962.

Primakoff, P., Myles, D.G., 2000. The *ADAM* gene family: surface proteins with adhesion and protease activity. Trends Genet. 16, 83–87.

Puente, X.S., Sanchez, L.M., Gutierrez-Fernandez, A., Velasco, G., Lopez-Otin, C., 2005. A genomic view of the complexity of mammalian proteolytic systems. Biochem. Soc. Trans. 33, 331–334.

Seals, D.F., Courtneidge, S.A., 2003. The ADAMs family of metalloproteases: multidomain proteins with multiple functions. Genes Dev. 17, 7–30.

Singh, R.S., Kulathinal, R.J., 2000. Sex gene pool evolution and speciation: a new paradigm. Genes Genet. Syst. 75, 119–130.

Stein, K.K., Go, J.C., Primakoff, P., Myles, D.G., 2005. Defects in secretory pathway trafficking during sperm development in Adam2 knockout mice. Biol. Reprod. 73, 1032–1038.

Stone, A.L., Kroeger, M., Sang, Q.X., 1999. Structure–function analysis of the ADAM family of disintegrin-like and metalloproteinase-containing proteins (review). J. Protein Chem. 18, 447–465.

Swanson, W.J., Vacquier, V.D., 2002. The rapid evolution of reproductive proteins. Nat. Rev. Genet. 3, 137–144.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Van Eerdewegh, P., et al., 2002. Association of the *ADAM33* gene with asthma and bronchial hyperresponsiveness. Nature 418, 426–430.

Van Wart, H.E., Birkedal-Hansen, H., 1990. The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. Proc Natl. Acad. Sci. U.S.A. 87, 5578–5582.

Wei, S., et al., 2010. Conservation and divergence of ADAM family proteins in the *Xenopus* genome. BMC Evol. Biol. 10, 211.

Weskamp, G., Blobel, C.P., 1994. A family of cellular proteins related to snake venom disintegrins. Proc. Natl. Acad. Sci. U.S.A. 91, 2748–2751.

White, J., Bridges, L., DeSimone, D., Tomczuk, M., Wolfsberg, T., 2005. Introduction to the ADAM family. In: Hooper, N.M., Lendeckel, U. (Eds.), The ADAM Family of Proteases, Proteases in Biology and Disease, Vol. 4. Springer, US, pp. 1–28.

Wolfsberg, T.G., Primakoff, P., Myles, D.G., White, J.M., 1995a. ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease domain: multipotential functions in cell–cell and cell–matrix interactions. J. Cell Biol. 131, 275–278.

Wolfsberg, T.G., et al., 1995b. ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. Dev. Biol. 169, 378–383.

Wyckoff, G.J., Wang, W., Wu, C.I., 2000. Rapid evolution of male reproductive genes in the descent of man. Nature 403, 304–309.

Yang, Z., 2007. PAML 4: phylogenetic analysis by maximum likelihood. Mol. Biol. Evol. 24, 1586–1591.

Zhu, G.Z., Lin, Y., Myles, D.G., Primakoff, P., 1999. Identification of four novel ADAMs with potential roles in spermatogenesis and fertilization. Gene 234, 227–237.

Zhu, G.Z., Myles, D.G., Primakoff, P., 2001. Testase 1 (ADAM 24) a plasma membrane-anchored sperm protease implicated in sperm function during epididymal maturation or fertilization. J. Cell Sci. 114, 1787–1794.

Zhu, G.Z., Gupta, S., Myles, D.G., Primakoff, P., 2009. Testase 1 (ADAM 24) a sperm surface metalloprotease is required for normal fertility in mice. Mol. Reprod. Dev. 76, 1106–1114.