

The IgE gene in primates exhibits extraordinary evolutionary diversity

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Received: 23 September 2011 / Accepted: 27 October 2011
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Abstract Membrane-bound IgE (mIgE) on B lymphocytes is essential for IgE production. Earlier studies showed that the ϵ chain of mIgE (m ϵ) on human B cells has a “long” isoform, with an extra “C ϵ mX” domain of 52 amino acid (aa) residues between the CH4 domain and the membrane-anchor segment, as compared to the conventional “short” isoform. Because C ϵ mX provides an antigenic site for targeting IgE-expressing B cells to down-regulate IgE production in patients with allergy, analysis of C ϵ mX in various animals is of great interest. Hence, we analyzed the ϵ Ig gene, in particular, its membrane exon regions encoding the membrane anchor peptide segment and C ϵ mX domain, of 26 species of the order Primates and 12 species of seven non-Primate orders using data obtained experimentally or retrieved from GenBank. Our analyses reveal the unexpected finding that the genes of three extant tarsier

species do not contain the membrane exons for mIgE. Another striking finding is that early evolved Strepsirhini primates such as lemurs and lorises do not have gene segments for the long isoform, whereas New World monkeys such as marmosets and squirrel monkeys allow the transcription of only the long isoform. In Old World monkeys and apes, including humans, the ϵ gene allows the transcription of both isoforms. This work thus reveals the dramatic differences in the gene segment encoding the m ϵ C terminal region among the four major primate lineages: the Strepsirhini primates, the tarsiers, New World monkeys, and Old World monkeys and apes/humans.

Keywords Membrane-bound IgE · ϵ Immunoglobulin gene · Membrane exons · Primates · Tarsiers · Diversity

Electronic supplementary material The online version of this article (doi:10.1007/s00251-011-0586-9) contains supplementary material, which is available to authorized users.

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Introduction

The membrane-anchor peptide segment, encoded by the “membrane exons,” is critical for the functions of membrane-bound immunoglobulins (Reth et al. 1995). This C-terminal segment of mIg, which encompasses extracellular, transmembrane, and cytoplasmic segments, plays various roles, including signal transduction from the mIg/B cell receptor complex (Reth et al. 1995; Batista et al. 1996). Disruptive targeting of the membrane exons of the μ chain of IgM, the γ chain of IgG, or the ϵ chain in mice causes deficiency of particular Ig classes (Kitamura et al. 1991; Achatz et al. 1997; Kaisho et al. 1997). Patients with mutations that hamper mIg expression suffer from general or selective antibody deficiency (Yel et al. 1996; Tashita et al. 1998).

IgE probably plays a role in parasite immunity (Capron and Dessaint 1992; Pinot de Moira et al. 2010), but in people living in modern environment and lifestyle, it is the central mediator of type-I hypersensitivity reactions, responsible for allergic asthma, allergic rhinitis, food allergy, and many other allergic diseases (Stone et al. 2010; Broide et al. 2011). The generally positive results of the various clinical studies of anti-IgE antibodies, including omalizumab and TNX-901 (Leung et al. 2003; Casale et al. 2006; Kaplan et al. 2008; Busse et al. 2011; Pace et al. 2011), have firmly established that IgE is an effective molecular target for therapeutic intervention of IgE-mediated allergic diseases (Holgate et al. 2005; Chang et al. 2007). These results have encouraged other approaches that aim at reducing IgE production.

In the RNA transcript of the ϵ gene of mouse and rat, the CH4 exon is spliced to the M1 exon, and the M1 exon is spliced to the M2 exon, in the process of generating m ϵ messenger RNA (mRNA) (Anand et al. 1997; Aveskogh and Hellman 1995; Hellman 1993). In human IgE-expressing B cells, the CH4 exon in the ϵ transcript is alternatively spliced to a site 156 bp upstream of its conventional M1 exon splicing acceptor site. The resultant m ϵ contains an extra domain of 52 aa residues, referred to as “C ϵ mX,” located between the CH4 and the membrane-anchor peptide (Fig. 1) (Peng et al. 1992). The existence of the C ϵ mX segment,

which is also referred to as “extracellular membrane proximal domain” in human m ϵ was also studied by other investigators (Batista et al. 1995; Zhang et al. 1992). The gene segment encoding C ϵ mX is referred to as “me.p” (Peng et al. 1992). The discovery of C ϵ mX, whose amino acid and nucleotide sequences are unique in protein and DNA databases, is of great interest because C ϵ mX provides an antigenic site for potential immunological targeting of IgE-expressing B cells to down-regulate IgE production in patients with allergy (Chang et al. 2007; Chen et al. 2010). The analysis of C ϵ mX in various animals thus provides information about potential suitable animal models for studies of anti-C ϵ mX agents. Herein, we report the unexpected findings concerning the extraordinary divergence among primate lineages in the membrane exon regions of the ϵ gene.

Materials and methods

PCR and DNA sequencing of primate IGHE genes

Whole-genome shotgun sequences in the GenBank were searched by running the NCBI BLAST program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) against the human Ig heavy ϵ chain (IGHE) gene. Gene fragments in harvested sequences, which are identical or highly homologous to human ones, were used to design primers for amplifying IGHE genes of non-human primates (see Supplementary Table S1). PCR was performed using GoTaq® Green Master Mix (Promega Corporation) with 50–100 ng genomic DNA (gDNA) or whole-genome amplification (WGA) templates and 0.2 μ M of each primer in a 25- μ l reaction mixture. Primer synthesis and DNA sequencing of PCR products were performed by Purigo Biotech Company and Mission Biotech Company, located in Taipei, under our instruction. Sources of DNA materials and the primers designed for amplifying the ϵ gene segments are listed in Supplementary Table S2, with GenBank accession numbers in brackets.

Analyzing IGHE loci by nucleotide BLAST

To identify discrete structural elements and to determine the homology of exons and other gene segments of IGHE loci, we analyzed their ϵ gene sequences using the BLAST program (<http://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>). The programs and analytical parameters selected for optimizing the BLAST results are listed in Supplementary Table S3.

Isolating ϵ gene-harboring clones in tarsier λ phage genomic libraries by plaque hybridization screening

λ Phage genomic libraries of a Philippine tarsier (*Carlito syrichta*) and a western tarsier (*Cephalopachus bancanus*)

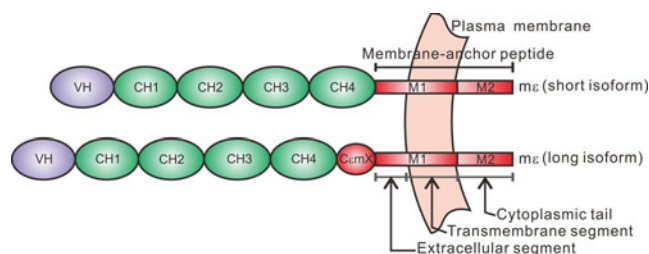


Fig. 1 Schematic representations of the structural domains and segments of the short and long isoforms of m ϵ . The long m ϵ isoform contains, as compared to the conventional short m ϵ isoform, a “C ϵ mX” domain, located between the CH4 domain and membrane-anchor peptide. The C ϵ mX domain is encoded by the gene segment, me.p shown in Fig. 3

were screened to identify IGHE gene-containing clones. For both libraries, digoxigenin (DIG)-labeled hybridizing probes were synthesized by PCR with 25 ng western tarsier genomic DNA (gDNA), the primer pair of universe Ce1 f02b and universe Ce4 r02c, and DIG DNA Labeling Mix (Roche Applied Science, Mannheim, Germany). Briefly, plaques were transferred onto positive-charged nylon membranes (Roche Applied Science, Mannheim, Germany), and the DNA in the lysed phage plaques was covalently cross-linked to the membrane by UV exposure. The membranes were hybridized with DIG-labeled probes at 53°C overnight. After both low and high stringency washes, identification of IGHE-specific plaques was carried out using peroxidase-labeled IgG.Fab anti-DIG (Roche Applied Science, Mannheim, Germany) with an electrochemiluminescence detection system following the manufacturer's protocol. Single-phage clones were purified with three to four rounds of screening employing the same procedure. The phage clones were cultured and their inserted gDNA extracted for cloning and sequencing.

For those studies involving using animal tissues, the authors obtained approval from regulatory committees for animal care and use of laboratory animals of their respective institutions.

Results

We analyzed the DNA sequences of the ϵ genes from 26 extant species of 16 families of the order Primates and 12 extant species of 11 families of seven other orders of the mammal class. The availability of DNA sequences for the major ϵ genomic segments for most of those species is summarized in Table 1. The phylogeny and molecular time scale of the four lineages of primate species studied, according to Perelman et al. (2011), are shown in Fig. 2. For each of the Hominidae, Cercopithecidae, and Tarsiidae families, three species were examined. The ϵ gene nucleotide sequences spanning from CH1 to M1, M2, and the poly (A) signal site were retrieved from existing databases for 21 species and determined experimentally using gDNA libraries or preparations derived by WGA for 17 species. For many of these species, samples from one to six individuals were employed. For all 34 species in the 27 families in Table 1, with the exception of three species, one from each of the Daubentoniidae, Cheirogaleidae, and Indriidae families, complete sequences from CH1 to M2 were available for analysis. Sequence entries and genetic resources of the species studied are listed in Supplementary Tables S1 and S2.

Strepsirhini primates and non-primate mammals do not have a gene segment for the long $m\epsilon$ isoform

The composite domains of the IGHE loci of primate and non-primate species studied were aligned using the BLASTN

program (<http://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>). In Fig. 3, segments with a background color exhibit detectable homology with corresponding regions in human ϵ gene, whereas those without a background color share no significant homology with any region in the human ϵ gene. The *me.p* reading frame is absent in *Mus musculus* (mouse), *Canis lupus familiaris* (dog), and *Equus caballus* (horse), and all other non-primate mammals examined. It is also absent in early evolved primates such as *Lepilemur ruficaudatus* (lemur) and *Otolemur crassicaudatus* (galago) belonging to the suborder Strepsirhini, which appeared ~87 million years ago (mya) (Perelman et al. 2011).

Neither the 109–158 bp gene segments immediately upstream of M1 in mice, dogs, and horses nor the 151–158 bp gene segment immediately upstream of M1 in lemurs and galagos is a reading frame. This gene segment has not evolved a splicing acceptor site, a branch point, and a polypyrimidine tract, required for the segment to be spliced during the formation of mature mRNA species. Nonetheless, the segment bears various degrees of sequence homology with *me.p* in infraorder Simiiformes. BLAST analyses of the *me.p* nucleotide sequence (Fig. 4a) yielded no homologous sequences in existing DNA databases, which suggests that *me.p* evolved from the intronic segment upstream of M1 and was not transposed from elsewhere.

New World monkeys, Old World monkeys, and apes possess *me.p*

The *me.p* reading frame, accompanied by an AG splicing acceptor site at its 5'-end and a branch point, located at 27–28 bp upstream of the acceptor site, is present in species of infraorder Simiiformes, which appeared about 40 mya (Perelman et al. 2011). These include New World monkeys (e.g., *Callithrix jacchus*, marmoset), Old World monkeys (e.g., *Macaca mulatta*, rhesus macaque), and apes/humans (Fig. 3). The evolution of *me.p* in the various species in the infraorder Simiiformes appears to provide a mechanism for these species to undergo an alternative splicing of the ϵ transcript and generate an additional isoform of the $m\epsilon$ chain (Fig. 1). Note that the splicing acceptor site at the 5'-end of M1 and both the branch point and the polypyrimidine tract inside the *me.p* segment in species of parvorder Catarrhini, including superfamily Cercopithecoidea (Old World monkeys) and superfamily Hominoidea (apes and humans), are preserved (Fig. 4b).

New World monkeys may not have the short $m\epsilon$ isoform

The evolution of *me.p* and the M1 gene segments in the parvorder Platyrrhini (New World monkeys) is remarkable. Analysis of the ϵ gene of all species in five families of parvorder Platyrrhini indicates that the corresponding

Table 1 Species and ε gene segments analyzed in this paper

Orders	Families	Binominal names	Segments with complete sequence									Poly (A)	I _α
			CH regions				intron	mε exons					
			1	2	3	4		me.p	me.1	me.2			
Primates	Daubentoniidae	<i>Daubentonia madagascariensis</i> (2)	p	●	●	●	p	D	●	●	n/d	n/d	
	Lemuridae	<i>Eulemur fulvus</i> (2)	●	●	●	●	●	D	●	●	n/d	n/d	
	Indriidae	<i>Propithecus verreauxi</i>	p	●	●	●	●	D	p	n/d	n/d	n/d	
	Cheirogaleidae	<i>Microcebus murinus</i> ^a	●	●	●	●	p	D	●	●	●	n/d	
	Lepilemuridae	<i>Lepilemur ruficaudatus</i>	●	●	●	●	●	D	●	●	●	n/d	
	Lorisidae	<i>Nycticebus coucang</i> (2)	●	●	●	●	●	D	●	●	●	n/d	
	Galagidae	<i>Otolemur crassicaudatus</i>	●	●	●	●	●	D	●	●	●	n/d	
	Tarsiidae	<i>Cephalopachus bancanus</i>	●	●	●	●	●	D	D	D	●	●	
		<i>Carlito syrichta</i>	●	●	●	●	●	D	D	D	●	●	
		<i>Tarsius dentatus</i>	●	●	●	●	●	D	D	D	●	●	
	Cebidae	<i>Saimiri sciureus</i> (6)	●	●	●	●	●	●	●	●	n/d	n/d	
	Aotidae	<i>Aotus azarae</i>	●	●	●	●	●	●	●	●	n/d	n/d	
	Callitrichidae	<i>Callithrix jacchus</i>	●	●	●	●	●	●	●	●	●	●	
	Atelidae	<i>Ateles geoffroyi</i>	●	●	●	●	●	●	●	●	n/d	n/d	
	Pitheciidae	<i>Pithecia pithecia</i>	●	●	●	●	●	●	●	●	n/d	n/d	
	Cercopithecidae	<i>Colobus guereza</i> (2)	●	●	●	●	●	●	●	●	n/d	n/d	
		<i>Macaca mulatta</i> ^a	●	●	●	●	●	●	●	●	●	●	
		<i>Erythrocebus patas</i> (6)	●	●	●	●	●	●	●	●	n/d	n/d	
		Hylobatidae	<i>Hylobates lar</i> (6)	●	●	●	●	●	●	●	●	n/d	n/d
		Hominidae	<i>Pongo abelii</i> ^a	●	●	●	●	●	●	●	●	●	●
	<i>Gorilla gorilla (gorilla)</i> ^a		●	●	●	●	●	●	●	●	●	●	
	<i>Homo sapiens</i> ^a		●	●	●	●	●	●	●	●	●	●	
Scandentia	Tupaiaidae		<i>Tupaia belangeri</i> ^a	●	●	●	●	●	●	●	n/d	n/d	
Rodentia	Caviidae	<i>Cavia porcellus</i> ^a	●	●	●	●	●	D	●	●	●	●	
	Cricetidae	<i>Cricetulus griseus</i> ^a	●	●	●	●	●	D	●	●	●	●	
	Muridae	<i>Mus musculus</i> ^a	●	●	●	●	●	D	●	●	●	●	
<i>Rattus norvegicus</i> ^a		●	●	●	●	●	D	●	●	●	●		
Lagomorpha	Leporidae	<i>Oryctolagus cuniculus</i> ^a	●	●	●	●	●	D	●	●	●	●	
Carnivora	Canidae	<i>Canis lupus (familiaris)</i> ^a	●	●	●	●	●	D	●	●	●	●	
	Mustelidae	<i>Mustela putorius (furo)</i> ^a	●	●	●	●	●	D	●	●	●	●	
Perissodactyla	Equidae	<i>Equus caballus</i> ^a	●	●	●	●	●	D	●	●	●	●	
Artiodactyla	Bovidae	<i>Bos taurus</i> ^a	●	●	●	●	●	D	●	●	●	●	
	Camelidae	<i>Lama pacos</i> ^a	●	●	●	●	●	D	●	●	●	●	
Monotremata	Ornithorhynchidae	<i>Ornithorhynchus anatinus</i> ^a	●	●	●	●	●	D	●	●	●	●	

Black dots denote that the sequences are complete. The 17 species listed in this table and additional four primate species not listed in this table (as discussed in the text) represents all of the species whose ε gene sequence is available in the database as of October 25, 2011. The sequences determined in this study, as summarized in Table S2, were deposited in GenBank. The numbers in the parentheses after the binominal names indicate the numbers of individuals whose DNA samples were prepared for analysis.

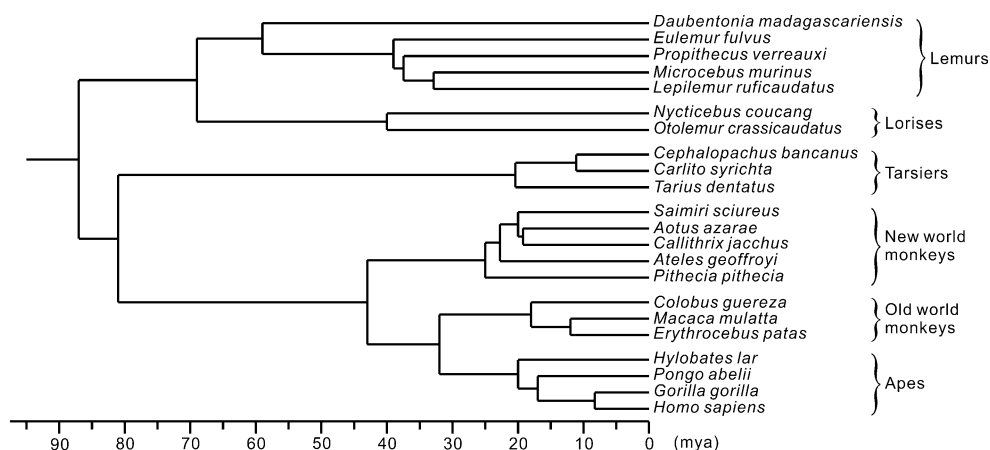
p partially sequenced, n/d not determined, D designates that the segment is devoid in the genome

^a Sequences were taken from GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>)

splicing acceptor site, AG, of M1 has mutated to CG or TG (Fig. 4b). A previous study showed that out of 22,815 mammalian splice junctions, 98.71% were canonical GT-AG, 0.56% were non-canonical GC-AG, and 0.73% were other

various pairs, each occurring <0.05% (Bursat et al. 2001). Our analysis revealed that in all other mammalian species, including platypus, horse, dog, mouse, lemur, loris, macaque, colobus monkey, gibbon, and human, the CH4-M1 splice

Fig. 2 The phylogeny and molecular time scales of the evolution of primates studied in this paper



junction is GT-AG. Thus, it is very likely that CH4-M1 splicing does not occur in the ϵ gene, and hence, the short isoform of $m\epsilon$ is not generated in the New World monkeys.

Old World monkeys express predominantly long $m\epsilon$ isoform

Although the ϵ gene in humans (parvorder Catarrhini) retains the canonical splicing acceptor site AG at M1, it expresses its

$m\epsilon$ product predominantly in the long isoform as revealed in the study with a human IgE-expressing B cell lines (Batista et al. 1996; Peng et al. 1992). Whether $m\epsilon$ chain is also expressed predominantly in other Old World monkeys, such as cynomolgus monkeys and rhesus macaques, remains to be determined. If so, the presence or absence of a canonical splicing acceptor site at M1 does not seem to make a substantial difference in $m\epsilon$ chain production. The data also

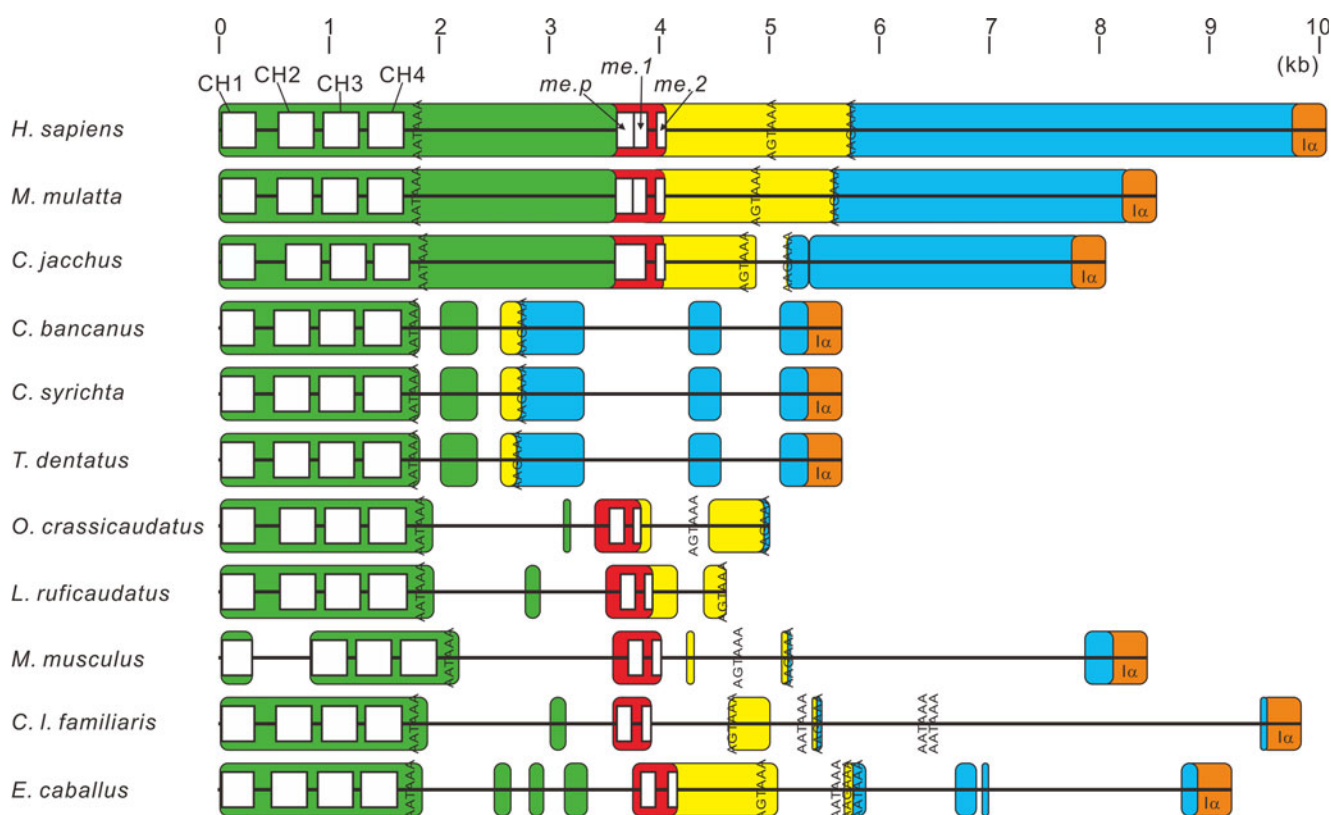


Fig. 3 Alignment of exons and homologous segments of the ϵ gene among the species studied in this paper. Putative poly (a) signal sites of the secretory and $m\epsilon$ are marked. The exons are designated as white boxes and introns as black lines. The gene segment spanning from the CH1 exon to the intron upstream of $me.p$ -M1 is in green, that

harboring $me.p$ -M1 and M2 in red, that harboring the 3'-end UTR inclusive of the AAGAAA poly (a) signal site in yellow, that harboring the intron downstream of the poly (a) site of the ϵ gene and upstream of the intervening promoter region of IgH α chain (I_α) (Nilsson et al. 1991) in cyan, and that corresponding to I_α in orange

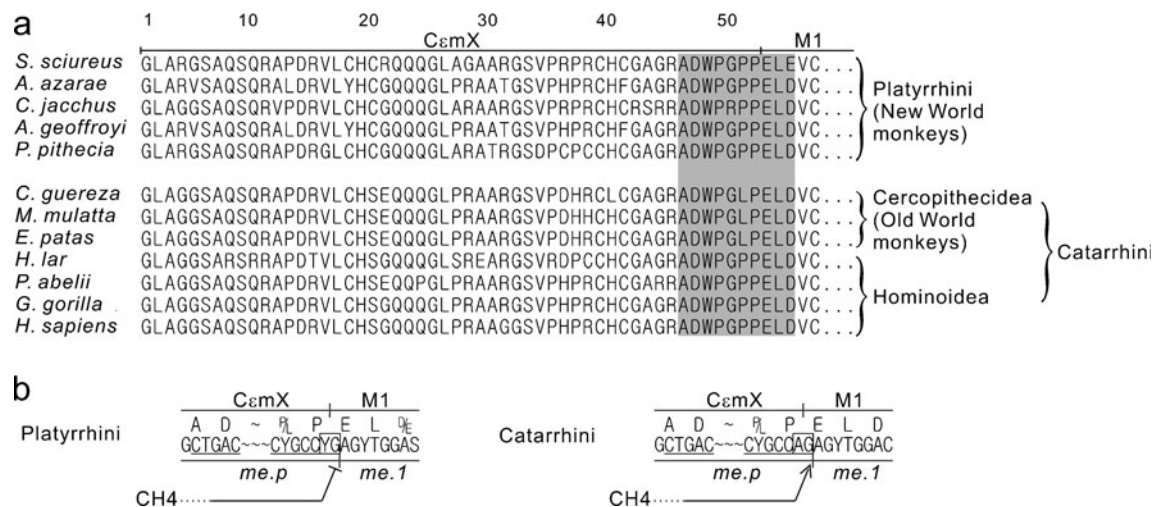


Fig. 4 **a** Amino acid sequence alignments of CεmX and an N-terminal segment of M1 of representative anthropoid species. The shaded segment represents the junction of CεmX and M1. **b** Amino acid and nucleotide sequence alignments at the junction of CεmX/me.p and M1/me.1, as

shaded in **a**. “Y” denotes pyrimidine nucleotides T or C, and “S” denotes C or G. The canonical and non-canonical splicing acceptor sites, AG and YG, respectively, are boxed. The consensus branch point, CTGAC, and polypyrimidine tract, CYGCC, are underlined

suggest that as the parvorders Platyrrhini and Catarrhini split from their common ancestors, the Platyrrhini line lost the canonical splicing acceptor site AG at M1. They do not suggest that their common ancestors evolved the *me.p* reading frame, and lost the canonical splicing acceptor site AG at M1 and the Catarrhini line regained the AG site at M1 later.

Tarsiers do not seem to have membrane exons of the ϵ Ig gene

The most striking findings concerning the evolution of the ϵ gene were found in tarsiers. Three genera of tarsiers are extant (Shekelle et al. 2010). One inhabits the southern Philippine islands and two are found in Indonesia. Because of the extraordinary findings of the ϵ gene of the tarsier in our initial study on one species, we extended our analysis to all three tarsier genera, including *C. syrichta* (Philippine tarsier), *C. bancanus* (Western tarsier from Sumatra and Borneo, Indonesia), and *Tarsius dentatus* (Eastern tarsier from Sulawesi, Indonesia). While the infraorder Tarsiiformes split from other anthropoid primate lineages about ~81 mya (Perelman et al. 2011), all three extant tarsier species analyzed in this paper diverged rather recently. The Eastern tarsiers (*T. dentatus*) and the Philippine-Western tarsiers (*C. syrichta* and *C. bancanus*) split about ~20 mya, while the latter two split ~10 mya (Merker et al. 2009; Perelman et al. 2011). The tarsier samples employed were a WGA preparation and a gDNA library for *C. syrichta*, a gDNA library, and a gDNA preparation for *C. bancanus* (Kawamura and Kubotera 2004), and a WGA preparation for *T. dentatus* (Merker et al. 2007). These tarsier DNA samples were prepared by four laboratories in three countries. Multiple

clones of the ϵ gene from each of those samples were isolated and their DNA sequences determined. Figure 5a shows the restriction enzyme maps for the three tarsier species. The sequence data indicate that CH1, CH2, CH3, and CH4 exons for all three tarsier species are intact reading frames with canonical splice donor and acceptor sites, 3'-end UTR, and AATAAA poly (A) signal site. The divergence of DNA sequences in the CH1–CH4 genomic regions among the three species (Fig. 5b) is consistent with the results of earlier phylogenetic analyses (Merker et al. 2009; Perelman et al. 2011).

The ϵ gene of the tarsiers is 3–4 kb shorter than that of other primate species and species of the orders Rodentia, Carnivora, and Perissodactyla analyzed herein (Fig. 3). The region harboring *me.p*, M1, and M2 exons and the intervening introns is entirely absent in the tarsier ϵ gene. The 3'-end UTR inclusive of the AAGAAA poly (A) signal site is about 1.6 kb in the human ϵ gene, but only 100–150 bp in tarsiers (Fig. 3). Another conspicuous alteration is that the canonical splicing donor site GT at the end of the CH4, found in the ϵ gene of all mammalian species, has mutated to GC in the tarsier ϵ gene (Fig. 5a). These findings indicate that deletion of a massive DNA segment occurred in the IGHE locus either when the infraorder Tarsiiformes emerged around 81 mya or sometime before the appearance of the three extant tarsier genera about 20 mya.

Discussion

Other than the 22 primate species discussed in Table 1, the gene segments encompassing the *me.p*, *me.1*, and *me.2* can

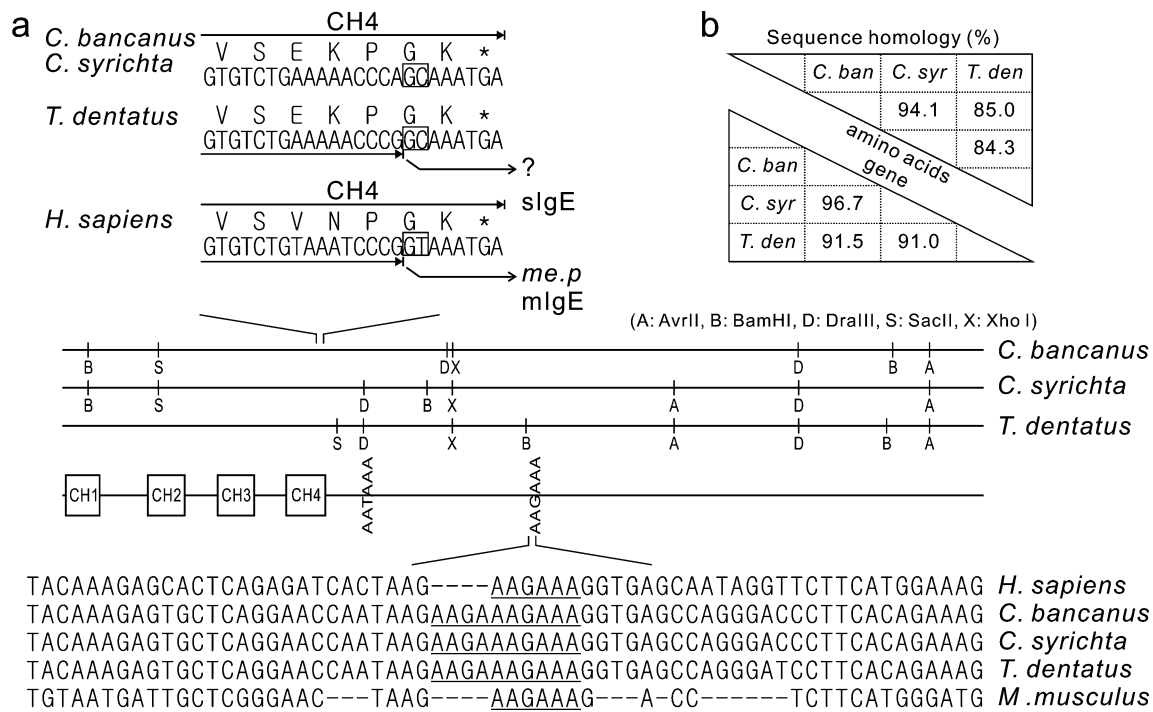


Fig. 5 a Restriction enzyme maps of the ϵ genomic segment of the three tarsier species studied in this paper and the loss of canonical splicing donor site at CH4. The splicing donor site, GT, at the 3' end of CH4 exon, found in all other primate species, has mutated to GC in the tarsiers. Remnant segments surrounding the external polyadenylation

signal site, AAGAAA (underlined), are also shown. **b** Degrees of homology of DNA sequence from CH1 exon to the first polyadenylation signal site, AATAAA and of amino acid sequence for the CH1–CH4 region among the three tarsier species

be found in the DNA databases for four other primate species, namely, chimpanzees (*Pan troglodytes*), northern white-cheeked gibbons (*Nomascus leucogenys*), crab-eating monkeys (*Macaca fascicularis*), and Hamadryas baboons (*Papio hamadryas*), all belonging to the Old World monkey lineage. The 156-bp coding sequence for C ϵ mX, the

splicing acceptor sites at the 5'-end of me.p and me.l, and the presence of the branch points are the same as described for rhesus macaque (*M. mulatta*) in Table 1 and Figs. 3 and 4. It should be noted that the ϵ chain membrane exon-containing shotgun contigs of chimpanzees and northern white-cheeked gibbons identified in our BLAST search do

Table 2 Occurrence of me isoforms in species analyzed in this study

Species	Order/Infraorder/Parvorder	me isoform
Non-primate mammals	Order	
Platypus	Monotremata	Short
Bovine, Alpaca	Artiodactyla	
Horse	Perissodactyla	
Dog, ferret	Carnivora	
Rabbit	Lagomorpha	
Rat, mouse, guinea pig, hamster	Rodentia	
Treeshrew	Scandentia	
Strepsirrhine primates	Infraorder/Parvorder	
Lemurs	Chiromyiformes/Lemuriformes/	Short
Lorises	Lorisiformes/	
Haplorrhine primates	Infraorder/Parvorder	
Tarsiers	Tarsiiformes/	None
New World monkeys	Simiiformes/Platyrrhini	Long
Old World monkeys, apes	Simiiformes/Catarrhini	Long>>short

^aThe species in the lineages of New World monkeys and Old World monkeys are listed in Fig. 2

not contain the exons for CH1–CH2–CH3–CH4 domains (http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome). It is possible that the identified membrane exons may be part of a pseudogene of ϵ chain in which the gene segment containing CH1–CH2–CH3–CH4 had been deleted (Kawamura and Ueda 1992).

The isoforms of the $m\epsilon$ Ig among the non-primate mammals and the major lineages of primates, namely, Strepsirhini primates, tarsiers, New World monkeys, and Old World monkeys and apes, based on the genomic analyses performed herein are summarized in Table 2. The finding that the New World monkeys can only produce the long isoform of $m\epsilon$ and not the short isoform should be verified by examining blood and lymphoid tissues from these animals. Our previous studies (Peng et al. 1992) showed that in human B cell lines expressing mIgE, the amount of mRNA for the long isoform of $m\epsilon$ is much more abundant than that for the short $m\epsilon$ isoform in Northern blotting analysis. They also showed that in human peripheral blood B lymphocytes, the mRNA for the long isoform of $m\epsilon$ is also predominant using real-time PCR. At present, the function of $C\epsilon mX$ is largely unknown; however, it is probably related to transduction of incoming signals from the mIgE/B cell receptor complex. The $C\epsilon mX$ sequences (Fig. 4a) reveal that the domain is positively charged in contrast to the adjacent extracellular segment of the membrane-anchor peptide, which is negatively charged (Major et al. 1996).

Because the long isoform is the predominant form of $m\epsilon$, $C\epsilon mX$ provides an attractive antigenic site for the targeting of mIgE-expressing B lymphoblasts and memory cells. Indeed, monoclonal antibodies specific for human $C\epsilon mX$, which mediates apoptosis and antibody-dependent cellular cytotoxicity on mIgE-expressing B cells, have recently been reported by our team and another group (Brightbill et al. 2010; Chen et al. 2010).

Our studies have shown that rodents do not contain $C\epsilon mX$ in their mIgE and therefore cannot be used as an animal model to study anti- $C\epsilon mX$ agents. However, when the human $C\epsilon mX$ gene element is knocked in into the mouse or rat genome, the mIgE expressed in these transgenic animals contains human $C\epsilon mX$. Hence, these genetically modified rodents can serve as appropriate animal models to study certain aspects of the anti- $C\epsilon mX$ monoclonal antibodies (Brightbill et al. 2010). Similarly, cynomolgus monkeys and rhesus macaques, which are often used as models in other studies, may also be used as models for anti- $C\epsilon mX$ agents.

Whether tarsiers do or do not express the $m\epsilon$ chain and mIgE and whether they are deficient in IgE production should be investigated by examining tissue samples from these animals. The endangered tarsiers are present in very small numbers in limited regions in the Philippines and

Indonesia. Because of the strict regulation in sampling and exporting tissues of endangered species, we are exploring the possibility of establishing cooperative programs with researchers in the Philippines or Indonesia for further investigating the findings concerning the ϵ gene of tarsiers.

Some human subjects with apparent normal immune functions are found to have serum IgE below detectable levels of immunoassays. Since omalizumab was approved for allergic asthma in 2003, some patients have used this IgE-neutralizing antibody for more than 5 years. These facts would suggest that IgE is dispensable for people living in modern environment (Cruz et al. 2007; Cooper et al. 2008). However, as IgE evolves with the mammals over the past 250 million years (Vernersson et al. 2002, 2004), it most likely serves an essential immune function. Thus, for animals living in primitive environment, IgE should play a vital role in immune defense. Studies by various investigators have found that the inability of transgenic mouse lines to express the $m\epsilon$ chain and mIgE have profoundly reduced blood IgE in the resting state and upon parasitic challenge (Achatz et al. 2008). It is of great interest to understand how the tarsiers compensate for the enormous mutation of losing membrane exons in the ϵ gene and how the mutated tarsiers gained advantage over the “wild-type” tarsiers as the infraorder Tarsiiformes evolved. Further studies on the evolution of IgE in primates may provide clues for understanding the regulation and function of this minute and yet powerful antibody class.

Acknowledgments This work is supported by a grant, NSC99-2320-B-001-006-MY3, from the National Science Council, Taiwan. We thank Dr. George Perry of Arizona State University for the gift of DNA samples from two aye-ayes and Dr. Harry Wilson for editing the manuscript.

Conflict of interest The authors have no financial conflicts of interest.

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