

SHORT REPORT

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# Adaptive evolution of bat dipeptidyl peptidase 4 (dpp4): implications for the origin and emergence of Middle East respiratory syndrome coronavirus

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## Abstract

**Background:** The newly emerged Middle East respiratory syndrome coronavirus (MERS-CoV) that first appeared in Saudi Arabia during the summer of 2012 has to date (20th September 2013) caused 58 human deaths. MERS-CoV utilizes the dipeptidyl peptidase 4 (DPP4) host cell receptor, and analysis of the long-term interaction between virus and receptor provides key information on the evolutionary events that lead to the viral emergence.

**Findings:** We show that bat *DPP4* genes have been subject to significant adaptive evolution, suggestive of a long-term arms-race between bats and MERS related CoVs. In particular, we identify three positively selected residues in DPP4 that directly interact with the viral surface glycoprotein.

**Conclusions:** Our study suggests that the evolutionary lineage leading to MERS-CoV may have circulated in bats for a substantial time period.

**Keywords:** MERS-CoV, Bats, Arms-race, Adaptive evolution, Emergence

## Main text

Middle East respiratory syndrome coronavirus (MERS-CoV) [1], first described by the World Health Organization (WHO) on 23rd September 2012 [2,3], has to date (20th September 2013) caused 130 laboratory-confirmed human infections with 58 deaths ([http://www.who.int/csr/don/2013\\_09\\_20/en/index.html](http://www.who.int/csr/don/2013_09_20/en/index.html)). MERS-CoV belongs to lineage C of the genus *Betacoronavirus* in the family *Coronaviridae*, and is closely related to *Tylonycteris* bat coronavirus HKU4 (BtCoV-HKU4), *Pipistrellus* bat coronavirus HKU5 (Bt-HKU5) [4,5] and CoVs in *Nycteris* bats [6], suggestive of a bat-origin [6]. Unlike severe acute respiratory syndrome (SARS) CoV which uses the angiotensin-converting enzyme 2 (ACE2) receptor for cell entry [7], MERS-CoV employs the dipeptidyl peptidase 4 receptor (DPP4; also known as CD26), and recent work has demonstrated that expression of both human and bat DPP4 in non-susceptible cells enabled viral entry [8].

Cell-surface receptors such as DPP4 play a key role in facilitating viral invasion and tropism. As a consequence, the long-term co-evolutionary dynamics between hosts and viruses often leave evolutionary footprints in both receptor-encoding genes of hosts and the receptor-binding domains (RBDs) of viruses in the form of positively selected amino acid residues (i.e. adaptive evolution). For example, signatures of recurrent positive selection have been observed in *ACE2* genes in bats [9], supporting the past circulation of SARS related CoVs in bats. To better understand the origins of MERS-CoV, as well as their potentially long-term (compared to short-term which lacks virus-host interaction) evolutionary dynamics with bat hosts [5,10], we studied the molecular evolution of *DPP4* across the mammalian phylogeny.

We first analyzed the selection pressures acting on bat *DPP4* genes using the ratio of nonsynonymous ( $d_N$ ) to synonymous ( $d_S$ ) nucleotide substitutions per site (ratio  $d_N/d_S$ ), with  $d_N > d_S$  indicative of adaptive evolution. The complete *DPP4* mRNA sequence of the common pipistrelle

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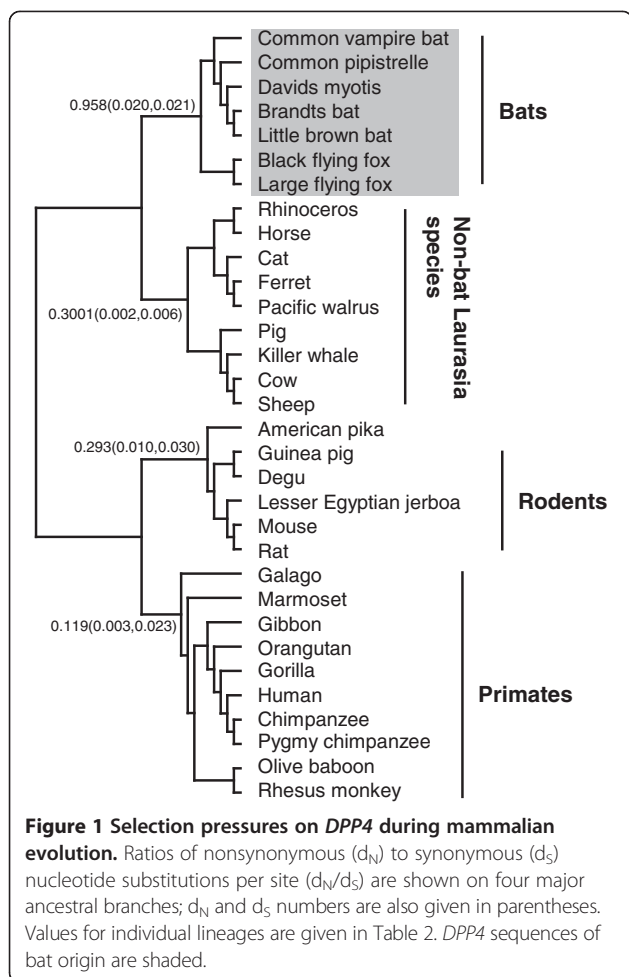
**Table 1 Sequences used in the evolutionary analysis of *DDP4***

Common name	Species name	Family	Accession no.
Sheep	<i>Ovis aries</i>	Bovidae	XM_004004660
Killer whale	<i>Orcinus orca</i>	Delphinidae	XM_004283621
Cow	<i>Bos taurus</i>	Bovidae	NM_174039
Pig	<i>Sus scrofa</i>	Suidae	NM_214257
Pacific walrus	<i>Odobenus rosmarus divergens</i>	Odobenidae	XM_004410199
Ferret	<i>Mustela putorius furo</i>	Mustelidae	DQ266376
Cat	<i>Felis catus</i>	Felidae	NM_001009838
Horse	<i>Equus caballus</i>	Equidae	XM_001493999
Rhinoceros	<i>Ceratotherium simum</i>	Rhinocerotidae	XM_004428264
Large flying fox	<i>Pteropus vampyrus</i>	Pteropodidae	ENSPVAG00000002634
Black flying fox	<i>Pteropus alecto</i>	Pteropodidae	KB031068
Common vampire bat	<i>Desmodus rotundus</i>	Phyllostomidae	GABZ01004546
Brandt's bat	<i>Myotis brandtii</i>	Vespertilionidae	KE161360
David's myotis	<i>Myotis davidii</i>	Vespertilionidae	KB109552
Little brown bat	<i>Myotis lucifugus</i>	Vespertilionidae	GL429772
Common pipistrelle	<i>Pipistrellus pipistrellus</i>	Vespertilionidae	KC249974
Guinea pig	<i>Cavia porcellus</i>	Caviidae	XM_003478564
Degu	<i>Octodon degus</i>	Octodontidae	XM_004629976
Lesser Egyptian jerboa	<i>Jaculus jaculus</i>	Dipodidae	XM_004651712
Mouse	<i>Mus musculus</i>	Muridae	BC022183
Rat	<i>Rattus norvegicus</i>	Muridae	NM_012789
Human	<i>Homo sapiens</i>	Hominidae	NM_001935
Chimpanzee	<i>Pan troglodytes</i>	Hominidae	GABE01002695
Pygmy chimpanzee	<i>Pan paniscus</i>	Hominidae	XM_003820939
Gorilla	<i>Gorilla gorilla gorilla</i>	Hominidae	XM_004032706
Orangutan	<i>Pongo abelii</i>	Hominidae	NM_001132869
Gibbon	<i>Nomascus leucogenys</i>	Hylobatidae	XM_003266171
Olive baboon	<i>Papio anubis</i>	Cercopithecidae	XM_003907539
Rhesus monkey	<i>Macaca mulatta</i>	Cercopithecidae	JU474559
Galago	<i>Otolemur garnettii</i>	Galagidae	XM_003795172
Marmoset	<i>Callithrix jacchus</i>	Cebidae	XM_002749392
American pika	<i>Ochotona princeps</i>	Ochotonidae	XM_004577330

(*Pipistrellus pipistrellus*) was downloaded from GenBank ([www.ncbi.nlm.nih.gov/genbank/](http://www.ncbi.nlm.nih.gov/genbank/)) along with that of the common vampire bat (*Desmodus rotundus*) from one transcriptome database (<http://www.ncbi.nlm.nih.gov/bioproject/178123>). These sequences were then used to mine and extract DPP4 mRNA transcripts from a further five bat genomes (Table 1) using tBLASTn and GeneWise [11]. The complete DPP4 genes of bats and non-bat reference genomes from a range of mammalian species (Table 1) were aligned using MUSCLE [12] guided by translated amino acid sequences ( $n = 32$ ; 727

amino acids). We then compared a series of models within a maximum likelihood framework [13], incorporating the published mammalian species tree [14-16]. This analysis (the Free Ratio model) revealed that the  $d_N/d_S$  value on the bat lineage (0.96) was four times greater than the mammalian average (Figure 1). The higher  $d_N/d_S$  ratios leading to bats (Table 2) during mammalian evolution accord with the growing body of data [5,6,17,18] that the newly emerged MERS-CoV ultimately has a bat-origin.

We next analysed the selection pressures at individual amino acid sites in bat DPP4. Using the Bayesian FUBAR



method [19] in HyPhy package [20], we identified six codons that were assigned  $d_N/d_S > 1$  with higher posterior probability (a strict cut-off of 95% in this analysis) (Table 3). To identify those sites under positive selection that may interact directly with MERS-CoV-like spike protein, bat *DPP4* (from the common pipistrelle) was modelled against the structure of the human *DPP4*/MERS-CoV spike complex [21] (Figure 2A). This revealed that three of the six positive selected residues (position 187, 288 and 392) were located at the interface between bat *DPP4* and MERS-CoV RBD (receptor binding domain) (Figure 2). These residues therefore provide direct evidence of a long-term co-evolutionary history between viruses and their hosts. We also observed several variable regions (Figure 2B) within the bat RBD, that may also have resulted from virally-induced selection pressure and which merit additional investigation in a larger data set.

Our analysis therefore suggests that the evolutionary lineage leading to current MERS-CoV co-evolved with bat hosts for an extended time period, eventually jumping species boundaries to infect humans and perhaps through an intermediate host. As such, the emergence of

**Table 2 Numbers of nonsynonymous ( $d_N$ ) and synonymous ( $d_S$ ) substitutions per site *DPP4* genes in different mammals**

Common name	$d_N$	$d_S$	$d_N/d_S$
Sheep	0.004	0.013	0.280
Killer whale	0.023	0.039	0.595
Cow	0.003	0.016	0.157
Pig	0.027	0.109	0.246
Pacific walrus	0.014	0.053	0.260
Ferret	0.015	0.064	0.235
Cat	0.021	0.081	0.258
Horse	0.016	0.055	0.290
Rhinoceros	0.017	0.044	0.385
Large flying fox	0.005	0.001	3.561
Black flying fox	0.004	0.008	0.487
Common vampire bat	0.042	0.125	0.500
Brandt's bat	0.006	0.012	0.463
David's myotis	0.010	0.028	0.380
Little brown bat	0.007	0.007	0.943
Common pipistrelle	0.031	0.066	0.470
Guinea pig	0.018	0.078	0.238
Degu	0.016	0.128	0.122
Lesser Egyptian jerboa	0.023	0.179	0.131
Mouse	0.019	0.093	0.206
Rat	0.027	0.110	0.248
Human	0.001	0.007	0.086
Chimpanzee	0.000	0.002	0.000
Pygmy chimpanzee	0.001	0.000	ND
Gorilla	0.003	0.004	0.863
Orangutan	0.002	0.000	ND
Gibbon	0.003	0.009	0.344
Olive baboon	0.000	0.005	0.000
Rhesus monkey	0.000	0.004	0.000
Galago	0.022	0.149	0.149
Marmoset	0.009	0.053	0.160
American pika	0.036	0.229	0.156

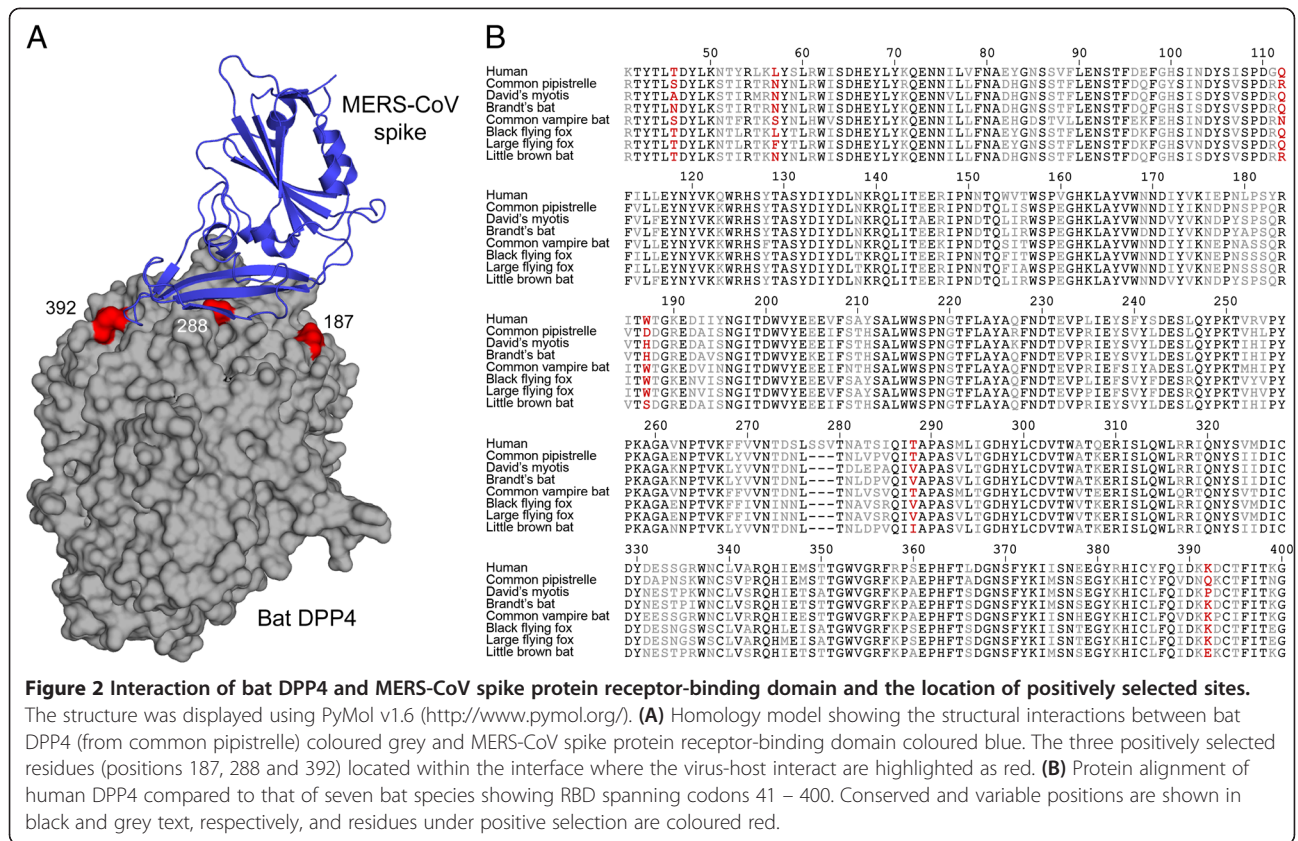
ND: Not determined because no synonymous substitutions are present.

**Table 3 Putatively positive selected *DPP4* codons in bats**

Codon position <sup>a</sup>	Posterior probability <sup>b</sup>	$d_N/d_S$
46	0.97	14.95
57	0.97	13.13
112	0.94	10.27
187	0.95	8.55
288	0.98	13.90
392	0.97	14.63

<sup>a</sup>Codon position corresponding to the human *DPP4* (NP\_001926) protein sequence.

<sup>b</sup>Posterior probability of residues assigned a  $d_N/d_S$  ratio greater than 1.



MERS-CoV may parallel that of the related SARS-CoV [22]. Although one bat species, *Tapozous erforatus*, in Saudi Arabia has been found to harbour a small *RdRp* (RNA-Dependent RNA Polymerase) fragment of MERS-CoV [17], a larger viral sampling of bats and other animals with close exposure to humans, including dromedary camels were serological evidence for MERS-CoV has been identified [23], are clearly needed to better understand the viral transmission route. Alternatively, it is possible that the adaptive evolution present on the bat DPP4 was due to viruses other than MERS-CoVs, and which will need to be better assessed when a larger number of viruses are available for analysis. Overall, our study provides evidence that a long-term evolutionary arms race likely occurred between MERS related CoVs and bats.

**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

JC and LFW designed the research. JC and JSE analysed the data. JC and ECH drafted the manuscript. All authors read and approved the final manuscript.

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