1 **Genomic insights into evolutionary journey of the porcine** 2 **endogenous retroviruses**

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15 **Abstract**

16 **Background:** Xenotransplantation may overcome significant shortage of human
 17 allotransplant. Porcine organs are considered favorable for xenotransplantation due to
 18 similar size and function to human organ. However, porcine endogenous retroviruses
 19 (PERVs) are potential infectious agents during xenotransplantation as they are able to
 20 infect and horizontally transfer among human cells. Furthermore, PERVs can be
 21 endogenized in pig genomes and are transmitted genetically in a Mendelian fashion. Here,
 22 we depict a complex evolutionary history of modern PERVs.

23 **Results:** We *in silico* mined 142 mammalian genomes and 14 pig genomes. This led to the
 24 documentation of 185 PERVs and a new viral cluster. Large-scale genomic alterations
 25 were found in most PERVs including many insertion-deletion events and which are
 26 suggestive of ancient origins, and pig genomes have been shaped by PERV-mediated
 27 genomic rearrangement during evolution. Notably, we found that lesser Egyptian jerboa
 28 and rock hyrax harbor ancestral PERV-related elements indicative of ancient cross-species
 29 transmission events from non-porcine species to pigs. A comprehensive analysis of these
 30 viral “fossils” suggested that recombination among non-porcine endogenous retroviruses
 31 led to the origination of PERVs.

32 **Conclusion:** For the first time, using large scale genomic mining, we decipher a complex
 33 evolutionary history for the PERVs. These new findings help us to understand the past of
 34 PERVs which pose the potential risk in clinical trials of xenotransplantation and provide
 35 novel insights into the origin and evolution of a human-infecting pathogen.

36

37 **Keywords:** porcine endogenous retrovirus, genomic rearrangement, cross-species
38 transmission, evolution, origin, recombination

39

40 **Background**

41 Xenotransplantation, the transplantation of tissues and organs from one species to another,
42 may alleviate shortages of human donor organs(1, 2). Porcine organs are suitable for
43 xenotransplantation due to their similar size and function to human organs, and the fact
44 that pigs can be bred in large numbers(3). However, porcine endogenous retroviruses
45 (PERVs) are able to infect and horizontally transfer among human cells, raising major
46 concerns about the safety of xenotransplantation(3, 4), particularly given that retroviruses
47 are often associated with severe infectious disease or oncogenic disease(3, 5-7).

48

49 PERVs are endogenous gammaretroviruses, and exist in the genomes of all pig strains(3,
50 8). The envelope (*env*) genes of three PERV classes (PERV-A, -B and -C) differ with
51 respect to the receptor-binding domain (RBD)(9). Although there is no evidence of PERV
52 transmission in patients receiving encapsulated pig islets(10-12), PERV-A and -B have
53 been observed to infect both human cells and pig cells while PERV-C infects only pig
54 cells(13). PERVs may also integrate into the human genome *in vitro*(14, 15). In pig cells,
55 PERV-C can recombine with the *env* of PERV-A to produce A/C recombinants, which can
56 infect human cells more efficiently(13). This increases the inherent risk in
57 xenotransplantation and xenogeneic cell therapies.

58

59 While several studies have examined the evolutionary relationships between PERVs and
60 other viruses, the origin and evolution of PERVs remains uncertain, particularly why they
61 are unable to infect humans(16, 17). At least two species that belong to the same order as
62 pigs, *Tayassu pecari* (of Eocene origin) and *Babryrousa babyrussa* (of Miocene origin)
63 lack PERVs(18). However, the common warthog (*Phacochoerus africanus*) carries
64 PERVs, suggesting that an ancestral porcine species carried PERVs(18). PERVs have two
65 different types of long terminal repeat (LTRs), one with a 39-bp repeat structure in the U3
66 region, and the other without this repeat structure(19, 20). The 39-bp repeats carried by
67 PERV-A and -B confer strong promoter activity and thus increase transcription(19, 20).
68 However, the 39-bp repeat structure is absent in some PERV-A and all PERV-C. These
69 PERVs thus have low transcriptional activity(19, 20). BLAST search analysis confirmed
70 that the R and U5 regions of the PERV LTRs are highly conserved in the pig and mouse
71 genomes (74–87% identity)(21). Indeed, the LTRs of PERV-A, -B and LTR-IS (a LTR
72 family found solely in the mouse genome) have similar structures(21). The conserved LTR
73 sequences found in pigs and mice might have originated from a common exogenous viral
74 element, but evolved independently(21). Using genome mining with all available
75 mammalian genomes, we clearly illustrate the evolutionary journey of the modern PERVs
76 and reveal that the genesis of PERVs is much more complex than previously thought.

77

78 **Results**

79 ***In silico* characterization of putative PERVs.**

80 Using previously reported PERV sequences as queries, we mined 14 pig genomes

(Additional files 2: Table S1) available in GenBank, and showed a detailed genome-wide distribution of full-length PERVs (i.e. containing two LTRs). We initially compiled a PERV data set that included 185 putative PERVs (containing at least one LTR) (Additional files 2: Table S2). A total of 84 classified (30 PERV-A, 39 PERV-B and 15 PERV-C), 18 unclassified full-length PERVs (i.e., lacking of env gene) were retrieved from pig genomes. We identified 2–10 full-length PERVs in each of 12 other pig breeds, including Meishan, Goettingen, and Large White. We removed 19 previously classified PERV sequences that were low quality fragments (> 200 “N” bases). Hence, the final data set comprised 65 high quality classified PERVs (27 PERV-A, 29 PERV-B, and 9 PERV-C). Their viral genomic structures are summarized in Figure 1.

Most of the PERVs exhibited large-scale genetic alterations induced by indels and stop codons (Fig. 1), suggestive of a relatively long evolutionary history. PERV LTRs were classified by the presence (LTR B) or absence (LTR A) of the 18 bp and 21 bp repeat structure reported previously(9, 19, 22). Three different type B LTRs in the PERV were identified, distinguished by the number of 18 bp and 21 bp repeat sequences: LTR B1 (two 18-bp and one 21-bp repeats), LTR B2 (three 18-bp and two 21-bp repeats), and LTR B3 (four 18 bp and three 21 bp repeats). Of the 65 high-quality PERVs we analyzed, we assigned 57, of which 32 (>55%) carried LTR A, 10 carried LTR B1, 13 carried LTR B2, and 2 carried LTR B3. LTR A was identified in PERV-A and -C, and LTR B1 was identified in PERV-A and -B. LTR B2 and LTR B3 were only identified in PERV-B. The remaining eight PERVs contained different types of 5'- and 3'- LTR which may reflect

103 recombination between PERVs with different LTR types (Fig. 1). For example, we
104 discovered one PERV (AEMK02000536.1) with LTR B2 at the 5' end and LTR A at the 3'
105 end (Fig. 1).

106

107 **Potential genomic rearrangement via PERVs.**

108 Retrovirus integration creates a short duplication called target site duplication (TSD)
109 flanking the LTR(23, 24). If chromosomal rearrangement through homologous
110 recombination between distant proviruses occurred, the flanked TSDs should be different,
111 as mentioned in a previous study of primate genomic rearrangement via ERVs (25). To
112 identify pig genomic rearrangement via PERVs, we firstly constructed a maximum
113 likelihood (ML) tree representing the 5'- and 3'- LTR sequences of full-length PERVs
114 (Additional files 1: Fig. S1). The phylogenetic tree was divided into three large clusters
115 (Additional files 1: Fig. S1), suggesting that three major integration events had occurred.
116 We collected PERVs with 5'- and 3'- LTR sequences did not clustered together in the
117 phylogenetic. Remarkably, 11 PERVs did not share the same TSD (4bp in length) (Table 1,
118 Additional files 2: Table S3), thus these PERVs could reflect porcine genomic
119 rearrangement during evolution.

120

121 **Table 1. PERVs with different target site duplications (TSDs).**

Name	Accession number	Divergent LTRs based on structure [*]	Divergent LTR based on tree	Flanking TSD ^{**}	
				5'	3'
AEMK02000536.1	AEMK02000536.1	yes	yes	AGCC	CTTT
CM000818.5_a	CM000818.5	no	yes	GTTC	CTTC

CM000826.5_c	CM000826.5	no	no	ACCA	AATC
CM000828.5_d	CM000828.5	no	yes	CCAC	CACC
KQ001967.1	KQ001967.1	no	yes	CCAC	CACC
LIDP01000017.1	LIDP01000017.1	no	yes	CCAC	CACC
LUXR01004647.1	LUXR01004647.1	yes	yes	GTTC	CTTC
LUXR01022139.1	LUXR01022139.1	yes	yes	CCAC	CACC
LUXX01045907.1	LUXX01045907.1	no	yes	CCAC	CACC
LUXX01080744.1	LUXX01080744.1	no	yes	GTTC	CTTC
LUXY01101100.1	LUXY01101100.1	no	yes	CCAC	CACC

122 *LTRs of PERVs comprise four types (LTR A, B1, B2, and B3). If two different types of

123 LTRs are flanking the PERV, the LTRs are considered "divergent".

124 **Only TSDs flanking the intact 5' and 3' LTRs sequences were analyzed

125

126 **Detection of PERV-related sequences in mammalian genomes.**

127 After screening 142 mammalian genomes (Additional files 2: Table S4) available on

128 GenBank using tBLASTn and choosing three major proteins (Gag, Pol and Env) of

129 PERVs as queries, a significant sequence (accession number: NW_004504334.1) in the

130 genome of lesser Egyptian jerboa (*Jaculus jaculus*) that exhibited strong sequence

131 similarity (for *gag* and *pol*: >75% nucleotide identity over 95% region; for *env*: >75%

132 nucleotide identity over 55% region) to PERVs. Using this PERV-related sequence as

133 query, three other possible PERV related sequences were identified in *J. jaculus* (accession

134 number: NW_004504375.1, NW_004504378.1, and NW_004504445.1) with >85%

135 nucleotide identity over 80% of the query sequence. The four PERV-related sequences

136 identified in *J. jaculus* were designated as eJRVs. These four significant hits located in

137 large scaffolds > 5 Mb in length (Additional files 2: Table S5), with several host genes

identified, indicated that the eJRV sequences were relatively reliable.

We were only able to identify one pair of eJRV LTRs. This full-length eJRV (containing 2 LTRs) (accession number: NW_004504334.1) is annotated in Additional files 1: Fig. S2.

The length of 3'-LTR of this eJRV is 674 bp while 5'-LTR is 932 bp with a 258 bp insertion. We aligned eJRV LTRs with PERV LTRs. The start of the U3 region and the end of the U5 region were distinct and not included in the alignment (Additional files 1: Fig. S3). The eJRV LTRs included a repeat structure (three 18 bp and two 21 bp repeat sequences) in the U3 region, identical to that of the PERV LTR B2. Furthermore, 3' LTR of eJRV had high identity with LTR B2 of PERV (~73%). Alignment analysis revealed a closer relationship between LTRs of the eJRV and LTR B2 of PERVs (Additional files 1: Fig. S3). Notably, the alignment of the conserved R region supported a close evolutionary relationship between the eJRV and PERVs (Fig. 2a). To highlight the similarity between PERVs and eJRVs we generated pairwise alignments of eJRV and PERV nucleotides using the full-length ERVs, and performed a sliding window analysis of these pairwise alignments (Fig. 2b)(26, 27). For comparison, we determined the similarity of HIV-1 provirus sequence to that of its closest relative (chimpanzee SIVcpz) (28, 29). Interestingly, *gag* and *pol* were more similar between eJRV and PERV-A, -B and -C than HIV-1 and SIVcpz (Fig. 2b). Therefore, our results indicate that eJRVs and PERVs are homologous. However, the RBD and the proline rich-region (PRR) of the surface subunit (SU) of *env* were dissimilar between eJRV and PERV-A, -B and -C, as also seen in HIV and SIVcpz. As RBD determined host range(30-33), this observation suggests that eJRVs and PERVs have distinct host ranges.

160

161 We used the RBD amino acid sequences from PERV-A, -B and -C as queries to screen for
162 homologous viral elements. The eight significant hits (>60% amino acid identity over 80%
163 region) were obtained in rock hyrax (*Procavia canpensis*) of *Procaviidae*, and all eight
164 hits flanking with genes located in large scaffolds >0.3 Mb in length (accession number:
165 KN678690.1, KN676491.1, KN678005.1, KN677924.1, KN676905.1, KN676182.1,
166 KN680906.1, and KN676638.1) (Additional files 2: Table S5). We examined the
167 sequences flanking the eight hits (especially *pol*), and found that ERVs including these hits
168 were endogenous gamma-retroviruses. These hits were therefore designated ePCRVs. We
169 aligned the RBDs of PERVs and ePCRVs, and found that ePCRVs were highly similar to
170 PERVs (Fig. 3). Pairwise comparisons revealed that ePCRV_1 and ePCRV_2 had a high
171 identity to PERV-B (63%) but a low identity with PERV-A, -C and PERV-IM (40–43%).
172 Therefore, the RBD of ePCRVs and PERV-B are homologous.

173

174 To characterize the relationship within eJJRVs, ePCRVs and PERVs, we inferred
175 phylogenetic trees of Gag, Pol and Env, first removing the variable RBD (Fig. 2c-e). Our
176 maximum likelihood (ML) phylogenetic tree revealed that eJJRVs and PERVs clustered
177 together with high bootstrap supports in three phylogenies (Fig. 2c-e), suggesting that they
178 shared the most recent common ancestry. However, the Gag of ePCRVs clustered with
179 eJJRVs and PERVs while Pol and Env (without RBD) of ePCRVs were distant related to
180 eJJRVs and PERVs which might indicate recombinant events among ancient retroviruses
181 (Fig. 2c-e).

182

183 Remarkably, a new lineage close to PERV-A and PERV-C was observed, named PERV-IM
184 (as designation of PERV-intermediate type), and presented in all 14 pig genomes (Fig. 2e,
185 Additional files 2: Table S2). The Env proteins of PERV-IMs showed relatively low
186 similarity to PERV-A, -B, and -C, and they were clearly distinct in the RBD region (Fig.
187 3).

188

189 Discussion

190 Using systematic large-scale genome mining, we analyzed the origin and evolution of the
191 modern PERVs. We found homologous LTRs of PERVs (~73% identity) in eight
192 *Muroidea* species (*Mus caroli*, *M. pahari*, *M. musculus*, *M. spretus*, *Apodemus speciosus*,
193 *A. sylvaticus*, *Rattus norvegicus*, and *Phodopus sungorus*). The coding genes (*gag*, *pol*,
194 and *env*) near these homologous LTRs were identified (Additional files 2: Table S6). Also,
195 ERVs in two *Muroidea* species (*M. musculus* and *R. norvegicus*) found in previous study
196 were also presented(34). However, phylogenetic analysis of the Gag (>220 amino acids),
197 Pol (>560 amino acids) and Env (>330 amino acids) sequences of these ERVs suggested
198 that they were distantly related to the modern PERVs (Additional files 1: Fig. S7). We
199 provided robust phylogenetic results that eJJRV shared the most recent ancestry with the
200 PERVs (Fig. 2), and ePCRv also contributed the origin of Gag (Additional files 1: Fig.
201 S7). Failure to detect any other eJJRV and PERV-related elements in the remaining rodent
202 genome screening suggests that the virus was not vertically transmitted, suggesting
203 ancient horizontal transmission occurred during evolution.

204

205 PERVs, eJRVs, and ePCRVs integrated into *Suidae*, *Dipodidae*, and *Procaviidae*,
 206 respectively. Miocene (23 - 5.33 MY) *Suidae* fossils have been found in East Africa,
 207 Europe and Asia (http://fossilworks.org/?a=taxonInfo&taxon_no=42381). Miocene
 208 *Dipodidae* fossils have been found in North Africa, Europe and Asia
 209 (http://fossilworks.org/?a=taxonInfo&taxon_no=41695); Pliocene (5.3 - 2.59 MY)
 210 *Dipodidae* fossils have been identified in East Africa, thus suggesting that the *Dipodidae*
 211 may have spread to East Africa during the Miocene. Miocene *Procaviidae* fossils have
 212 been found in South of Africa and East Africa
 213 (http://fossilworks.org/?a=taxonInfo&taxon_no=43293). According to the current fossil
 214 records, the only shared region for Miocene *Dipodidae*, *Procaviidae* and *Suidae* fossils is
 215 East Africa. It is likely that ancestral PERV might be derived from two ancient retroviruses
 216 carried by *Dipodidae* and *Procaviidae*, denoted as JRV and PCRV in the illustration (Fig.
 217 4), in East Africa during the Miocene time. The ancestral PERV then split into different
 218 classes (PERV-A, -B, -IM and PERV-C), in which the PERV-B diverged earlier than other
 219 classes of PERVs (Fig. 4).

220

221 We also discovered a new class of PERV named PERV-IM and they are presented in all
 222 pig genomes we scanned (Additional files 2: Table S1). It remains unknown whether
 223 PERV-IMs are able to infect human cells. However, it is possible that non-human trophic
 224 PERV was able to participate in recombination, leading to human trophic retroviruses(35,
 225 36). The identification of the new PERVs is crucial for future assessment of pathogen

226 transmission during xenotransplantation.

227

228 **Conclusions**

229 For the first time, we decipher a complex evolutionary history for the PERVs. The
230 ancestral PERV might be derived from two ancient retroviruses carried by non-porcine
231 species. We also suggest that pig genomes have been shaped by PERVs, as specifically
232 reflected by PERV-associated genomic rearrangement that have occurred during porcine
233 evolution. In a word, modern PERVs have a complex evolutionary history than previously
234 thought prior to their appearance in pigs.

235

236 **Materials and methods**

237 ***In silico* identification of PERV and PERV-related proviruses.**

238 To identify PERV-related elements in *Sus scrofa*, tBLASTn (37) was used and the amino
239 acid sequences of Gag, Pol and Env of 20 representative PERV proviruses (accession
240 numbers: HQ536016.1, HQ536015.1, HQ536013.1, KC116220.1, AY570980.1,
241 HQ540592.1, HQ536007.1, AX546209.1, AF435967.1, AY953542.1, HQ540591.1,
242 AY099323.1, AJ133817.1, EU523109.1, EF133960.1, AY056035.1, AY099324.1,
243 A66553.1, HQ536011.1, and HQ536009.1) were chosen as queries to screen the 14 pig
244 genomes available in GenBank that were released before November 2017. A 50% identity
245 over 50% of the match region was used to filter significant hits. It has been shown that
246 PERVs harbor two LTR structures, one with and one without a repeat structure in the U3
247 region(9, 19). Using two typical LTRs as queries we extended flanking sequences of

coding domains of PERVs to identify LTRs with BLASTn, and TSDs were used to define PERV boundaries. LTR lengths were defined as 100–1,000 bp. PERVs with at least one LTR and one coding gene were used in the evolutionary analysis.

To identify PERV-related proviruses in mammals, tBLASTn was used with the queries described above in 20 representative PERV proviruses to search the 142 mammal genomes available in GenBank as of November 2017. A 50% identity over 80% region was used to filter significant hits. LTRs were identified using LTR finder(38), LTRharvest (39) and BLASTn. LTR lengths were also defined as 100–1,000 bp.

Detection of potential genomic rearrangement via PERVs.

To search for proviruses involved in recombination and genomic rearrangement, we constructed a maximum likelihood (ML) tree of the 5'- and 3'- LTRs of full-length PERVs using PhyML 3.1(40) with GTR+I+ Γ nucleotide substitution model. LTRs less than 250 bp were not considered. Sequence alignment was performed with MAFFT 7.222(41).

Phylogenetic analyses.

To determine the evolutionary relationship among PERVs, eJRVs, ePCRVs and representative gammaretroviruses (Additional files 2: Table S7), phylogenetic trees were inferred using the amino acid sequences of full-length PERVs and PERVs with one LTR and at least one coding gene. All Gag, Pol and Env protein sequences (Dataset S1) were aligned in MAFFT 7.222 and confirmed manually in MEGA7(42). The phylogenetic

270 history of these gammaretroviruses was then determined using the maximum likelihood
271 (ML) method available in PhyML 3.1(40), incorporating 100 bootstrap replicates to assess
272 node robustness. The best-fit JTT+ Γ amino acid substitution model was selected for Gag,
273 Pol and JTT+ Γ +I for Env using the ProtTest 3.4.2(43).

274

275 **Additional Files**

276 **Additional files 1. Fig. S1.** Maximum likelihood (ML) tree of the 5' and 3' LTRs of all
277 full-length PERVs. **Fig. S2.** Detailed descriptions of eJRV genome. **Fig. S3.** The
278 alignment of LTRs of PERVs and eJRV. **Fig. S4.** The complete phylogenetic tree of Gag.
279 **Fig. S5.** The complete phylogenetic tree of Pol. **Fig. S6.** The complete phylogenetic tree of
280 Env. **Fig. S7.** The phylogenetic trees of Gag, Pol and Env of Muroidea ERVs, eJRVs,
281 ePCRVs, PERVs and representative retroviruses.

282 **Additional files 2. Table S1.** The information of pig, rodent, and rock hyrax genomes
283 used for data mining. **Table S2.** The information of full-length and near full-length
284 PERVs. **Table S3.** The recombination-related information of full-length PERVs shown in
285 Fig. S1. **Table S4.** The information of 142 mammals used for PERV-like sequences
286 mining. **Table S5.** The information of genes flanking the eJRVs and ePCRVs. **Table S6.**
287 The information of full-length and near full-length Muroidea ERVs. **Table S7.** The
288 information of representative retroviruses used for phylogenetic analysis.

289 **Additional files 3. Data set S1.** The alignments used to build the phylogenetic trees of
290 Gag, Pol and Env represented in Fig. S4, S5, and S6, respectively. **Data set S2.** The
291 alignments used to build the phylogenetic trees of Gag, Pol and Env represented in Fig.

292 S7.

293

294 **Declarations**

295 **Ethics approval and consent to participate.** Not applicable.

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300 is described in the main text (and additional files)

301 **Author contributions.** J.C. conceived and designed the research. Y.C. and M.C.
302 conducted the analyses. X.D. participated in the analysis of full-length PERVs. J.C.
303 supervised the whole project. All authors participated in the project discussion and
304 manuscript preparation.

305 **Competing interests.** The authors declare that they have no competing interests

306

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426 **Fig. 1 PERV proviruses in porcine genomes.** (A) Genomic structure of PERVs,
427 including *gag*, *pol* and *env* genes and LTRs. Proviruses of PERV-A and PERV-A like (B),
428 PERV-B and PERV-B like (C) and PERV-C and PERV-C like (D) groups depicted based
429 on reference PERV-A (accession number: AF435967.1), PERV-B (accession number:
430 EU523109.1) and PERV-C (accession number: HQ536015.1). LTRs of PERVs were
431 classified by the presence (type B) or absence (type A) of the 18 bp and 21 bp repeat
432 structure. Type B LTRs were divided into three subtypes (LTR B1, LTR B2 and LTR B3).
433 LTR A, B1, B2 and LTR B3 are presented in white, light gray, dark gray and black,
434 respectively. Insertions and deletions (< 50 bp) are depicted with blue and red flags,
435 respectively. Larger insertions (>50 bp) are labeled with green arrow. Large deletions (>50
436 bp) are shown without lines. Stop codons are showed with a black flag. (Du, Duroc pig;
437 Wu, Wuzhishan pig; El, Ellegaard pig; Ti, Tibetan pig; Go, Goettingen pig; Me, Meishan
438 pig; Ro, Rongchang pig; Ha, Hampshire pig; Lan, Landrace pig; Pi, Pietrain pig; Ba,
439 Bamei pig; Be, Bekshire pig; Lar, LargeWhite pig; Ji, Jinhua pig).

440

441 **Fig. 2. Comparison of PERVs, eJRVs and ePCRVs.** (A) Alignment of R region of LTR
442 in eJRV and PERVs. (B) Sliding window analysis of percent sequence identity along
443 pairwise alignments of proviruses without LTRs. Phylogenetic trees of Gag (C), Pol (D)
444 and Env (E) inferred using the amino acid sequences of PERVs, eJRVs, ePCRVs and
445 other representative gammaretroviruses (Table S7). Bootstrap values < 70% are not shown
446 in phylogenetic trees. Trees were rooted to Reticuloendotheliosis virus (REV). The

complete phylogenetic trees of Gag (**C**), Pol (**D**) and Env (**E**) are presented in additional files 1: Fig. S4-S6, respectively. All abbreviations can be found in Table S7.

Fig. 3. Amino acid sequence comparison of the RBD of PERVs and ePCRVs. Two ePCRVs (ePCRV_1(accession number: KN678005.1) and ePCRV_2 (accession number: KN678690.1)) were aligned to PERV-A, -B, -C, and the newly discovered PERV-IM.

Fig. 4. Scenario of genesis of PERVs. A schematic representation of PERV evolutionary history is shown, summarizing our hypothesis regarding the origin and evolution of PERVs. Arrow on the left indicates putative timeline of each type of ERVs. Different background colors illustrate putative evolution periods of modern PERVs. JJRV, PCRV, ancestral PERV represent the exogenous forms of the retroviruses from their hosts. Solid blue arrows show origination and dotted blue arrows show speciation. Abbreviation: RBD, receptor binding domain; LTR, long terminal repeat; Gag, group antigens gene.

| Insertion
 | Deletion
 | Large Insertion
 | Stop codon

LTR A
 LTR B1
 LTR B2
 LTR B3

1

40

ePCRv_1
ePCRv_2
PERV-B
PERV-A
PERV-IM
PERV-C

P	S	A	-	-	K	S	T	P	P	N	L	M	R	S	Y	G	F	Y	S	C	P	G	-	H	N	K	G	R	H	C	G	G	T	G	S	H	Y	C	G
P	S	A	-	-	K	S	T	P	P	N	L	V	R	S	Y	G	F	Y	S	C	P	G	-	N	N	K	G	R	H	C	G	G	A	G	S	Y	Y	C	G
P	A	V	-	-	K	S	T	P	P	N	L	V	R	S	Y	G	F	Y	C	C	P	G	-	T	E	K	E	K	Y	C	G	G	S	G	E	S	F	C	R
P	G	L	N	D	Q	A	T	P	P	D	V	L	R	A	Y	G	F	Y	V	C	P	G	P	P	N	N	E	E	Y	C	G	N	P	Q	D	F	F	C	K
P	G	L	N	T	M	S	T	P	P	D	I	L	H	A	Y	G	F	Y	V	C	P	G	P	P	N	N	E	K	Y	C	G	N	P	R	D	F	F	C	K
P	S	L	-	-	T	S	P	P	D	I	L	H	A	H	G	F	Y	V	C	P	G	P	P	N	N	G	K	H	C	G	N	P	R	D	F	F	C	K	

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S	W	S	C	V	T	S	N	D	G	D	W	K	W	G	I	R	K	Q	D	L	L	K	F	S	F	V	N	P	G	P	G	K	H	V	T	M	E	-	-
S	W	S	C	V	T	S	N	D	G	D	W	K	W	G	V	G	N	R	D	L	L	K	F	S	F	V	N	P	G	P	G	K	H	V	T	M	E	-	-
R	W	S	C	V	T	S	N	D	G	D	W	K	W	P	I	S	L	Q	D	R	V	K	F	S	F	V	N	S	G	P	G	K	Y	K	V	M	K	-	-
Q	W	S	C	I	T	S	N	D	G	N	W	K	W	P	V	S	Q	Q	D	R	V	S	Y	S	F	V	N	N	P	T	S	Y	N	Q	F	N	Y	G	H
E	W	S	C	V	T	S	N	-	G	R	Y	K	W	P	T	S	R	Q	D	R	A	S	F	S	F	V	N	N	Y	T	S	S	D	Q	L	N	Y	-	-
Q	W	N	C	V	T	S	N	D	G	Y	W	K	W	P	T	S	Q	Q	D	R	V	S	F	S	Y	V	N	T	Y	T	S	S	E	Q	F	N	Y	-	-

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-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	Y	R	D	K	P	C	S	Q	K	D	L	D	Y	V	K	I	Q	F	T	E	T	G	K	T	D
-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	Y	R	D	K	P	C	S	Q	K	D	L	D	Y	V	K	I	Q	F	T	E	T	G	K	T	D
-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	Y	K	D	K	S	C	S	P	S	D	L	D	Y	L	K	I	S	F	T	E	K	G	K	Q	E
G	R	W	K	D	W	Q	Q	R	V	Q	K	D	V	R	N	K	Q	I	S	C	H	S	L	D	L	D	Y	L	K	I	S	F	T	E	K	G	K	Q	E
-	-	L	D	W	-	-	-	-	-	Q	R	E	G	K	Y	K	Q	I	S	C	S	P	S	D	L	D	Y	L	K	I	S	F	T	E	K	G	E	Q	Q
-	-	L	T	W	-	-	-	-	-	-	-	-	I	R	T	G	S	P	K	C	S	P	S	D	L	D	Y	L	K	I	S	F	T	E	K	G	K	Q	E

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P	-	S	K	W	V	D	G	L	T	W	G	I	V	F	W	K	Y	S	R	G	E	-	G	S	G	L	H	V	R	L	N	I	K	R	-	-	P	
T	-	S	K	W	I	N	G	L	T	W	G	V	V	F	W	K	Y	G	G	G	V	-	G	S	S	L	H	I	R	L	K	I	E	S	-	-	P	
N	I	Q	K	W	I	N	G	M	S	W	G	I	V	F	Y	K	Y	G	G	G	A	-	G	S	T	L	T	I	R	L	R	I	E	T	G	T	E	
N	I	Q	K	W	V	N	G	I	S	W	G	I	V	Y	Y	G	G	S	G	R	K	K	-	G	S	V	L	T	I	R	L	R	I	E	T	Q	M	E
N	I	-	K	W	I	N	G	M	S	W	G	I	V	F	Y	R	G	S	G	E	E	-	R	S	I	L	T	I	H	L	R	I	E	T	Q	M	E	
N	I	L	K	W	V	N	G	M	S	W	G	M	V	Y	Y	G	G	S	G	K	Q	P	G	S	I	L	T	I	R	L	K	I	-	N	Q	L	E