**Evolution of p53 pathway-related genes provides insights into anticancer mechanisms of natural longevity in cetaceans**

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**Table S3 Evidence of positive selection in cetaceans using free-ratio and branch-site model analysis in PAML**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** |  | **Models** | **-lnL** a | **2 ΔLnL** b | **ω** | **P value** c | **Positive selective branches/site** | **Radical changes in AA properties** d |
|  | **Branch model** | | | | |  |  |  |
| ***APAF1*** |  | one ratio | 7335.255476 |  | 0.2724 | P = 0.008 |  |  |
|  |  | free ratio | 7363.029643 | 55.5483 | ω variation for each branch |  | *T. aduncus*; LCA of *M. novaeangliae* |  |
| ***AIFM2*** |  | one ratio | 3371.39412 |  | 0.3128 | P = 0.0410 | *G. melas*; *P. sinus* |  |
|  |  | free ratio | 3347.203975 | 48.3803 | ω variation for each branch |  |  |  |
| ***CASP8*** |  | one ratio | 3114.260535 |  | 0.4487 | P = 0.0005 | *L. obliquidens*; LCA of delphinids |  |
|  |  | free ratio | 3081.177543 | 66.1660 | ω variation for each branch |  |  |  |
|  | **Branch-site model**  **Foreground branch: *G. melas*** | | | | | |  |  |
|  |  |  |
| ***AIFM2*** |  | Null (Ma0) | 3029.974424 |  | ω0 = 0.00000 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 3024.030126 | 11.8885 | ω0 = 0.00000 ω1 = 1.00000 ω2 = 999.00000 | P = 0.0006 | 459 | RF; Pc; αc; K0 |
|  | **Foreground branch: LCA of *P. phocoena*** | | | | | |  |  |
| ***TP53I3*** |  | Null (Ma0) | 2142.912639 |  | ω0 = 0.00000 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 2140.398476 | 5.0283 | ω0 = 0.00000 ω1 = 1.00000 ω2 = 99.13473 | P = 0.0249 | 236  254 | αc; Et  pHi; Mv; Mw; V0 |
|  | **Foreground branch: *B. acutorostrata*** | | | | |  |  |  |
| ***CCNE1*** |  | Null (Ma0) | 1969.5177 |  | ω0 = 0.00000, ω1 = 1.00000, ω2 = 1.0000 |  |  |  |
|  |  | Alternative (Ma) | 1966.2617 | 6.5118 | ω0 = 0.00000 ω1 = 1.00000 ω2 = 999.00000 | P = 0.0107 | 339 | NA |
| ***TP53*** |  | Null (Ma0) | 2155.1763 |  | ω0 = 0.00000 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 2152.7685 | 4.8155 | ω0 = 0.00000 ω1 = 1.00000 ω2 = 999.00000 | P = 0.0282 | 18 | NA |
|  | **Foreground branch: LCA of Phocoenidae and Monodontidae** | | | | |  |  |  |
| ***GTSE1*** |  | Null (Ma0) | 5554.4296 |  | ω0 = 0.04091, ω1 = 1.00000, ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 5551.5030 | 5.7850 | ω0 = 0.00000 ω1 = 1.00000 ω2 = 185.90141 | P = 0.0053 | 522 | Pk’; Ra |
|  | **Foreground branch: *L. vexillifer*** | | | | |  |  |  |
| ***SIVA1*** |  | Null (Ma0) | 1059.1290 |  | ω0 = 0.07849 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 1056.7690 | 4.7198 | ω0 = 0.08060 ω1 = 1.00000 ω2 = 38.82668 | P = 0.0298 | 21 | Bl |
|  | **Foreground branch: *P. catodon*** | | | | |  |  |  |
| ***TP73*** |  | Null (Ma0) | 3894.0871 |  | ω0=0.00000 ω1=1.00000 ω2=1.00000 |  |  |  |
|  |  | Alternative (Ma) | 3890.4768 | 7.2207 | ω0 = 0.00000 ω1 =1.00000 ω2 = 20.07134 | P = 0.0072 | 506 | pHi |

Physicochemical amino acid properties available in TreeSAAP are as following: α−helical tendencies (Pα); Average # surrounding residues (Ns); β-structure tendencies (Pβ); Bulkiness (Bl); Buriedness (Br); Chromatographic index (RF); Coil tendencies (Pc); Composition (c); Compressibility (K0); Equil. Const. – ioniza., COOH (Pk’); Helical contact energy (Ca); Hydropathy (h); Isoelectric point (pHi); Long-range n.b. energy (El); Mean r.m.s. fluctuat. displace. (F); Molecular volume (Mv); Molecular weight (Mw); Normal. consensus hydrophob. (Hnc); Partial specific volume (V0); Polar requirement (Pr); Polarity (p); Power to be – C-term., α-helix (αc); Power to be – middle, α-helix (αm); Power to be – N-term., α-helix (αn); Refractive index (µ); Sh.- & med.-range n.b. energy (Esm); Solvent accessible reduct. ratio (Ra); Surrounding hydrophobicity (Hp); Thermodyn. transfer hydrophob. (Ht); Total n.b. energy (Et); Turn tendencies (P)

Note: a, ln L is the log-likelihood score; b, twice the difference in ln L between the two models compared; c likelihood ratio test p-values were adjusted for multiple testing with FDR method; d, radical changes in amino acid properties under category 6-8 were detected in TreeSAAP. LCA: last common ancestor.

**Table S4 Evidence of positive selection using the aBSREL**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **Log (L)** | **AIC-c** | **Branch** | **P-value** |
| ***TP73*** | -3958.21 | 7994.54 | *M. novaeangliae* | 0.0011 |
| ***GTSE1*** | -5646.58 | 11371.25 | LCA of Phocoenidae and Monodontidae | 0.0198 |

Note: LCA: last common ancestor; Log (L): Log likelihood of model fit; AIC: Small-sample corrected Akaike information Score

**Table S5 Evidence of positive selection using the BUSTED**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Model** | ***log* L** | **#par.** | **Branch set** | **ω1** |  | **ω2** |  | **ω3** | ***P*-value** |
| ***TP73*** | Unconstrained | -3869.6 | 59 | FG | 0.00 (59.75%) | | 0.00 (26.62%) | | 8.71 (13.63%) |  |
|  |  |  |  | BG | 0.00 (0.41%) | | 0.60 (99.57%) | | 10000 (0.02%) | 0.000 |
|  | Constrained | -3877.3 | 58 | FG | 0.00 (29.18%) | | 1.00 (42.64%) | | 1.00 (28.18%) |  |
|  |  |  |  | BG | 0.60 (99.46%) | | 1.00 (0.52%) | | 10000 (0.02%) |  |

Note: log L, log-likelihood values, # par., the number of parameters.

**Table S6 Evidence of positive selection in mammal using free-ratio and branch-site model analysis in PAML**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** |  | **Models** | **-lnL** a | **2 ΔLnL** b | **ω** | **P value** c | **Positive selective branches/site** | **Radical changes in AA properties** d |
|  | **Branch model** | | | | |  |  |  |
| ***CASP8*** |  | one ratio | 9732.3183 |  | 0.3606 | P = 0.0019 | LCA of Primates; |  |
|  |  | free ratio | 9702.9048 | 58.8271 | ω variation for each branch |  |  |  |
| ***CCNE1*** |  | one ratio | 6051.7209 |  | 0.1099 | P < 0.0100 | *A. melanoleuca* |  |
|  |  | free ratio | 5993.6859 | 116.0699 | ω variation for each branch |  |  |  |
| ***TP53*** |  | one ratio | 7174.7654 |  | 0.2315 | P < 0.0100 | *G. gorilla*; LCA of Primates; *M. brandtii* |  |
|  |  | free ratio | 7124.3542 | 100.8226 | ω variation for each branch |  |  |  |
|  | **Branch-site model**  **Foreground branch: LCA of Primates** | | | | | |  |  |
|  |  |  |
| ***CASP8*** |  | Null (Ma0) | -9421.5260 |  | ω0 = 0.10392 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 9418.1562 | 6.7396 | ω0 = 0.10489 ω1 = 1.00000 ω2 = 15.99601 | P = 0.0094 | 221  315 | Pα; Pc; P  F; Ht |
| ***TP73*** |  | Null (Ma0) | 8843.1918 |  | ω0 = 0.04235 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 8840.1222 | 6.1393 | ω0 = 0.04239 ω1 = 1.00000 ω2 = 998.99972 | P = 0.0132 | 427 | Bl |
|  | **Foreground branch:** ***B. taurus*** | | | | | |  |  |
| ***CASP8*** |  | Null (Ma0) | 9421.3911 |  | ω0 = 0.10510 ω1=1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 9416.6020 | 9.5780 | ω0 = 0.10608 ω1 = 1.00000 ω2 = 49.40619 | P = 0.0020 | 433 | pHi |
| ***TP53*** |  | Null (Ma0) | 7038.9944 |  | ω0 = 0.08060 ω1 = 1.00000 ω2 = 32.60446 |  |  |  |
|  |  | Alternative (Ma) | 7036.5168 | 4.9551 | ω0 = 0.08060 ω1 = 1.00000 ω2 = 32.60446 | P = 0.0260 | 309 | h; Br;K0; pHi |
|  | **Foreground branch: *M. brandtii*** | | | | |  |  |  |
| ***TP73*** |  | Null (Ma0) | 8815.0742 |  | ω0 = 0.03926 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 8786.7094 | 56.7297 | ω0 = 0.03971 ω1 = 1.00000 ω2 = 999.00000 | P < 0.0100 | 157  164  217  479 | NA  Pα  P  RF; Pk’; Ra; P |
|  | **Foreground branch: *H. glaber*** | | | | |  |  |  |
| ***CD82*** |  | Null (Ma0) | 4613.5209 |  | ω0 = 0.07689 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 4610.9476 | 5.1465 | ω0 = 0.07764 ω1 = 1.00000 ω2 = 6.72069 | P = 0.0233 | 39  143 | NA  NA |
|  | **Foreground branch: LCA of *M. brandtii*** | | | | |  |  |  |
| ***TP53*** |  | Null (Ma0) | 7039.6762 |  | ω0 = 0.08059 ω1 = 1.00000 ω2 = 1.00000 |  |  |  |
|  |  | Alternative (Ma) | 7037.4689 | 4.4146 | ω0 = 0.08428 ω1 = 1.00000 ω2 = 67.82093 | P = 0.0356 | 287 | Pr; αc |

Physicochemical amino acid properties available in TreeSAAP are as following: α−helical tendencies (Pα); Average # surrounding residues (Ns); β-structure tendencies (Pβ ); Bulkiness (Bl); Buriedness (Br); Chromatographic index (RF); Coil tendencies (Pc); Composition (c); Compressibility (K0); Equil. Const. – ioniza., COOH (Pk’); Helical contact energy (Ca); Hydropathy (h); Isoelectric point (pHi); Long-range n.b. energy (El); Mean r.m.s. fluctuat. displace. (F); Molecular volume (Mv); Molecular weight (Mw); Normal. consensus hydrophob. (Hnc); Partial specific volume (V0); Polar requirement (Pr); Polarity (p); Power to be – C-term., α-helix (αc); Power to be – middle, α-helix (αm); Power to be – N-term., α-helix (αn); Refractive index (µ); Sh.- & med.-range n.b. energy (Esm); Solvent accessible reduct. ratio (Ra); Surrounding hydrophobicity (Hp); Thermodyn. transfer hydrophob. (Ht); Total n.b. energy (Et); Turn tendencies (P)

Note: a, ln L is the log-likelihood score; b, twice the difference in ln L between the two models compared; c likelihood ratio test p-values were adjusted for multiple testing with FDR method; d, radical changes in amino acid properties under category 6-8 were detected in TreeSAAP. LCA: last common ancestor.

**Table S9. GenBank accession numbers for the *Tursiops truncates* genes used for TBLASTN**

|  |  |
| --- | --- |
| **Gene** | **Accession numbers** |
| *ADGRB1* | XM\_033842849.1 |
| *AIFM2* | XM\_033841541.1 |
| *APAF1* | XM\_019918393.2 |
| *ATM* | XM\_033862082.1 |
| *ATR* | XM\_019934297.2 |
| *BAX* | XM\_033845723.1 |
| *BBC3* | XM\_033844600.1 |
| *BID* | XM\_033867288.1 |
| *CASP8* | XM\_019939139.2 |
| *CASP9* | XM\_033842913.1 |
| *CCND1* | XM\_033861345.1 |
| *CCND2* | XM\_033867173.1 |
| *CCND3* | XM\_033864265.1 |
| *CCNE1* | XM\_019941011.2 |
| *CCNE2* | XM\_033843339.1 |
| *CCNG2* | XM\_033857152.1 |
| *CD82* | XM\_033860501.1 |
| *CDKN1A* | XM\_033864359.1 |
| *CDKN2A* | XM\_033858018.1 |
| *CHEK2* | XM\_033837913.1 |
| *COP1* | XM\_019952340.2 |
| *DDB2* | XM\_004322699.2 |
| *FAS* | XM\_019941497.2 |
| *GADD45B* | XM\_033854871.1 |
| *GADD45G* | XM\_004318303.3 |
| *GORAB* | XM\_004317872.3 |
| *GTSE1* | XM\_019933139.2 |
| *IGF1* | XM\_004323127.3 |
| *MDM2* | XM\_033866334.1 |
| *PIDD1* | XM\_033861465.1 |
| *PMAIP1* | XM\_019937161.2 |
| *PPM1D* | XM\_019945482.1 |
| *PTEN* | XM\_019938503.2 |
| *RPRM* | XM\_004319723.3 |
| *SERPINB5* | XM\_019937194.2 |
| *SERPINE1* | XM\_033840247.1 |
| *SESN1* | XM\_019929540.2 |
| *SESN2* | XM\_033839721.1 |
| *SESN3* | XM\_033862123.1 |
| *SHISA5* | XM\_019946704.2 |
| *SIVA1* | XM\_004322704.3 |
| *TP53* | XM\_019944223.2 |
| *TP53I3* | XM\_033838209.1 |
| *TP73* | XM\_033844567.1 |
| *TSC2* | XM\_033839628.1 |
| *ZMAT3* | XM\_019946249.2 |