**Virologica Sinica**

**Supplementary Data**

**Domesticated HERV-W env contributes to the activation of the small conductance Ca2+-activated K+ type 2 channels via decreased 5-HT4 receptor in Recent-Onset schizophrenia**

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**Fig. S1** The transfection efficiency of HERV-W env in SHSY5Y cells. **A** Representative real-time PCR results for HERV-W env in SH-SY5Y. **B** Representative Western blotting results for HERV-W env in SH-SY5Y. Data are presented as the mean ± SD. \*\**P* < 0.01, \*\*\**P* < 0.001 compared to pCMV, each experiment was performed three times. Statistical analysis: one-way ANOVA

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**Fig. S2** HERV-W env directly interacts with 5-HT4R in HEK293T cells. **A** Coimmunoprecipitation assays (COIP) were performed with anti-HA-HERV-W env or anti-Flag-5-HT4R antibodies by Western blotting in HEK293T cells. **B** STED images of HERV-W env-TM (green) and 5-HT4R (red). (b1-b4) these experiments were performed in HEK293T cells. All experiments were repeated three times.

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**Fig. S3** The expression levels of HERV-W env and 5-HT4R were detected in cells cotransfected with HERV-W env and pENTER-5-HT4R. **A** Real-time PCR results for HERV-W env and 5-HT4R in SH-SY5Y. **B** Expression of HERV-W env and 5-HT4R proteins were detected by Western blot analysis. Data are presented as the mean ± SD. \**P* < 0.05; \*\**P* < 0.01, each experiment was performed three times. Statistical analysis: one-way ANOVA.

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**Fig. S4** The expression levels of HERV-W env and 5-HT4R were detected in cells by applying the 5-HT4R agonist RS67333 and HERV-W env. **A** Real-time PCR results for HERV-W env and 5-HT4R in SH-SY5Y. **B** Western blot analysis of HERV-W env and 5-HT4R expression in SH-SY5Y. Data shown are the mean ± SD and represent three independent experiments. Statistical analysis: one-way ANOVA (\**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001).

Supplementary Table S1. The demographic data of healthy controls and schizophrenia patients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Gender | Age (years) | BMI (body mass index) | Education (years) |
| Control 1 | Female | 54 | 21.08281 | 5 |
| Control 2  Control 3  Control 4  Control 5  Control 6  Control 7  Control 8  Control 9  Control 10  Control 11  Control 12  Control 13  Control 14  Control 15  Control 16  Control 17  Control 18  Control 19  Control 20  Control 21  Control 22  Control 23  Control 24  Control 25  Control 26  Control 27  Control 28  Control 29  Control 30  Control 31  Schizophrenia 1  Schizophrenia 2  Schizophrenia 3  Schizophrenia 4  Schizophrenia 5  Schizophrenia 6  Schizophrenia 7  Schizophrenia 8  Schizophrenia 9  Schizophrenia 10  Schizophrenia 11  Schizophrenia 12  Schizophrenia 13  Schizophrenia 14  Schizophrenia 15  Schizophrenia 16  Schizophrenia 17  Schizophrenia 18  Schizophrenia 19  Schizophrenia 20  Schizophrenia 21  Schizophrenia 22  Schizophrenia 23  Schizophrenia 24  Schizophrenia 25  Schizophrenia 26  Schizophrenia 27  Schizophrenia 28  Schizophrenia 29  Schizophrenia 30  Schizophrenia 31  Schizophrenia 32  Schizophrenia 33 | Female  Male  Male  Male  Male  Female  Male  Female  Female  Male  Male  Female  Female  Female  Male  Female  Male  Female  Female  Male  Male  Male  Female  Female  Female  Female  Male  Female  Female  Female  Female  Male  Female  Male  Male  Female  Female  Female  Female  Female  Male  Female  Female  Male  Male  Male  Female  Female  Male  Female  Male  Female  Female  Male  Male  Female  Male  Female  Female  Female  Male  Female  Male | 28  36  33  38  46  43  55  65  33  61  33  47  25  57  64  59  58  44  35  30  23  46  31  55  50  65  52  39  31  37  56  39  27  49  45  34  66  24  46  55  36  47  25  60  56  20  29  45  51  30  37  44  33  39  52  22  29  60  65  31  30  28  37 | 19.72104  20.67901  23.72281  23.40751  22.59814  24.30462  24.30462  19.31295  22.14533  25.43269  22.64219  25.91068  22.67995  20.83  24.84098  15.6157  23.29123  19.94321  15.05969  20.51509  23.09541  25.30864  22.94812  20.68515  16.02307  22.10029  25.25952  16.75729  23.66524  27.46914  25.39022  23.54788  18.28989  19.60716  21.29529  24.85907  20.06095  17.47472  20.9042  23.73324  21.46194  23.33768  21.75547  23.33547  26.3656  21.20099  19.59646  17.56965  23.87543  16.93703  19.47078  22.67995  22.50693  23.84978  22.40818  23.4375  23.93948  24.74745  19.59646  17.36111  22.72044  17.36111  23.35564 | 12  16  12  16  12  9  9  12  16  5  9  12  16  12  9  3  12  12  16  16  16  12  12  3  5  9  3  16  16  9  12  12  16  16  12  16  5  12  9  12  16  12  12  5  12  12  16  12  9  12  16  9  16  16  9  9  16  12  9  12  16  16  16 |

**Supplementary Table S2.** Comparison of demographic and clinical characteristics between the normal /onset schizophrenia groups in Chinese (yellow race).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Onset Schizophrenia (N = 33) | | Healthy controls (N = 31) | | Analysis | |
|  | Median | Range | Median | Range | Z | *P* |
| Age (years) | 41 | 20–66 | 44 | 23–65 | 1.96 | 0.18 |
| Education (years) | 12 | 5–16 | 11 | 3–16 | 1.96 | 0.08 |
| BMI (body mass index) | 21.6 | 16.9–26.4 | 22 | 15.1–27.5 | 1.96 | 0.86 |
|  | N | % | N | % | *χ2* | *P* |
| Gender |  |  |  |  |  |  |
| Female | 19 | 58 | 17 | 55 | 0.825 | 1 |
| Male | 14 | 42 | 14 | 45 |
| Smoking status |  |  |  |  |
| Yes | 12 | 36 | 13 | 42 | 0.648 | 0.7983 |
| No | 21 | 64 | 18 | 58 |

Supplementary Table S3. Information on the representative retroviruses.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Virus name | Genus | Abbreviation | Natural host | Accession No. |
| Human endogenous retrovirus H\_10610\_100012\_con\_env | ClassI | HERV-H\_env | Human | Ref1 |
| HERV9\_10320\_100006\_con\_env | ClassI | HERV9\_env | Human | Ref1 |
| HERVW\_10310\_100019\_con\_env | ClassI | HERV-W\_env | Human | Ref1 |
| HERVADP\_10420\_100007\_con\_env | ClassI | HERVADP\_env | Human | Ref1 |
| Human endogenous retrovirus T\_10110\_100018\_con\_env | ClassI | HERV-T\_env | Human | Ref1 |
| HERVIP\_10410\_100015\_con\_env | ClassI | HERVIP\_env | Human | Ref1 |
| HERVFA\_10630\_100009\_con\_env | ClassI | HERVFA\_env | Human | Ref1 |
| HERVFB\_10640\_100010\_con\_env | ClassI | HERVFB\_env | Human | Ref1 |
| HERVFC\_10650\_100011\_con\_env | ClassI | HERV-FC\_env | Human | Ref1 |
| hervh48\_10620\_100013\_con\_env | ClassI | hervh48\_env | Human | Ref1 |
| prima41\_10720\_100035\_con\_env | ClassI | prima41\_env | Human | Ref1 |
| HERV1\_10240\_100002\_con\_env | ClassI | HERV1\_env | Human | Ref1 |
| HERV3\_10230\_100004\_con\_env | ClassI | HERV3\_env | Human | Ref1 |
| HERV4\_10760\_100005\_con\_env | ClassI | HERV4\_env | Human | Ref1 |
| Human endogenous retrovirus I\_10250\_100014\_con\_env | ClassI | HERVI\_env | Human | Ref1 |
| Human endogenous retrovirus E\_10210\_100008\_con\_env | ClassI | HERV-E\_env | Human | Ref1 |
| PABL\_10750\_100033\_con\_env | ClassI | PABL\_env | Human | Ref1 |
| HUERSP3\_10930\_100031\_con\_env | ClassI | HUERSP3\_env | Human | Ref1 |
| HERVL\_30010\_100016\_con\_env | ClassII | HERVL\_env | Human | Ref1 |
| HML2\_20020\_100022\_con\_env | ClassII | HML2\_env | Human | Ref1 |
| HERVL32\_30220\_1946\_bre\_env | ClassIII | HERVL32\_env | Human | Ref1 |
| HERVS\_30200\_100017\_con\_env | ClassIII | HERVS\_env | Human | Ref1 |
| Friend murine leukemia virus | Gammaretrovirus | F MuLV | Mouse | NC\_001362.1 |
| Rauscher murine leukemia virus | Gammaretrovirus | R-MuLV | Mouse | U94692.1 |
| Mus dunni endogenous retrovirus | Gammaretrovirus | MDEV | Mouse | AF053745 |
| Koala retrovirus | Gammaretrovirus | KoRV | Phascolarctos cinereus | KC779547.1 |
| envelope protein Mab3 | Gammaretrovirus | Mab3\_env | Mabuya | ATY46613.1 |
| envelope protein Mab4 | Gammaretrovirus | Mab4\_env | Mabuya | ATY46614.1 |
| Gibbon ape leukemia vinus | Gammaretrovirus | GALV | Gibbon | NC\_001885.3 |
| Avian leukemia vinus | Alpharetrovinus | ALV | Chicken | NC\_015116 |
| Avian endogenous retrovirus EAV-HP | Alpharetrovirus | EVA\_ HP | Chicken | NC\_005947.1 |
| Coturnix\_japonica\_BASJ02003037.1 | Alpharetrovirus | Cja\_BASJ02003037.1 | Coturnix japonica | Ref2 |
| Rous sarcoma vinus | Alpharetrovinus | RSV | Chicken | NC\_001407.1 |
| Tympanuchus\_cupido\_MOXI01007493.1\_cj | Alpharetrovirus | Tcu\_MOXI01007493.1 | Tympanuchus cupido | Ref2 |
| Callipepla\_squamata\_MCFN01002646.1 | Alpharetrovirus | Csq\_MCFN01002646.1 | Callipepla squamata | Ref2 |
| Human immunodeficiency virus 1 | Lentivirus | HIV1 | Human | NC\_001802.1 |
| Human immunodeficiency virus 2 | Lentivirus | HIV2 | Human | NC\_001722.1 |
| Mouse mammary tumor virus | Betaretrovirus | MMTV | Mouse | NC\_001503 |
| Human T-lymphotropic virus 1 | Deltaretrovirus | HTLV1 | Human | NC\_001436 |
| Human T-lymphotropic virus 3 | Deltaretrovirus | HTLV3 | Human | DQ093792.1 |
| Human T-lymphotropic virus 4 | Deltaretrovirus | HTLV4 | Human | NC\_011800.1 |
| LTR46\_10660\_100032\_con\_env | Unclassifiable | LTR46\_env | Human | Ref1 |

Note: Ref1, refer to the reference (Vargiu *et al.*, 2016); Ref2, refer to the reference (Chen and Cui, 2019).

Supplementary Table S4. Primer sequences used in plasmid constructs

|  |  |  |
| --- | --- | --- |
| Name |  | Primer sequence (5′– 3′) |
| SK2-promoter-Full length a  SK2-promoter-truncation 1 b | F-  R-  F-  R- | GGGGTACCGCGCCTTTCTCTTTTCTCCT  CTAGCTAGCAGCAGCAGGGACAGGTTATC  GGGGTACCTCCCCGCAGGTGATGTCAT  CTAGCTAGCAGCAGCAGGGACAGGTTATC |
| SK2-promoter-truncation 2 c | F-  R- | GGGGTACCAAACCCCATTGCAGTTCCAG  CTAGCTAGCAGCAGCAGGGACAGGTTATC |
| SK2-promoter-truncation 3 d  SK2-promoter-truncation 4 e | F-  R-  F-  R- | GGGGTACCGCGCCTTTCTCTTTTCTCCT  CTAGCTAGCCCGAGACTGAGTTTGCGGC  GGGGTACCTCCCCGCAGGTGATGTCAT  CTAGCTAGCCCATGTCAGGGCTGTTAAAGC |

Note: a, SK2-promoter-Full length (from -572 to +90); b, SK2-promoter- truncation 1 (from -364 to +90); c, SK2-promoter- truncation 2 (from -175 to +90); d, SK2-promoter-truncation 3 (from -572 to -365); e, SK2-promoter-truncation 4 (from -364 to -176).

Supplementary Table S5. Primer sequences used in real-time quantitative PCR.

|  |  |  |
| --- | --- | --- |
| Name |  | Primer sequence (5′ – 3′) |
| GAPDH (NM\_002046.7)  HERV-W env (NM\_014590.4) | F-  R-  F-  R- | ATGACATCAAGAAGGTGGTG  CATACCAGGAAATGAGCTTG  CCATGCCGCTGTATGACCAG a  GGGTTCCCTTAGAAAGACTCCT a |
| 5-HT4R (NM\_000870.7) | F-  R- | TTATGGGGAGGTGTTTTGTCTT  GCAGAGGG GTCATCTTGTTC |
| SK2 (NM\_021614.4) | F-  R- | TGGTAGCTGTAGTGGCAAGGA  TGTTTCCCTGAGTACATTGGC |

Note: a, the primer was used to amplify the mRNA of HERV-W env by RT-qPCR as described previously (Wang *et al.*, 2018).

**References:**

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Vargiu, L., Rodriguez-Tome, P., Sperber, G. O., Cadeddu, M., Grandi, N., Blikstad, V., Tramontano, E., Blomberg, J., 2016. Classification and characterization of human endogenous retroviruses; mosaic forms are common. Retrovirology.13, 7.

Wang, X., Liu, Z., Wang, P., Li, S., Zeng, J., Tu, X., Yan, Q., Xiao, Z., Pan, M., Zhu, F., 2018. Syncytin-1, an endogenous retroviral protein, triggers the activation of CRP via TLR3 signal cascade in glial cells. Brain Behav. Immun.67, 324-334.