**A patient-enriched *MEIS1* coding variant causes a restless legs syndrome-like phenotype in mice**

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Table S1. Breeding statistics

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | | | | Average ± SD | | |
|  | **No of pairings** | **Total offspring** | **Total males** | **Total females** | **Litter size** | **Male offspring** | **Female offspring** |
| **WT x WT\*** | 17 | 124 | 58 | 66 | 7 ± 1.9 | 3 ± 1.7 | 4 ± 1.5 |
| **WT x HET\*** | 22 | 162 | 89 | 73 | 7 ± 2.7 | 4 ± 2.3 | 3 ± 2 |
| **HET x HET** | 119 | 780 | 398 | 382 | 7 ± 2.3 | 3 ± 1.6 | 3 ± 1.7 |
| **HET x HOM** | 12 | 69 | 37 | 32 | 6 ± 1.6 | 3 ± 1.8 | 3 ± 1.8 |
| **HOM x HOM** | 25 | 143 | 73 | 70 | 6 ± 2.7 | 3 ± 1.5 | 3 ± 2 |
| **WT x HOM** | 3 | 22 | 9 | 13 | 7 ± 1.5 | 3 ± 1.7 | 4 ± 2.1 |

\* Of these pairings, 1 instance in each of 1 pup mortality after birth

Table S2A. WR activity measures

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Young | | | | | Aged | | | | |
|  | **WT (n=7)** | | ***meis1*R272H/R272H (n=7)** | | **P value** | **WT (n=6)** | | ***meis1*R272H/R272H (n=9)** | | **P value** |
| **Light Activity** | 70.41 ± | 9.33 | 60.47 ± | 7.50 | >0.9 | 29.86 ± | 6.73 | 53.81 ± | 9.16 | 0.99 |
| **Dark Activity** | 2058.28 ± | 288.28 | 1508.56 ± | 167.45 | **0.02** | 907.41 ± | 179.08 | 1078.61 ± | 181.37 | 0.80 |
| **24h** | 1064.34 ± | 147.19 | 784.51 ± | 85.25 | 0.46 | 468.63 ± | 92.55 | 566.21 ± | 94.42 | 0.95 |
| **Dawn (ZT23-0)** | 316.12 ± | 63.33 | 166.11 ± | 25.43 | 0.86 | 266.72 ± | 89.02 | 362.01 ± | 64.12 | 0.95 |
| **Dusk (ZT11-13)** | 1522.47 ± | 193.11 | 1203.68 ± | 121.74 | 0.38 | 763.10 ± | 168.59 | 1055.74 ± | 175.10 | 0.41 |

Table S2B. RER and distance from indirect calorimetry

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Calorimetry | | | | | Distance (m) | | | | |
|  | **WT (n=15)** | | ***meis1*R272H/R272H (n=15)** | | **P value** | **WT (n=15)** | | ***meis1*R272H/R272H (n=15)** | | **P value** |
| **Light Activity (9h)** | 0.80 ± | 0.01 | 0.81 ± | 0.01 | >0.9 | 207.33 ± | 24.5 | 211.57 ± | 21.3 | >0.9 |
| **Dark Activity (12h)** | 0.84 ± | 0.01 | 0.87 ± | 0.02 | 0.85 | 316.65 ± | 31.1 | 325.56 ± | 30.5 | >0.9 |
| **21h** | 0.82 ± | 0.01 | 0.85 ± | 0.01 | >0.9 | 269.61 ± | 27.5 | 276.71 ± | 25.2. | >0.9 |
| **Dawn (ZT23-0)** | 0.81 ± | 0.01 | 0.84 ± | 0.02 | 0.74 | 217.94 ± | 39 | 243.3 ± | 38.8 | >0.9 |
| **Dusk (ZT11-13)** | 0.82 ± | 0.01 | 0.85 ± | 0.01 | >0.9 | 362.79 ± | 59.7 | 312.95 ± | 30.6 | >0.9 |
|  |  |  |  |  |  |  |  |  |  |  |
| **Body weight (g)** | 23.3 ± | 0.4 | 23.09 ± | 0.77 | >0.9 |  |  |  |  |  |
| **Food Intake (g)** | 1.19 ± | 0.16 | 1.59 ± | 0.23 | >0.9 |  |  |  |  |  |
| **Water Intake (g)** | 1.39 ± | 0.25 | 1.67 ± | 0.32 | >0.9 |  |  |  |  |  |

Table S3. Clinical chemistry

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **WT (n=15)** | | ***meis1*R272H/R272H (n=15)** | | **P value** |
| **Calcium [mmol/l]** | 2.35 ± | 0.01 | 2.33 ± | 0.03 | >0.9 |
| **Inorganic phosphate [mmol/l]** | 2 ± | 0.52 | 2.31 ± | 0.44 | >0.9 |
| **ALP [µmol/l]** | 105.73 ± | 12.59 | 108.9 ± | 4.07 | 0.6 |
| **Iron [µmol/l]** | 23.59 ± | 1.1 | 25.32 ± | 3.06 | 0.96 |
| **Unsaturated iron binding capacity [µmol/l]** | 37.1 ± | 1.49 | 36.6 ± | 3.7 | >0.9 |
| **Total iron binding capacity [µmol/l]** | 60.7 ± | 3.9 | 62 ± | 1.7 | 0.69 |
| **Calc transferrin saturation [%]** | 39 ± | 7.8 | 40.9 ± | 5.2 | 0.79 |

Table S4A. Wake activity measures in piezo sleep screen

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Young | | | | | Aged | | | | |
|  | **WT (n=10)** | | ***meis1*R272H/R272H (n=10)** | | **P value** | **WT (n=8)** | | ***meis1*R272H/R272H (n=11)** | | **P value** |
| **Light Activity** | 40.84 ± | 2.23 | 41.45 ± | 1.38 | >0.9 | 44.85 ± | 1.54 | 44.91 ± | 1.42 | >0.9 |
| **Dark Activity** | 84.34 ± | 1.50 | 88.63 ± | 1.42 | >0.9 | 81.64 ± | 1.82 | 84.42 ± | 1.50 | >0.9 |
| **24h** | 62.59 ± | 1.40 | 65.04 ± | 1.16 | >0.9 | 63.25 ± | 1.50 | 64.67 ± | 1.09 | >0.9 |
| **Dawn (ZT23-0)** | 81.84 ± | 3.84 | 91.33 ± | 3.85 | 0.10 | 72.44 ± | 4.07 | 83.63 ± | 2.95 | **0.029** |
| **Dusk (ZT11-13)** | 46.54 ± | 3.40 | 47.91 ± | 2.29 | >0.9 | 46.30 ± | 4.37 | 49.32 ± | 1.44 | >0.9 |

Table S4B. State transitions in piezo sleep screen

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Young | | | | | Aged | | | | |
|  | **WT (n=10)** | | ***meis1*R272H/R272H (n=10)** | | **P value** | **WT (n=8)** | | ***meis1*R272H/R272H (n=11)** | | **P value** |
| **Light Activity** | 93.1 ± | 8.47 | 87.2 ± | 7.45 | >0.9 | 123.6 ± | 6.28 | 101.4 ± | 6.16 | 0.12 |
| **Dark Activity** | 62.4 ± | 12.22 | 46.9 ± | 7.55 | >0.9 | 70.4 ± | 8.42 | 59.45 ± | 4.98 | >0.9 |
| **24h** | 157.5 ± | 18.46 | 136.1 ± | 12.97 | 0.54 | 196.0 ± | 14.07 | 162.82 ± | 9.94 | **0.005** |
| **Dawn (ZT23-0)** | 13.5 ± | 2.68 | 10.5 ± | 3.26 | >0.9 | 17.9 ± | 3.78 | 13.0 ± | 1.76 | >0.9 |
| **Dusk (ZT11-13)** | 11.4 ± | 2.17 | 9.3 ± | 1.21 | >0.9 | 13.9 ± | 1.81 | 10.7 ± | 1.90 | 0.79 |

Table S5. Breathing activity (Hz) measures in piezo sleep screen

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Young | | | | | Aged | | | | |
|  | **WT (n=10)** | | ***meis1*R272H/R272H (n=10)** | | **P value** | **WT (n=8)** | | ***meis1*R272H/R272H (n=11)** | | **P value** |
| **Light Activity** | 3.21 ± | 0.05 | 3.13 ± | 0.06 | >0.9 | 3.10 ± | 0.05 | 3.15 ± | 0.06 | >0.9 |
| **Dark Activity** | 2.82 ± | 0.08 | 2.77 ± | 0.10 | >0.9 | 2.74 ± | 0.12 | 3.05 ± | 0.08 | 0.17 |
| **24h** | 3.03 ± | 0.05 | 2.98 ± | 0.07 | >0.9 | 2.98 ± | 0.08 | 3.10 ± | 0.07 | >0.9 |
| **Dawn (ZT23-0)** | 3.06 ± | 0.13 | 2.98 ± | 0.09 | >0.9 | 2.87 ± | 0.18 | 3.17 ± | 0.13 | 0.19 |
| **Dusk (ZT11-13)** | 3.02 ± | 0.09 | 3.11 ± | 0.07 | >0.9 | 3.11 ± | 0.11 | 3.21 ± | 0.07 | 0.79 |
| ***Low breathing rate episodes (sec/hr)*** | | |  |  |  |  |  |  |  |  |
| **Light Activity** | 27.82 ± | 4.11 | 23.16 ± | 3.37 | 0.64 | 14.04 ± | 1.83 | 15.01 ± | 1.71 | >0.9 |
| **Dark Activity** | 4.88 ± | 1.21 | 4.22 ± | 1.1 | >0.9 | 3.2 ± | 1.2 | 4.23 ± | 0.75 | >0.9 |
| **24h** | 16.35 ± | 2.3 | 13.69 ± | 2.16 | >0.9 | 8.6 ± | 1.13 | 9.62 ± | 1.1 | >0.9 |