Renal damage-induced hepcidin accumulation contributes to anemia in angiotensinogen-deficient mice

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**Table S1 – List of primer pairs used for mRNA quantification by RT-qPCR.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Target, *Gene*** | **Sequence (5' to 3')** | **Sense** | **Amplicon (bp)** |
| 18s, *Rn18s* | TTGATTAAGTCCCTGCCCTTTGT | Forward | 75 |
|  | CGATCCGAGGGCCTCACTA | Reverse |  |
| β-actin, *Actb* | CTGGCCTCACTGTCCACCTT | Forward | 61 |
|  | CGGACTCATCGTACTCCTGCTT | Reverse |  |
| BMP6, *Bmp6* | AGAAGCGGGAGATGCAAAAGG  | Forward | 211 |
|  | GACAGGGCGTTGTAGAGATCC  | Reverse |  |
| Dcytb, *Cybrd1* | TTCCTGTCGGTGATCTTCGTG  | Forward | 203 |
|  | CCGGCATGGATGGATTTCATC  | Reverse |  |
| DMT1, *Slc11a2* | TACCTAGACCCAGGAAACATCG  | Forward | 132 |
|  | CACTCCAAGTCTCGCTGCAA  | Reverse |  |
| Erythropoietin, *Epo* | ACTCTCCTTGCTACTGATTCCT  | Forward | 123 |
|  | ATCGTGACATTTTCTGCCTCC | Reverse |  |
| Ferroportin, *Slc40a1* | CTACCATTAGAAGGATTGACCAGCTA  | Forward | 82 |
|  | ACTGGAGAACCAAATGTCATAATCTG  | Reverse |  |
| FTH, *Fth1* | TGATGAAGCTGCAGAACCAG | Forward | 105 |
|  | GTGCACACTCCATTGCATTC  | Reverse |  |
| FTL, *Ftl1* | AATGGGGTAAAACCCAGGAG  | Forward | 76 |
|  | AGATCCAAGAGGGCCTGATT  | Reverse |  |
| Hepcidin, *Hamp1* | AGGGCAGACATTGCGATACC | Forward | 101 |
|  | GCAACAGATACCACACTGGGA | Reverse |  |
| HIF-2α, *Epas1* | CTGAGGAAGGAGAAATCCCGT  | Forward | 161 |
|  | TGTGTCCGAAGGAAGCTGATG  | Reverse |  |
| Interleukin-6, *Il6* | CTGCAAGAGACTTCCATCCAGTT | Forward | 70 |
|  | GAAGTAGGGAAGGCCGTGG | Reverse |  |
| KIM-1, *Havcr1* | TCAGAAGAGCAGTCGGTACAAC | Forward | 220 |
|  | TGTAGCTGTGGGCCTTGTAGT  | Reverse |  |
| Nephrin, *Nphs1* | GTGCCCTGAAGGACCCTACT | Forward | 169 |
|  | CCTGTGGATCCCTTTGACAT | Reverse |  |
| NGAL, *Lcn2* | CCATCTATGAGCTACAAGAGAACAAT  | Forward | 89 |
|  | TCTGATCCAGTAGCGACAGC | Reverse |  |
| Podocin, *Nphs2* | CTTGGCACATCGATCCCTCA | Forward | 198 |
|  | CGCACTTTGGCCTGTCTTTG  | Reverse |  |
| RPB1, *Polr2a* | CAAGAGAGTGCAGTTCGGAGT | Forward | 93 |
|  | CCCTCCGTTGTTTCTGGGTATTT  | Reverse |  |
| TFR, *Tfrc* | ATGCCGACAATAACATGAAGGC | Forward | 136 |
|  | ACACGCTTACAATAGCCCAGG | Reverse |  |



**Figure S1 – Capillary hematocrit.** Measured hematocrit in males (**A**) and females (**B**). Values are mean ± SD \*\*\**P*<0.001 *vs* Agt-Het (Student’s *t* test).

**Table S2 - Hematology of Agt-KO female.**

|  |  |  |
| --- | --- | --- |
| **Parameter**, *unit* | **Agt-Het**, *n=7* | **Agt-KO**, *n=9* |
| **Hematocrit**, *%RBC* | 47.1 ± 3.0 | 42.0 ± 1.7\*\*\* |
| **RBC**, *M/uL* | 9.5 ± 0.4 | 8.9 ± 0.3\*\* |
| **MCV**, *fL* | 49.7 ± 1.3 | 47.0 ± 1.3\*\*\* |
| **Hemoglobin**, g*/dL* | 14.2 ± 0.6 | 12.7 ± 0.5\*\*\*\* |
| **MCH**, *pg* | 15.0 ± 0.2 | 14.3 ± 0.3\*\*\* |
| **MCHC**, *g/dL* | 30.2 ± 0.6 | 30.4 ± 0.5 |
| **Reticulocytes**, *K/uL* | 496 ± 97 | 409 ± 146 |
| **Platelets**, *K/uL* | 973 ± 106 | 1087 ± 181 |
| **WBC**, *K/uL* | 3.8 ± 0.8 | 4.37 ± 1.0 |

Values are mean ± SD \*\**P*<0.01, \*\*\**P*<0.001, \*\*\**P*<0.0001 vs Agt-Het (Student’s t test). RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT= platelets, WBC = white blood cell.



**Figure S2 – Plasma iron and iron homeostasis parameters, and tissue iron in females.** Iron levels in plasma (**A**). Transferrin levels in plasma (**B**). Plasma calculated TIBC, total iron binding capacity (**C**). Plasma UIBC, unsaturated iron binding capacity (**D**). Transferrin saturation in plasma (**E**). Ferritin levels in plasma (**F**). Concentration of iron in liver (**G**) and spleen (**H**). Values are mean ± SD \**P*<0.05, \*\**P*<0.01 *vs* Agt-Het (Student’s *t* test).



**Figure S3 – Duodenal ferritin expression in males.** Light chain ferritin, Ftl1(**A**)and heavy chain ferritin, Fth1(**B**)mRNA quantification in duodenum. Values are mean ± SD.



**Figure S4 – Renal function markers in females.** Creatinine **(A)** and urea **(B).** Values are mean ± SD. \*\*\*\**P*<0.0001 *vs* Agt-Het (Student’s *t* test).