**Shedding a new light on Huntington's disease:**

**how blood can both propagate and ameliorate disease pathology**

#Marie Rieux1,2, #Melanie Alpaugh1,3, Giacomo Sciacca1,3, Martine Saint-Pierre1, Maria Masnata1,3, Hélèna L. Denis1,3, Sébastien A. Lévesque1,2, Frank Herrmann4, Chantal Bazenet4, Alexandre P. Garneau5,6, Paul Isenring5, Ray Truant7, Abid Oueslati1,2, Peter V. Gould8, Anne Ast9, Erich E. Wanker9, Steve Lacroix1,2 and \*Francesca Cicchetti1,3

*#Equal contribution*

*\*Corresponding author*

**Supplementary Materials and Methods**

**Table S1. Details of western blot protocols**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | mHTT within erythrocytes | mHTT within organs | Microglial  analyses | All other  western blots |
| Protein quantity | 40 µg | 30 µg | 40 µg | 30 µg |
| Gel | Precast  4 % - 12 % | 6 % SDS-Page | 14 % SDS-Page | 10 % SDS-Page |
| Migration | 200 V – 25 min | 110 V – 3 h | 100 V – 15 min  40 V – 1 h  +15 V every 7 min until 100 V  100 V – 1 h | 100 V – 1 h 50 |
| Membrane | PVDF | Low fluorescence PVDF | Nitrocellulose | PVDF |
| Transfer | O/N - 4°C  15 V  1X transfer buffer\* | O/N - 4°C  20 V  Towbin transfer buffer containing 0.01 % SDS and 16 % methanol | O/N - 4°C  20 V  1X transfer buffer\* | 130 V – 1 h  1X transfer buffer\* |
| Blocking solution | 5 % milk  0.5 % BSA in PBST | 2.5 % BSA in TBS | 5 % milk  1 % BSA in PBST | 5 % milk  0.5 % BSA in PBST |
| Imaging system | myECL imager | Odyssey Imaging System | myECL imager | myECL imager |

\*1X transfer buffer contained 20 % methanol, 25 mM C6H13NO4, 25 mM C8H19NO5, 1 mM EDTA and 1.3 mM NaHSO3. **Abbreviations**: BSA, bovine serum albumin; h, hour; HTT, huntingtin; m, mutant; min, minute; O/N, overnight; PBST, phosphate-buffered saline containing 0.1 % Tween 20; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate; TBS, tris- buffered saline; V, volt.

**Table S2. Antibodies used for filter retardation assay or western blotting**

|  |  |  |  |
| --- | --- | --- | --- |
| Primary antibody | Dilution | Company | Catalog Number |
| Aggregated HTT | 1:500 | Merck-Millipore | #MAB5374 |
| Calretinin | 1:1000 | SWANT | #CR7697 |
| Claudin-5 | 1:500 | Merck-Millipore | #ABT45 |
| Cytochrome c | 1:1000 | Abcam | #Ab13575 |
| DARPP32 | 1:1000 | Cell signaling | #2306S |
| GAD65/67 | 1:1000 | Sigma-Aldrich | #G5163 |
| GAPDH | 1:5000 | ABM | #G041 |
| GFAP | 1:1000 | Sigma-Aldrich | #G3893 |
| IBA1 | 1:500 | Wako | #016-20001 |
| ICAM-1 | 1:1000 | Abcam | #Ab153749 |
| NeuN | 1:5000 | Merck-Millipore | #MAB377 |
| Polyglutamine | 1:1000 | Merck-Millipore | #MAB1574 |
| TOM40 | 1:500 | Santa-Cruz | #sc-365467 |
| Total HTT | 1:1000 | Merck-Millipore | #MAB2166 |
| Total HTT (N18) | 1:1000 | N/A | N/A |
| VGLUT1 | 1:1000 | NeuroMAB | #75-066 |
| Vinculin | 1:5000 | Abcam | #Ab129002 |
| Secondary antibody | **Dilution** | **Company** | **Catalog Number** |
| Donkey anti-mouse  IRDye 680RD | 1:20000 | LI-COR Biotechnology | #925-68072 |
| Donkey anti-mouse IRDye 800RD | 1:10000 | LI-COR Biotechnology | #925-32212 |
| Donkey anti-rabbit IRDye 680RD | 1:10000 | LI-COR Biotechnology | #925-68073 |
| Goat anti-mouse HRP | 1:25000 | Jackson Immunoresearch | #115-035-166 |
| Goat anti-rabbit HRP | 1:25000 | Jackson Immunoresearch | #115-035-144 |

**Abbreviations**: DARPP32, Dopamine- and cAMP-regulated phosphoprotein 32; GAD65/67, Glutamic acid decarboxylase 65/67; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GFAP, Glial fibrillary acidic protein; HRP, horseradish peroxidase; HTT, huntingtin; ICAM-1, intercellular adhesion molecule; IBA1, Ionized calcium binding adaptor molecule; NeuN, neuronal nuclei; TOM40, translocase of the mitochondrial outer membrane 40; VGLUT1, vesicular glutamate transporter 1.

**SUPPLEMENTARY FIGURE LEGENDS**

**Figure S1. Parabiosis - proof of concept**. (**a**)Timeline of GFP-WT parabiosis experiments. (**b**) GFP+ leukocytes detected at the indicated time point. N=4. Data are expressed as mean ± SEM with data points indicating values for each animal. Statistical analyses were performed using a Mann-Whitney test*.***Abbreviations**: d, day; GFP, Green Fluorescent Protein; WT, wild-type.

**Figure S2. Assessment of mHTT aggregates/mRNA within peripheral organs of parabionts.** Quantifications and representative immunoblots (showing two animals per group) of SDS-insoluble mHTT aggregates detected in the (**a**) liver, (**b**) kidney and (**c**) muscle. Relative quantification of *HTT/mHTT* RNA levels in the (**d**) liver and (**e**) kidney by RT-qPCR. Data are expressed as mean ± SEM with data points indicating values for each animal. N=2 for same genotype pairs and n=5 for multi-genotype pairs. Statistical analyses were performed using a two-way ANOVA with Sidak’s post-hoc tests. \*\*p<0.01; \*\*\*p<0.001. **Abbreviations**: HD, Huntington's disease; HTT, huntingtin protein; m, mutant; NP, non-parabiotic mice; N18, anti-huntingtin protein antibody; PolyQ, polyglutamine stretch of mHTT protein; RQ, relative quantification; WT, wild-type.

**Figure S3. Impact of parabiosis on cortical vasculature in parabionts**.(**a**)Quantificationand representative images of mean fluorescence intensity for Collagen IV at 12 months post-surgery. (**b**) Density, (**c**) number, (**d**) diameter and (**e**) vessel population by diameter were assessed as well as (**f**) branching and (**g**) branch length. (**h**) Representative images of cortical vasculature and (**i**) depiction of quantification methods. Scale bars = (**a**) 25 μm, (**h-i**) 40 μm, (**i**, high magnification) 20 μm. Data are expressed as mean ± SEM with data points indicating values for each animal. N=2 for same genotype pairs and n=5 for multi-genotype pairs. Statistical analyses were performed using a two-way ANOVA with Tukey’s or Sidak’s post-hoc tests. For (**d-f**), unpaired t-tests were completed. \*p<0.05; \*\*p<0.01. **Abbreviations**: HD, Huntington’s disease; WT, wild-type.

**Figure S4.** **Impact of parabiosis on cerebral HTT protein and mRNA levels of parabionts**. Quantifications and immunoblots of soluble HTT content in the (**a**) striatum and (**b**) hippocampus at 12 months post-surgery. (**c**) Quantifications and representative images (showing two animals per group) of filter retardation assay of striatal samples 9 and 12 months post-surgery. (**d**) Relative quantification of *HTT/mHTT* RNA levels in the hippocampus by RT-qPCR. Data are expressed as mean ± SEM with data points indicating values for each animal. N=2 for same genotype pairs and n=9 for multi-genotype pairs. For (**a-b**), statistical analyses were performed using a two-way ANOVA with Tukey’s or Sidak’s post-hoc tests. To analyze wtHTT content in HD groups in (**b**), a one-way ANOVA with Holm-Sidak’s post-hoc test was used. For (**c**), unpaired t-tests were completed. \*p<0.05;\*\*p<0.01; \*\*\*p<0.001; \*\*\*\*p<0.0001. **Abbreviations**: EM48, anti-mutant huntingtin protein antibody; HD, Huntington's disease; HTT, Huntingtin protein; kDa, kilodalton; m, mutant; NP, non-parabiotic mice; N18, anti-huntingtin protein antibody; RQ, relative quantification; WT, wild-type.

**Figure S5. Impact of parabiosis on cell populations of the CNS**. Representative photomicrographs and quantifications of stereology are shown for (**a**) interneurons (calbindin+ cells) in the cortex, (**b**) projection neurons (DARPP32+ cells) in the striatum and (**c-d**) microglia (Iba1+ cells) in the cortex and striatum. Scale bars = 50 μm. Data are expressed as mean ± SEM with data points indicating values for each animal. N=2 for same genotype pairs and n=4 for multi-genotype pairs. Statistical analyses were performed using a two-way ANOVA followed by Tukey’s or Sidak’s post-hoc tests. **Abbreviations:** CB, calbindin; CNS, central nervous system; DARPP32, Dopamine- and cAMP-regulated phosphoprotein 32; HD, Huntington's disease; Iba1, Ionized calcium binding adaptor molecule; NP, non-parabiotic mice; WT, wild-type.

**Figure S6. Absence of overt inflammatory response in parabionts.** Quantifications and representative immunoblots of (**a**) Iba1 as a microglial marker and (**b**) GFAP as an astrocytic marker in the cortex of parabionts 9 months post-surgery. Data are expressed as mean ± SEM with data points indicating values for each animal. N=2 for same genotype pairs and n=7 for multi-genotype pairs. Statistical analyses were performed using a two-way ANOVA with Tukey’s or Sidak’s post-hoc tests.**Abbreviations**: GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; GFAP, Glial fibrillary acidic protein; HD, Huntington's disease; Iba1, Ionized calcium binding adaptor molecule; kDa, kilodalton; NP, non-parabiotic mice; WT, wild-type.

**Figure S7. Hemodynamic measures in WT and HD mice.** (**a**) Systolic, (**b**) diastolic and (**c**) mean arterial blood pressure, such as (**d**) heart rate, were estimated through a tail cuff apparatus. Data are expressed as mean ± SEM with data points indicating values for each animal. N=6 per group. Statistical significance was assessed by an unpaired t-test. \*\*p<0.01. **Abbreviations:** HD, Huntington's disease; min-1, per minute; mmHg, millimeter of mercury; NP, non-parabiotic mice; WT, wild-type; zQ175, mouse model of Huntington’s disease.

**Figure S8. Assessment of inflammatory cytokines in parabionts.** Levels of IL-4, IL-5, IL-6, IL-10 and TNF-α were evaluated in the (**a**) plasma 9 and 12 months post-surgery, and in the (**b**) liver and (**c**) kidney of the parabionts 12 months post-surgery. Data are expressed as mean ± SEM with data points indicating values for each animal. N=2 for same genotype pairs and n=9 for multi-genotype pairs. Statistical analyses were performed using a two-way ANOVA with Tukey’s or Sidak’s post-hoc tests. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001. **Abbreviations:** HD, Huntington’s disease; IL, interleukin; ml, milliliter; NP, non-parabiotic mice; pg, picogram; TNF, tumor necrosis factor; WT, wild-type.