

# Influencia del sexo, la edad y la diabetes en las modificaciones del transcriptoma y proteoma tras la isquemia cerebral

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*(Ramiro et al. BMC Neuroscience. 2023)*

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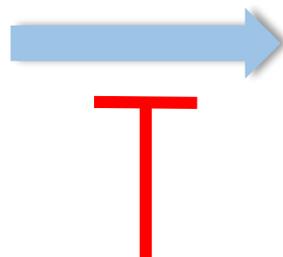
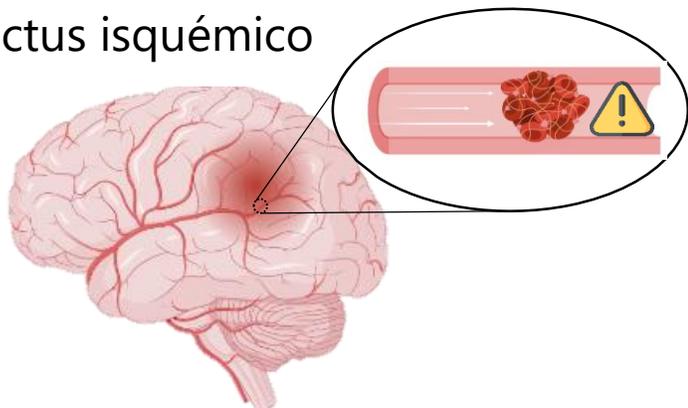
Laboratorio de Investigación Neurovascular

Vall d'Hebron Institut de Recerca (VHIR), Barcelona

Madrid, 24/03/2023

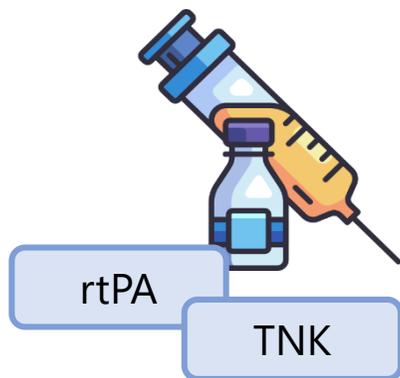


Ictus isquémico

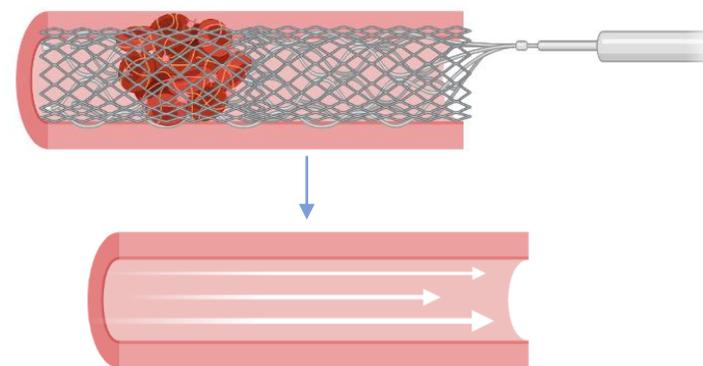


Terapias de reperfusión

Tratamiento farmacológico



Trombectomía mecánica



> **1.000** agentes neuroprotectores **han fallado** en la práctica clínica

Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies

Develop new neuroprotective strategies for ischemic stroke

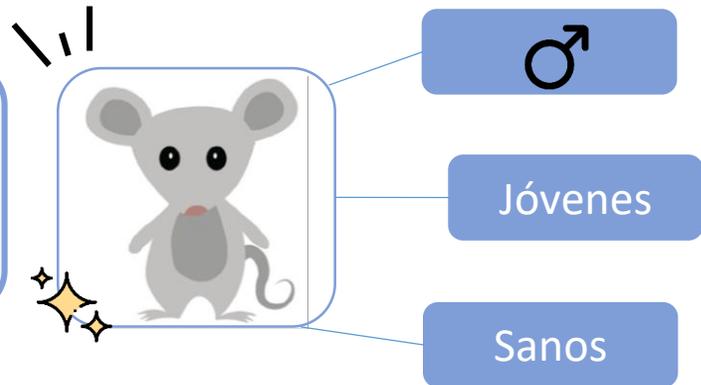
The neuroprotective effect of Apelin on ischemic stroke: activating AMPK/GSK3β/Nrf2 signaling

Glucagon-like peptide-1 receptor agonists as neuroprotective agents for ischemic stroke: a systematic review

Glucagon-like peptide-1 receptor agonist suppresses ferroptosis induced by cerebral ischemia reperfusion in MCAO/R rats

**BENCH TO BEDSIDE**

Modelos animales utilizados en experimentación **no representan** adecuadamente las características de los pacientes.



Stroke Treatment Academy Industry Roundtable

**STAIR**

Ambos sexos

Edad avanzada

Comorbilidades

♀ ♂ ≈

**Objetivo:** Explorar los cambios **proteómicos** y **transcriptómicos** a nivel cerebral desencadenados durante la fase **hiperaguda** de la isquemia cerebral en ratones:

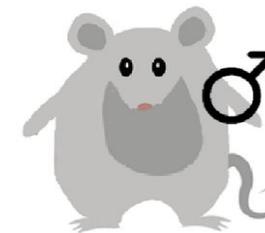


8 jóvenes (8-12w)  
4 sham

*Simats et al (2020)*



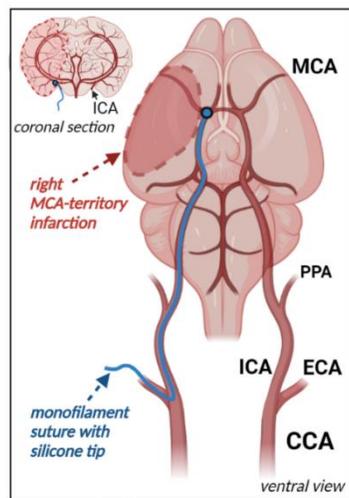
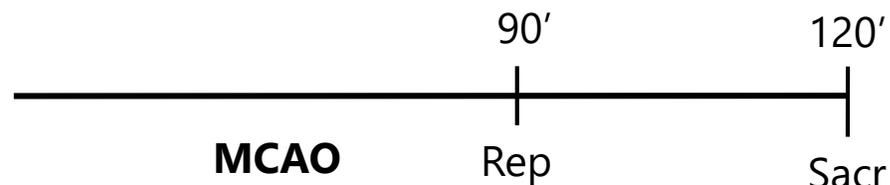
8 jóvenes  
4 sham



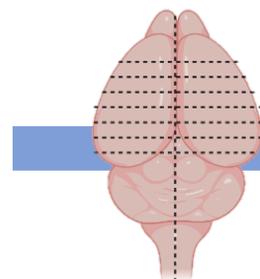
8 jóvenes db/db  
4 sham



8 edad avanzada (18 m)  
4 sham



*Lemmerman et al (2022)*

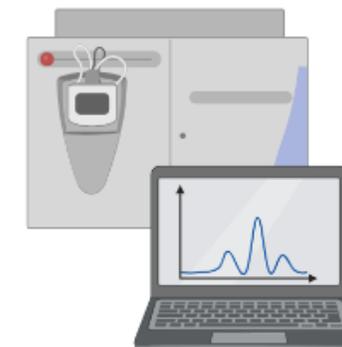


Proteínas y RNA

Estudio transcriptómico y proteómico

MS

Microarrays



## TRANSCRIPTÓMICA

## Genes diferencialmente expresados (DEG)

**Table 1** Top 15 differentially expressed genes (FDR < 0.25) between the infarcted hemisphere and the contralateral healthy hemisphere 2 h after cerebral ischemia induction. Genes that are differentially expressed in all the groups of animals are highlighted in bold. FDR: false discovery rate; logFC: Logarithmic fold change

| Young male mice, <i>males</i> |        |          |          | Young female mice, <i>females</i> |         |          |         | Diabetic young male mice, <i>diabetic</i> |        |          |          | Aged male mice, <i>old</i> |         |          |          |
|-------------------------------|--------|----------|----------|-----------------------------------|---------|----------|---------|---|--------|----------|----------|----------------------------|---------|----------|----------|
| SYMBOL                        | logFC  | P.Value  | FDR      | SYMBOL                            | logFC   | P.Value  | FDR     | SYMBOL                                    | logFC  | P.Value  | FDR      | SYMBOL                     | logFC   | P.Value  | FDR      |
| <b>CCL3</b>                   | 2.4958 | 1.73E-15 | 8.80E-12 | <b>ATF3</b>                       | 1.4557  | 1.42E-06 | 0.00284 | <i>HSPA1A</i>                             | 2.4544 | 1.02E-09 | 5.17E-06 | <b>NPAS4</b>               | 2.9274  | 1.53E-08 | 7.75E-05 |
| <i>FOSB</i>                   | 1.9774 | 7.77E-12 | 1.58E-08 | <i>PTGS2</i>                      | 1.5976  | 1.76E-06 | 0.00284 | <i>FOS</i>                                | 2.4266 | 9.29E-09 | 1.63E-05 | <b>CCL3</b>                | 2.0499  | 1.08E-07 | 2.74E-04 |
| <i>CCN1</i>                   | 1.8493 | 1.10E-11 | 1.58E-08 | <i>CCRL2</i>                      | 1.1296  | 2.21E-06 | 0.00284 | <i>CCN1</i>                               | 1.9876 | 9.61E-09 | 1.63E-05 | <i>FOS</i>                 | 1.6891  | 8.48E-07 | 0.00143  |
| <i>FOS</i>                    | 2.1172 | 1.25E-11 | 1.58E-08 | <i>FOS</i>                        | 1.5602  | 2.24E-06 | 0.00284 | <b>CCL3</b>                               | 2.6185 | 1.55E-08 | 1.97E-05 | <i>IL1A</i>                | 1.7778  | 2.60E-06 | 0.00329  |
| <b>NPAS4</b>                  | 2.6951 | 7.08E-11 | 7.18E-08 | <b>CCL3</b>                       | 2.1777  | 2.93E-06 | 0.00298 | <i>FOSB</i>                               | 2.1383 | 4.27E-08 | 4.33E-05 | <i>PTGS2</i>               | 1.3054  | 5.90E-06 | 0.00599  |
| <b>ATF3</b>                   | 1.6062 | 1.09E-09 | 9.22E-07 | <b>NPAS4</b>                      | 2.2737  | 4.40E-06 | 0.00372 | <i>GADD45G</i>                            | 1.7414 | 5.81E-08 | 4.91E-05 | <i>FOSB</i>                | 1.5304  | 9.64E-06 | 0.00815  |
| <i>JUNB</i>                   | 1.4066 | 2.36E-09 | 1.67E-06 | <i>ADAMTS1</i>                    | 1.0314  | 6.65E-06 | 0.00482 | <i>IER2</i>                               | 1.2020 | 9.91E-08 | 7.18E-05 | <i>DUSP6</i>               | 0.9922  | 3.29E-05 | 0.02382  |
| <i>RGS2</i>                   | 1.4974 | 2.80E-09 | 1.67E-06 | <i>CCN1</i>                       | 1.3156  | 9.48E-06 | 0.00601 | <i>THBS1</i>                              | 1.8039 | 1.14E-07 | 7.22E-05 | <i>HSPA1A</i>              | 1.6433  | 7.39E-05 | 0.04390  |
| <i>GADD45G</i>                | 1.3902 | 2.96E-09 | 1.67E-06 | <i>NR4A1</i>                      | 1.0643  | 1.54E-05 | 0.00868 | <b>NPAS4</b>                              | 3.1196 | 3.98E-07 | 2.25E-04 | <i>JUN</i>                 | 0.8117  | 8.45E-05 | 0.04390  |
| <i>HSPA1A</i>                 | 1.5433 | 3.32E-09 | 1.68E-06 | <i>FOSB</i>                       | 1.5493  | 1.88E-05 | 0.00951 | <i>DUSP6</i>                              | 1.1689 | 5.27E-07 | 2.67E-04 | <i>CCL4</i>                | 1.1057  | 8.66E-05 | 0.04390  |
| <i>EGR4</i>                   | 1.4966 | 8.59E-09 | 3.79E-06 | <i>RND3</i>                       | 0.8880  | 2.34E-05 | 0.01079 | <b>NR4A1</b>                              | 1.6955 | 7.58E-07 | 3.49E-04 | <i>FKBP11</i>              | -1.0158 | 1.84E-04 | 0.08205  |
| <i>NR4A1</i>                  | 1.4166 | 8.97E-09 | 3.79E-06 | <i>RGS1</i>                       | 1.1871  | 2.88E-05 | 0.01216 | <i>EGR4</i>                               | 1.6425 | 9.33E-07 | 3.94E-04 | <i>GM9958</i>              | -0.9378 | 2.12E-04 | 0.08205  |
| <i>PTGS2</i>                  | 1.3968 | 1.51E-08 | 5.88E-06 | <i>GADD45G</i>                    | 1.0496  | 3.97E-05 | 0.01547 | <i>RGS2</i>                               | 1.3091 | 1.09E-06 | 4.23E-04 | <b>NR4A1</b>               | 1.1258  | 2.21E-04 | 0.08205  |
| <i>BTG2</i>                   | 1.2922 | 2.79E-08 | 1.01E-05 | <i>DUSP6</i>                      | 0.8763  | 4.39E-05 | 0.01547 | <i>JUN</i>                                | 1.1095 | 1.17E-06 | 4.23E-04 | <i>CCRL2</i>               | 0.9681  | 2.27E-04 | 0.08205  |
| <i>EGR2</i>                   | 1.3976 | 4.45E-08 | 1.51E-05 | <i>GALNT12</i>                    | -1.1040 | 4.58E-05 | 0.01547 | <i>AKAP12</i>                             | 0.9770 | 1.51E-06 | 5.10E-04 | <i>CCN1</i>                | 1.2480  | 2.56E-04 | 0.08651  |

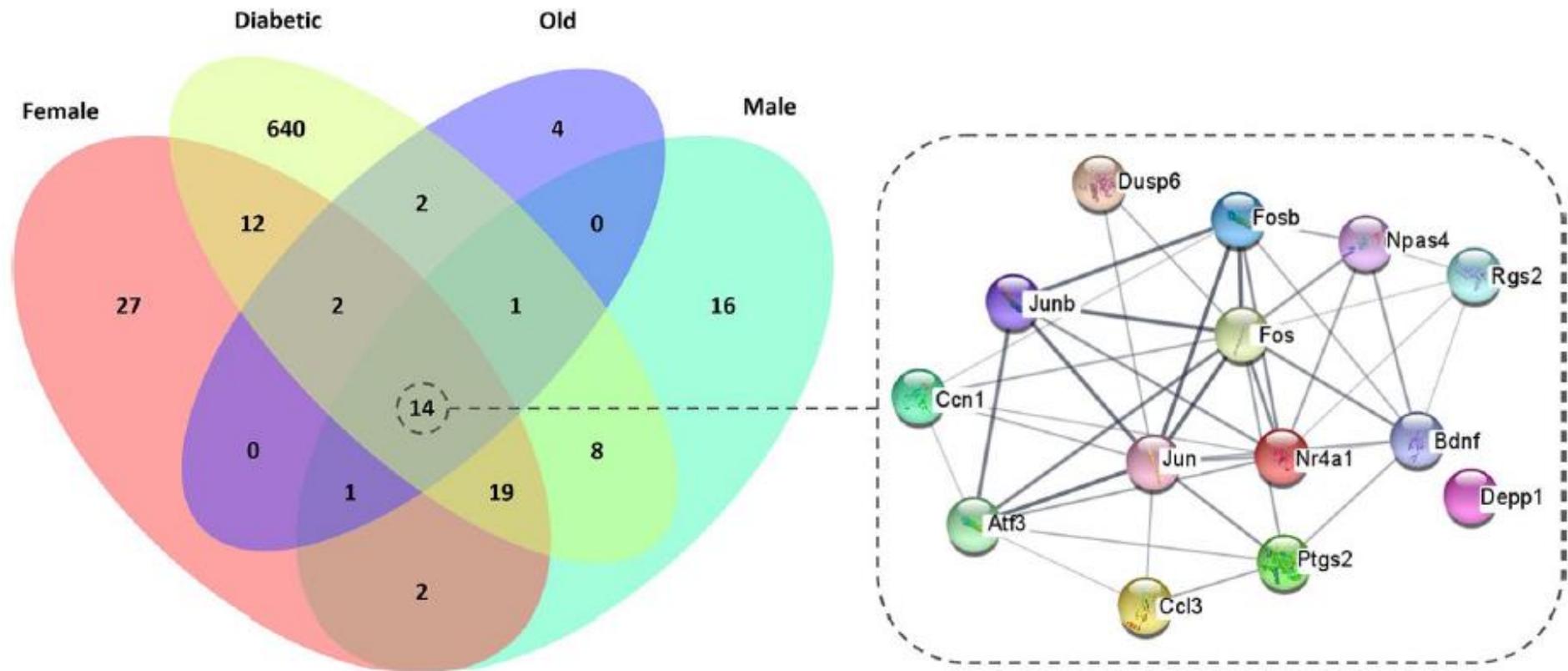
## PROTEÓMICA

## Proteínas diferencialmente expresadas

**Table 2** Top 15 proteins differentially expressed (FDR < 0.25) between the ipsilateral hemisphere and the contralateral hemisphere 2 h after cerebral ischemia induction FDR false discovery rate; logFC Logarithmic fold change

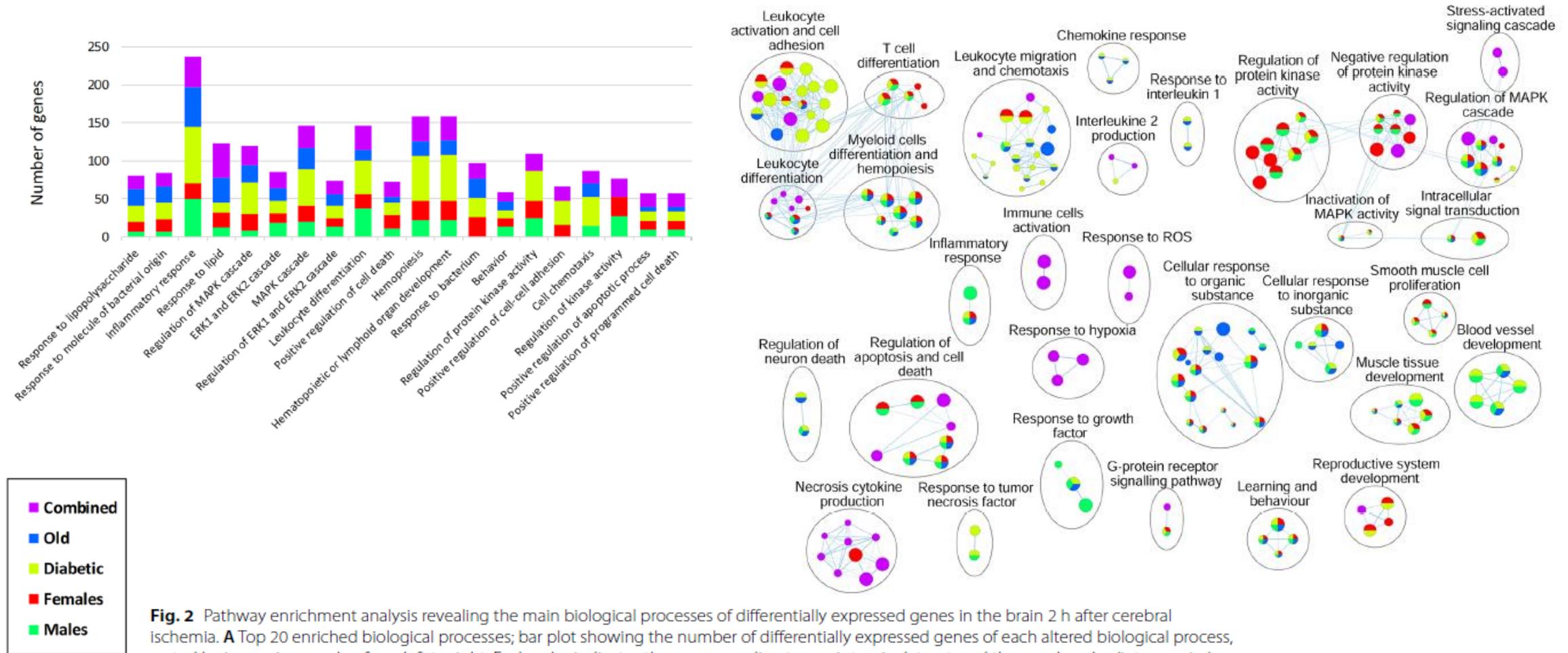
| Young male mice, <i>males</i> |         |          |          | Young female mice, <i>females</i> |         |          |        | Diabetic young male mice, <i>diabetic</i> |         |          |          | Aged male mice, <i>old</i> |       |         |     |
|-------------------------------|---------|----------|----------|-----------------------------------|---------|----------|--------|---|---------|----------|----------|----------------------------|-------|---------|-----|
| UNIPROT                       | logFC   | P.Value  | FDR      | UNIPROT                           | logFC   | P.Value  | FDR    | UNIPROT                                   | logFC   | P.Value  | FDR      | UNIPROT                    | logFC | P.Value | FDR |
| Q8CI32/Bag5                   | -0.3250 | 2.82E-08 | 7.01E-05 | Q8BKX1/Baiap2                     | 0.4799  | 1.62E-05 | 0.0413 | Q8VBY2/Camkk1                             | -0.4021 | 1.55E-07 | 3.85E-04 |                            |       |         |     |
| P11798/Camk2a                 | -0.2119 | 9.70E-05 | 0.1205   | Q8CGY8/Ogt                        | -0.2743 | 1.29E-04 | 0.1068 | O70433/Fhl2                               | -0.6287 | 5.24E-07 | 6.51E-04 |                            |       |         |     |
| Q6PHZ2/Camk2d                 | -0.1114 | 3.09E-04 | 0.2427   | Q920P5/Ak5                        | -0.2659 | 1.43E-04 | 0.1068 | Q62318/Trim28                             | -0.2786 | 1.21E-06 | 0.0010   |                            |       |         |     |
| O35927/Ctnnd2                 | -0.0972 | 3.91E-04 | 0.2427   | F6SEU4/Syngap1                    | 0.4880  | 1.93E-04 | 0.1068 | Q8R570/Snap47                             | -0.4342 | 6.25E-06 | 0.0037   |                            |       |         |     |
|                               |         |          |          | P68404/Prkcb                      | -0.2485 | 2.09E-04 | 0.1068 | P27005/S100a8                             | 0.7479  | 7.42E-06 | 0.0037   |                            |       |         |     |
|                               |         |          |          | P08414/Camk4                      | -0.3514 | 4.61E-04 | 0.1680 | P08414/Camk4                              | -0.5602 | 9.31E-06 | 0.0039   |                            |       |         |     |
|                               |         |          |          | Q9Z2Y3/Homer1                     | 0.2486  | 5.01E-04 | 0.1680 | Q64337/Sqtm1                              | -0.4070 | 1.33E-05 | 0.0041   |                            |       |         |     |
|                               |         |          |          | E9Q0K9/Elmod1                     | -0.3820 | 5.27E-04 | 0.1680 | Q9WV34/Mpp2                               | -0.2949 | 1.34E-05 | 0.0041   |                            |       |         |     |
|                               |         |          |          | Q8BFT9/Svop                       | 0.2494  | 7.22E-04 | 0.2046 | Q8BFR5/Tufm                               | -0.3072 | 1.68E-05 | 0.0046   |                            |       |         |     |
|                               |         |          |          | Q61329/Zfhx3                      | 0.1805  | 8.62E-04 | 0.2200 | Q8K0E8/Fgb                                | 0.8275  | 1.99E-05 | 0.0049   |                            |       |         |     |
|                               |         |          |          | Q8VEA4/Chchd4                     | 0.2649  | 1.01E-03 | 0.2201 | Q3UHD6/Snx27                              | -0.5525 | 2.30E-05 | 0.0049   |                            |       |         |     |
|                               |         |          |          | E9QK62/Ngef                       | -0.2693 | 1.04E-03 | 0.2201 | Q68FF6/Git1                               | -0.4539 | 2.38E-05 | 0.0049   |                            |       |         |     |
|                               |         |          |          | P97822/Anp32e                     | -0.1824 | 1.19E-03 | 0.2332 | Q920P5/Ak5                                | -0.5258 | 2.73E-05 | 0.0052   |                            |       |         |     |
|                               |         |          |          | O88737/Bsn                        | 0.2131  | 1.28E-03 | 0.2332 | P68404/Prkcb                              | -0.2960 | 3.87E-05 | 0.0069   |                            |       |         |     |
|                               |         |          |          |                                   |         |          |        | P04627/Araf                               | -0.4653 | 4.54E-05 | 0.0070   |                            |       |         |     |

## Análisis integrativo de los resultados transcriptómicos

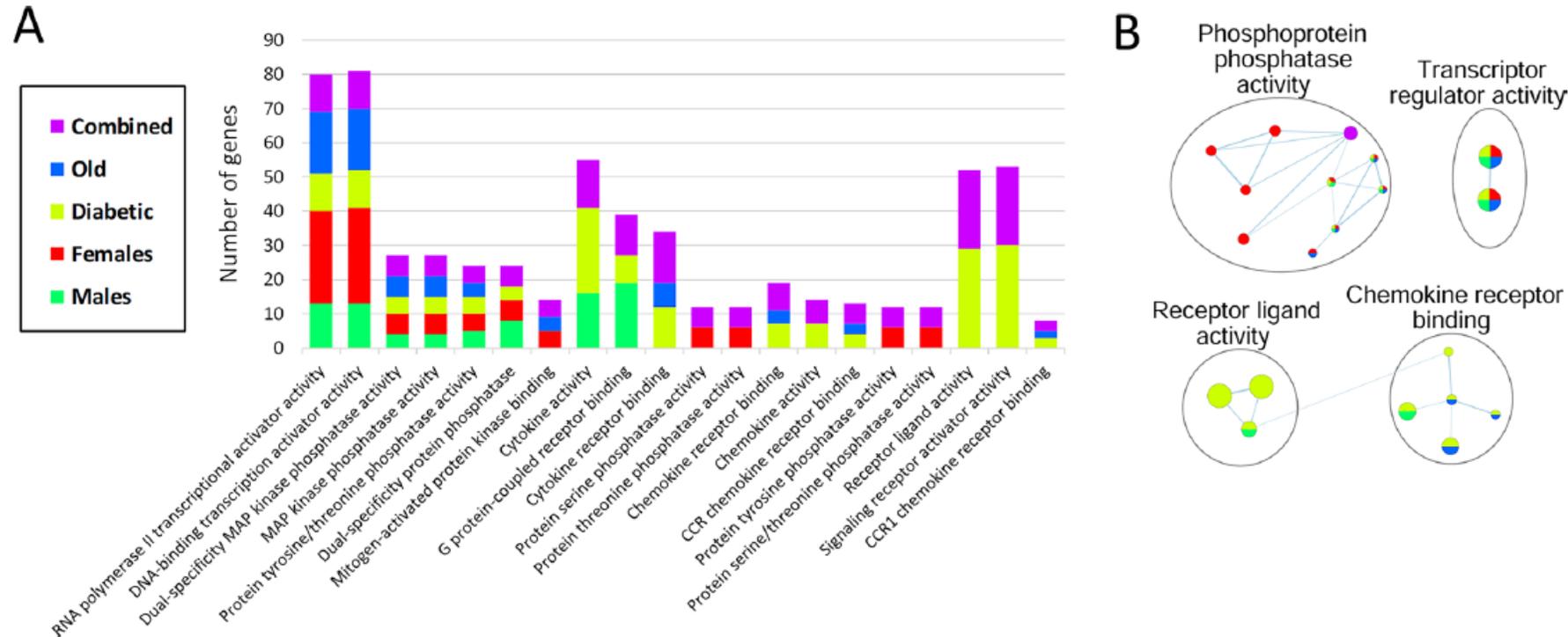


**Fig. 1** Venn diagram of differentially expressed genes in the ischemic brain (FDR < 0.25) in all studied groups. The 14 common differentially expressed genes are shown through a STRING network revealing the main interactions between molecules. The strength of the correlation between two genes (nodes) is reflected by the thickness of the edge. Diabetic: diabetic young male mice; Female: young female mice; Male: young male mice; Old: aged male mice

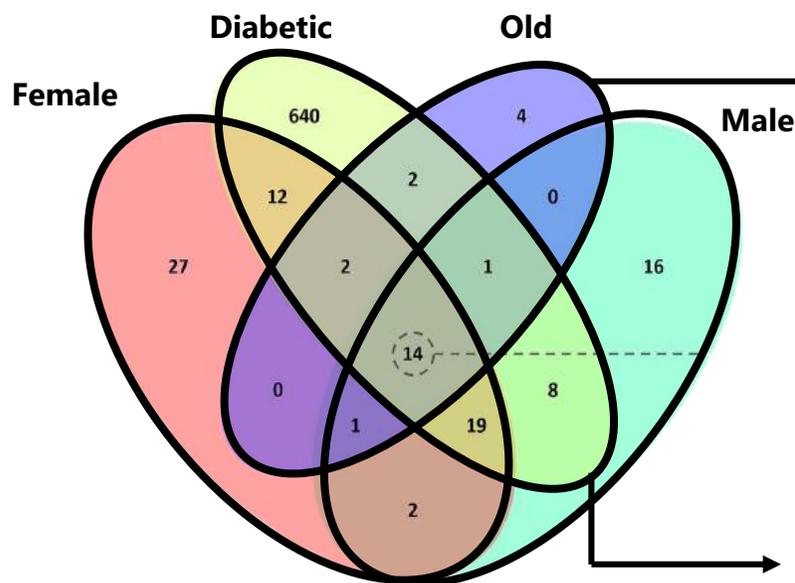
## Análisis integrativo de los resultados transcriptómicos: *procesos biológicos*



## Análisis integrativo de los resultados transcriptómicos: *funciones moleculares*



## TRANSCRIPTÓMICA



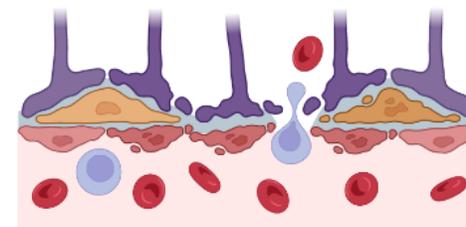
Edad

- “Ratones de edad avanzada ↑ DEG 3 días después del evento isquémico” *Androvic P, et al (2020)*
- En la fase hiperaguda (2h) los ratones de edad avanzada tienen ↓ DEG que los jóvenes  
→ La respuesta transcripcional tras la isquemia es un proceso lento que se puede exacerbar en tiempos más tardíos.

Comorbilidades

Obesidad, diabetes e hipertensión → factores de riesgo para presentar peor outcome

- Ratones diabéticos ↑ DEG → activación y modulación de procesos biológicos:
  - Adhesión celular
  - Activación leucocitaria

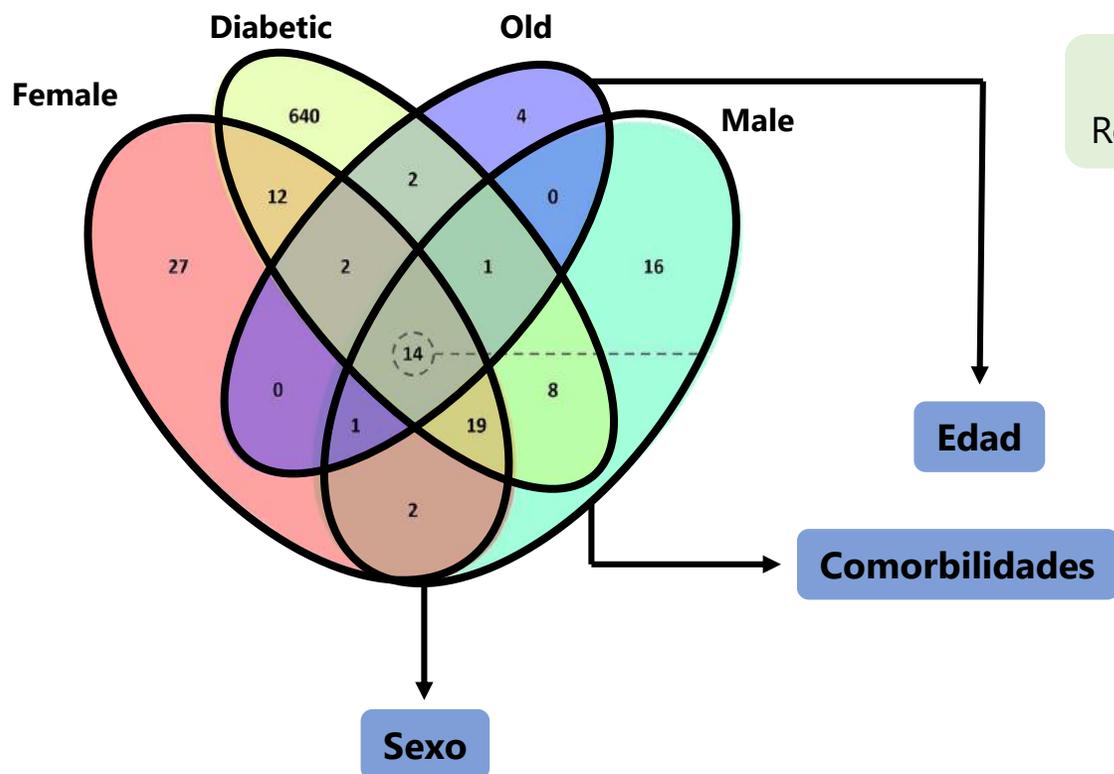


Daño en la BHE

Sexo

- Factores genéticos/epigenéticos
- Activación diferencial de programas de muerte celular
- Respuestas del SI

**TRANSCRIPTÓMICA – ANÁLISIS VÍAS ENRIQUECIMIENTO**



DEG ♂ > ♀

Alteración debido a hormonas

**INFLAMACIÓN**  
Rol dual en la respuesta al ictus

**HEMOPOYESIS**  
Tras el ictus ↑ las células innatas del SI (HSC)

↑ daño BHE:  
transformación hemorrágica,  
daño por reperusión, edema...

↓ funcionalidad de las HSC  
↓ células T



La respuesta molecular al ictus varía en función del fenotipo de los animales, lo que refuerza la necesidad de seguir las recomendaciones STAIR a la hora de diseñar futuros estudios para mejorar la traducción "*from bench to bedside*".

Stroke  
Treatment  
Academy  
Industry  
Roundtable



Ambos sexos

Edad avanzada

Comorbilidades

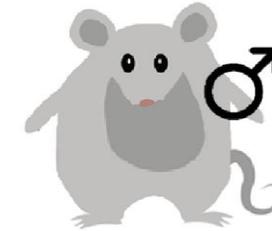
**1) Variabilidad inter-ensayo****2) Consideración de otros fenotipos****3) Extrapolación temporal ratón – humano ?****4) Evaluación de los cambios a nivel proteómico en etapas tempranas****5) No evaluación del volumen de infarto**

8 jóvenes (8-12w)  
4 sham

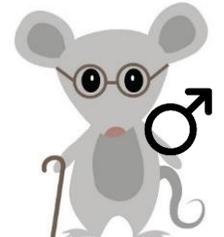
*Simats et al (2020)*



8 jóvenes  
4 sham



8 jóvenes db/db  
4 sham



8 edad avanzada (18 m)  
4 sham



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Daisy Guamán  
Berta Paez  
Alejandro Ballvé

**Investigadora post-doctoral**

Anna Bonaterra

**Estadístico**

Marcel Lamana

**Enfermero**

Jesús Pizarro