A deeper dive into DCS resistant rats miRnome and transcriptome pathway analysis.

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Introduction / Background: Susceptibility to decompression sickness is characterized by a wide interindividual variability which origin is still poorly understood. This hampers reliable prediction of DCS by decompression algorithms. We previously selectively bred rats with at least a 3-fold greater resistance to DCS than standard ones after 6 generations. In order to better understand DCS mechanisms, we conducted a *Reactome* Pathway analysis from hepatic transcriptome (on microarray) and circulating miRnome (on PCR plates). Our transcriptomic results are pointing to inflammatory and immune responses with gender differences, while our miRnome results show no gender differences and mainly point to 3 pathways: miRs biogenesis, Miro GTPase Cycle and signaling by RAS-like families.

Materials and Methods: We used a transcriptomic approach (on microarray from liver samples) coupled and with a circulating miRnome study (PCR plates); from 4 males, 4 females Wistar rats) compared to DCS-resistant rats (4 males, 4 females) Bioinformatic tools : we used *Reactome* for both and we added *GeneCards* for the transcriptome part.

Results: Our hepatic transcriptome results highlight similar pathways related to the differentially expressed genes. Among the major pathways to which they point, we can cite inflammatory or immune responses (Tef, Vsig4, Nr1d1, Zcchc7, Cyp2e1, Cyp1a1, or Fnbp4), Circadian rhythm, (which might also influence inflammatory processes with Arntl, Nr1d1, Per2, Nfil3, or Nr1d2), complement cascade (Vsig4, Arntl or Nr1d1) cell signaling (Vsig4, Arntl, Fnbp4, Sstr3, Epha5, Amtn, Zcchc7, Nr1d1 or Rgs2), or cytoskeleton dynamics for Fnbp4.

Among the 51 miRs differentially expressed in our circulating miRnome results, 50 showed the same 3 pathways: miRs biogenesis, Miro GTPase Cycle and Signaling by Rho GTPases, Miro GTPases and RHOBTB3 (which are part Ras family or RAS-like families). The last miR, rno-miR-1-3p, seems to be also involved in 4 other pathways: Progressive trimming of alpha-1,2-linked mannose residues from Man9/8/7GlcNAc2 to produce Man5GlcNAc2, CLEC7A (Dectin-1) induces NFAT activation, CLEC7A (Dectin-1) signaling and Synthesis, secretion, and inactivation of Glucagon-like Peptide-1 (GLP-1).

Summary / Conclusions: For the first time on our resistant rats' studies, we obtain similar pathways from males and females, and these results might help to better understand the gene expression and phenotype differences from our previous studies.

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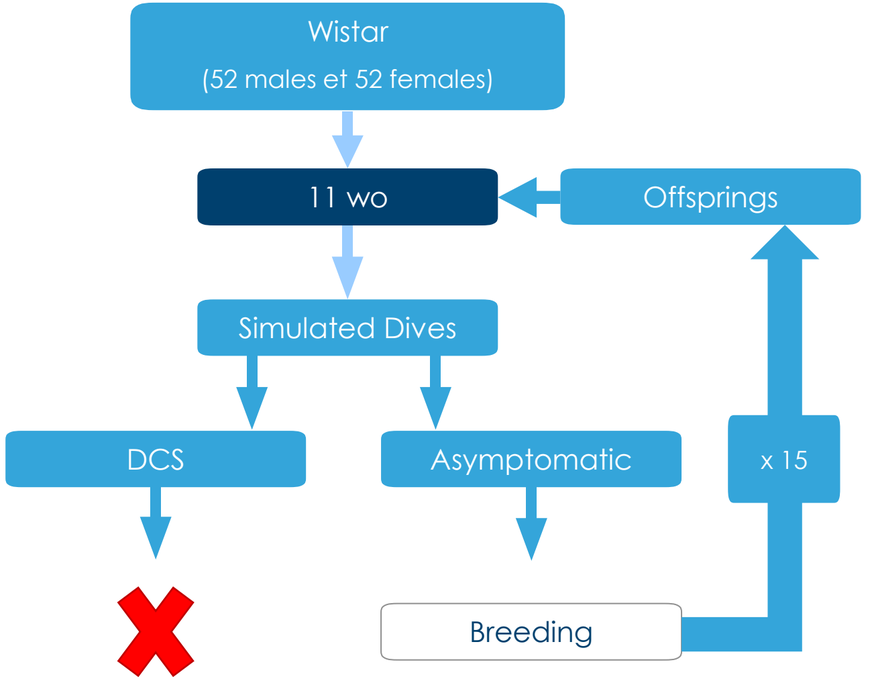
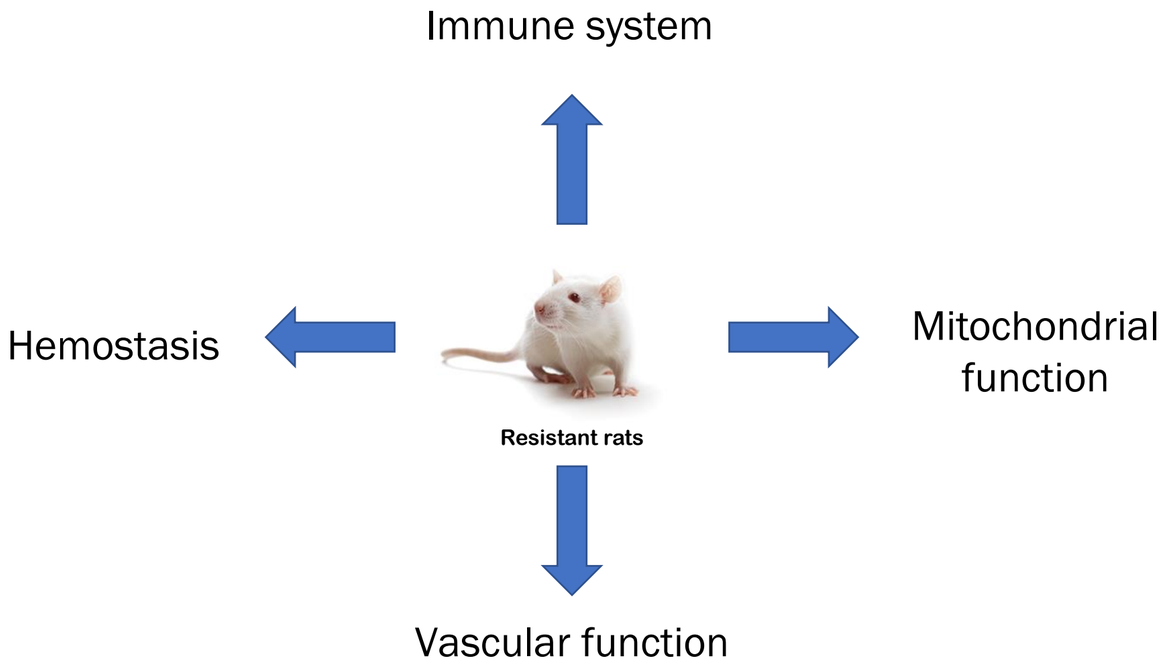
Selection protocol



Phenotypic characterization

<u>Objective:</u>

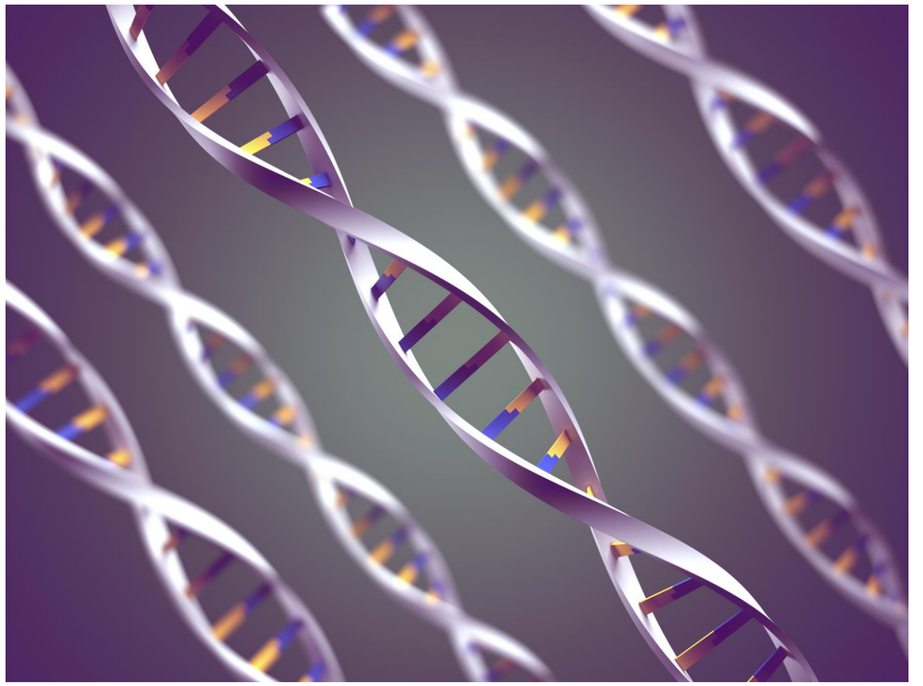
- Comparison between Resistant vs Wistar rats
- From genes to phenotype ?



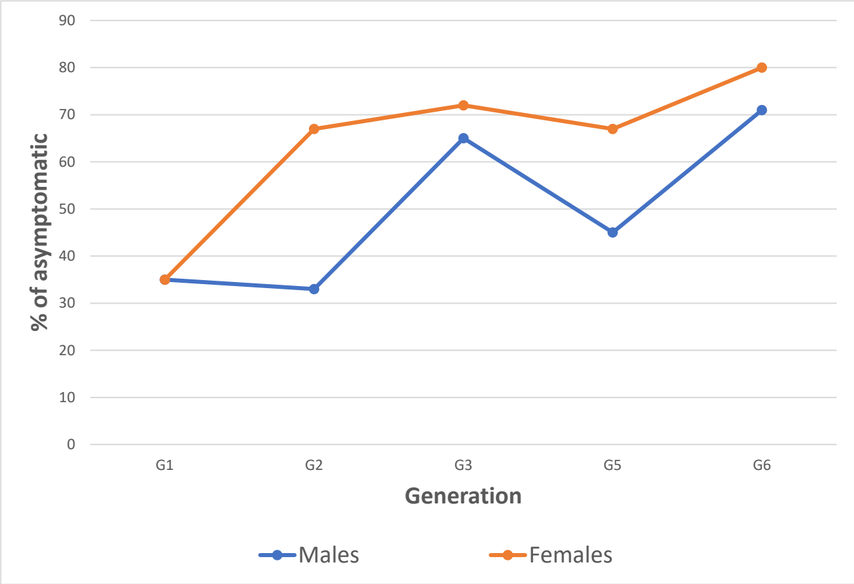
Molecular characterization

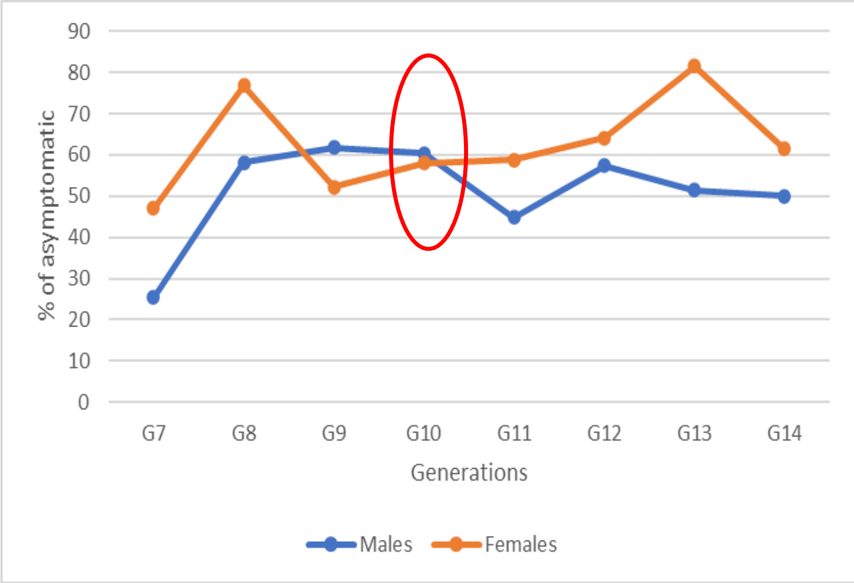
www.biorigami.com
QIAGEN NGS

- Enzymatic activities of antioxidant genes
- Transcriptomic study (liver)
- Epigenetic study (blood)
  - miRnome



AIPMT Biology: Mastering the Genetic Code | Testprep Content Hub (meritnation.com)





	Res M	Res F	Res
body mass	-	-	-
Neutrophils	+		
Neutro/Lymphocytes			+
coag factor X	+		+
mito O_2 cons	-		-
endo ind vasodil	-	-	-
SNP on CHR X		≠	≠
SNP regulating MyD88 or NFKB1	≠		≠
fecal metabolomic signature	≠	≠	≠
MBP after Ach admin	-	-	-
DBP and MBP after NorA	-	-	-

Lautridou et al, *Med.Sci. Sports Exerc,* 2017

Lautridou, Dugrenot et al, *J Appl Physiol,* 2020

Vallée et al, *Sc. Reports*, 2021

Dugrenot et al, *DHM*, 2022

Vallée et al, *Frontiers Physiol*, 2023

Dugrenot et al, *Comms Bio*, 2024

Hepatic transcriptome

Res F (n = 4)	Res M (n = 4)	Std F (n = 4)	Std M (n = 4)

Anesthesia and analgesia

↓

Liver Sampling (- 80 °C)

↓

Grindings and purification

↓

Extraction, RT and amplification

↓

DNA chips reading and PCR validation

https://www.agilent.com/en/product/gene-expression-microarray-platform/gene-expression-exon-microarrays/human-microarrays/human-gene-expression-microarrays-228462

Study of circulating miRnome

Res F (n = 4) | Res M (n = 4) | Std F (n = 4) | Std M (n = 4)

Anesthesia and analgesia

↓

Liver Sampling (- 80 °C)

↓

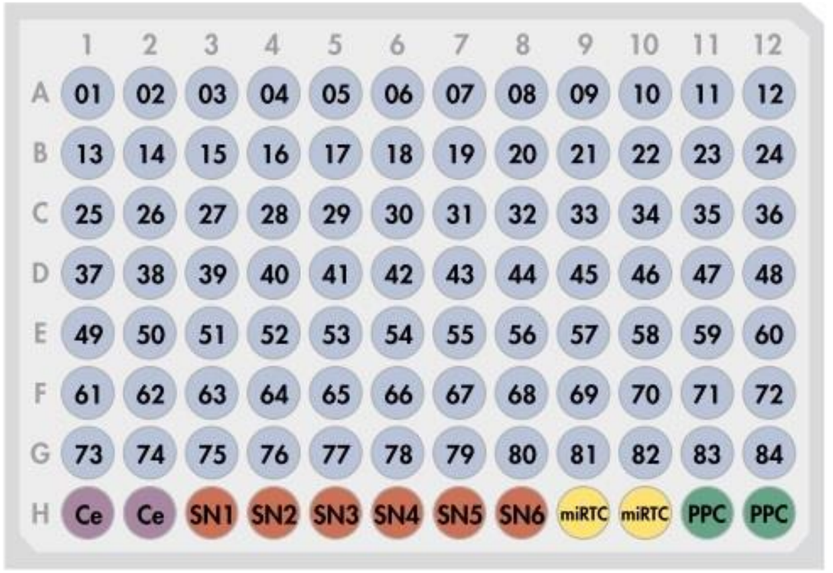
Grindings and purification

↓

Extraction, RT-qPCR

↓

PCR plates reading



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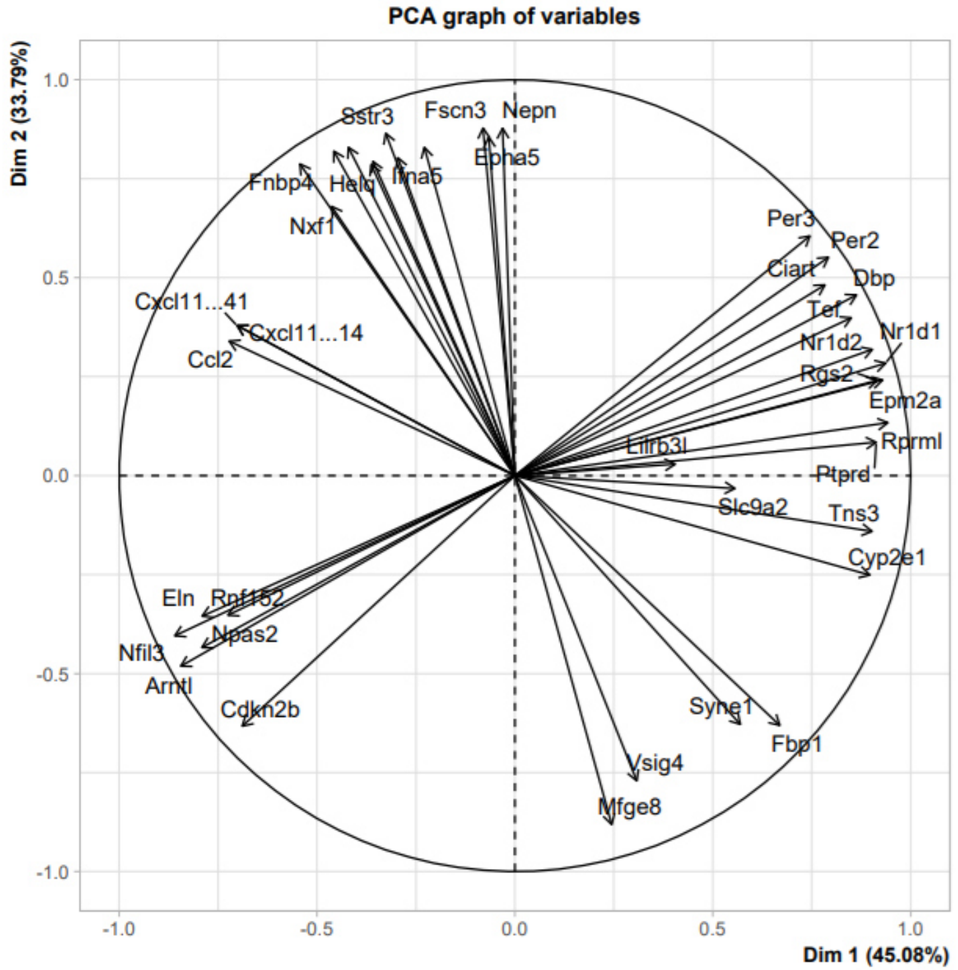
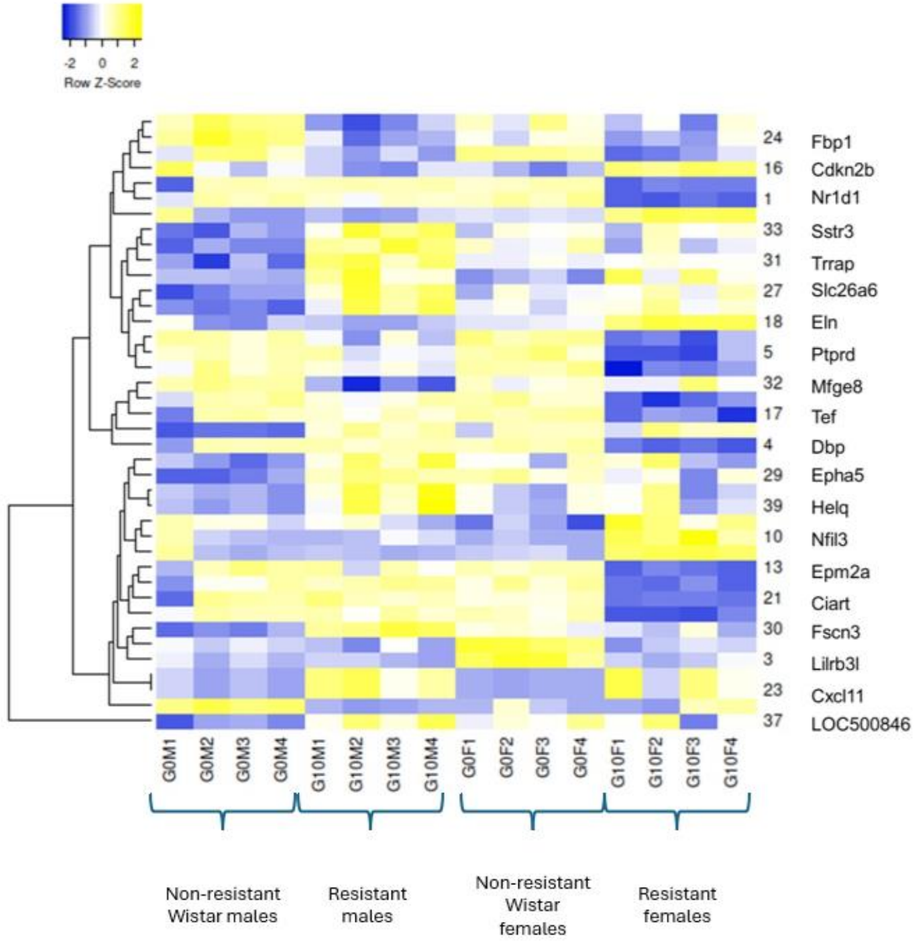
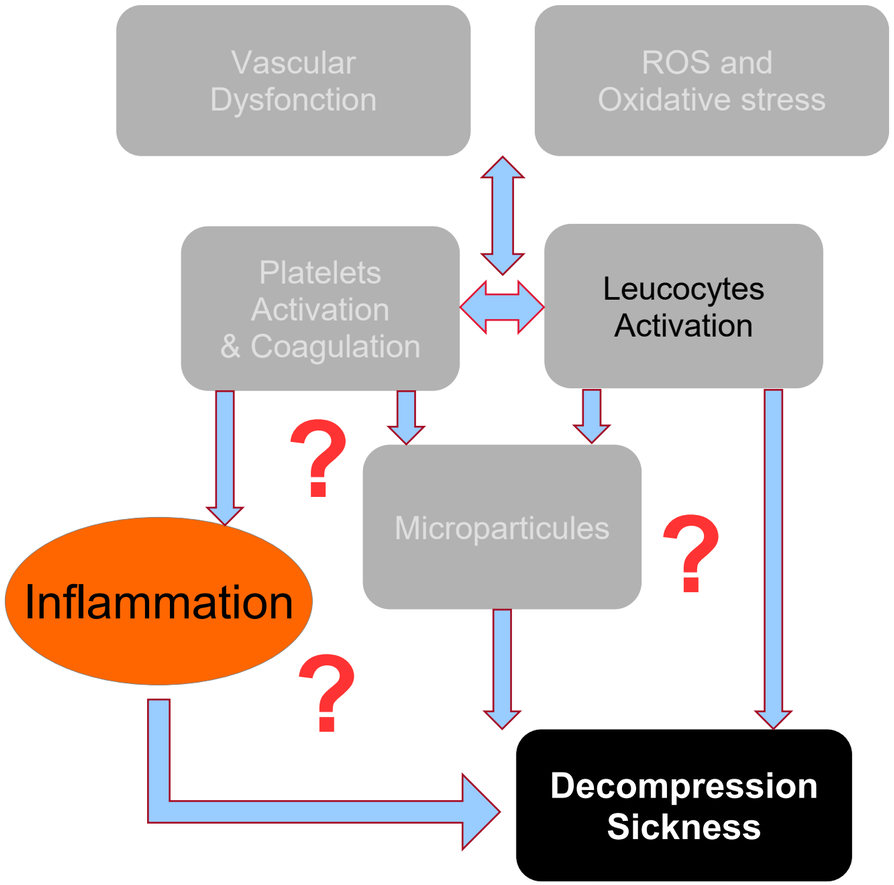


Principal Component Analysis of the most significant differentially expressed genes
(Graph of variables or correlation circle; n=4).



Clustered Heat Map of the most significant differentially expressed genes (n=4).

- Leucocytes count (↗ neutrophils)
- **Rno-miR-128-3p** → Nxf1 → **activation of TLRs 2 and 4 through HSP70**
- **HSP70** is involved in the quaternary structure of the TTR
- **rno-miR-10b-5p** → Nr1d2 (inflammation, mitochondrial biogenesis, etc.)



miRnome vs-transcriptome

inflammation, cell signaling and motility

Gene Ontology

circadian rhythm and inflammation

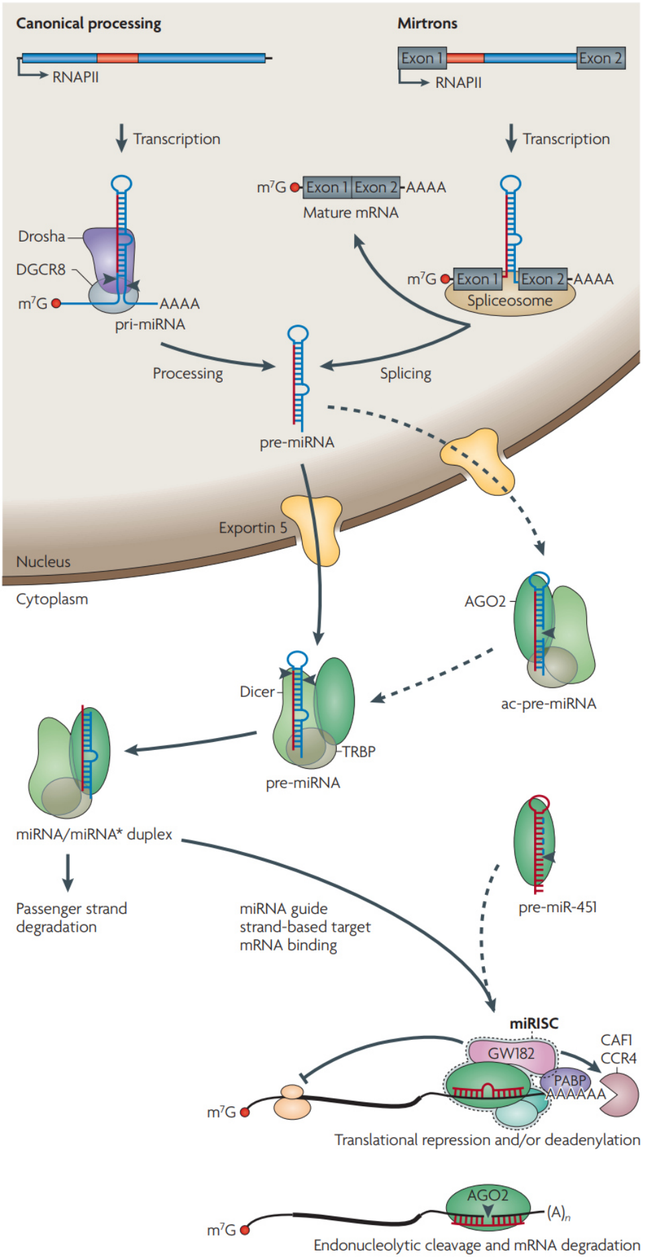
Pathway Analysis (*Reactome*)

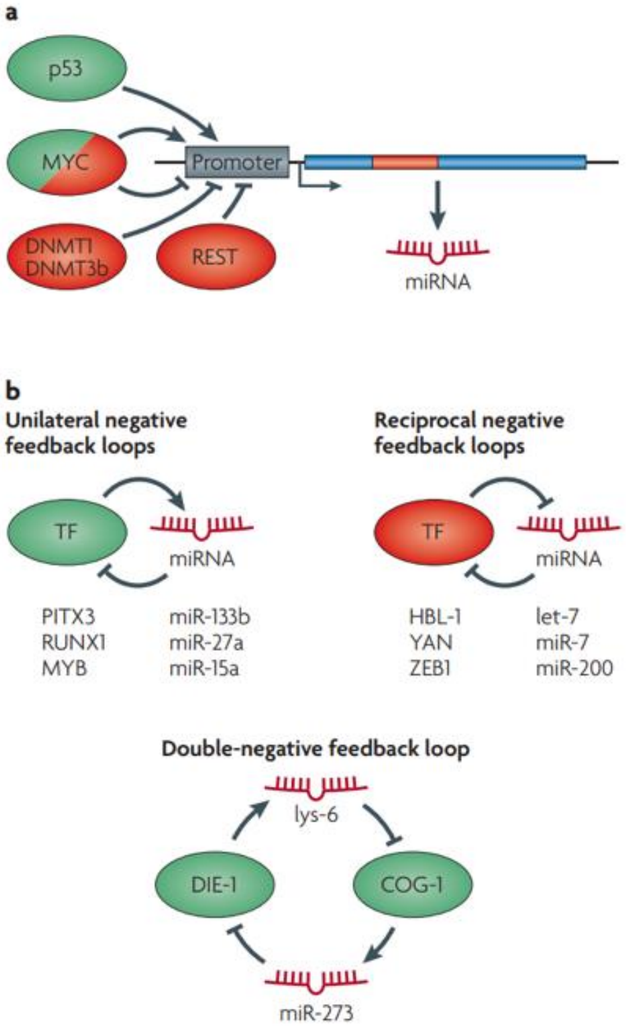
- Hepatic Transcriptome
  - Immune responses (*Tef, Vsig4,Nr1d1, Zcchc7, Cyp2e1, Cyp1a1, Fnbp4*)
  - Circadian rhythm (*Arntl, Nr1d1, Per2, Nfil3, Nr1d2*)
  - Cell Signaling (*Vsig4, Arntl, Fnbp4, Sstr3, Epha5, Amtn, Zcchc7, Nr1d1, Rgs2*)

- Circulating miRnome
  - 50 of the 51 differentially expressed miRs show the same 3 pathways:
    - miRs biogenesis
    - Miro GTPase Cycle and Signaling by Rho GTPases
    - Miro GTPases and RHOBTB3 (Ras and Ras-like families)
  - Rno-miR-1-3p also involved in 4 other pathways

- If we extend this analysis to human miRs, we obtain about 30 more pathways, mostly involved in cell migration, innate immune responses, ROS production or cell signaling

miRs 101





Krol, et al, *Nature Reviews Genetics* 11, nº 9 (septembre 2010): 597-610.

miRs :

- About 20 bp
- Originate from a coding sequence or an Intron
- Matured in the cytoplasm
- 30 to 50% of protein coding genes are targeted by miRs
- Each gene might be regulated by many miRs
- Each miR has up to hundred targets

Actions :

- Translational repression
- Target degradation
- Regulated by enhancers and silencers
- Involved in retro-control loops

miRs and sex differences

- Sex (= biological determinants) ≠ Gender (include socio-cultural matter)

- High number of miRs on the X CHR
  - 118 compared to 2 on the Y CHR
    - Most of them are involved in immunity regulation
  - An average of 40 to 50 miRs on the autosomes
- “The female immune system appears more efficient in a number of species, including humans”, *Klein and Flanagan, 2016.*

Learn with Posters : Difference between Sex and Gender - Fuzia

Ophn1 gene

- At the 6th generation, we observed an allele frequency shift on a SNP located on the X CHR. 2 genes are located in 5’ and 3’ of this SNP:
  - The Ar gene (for Androgen Receptor) is involved in signaling pathways: TGF-beta and G protein-coupled receptors (GPCR).
  - The Ophn1 gene interacts with Rho proteins and plays a role in transduction signals, cell migrations, inflammation and apoptosis, as well on platelet aggregation and thrombosis.

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RESEARCH ARTICLE

Physiological characteristics associated with increased resistance to decompression sickness in male and female rats

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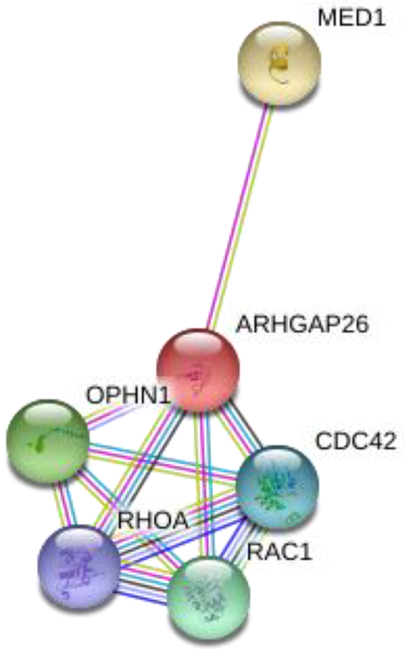
Table 1. *Sequence and chromosomal localization of the polymorphic SNPs*

Chromosome	Localization	Sequence
9	28408403–28408483	ACCCCACCCCAACATTCTCCTTTAAAAACACTCCGAATCC[C/T]GCCTATGACTGTCATTTTCTATCCTGCTCATTTAGAAGTT
10	33090635–33090715	CTGAAAAATTTGCTATCAAAGACTTAATGACTATTTAAAA[T/C]CTCTTTCTATTCTTTGAGAGTTCGTATATGAGTATAATGT
11	20635967–20636047	TGACATAAGCATTGTATATACATTGTCAGCATTCACAGCA[T/C]CTTAAAATAATATATACAAAACAGAATCAATTTGAAAATA
12	24177615–24177695	GACTCCAGGCTGAAGCTGAGTCCTTATGGGAGATGGAGGA[T/C]GGCAGGGTCTGAGGCGCTGAGAGCCCCAGACACACTAGCC
15	21828053–21828133	ATCCGTGACGCGGCCTTGGAGAGGGTCTGCTGATAGTGAT[A/G]ACTGAGCCAAAAAGAAAGAGTAGTGTAGAGTGGACAAGGT
17	31376660–31376740	AATAATATGGTAGAGCCTAAATCATCATGGAGTTTATCTC[T/C]CACACTTTGTCATATATGAGTCTGACCAGAGCTCCCCAGT
18	16921137–16921217	TGCAACTACATCACACTGAGGTTCCTCACAGCCCTCACCT[A/G]GCTGCTGAGTCCTCGGTTATTGCTAGCTGCCTTCTGGGAG
X	63759801–63759881	AACCACATTGAGGTGTGATTTTGCAAATTGTCCATATCAC[A/G]ACCTTCACCGTTTGTCACCCAGTTCTCTAATCCAAGTTAC
X	37110132–37110212	ACTGCATGTTCCAGGTAAAATGTGTTTCTAGGTCTTGGTA[A/G]ATAACACAGAAGAATGAGAATTGACTAGAAGGAAGGGCTG
X	16143211–16143131	TGGGTATGGGGGCATGGGAGACACAGAAGGACAGAAAAAC[A/G]GAACAGCAGCCTAAGGTTCCTGTGTAATGTCAGTTCTTAA
X	105875099–105875019	TCCACAATGGAACATACACACTGTGGGTCTCAGCATAAGA[T/C]GTGGGTGGGGCCTGGCCAGATAGCTGGAAGAAACAATACT
X	89753705–89753785	AGTGTATAATATGAGAACGTTCTCATTAGTAGTTATTATT[C/T]CATGACTATCAATGATAGAAACCACTGACTTAAATTCTT
X	80329108–80329188	AGTTTGTGCATTAGTGTTAATCTTATGATGTTGTTTCTTC[T/C]GTAACAATATCATGGGTTTCAAAATTAAGAAACTAATAAT
X	130861798–130861878	TCCTTATACATCTTTGTGGACTGTAATAGCCTTTCTTAGG[G/A]AGAGATTATTGTAAATGAAGCGCTAATTGCAGTCTACTTC
X	18038405–18038485	GGGCTCTTCTTACTGATTTATTCAATTAAAAGAGTATTTT[T/C]ATCTCAGGTTCTCTAATAAATGAAACATAACAATGTTTCT
X	86039984–86040064	CTCAACGTCTGAATTGAACTTCTACCAGGACTGTCCTACA[C/T]TCTGGAAACATATGTATCTTTTTTAGTAAGAAGAATGTGA
X	93189573–93189653	GAAAAGTCCAGAAGTTGCTGCATATTTTCTAAGCAATTAG[T/G]TGGTTCTGGGTGTGCCCTCTCTGGCTCTACTGTAAAATTA
X	134914274–134914354	TGTAACATTATAACACAGAAGAGACAATCTAATACAGCTG[T/C]AAAGTCAAGGGAAGTGATTCCTAGAAGAAAGGACACTTAA

SNPs, single nucleotide polymorphisms.

Ophn1 gene

- Interacts with Rho proteins which are part of the Ras-like families.
  - The Ras superfamily refers to a large group of GTPases, including Ras, Rho, Ran, Rab, Rheb, and ARF.
  - Originally discovered to be mutated in cancer, and now known to also regulate non-oncogenic processes such as immunity and inflammation.
- The human **Oligophrenin-1** (OPHN1) gene is also located on the CHR X
- All the differentially expressed miRs were involved in Rho and Ras-like signaling
  - “The ability of miRs to regulate such a vast amount of the genome with a high degree of specificity makes them perfectly poised to play a critical role in programming of the sexually dimorphic brain”, *Morgan and Bale, 2012.*

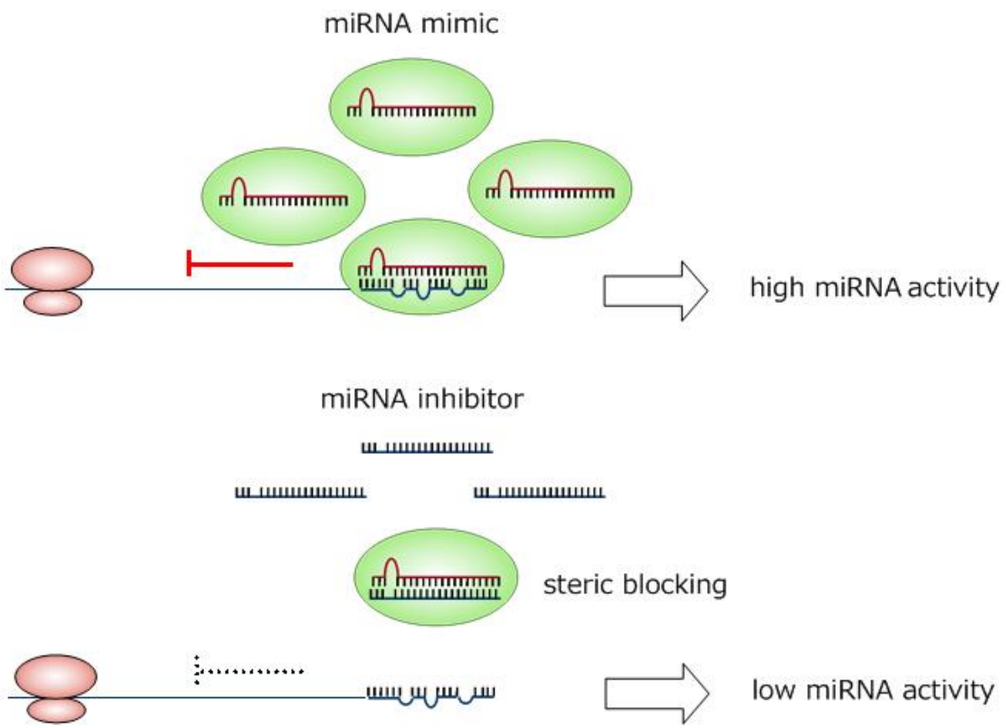


Conclusion

	Res M	Res F	Res
body mass	-	-	-
Neutrophils	+		
Neutro/Lymphocytes			+
coag factor X	+		+
mito O_2 cons	-		-
endo ind vasodil	-	-	-
SNP on CHR X		≠	≠
SNP regulating MyD88 or NFKB1	≠		≠
fecal metabolomic signature	≠	≠	≠
MBP after Ach admin	-	-	-
DBP and MBP after NorA	-	-	-

- SNP on CHR X allelic frequency was not modified in Resistant males
- SNP regulating MyD88 or NFKB were not modified in Resistant females
- Can the miRs explain why so many male vs female differences in our resistant animals AND their common resistance to DCS?

Perspectives



https://upload.wikimedia.org/wikipedia/commons/a/a9/MiRNA_mimic_and_miRNA_inhibitor.png

miRs mimics and antago-miRs

→ rno-miR-128-3p ?

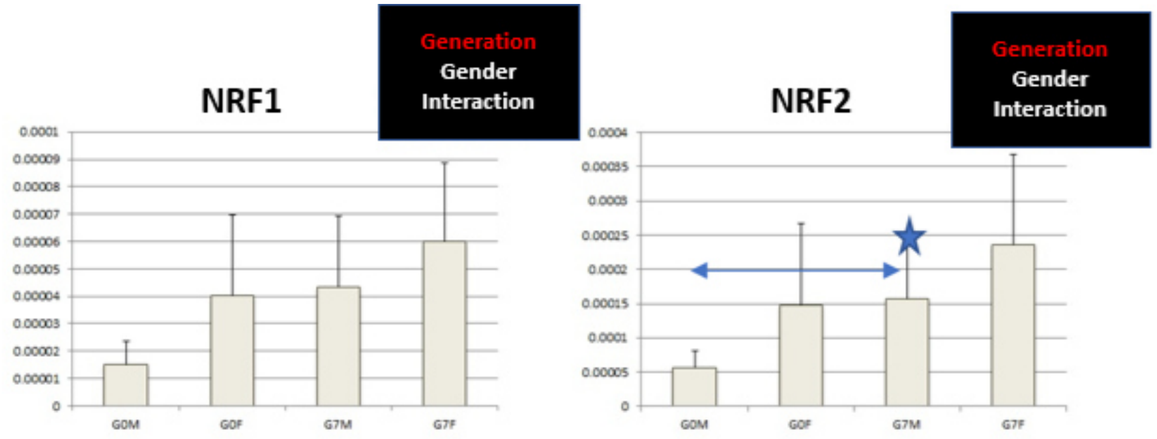
→ rno-miR-10b-5p ?

→ rno-miR-200c-3p ?

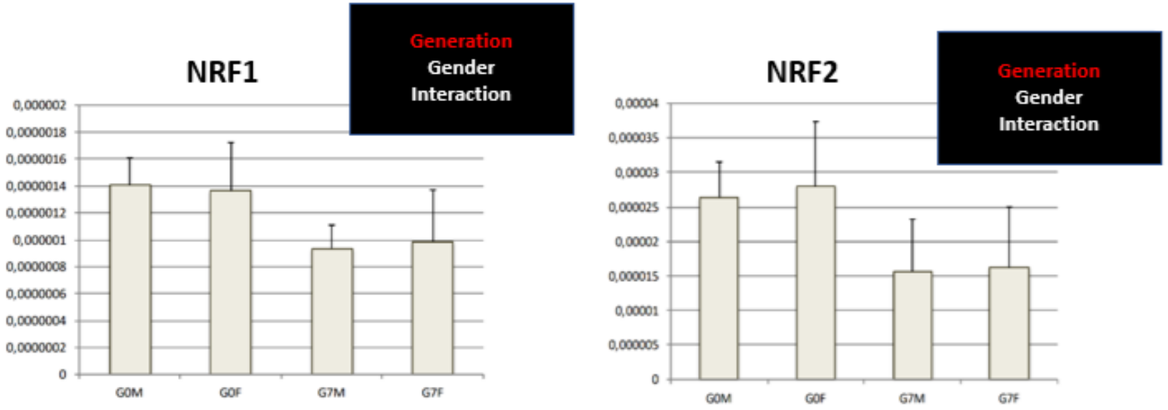
Effects and the transcriptome and the phenotype?

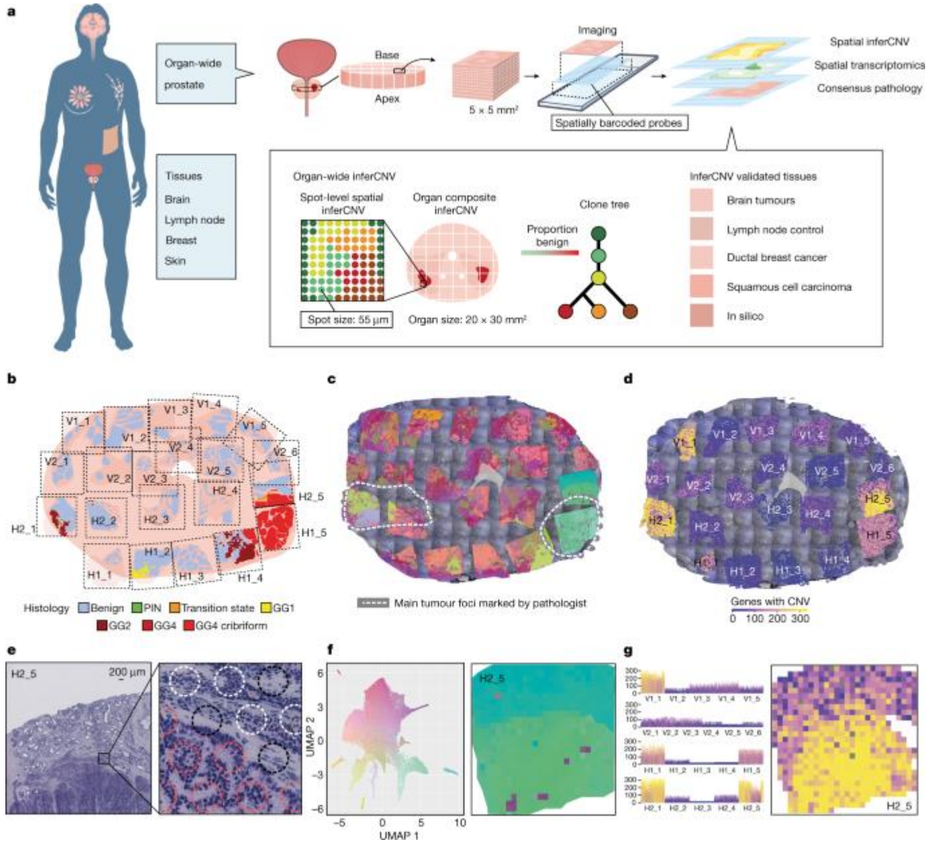
Perspectives

Lung's antioxidant mRNAs



Liver's antioxidant mRNAs





Erickson, A., He, M., Berglund, E. *et al.* Spatially resolved clonal copy number alterations in benign and malignant tissue. *Nature* **608**, 360–367 (2022). https://doi.org/10.1038/s41586-022-05023-2

Perspectives

Most Women SCUBA Diving Together: World Record Attempt - Indonesia Travel

- Are women really more at risk regarding to DCS? Or the opposite?
- What is the role of the Immune system in that possible DCS susceptibility difference?
- What is the role of the mitochondrial DNA transmission in DCS susceptibility?

Thank you for your attention!

1/

Hello everybody,
My name is Emmanuel Dugrenot, I am a senior researcher at the Divers Alert Network, I have no conflict of interest to declare and today I am going to present a work on Decompression sickness (or DCS) resistant rats' miRnome and transcriptome pathway analysis, with ORPHY's laboratory at the University of Western Brittany.

This work is the continuation of what I presented the past 2 years at EUBS and UHMS, and sorry if you get bored of them, but I will again talk about the rats we have selected for their resistance to DCS.

2/

So, once more! To better understand DCS mechanisms, our team embarked on the selection of animals more resistant to DCS. For this, we started from a pool of Wistar rats (52 males and 52 females), and we submitted them to a simulated dive at 11 weeks, in order to select asymptomatic individuals for them to breed with each other, and this from generation to generation, until the 15th generation.

3/

Our main objective was to compare the resistant rats with the Wistar strain they came from, trying to go from the genotype to phenotype, with a particular emphasis on the Immune system, the Hemostasis, the Mitochondrial and the Vascular functions.

4/

Concerning the Molecular characterization, we focused on:

5/

After 3 generations only, we halved the DCS rate in our rats and almost divided it by 3 after 6, so we decided to harden the dive profile to increase the selection pressure.

After 8 generations, the resistance seemed to be stable and the results I am going to present were obtained at the 10^{th} generation.

We conducted different comparisons along the selection process, and as you can see on this table:

As you can see we obtained many males vs females differences in our results, and most of them are already published or about to be, so I invite you to read these publications if you want to know more about our resistant rats.

6/

Let's go back on our mRNA and miRNAs pathway now

7/

We combined the static hepatic transcriptome study with the circulating miRnome

8/

As you can see here from this correlation circle and the Clustered Heatmaps obtained from the more differentially expressed genes

The PCA showed that Dimensions 1 and 2 accounted for about 80% of the variability (respectively 45.08 and 33.79%) for the 40 DEGs we have selected among the rat transcriptome, based on their higher significance

We can clearly distinguish 4 groups of genes in the correlation circle and the clustered heat map

9/

As I already presented in Prague our results are mainly pointing to inflammation and the immune system with some miRs targeting inflammatory pathways

10/

And now if we take a closer look at the Pathway Analyses from our results, we observe for the first time a pattern that is coherent in males and females and that can also explain the males vs females differences we previously observed.

11/

Just in case some of you are not too familiar with miRNAs, they are

12/

Their mechanism of action might be important here, as they play a major role in the immune system efficiency

13/

If we go back to our previous results

14/

And interestingly, Ophn1 gene

15/

In conclusion, during our DCS resistant rats selection, we can wonder if the miRs can explain…

As

And

16/

In perspectives now, if we want to better understand the role of these miRs on DCS susceptibility, we can

17/

The ignition site of DCS also remains unclear, and as we previously observed…

So we can also think about spatial omics approaches to better understand DCS pathophysiology.

18/

Based on these results obtain from rats, we can also start thinking about how we can transfer some of those results to human as it is still unclear if women are really more at risk than men, as some studies will tend to show they are and some other they are not

19/

That’s all for me today. Thank you very much for your attention, I will be happy to take any question at the end of this session, or later during any coffee or lunch break.