

# Serum miR-30 is a potential biomarker for hepatic copper accumulation in Labrador retrievers

E.R. den Boer<sup>1\*</sup>, Y.S. Roelen<sup>2\*</sup>, B. Spee<sup>1</sup>, M.E. van Wolferen<sup>1</sup>, H.Fieten<sup>1</sup>

<sup>1</sup>Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University

<sup>2</sup>Anicura 'De Tweede Lijn', Zwolle, The Netherlands

\*Both authors contributed equally

## Background

- **Copper associated hepatitis in Labrador retrievers** is a hereditary disease with a complex genetic background and a female predisposition
- **The disease is progressive:**
  - Stage 1: copper build-up in the liver
  - Stage 2: Development of chronic hepatitis
  - Stage 3: Liver failure and liver cirrhosis
- Treatment outcome is highly dependent on early diagnosis
- **Diagnosis is often delayed** due to a late onset of clinical signs and because a **liver biopsy is needed** for diagnosis

- Liver biopsies are **invasive** for the patient and **costly** for the owner
- Treatment monitoring needs regular liver biopsies
- A **blood biomarker specific for hepatic copper levels** would simplify diagnosis and treatment monitoring
- **MiRNAs are non-coding RNAs which regulate post-transcriptional gene expression** and are released from tissues into the bloodstream
  - Increasingly studied as **biomarkers for hepatic disease**
  - Potentially **cheap and non-invasive biomarkers** for diagnosis and disease monitoring



Figure 1 Liver cirrhosis due to copper associated hepatitis

## Aim: To identify serum miRNAs associated with hepatic copper accumulation in Labrador retrievers

### Results

- The miRNA array detected 186 (67.1%) from the 277 tested miRNAs
- From these 186 miRNAs, 6 were selected to be validated in the replication cohort
- miRNA miR-30b was significantly associated with hepatic copper levels in both the array cohort and the replication cohort

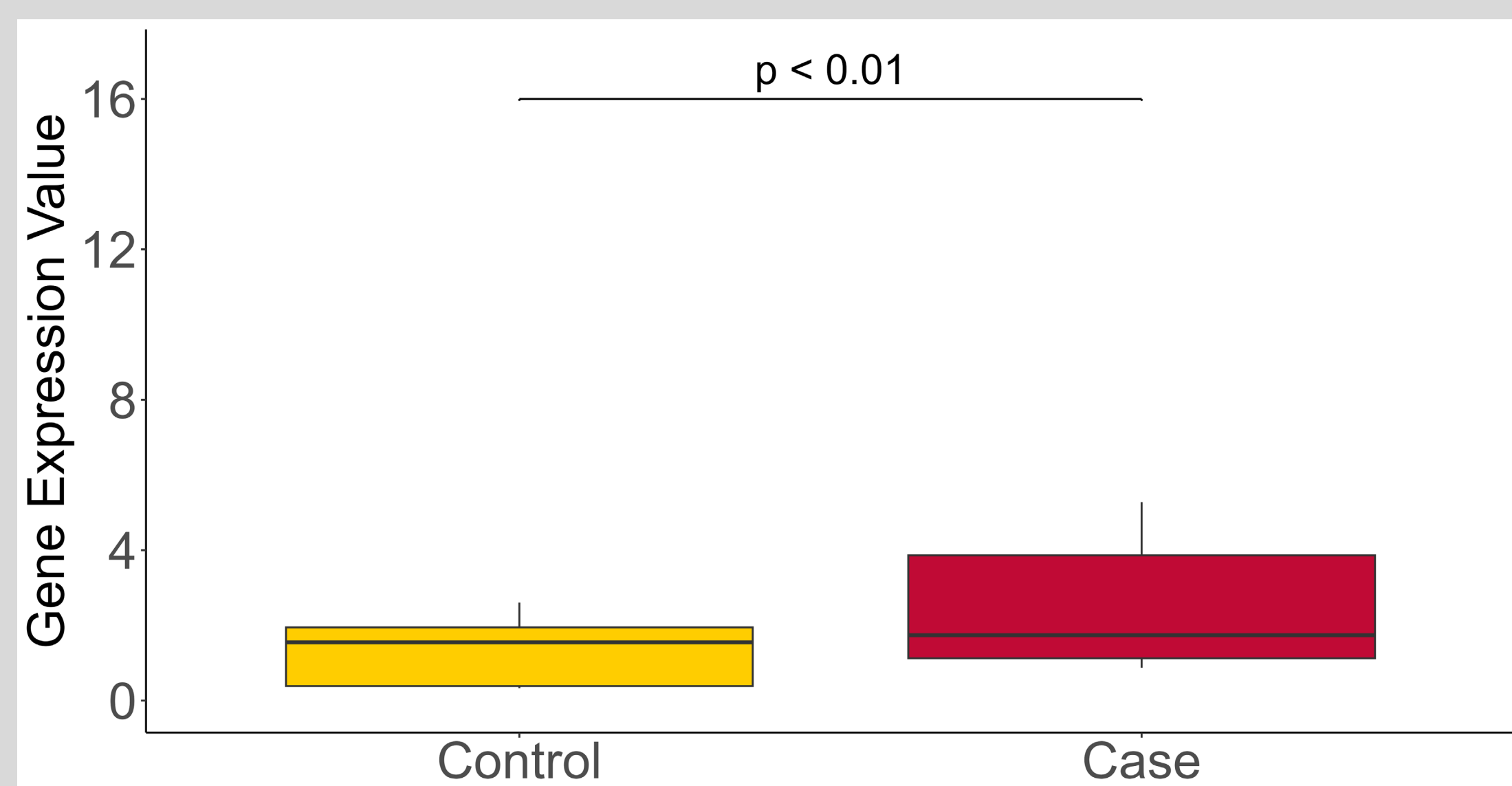


Figure 2 MiRNA 30b Gene Expression Values detected in the case (n=5) and control (n=5) group in the Canine miRNA PCR array

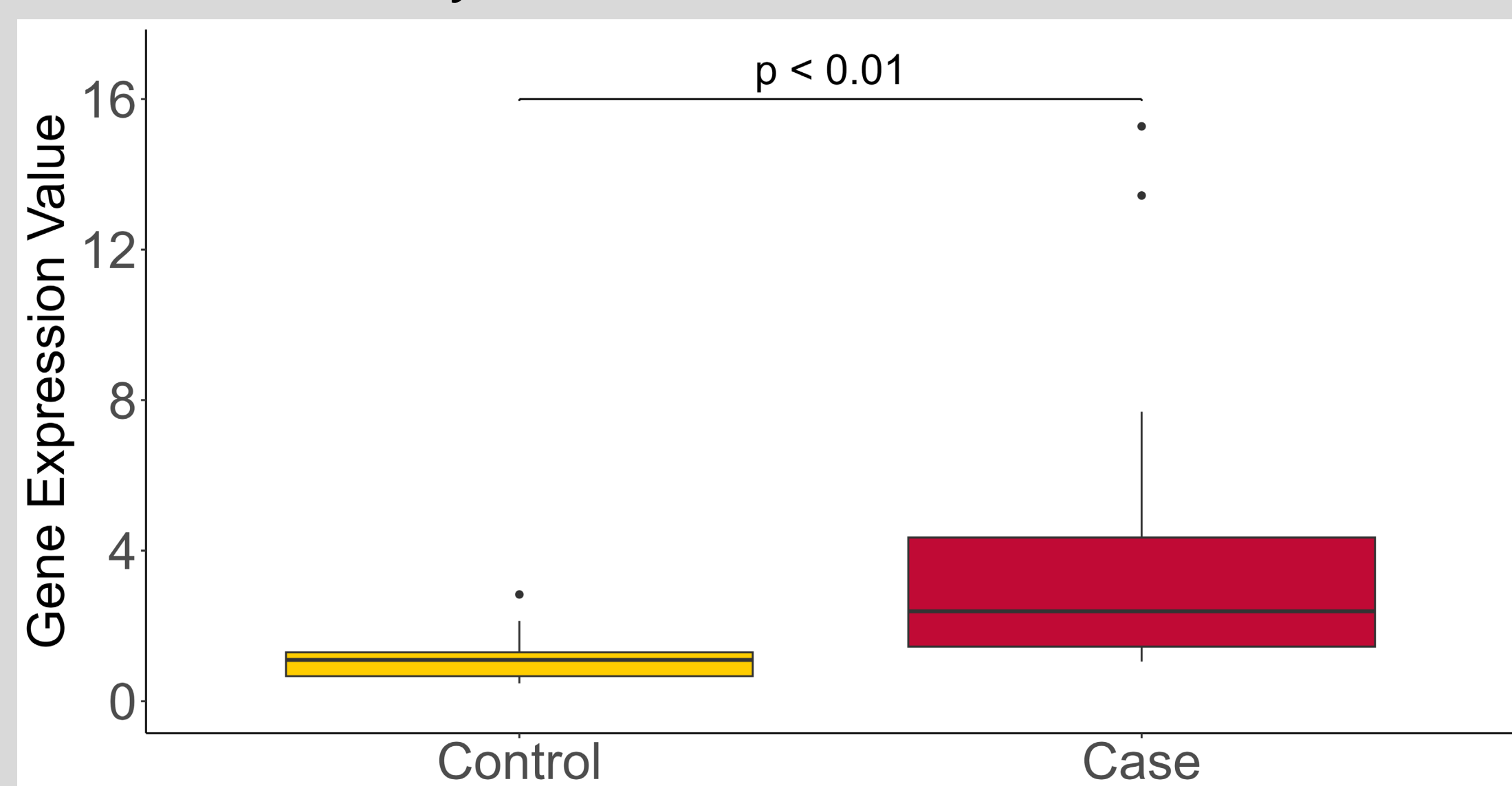


Figure 3 MiRNA 30b Gene Expression Values detected in the case (n=18) and control (n=13) group in the replication cohort

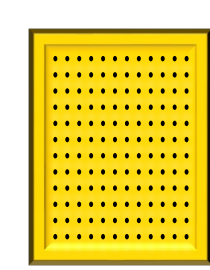
### Materials and methods

#### Study population selection:



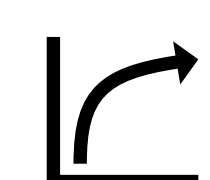
- Labrador retrievers from a patient database with liver biopsies
- Control: Normal hepatic copper levels and no liver disease
- Case: Elevated hepatic copper levels with and without hepatitis
- All dogs: Serum stored at  $-80^{\circ}\text{C}$  available

#### Canine miRNA PCR array of 277 miRNAs in 5 cases and 5 controls



- Statistics:  $\Delta\Delta\text{CT}$  method with Student's t-test
- Selection of upregulated miRNAs:
  - >2-fold upregulated and  $p\text{-value} < 0.05$
  - >4-fold upregulated

#### Replication of selected upregulated miRNAs in 18 cases and 13 controls



- Analysis of miRNA quantity: Quantitative Real-time PCR
- Statistics:  $\Delta\Delta\text{CT}$  method with Mann-Whitney U test and Bonferroni correction for multiple testing, adjusted  $p\text{-value} < 0.05$  was considered significant

MiRNA	Results array		Results replication cohort		
	Fold change	p-value	Fold change	Unadjusted p-value	Adjusted p-value
miR-28	9.51	0.061	1.27	0.068	0.34
miR-30b	2.04	0.0090	2.17	0.00035	0.0018
miR-331	4.13	0.73	1.39	0.046	0.23
miR-411	10.58	0.041	Not detected	NA	NA
miR-502	6.02	0.0027	1.59	0.022	0.11
miR-551b	8.17	0.020	1.18	0.12	0.60

Table 1 Results from the six miRNAs that were either >2-fold upregulated with a  $p\text{-value} < 0.05$  or were >4-fold upregulated in the cases compared to the controls in the miRNA array. Results are shown for the array and replication analysis.

### Discussion

- Interestingly, MiR-30b hasn't been previously identified as a miRNA with a role in copper metabolism, this study indicates a previously unknown gene regulation function of this miRNA.
- A limitation of our study was the sample size and an overrepresentation of female cases due to limited patient availability for this study.

**Conclusion:** MiRNA miR-30b is a promising biomarker for hepatic copper levels in Labrador retrievers which warrants further research in a larger cohort to determine specificity and sensitivity for elevated hepatic copper levels in dogs.

