

# Molecular Analysis of microRNA expression profiles in *AIP* mutation positive somatotropinomas

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## OBJECTIVES

### •Overall aim:

Discover biologically relevant microRNAs (miRNAs) to gain insights into the molecular pathogenesis of pituitary adenomas associated with mutations in the aryl hydrocarbon receptor interacting protein (AIP)

### •Specific aims:

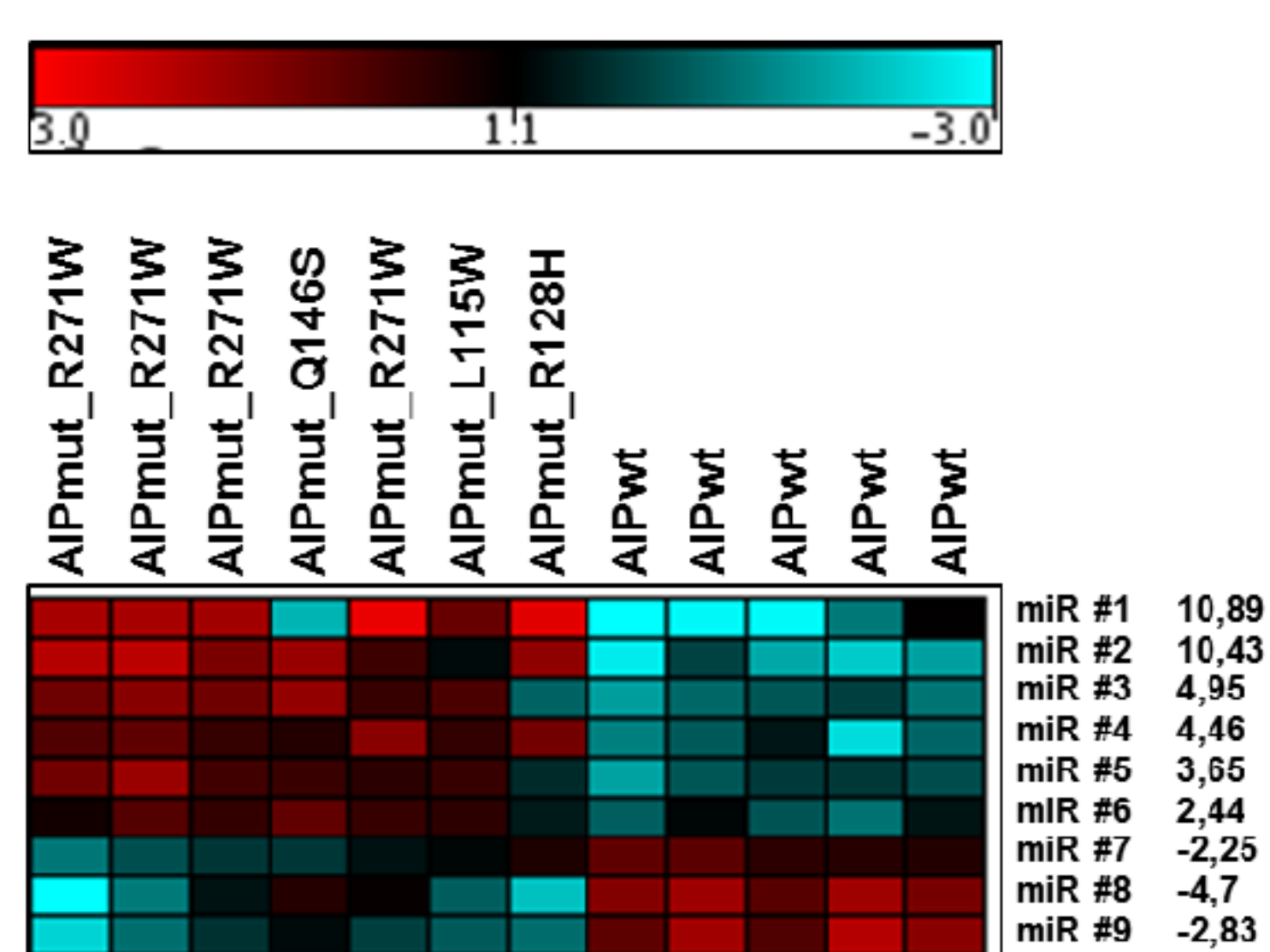
Determine miRNA expression profiles of *AIP* mutation-positive (AIPmut) and *AIP* mutation-negative (AIPwt) somatotropinomas

Compare the profiles of these two tumor groups and identify differentially expressed miRNAs

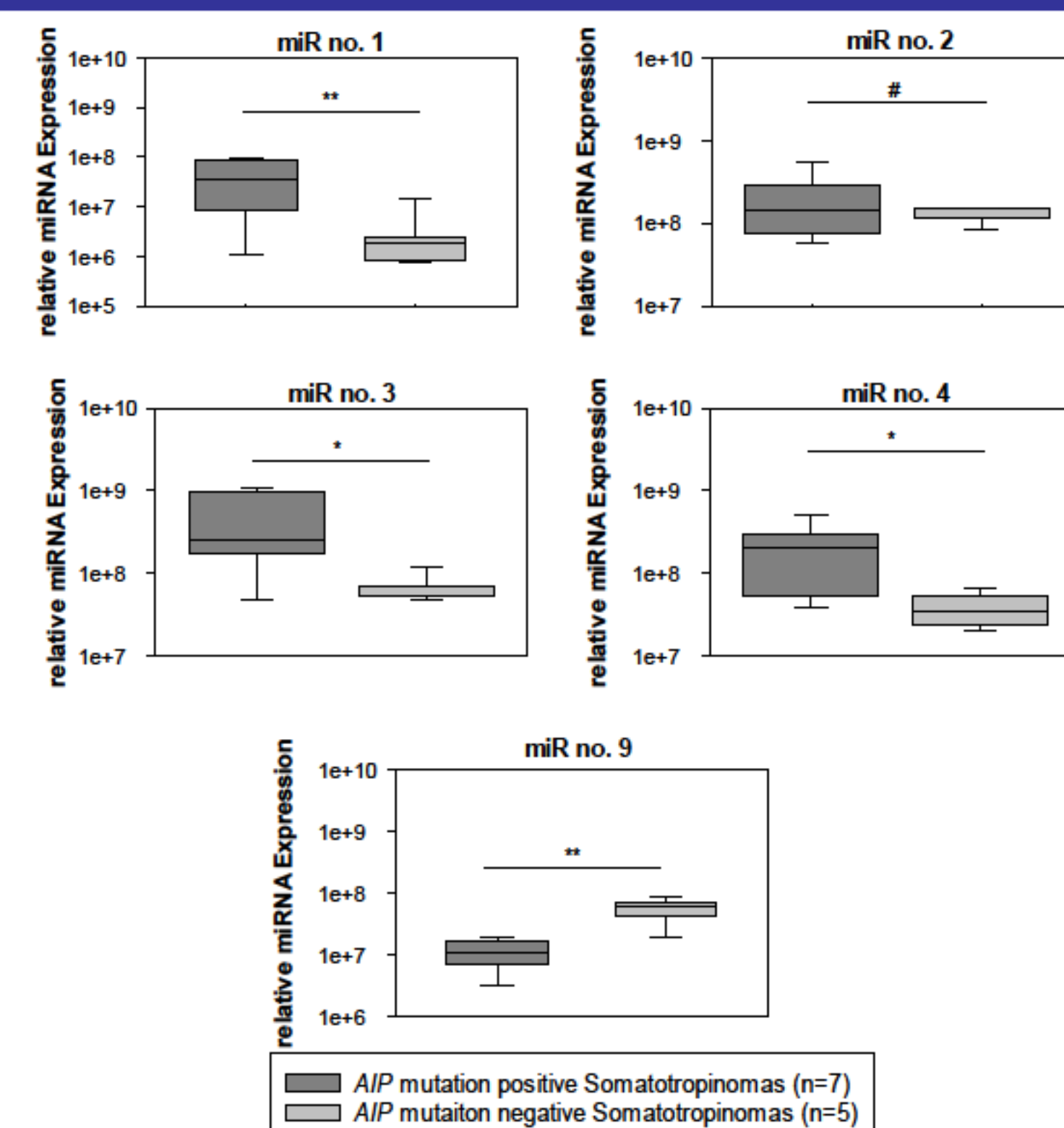
## METHODS

- Formalin fixed, paraffin-embedded (FFPE) tumor tissues were collected: 7 AIPmut and 5 AIPwt somatotropinomas. RNA was isolated and was hybridised onto GeneChip microRNA 1.0 arrays from Affymetrix
- miRNAs were filtered for detection, which needed to occur in at least 4 out of 7 AIPmut and in at least 3 out of 5 AIPwt specimens. After Benjamini-Hochberg correction (1), a cut-off p-value of <0.05 and an expression value >1.5 fold were chosen. Nine miRNAs satisfied these criteria and were further analyzed utilising the statistical programming environment R implemented in CARMAweb (2, 3).
- Five miRNAs among those most differentially expressed between the two sample groups were selected to validate the array results by performing quantitative (q) RT-PCR
- The 9 most differentially expressed miRNAs between AIPmut *versus* AIPwt somatotropinomas were analyzed by QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) to gather clues about their function.
- Mouse embryonic fibroblasts (MEFs) from *Aip* knockout mice were transfected with vectors expressing the human AIP\_R271W mutation or AIP\_wt and then the expression of selected miRNAs was assessed by qRT-PCR

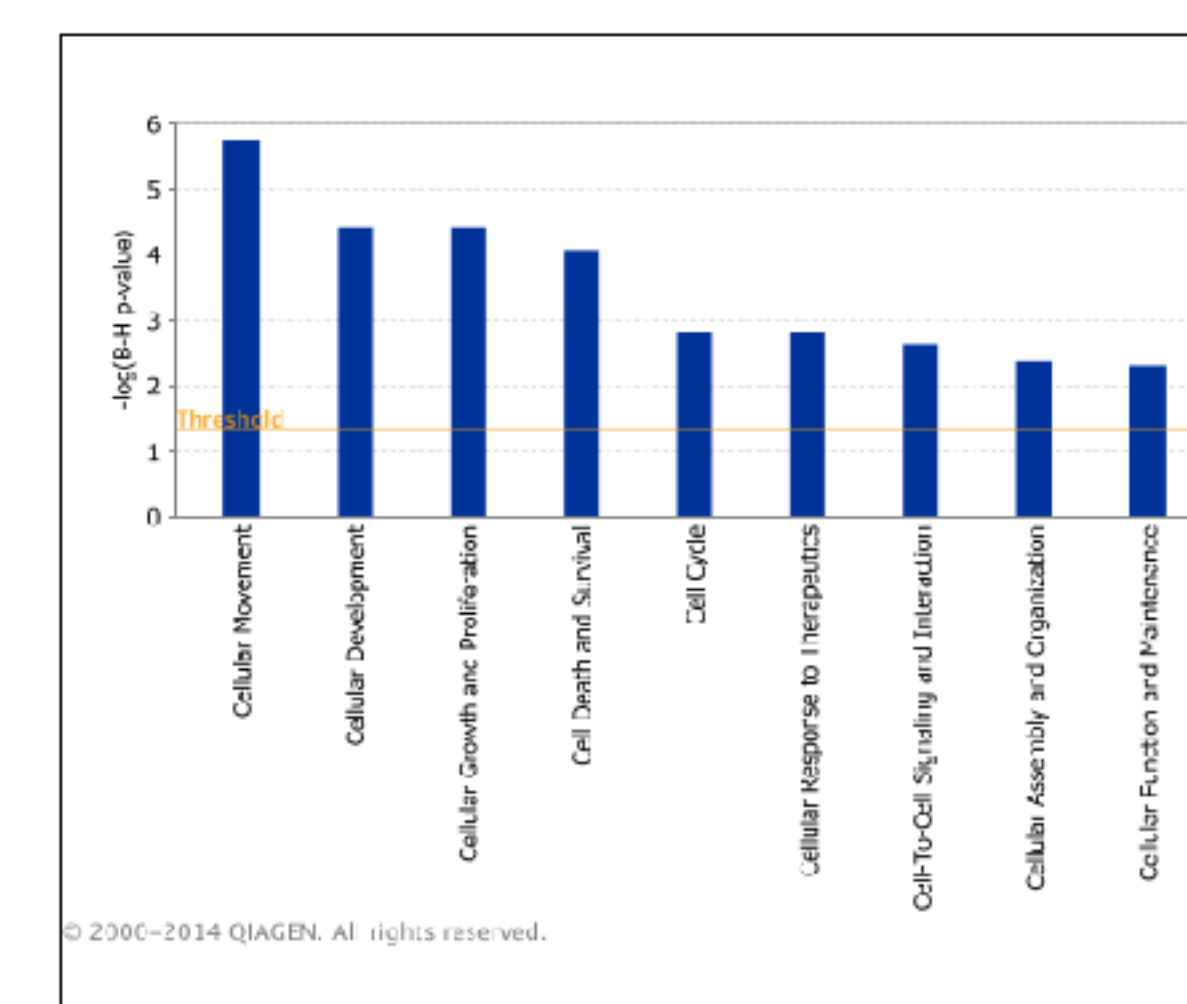
## RESULTS



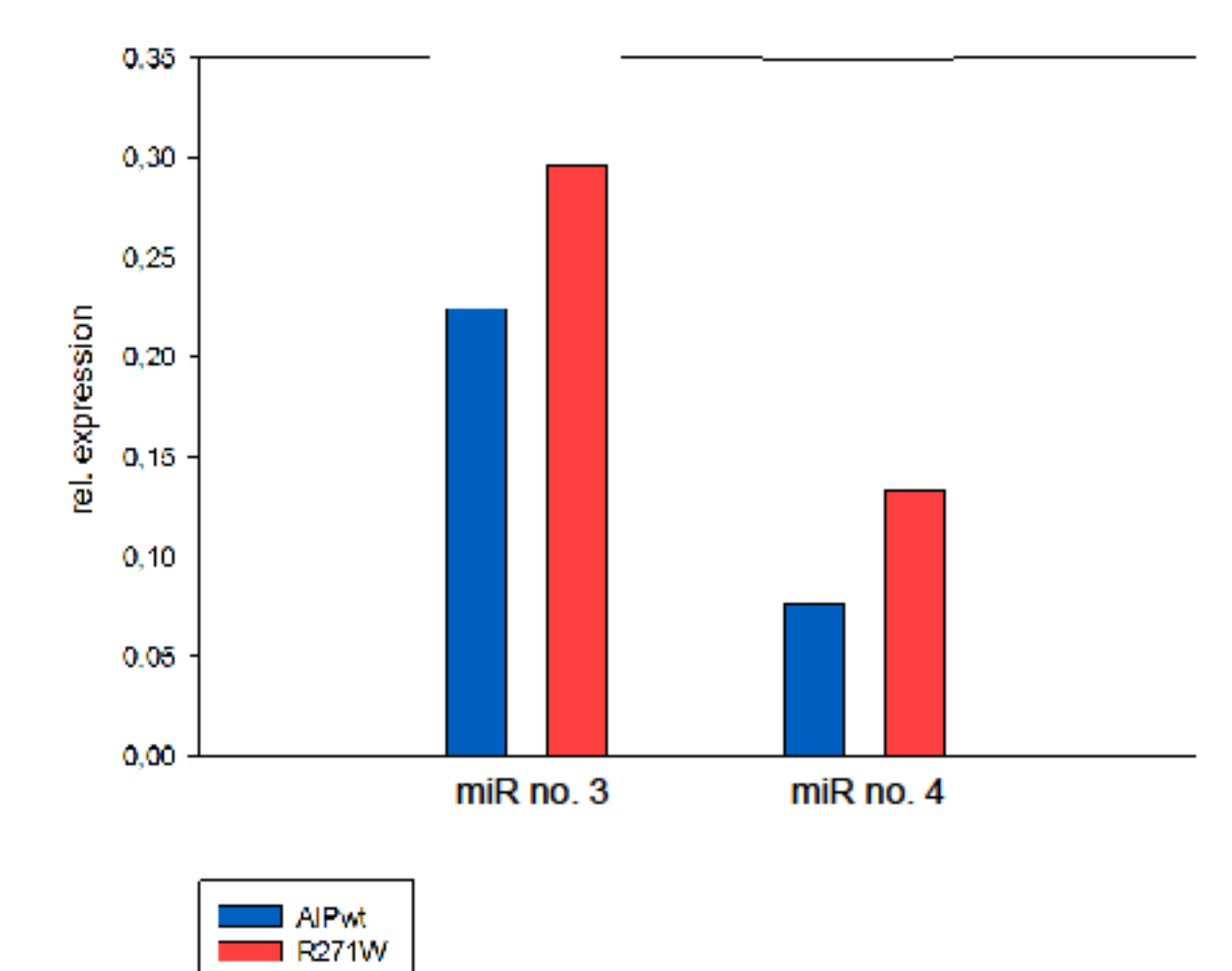
**Figure 1.** Heat map of the expression values of the 9 most differentially expressed miRNAs is shown. These miRNAs separate the AIPmut from the AIPwt somatotropinomas. Red (blue) indicates higher (lower) expression level with respect to the median across all samples and the log2 scale is provided above. The mutations in AIP are indicated as well as the fold change for each miRNA in AIPmut *versus* AIPwt tumors (on the right).



**Figure 2.** Five of the differentially expressed miRNAs were selected for validation by qRT-PCR using individual TaqMan assays. Four of them were found to be differentially expressed in AIPmut and AIPwt samples similar to the array analysis. \*, P<0.05; \*\* P<0.001.



**Figure 3.** IPA analysis of the 9 differentially expressed miRNAs suggests that they are involved in: "Cellular Movement" (the most significantly affected gene ontology functional category); "Cellular Growth and Proliferation", "Cell Death and Survival" and "Cell Cycle" (cancer-related processes).



**Figure 4.** Mouse embryonic fibroblasts from *Aip* knockout mice were transfected with vectors expressing the human AIP\_R271W (red) mutation or AIPwt (blue). RNA was extracted and the expression of 2 differentially expressed miRNAs (i.e. miR no. 3 and 4) was assessed by qRT-PCR. The expression of these 2 miRNAs is regulated by AIPmut.

## CONCLUSIONS

- The fact that we could identify miRNAs differentially expressed between AIPmut *versus* AIPwt somatotropinomas demonstrates that mutation of AIP affects the miRNA signature of somatotropinomas
- In vitro* studies showed that overexpression of AIPmut cDNA (bearing the R271W mutation) associates with changes in the expression of miRNA no. 3 and no. 4.
- "IPA downstream effects analysis" suggests that the miRNAs differentially expressed between the two sample groups are involved in the regulation of cancer relevant processes such as cell motility, growth and proliferation and warrant further studies to identify their target genes in pituitary adenomas

## References

- Benjamini, Y. and Y. Hochberg, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, 1995. **57**(1): p. 289-300.
- Rainer, J., et al., CARMAweb: comprehensive R- and bioconductor-based web service for microarray data analysis. *Nucleic Acids Research*, 2006. **34** (suppl 2): p. W498-W503.
- R Development Core Team, R: A Language and Environment for Statistical Computing. 2008, R Foundation for Statistical Computing, Vienna, Austria.

