1. Introduction
Heterozygote germline mutations in the aryl-hydrocarbon receptor interacting protein (AIP) gene play a role in the pathogenesis of pituitary adenoma development in familial isolated pituitary adenoma (FIPA) as well as simplex pituitary adenoma cases. AIP mutation positive patients develop often aggressively growing tumours in early teenage years and often show invasion at the time of diagnosis as well as poor response to somatostatin analogues than sporadic tumours 1,2.

2. Aims
The aim of this study was to perform comparative gene expression analysis of AIP mutation-positive (AIPpos) pituitary adenomas to discover the genes/pathways responsible for the aggressive clinical phenotype of these tumours.

3. Methods
Gene expression analysis on normal pituitary, AIP mutation positive, familial AIPheg as well as sporadic somatotrophinomas (n=25) using the Affymetrix human Gene Chip HG-U133 Plus 2.0 array. Ingenuity Pathway Analysis (IPA) tool was used for pathway analysis. Differential expression of selected genes was validated by RT-qPCR and immunohistochemistry. In vitro stimulation of epithelial-to-mesenchymal transition (EMT) was performed on stable AIP-knockdown cells using forskolin and assessed the EMT markers by Western blotting. In vitro invasion assay was performed on AIP siRNA-knocked down BxPC3 cells using BioCoat-Matrigel invasion chambers.

4. Ingenuity Pathway Analysis
Epithelial to Mesenchymal Transition (EMT) pathway in
AIPpos somatotroph tumours

5. Validation by RT-qPCR
Validation of five downregulated (CDH1, CTNNB1, ESRP1, PERP and EPCAM) and one upregulated (ZEB1) genes (P ranging <0.05 to < 0.0001).

6. Validation by RT-qPCR (cont.)
Representative images:
Normal pituitary (left panel), AIPpos GH (middle panel) and sporadic GH (right panel)

7. Validation by IHC
Validation at protein level for four downregulated (E-cadherin, Beta-catenin, ESRP1 and PERP) and one upregulated (ZEB1) genes (P ranging <0.05 to < 0.0001).

7. Validation by IHC (cont.)

8. In vitro stimulation of EMT

9. Invasion assay

10. Summary and Conclusions
One of the top altered pathways in AIPpos adenomas was the EMT pathway. Genes related to EMT, such as epithelial markers (CDH1, CTNNB1, ESRP1 and EPCAM), transcriptional regulator (ZEB1) and post-transcriptional regulator (ESRP1 and ESRP2) all appear to be significantly deregulated.

The cAMP pathway has tissue specific regulation on cell proliferation and possibly on EMT. We hypothesise that increased levels of cAMP could stimulate EMT in the pituitary, while it inhibits in other cell types 4.

In vitro EMT stimulation lead to induction of EMT as indicated by down-regulation of epithelial marker and up-regulation of mesenchymal marker (ZEB1) as well as an increase in actin stress fibers formation. Invasion assay revealed that AIP silencing led to an increase in invasion compared to non-targeting siRNA.

This novel potential mechanism of the regulation of EMT/or switching the cellular phenotype from ‘epithelial’ to ‘mesenchymal like’ through AIP may thus be important for acquiring an invasive phenotype.

11. References

12. Acknowledgement
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