

# COMBINED CLINICAL AND GENE EXPRESSION PROFILING IN HUMAN ACTH-SECRETING PITUITARY TUMORS

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## Introduction

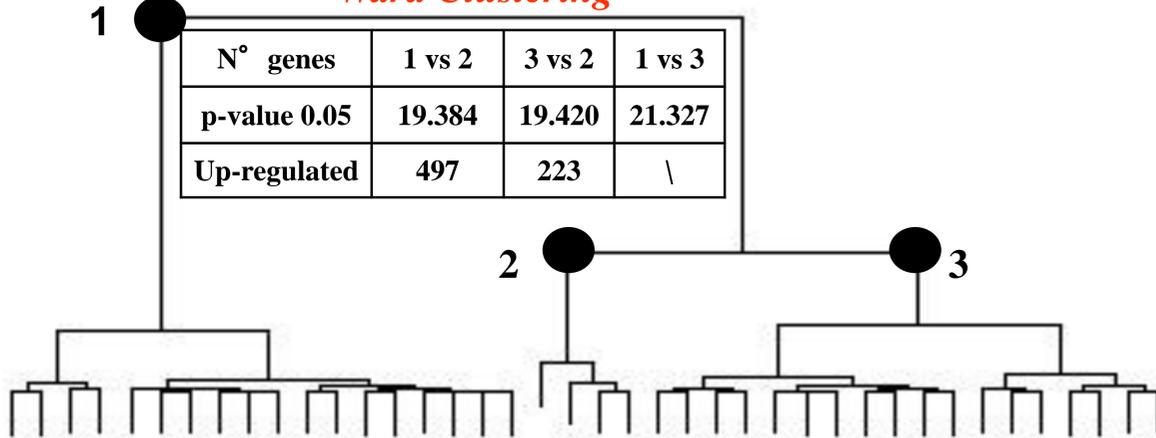
We have previously reported on the **considerable variability** in **ACTH-secreting pituitary adenomas** as regards **responses to major modulators** *in vitro* (Pecori Giraldi et al *Journal of Neuroendocrinology* 2011).

**Aim** of this study is to correlate **transcriptome** expression pattern in archival **human ACTH-secreting adenomas** with **clinical features** of patients prior to and after surgery.

**Methods.** **Forty human ACTH-secreting pituitary adenoma** formalin-fixed paraffin-embedded specimens were cut into 20  $\mu$ m thick sections and RNA extracted using Recover All Total Nucleic Acid Isolation Kit (Invitrogen, Carlsbad CA, USA). RNA (300 ng) was hybridized to **Human HT-12V4 expression bead Chip** (approx 29000 transcripts) and analyzed with WG-DASL-HT assay (Illumina, San Diego CA, USA). Patients' clinical charts were reviewed and data analyzed by **Principal Component Analysis** (JMP, Statistical Discovery, SAS Institute, Cary NC, USA). **Combined clinical and expression analysis** was performed on R-studio and **functionality** of identified genes assessed by DAVID and Cytoscape.

## Results

### Ward Clustering



Clinical and expression data clustered in **three major groups**, with 18, 4 and 18 patients, respectively. 1259 genes were significantly expressed ( $p < 0.001$ ) and clinical variables which proved predictive of clustering were **adenoma size** and **plasma ACTH concentrations**.

### 1 vs 2: 487 up-regulated genes

Annotation Cluster	Enrichment Score	Count	PValue
GOTERM_CC_FAT	2.6 cytoplasmic vesicle part	9	9,3E-02
GOTERM_BP_FAT	2.43 response to extracellular stimulus	13	0,007
GOTERM_MF_FAT	2.3 RNA-dependent ATPase activity	5	0,002
GOTERM_BP_FAT	2.07 regulation of phosphorylation	20	0,019
GOTERM_MF_FAT	1.65 metalloenzyme regulator activity	3	0,02
GOTERM_BP_FAT	1.58 endoplasmic reticulum unfolded protein response	4	0,01
GOTERM_BP_FAT	1.57 negative regulation of phosphorylation	5	0,023
GOTERM_BP_FAT	1.49 muscle tissue development	8	0,03
SP_PIR_KEYWORDS	1.46 helicase	8	0,03
GOTERM_BP_FAT	1.35 positive regulation of protein metabolic process	13	0,02

### 3 vs 2: 233 up-regulated genes

Annotation Cluster	Enrichment Score	Count	PValue
GOTERM_MF_FAT	2.08 acid-amino acid ligase activity	8	0,003
GOTERM_MF_FAT	1.69 nucleotide binding	32	0,023
GOTERM_BP_FAT	1.64 regulation of protein ubiquitination	6	0,004
GOTERM_BP_FAT	1.47 negative regulation of cell migration	4	0,020
GOTERM_BP_FAT	1.45 macromolecule catabolic process	15	0,03
GOTERM_BP_FAT	1.40 negative regulation of cellular protein metabolic process	7	0,010
GOTERM_BP_FAT	1.3 protein ubiquitination	5	0,033

Differential expression analysis among clusters revealed significant expression of genes annotated to functions including **granule lumen** (enrichment score 2.6), **phosphorylation** (enrichment score 2.07), **aminoacid ligase** (enrichment score 2.08) and **ubiquitin pathway** (enrichment score 1.8).

**Conclusions.** **Combined clinical and gene expression** analysis of **human ACTH-secreting adenomas** allowed the identification of **three major clusters**. Functional annotations revealed the **involvement of distinct pathways** in individual clusters, paving the way to a **greater understanding of the variability** of human corticotrope tumors.