ISPA - Etude génomique et génétique intégrée de l'hyperaldostéronisme primaire: implications physiopathologiques et pronostiques

ISPA - Integrated study of primary aldosteronism: from genetics and genomics to physiopathology and prognosis

Participating teams

Team 1: Maria-Christina Zennaro (Principal Investigator)
INSERM U772, Collège de France, Université Paris Descartes, HEGP, Paris

Team 2 : Arndt Benecke
Institut des Hautes Etudes Scientifiques & I.R.I. CNRS FRE2963, Bures sur Yvette

Team 3 : Tchao Meatchi
Service d'anatomie pathologique, HEGP, Paris

Team 4 : Hervé Lefebvre
Service d'Endocrinologie, CHU Rouen; INSERM, U413, Mont-Saint-Aignan

Team 5 : Eric Clauser
Department of Endocrinology, Metabolism and Cancer, Institut Cochin – INSERM U567 – CNRS UMR8104 - Paris Descartes University, Paris

Team 6 : Marc Lathrop
Centre National de Génotypage, Evry

Primary aldosteronism (PAL) - Background

- **Prevalence** : ~6 % HTA (Plouin 2004, Mulatero 2004)
- **Cardiovascular severity** (Rossi, 1997; Milliez 2005; Stowasser 2005)

- **Different forms**:
  - ✓ Conn's adenoma (APA, 30-50%)
  - ✓ Bilateral adrenal hyperplasia (BAH, 30-50%)
  - ✓ Primary unilateral adrenal hyperplasia (5-10%)
  - ✓ Carcinoma

- **Pathophysiology**

  - ✓ Comparative genomic hybridization (CGH): little changes (chr. 4q and 17, *Sidhu et al, 2004*)
  - ✓ Coordinated overexpression of genes involved in steroidogenesis and in cholesterol and electron supply to the cell (*Assié et al, 2005, Basset et al 2005*)
  - ✓ Alterations in transcription factors that enhance expression of steroid metabolizing enzymes (Nurr1, DAX-1, *Basset et al 2005*)
  - ✓ Overexpression of legitimate and illegitimate membrane receptors
    -HTR4-R, LH-R, GnRH-R, GPR37, GRM3 (*Cartier et al 2005, Saner-Amigh et al 2006, Ye et al, 2007*)

Aim of the study

We make the hypothesis that activation of particular signalling pathways or transcriptional cascades, via qualitative (mutations) or quantitative (expression) molecular changes may be responsible for the development of PAL.

The aim of our project is to identify these abnormalities by using a combined approach, integrating genomics and genetics with pathological, cellular and molecular investigations.

Our project includes four research axes:

1. Transcriptional profiling of PAL and correlation of gene expression signatures with the pathological profile and therapeutic outcome after surgery
2. Study of the role of mast cells in the pathogenesis of PAL
3. Identification of susceptibility genes by whole genome association studies
4. Analysis of signalling pathways and transcriptional cascades in model systems

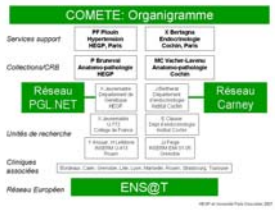
Expected outcome

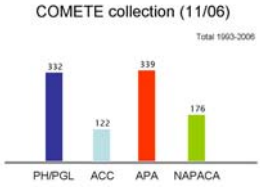
The project will allow to gain better insight into the pathogenic mechanisms involved in the development of PAL, possibly leading to the identification of susceptibility genes and new therapeutic targets.

Furthermore, the identification of molecular signatures correlated with therapeutic outcome, the analysis of new factors regulating steroidogenesis and proliferation in glomerulosa cells and the characterisation of relevant pathways, might open new perspectives in the development of novel strategies for therapeutic intervention and follow-up after surgery.

The originality of our strategy stems from the interdisciplinary approach, which integrates the competences of basic researchers, mathematicians and clinicians, allowing to explore all the aspects of the project and the transfer of relevant results to clinical practice.

The COMETE network : (COrtico and MEdullo-surrenale: les Tumeurs Endocrines)





Description of the project

Transcriptional profiling of PAL

Team 1 (MC. Zennaro, SK. Tareen), team 2 (A. Benecke, FX. Pellay, G. Brysbeart), team 4 (H. Lefebvre, E. Louiset), team 5 (E. Clauser)

To establish the transcriptional profiles of adrenal aldosterone producing adenomas and hyperplasias and to identify molecular gene expression signatures correlated with the pathological profile and therapeutic outcome after surgery.

Samples
- 100 PAL samples (COMETE network)
- 20 normal adrenal cortices (pieces from expanded nephrectomy for kidney carcinoma, tumor library of the CHU of Rouen)
- Replication of relevant gene signatures on 100 additional PAL samples

Methodology
- Transcriptional profiling: AB1700 platform (IRI, Lille, CNRS FRE2963)
- Replication studies: high throughput Taqman assays on AB7900 platform

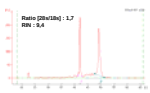


Figure 1. Example of quality control analysis of RNA of PAL samples

Figure 2. AB1700 platform and microarrays

Evaluation criteria
- comparison and clustering of individual transcriptional profiles
- analysis of transcriptional profiles with regard to pathological features and blood pressure outcome after surgery
- comparison of transcriptional profiles of PAL tissue and normal adrenal tissue
- Building global signalling pathways and transcriptional cascades from gene expression profiles

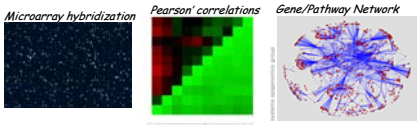


Figure 3. Data acquisition and transcriptional profile analysis

Role of mast cells in the physiopathology of PAL

Team 4 (E. Louiset, C. Duparc, V. Perraudin, H. Lefebvre, J-M. Kuhn)

To characterize mast cells present in the normal adrenal gland and APAs and to investigate the influence of mast cells on the steroidogenic and mitogenic activities of aldosterone-producing adrenocortical cells.

Methodology
- Characterization of mast cells and connexion with sympathetic nerve terms by immunohistochemistry
- Regulation of 5-HT release by cultured mast cells derived from normal adrenocortical and APA tissues
- Influence of mast cells on the steroidogenic and mitogenic activities of the zona glomerulosa (co-cultures)
- Clinical studies in patients with PAL, using antagonists of substance P receptor or 5HT4-R

Evaluation criteria
- 5-HT release by cultured mast cells derived from normal adrenocortical and APA tissues
- determination of aldosterone concentrations in culture medium and BrDU incorporation in co-cultured adrenocortical cells
- Inhibiton of aldosterone production in patients with aldosterone-producing adrenocortical adenomas

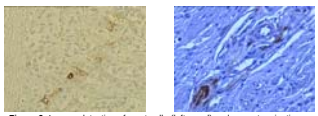


Figure 6. Immunodetection of mast cells (left panel) and nerve terminations (right panel)

Figure 7. Immunodetection of mast cells in PAL tissues showing immunostaining in the tumoral (left panel) or in the peritumoral (right panel) tissue

Pathological analysis of aldosterone producing tumors and hyperplasias

Team 3 (T. Meatchi), team 4 (E. Louiset, V. Perraudin, H. Lefebvre)

To describe detailed morpho-pathological criteria of PAL (APA and unilateral primary adrenal hyperplasia).

Methodology
- Standard pathological examination: analysis of the type of tumour or hyperplasia, evaluation of cell types, degree of nodularity, peritumoral morphology
- Analysis of extratumoral tissue: quantification of glomerular hyperplasia, nodulation and vascularization
- Immunohistochemistry : detection of mast cells, nerve terminations and chromaffin cells

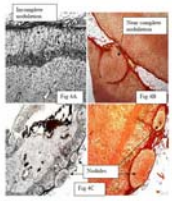


Figure 4. Nodulation score

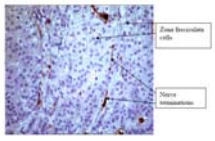


Figure 5. Nerve terminations between zona fasciculata cells

Identification of susceptibility genes in PAL

Team 1 (X. Jeunemaitre, MC. Zennaro) and Team 6 (M Lathrop)

To identify susceptibility genes for the development of PAL

Samples
- 500 PAL samples (COMETE network, 250 APA, 250 BAH)
- Control population:
  - 800 Essential HTN (HYPERGENE)
  - 1500 population-based controls (SUVIMAX)
- Replication study on 500 additional PAL samples (250 APA, 250 BAH) trough national DNA-PAL repository and international collaborations (ENS@T)

Methodology
- Illumina platform (300K, CNG Evry)
- Statistical analysis using SAS, Snpgwa, PLINK

Evaluation criteria
- Detection of common genetic variants that confer a risk stronger than a predetermined odds ratio (fixed a priori to 3 and 6 at the heterozygous and homozygous state)
- Replication of regions with SNPs conferring such a risk from a second set of 500 patients with PAL

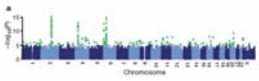


Figure 8. Example of results obtained by genome-wide association studies. Green dots indicate SNPs with a P value <1X10^{-5}.

Analysis of signalling pathways in model systems

Team 5 (E. Clauser), team 4 (H. Lefebvre, E. Louiset)

To give a pathophysiological sense to the qualitative (gene mutation) or quantitative (transcriptional profiles) molecular alterations observed in PAL.

Methodology
- inactivation (siRNAs) and/or activation (overexpression) of potential causal genes or pathways in different cell types
- Long-term development of similar animal models (transgenesis)

Evaluation criteria
- Comparison of growth and steroidogenesis in adrenal cell cultures with or without overexpression or inactivation of the gene of interest
- Parallel analysis of the signalling pathways
- Production of aldosterone and cAMP by cultured aldosterone-producing adenoma (APA) cells.
- Measurement of membrane ion currents in cultured APA cells

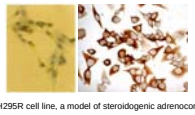


Figure 9. H295R cell line, a model of steroidogenic adrenocortical cells