ECE 2015 – GP-29-03– *Endocrine tumours and neoplasia: adrenal tumours.*

LMU KLINIKUM DER UNIVERSITÄT MÜNCHEN

CAMPUS INNENSTADT
Medizinische Klinik und Poliklinik IV
Endocrine Research Department

Prof. Dr. med. Felix Beuschlein

Immunoexpressions of CYP11B2 and HSD3B2 in genetically characterized aldosterone producing adenomas

Christian Gebhard[1], Yara Rhayem[1], Anna Dietz[1], Anna Riester[1], Constanze Hantel[1], Marion Schuster[1], Tim M. Strom[2,3]
Celso Gomez-Sanchez[4,5], Martin Reincke[1], Felix Beuschlein[1]

[1]Medizinische Klinik und Poliklinik IV, Ludwig-Maximilians-University, Munich, Germany , [2]Institute of Human Genetics, Helmholtz Zentrum Munich, Germany [3]Institute of Human Genetics, Technische Universität München, Munich, Germany, [4]Division of Endocrinology, G.V. (Sonny) Montgomery VA Medical Center, Jackson, MS, USA [5]Department of Medicine-Endocrinology, University of Mississippi Medical Center, Jackson, MS, USA.

Introduction and Objectives

Somatic mutations of *KCNJ5* (1), *CACNA1D* (2), *ATP1A1* and *ATP2B3* (3) have been shown to be involved in the formation of aldosterone producing adenoma (APA). This study aimed to investigate in paraffin-embedded APA tissues, the immunoexpression of CYP11B2, the rate-limiting enzyme for aldosterone production and HSD3B2, the prevalent isoform of 3β-HSD found in APA, and to relate these findings to mutation status, histopathology of APA and biochemical and clinical outcome of adrenalectomy.

Methods

Subjects and human adrenal tissue

Patients (n= 59) were diagnosed with PA by AVS and CT-scan according to the Endocrine Society Practice Guidelines (4) and were included in the German Conn's Registry. After unilateral adrenalectomy the adrenal tissue was histologically confirmed as APA. All patients gave written informed consent and the study was approved by the ethics committee of the University of Munich. Biochemical and clinical data was prospectively collected from PA diagnosis date up until 16.8 ±11.3* months following adrenalectomy.

Histological and immunohistochemical staining analysis

32 out of 59 genotyped APA presented available paraffin-embedded tissues and were selected for functional immunohistochemical staining analysis. Hematoxylin and eosin (H&E) staining was performed with routine protocol to determine cell composition. Immunostaining was performed for CYP11B2 using the ImmPRESS Reagent (Vector laboratories) and for HSD3B2 using the Vectastain ABC Elite Kit (Vector laboratories). Primary antibodies used in immuno-histochemistry are listed in table 1. The antigen-antibody complex was visualized with 3,3´-diaminobenzidine solution (DAB) and counterstained with hematoxylin. Double immunostaining was performed using Polink DS-MR-Hu C1 kit (GBI Labs). Semi-quantitative immunohistochemical evaluation was assessed using McCarty´s H-scoring system and statistical analysis was performed using Mann-Whitney multiple comparison test.

APA Genotyping

Genotyping was performed by direct bidirectional Sanger sequencing or whole exome sequencing. Tissues were divided into five groups according to their mutation status: *KCNJ5, CACNA1D, ATP1A1, ATP2B3* or *Wild type* (*WT* - defined as the absence of candidate gene mutation).

Table 1
Primary antibodies used in immunohistochemical studies of APAs.

Antibody	Source
Anti-hCYP11B2 clone 41-17 (Mouse monoclonal)	Celso Gomez-Sanchez *et al.*, 2014 (5)
Anti-hHSD3B2 ref. Ab154385 (Rabbit polyclonal)	Abcam

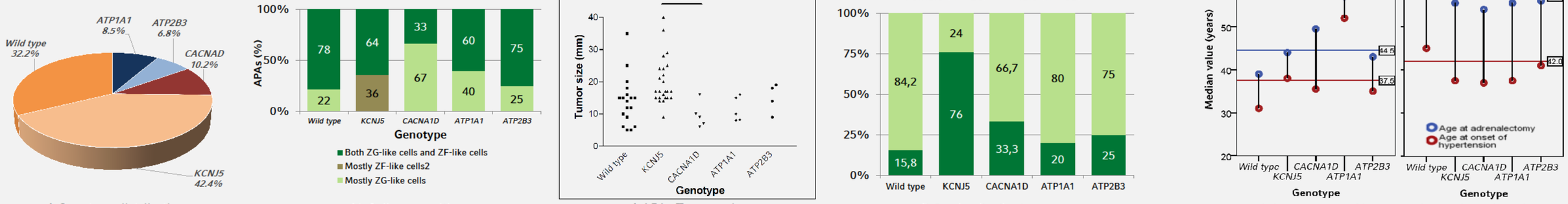
** (values are expressed as mean ± SD unless specified otherwise)*

Results

Genetic and histopathological characterization of APA cases

68% of APAs presented with a somatic mutation, most frequently *KCNJ5* (42.4%). Histomorphological analysis showed that a majority of APAs consisted of both *zona fasciculata*-like (*ZF* -like) clear cortical cells and *zona glomerulosa*-like (*ZG*-like) compact cells, except for *CACNA1D*-mutated APAs, where 67% presented only *ZG*-like cells. *CACNA1D*-mutated tumours presented significantly smaller tumour size (P<0.05) than *KCNJ5*-mutated APAs. The gender distribution shows that mostly men are affected by APAs, except for KCNJ5-mutated APA, which were predominantly seen in women (76%). Earlier onset of hypertension is observed in women (median age: 37.5 ± 8.3 years *vs* 42 ± 9.4 for men), while longer duration of hypertension before adrenalectomy is observed in men (median duration: 13 ± 16.3 years *vs* 6.5 ± 16.3 for women) (Fig. 1).

Fig.1: Characteristics of APA cases



a) Genotype distribution **b)** Cell composition **c)** APAs Tumour size **d)** Gender distribution **e)** Age at onset of hypertension and at adrenalectomy

Immunoexpression of CYP11B2 *and* HSD3B2 *in genetically characterized APAs*

APAs with a somatic mutation presented CYP11B2 positive clusters or scattered cells (Fig. 2a).*WT*-APAs were weakly or negatively stained for CYP11B2 (Fig. 2b) while the adjacent *ZG* presented CYP11B2-positive clusters (Fig. 2h). The mean H-scores for CYP11B2 (Fig. 2c) was significantly different between *WT*-APAs and mutated APAs (P<0.05). APA lesions presented wider distribution of HSD3B2 independently of their mutation status (Fig. 2 d,e) and no significant difference was noted in HSD3B2 immunoexpression regarding the APAs genotype (Fig. 2f). Expression patterns of CYP11B2 and HSD3B2 in APA were visualized using double immunostaining (Fig. 2g).

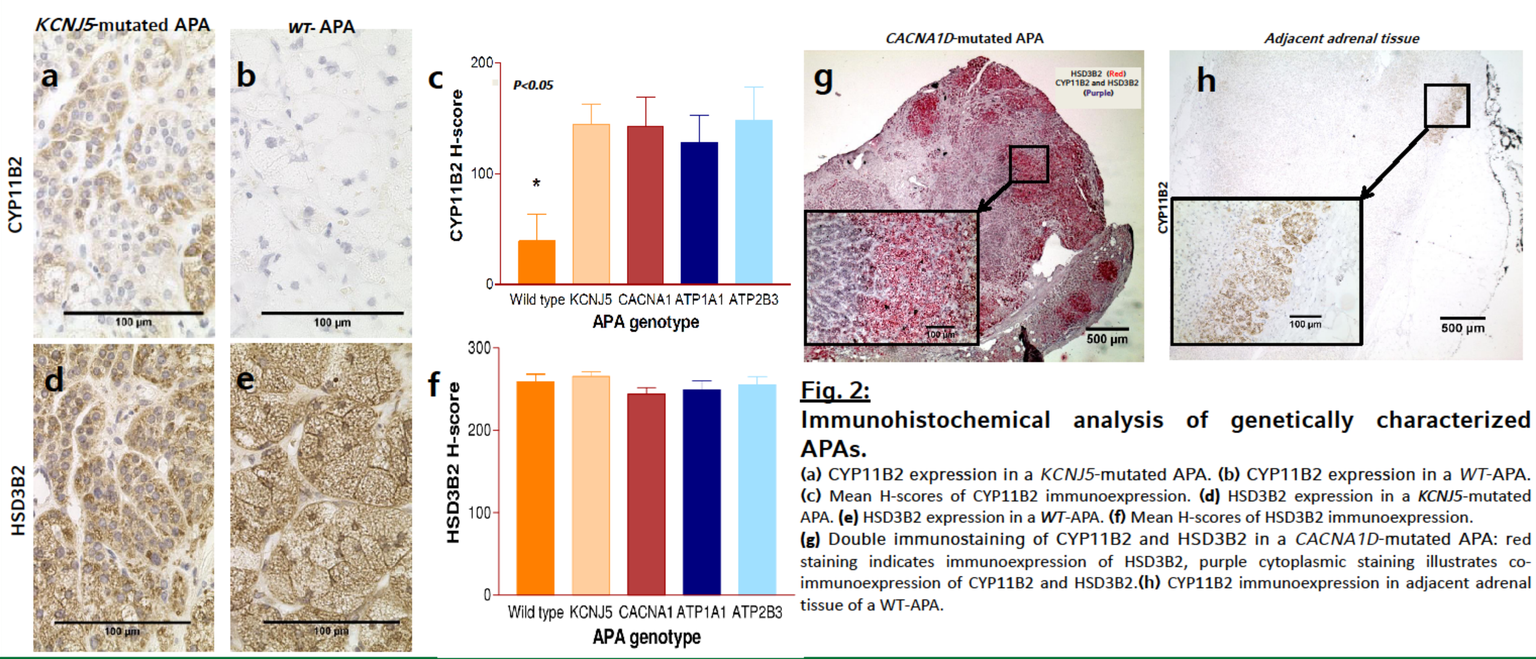


Fig. 2:
Immunohistochemical analysis of genetically characterized APAs.
(a) CYP11B2 expression in a *KCNJ5*-mutated APA. **(b)** CYP11B2 expression in a *WT*-APA. **(c)** Mean H-scores of CYP11B2 immunoexpression. **(d)** HSD3B2 expression in a *KCNJ5*-mutated APA. **(e)** HSD3B2 expression in a *WT*-APA. **(f)** Mean H-scores of HSD3B2 immunoexpression. **(g)** Double immunostaining of CYP11B2 and HSD3B2 in a *CACNA1D*-mutated APA: red staining indicates immunoexpression of HSD3B2, purple cytoplasmic staining illustrates co-immunoexpression of CYP11B2 and HSD3B2.**(h)** CYP11B2 immunoexpression in adjacent adrenal tissue of a WT-APA.

Biochemical and clinical outcome of adrenalectomy

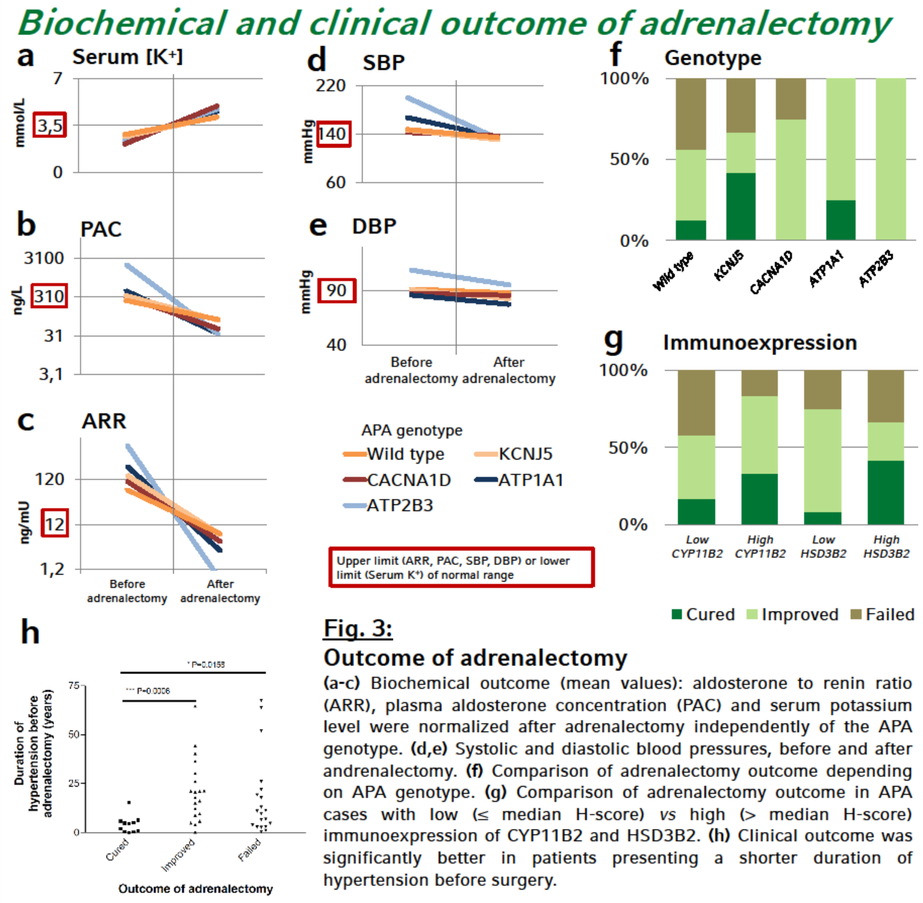


Fig. 3:
Outcome of adrenalectomy
(a-c) Biochemical outcome (mean values): aldosterone to renin ratio (ARR), plasma aldosterone concentration (PAC) and serum potassium level were normalized after adrenalectomy independently of the APA genotype. **(d,e)** Systolic and diastolic blood pressures, before and after andrenalectomy. **(f)** Comparison of adrenalectomy outcome depending on APA genotype. **(g)** Comparison of adrenalectomy outcome in APA cases with low (≤ median H-score) *vs* high (> median H-score) immunoexpression of CYP11B2 and HSD3B2. **(h)** Clinical outcome was significantly better in patients presenting a shorter duration of hypertension before surgery.

Conclusions

Our findings suggest that mutated APAs present a significantly higher CYP11B2 immunoreactivity, compared to *wild type*-APAs. In our analyzed APA cases, neither immunoreacivities of HSD3B2 or CYP11B2, nor genotype seem to be correlated with post-adrenalectomy outcome in primary aldosteronism.

Funding: *ANR-DFG Genetik und Pathophysiologie des primären Aldosteronismus BE 2177/13-1*

References

1/ Choi M. et *al.*, ***K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension.*** Science. 2011 Feb 11;331(6018):768-72.PMID:21311022.
2/ Scholl U. et *al.*, ***Somatic and germline CACNA1D calcium channel mutations in aldosterone-producing adenomas and primary aldosteronism.*** Nat Genet. 2013 Sep; 45(9):1050-4.PMID:23913001.
3/ Beuschlein F. et *al.*, ***Somatic mutations in ATP1A1 and ATP2B3 lead to aldosterone-producing adenomas and secondary hypertension.*** Nat Genet. 2013 Apr; 45(4):440-4, 444e1-2.PMID:23416519.
4/ Funder JW. et *al.*, ***Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline.*** J Clin Endocrinol Metab. 2008 Sep; 93(9):3266-81. doi: 10.1210/jc.2008-0104. PMID: 18552288.
5/ Gomez-Sanchez CE. et *al.*, ***Development of monoclonal antibodies against human CYP11B1 and CYP11B2.*** Mol Cell Endocrinol. 2014 Mar 5; 383(1-2):111-7. PMID:24325867.

17 ECE 3--GP-29
Endocrine tumours
Yara Rhayem
DOI: 10.3252/pso.eu.17ece.2015
Poster presented at: ECE2015 16-20 May 2015 DUBLIN, IRELAND 17th EUROPEAN CONGRESS OF ENDOCRINOLOGY
PosterSessionOnline.com