Immunoeexpressions of CYP11B2 and HSD3B2 in genetically characterized adrenocortical adenomas

Christian Gebhard1, Yara Rhayem1, Anna Dietz1, Anna Riester1, Constanze Hantel1, Marion Schuster1, Tim M. Strom2,3
Celso Gomez-Sanchez4, Martin Reincke1, Felix Beuschlein1

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

Introduction and Objectives

Somatic mutations of KCNQ1 (IT), CACNA1D (QT), ATP3A1 and ATP2B3 (I) have been shown to be involved in the formation of adrenocortical producing adenoma (AAP). This study aimed to investigate in postfil-

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 Cushing’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 Kit (Vector Laboratories) and for HSD3B2 using the Vectastain ABC Elite Kit (Vector Laboratories). Primary antibodies used in immunohistochemistry 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 Polik-Ds-Gr-Hu C1 kit (DQB Labs). Semi-quantitative immunohistochemical evaluation was assessed using Munic-Carty’s H-scor-ping system and statistical analysis was performed using Munic-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: KCNQ1, CACNA1D, ATP3A1, ATP2B3 or Wild type (WT - defined as the absence of candidate gene mutations).

_results

68% of APAs presented with a somatic mutation, most frequently KCNQ1 (42%). 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 tumours, where 67% presented only ZG-like cells. CACNA1D-mutated tumours presented significantly smaller tumour size (P=0.051 than KCNQ1-mutated APAs. The gender distribution shows that mostly men are affected by APAs, except for KCNQ1-mutated APA, which were predominantly seen in women (76%). Earlier onset of hypertension is observed in men (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 ± 6.3 years vs 6.5 ± 16.3 for women) (Fig. 1).

Immunoexpressions of CYP11B2 and HSD3B2 in genetically characterized APAPs

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 2G presented CYP11B2-positive clusters (Fig. 2b). 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. 2d) and no significant difference was noted in HSD3B2 immunoexpression regarding the APAs genotype (Fig. 2e). Expression patterns of CYP11B2 and HSD3B2 in APA were visualized using double immunostaining (Fig. 2g).

Conclusions

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

References

CAMPUS INNENSTADT
Medizinische Klinik und Poliklinik IV
Endocrine Research Department
Prof. Dr. med. Felix Beuschlein


DOI: 10.3252/pso.eu.17ece.2015

Endocrine tumours

Yara Rhayem

DOI: 10.3252/pso.eu.17ece.2015

Poster presented at:


DOI: 10.3252/pso.eu.17ece.2015

Endocrine tumours

Yara Rhayem

DOI: 10.3252/pso.eu.17ece.2015

Poster presented at: