Klinikum der Universität München · Campus Innenstadt
Medizinische Klinik · Ziemssenstraße 1 · 80336 München

To Mrs. Blanche Facchini
ESF Liaison Officer
European Science Foundation

Klinikdirektion
Prof. Dr. med. Martin Reincke

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80336 München

Ihr Zeichen: Unser Zeichen: MR/Spi

München, 10.11.2011

International Conference Progress in Primary Aldosteronism (PiPA-3), München, 03/07/2013 - 05/07/2013; ENSAT - Science Meeting 4830

Dear Mrs. Facchini,

Enclosed you will find the revised scientific report of the above mentioned meeting. I have structured the text according to the ESF rules.

I Summary (up to one page)

From July 3rd to July 5th 2013 the symposion Progress in Primary Aldosteronism 3 (PIPA-2) was held in Munich. In contrast to PIPA1, which took place in 2009 and was only funded by the German Research Organisation (DFG) and the Carl Friedrich von Siemens Foundation (CFvSF), and PIPA2 which was co-sponsered by DFG and ESF, PIPA3 was only sponsored by ESF (travel, hotel, logistics). Because of a generous contribution of the CFvSF, the beautiful location at the Castle Nymphenburg was free of charge. In addition, the CFvSF sponsored all the meals including 2 dinners. Therefore, expenses for catering and room rent were zero, and the funds applied for were used mainly for transportation. This explains why PIPA3 needed less funding from ESF than initially requested.

The symposion started with a half-day workshop dedicated to the genetics of bilateral adrenal hyperplasia attended by a total of 58 participants. The symposion itself covered all aspects of primary aldosteronism (genetics, pathophysiology, epidemiology, diagnosis, differential diagnosis, comorbidities, and treatment). Including speakers, 77 persons participated in the symposion coming from Germany, France, Italy, UK, Norway, Sweden, USA, Australia, Greece Russia, China, Japan, Brazil).

12 free communications from young investigators were selected for oral presentations, additional 11 posters were presented.

II, Description of the scientific content of and discussions at the event (up to four pages);

Being dismissed as a rare endocrine condition for many decades, primary aldosteronism (PA) is accepted as the most frequent and most important cause of a secondary arterial hypertension, certainly partly due to the widespread introduction of the aldosterone to renin ratio (ARR) as screening instrument. This is the rational to dedicate a scientific symposion such as PIPA-3 to this topic. Several evolving aspects were discussed during the meeting:

1. With a prevalence of 5% of hypertensive subjects in primary care and 10% of hypertensives in referral centers, screening strategies should be extended in designated risk populations. Primary aldosteronism is even more likely to be found in patients with resistant hypertension (10-30%) and patients with hypokalemic hypertension (>60%). With a growing awareness about the cardiovascular and metabolic comorbidities of sustained aldosterone hypersecretion, and the concomitant possibility of reversing these consequences through an early detection and therapy, the diagnosis of PA should not be delayed.
2. Besides rare familial forms, unilateral adrenal hyperplasia and aldosterone-producing carcinoma, the two main causes of PA are aldosterone producing adenoma (APA) and idiopathic bilateral adrenal hyperplasia (IHA), accounting for more than 95% of the cases. Although there has been significant progress in the understanding of the pathophysiology of primary aldosteronism, the accuracy of diagnostic tests and the outcome of various therapeutic measures, there are still many areas of uncertainty.
3. Recent reports identified mutations in the potassium channel *KCNJ5* as cause of familial and sporadic forms of primary aldosteronism and estimated the proportion of APAs caused by those mutations at 30-40% (Choi et al, Science. 2011 Feb 11;331(6018):768-72). Molecular mechanisms of autonomous aldosterone secretion were additionally highlyted by results of exome sequencing of APA in a large European consortium which is based on our ENSAT-ESF funding (Beuschlein et al., Nat Genet. 2013 Feb 17. doi: 10.1038/ng.2550). Somatic hot spot mutations in Na^+,K^+-ATPase (*ATP1A1*) and Ca^{2+}-ATPase (*ATP2B3*) genes were identified. These ATPases are expressed in adrenal cells and control Na^+, K^+ and Ca^{2+} homeostasis. Loss of pump activity and a strongly reduced affinity for K^+ was demonstrated by functional *in vitro* experiments for *ATP1A1* mutants. Electrophysiological *ex vivo* studies on primary adrenal adenoma cells further provided evidence for inappropriate depolarization of cells with ATPase mutations. In a large collection of 309 APA samples, 16 (5.2%) somatic mutations were found in *ATP1A1* and 5 (1.6%) in *ATP2B3*. Mutation carriers displayed male dominance

and increased plasma aldosterone and lower potassium compared with non-carriers.

Based on these results the third symposium on Progress in Primary Aldosteronism (PIPA-3) took place in July 2013 in the same location as before: the Carl Friedrich von Siemens Foundation. The faculty of PIPA-3 was based on that of the highly successful PIPA-1 (in 2009) and PIPA-2 (in 2011, co-funded by ESF) meetings.
The symposion PIPA-3 had the main focus on:

1. Genetics of primary aldosteronism. Recent publications have highlighted the genetic nature of primary aldosteronism. The symposium reviewed the most recent developments by inviting the top researcher from Europe and around the world. All major players from ENSAT-ESF countries will be invited. We also had presentations by the group of Morris Brown, Cambridge, UK, which presented unpublished data regarding a new genetic hotspot in the CACNAD1 gene.
2. Pathophysiology of aldosterone excess: main purpose of this session was to translate the consequences of somatic and germ-line mutations identified in PA into pathophysiologic concepts explaining aldosterone excess and tumor formation. Important presentations were given by Richard Warth, Maria Christina Zennaro, Paolo Mulatero and William Rainey.
3. Idiopathic bilateral adrenal hyperplasia: this subtype of PA has been an enigma since first description. Aim of this session is to show different concepts on potential mechanism leading to IHA. In addition, a workshop is going to address the genetics of PA. leading scientists, such as Xavier Jeunemaitre and Celso Gomez-Sanchez presented their view. In addition, we had presentations covering super-selective AVS and spontaneous remission of IHA.
4. Complications of PA and more
   Several worl class speaker presented their data, including John Funder who discussed his view on the ‘epidemics’ of PA

The final program is included. The invited speakers covered a wide range of topics, starting with basic research and ending in clinical management of primary aldosteronism. Throughout the meeting it remained a special feature that the speakers presented mainly unpublished data which had not been presented at scientific meetings previously. Therefore, it stimulated intense discussions. The session dedicated to free communications and posters was a special success. We could grant 23 travel awards to young scientists below the age of 35 years. The presentations were of extraordinary quality steering an intense discussion. The feedback given to the young investigators by the discussants and seniors was a special reward in the sense of mentorship.

The workshop on genetics of IHA had the following program:
Introduction: F. Beuschlein
Whole Genome Sequencing: T. Strom
Prevalences, Genetics
- Prevalence in China: Zhengpei Zeng
- Prevalence in Japan: H. Shibata
- Prevalence in Germany: M. Reincke
- Prevalence in Australia: M. Stowasser
- Prevalence in France: MC Zennaro
- Prevalence in Italy: P Mulatero
Study Outline and Governance of Collaborative Approach

After an introductory talk on the different genetic techniques presented by Tim Strom the participants received an overview on the different screening strategies and prevalences of PA around the world. In the final discussion there was general consensus that it will be very fruitful to collaborate world-wide on elucidating the genetic mechanism of IHA. An outline of the work plan is included below. In summary, it was an excellent meeting with outstanding speakers and presentations.

Because food and beverages were covered by the Carl Friedrich von Siemens Stiftung, the resulting costs were much lower than expected. In total, we needed 31.094,15 € from ESF, of this 21.125,32 for travel costs, 7.583,43 € for lodging, and 2385, 40 € for rent of poster walls and secretary assistance. I would like to thank ESF for its generous support in the name of all participants.

III. Assessment of the results and impact of the event on the future directions of the field (up to two pages);

The success and impact of PIPA-3 can be summarized as follows:

1. Co-operations initiated during the workshop on IHA

1. PIPA-3 was very successful in terms of collaboration. The workshop ended with general agreement that a large multi-centric study on the genetics of IHA should be initiated. The following countries agreed to participate:
    a. Germany
    b. France
    c. Italy
    d. Slovenia

e. Greece
f. UK
g. Sweden
h. Norway
i. China
j. Japan
k. Australia

This initiative will have the following governance structure:

a.) all participating centers will agree on a common definition on PA
b.) The scientific organization will be done by the Munich center
c.) The genetic analysis will be done decentrally
d.) Financial support will be perused on a national and internally level

2.) Another feature of PIPA-3 was the highly scientific atmosphere of the meeting leading to several multi-national initiatives and collaborations. As far as our center is involved they address genetic studies in sporadic PA, adrenal vein sampling studies, molecular biology studies in adrenal tissues, and genetic studies in families with PA.

In summary, I am convinced that the symposion PIPA-3 served very much its intended purpose.

Please let me know if I can of any further assistance.

Best regards

Prof. Dr. med. Martin Reincke
Direktor Medizinische Klinik und Poliklinik IV

Medizinische Klinik und Poliklinik IV
Ludwig-Maximilians-Universität
Department of Internal Medicine

Conference Program and Abstracts

Progress in Primary Aldosteronism 3

- From basic research to clinical evidence -

July 3rd-5th, 2013

Carl Friedrich von Siemens Stiftung
Südliches Schlossrondell 23
D-80638 Munich

We gratefully acknowledge the support

of this symposium received from

EUROPEAN SCIENCE FOUNDATION
SETTING SCIENCE AGENDAS FOR EUROPE

Carl Friedrich von Siemens Stiftung

Welcome

July 3, 2013

Dear Participants of the 3rd Symposium on Progress in Primary Aldosteronism,

The understanding of genetics and pathophysiology of primary aldosteronism has made significant progress since the symposium PIPA 2 held in May 2011. Several hallmark manuscripts have been published since, demonstrating that primary aldosteronism is (in part) a channelopathy. *We* are grateful to announce the follow-up meeting PIPA 3 which will take place in the same location as before, the *Carl Friedrich von Siemens Stiftung* in München. The faculty will be international with experts from around the world. Plenty of time is reserved for discussion. Investigators will have the opportunity to present their most recent data as short orals or as posters. One session will be dedicated to a new international initiative, a collaborative project to elucidate the genetics of idiopathic adrenal hyperplasia. We cordially invite you and would be glad if you participate in this prime scientific event.

Sincerely,

Martin Reincke Felix Beuschlein

for the Organizing Committee

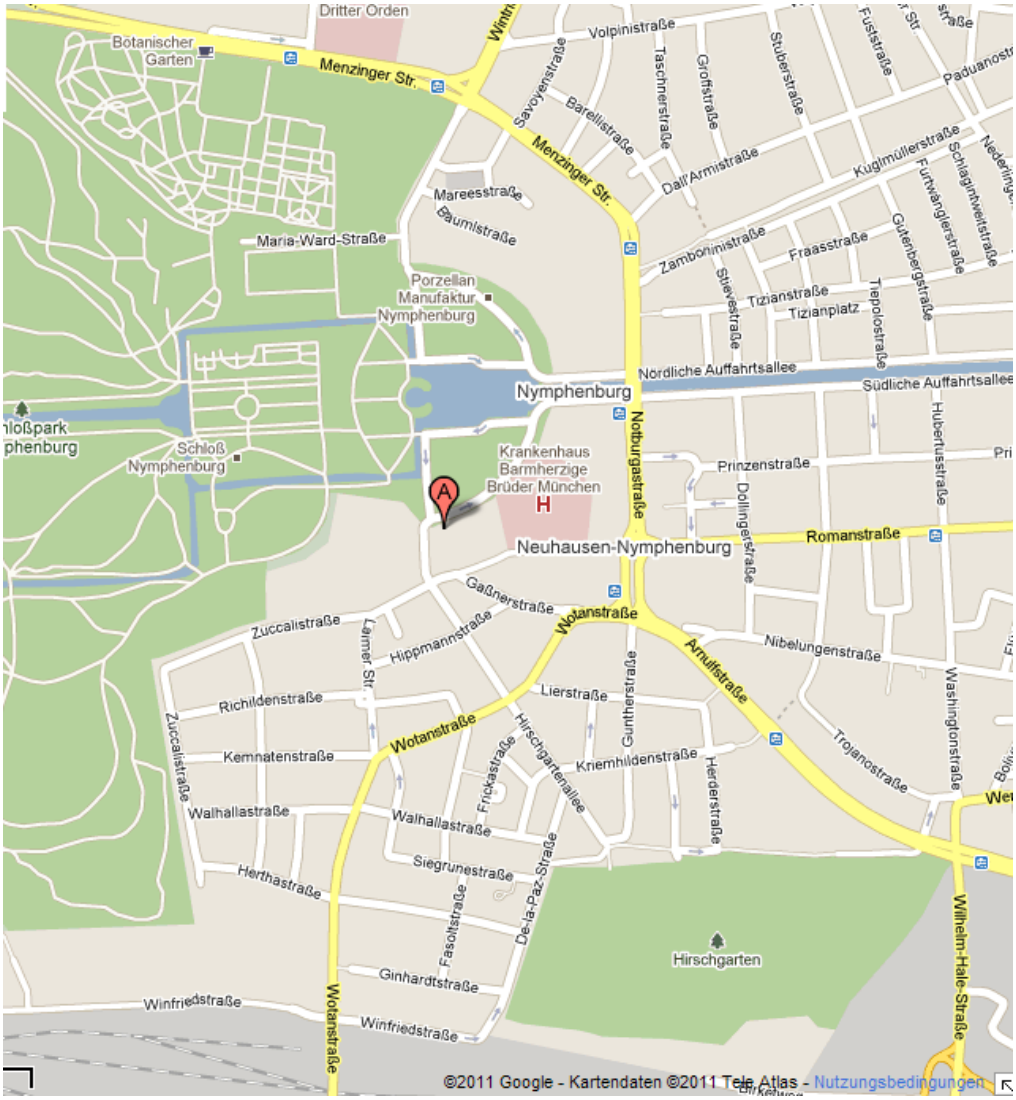
Content

Directions

Conference Address

A: Carl Friedrich von Siemens Stiftung
Südliches Schlossrondell 23
80638 Munich
Tel: +49 (0)89 / 178 033 0

Free WLAN!

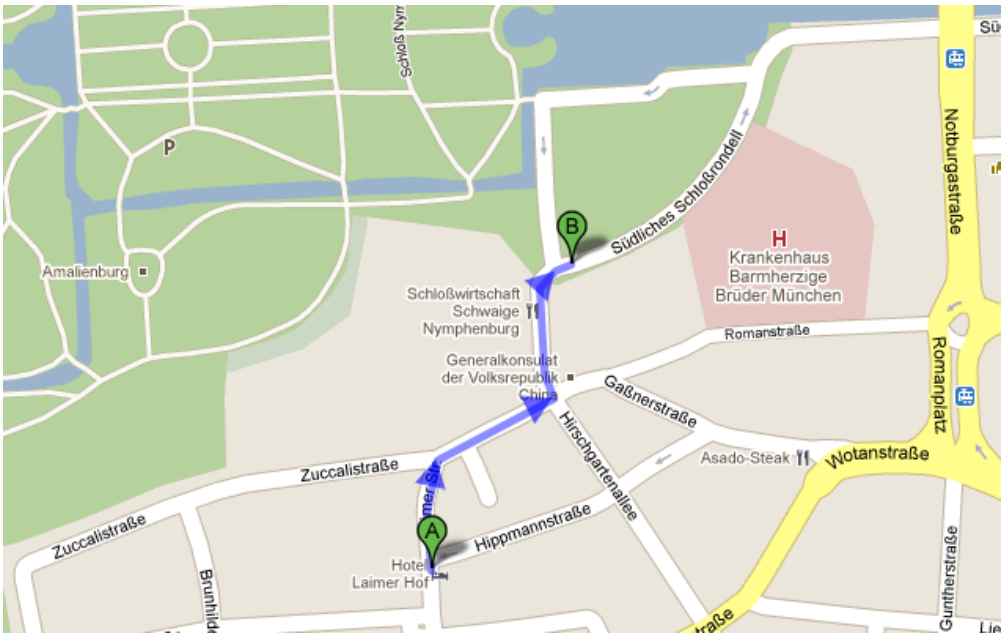


If you are lost, please call:
Dr. med Anna Riester
+49 (0)151 144 620 86

Emergency call:	112
Police:	110

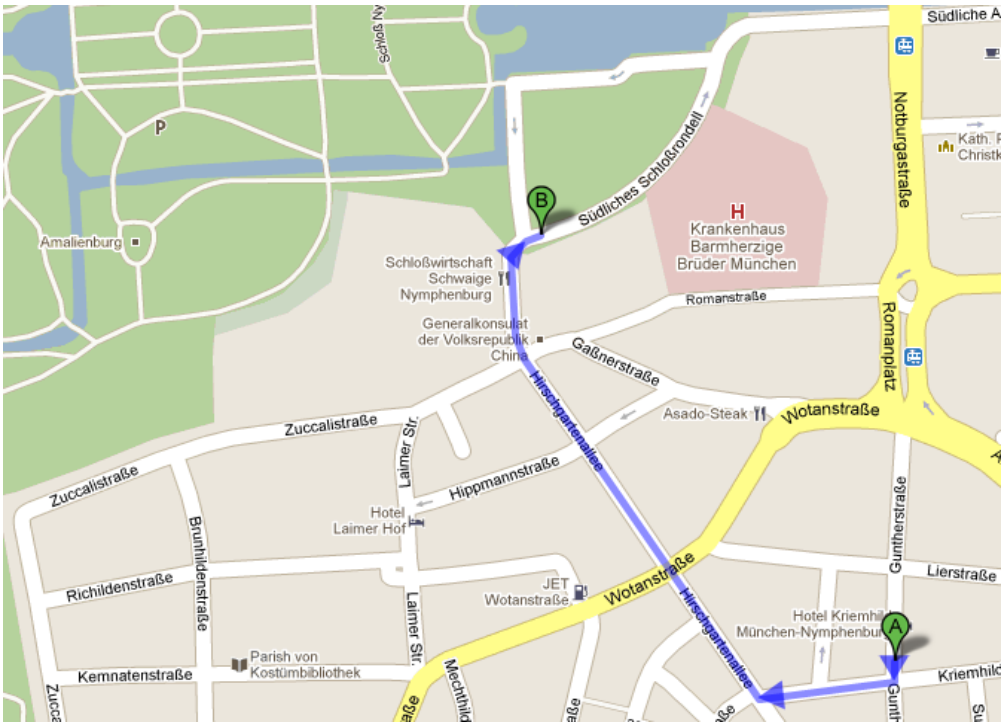
Hotels

Hotel Laimer Hof, Laimer Str. 40, 80639 Munich, +49 (0) 89/178 038 0



A: Hotel Laimer Hof
B: Symposium
Walking time: 5 minutes

Hotel Kriemhild, Guntherstr. 16, 80639 Munich, +49 (0) 89/ 171 117 0

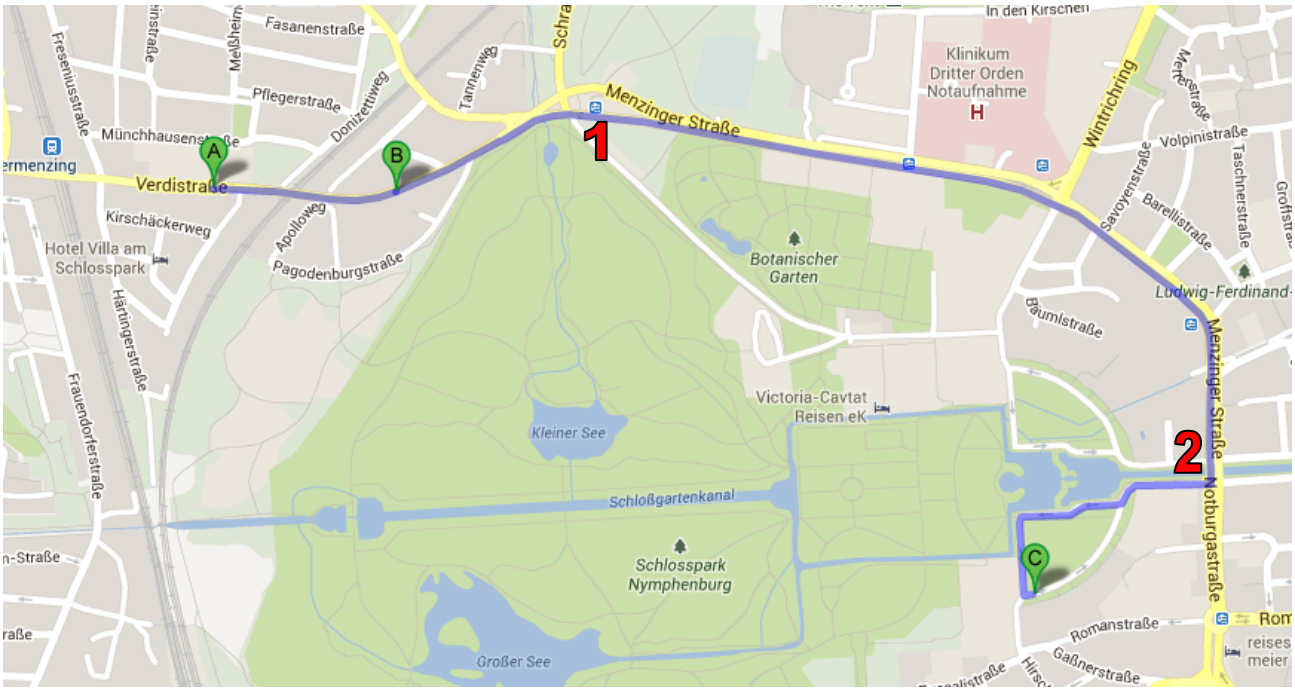


A: Hotel Kriemhild
B: Symposium

Walking time: 10 minutes

Hotel Amalienburg, Amalienburgstr. 24-26, 81247 Munich, +49 (0) 89/891 155 0

Hotel Aida, Verdistr. 9, 81247 Munich, +49 (0) 89/ 895 569 500

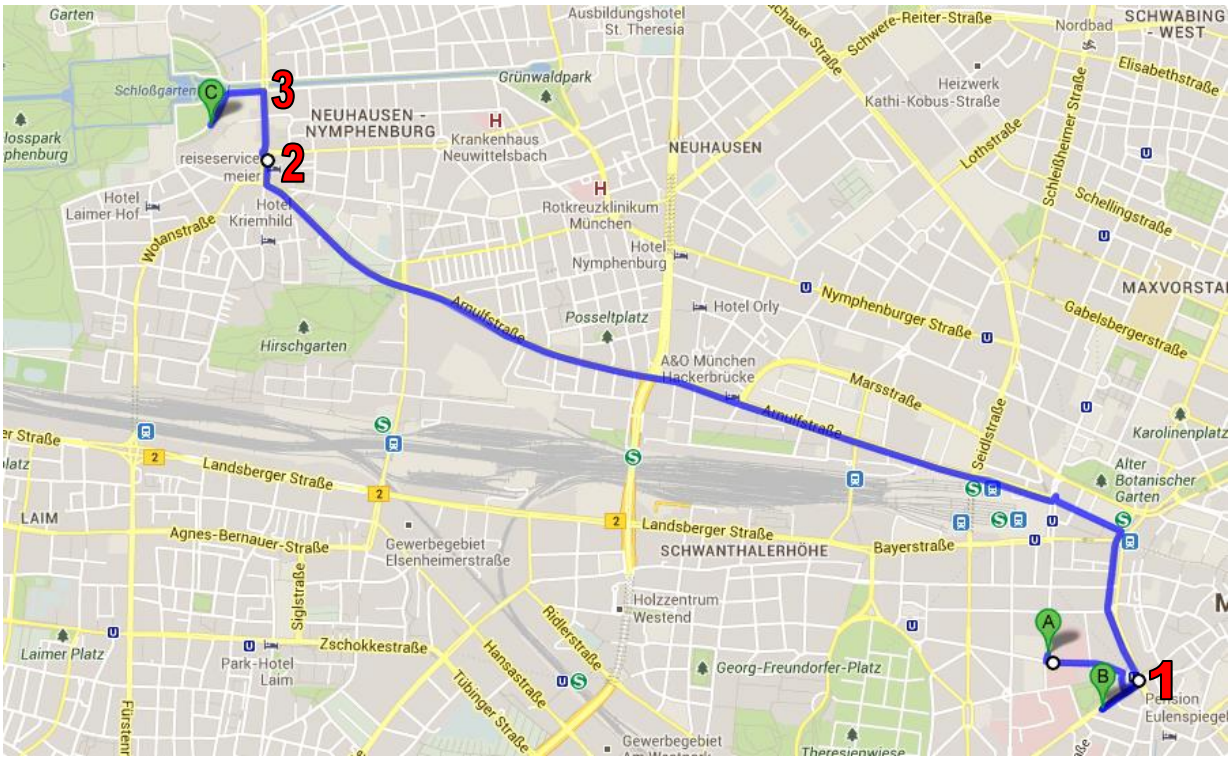


A: Hotel Aida
B: Hotel Amalienburg
C: Symposium

Walking time: 30 minutes
Or: Take the tramway "Tram 17", direction Effnerplatz, from the station "Amalienburgstraße" (1) to the station " Schloss Nymphenburg" (2)

Hotel Kraft, Schillerstr. 49, 80336 München, +49 (0) 89/ 550 594 0

Carathotel, Lindwurmstr. 13, 80337 München, +49 (0) 89/ 230 380



A: Hotel Kraft
B: Carathotel
C: Symposium

Take the tramway “Tram 17”, direction Amalienburgstr, from the station “Sendlinger Tor” (1) to the station “Romanplatz” (2) or “Schloss Nymphenburg” (3)
or
Take the tramway “Tram 16”, direction Romanplatz, from the station “Sendlinger Tor” (1) to the station “Romanplatz” (2)

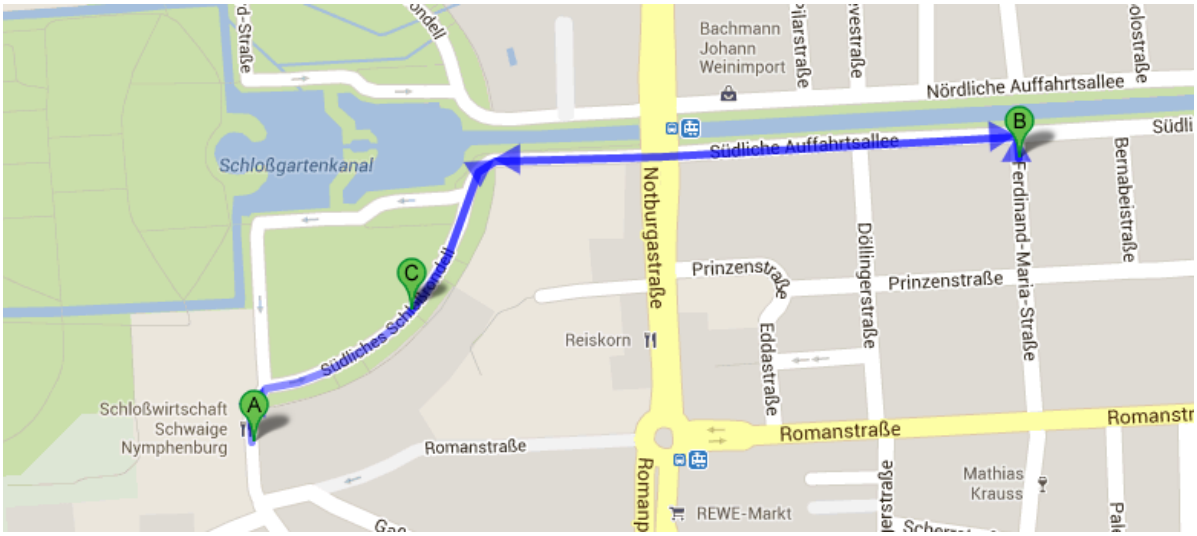
Adresses for Dinner:

Wednesday, July 3rd 2013

Restaurant Schwaige at Schloss Nymphenburg
Schloss Nymphenburg 30
80638 Munich
+49 (0) 89/ 120 208 90

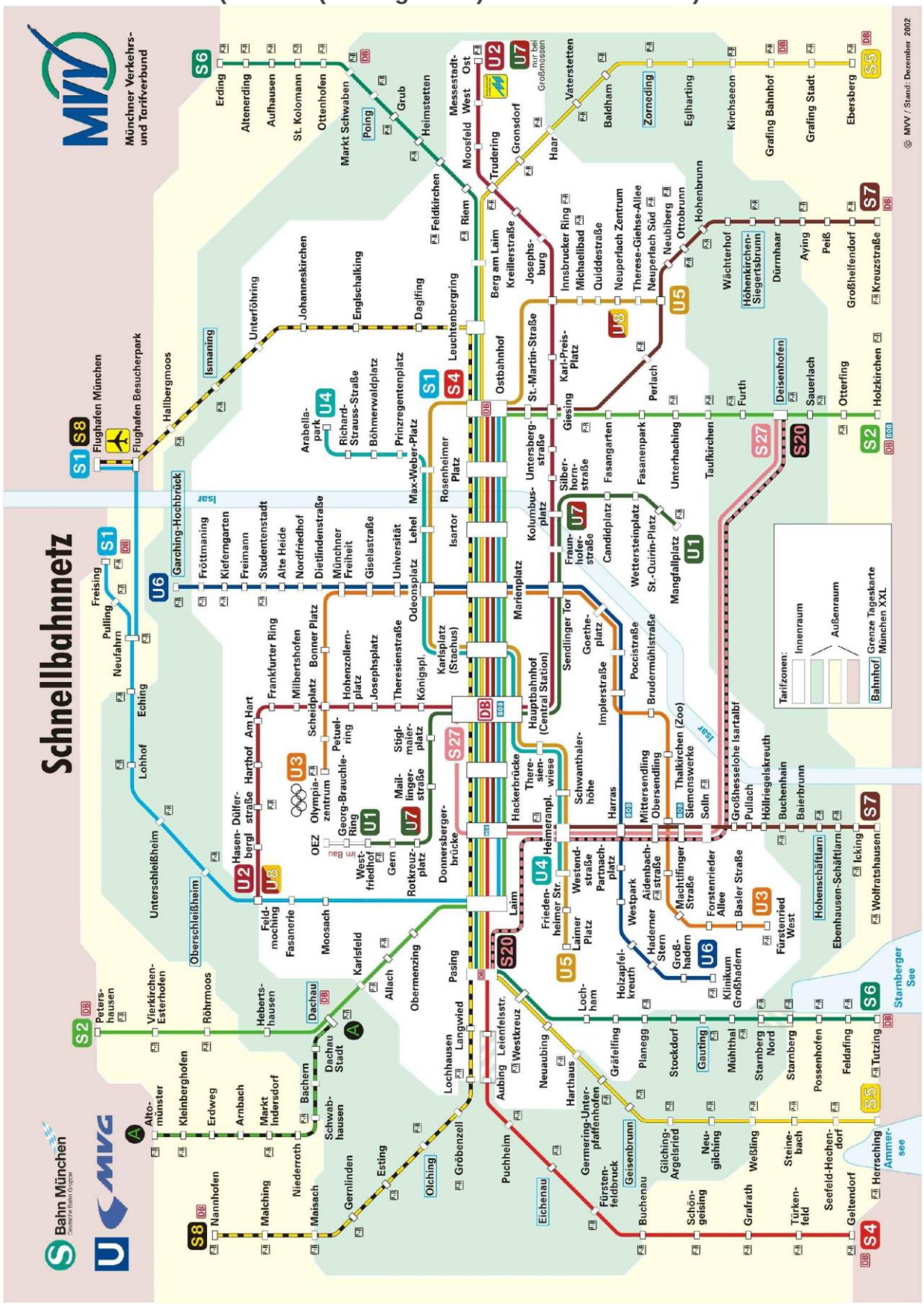
Thursday, July 4th 2013

Restaurante Canal Grande
Ferdinand-Maria-Straße 51
80639 Munich
+49 (0)89/ 174 565



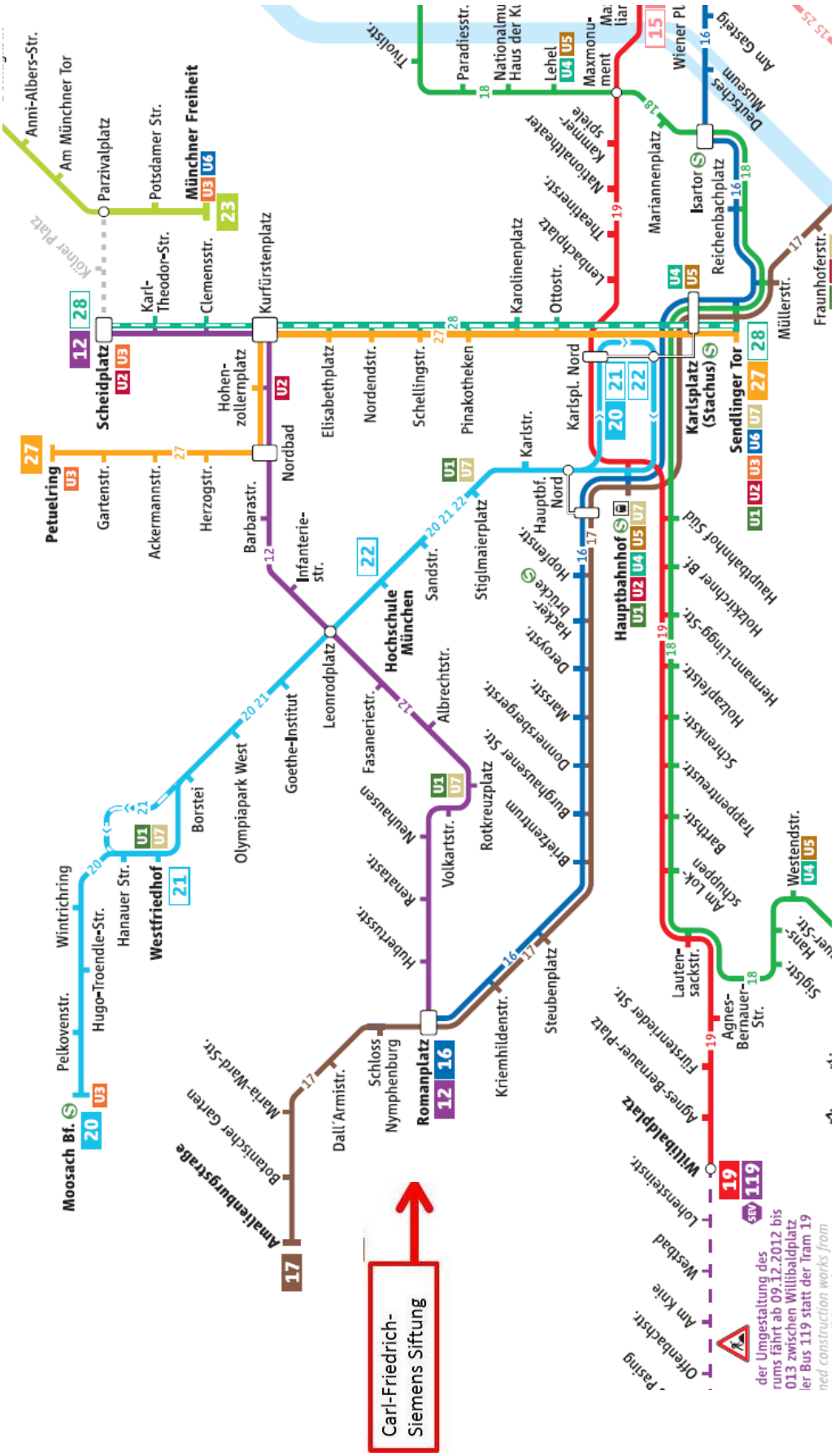
A: Symposium
B: Restaurant Canal Grande
C: Restaurant Schwaige

Urban rail network (U-Bahn (Underground) and S-Bahn Lines)



http://www.mvv-muenchen.de

Tram


Tram
Anni-Albers-Str.
Am Münchner Tor
Parzivalplatz
Potsdamer Str.
Münchner Freiheit
Kölner Platz
Karl-Theodor-Str.
Clemensstr.
Kurfürstenplatz
Scheidplatz
Hohen-zollernplatz
Elisabethplatz
Nordendstr.
Schellingstr.
Pinakotheken
Karolinenplatz
Ottostr.
Theatinerstr.
Nationaltheater
Kammer-spiele
Lehel
Maxmonu-ment
Paradiesstr.
Tivolistr.
Mariannenplatz
Isartor
Reichenbachplatz
Müllerstr.
Fraunhoferstr.
Deutsches Museum
Am Gasteig
Karlsplatz (Stachus)
Sendlinger Tor
Karlspl. Nord
Karlstr.
Petuelring
Gartenstr.
Ackermannstr.
Herzogstr.
Barbarastr.
Nordbad
Infanterie-str.
Leonrodplatz
Goethe-Institut
Olympiapark West
Borstei
Westfriedhof
Wintrichring
Pelkovenstr.
Hugo-Troendle-Str.
Hanauer Str.
Moosach Bf.
Hochschule München
Sandstr.
Stiglmaierplatz
Hauptbf. Nord
Hauptbahnhof
Hopfen-str.
Hacker-brücke
Destouchestr.
Marsstr.
Donnersbergerstr.
Burghausener Str.
Briefzentrum
Steubenplatz
Kriemhildenstr.
Fasanerie-str.
Albrechtstr.
Rotkreuzplatz
Volkartstr.
Neuhausen
Renatastr.
Hubertusstr.
Schloss Nymphenburg
Romanplatz
Dall'Armistr.
Amalienburgstraße
Botanischer Garten
Marta-Ward-Str.
Hauptbahnhof Süd
Holzkirchner Bf.
Hermann-Lingg-Str.
Holzapfelstr.
Schrenkstr.
Trappentreustr.
Bartstr.
Am Lok-schuppen
Lauten-sackstr.
Agnes-Bernauer-Str.
Fürstenrieder Str.
Agnes-Bernauer-Platz
Willibaldplatz
Lotheinstein-str.
Westend
Am Knie
Ohlenbachstr.
Pasing
Westendstr.
Hans-Sigl-Str.
der Umgestaltung des
rums fährt ab 09.12.2012 bis
013 zwischen Willibaldplatz
ler Bus 119 statt der Tram 19
ned construction works from
Carl-Friedrich-Siemens Siftung

Conference Program

Wednesday, July 3rd 2013

14.00- Workshop on Genetics of *Idiopathic*
18.00 *Adrenal Hyperplasia*
Speakers from Japan, China, Australia, US, Europe

14.00	**Introduction**: F. Beuschlein
14.30	**Whole Genome Sequencing**: T. Strom
15.00	**Prevalences and Genetics of PA/IHA**
	-in Australia: M. Stowasser
	-in France: M.C. Zennaro
	-in Italy: P. Mulatero
	-in Germany: M. Reincke
	-in China: Z. Zeng
	-in Japan: H. Shibata
17.30	Study Outline and Governance of Collaborative Approach
19.00	***Welcome and Get Together***
	Restaurant Schwaige at Schloss Nymphenburg

Thursday, July 4th 2013

08:00	**Registration**
08:30	**Welcome Address**
	M. Reincke, Munich

Session I Genetics and Pathophysiology
Chairs: MC Zennaro; J. Funder

08.35	**ATPase Mutations**
	Felix Beuschlein, Germany
09.00	**Genetics and clinical study of primary aldosteronism in Peking**
	Zhengpei Zeng; Peking; China
09.25	**Gain-of-Function mutations affecting Na+ and Ca++: Genotype-Phenotype differences among APA**
	Morris Brown, Cambridge, UK
09.50	**Aldosterone-Induced Cardiovascular Damage: the Critical Role of Salt**
	Michael Stowasser, Brisbane, Australia
10.15	**Familial Aldosteronism 3: What is new?**
	Paolo Mulatero, Turino, Italy

11.00 *Coffee Break*

Session II Genetics and Pathophysiology
Chairs: M. Bidlingmaier ; X. Jeunemaitre

11.30 **KCNJ5 Mutations and Cell Proliferation**
William Rainey, Ann Arbor, USA

11.55 **Aldosterone Excess in APA: a Channelopathy?**
Richard Warth, Regensburg, Germany

12.20 **Mouse Models for PA**
Ariadni Spyroglou, Munich, Germany

12.45 **From Gene Expression to Pathophysiological Models**
Maria-Christina Zennaro, Paris, France

13.10 *Lunch and Poster Viewing*

Free Communications from all fields of PA I
Chairs: F. Fallo, H. Shibata

14.30 **Technical aspect of adrenal venous sampling -Anatomical variants, catheter selection, and segmental AVS-**
Kei Takase, Miyagi, Japan

14.40 **Adrenal venous sampling versus I-131 NP-59 scintigraphy for primary aldosteronism – a review of the first five years in the national referral center**
Tomaz Kocjan, Ljubljana, Slovenia

14.50 **KCNJ5 Mutations: Implications for clinical care**
Fumitoshi Satoh, Sendai, Japan

15.00 **Influence of somatic mutations on the saline infusion test and salivary aldosterone**
Anna Riester, Munich, Germany

15.10 **Therapy of primary aldosteronism improves glucose homeostasis - results from the prospective German Mephisto study**
Gregor Hanslik, Berlin, Germany

15.20 **Cardiovascular complications in primary aldosteronism**
Masao Omura, Yokohama, Japan

15.30 *Coffee Break*

Free Communications from all fields of PA II
Chairs: M. Fassnacht; F. Satoh

16.00 **Gain-Of-Function Somatic Mutation Of The Na^+/K^+-ATPase α1 Subunit In A Zona Glomerulosa-Like Subtype Of Aldosterone-Producing Adenomas (APAs)**
Elena Azizan, Cambridge, UK

16.10 **Immunohistological diagnosis of aldosterone producing adenoma and KCNJ5 mutation status**
Sheerazed Boulkroun, Paris, France

16.20 **Twenty-five-fold up-regulation of a leucine-rich repeat containing G proteincoupled receptor (LGR5) in human adrenal zona glomerulosa and its potential role in cell proliferation and apoptosis**
Haris Shaikh Lalarukh, Cambridge, UK

16.30 **Oxidative stress in patients affected by Primary Aldosteronism**
Luigi Petramala, Roma, Italy

16.40 **Marinobufagenin in essential hypertension and primary aldosteronism: a cardiotonic steroid with clinical and diagnostic implications. The Graz Endocrine Causes of Hypertension (GECOH) Study**
Martin Gaksch, Graz, Austria

16.50 **Aldosterone and Parathyroid**
Vanessa Ronconi, Ancona, Italy

Session III Pathophysiology
Chairs: W. Rainey; M. Stowasser

17.00 **Myocardial Fibrosis in Primary Aldosteronism**
John Connell, Dundee, UK

17.25 **CYP 11B1 and B2 Immunohistochemistry of the Adrenal Gland**
Celso Gomez-Sanchez, Jackson, US

17.50 **From Low Renin Hypertension to IHA**
Xavier Jeunemaitre, Paris, France

20.00 ***Dinner***
Restaurante Gran Canale

Friday, July 5th 2013

Session IV Idiopathic Hyperaldosteronism
Chairs: F. Beuschlein; GP Rossi

08.30 **Superselective AVS and Adrenal Sparing Surgery**
Fumitoshi Satoh, Sendai, Japan

08.55 **Plasma Metanephrines during AVS**
Holger Willenberg, Düsseldorf, Germany

09.20 **Spontaneous Remission in IHA?**
Evelyn Fischer, Munich, Germany

09.45 **Adipocyte-derived Factors as a Potential Cause of IHA**
Hirotaka Shibata, Oita, Japan

10.10 *Coffee Break*

Chairs: F. Mantero, Z. Shafigullina

10.40 **PA in Hypertension: more frequent than currently assumed?**
George Piaditis, Athens, Greece

11.05 **Diagnostic Criteria and Prevalence of IHA (in Europe)**
Laurence Amar, Paris, France

Session V Complications of PA and more
Chairs: P. Mulatero; L. Amar

11.30 **Is PA ~30% of Hypertension?**
John Funder, Melbourne, Australia

11.55 **Neoplasms in Patients with PA**
Katharina Lang, Würzburg, Germany

12.20 **Regression of Left Ventricular Hypertrophy during Treatment**
Gian-Paolo Rossi, Padua, Italy

12.45 **Metabolic Syndrome**
Francesco Fallo, Padua, Italy

13.10 **Closing Remarks and Farewell**
Martin Reincke, Munich, Germany

13.15 Lunch

Poster

P1 **Highly selective expression of DACH1 in the zona glomerulosa of human adrenal glands, and a putative role in regulation of aldosterone production**
Junhua Zhou, University of Cambridge, UK

P2 **Adrenal steroidogenic profiles through LC-MS/MS in patients with primary aldosteronism during selective adrenal vein sampling**
Gilberta Giacchetti, Ancona, Italy

P3 **18-hydroxycorticosterone and 11-deoxycorticosterone in the diagnosis of primary aldosteronism**
Dina Protashchik, Saint-Petersburg, Russia

P4 **Prospective analysis of the saline infusion test regarding sub-differentiation and outcome in primary aldosteronism**
Marianne Weigel, Berlin, Germany

P5 **Lack of influence of somatic mutations on steroid gradients of adrenal vein sampling in aldosterone producing adenomas: results of the German Conn's registry – Else Kröner-Fresenius Hyperaldosteronismus Registry**
Andrea Osswald, Munich, Germany

P6 **Sleep quality in patients with primary aldosteronism**
Franziska Hanusch, Berlin, Germany

P7 **Aldosterone hyperesponse to ACTH stimulation in a hypertensive population: a new clinical entity?**
Athina Markou, Athens, Greece

P8 **Diagnosis and treatment for bilateral aldosterone-producing adenomas**
Masao Omura, Yokohama, Japan

P9 **Clinical strategy for recurrent APA with segmental AVS**
Yoshikiyo Ono, Sendai, Japan

P10 **Intermittent hyperaldosteronism in a young patient with an adrenal micronodule – a diagnostic challenge**
Monica Livia Gheorghiu, Bucharest, Romania

P11 **Rapid testing in adrenal venous sampling**
Holger S Willenberg, Düsseldorf, Germany

Organizing Committee

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John Connell
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Abstracts

Free Communications from all fields of PA I

Thursday, 1st July,

2.30 pm - 3.30 pm

Technical aspect of adrenal venous sampling
--- Anatomical variants, catheter selection, and segmental AVS---

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OBJECTIVE. To describe our method of performing AVS utilizing MDCT information, pre-shaped adrenal venous catheters, and segmental venous catheterization.

MATERIALS AND METHODS. Post-contrast four-phase dynamic MDCT were obtained using a 64-detector row helical CT scanner in patients with primary aldosteronism to visualize adrenal venous anatomy. The scan delay was set by means of an automatic triggering system (SureStart; Toshiba). When the attenuation value at the level of the ascending aorta reached a preset threshold (an absolute attenuation value of 100 HU), Early-arterial-phase scanning automatically started. Late-arterial-phase scanning was begun immediately after the completion of the early arterial scanning. Venous phase scanning was begun 15 seconds after the completion of the late arterial scan followed by delayed-phase scanning 2 minutes after start of the injection. Multiplanar images were analyzed by workstation.
We used five types of adrenal venous catheters ("Adselect", Hanako, Tokyo, Japan). We selected the type of the catheter based on the adrenal venous anatomy visualized by MDCT. Type 1 catheter was used for the common anatomy of the right adrenal vein (RAV) which runs medially to join lateral posterior quadrant of the IVC. Type 2 was used for RAV which runs cranially. Type 3 catheter was used for RAV which joins medial posterior quadrant of the IVC. Type 4 catheter was used for RAV which runs laterally to join the IVC. Type 5 catheter was used for RAV-AHV common trunk. From 2011 to 2012, segmental AVS was performed in 65 patients using a high-flow type of microcatheter inserted into three tributaries on each side.

RESULTS
The right adrenal vein was detected in all patients. The right adrenal vein formed a common trunk with an accessory hepatic vein in 16%. The orifice was located between the level of vertebrae T10 and L2. The right adrenal vein joined the inferior vena cava in the right posterior quadrant in 98% and in the left posterior quadrant in 2%. The transverse direction from the inferior vena cava was posterior and rightward in 78% and posterior and leftward in 22%, and the vertical direction from the inferior vena cava was caudal in 68% and cranial in 19 patients 32%. These results coincided with angiographic diagnosis. In all patients, right adrenal venous blood was successfully sampled. Other cases of variant adrenal veins including duplicated adrenal vein, occluded adrenal vein, and splenorenal shunt were also detected. Based on the results of segmental AVS procedure, the numbers of patients diagnosed with unilateral APA, IHA and bilateral APAs were, 31 (49%), 22 (39%), and 12 (19%), respectively.

CONCLUSION. MDCT enabled the identification of the RAV and delineation of its anatomy. Use of our adrenal venous catheter based on the preoperative RAV information helps accurate catheterization of the RAV for adrenal venous sampling. Segmental AVS allowed the identification of the location of intra-adrenal aldosterone hypersecretion. Superselective AVS has the potential to diagnose patients with bilateral APA who otherwise might be misdiagnosed.

Adrenal venous sampling versus I-131 NP-59 scintigraphy for primary aldosteronism – a review of the first five years in the national referral center

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Background. For optimal treatment of primary aldosteronism (PA) it is crucial to distinguish between unilateral adenoma (APA) and bilateral hyperplasia (IHA) of the adrenal cortex. Traditionally our decision for operation was usually based on positive adrenal 131-I NP-59 scintigraphy with dexamethasone suppression. Due to insufficient performance of the method selective adrenal venous sampling (AVS) was introduced in diagnostics of PA in 2004.
Aim. By comparison of scintigraphy and AVS results with final diagnosis we tried to confirm that introduction of AVS significantly improved distinguishing between APA and IHA and consequently facilitated the selection of patients who would benefit from surgical treatment.
Methods. A retrospective review of the 5-year period (from year 2004 to 2008) was done on a sample of all 58 patients with PA at our department who had a scintigraphy and/or AVS performed during the listed period. Special attention was paid to concordance of these two methods' results with final diagnosis. For AVS the success rate and frequency of possible complications were also checked. Final diagnosis in operated patients was made on the basis of the operation success, and in other patients on the basis of a successfully performed AVS. Concordance of diagnostic methods (which both give binary outcome) was compared with the help of Cohen's kappa coefficient.
Results. There were 37 males and 21 females with average age of 51±11.8 years in our group of 58 patients. AVS was performed in 11 operated patients with a specificity of 100%, while estimation of the kappa coefficient was not possible. During our review the success rate of AVS was 66% (in 25 patients out of 38), although in seven patients the procedure had to be repeated. One patient suffered a complication with adrenal hemorrhage (2.2%). Concordance of the scintigraphy with the final diagnosis of APA was according to the Cohen's kappa coefficient statistically not only not significantly better than chance, but also worse (-0,250; p = 0,521). Scintigraphy results were concordant to AVS results only in 11% of cases.
Conclusions. AVS proved itself to be a method which can distinguish APA and IHA with 100% specificity when successful. On the other hand, scintigraphy of the adrenal cortex seems to be not sufficiently reliable for this purpose. When performed by an experienced radiologist AVS is sufficiently safe for routine use. The success rate of AVS performance at our center was slightly below average in the first five years.

KCNJ5 Mutations: Implication for Clinical Care?

Ono Y[1], Nakamura Y[2], Felizola SJA[2], Morimoto R[1], Kudo M[1], Iwakura Y[1], Ito S[1], Rainey WE[3], Sasano H[2], and Satoh F[1]

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Context: Primary aldosteronism is worldly accepted as a major cause of secondary hypertension. Recently two gain-of-function somatic missense mutations (G151R or L168R) were found in the potassium channel KCNJ5 gene (kir3.4) in aldosterone producing adenomas (APAs). KCNJ5 mutations, which alter the channel selectivity filter of the encoded potassium channel and result in a calcium-mediated increase in aldosterone production, have been reported to cause familial hyperaldosteronism type III and approximately 40% of sporadic APAs. Interestingly, higher incidents of KCNJ5 mutations were reported in Japanese population with APAs.

Objective: To determine the effects of KCNJ5 mutations of APAs on clinical characteristics and steroidogenic enzyme gene expression associated with aldosterone production.
Methods: We sequenced KCNJ5 cDNA and performed real-time qPCR to investigate steroidogenic enzyme mRNA using APA tissues of 73 patients with APAs. We also compared clinical characteristics between the patients with and without mutations.

Results: Of the 73 patients with APAs, 53 (72.6%) had two somatic mutations of KCNJ5 (G151R or L168R) and they demonstrated significantly higher mRNAs expression of CYP11B2 and significantly lower mRNAs expressions of CYP11B and CYP17A compared to the wild type 20 patients.
Plasma aldosterone concentrations (PAC) and aldosterone/renin activity ratio (ARR) were significantly higher, and serum potassium concentrations and plasma renin activity were significantly lower in the mutated group.

Conclusion: We found the higher incident of KCNJ5 mutation in Japanese patients with APAs and the patients with mutation demonstrated higher aldosterone production from tumors.

Influence of somatic mutations on saline infusion test and salivary aldosterone

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Saline Infusion Test (SIT) is one of the most used confirmation test to diagnose primary aldosteronism. Recently, somatic mutations in aldosterone producing adenomas (APA) have become a focus of research. We previously identified somatic KCNJ5 K^+-channel and ATPase gene mutations in approximately 50% of APAs. However, it is unclear whether this mutations lead to specific biochemical alterations in parameters of the saline infusion test or in salivary aldosterone 24h-profiles.

To analyse the influence of somatic mutations on saline infusion test, 108 patients with APAs diagnosed according to the German Conn Registry standard were studied. All subjects underwent saline infusion test for confirming the aldosteronism and also technically successful adrenal vein sampling. In 43 patients aldosteronism was caused by bilateral hyperplasia. The other patients had unilateral adenomas, which were analysed for KCNJ5 mutation (n=20, 31 % of all APA) and ATPase mutation (n=10, 15 %) in tumor tissue. In 35 adenomas none of these mutations could be found.

Comparison of all 4 groups showed differences in plasma aldosterone before (P<0.01) and after (P<0.001) saline infusion (s. table 1). Especially, adenomas with ATPase mutation showed higher levels of baseline aldosterone in comparison to bilateral hyperplasia (P<0.01), whereas after saline infusion test adenomas with KCNJ5 mutations had significantly higher aldosterone values than bilateral disease (P<0.001). Comparing aldosterone in salivary day profile the value at 8 am differs between the 4 groups (P<0.05) and ATPase mutation carriers had significantly higher salivary aldosterone values than patients with bilateral hyperplasia (250±47 vs. 106±12 ng/l, P<0.05).

	Aldo prae SIT (ng/l)	Aldo post SIT (ng/l)	Salivary Aldo 8 am (ng/l)	Salivary Aldo 8 pm (ng/l)
P (all groups)	*<0.01*	*<0.001*	*<0.05*	*0.64*
Bilateral hyperplasia	209±22	102±9	106±12	61±8
APA with KCNJ5-/-,ATPase-/-	283±24	178±19	120±21	70±13
APA with KCNJ5 mutation	316±36	220±25	132±30	56±9
APA with ATPase mutation	441±91	193±53	250±50	118±41

Table 1: Plasma aldosterone before and after saline infusion and in salivary aldosterone at 8 am and 8 pm in all 4 groups (mean±standard error). Data were compared using Kruskal-Wallis-Test.

In summary, ATPase mutations seem to have an impact on salivary and plasma aldosterone levels. In the future, prediction of mutations based on clinical parameters might be possible in specific cases and should be analysed with a higher number of patients.

Therapy of primary aldosteronism improves glucose homeostasis - results from the prospective German Mephisto study.

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Background: It is widely known that patients suffering from primary aldosteronism (PA) have a higher risk of comorbidities than patients with essential hypertension (EH). Recent data might suggest that diabetes mellitus type 2 (DM2) is more prevalent in patients with PA than in patients with EH. No data exists regarding pre-diabetes conditions such as impaired glucose tolerance (IGT) in patients with PA.

Objective: 1) To identify and to evaluate the glucose homeostasis in PA patients; 2) To evaluate changes in glucose homeostasis before and after treatment initiation of PA

Methods: PA patients were recruited as part of the Mephisto-Study (German Conn's Registry). Prospective patients were evaluated before treatment (at diagnosis) and every six months thereafter. Assessment included metabolic parameters such as oral glucose tolerance test (oGTT).

Results: 162 prospective PA patients were included in this analysis. Using the oGTT we identified 104 patients (64.2%) with a normal glucose tolerance (NGT), 43 (26.5%) with an IGT and 15 (9.3%) were identified as DM2 patients. Patients with a pathological glucose tolerance (PGT: IGT and DM2) had higher triglycerides (131.6 ± 79.7 vs 105.3 ± 53.3; $p<0.05$), higher body-mass index (BMI) (28.9 ± 4.3 vs 27.5 ± 4.5; $p<0.05$), higher waist-to-hip ratio (WHR) (1.00 ± 0.15 vs 0.92 ± 0.09; $p<0.05$) and lower initial potassium levels (3.26 ± 0.47 vs 3.50 ± 0.50, $p < 0.01$) compared to NGT patients. Consequently hypokaliemic PA patients showed significantly higher 2h glucose (141.9 ± 47.6 vs 118.0 ± 47.4; $p<0.01$) and insulin levels (104.3 ± 89.9 vs 57.8 ± 74.4; $p<0.001$) in oGTT testing at baseline. 43 PA patients were re-evaluated 12 months after treatment initiation with oGTT: 35 patients had NGT (n=30 at baseline) and 8 IGT (n=13 at baseline; $p<0.01$). The baseline IGT subgroup showed significantly lower 2h glucose levels in oGTT testing at follow-up (158.8 ± 16.7 vs 140.6 ± 25.1; $p<0.05$). In addition the potassium level was significantly higher at follow-up (3.07 ± 0.38 vs 4.10 ± 0.52; $p<0.001$). BMI and WHR showed no changes. This improvement was not dependent on type of therapy (operation vs medical therapy).

Conclusions:
In PA patients DM2 and IGT are diagnosed often. Therapy of PA improved significantly glucose tolerance. Hypokaliemia might influence glucose homeostasis in PA patients.

Cardiovascular complications in primary aldosteronism.

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Objective: Primary aldosteronism (PA) is well known to easily induce hypertension and organ damages (cerebral hemorrhage, cerebral infarction, myocardial infarction, cardiomegaly, arrhythmia, renal insufficiency, etc.). Thus, we attempted to examine the clinical characteristics of cardiovascular complications just before surgical or medical treatment, and also analyzed cure rate of hypertension after unilateral adrenalectomy in PA-patients.

Methods: We enrolled 400 PA-patients who had been definitely diagnosed after detecting positive confirmatory tests according to the Guideline for PA from Japan Endocrine, such as screening by the ratios of PAC to PRA (ARR) being 20ng/dl per ng/ml/h or higher. Hyper-secretion of aldosterone from unilateral or bilateral adrenal glands was also confirmed by ACTH-stimulated adrenal venous sampling (ACTH-AVS) in all 400 PA-patients. Among 261 patients with unilateral PA confirmed by ACTH-AVS, 214 were treated by unilateral adrenalectomy and aldosterone-producing adenoma was diagnosed by pathological examinations of resected adrenal glands. One hundred thirty-nine patients were clinically diagnosed as IHA by ACTH-AVS. We examined them whether or not they complicated with cardiovascular diseases (CVD), stroke and CKD. Moreover, we analyzed 214 cases to clarify changes in hypertension after treating by unilateral adrenalectomy.

Results: There were 90 cases among 400 PA-patients, showing any complications. Logistic analysis revealed that cardiovascular complications, including CVD, stroke and CKD are significantly related to the duration of hypertension, but not to the levels of PAC, PRA, urinary aldosterone, serum potassium and blood pressure. No ant-hypertensive drugs were necessary in 140 cases among 214 cases one year after surgery. Multiple regression analysis revealed that BMI, duration, Cr and tumour size were independent positive contributors to uncured hypertension after surgery; however, blood pressure and PAC were statistically excluded.

Conclusion; The present data clearly demonstrated that the duration of hypertension is the most important risk factor for inducing cardiovascular complications in PA. Thus we should definitely diagnose PA among hypertensives as earlier as possible. Obese must be avoided when PA is diagnosed, and aldosterone-producing microadenoma, that is usually undetectable by CT, should be precisely diagnosed by adrenal vein sampling.

Abstracts

Free Communications from all fields of PA II

Thursday, 1st July,

4.00 pm – 5.00 pm

Gain-Of-Function Somatic Mutation Of The Na^+/K^+-ATPase α1 Subunit In A Zona Glomerulosa-Like Subtype Of Aldosterone-Producing Adenomas (APAs)

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Phenotypic differences between APAs with or without activating mutations of KCNJ5 prompted us to look for novel somatic mutations in APAs with gene expression profiles and histological features resembling normal adrenal zona glomerulosa (ZG) (Azizan et. Al, 2012). 10 such APAs were sent to BGI (Shenzen, China) for whole exome sequencing along with paired germline DNA for 9 of the APAs. Replication of novel mutations was sought in two independent (Czech and Dutch) cohorts. APAs with novel mutations were compared histologically (cell density, number of spironolactone bodies) and immunohistochemically with KCNJ5-mutant APAs; transcriptomes of the genotypically different APAs were compared by microarray both with each other and with adjacent ZG and ZF from which RNA was separately collected by laser capture microdissection (LCM). The function of expressed mutants was studied in *Xenopus* oocytes and human adrenocortical (H295R) cells.

Exome sequencing showed nine of the 10 APAs to have a novel somatic mutation in genes encoding a subunit of either a Na^+,K^+-ATPase (n=4) or voltage-dependent Ca^{2+} channel (Ca_v)(n=5). Two of the mutations occurred twice, including the recently discovered L104R mutation of *ATP1A1 (Beuschlein et al., 2013)*. This, and a 100-104del mutation spanning the same L104 residue, not only blocked Na/K transport but caused a large inward leak of Na^+ and H^+ in oocytes; ATP1A1 L104R caused a 2-3 fold increase in aldosterone secretion and CYP11B2 expression in H295R cells. 5/39 APAs in the Czech cohort diagnosed in some cases by adrenal vein sampling (AVS) alone (i.e. with normal adrenal CT), had the L104R (n=4) or Ca_v mutations, compared to only 2/50 patients in the Dutch cohort. The function of the Ca_v mutations is not yet certain; but their conserved sites, and recurrence of three out of the four, points to a probably causal role. All APAs with the novel ATPase or Ca_v mutations were <2 cms in diameter (eight were $\leq$ 1cm), and had >40% compact cells. 9/13 had spironolactone bodies *cf.* 8/20 KCNJ5 mutant APAs (average counts of respectively, 40$\pm$18 *cf.* 1$\pm$1; p=0.01). Unsupervised cluster analysis of the microarray separated KCNJ5 mutant APAs from those with the new mutations, and qPCR confirmed a cluster of genes which were more highly expressed both in normal ZG than ZF, and in APAs with the new mutations than the KCNJ5 mutants. IHC showed the expression of the Na^+,K^+-ATPase and Ca_v protein were themselves more abundant in ZG than ZF with higher expression also in the APAs with the new mutations than in KCNJ5 mutants.

The new mutations, and genotype:phenotype correlations, point to a semi-distinct type of APA, possibly arising in ZG. Its higher frequency in cohorts where a normal CT or MRI does not exclude further investigation for unilateral primary hyperaldosteronism, suggest that the 'ZG-like' APAs can be easily overlooked, with a tendency to smaller size perhaps due in part to their more compact cells. The dominant nature of the L104R mutation, despite abundance of wild-type enzyme, is likely due to a depolarising effect of the inward leak current.

Immunohistological diagnosis of aldosterone producing adenoma and KCNJ5 mutation status.

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Background. Primary aldosteronism (PA) is the most common and curable form of hypertension, due to autonomous aldosterone production. Despite progress in the management of patients with PA, critical issues related to diagnosis still persist. Recurrent somatic mutations of the *KCNJ5* gene, coding for the potassium channel GIRK4, have been implicated in the formation of aldosterone producing adenoma (APA). These mutations are present in about 40% of APA. We have shown that while GIRK4 is expressed in APA and in the adjacent zona glomerulosa, significantly lower levels are detected in APA harboring *KCNJ5* mutations.
The current pathological diagnosis of APA is based on the description of nodules and/or hyperplasia in the resected adrenal gland, which together with normalization of aldosterone levels after surgery is indicative of aldosterone overproduction. However, no functional histopathological marker is available to unequivocally identify the presence and sites of aldosterone production. The use of specific antibodies against aldosterone synthase (AS) and 11β-hydroxylase has recently been reported for diagnosis of PA by immunohistochemistry. However it has been previously shown that not all APA do express high levels of AS. Furthermore, routine diagnosis in a hospital setting requires commercial antibodies available in large amounts.
Objective. The aim of our study was to investigate the possibility of using different combinations of antibodies to: (1) detect aldosterone producing sites in resected adrenals from patients with PA; (2) distinguish APA from non-producing adenomas (NPA) and cortisol-producing adenoma (CPA); (3) identify APA harboring GIRK4 mutations.
Methods. Adrenals from 18 patients with APA, 18 patients with cortisol producing adenoma (CPA) and 13 patients with NPA were investigated. Consecutive paraffin-embedded sections of APA and the adjacent (ADJ) non-tumoral adrenal cortex were stained with commercial antibodies against Dab2 (a marker of zona glomerulosa cells which is also expressed in APA) and GIRK4. *In situ* hybridization for *Cyp11B1* (coding for 11β-hydroxylase) and *Cyp11B2* (coding for AS) was performed to confirm functional nodules. Expression was analyzed as a qualitative trait.
Results. Dab2 expression was detected at the membrane in 12 out of 18 APA, two NPA and two CPA (p=0.0005). All APA showed positive membranous labeling for GIRK4; similar labeling was observed in five NPA and 4 CPA (p<0.0001). A majority of NPA and CPA showed cytoplasmic GIRK4 expression. The sensitivity of Dab2 staining for diagnosis of APA was 66.6% and the specificity was 87%; the sensitivity and specificity of membrane GIRK4 staining were 100% and 66.6%, respectively. If considering Dab2/GIRK4 double staining at the membrane, 12 APA, one NPA and one CPA stained positive for both markers. Remarkably, GIRK4 expression was significantly lower in APA harboring *KCNJ5* mutation compared to those without mutation (p=0.0377). Immunostaining allowed to discriminate APA harboring or not *KCNJ5* mutations with sensitivity of 76.9% and specificity of 72.7%.
Conclusion. We show that GIRK4 immunostaining is more sensitive to discriminate aldosterone and non secreting adenoma, while Dab2 immunohistochemistry has a higher specificity. Our results also suggest that GIRK4 expression levels in APA could be predictive of mutational status of the tumor.

Twenty-five-fold up-regulation of a leucine-rich repeat containing G proteincoupled receptor (LGR5) in human adrenal zona glomerulosa and its potential role in cell proliferation and apoptosis

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Background: Human adrenal zona glomerulosa has high apoptotic and cell turnover rates (Wolkersdorfer et al., 1996). In a microarray comparison of zona glomerulosa (ZG) vs zona fasciculata (ZF), several candidates of interest within the WNT/β-catenin signalling pathway were upregulated in the ZG.
Aims: To determine genes which are upregulated in human ZG which may contribute to increased cell turnover, and then investigate the expression and function of the encoded proteins.
Methods: Laser capture microdissection (LCM) was used to selectively extract RNA from ZG and ZF of 20 patients undergoing adrenalectomy. 13 adrenals were adjacent to an aldosteronoma and seven to a phaeochromocytoma. The LCM isolates were analysed by the probeset of GeneChip® Human Gene 1.0 ST Array. Selected significant differences between ZF and ZG were confirmed by qPCR. Immunohistochemistry (IHC) was then performed to confirm ZG distribution. The cognate ligands for LGR5, R-spondin homologs 1 & 3 (RSPO1 & RSPO3) were used to assess consequences of LGR5 activation on aldosterone production.
Results: LGR5 was the most upregulated gene in ZG, with a 25-fold change (P =10-23) vs. paired ZF. RSPO3 was also highly expressed in ZG, with a fold change of 5.27 (P =10-11). LGR5 is a well-described intestinal stem cell marker and a target gene for WNT signalling. IHC confirmed selective localisation of both LGR5 and RSPO3 in the ZG of the adrenal. TUNEL staining showed the high rate of apoptosis in the ZG (Figure 1); Ki67 staining showed some punctuate and dispersed staining. In both primary human adrenal and H295R adrenocortical cells, RSPO1 or RSPO3 at low, subnanomolar concentrations caused a dramatic suppression of aldosterone below detection levels, but a dose-related recovery at concentrations 10-8 to 10-7M. This stimulation was potentiated by Angiotensin II and WNT4.
Conclusion: The abundance of LGR5 in human ZG not seen in other species, its role in the WNT/ β-catenin pathway, and response to low-concentrations of RSPO1 or 3, all suggest a prominent role in the high cell turnover of human ZG. We speculate that the pathway is important in the adaptive response to humans' high salt intake, and that the increased turnover is a precursor to the high prevalence of adrenocortical adenomas

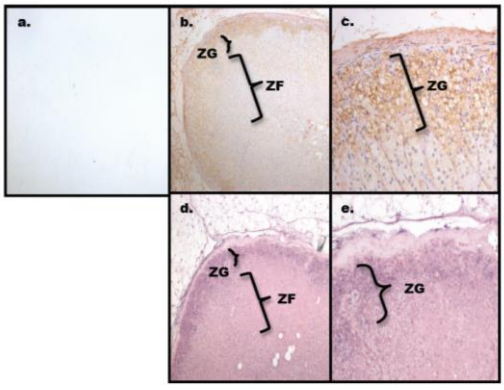

Figure 1: Immunohistochemistry of 4µ paraffin embedded sections of human adrenal. (a) No primary antibody; (b)-(c) LGR5 primary antibody, DAB-peroxidase, at 4x and 10x; (d)-(e) TUNEL staining for apoptosis of the same sections, at 4x and 10x. ZG= zona glomerulosa and ZF= zona fasciculata.

Oxidative stress in patients affected by Primary Aldosteronism

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Introduction: Primary aldosteronism (PA), one of the most common form of secondary hypertension, is associated with a significant increase of global cardiovascular risk (CVD) (ischemic heart, cerebrovascular events, arrhythmias) (RR 4.6). The specific treatment of the PA (surgical in adrenal adenoma -APA- or pharmacological in idiopathic forms -IHA) significantly reduces CVD risk. Recently, in addition to high blood pressure values and direct action of aldosterone, newly mechanisms are involved in the development of increased CVD risk and organe damage in PA subjects, such as metabolic, endothelial and coagulation alterations.

Aim of the study: evaluate parameters of oxidative stress in 38 patients (21 M, 17 F, mean age 53.3 ± 4.7 years) with PA [11 APA (4M, 7F, mean age 50.2 ± 4.5 years) and 27 IHA (17M, 10F, mean age 54.5 ± 5.3 years)] at diagnosis and after specific treatment (surgical or pharmacological), respect with control group of 41 patients with low renin essential hypertension (IEBR) (21M, 20F, mean age 49 ± 7.4 years).

Results: PA subjects (both APA or IHA) showed significantly increase of plasma levels of NADPH oxidase (NOX_2) and urinary isoprostanes (34.9±4.3 µg/dl and 216.3±15.7 ng/mg, respectively; $p<0.05$) than IEBR subjects (27.1±3.7 µg/dl and 144.8±9.4 ng/mg, respectively; $p<0.05$). In APA patients undergoing to adrenalectomy, after 6 months follow-up, we observed reduction of both circulating levels of NOX_2 (29±2.1 µg/dl vs 22,4±1.7 µg/dl; $p<0.05$) and urinary levels of isoprostanes (221.1±10.5 ng/mg vs 132.6±8.7 ng/mg; $p<0.05$).

Conclusions: our study firstly shows an increased oxidative stress in PA subjects, characterized by an increase in serum levels of NOX_2 and urinary excretion of isoprostanes. After adrenalectomy in APA patients, we found reduction of serum NOX_2 and urinary isoprostanes and normalization of blood pressure values.

Marinobufagenin in essential hypertension and primary aldosteronism: a cardiotonic steroid with clinical and diagnostic implications The Graz Endocrine Causes of Hypertension (GECOH) Study

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The cardiotonic steroid marinobufagenin (MBG) is increasingly suggested to be responsible for some of the cardiovascular injury that has been previously attributed to aldosterone. We examined the clinical correlates of circulating and urinary MBG concentrations in hypertensive patients and tested the hypothesis that MBG serves as a reliable diagnostic tool for detecting primary aldosteronism (PA).
Plasma and (spot) urinary MBG concentrations (0.46 ± 0.28 and 1.29 ± 0.65 nmol/L, respectively) were measured in the morning fasting samples in 10 patients with PA and 20 essential hypertensive controls matched for age, gender, renal function, urinary sodium and intake of antihypertensive medication (mean age: 50.6 y; 63.3% women).
Plasma/urinary MBG were significantly associated with age, plasma aldosterone, aldosterone to active renin ratio (AARR), systolic/diastolic blood pressure, estimated GFR, mean carotid intima-media thickness, NT-pBNP, urinary sodium/potassium and urinary albumin/protein excretion.
After adjustment for multiple confounders, plasma (P = 0.026) but not urinary MBG (P = 0.190) levels were significantly higher in patients with PA compared to EH.
ROC analysis yielded a greater AUC for plasma MBG compared to the AARR, PAC, urinary MBG and serum potassium levels for detecting PA.
Youden´s Index analyses yielded the optimal plasma MBG cut-off score for diagnosing PA at 0.49 nmol/L with specificity and sensitivity values of 0.90 and 0.85, respectively, which were higher than those at the optimum AARR cut-off.
In a well-characterized cohort, values of plasma /urinary MBG were significantly related to clinical correlates of cardiovascular and renal disease. Plasma MBG emerged as a valuable alternative to the AARR for screening of PA.

Aldosterone and Parathyroid Hormone, the unlikely couple

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Recent studies have shown that aldosterone induces urinary calcium excretion leading to a reduction of calcemia with consequent secondary hyperparathyroidism and bone mineral density (BMD) loss. In patients with primary aldosteronism (PA) this picture of hyperparathyroidism is significantly improved by treatment with adrenal surgery or with mineralocorticoid receptor antagonists. On these premises, the aim of the present study was to evaluate calcium and phosphate metabolism parameters in PA patients, compared with patients with essential hypertension (EH) and the effect of treatment of aldosterone excess on bone health in PA patients. In addition, in order to better define the bidirectional link existing between aldosterone and PTH, gene expression studies of the PTH recetopr type 1 were performed in adrenal samples of patients with Conn's adenoma.

Subjects and Methods: A total of two-hundred and twenty six patients were studied: 116 with PA (46 with an aldosterone producing adenoma-APA and 70 with bilateral adrenal hyperplasia-IHA), and 110 patients with EH. In 40 patients with PA we evaluated biochemical parameters and bone mass, using the dual-energy x-ray absorptiometry (DXA), at baseline and after a mean follow-up of 24 months from treatment. PTHR1 gene expression was evaluated by real time PCR in 18 APA samples and in 18 peri-APA tissues.

Results: In PA patients, compared with EH, PTH levels and urinary calcium excretion significantly increased, while serum calcium significantly decreased with comparable vitamin D levels. At follow-up in PA patients, PTH levels were significantly reduced compared with basal evaluation, despite similar vitamin D amounts.
At follow-up, we observed a significant improvement of the Z-score at lumbar spine, at femoral neck and at total hip sites. Compared with the peri-APA, APA samples displayed a reduced PTHR1 gene expression (RQ 0,534±0,647 vs 1,861±2,516 $p<0,05$).

Conclusions: Our results support previous data showing secondary hyperparathyroidism in PA patients, which is reversible after treatment. Moreover, this targeted treatment appears to be able to determine a significant improvement of BMD both at spine and hip sites.
Finally, preliminary data show a down-regulation of *PTHR1* gene expression in Conn's adenoma compared with the peri-APA tissue, that could be due to the hyperparathyroidism that characterize PA. Further studies in this light are needed.

Abstracts

Poster

Thursday, 1st July – Friday, 2nd July

Highly selective expression of DACH1 in the zona glomerulosa of human adrenal glands, and a putative role in regulation of aldosterone production

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Background: Genetic, transcriptome and histological analyses suggest that small, frequently overlooked APAs resembling normal zona glomerulosa (ZG) may be at least as common as the more classical zona fasciculata (ZF)-like APAs ([1] and Azizan et al, this meeting). We wished to determine whether there are signature genes for ZG that might provide evidence of ZG-origin of ZG-like APAs, and provide clues why such APAs are so common.

Methods: 1) A microarray assay was performed using 14 trios of ZF, ZG and APA, and a further seven pairs of ZF and ZG adjacent to a phaeochromocytoma. RNA extracted by laser capture microdissection was analysed on the Affymetrix Human Genome U133 Plus 2.0 Array. Validation by qPCR was performed of selected genes upregulated in ZG. 2) Some of these were further analysed for protein expression, determined by immunohistochemistry and western blotting in eight adrenals. 3) The consequence of protein deletion was assessed in HAC-15 adrenocortical cells using ON-TARGETplus siRNA and relevant controls, with aldosterone production as primary outcome.

Results: 1) Expression of 28 genes was at least 5-fold higher in ZG than ZF. Several genes were >10-fold abundant, including *LGR5*, *VSNL1*, *NEFM*, *VCAN*, *DACH1*, and *NR4A2*. *DACH1* was 14.4 fold higher expressed in ZG vs. ZF ($P=1.4\times10^{-21}$) and 3.04 fold up-regulated in ZG vs. APA ($P=2.2\times10^{-7}$) 2) qPCR confirmed 20 to 50-fold higher expression of the top six genes on microarray. *DACH1* showed an average of 30.3 and 4.1 fold up-regulation in ZG vs. ZF and APA vs. ZF. qPCR for *CYP11B1*, *CYP11B2* and *CYP17A1* expression levels in ZF and ZG confirmed the purity of each cell population. 3) IHC was undertaken for *DACH1* and *VSNL1*, showing highly selective staining of ZG in all 8 adrenals (Figure 1). Western blot supported IHC. 4) mRNA of *DACH1* in Si-*DACH1* cells was silenced by 68.8% compared to non-targeting treatment. This was associated with a significant increase of aldosterone compared to Si-*GAPDH*, Si-non-targeting and untreated groups (n=16, *P*=0.05).

Conclusion: Several unexpected genes are markedly over-expressed in human ZG, which did not feature in a similar recent study of rat adrenal.[2] *DACH1* plays an important role in cell fate determination and apoptosis, and over-expression down-regulates WNT pathway genes. We speculate that its high expression contributes to the high apoptotic rate in human ZG,[3] where aldosterone secretion is less important than in salt-poor species, and that down-regulation of *DACH1* may contribute to APA formation.

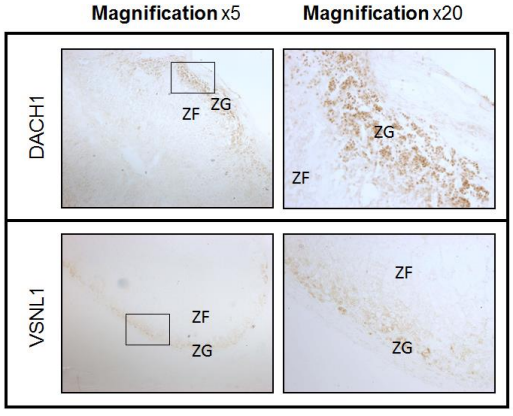

Figure 1. IHC of *DACH1* and *VSNL1*

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Adrenal steroidogenic profiles through LC-MS/MS in patients with primary aldosteronism during selective adrenal vein sampling

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To the aim of studying the adrenal steroidogenic pattern in patients with primary aldosteronism we used a quantitative and simultaneous determination of steroids with a high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). This technique is characterized by high sensitivity and specificity.
Serum samples were obtained during selective adrenal vein sampling (AVS), with continuous infusion of ACTH, in two patients with aldosterone producing adenoma (APA) and in two patients with idiopathic hyperaldosteronism (IHA).
Mean blood pressure values were 180±28 and 100±14 mmHg in APA and 155±7 and 97±10 mmHg in IHA patients; serum potassium levels were 3,2±0,2 in APA and 3,4±0,4 mEq in IHA.
Both APA patients had an adrenal left mass at CT evaluation; the AVS confirmed an aldosterone oversecretion in the left side and, after adrenalectomy, both patients were cured from PA.
The LC-MS/MS showed higher amounts of aldosterone precursors during ACTH stimulation in the adrenal gland with the mass, compared to the controlateral gland. 11-deoxycorticosterone (DOC) values were 19±14 ng/ml in the left adrenal vein, 1,2±1,1 ng/ml in the right adrenal vein and 13,8±6,3 ng/ml in the inferior vena cava. Similarly, 18-OH DOC values were 12±0,4 ng/ml in the left adrenal vein, 2,5±3,1 ng/ml in the right adrenal and 4,8±5,8 ng/ml in the inferior vena cava. Corticosterone levels were 607±412 ng/ml in the left adrenal vein, 211±280 ng/ml in the right adrenal vein and 206±241 ng/ml in the inferior vena cava.
Adrenal steroid pattern in patients with IHA showed less significant differences.
In conclusion, preliminary data show that aldosterone precursors are higher in the adrenal gland with the aldosterone producing adenoma and that there is their concomitant suppression in the controlateral adrenal gland.
In order to better define the adrenal steroidogenic pattern in aldosteronism and to confirm these preliminary results, it will be necessary to analyze a larger cohort of patients with PA.

18-hydroxycorticosterone and 11-deoxycorticosterone in the diagnosis of primary aldosteronism

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Introduction. Primary aldosteronism (PA) is considered the most frequent cause of secondary hypertension. According to Endocrine Society clinical practice guidelines for case detection, diagnosis and treatment of patients with primary aldosteronism, the screening for PA is based on aldosterone-renin ratio (ARR). The next step is confirmation of diagnosis by one of four recommended tests but a preference of a test is still controversial. The final step is a subtype diagnosis. The gold standard for differentiation is adrenal vein sampling (AVS), but it is an invasive and expensive procedure and is not always informative and accessible for many clinics. These challenges in the diagnostic process have prompted ongoing search for alternative or complementary tests.
11-deoxycorticosterone (DOC) and 18-hydroxycorticosterone (18-OHB) are precursors of aldosterone with various affinities for the mineralocorticoid receptor (MR) which are the result of conversion of progesterone by aldosterone synthase, although small amounts may be produced by the 11beta-hydroxylase.

Objective: The aim of this study was to estimate precursors of aldosterone in plasma and urine in patients with PA and low-renin hypertension (LRH).

Patients and methods: We examined 128 patients with LRH and 30 healthy subjects without hypertension. We measured plasma levels of aldosterone (PAC) and renin activity (PRA) by RIA. Intermediates of steroidogenesis were assessed by means of high-performance liquid chromatography (HPLC) including measurement of plasma cortisol (F), cortisone (E), corticosterone (B), 18-hydroxycorticosterone (18-OHB), 11-dehydrocorticosterone (A), 11-deoxycorticosterone (DOC), 11-deoxycortisol (S) and urinary excretion of 18-hydroxycorticosterone (18-OHB). All patients underwent saline infusion test (SIT) and computed tomography (CT). Patients with elevated cortisol, metanephrine and normetanephrine were excluded.

Results: PAC/PRA ratio was positive in 76 patients with LRH. The diagnosis of PA among them was based on pathological results of SIT and CT. Fourteen patients suspected with bilateral adrenal hyperplasia (BAH) underwent AVS. All patients with positive ARR were subsequently divided into 3 groups: low-renin essential hypertension (n=43), patients with aldosterone-producing adenoma (APA) (n=21) and BAH (n=12). Patients with PA were found to have significantly higher concentrations of plasma 18-OHB, DOC and urinary excretion of 18-OHB compared with LRH and normal groups. Moreover, patients with APA showed significantly higher levels of plasma and urine 18-OHP than BAH patients.
Conclusion: The determination of 18-OHB and DOC may be a useful test for the detection and subtype diagnosis of PA as they are elevated in patients with APA. Our results stress the need for long-term prospective studies for estimating the role of measurement of precursors of aldosterone in the diagnostic of PA.

Prospective analysis of the saline infusion test regarding sub-differentiation and outcome in primary aldosteronism

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Introduction: To date no gold standard confirmatory test has been identified in the diagnosis of primary aldosteronism (PA). The most widely used confirmatory test in the German data base network (Conn-Registry) is the saline infusion test (SIT). The SIT results are judged as follows: post-test aldosterone levels <50 ng/l exclude PA, whereas levels >50 ng/l confirm the diagnosis. In the past results between 50-100 ng/l were considered as indeterminate (guideline of American Endocrine Society, 2008). To date there is no outcome data from any confirmatory test available regarding sub-differentiation, treatment, and severity of the disease and comorbidities in PA.

Patients and Methods: PA patients were recruited as part of the Mephisto-Study (German Conn's Registry). Prospective patients were evaluated before treatment (at diagnosis) and every six months thereafter. Assessment included complete PA testing, metabolic and hormonal parameters as well as documentation of comorbidities.

Results: This prospective analysis included 264 patients (98 males, 166 females) who underwent the SIT at the baseline prior to PA treatment. The mean age was 53.1±12.1; the mean duration of hypertension was 13.7±12.2 years. The baseline mean systolic blood pressure (BP) amounted to 146.5±19.8mmHg and the mean diastolic BP 92.3±12.0mmHg.
The mean pre-treatment potassium value was at 3.43±0.58mmol/l. 132 of these patients completed the follow up visit.
We divided the cohort into two groups: 70 patients with post-SIT aldosterone levels 50-100 ng/l (53males and 17females) and 170 patients with post-SIT aldosterone levels >100 ng/l (98males and 72females). Both groups were evaluated before treatment, and 12 months after treatment initiation. The mean age was 53.6±17.0 vs. 52.8±12.7. The average duration of hypertension was 15.2±12.7 vs. 13.6±12.1 years. At baseline the mean systolic blood pressure was 142.0±15.0 vs. 148.9±16.7 mmHg, the mean diastolic blood pressure was 90.5±10.8 vs. 93.1±12.2mmHg. The initial potassium levels were 3.48±0.42 vs. 3.37±0.64 mmol/l. Pre-/posttest parameters of SIT (Aldosterone, Renin, Aldosterone-Renin-Ratio (ARR),Cortisol, Aldosterone-Cortisol-Ratio (ACR)) and the outcome regarding sub-differentiation, treatment and comorbidities are currently be analyzed.

Conclusions: This study will provide outcome data regarding PA patients with post-SIT aldosterone levels above and below 100ng/l at baseline. We did not find significant differences between the groups.

Lack of influence of somatic mutations on steroid gradients of adrenal vein sampling in aldosterone producing adenomas: results of the German Conn's registry – Else Kröner-Fresenius Hyperaldosteronismus Registry

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Adrenal vein sampling is a technically demanding procedure required for identification of suitable candidates for unilateral adrenalectomy in primary aldosteronism. Recently, somatic KCNJ5 K^+-channel mutations in aldosterone producing adenomas have been shown to influence steroid gradients during adrenal vein sampling. These and other recently identified genetic modifiers might impact the final diagnosis and treatment of affected patients.

To analyze the influence of somatic KCNJ5-, ATP1A1 and ATP2B3 mutations on gradients in adrenal vein sampling, 59 patients with aldosterone producing adenomas diagnosed according to current standards were studied. All subjects underwent successful adrenal vein sampling (adrenal vein cortisol to peripheral cortisol ≥2) and had a mutation analysis of their tumor tissue. The mutation status of the aldosterone producing adenomas was: 19 KCNJ5 mutations, 8 ATPase mutations (5 ATP1A1, 3 ATP2B3) and 32 with none of these mutations. The lateralization index (defined as the ratio between aldosterone to cortisol of the side of the adenoma to aldosterone to cortisol of the contralateral side) and the contralateral suppression index (defined as the ratio of aldosterone to cortisol of the contralateral side and aldosterone to cortisol in the periphery) were calculated in the KCNJ5 mutated APAs, the ATPase mutated APAs and in the KCNJ5/ATPase mutation negative APAs.

Lateralization indices of ATPase mutation carriers showed a median of 19.9 compared to 16.0 in KCNJ5 mutation carriers and 20.5 in KCNJ5/ATPase negative patients. Contralateral suppression indices of ATPase mutated patients showed a median of 0.1 compared to a median of 0.4 in KCNJ5 mutation carriers and 0.2 in KCNJ5/ATPase negative patients. The differences between the genetic groups were not statistically significant.
In conclusion, we did not find evidence for a clinically important impact of mutational status on steroid gradients during adrenal vein sampling.

Sleep quality in patients with primary aldosteronism

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Introduction: In subjects at high risk for sleep apnea (SA), aldosterone concentrations correlate with severity of SA and primary aldosteronism (PA) is more often diagnosed. Patients with PA show a high prevalence for SA. Treatment of PA either by adrenalectomy (ADX) or mineralocorticoid receptor (MR) blockade is thought to abolish the increased comorbidities. No data is available regarding effectiveness of different PA treatments on quality of sleep.

Patients and methods: This prospective multi-center study included 15 patients with newly diagnosed PA evaluated before and0.7 ± 0.2 years after treatment initiation and a second cohort including 81 patients who were evaluated 5.3 and 6.8 years after treatment initiation. Biochemical parameters, 24h blood pressure and three validated self-assessment questionnaires (Giessen Complaint List (GBB-24), Epworth Sleepiness Scale (ESS), Pittsburgh Sleep Quality-Index (PSQI)) were analysed.

Results: Z-scores of exhaustion tendency of GBB significantly improvedin newly diagnosed PA patients after treatment initiation (1.8±1.4 vs. 1.0±1.2, p=0.034). In the second cohort no differences were found in GBB-24, ESS and PSQI. No differences were found in all three questionnaires depending on type of PA therapy. However, PSQI showed a significant worse outcome ($p<0.005$) in female than in male patients (8.7±3.6 vs 5.7±4.2), independent of the type of therapy.

Conclusions: For the first time we analysed quality of sleep in patients with PA demonstrating that therapy initiation improves exhaustion tendency. Surprisingly, female PA patients showed significant more sleep disturbances than male PA patients several years after treatment initiation.

Aldosterone hyperesponse to ACTH stimulation in a hypertensive population: a new clinical entity?

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Background: Aldosterone secretion is regulated by angiotensin II, K^+ and adrenocorticotrophin (ACTH). We have previously observed a subset of patients without primary aldosteronism (PA), who exhibit an exaggerated aldosterone response to ACTH stimulation.
Objective: To investigate 1) the effect of ACTH stimulation in aldosterone and cortisol secretion in hypertensive patients without PA; 2) identify hyperesponsive patients and evaluate their response to targeted therapy with mineralocorticoid receptor antagonists (MRA); and 3) identify potential pathogenetic mechanisms at the molecular level.

Patients and Methods: 104 hypertensive individuals and 61 normotensive controls with normal adrenal computed tomography (CT) scan findings and normal aldosterone suppression on fludrocortisones dexamethasone suppression test (FDST), underwent a low dose (0.03 μg) ACTH-stimulation and an exercise (as per Bruce protocol) test. Hypertensive patients that exhibited an exaggerated aldosterone response according to the cut offs obtained from controls, also underwent 1) targeted treatment with MRA 2) genomic DNA analysis of genes known to be implicated in aldosterone secretion (*CYP11B1/CYP11B2* chimeric gene and KCNJ5). In the presence of mutations, electrophysiological studies were performed to determine their functionality through the effect on the membrane reversal potentials.

Results: Using the 97.5% percentiles from controls the post-ACTH stimulation cut-off levels for aldosterone and aldosterone-to-renin ratio (ARR) were defined as 1300pmol/L and 77pmol/mIU, respectively. Based on the combination of these cut-offs, 25 (24%) patients obtained an aldosterone hyper-response (HYPER-group) compared to 79 (76%), who had a response similar to controls (ESHT group). HYPER-group patients had significantly higher baseline aldosterone concentrations ($p<0.0001$ and $p<0.0001$ respectively) and ARR ($p<0.001$ and $p<0.0001$, respectively) compared to the ESHT- and control-groups, and higher 24hr urine K^+ ($P<0.001$) compared to controls. Following ACTH-stimulation, HYPER-patients obtained significantly higher area under the curve aldosterone concentrations ($p<0.0001$ and $p<0.0001$, respectively) and ARR ($p<0.0001$ and $p<0.0001$, respectively) compared to the ESHT- and control-group; there was no difference in AUC cortisol and the baseline ACTH, renin levels.
Following the exercise test, HYPER-patients obtained significantly higher aldosterone concentrations ($p<0.01$ and $p<0.001$, respectively), ARR ($p<0.05$ and $p<0.001$, respectively) and aldosterone/ACTH ratio ($p<0.01$ and $p<0.001$, respectively) compared to the ESHT- and control-groups. There was no difference in mean ACTH, cortisol and renin levels among the three groups. After changing previous antihypertensive treatment of the HYPER group to a MRA, systolic and diastolic blood pressure became significantly lower ($p<0.0001$ and $p<0.0001$, respectively).
Two novel *KCNJ5* heterozygous mutations (p.V259M c.775G>A in exon 2 and the p.Y348N, c.1042T>A in exon 3) were detected in the HYPER-group. Electrophysiological studies revealed that compared with the wild-type protein, the mutant Y348N showed greater rectification and a significantly less negative reversal potential (-1.3 ± 11.8 mV), while V259M appeared more rectified, however, the reversal potential was not significantly different (-18.7 ± 10.5 mV).

Conclusions: Among hypertensive patients without PA there is a group that exhibits an aldosterone hyper-response to ACTH-stimulation that could potentially benefit from treatment with MRA. The underlying pathogenetic mechanism remains to be elucidated, and could be related to acute or chronic stress; however, in a subset specific alterations of the KCNJ5 gene may be implicated.

Diagnosis and treatment for bilateral aldosterone-producing adenomas

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Introduction; Differential diagnosis of surgically curable bilateral aldosterone-producing adenomas (Blt-APAs) from medically treatable idiopathic hyperaldosteronism is difficult, because these cases demonstrate bilateral hyperaldosteronemia when examined by the usual method of adrenal venous sampling (AVS). We examined the prevalence of Blt-APAs among primary aldosteronism (PA) complicating unilateral adrenal tumor by super-selective ACTH-stimulated AVS (SS-ACTH-AVS) and treated them by unilateral partial adrenalectomy.

Cases and Methods; One-hundred two PA-cases with CT-detectable unilateral adrenal tumors were examined by SS-ACTH-AVS. We diagnosed hyper-secretion of aldosterone when concentrations of aldosterone sampled at intra-adrenal tributary veins were more than 1400ng/dl. Blt-APAs were diagnosed when concentrations of aldosterone were more than 1400ng/dl at one or two tributary veins and less than 1400ng/dl at the others of each adrenal gland sampled by SS-ACTH-AVS. IHA was diagnosed when concentrations of aldosterone were more than 1400ng/dl at all tributary veins sampled.

Results; Forty-eight cases among 102 PA-patients with unilateral adrenal tumor demonstrated bilateral hyperaldosteronism (Blt-PA). Eighteen cases were diagnosed as Blt-APAs, 24 cases as IHA with non-functioning adenoma (NF) and 6 cases as IHA with cortisol producing adenoma causing subclinical Cushing's syndrome (CPA) by SS-ACTH-AVS. Fourteen cases with Blt-APAs and 4 cases with IHA complicating CPA were treated by unilateral partial resection of adrenal glands possessing CT-detectable tumor. Histo-chemical staining of CYP11B2 and CYP11B1 clearly demonstrated CYP11B2-positive cells in 14 APAs and CYP11B2 positive aldosterone-producing cell clusters around 4 CYP11B1 positive CPAs. Hyperaldosteronism of 14 cases with Blt-APAs was improved, and blood pressure in 8 cases was normalized one year after unilateral partial adrenalectomy.

Conclusion; Prevalence of Blt-PA among all PA-cases demonstrating unilateral adrenal tumor by CT is 48%. Cases with Blt-PA are usually medically treated. However, surgically-treatable Blt-APAs was 29% among Blt-PA. Therefore, we should perform SS-ACTH-AVS especially in cases with Blt-PA complicating unilateral adrenal tumor, and we should treat cases with Blt-APAs by unilateral partial adrenalectomy.

Clinical strategy for recurrent APA with segmental AVS

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Background In many cases of hypertensive patients with primary aldosteronism (PA) which are diagnosed as unilateral aldosterone producing adenoma (APA) may be cured by adrenalectomy. However, there are a few cases of recurrent APA in the contralateral adrenal gland. We experienced recurrent APA case, diagnosed with segmental adrenal venous sampling (AVS) and treated by partial adrenalectomy.
Case 56 y.o. Male
History of present illness At the age of 30, hypertension had been pointed out by GP and he had started taking anti-hypertensive drugs. At the age of 35, a right adrenal tumor had been detected by CT, and he had been diagnosed to have PA at some hospital. AVS based right adrenalectomy had been performed at another hospital. Most of anti-hypertensive drugs had been discontinued after the surgery. At the age of 56, a GP pointed out hypokalemia of 2.8 mmol/l and CT showed the contralateral (i.e. left) adrenal tumor. He was referred to Tohoku University hospital for further investigation. Regardless of taking spironolactone 75mg/day, olmesartan 30mg/day and cilnidipine 10mg/day, his blood pressure (BP) was not controlled of 178/110 mmHg. After replacement of the medication to four agents of Ca antagonists and alpha adrenergic blockers, we found baseline plasma aldosterone concentration (PAC) and plasma renin activity (PRA) were 50.8 ng/dl and 0.8 ng/ml/hr, respectively, and PAC/PRA of 41.7 with captopril challenge test. Overnight 1mg dexamethasone suppression test resulted in cortisol level of 0.8 µg/dl. CT showed a left adrenal tumor with diameter of 22 mm and no residual adrenal tissue at right adrenal bed. We performed segmental AVS using micro-catheter and cosyntropin stimulation. We obtained samples drainer veins of both the tumor and an attached gland. With continuous infusion of cosyntropin, PAC/Cortisol (A/C) obtained from the tumor drainer was 28.2, in contrast to that obtained from the attached adrenal of 0.62. Based on the finding that A/C of the attached adrenal was lower than that obtained from a peripheral vein of 5.3. We interpreted that aldosterone secretion from the attached tissue was suppressed due to the autonomous secretion of aldosterone from the tumor, and diagnosed that resection of the left tumor was indicated. He underwent laparoscopic left partial adrenalectomy with no complications. The pathological diagnosis showed that, most of the tumor cells were clear-type and strongly positive for HSD-3B whereas negative for CYP17A. Paradoxical hyperplasia was detected in the adjacent non-hyperplastic adrenal gland. We diagnosed the tumor as APA. After the left partial adrenalectomy, the number of anti-hypertensive agents were reduced from 5 to 2 to obtain appropriate BP control and replacement of glucocorticoid was withdrawn 4 months after the surgery.
Summary We here report a case of a recurrent APA originated from a contralateral adrenal gland 21 years after removal of a first APA. With the advent of segmental AVS-based normal adrenal-sparing surgery, excess secretion of aldosterone was successfully corrected and reserve of cortisol secretion was recovered with a short-term period of replacement. Segmental AVS-based approach might have a role in discriminating tumor from hyperplastic lesion in preoperative settings and in making an indication of partial adrenalectomy in patients with recurrent APA or bilateral APAs.

Intermittent hyperaldosteronism in a young patient with an adrenal micronodule – a diagnostic challenge

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Introduction. Primary aldosteronism has to be excluded in any patient with an adrenal incidentaloma and hypertension. The considerable variability of aldosterone and renin assays may influence the diagnosis.

Case presentation. A 36 years-old lady with depressive disorder and anxiety was evaluated for hypertensive paroxysms up to 160/100 mmHg. She complained about episodes of headaches, muscular cramps and fatigue, palpitations, cold sweats, developed during the last 2 years. She smokes, has chronic professional stress and has a family history for hypertension. The initial evaluation showed normal serum kalium (4 mmol/l) and sodium, extremely high urinary aldosterone 13740.6 µg/24h (N 2,2 – 21.41), normal direct plasma renin 14,1 pg/ml (2,6 – 27), normal urinary and 1 mg – dexamethasone suppressed cortisol and free plasma metanephrines. Plasma aldosterone was increased 388.9 pg/ml (N 3.8 – 313.3), plasma renin normal 11.9 pg/ml (N 2.64 – 27.66), aldosterone/renin ratio (ARR) increased 32.7 (n < 20). CT scan showed a hyperplasic left adrenal with a 0.7/0.6 cm nodule. A 3 day-oral salt loading test showed normal recumbent aldosterone before the test (82.12, normal 10 – 160) without adequate suppresion < 50 pg/ml (110.6 pg/ml). Six month later, recumbent aldosterone was repeatedely increased (176 – 187 pg/ml), with normal renin, ARR varied between 31.7 and 11 (N<20), and a standard saline infusion test showed adequate suppresion. The patient had episodes of hypotension on betablockers, then spironolactone 100 mg/day was started. A 24h monitoring showed similar blood pressure profile on spironolactone (including a day-time 200 mmHg paroxysm) compared to the initial recording without treatment. After 45 months of follow-up, the adrenal imaging was similar with the initial one and recumbent aldosterone and renin were normal, with suppression after saline infusion test to 71 pg/ml and 1.7 pg/ml, respectively. The patient continued to have the same symptoms as before, in episodes of variable intensity and frequency, which were not consistently ameliorated with antidepressant and spironolactone treatment.
Discussion. The variability of the blood levels of aldosterone may be due to the assays' variability or to an intermittent hyperaldosteronism. Aldosterone excess has been associated with depression and anxiety in humans and induces depression-like behaviours and relevant gene expression changes in animals. Normalization of aldosterone secretion has been recently shown in about 60% of the patients with non-tumoral primary aldosteronism treated medically for at least 3 years.

Conclusion. In this young hypertensive patient with an adrenal micronodule, intermittently elevated aldosterone with normal kalium and renin and variable response to saline confirmatory tests made the diagnosis of primary hyperaldosteronism uncertain. If the associated mood disorder influences the clinical picture or is the result of hyperaldosteronism it is yet unknown.

Rapid testing in adrenal venous sampling

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Immediate determination of cortisol improves success rates in adrenal venous sampling. However, although termed as "rapid cortisol test" (RCT), the procedure is time-consuming and lasts between 30 and 60 minutes.
We have studied faster methods in assessing secretory products of adrenal cells, including the adrenochrome-based colorimetric (ABC) test, a rapid epinephrine immunoassay (REI), cross-reactivity of an metamphetamine drug test (MDT) with catecholamins, determination of ascorbic acid (DVC), and tetrazolium blue staining of steroid hormones (TZB).

While the RCT took in minimum 40 minutes, other tests, such as the ABC (max. 15 min), the MDT (max 10 min), DVC (max. 10 min) and the TZB (max. 15 min) were much faster. However, sensitivity and accuracy rates remained as low as maximal 79 % in the fast tests, including the REI, while the RCT rendered an accuracy rate of 96%.

Therefore, more modern techniques will have to be employed during rapid assessment of epinephrine or cortisol and the tests described herein need still further refinement. One such test could be a cell-based assay employing cAMP-specific fluorescence. Until its evaluation, the RCT is to be performed without alternative.


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