International Symposium on Familial Amyloidotic Polyneuropathy

International Symposium on Liver Transplantation in Familial Amyloidotic Polyneuropathy

Rio de Janeiro, Brazil, November 10 to 13, 2013
IX International Symposium on Familial Amyloidotic Polyneuropathy (ISFAP) and the
VIII International Symposium on Liver Transplantation in Familial Amyloidotic Polyneuropathy

Program and Abstract

Auditório do Centro de Convenções Antônio Seabra Moggi
Rio de Janeiro, RJ, Brazil
November 10th to 13th, 2013
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Cover Page: Alexandre T. Bando
Welcome to Brazil!

Welcome to Rio de Janeiro!

On behalf of the organizing committee, we sincerely welcome you to the IX International Symposium on Familial Amyloidotic Polyneuropathy (ISFAP) and the VIII International Symposium on Liver Transplantation in Familial Amyloidotic Polyneuropathy, to be held in Rio de Janeiro, Brazil, November 10th to 13th, 2013.

These symposia aim to promote a lively discussion around selected aspects of the most recent advances in understanding the mechanisms behind Familial Amyloidotic Polyneuropathy (FAP), as well as new biomarkers and early indicators of disease progression and disease staging, ending with a section devoted to treatments -- including liver transplantation and promising new drugs. We welcome the opportunity to bring together colleagues from Latin America and other regions with investigators from well-known referral centers, with the aim of fostering new and fruitful scientific collaborations.

This is the first time that these symposia will be held outside the well-known research centers of Europe, Japan or the USA. It is a tremendous challenge for us to match the quality of the previous symposia organized by our colleagues, and we look forward to it. We are sure that you will enjoy not only the scientific program but also the beauty of Rio de Janeiro! If we are successful, at the end of the meeting you will understand why we are proud to host this event, and why this city is called “Cidade Maravilhosa”.

Sincerely,

Márcia Waddington Cruz and Débora Foguel

Organizers
Committee

Advisory Board
Merrill Benson (Indiana University, USA)
Maria João Saraiva (Instituto de Biologia Molecular e Celular, IBMC, Portugal)
Per Westermark (Uppsala University, Sweden)
Joel Buxbaum (The Scripps Research Institute, San Diego, USA)
Jeffery Kelly (The Scripps Research Institute, San Diego, USA)
Claudio Rapezzi (University Hospital S. Orsola-Malpighi, Bologna, Italy)
Teresa Coelho (Hospital Santo Antônio, Porto, Portugal)
Isabel Maria Conceição (Hospital de Santa Maria, Lisboa, Portugal)
Yukio Ando (Kumamoto University, Japan)
Violaine Planté Bordeneuve (CHU Henri Mondor, Créteil, France)
Gerard Said (Hôpital de la Salpêtrière, France)
Arie Stangou (Queen Elizabeth Hospital, UK)
Giampaolo Merlini (University of Pavia, Italy)
David Adams (CHU Bicêtre, Université Paris Sud, Paris, France)
Ole Suhr (Umea University Hospital, Sweden)
Rodnei Falk (Brigham and Women’s Hospital, USA)
Matthew Maurer (Columbia University, USA)
Eduardo Barroso (Hospital Cury Cabral, Portugal)
Bo-Goran Ericzon (Karolinska Institutet, Sweden)
Shu-Ichi Ikeda (Shinshu University Hospital, Matsumoto, Japan)
David Azoulay (CHU Henri Mondor, Créteil, France)

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Débora Foguel (IBQM, UFRJ)
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Priscila Ferreira (IBQM, UFRJ)
Estefânia Azevedo (IBQM, UFRJ)
Fernando Palhano (IBQM, UFRJ)
Cintinha Lima (IBQM, UFRJ)
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Shenia Colnaghi Sbardelloto Novis (HUCFF, UFRJ)
Renata Barbosa Vasconcelos (HUCFF, UFRJ)
Joaquim Ribeiro (HUCFF, UFRJ)

Organization Support:
Brazilian Society for Biochemistry and Molecular Biology (SBBq)
Cynthia Sayuri Bando, Executive Secretary
General Information

The congress venue is
Auditório do Centro de Convenções Antônio Seabra Moggi
Petrobras - Cenpes
Av. Horácio Macedo, 1021 - Portaria 5
Cidade Universitária - Rio de Janeiro – RJ

Obs: Shorts, mini-skirts, sleeveless shirt, flip-flops will not be allowed in the event. Sneakers or shoes are required.

Transportation
From Hotel to Congress Venue
The event will provide buses for congress venue.
The itinerary will be from Hotel Atlantica Windsor, Hotel Excelcior, Hotel Leme and Hotel Plaza to Auditorium and back. The time table will be available during de congress.

The Multimedia Desk Room is located on Petrobras-Cenpes, Room 1
Please bring your presentation on a USB memory stick before the start of each session.
Save it to a file titled with your name.
We provide Windows 7 Power Point 2010 for your presentation.
You may use your own PC or Mac laptop as well. For Mac, you may need to bring an adaptor.
Please check the time for your presentation at the updated scientific program.
Note that the presentation time includes also the time for questions.

Poster Session
All posters MUST BE fixed on Monday in the morning and you should present according to the following days:
November 11th, 2013
17:05-18:05 - Poster Session 1 – Cardiology (C) and Basic Research (D)
November 12th, 2013
17:30-18:30 - Poster Session 2 - Transplant (A), Treatment (B) and Clinic (E)

Medical Center: During the event there is an intensive-care unit in the Petrobras-Cenpes and a Medical Center at room 1 with 1 doctor and 1 nurse which will be available for emergency first aid.

Badges
SBBq staff will provide badges for all delegates and accompanying persons. Access to scientific sessions and other activities, including social events, will be denied unless badges are used. Badges contribute greatly to promote personal contacts and also guarantee the full attention of the Congress Personnel, as well as security control. Upon losing the badge, a duplicate will be provided at the Secretariat at cost of US$ 50,00.

Social Program
November 10th: The Opening Cocktail will be at Atlantic Windsor Hotel on from 7pm to 9pm.
November 11th: Special Dinner will be at “Porçao” Restaurant, from 7pm to 9:30 pm.
November 12th: Rio Scenarium, from 7 pm to 10 pm
November 13th: City tour to Sugar Loaf Mountain, from 3:30 pm to 7 pm

For all activities the bracelet is required and the bus will leave from Petrobras-Cenpes.

Lunch
Lunch will be served at the congress venue on Monday (11th), Tuesday (12th) and Wednesday (13th).

ISFAP Secretariat
Is located on Petrobras-Cenpes, Room 1
agradecimentos
Index

Index..............................................................................................................................................7
Scientific Program...........................................................................................................................8
Opening Lecture ............................................................................................................................15
Key note Lectures ..........................................................................................................................17
Symposia .........................................................................................................................................23
Oral Presentation ............................................................................................................................35
Poster Presentation ..........................................................................................................................45
   A - Liver Transplantation .............................................................................................................46
   B - Treatment .............................................................................................................................52
   C - Cardiology ...........................................................................................................................56
   D - Basic Research .......................................................................................................................74
   E - Clinic ...................................................................................................................................85
Author’s Index ..............................................................................................................................101
Scientific Program

Monday – November 11th, 2013

09:00-09:25  Opening remarks

Debora Foguel, Institute of Medical Biochemistry, UFRJ, Brazil
Marcia Waddington Cruz, University Hospital Clementino Fraga Filho, UFRJ, Brazil
Chairpersons of the meeting
Elza Dias Tosta, President of the Brazilian Academy of Neurology
Merrill Benson, President of the International Society of Amyloidosis

09:25-09:50  Opening Lecture
Chairperson: Debora Foguel, Institute of Medical Biochemistry, UFRJ, Brazil

Genetically determined transthyretin levels, transthyretin stability, and risk of vascular disease
Anne Tybjaerg-Hansen, Copenhagen University Hospital, Denmark

09:50-10:15  Key Note Lecture 1
Chairperson: Jeffery Kelly, The Scripps Research Institute, USA

Amyloidosis from a biochemical perspective: From TTR production to amyloid deposit and disease
Debora Foguel, Institute of Medical Biochemistry, UFRJ, Brazil

10:15-10:35  Coffee Break

10:35-11:50  Symposium 1 - Amyloidogenesis, Cell Toxicity and Animal Models
Chairperson: Maria João Saraiva, Institute for Molecular and Cell Biology, IBMC, Portugal

SP.1-1  Wild-type ATTR amyloidosis (senile systemic amyloidosis) – a disease related to late-onset ATTR V30M amyloidosis and with consequences for peripheral nerves
Per Westmark, Uppsala University, Sweden

SP.1-2  Clearance of extracellular misfolded proteins in systemic amyloidosis: experience with transthyretin
Maria João Saraiva, Institute for Molecular and Cell Biology, Portugal

SP.1-3  The Anti-Amyloidogenic effects of TTR in vivo and in vitro
Joel Buxbaum, The Scripps Research Institute, USA

11:50-12:15  Key Note Lecture 2
Chairperson: Debora Foguel, Institute of Medical Biochemistry, UFRJ, Brazil

Current Insights into Quantifying Transthyretin Aggregation-associated Pathology and its Amelioration with Tafamidis
Jeffery Kelly, The Scripps Research Institute, USA

12:15-13:05  Symposium 2- Early Markers of the Disease. The Ideal Moment to Treat.
Chairperson: Yukio Ando, Department of Neurology, Kumamoto University, Japan

SP.2-1  Early Markers of the Disease. The Ideal Moment to Treat: Neurological Markers
Isabel Conceição, Hospital de Santa Maria, Portugal

SP.2-2  Early markers of the disease: The ideal moment to treat.
Mathew Maurer, Columbia University Medical Center, USA
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>13:05-13:35</td>
<td>Oral Presentation 1&lt;br&gt;Chairperson: <strong>Per Westmark</strong>, Uppsala University, Sweden</td>
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<tr>
<td></td>
<td><strong>OP.1-01</strong>&lt;br&gt;<em>Gene Expression Profile in Hereditary Transthyretin Amyloidosis: Differences In Targeted and Source Organs</em>&lt;br&gt;Nina Norgren, Umea University, Sweden</td>
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<td><strong>OP.1-02</strong>&lt;br&gt;<em>Involvement of M2 macrophages in the pathogenesis of FAP and efficacy of human iPS cell-derived macrophages in the treatment</em>&lt;br&gt;Genki Suenaga, Kumamoto University, Japan</td>
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<td><strong>OP.1-03</strong>&lt;br&gt;<em>Nerve injury in a mouse model of FAP leads to increased local expression of TTR and decreased regeneration</em>&lt;br&gt;Nádia Pereira Gonçalves, Instituto de Biologia Molecular e Celular, Portugal</td>
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<tr>
<td>13:35-14:30</td>
<td>Lunch</td>
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<tr>
<td>14:30-14:55</td>
<td>Key Note Lecture 3&lt;br&gt;Chairperson: <strong>Elza Dias Tosta</strong>, President of the Brazilian Academy of Neurology</td>
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<td><em>FAP diagnosis from clinical suspicion to confirmation</em>&lt;br&gt;Gerard Said, Hospital de la Salpetrière, France</td>
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<tr>
<td>14:55-16:05</td>
<td>Symposium 3 - Difficulties in the Diagnostic of FAP&lt;br&gt;Chairperson: <strong>Leila Chimelli</strong>, National Cancer Institute and UFRJ, Brazil</td>
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<td><strong>SP.3-1</strong>&lt;br&gt;<em>Differential diagnosis with hansen disease neuropathy</em>&lt;br&gt;Wilson Marques Junior, Department of Neuroscience and Behavioral Sciences- USP, Brazil</td>
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<td><strong>SP.3-2</strong>&lt;br&gt;<em>Differential diagnosis with CIDP</em>&lt;br&gt;Osvaldo Nascimento, Department of Neurology -UFF, Brazil</td>
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<td><strong>SP.3-3</strong>&lt;br&gt;<em>Familial Amyloid Neuropathy: Differential Diagnosis with Charcot-Marie-Tooth Neuropathy</em>&lt;br&gt;Mario Saporta, Institute of Biomedical Sciences-UF RJ, Brazil</td>
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<td><strong>SP.3-4</strong>&lt;br&gt;<em>Differential diagnosis with diabetes neuropathy</em>&lt;br&gt;Amilton Antunes Barreira, Department of Neuroscience and Behavioral Sciences- USP, Brazil</td>
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<tr>
<td>16:05-16:20</td>
<td>Coffee break</td>
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<tr>
<td>16:20-16:40</td>
<td>Key Note Lecture 4&lt;br&gt;Chairperson: <strong>Sergio Novis</strong>, University Hospital Clementino Fraga Filho, UFRJ, Brazil</td>
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<td><em>Historical aspects of FAP in Brazil</em>&lt;br&gt;Marcos Raimundo Gomes de Freitas, Health Sciences Center, Neurology Service , UFF, Brazil</td>
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<tr>
<td>16:40-17:05</td>
<td>Key Note Lecture 5&lt;br&gt;Chairperson: <strong>Giampaolo Merlini</strong>, Amyloidosis Research and Treatment Center, University of Pavia, Italy</td>
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<td><em>Lessons learned from AL and AA amyloidosis</em>&lt;br&gt;Merril Benson, Indiana University, USA</td>
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<tr>
<td>17:05-18:05</td>
<td>Poster session 1 – Cardiology (C) and Basic Research (D)</td>
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<tr>
<td>18:05</td>
<td>Departure</td>
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<td>19:00-21:30</td>
<td>Barbecue dinner</td>
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**Tuesday – November 12th, 2013**

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<tr>
<th>Time</th>
<th>Session</th>
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<tbody>
<tr>
<td>09:00-09:25</td>
<td>Key Note Lecture 6</td>
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<td></td>
<td>Chairperson: Onur Karayal, Medical Director, Pfizer Inc; THAOS Medical Lead</td>
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<td></td>
<td>The transthyretin amyloidosis outcomes survey (THAOS): a latin american perspective</td>
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<td>Marcia Waddington Cruz, University Hospital Clementino Fraga Filho, UFRJ, Brazil</td>
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<tr>
<td>09:25-10:40</td>
<td>Symposium 4 - ATTR: An Overview of Clinical and Demographic Aspects</td>
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<td></td>
<td>Chairperson: Shu-Ichi Ikeda, Shinshu University Hospital, Matsumoto, Japan</td>
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<td>Clinical and demographic aspects of ATTR amyloidosis in Italy: Genetic heterogeneity and novel</td>
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<td>therapeutic approaches</td>
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<td></td>
<td>Giampaolo Merlìni, Amyloidosis Research and Treatment Center, University of Pavia, Italy</td>
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<td>French Reference Center for FAP and other rare Peripheral Neuropathies, NNERF. French Network</td>
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<td>for FAP, CORNAML.</td>
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<td>David Adams, CHU Bicêtre, Université Paris Sud, France</td>
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<td>ATTR: Clinical and demographic characterization of the Swedish ATTR V30M population</td>
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<td>Ole Suhr, Umea University Hospital, Sweden</td>
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<td>10:40-11:00</td>
<td>Coffee Break</td>
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<tr>
<td>11:00-11:50</td>
<td>Symposium 4 - ATTR: An Overview of Clinical and Demographic Aspects</td>
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<td>Chairperson: Isabel Maria Santos Conceição, Santa Maria Hospital, Portugal</td>
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<tr>
<td></td>
<td>Familial Amyloid Polyneuropathy TTRMet30 in Portugal: an overview</td>
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<td>Teresa Coelho, Santo Antônio Hospital, Portugal</td>
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<td>Familial Amyloidotic Poluneuropathy in Japan: genotypes and phenotypes</td>
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<td>Yukio Ando, Department of Neurology, Kumamoto University, Japan</td>
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<tr>
<td>11:50-12:15</td>
<td>Key Note Lecture 7</td>
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<td>Chairperson: Merrill Benson, Indiana University, USA</td>
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<td></td>
<td>Endemic and non-endemic areas of FAP in Japan. Late and early onset cases.</td>
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<td>Shu-Ichi Ikeda, Shinshu University Hospital, Matsumoto, Japan</td>
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<tr>
<td>12:15-13:30</td>
<td>Symposium 5. ATTR Presenting as a Cardiac Disease</td>
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<td>Chairperson: Mathew Maurer, Columbia University Medical Center, USA</td>
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<td>Familial amyloidotic cardiomiopathy, FAC</td>
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<td>Claudio Rapezzi, University Hospital S. Orsola-Malpighi, Italy</td>
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<td>Senile systemic amyloidosis, SSA</td>
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<td>Rodney Falk, Brigham and Women's Hospital, USA</td>
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<td>New insights in the diagnosis approach of TTR-FAP</td>
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<td>Violaine Planté-Bordeneuve, CHU Henri Mondor, Paris.</td>
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<tr>
<td>13:30-14:35</td>
<td>Lunch</td>
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<td>14:35-15:00</td>
<td>Keynote Lecture 8</td>
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<td></td>
<td>Chairperson: Marcia Waddington Cruz, University Hospital Clementino Fraga Filho, UFRJ, Brazil</td>
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<td>Artificial neural networks as a tool to evaluate disease progression in ATTR and other diseases</td>
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<td>José Manoel Seixas, Alberto Luiz Coimbra Institute of Pos Graduation and Engineering Research-COPPE/UFRJ, Brazil</td>
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<tr>
<td>15:00</td>
<td>Welcome and opening remarks</td>
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<tr>
<td>15:10</td>
<td>Identifying the genetic links of TTR-FAP</td>
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<td>15:30</td>
<td>Identifying the early symptoms of TTR-FAP</td>
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<td>15:50</td>
<td>TTR-FAP: Strategies for early diagnosis in clinical practices</td>
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<tr>
<td>16:10</td>
<td>Panel discussion, audience questions, and summary of key points</td>
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<tr>
<td>15:00</td>
<td>Oral presentation 2 –</td>
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<tr>
<td>15:01</td>
<td>Transthyretin familial amyloid polyneuropathy in Bulgaria</td>
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<td>15:02</td>
<td>Quantitation of sudomotor innervation in skin biopsies of familial amyloid</td>
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<td>15:03</td>
<td>Increased circulating plasma ghrelin concentrations in ATTRV30M amyloidosis</td>
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<td>15:04</td>
<td>Pre-clinical identification of TTR-related amyloidosis, Senile Systemic</td>
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<td>15:05</td>
<td>A test to diagnose and measure cardiac amyloid in AL and ATTR amyloidosis</td>
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<td>15:06</td>
<td>Ex vivo characterization of transthyretin cardiac amyloidosis, TTR-CA using</td>
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<td>15:07</td>
<td>Rio Scenarium</td>
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**Chairperson:** Roy Freeman, Beth Israel Deaconess Medical Center, USA

**Chairperson:** Claudio Rapezzi, University Hospital S. Orsola-Malpighi, Italy
### Wednesday – November 13th, 2013

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Chairperson</th>
<th>Details</th>
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<tbody>
<tr>
<td>09:00-09:25</td>
<td><strong>Key Note Lecture 9</strong></td>
<td>Joel Buxbaum, The Scripps Research Institute, USA</td>
<td>Liver transplantation and hereditary transthyretin amyloidosis. Report from the FAPWTR Bo-Goran Ericzon, Karolinska Institute, Sweden</td>
</tr>
<tr>
<td>09:25-10:15</td>
<td><strong>Symposium 7 - Liver Transplantation, LT Exchanging Experiences</strong> 25’ each</td>
<td>Joaquim Ribeiro, University Hospital Clementino Fraga Filho-UFRJ, Brazil</td>
<td>LT Experience of Lisbon. Curry Cabral Hospital. Eduardo Barroso, Transplantation Unit, Hospital Curry Cabral, Portugal</td>
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<td>Daniel Azoulay, CHU Henri Mondor, Cretéil, France</td>
<td>Experience from France</td>
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<td>10:15-10:30</td>
<td><strong>Coffee Break</strong></td>
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<tr>
<td>10:30-11:45</td>
<td><strong>Symposium 7 - Liver Transplantation, LT Exchanging Experiences</strong> 25’ each</td>
<td>Eduardo Barroso, Transplantation Unit, Hospital Curry Cabral, Portugal</td>
<td>Overview of liver transplantation for FAP in São Paulo Paulo Massarolo, Universidade de São Paulo, Brazil</td>
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<td>Joaquim Ribeiro, University Hospital Clementino Fraga Filho-UFRJ, Brazil</td>
<td>The Domino Recipient</td>
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<td>Arie Stangou, Institute of Liver Studies, United Kingdom</td>
<td>Tafamidis treatment for the domino recipient</td>
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<tr>
<td>11:45-12:15</td>
<td><strong>Oral Presentation 3</strong></td>
<td>Ole Suhr, Umea University Hospital, Sweden</td>
<td>Steady amyloid turn-over after liver transplantation in FAP patients Masahide Yazaki, Shinshu University School of Medicine, Japan</td>
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<td>Long term stability of TTR-FAP after Liver transplantation a 20 years’ experience of the French reference center for FAP David Adams, Université Paris Sud, France</td>
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<td>Outcome of liver transplantation for non-ATTR V30M: report from the FAP World Transplant registry, FAPWTR Ole Suhr, Umea University Hospital, Sweden</td>
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<tr>
<td>12:15-13:05</td>
<td><strong>Symposium 8 - Drug Treatment: New perspectives 25’ each</strong></td>
<td>Gerard Said, Hospital de la Salpetrière, France</td>
<td>Summary data from Tafamidis treatment Teresa Coelho, Santo António Hospital, Portugal</td>
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<td>Summary Data from Phase II ALN-TTR02 RNAi Treatment for ATTR Jared Gollob, Alnylam Pharmaceuticals Inc, USA</td>
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<tr>
<td>13:05-14:00</td>
<td><strong>Lunch</strong></td>
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### Wednesday – November 13th, 2013

<table>
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<tr>
<th>Time</th>
<th>Session Title</th>
<th>Chairperson/Institution</th>
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<tr>
<td>14:00-14:50</td>
<td><strong>Symposium 8 - Drug Treatment: New Perspectives</strong></td>
<td>Marcia Waddington Cruz, University Hospital Clementino Fraga Filho, UFRJ, Brazil</td>
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<tr>
<td>14:50-15:20</td>
<td><strong>Oral Presentation 4</strong></td>
<td>Teresa Coelho, Santo Antônio Hospital, Portugal.</td>
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<tr>
<td>15:20-15:40</td>
<td><strong>Highlights of the symposium and closing remarks</strong></td>
<td>Merrill Benson, President of the International Society of Amyloidosis</td>
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<td>15:40-15:50</td>
<td><strong>Closing remarks</strong></td>
<td>Debora Foguel, Institute of Medical Biochemistry, UFRJ, Brazil; Marcia Waddington Cruz, University Hospital Clementino Fraga Filho, UFRJ, Brazil</td>
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<tr>
<td>15:50-19:00</td>
<td><strong>City tour to Sugar Loaf Mountain</strong></td>
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**SP.8-3**
*Summary data from phase II ASO treatment for FAP*
Elizabeth Ackerman, Isis-GSK Pharmaceuticals Inc, USA

**SP.8-4**
*Summary data from Diffusional phase III trial*
John Berk, Boston University, USA

**OP.4-01**
*Molecular Tweezers targeting Transthyretin aggregation: therapeutic applications on Familial Amyloidotic Polyneuropathy*
Nelson Ferreira, Universidade do Porto, Portugal

**OP.4-02**
*Gene therapy approach to familial amyloidotic polyneuropathy*
Ana Rita Batista, Universidade do Porto, Portugal

**OP.4-03**
*A phase II study of doxycycline plus tauroursodeoxycholic acid in transthyretin amyloidosis*
Laura Obici, Fondazione IRCCS Policlinico San Matteo, Italy

*5 minutes presentation and 5 minutes discussion*
Opening Lecture
OL.1 - GENETICALLY DETERMINED TRANSTHYRETIN LEVELS, TRANSTHYRETIN STABILITY, AND RISK OF VASCULAR DISEASE

Louise Stig Hornstrup¹, Ruth Frikke-Schmidt¹,³, Raul Campos², Per Hammarström², Børge G. Nordestgaard⁴,⁵, Anne Tybjærg-Hansen¹,³,⁵

¹Department of Clinical Biochemistry, Rigshospitalet; ²Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping, Sweden; ³The Copenhagen General Population Study and ⁴Department of Clinical Biochemistry, Herlev Hospital; ⁵The Copenhagen City Heart Study, Frederiksberg Hospital; ¹,³,⁵Copenhagen University Hospitals and Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

Background: Transthyretin can cause amyloidosis due to destabilization of transthyretin tetramers in plasma. Whether lifelong genetic destabilization of transthyretin, due to mutations in TTR, associates with low transthyretin levels and with increased risk of vascular disease in the general population is unknown. We tested this hypothesis. Methods: We first resequenced TTR in 10,513 participants in a general population study, the Copenhagen City Heart Study, and genotyped all identified nonsynonymous variants in the Copenhagen General Population Study(n=58,343). Second, we tested whether mutations in TTR were associated with transthyretin levels to the extent predicted by their stability score, and with risk of vascular disease. Results: We identified ten rare(minor allele frequency ≤0.2%) nonsynonymous variants in TTR in a total of 385 participants(1:170), and one common variant(7.4%). Compared to wildtype, carriers of destabilizing variants had lower plasma transthyretin levels(mean reduction: 37%), whereas carriers of a stabilizing variant, T119M, had higher levels(mean increase: 26%) (P for trend<0.0001; stabilizing versus wildtype versus destabilizing variants). The cumulative incidence of vascular disease as a function of age and TTR genotype increased from stabilizing to wildtype to destabilizing variants, and with decreasing transthyretin levels(log rank P=0.004). Compared to wildtype, the multifactorially adjusted hazard ratios for vascular disease were 0.70(95% CI:0.51-0.97) and 1.73(1.06-2.83) for stabilizing and destabilizing variants, respectively. Conclusion: Lifelong genetic destabilization of transthyretin is associated with decreased plasma transthyretin levels and with increased risk of vascular disease. This is clinically important, because transthyretin stabilizing drugs are currently under development for the treatment of transthyretin amyloidosis.
Key note Lectures
KN.01 - THE ROLE OF MICROGLIA AND NEUTROPHILS IN TTR-RELATED AMYLOIDOSES

Debora Foguel, Estefania Azevedo, José Henrique Ledo, Carolina Braga, Elvira Saraiva, Sergio T. Ferreira and Fernando L. Palhano
Instituto de Bioquímica Médica - Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590

Neutrophil extracellular traps (NETs) are key players in a death mechanism in which neutrophils release DNA traps decorated with proteins such as elastase and histones to entangle pathogens. We asked whether NETs are triggered by amyloid fibrils, reasoning that since proteases are present in NETs, protease digestion of amyloid may generate soluble, cytotoxic species. We show that amyloid fibrils from three different sources (α-synuclein, Sup35 and transthyretin) induced NADPH oxidase-dependent NETs in vitro from human neutrophils. Surprisingly, NET-associated elastase digested amyloid fibrils into short species that were cytotoxic for BHK-21 and HepG2 cells. We observed in situ, NETs in amyloidotic deposits from amyloidotic patients which co-localized with amyloid deposits. These data reveal that NETs, so far described to be elicited by pathogens, can also be triggered by amyloid fibrils. Moreover, the involvement of NETs in amyloidoses might be crucial for the production of toxic species derived from fibril fragmentation. In a second approach, we have investigated the pathogenic mechanism behind oculoleptomeningeal amyloidosis (OA) caused by fibrillation of A25T. We have showed that fibrils of A25T activate microglia leading to secretion of TNF-α, IL-6 and nitric oxide. We further found that A25T amyloid fibrils induce Akt activation, culminating in NFκB translocation to the nucleus of microglia. While A25T fibrils are not directly toxic to neurons, exposure of neuronal cultures to conditioned media of fibril-activated microglia causes synapse loss culminating in extensive neuronal death via apoptosis. Finally, intracerebroventricular (i.c.v.) injection of A25T fibrils caused microgliosis, increased brain TNF-α and IL-6 levels and cognitive deficits in mice, which could be prevented by minocycline. These results indicate that A25T fibrils act as inflammatory agents in OA, activating microglia and causing neuronal damage through inflammation.

KN.02 - CURRENT INSIGHTS INTO QUANTIFYING TRANSTHYRETIN AGGREGATION-ASSOCIATED PATHOLOGY AND ITS AMELIORATION WITH TAFAMIDIS

Justin Chapman¹, Irit Rappley², Cecilia Monteiro²,³, Marta Navais³, Teresa Coelho³, Xin Jiang¹, and Jeffery W. Kelly¹,²
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Recently, we showed that Tafamidis, a small molecule kinetic stabilizer of Transthyretin, dramatically slows the progression of Familial Amyloid Polyneuropathy in a placebo controlled clinical trial of 18 month duration (1), and in a 12 month follow-on open label study (2). This study presents the first pharmacologic evidence supporting the hypothesis that the process of active transthyretin aggregation causes the degeneration of post-mitotic tissue (i.e. the peripheral nervous system, the autonomous nervous system, and the heart) in the human amyloidoses. To further test the hypothesis that active TTR aggregation causes post-mitotic tissue degeneration, we developed four new clinical assays to measure tafamidis plasma concentration, to quantify tafamidis-associated kinetic stabilization of tetrameric transthyretin, and to determine the concentration of soluble misfolded TTR levels and natively folded TTR tetramer levels in plasma. The results of these assays before and after dosing Portuguese patients with Tafamidis post regulatory agency approval will be presented. References: (1) Coelho, T.; Maia, L.F.; Martins da Silva, A.; Cruz, M.W.; Planté-Bordeneuve, V.; Lozoner, P.; Suhr, O.B.; Campistol, J.M.; Conceição, I.; Schmidt, H.; Trigo, P. Kelly, J.W.; Labaudiniere, R.; Chan, J., Packman, J., Wilson, A.; Grogan, D.R. “Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial” Neurology 2012, 79, 785-792. (2) Coelho, T.; Maia, L.F.; Martins da Silva, A.; Cruz, M.W.; Planté-Bordeneuve, V.; Suhr, O.B.; Conceição, I.; Schmidt, H. H. J.; Trigo, P. Kelly, J.W.; Labaudiniere, R.; Chan, J., Packman, J.; Grogan, D.R. “Long-term Effects of Tafamidis for the Treatment of Transthyretin Familial Amyloid Polyneuropathy” J Neurol. 2013 Aug 22. [Epub ahead of print]
FAP is characterized by the association of a sensory-motor and autonomic polyneuropathy with identified amyloidogenic TTR mutation. In some patients however cardiac manifestations predominate. In patients with a known family history, the diagnosis of TTR FAP can be reached quickly after first neuropathic manifestations. The difficulty in this setting can be related to the anxiety of carriers of the amyloidogenic mutation aware of the need for an early diagnosis to increase efficacy of treatments. Investigations will be necessary to confirm neurological deficit before initiating specific treatment. In the so-called sporadic cases of TTR FAP absence of family history for amyloidosis accounts for the usual delay to diagnosis. When sensory-motor manifestations predominate, four limb involvement with mixed axonal and demyelinating features at EMG, CIDP is a common misdiagnosis especially in patients with little or no autonomic dysfunction. In this setting syncopes, orthostatic hypotension, cardiomyopathy; intracardiac conduction block; diarrhoea, vomiting, sexual impotence or carpal tunnel syndrome raise suspicion of amyloid polyneuropathy. In non-diabetic patients with a progressive axonal polyneuropathy with predominant small fiber involvement, the diagnosis of FAP should be considered, especially when associated with autonomic dysfunction, cardiac manifestation, or carpal tunnel syndrome. Amyloid can be visualized in nerve biopsy specimens, in muscle specimens, in skin biopsy, salivary glands, or abdominal fat. Negative biopsy findings do not rule out amyloidosis. Abnormal deposits must be characterized by Congo red affinity or thioflavin T. EM examination shows the fibrillar aspect of amyloid substance, made up of unbranched fibrils of 10 nm diameter with parallel dense borders. Mass-spectroscopy-based proteomic analysis can be used to identify the amyloid type. Immuno-labelling with anti-TTR antibody favors the genetic origin of the disease, but DNA testing remains mandatory. After detection of amyloid in biopsy specimens, the diagnosis of light-chain amyloidosis is often considered because of the high incidence of monoclonal gammopathies associated with CIDP in the elderly. In this setting immunolabelling can be misleading and mass spectroscopy can be useful but TTR gene sequencing must be done in all cases.

Amyloidosis is often very difficult to diagnose in a timely fashion. Many more prevalent diseases tend to mimic the signs and symptoms of amyloidosis and the correct diagnosis typically waits for tissue biopsy. After a diagnosis of amyloidosis is made an even more difficult diagnostic challenge may present itself. “What type of amyloidosis is this?” Several factors have brought this question to the forefront of present day diagnosis: 1. New diagnostic techniques identify increased numbers of patients with amyloidosis earlier in the course of disease. 2. Identification of new types of amyloidosis requires wider knowledge of each type: similarities and differences. 3. Development of specific therapies demands correct diagnosis. Too many FAP patients have received chemotherapy. 4. While new diagnostic techniques increase chances for correct diagnosis, decreasing autopsy rates may cloud perspective of our diagnostic failures. How we can address these issues is worthy of contemplation.
**KN -06 - THE TRANSTHYRETIN AMYLOIDOSIS OUTCOMES SURVEY (THAOS): A LATIN AMERICAN PERSPECTIVE**

Márcia Waddington-Cruz on behalf of the THAOS investigators  
*Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*

**Objective:** To present an overview of the THAOS patient registry with a focus on genotypes and phenotypes observed in Latin American countries and to describe how this growing data collection is helping to define the natural history of transthyretin amyloidosis (ATTR).  

**Background:** The natural course of ATTR is poorly defined and its characterization is complicated by the extreme heterogeneity in genotype and phenotype as well as by its relatively low prevalence. The global, non-interventional THAOS patient registry was established in 2007 to collect and analyze data on symptom occurrence and progression and on the effects of disease modifying treatments in a large, diverse patient population.  

**Methods:** THAOS is a longitudinal, observational registry open to all symptomatic individuals with confirmed hereditary or acquired ATTR, as well as to asymptomatic carriers of known pathogenic TTR mutations. Patient information and data from various standard assessments are obtained during clinical evaluations and recorded by the treating investigator using an interactive, Web-based system.  

**Results:** As of June 2013, a total of 1744 subjects from 17 different countries were enrolled in THAOS. 64 different TTR genotypes were represented. Most patients enrolled from Brazil (94/105) and Argentina (38/40) carried the Val30Met mutation, whereas the Ser50Arg mutation (27/34) was predominant in Mexico. Certain genotypes were generally associated with predominantly neuropathic (e.g., Val30Met) or predominantly cardiac (e.g., Val122Ile or wild-type) symptom presentation. Analysis of the prevalence of ATTR symptoms in patients from major genotype groups categorized by the duration of their symptoms provides first insights into the natural course of ATTR.  

**Conclusions:** The large collection of data from the THAOS register provides a unique opportunity to improve our understanding of the diverse presentations and the natural disease progression of ATTR.

**Disclosure:** Data presented in this abstract are derived from the THAOS registry, which is sponsored by Pfizer Inc.

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**KN -07 - ENDEMIC AND NONENDEMIC AREAS OF FAP IN JAPAN: EARLY ONSET VERSUS LATE ONSET CASES**

Shu-ichi Ikeda  
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Familial amyloid polyneuropathy (FAP) is caused by a mutation in the transthyretin (TTR) gene, which produces an amyloidogenic variant form of TTR (ATTR). The clinical phenotypes of ATTR type FAP have been shown to vary in different kindreds or individuals with diverse gene mutations. Even in FAP with ATTR Val30Met, the clinical picture is considerably different between patients originating from endemic foci and those with non-endemic origins: the latter group of patients shows a late onset, male predominance and obscure family history. Additionally, apparent dissociated sensory loss and serious autonomic dysfunctions are not usually seen in the latter. Since many neurologists are more familiar with classic concept of FAP, patients with non-familial ATTR Val30Met type FAP often receive an incorrect diagnosis initially. The vast majority of FAP patients whose DNA samples were recently referred to our institution were ATTR Val30Met type with non-endemic origins and thus the number of patients with this disease seems to be much higher than previously recognized. In the classic phenotype of FAP with an early onset it is emphasized that sensory neuropathy starts in the legs and shows progression in ascending fashion and that motor symptoms appear a few years later. However, in ATTR Val30Met type FAP with a late onset, sensory and motor symptoms in both the upper and the lower limbs appear within a short period, and in some patients upper limb symptoms precede lower limb symptoms. The mean duration of the disease onset to death is 7.3 years, which is shorter than that in ATTR Val30Met type FAP with an early onset (range 10-12 years). Both findings are new information that should be added to the category of ATTR type FAP. FAP is now a treatable disease: TTR-derived amyloid deposits in vivo are not always stable and can turn over dynamically. In addition to liver transplantation some drugs that prevent the formation of ATTR-derived amyloid fibrils are expected to modify the course of the disease in FAP patients.
KN-08 - ARTIFICIAL NEURAL NETWORKS AS A TOOL TO EVALUATE DISEASE PROGRESSION IN ATTR AND OTHER DISEASES

J.M. Seixas, F.G.T. Machado, V.B. Costa, Márcia W. Cruz, D.Foguel
Federal University of Rio de Janeiro

Computational intelligence tools have successfully been applied in health to a variety of topics. They are bio-inspired algorithms that proved to be efficient for data mining, pattern recognition, data clustering and visualization. In this work, an index for PAF patient evaluation is developed using artificial neural networks (ANNs), which are trained through a non-supervised learning method. ANNs are inspired in the human brain and process data in a nonlinear manner, so that high-order statistics can be accessed. Using a database for Brazilian patients, the Self-Organizing Map (SOM) is applied to map high-dimensional data (signals, symptoms and some exam results) onto two dimensions, which help data interpretation and post-processing. In this map, a clustering procedure is developed to group patients into five classes, which are analyzed in terms of walking disability range. Results show that ANN is capable to capture the main features of the database and helps evaluating how the disease evolves according to patient first visit data.

KN-09 - LIVER TRANSPLANTATION AND HEREDITARY TRANSTHYRETIN AMYLOIDOSIS. REPORT FROM THE FAPWTR

Bo-Goran Ericzon
Karolinska University Hospital Huddinge (Stockholm, Sweden)

Until recently, liver transplantation (Ltx) was the only available treatment for familial amyloidotic polyneuropathy (FAP), but in the last years several pharmaco-therapeutic approaches have emerged hoping to halt disease progression. Ltx as the golden standard of treatment is evaluated in a 20 years perspective by analysis of the FAP world transplant registry (FAPWTR). From April 1990 until December 2012, data from 77 liver transplant centers in 19 countries have been accumulated. Approximately 125 liver transplants are performed yearly worldwide. The Registry holds a total of 2044 patients undergoing 2236 Ltx. 586 patient deaths were reported. Ninety-seven Ltx were performed in combination with a heart or a kidney. Patients undergoing combined Ltx were generally older than those only subjected to Ltx and with a non-Val30Met mutation. The overall 20 year survival after tx, all mutations included, was 55.3%. Expected mortality rate decreased on average by approximately 4% per year between 1990 and 2010. In a multivariate analysis modified body mass index (mBMI), early onset of disease, disease duration before Ltx and Val30Met versus non Val30Met were independent significant factors for survival after Ltx. Survival of patients with onset of disease after age 50 was significantly reduced when compared to early onset patients, and most pronounced in the male subgroup. Thus, expected mortality rate in late onset male patients was 137% of that of late onset female patient mortality (p<0.05). Early onset patients (all mutations) had an expected mortality rate of 38% of that of the late onset group (p<0.01). Furthermore, Val30Met patients had an expected mortality rate of 61% of that of non Val30Met patients (p<0.01). With each year of increase in duration of disease before Ltx, the expected mortality increased by 11% (p<0.01). Overall, with each unit of increase in mBMI at Ltx, the expected mortality decreased by 0.12% (p<0.01). Twenty-two percent of the death causes were related to cardiovascular disease, thus significantly more common than usually seen in Ltx for end stage liver disease. Conclusion: Long term survival after Ltx for FAP is excellent. A good nutritional status, short duration of disease at the time of Ltx and early onset of disease were significant independent factors for survival. Val30Met patients had significantly better outcome when compared to non-Val30Met patients. The risk of delaying Ltx by testing alternative treatments needs consideration.
Symposia
SP.01-1 - WILD-TYPE ATTR AMYLOIDOSIS (SENILE SYSTEMIC AMYLOIDOSIS) – A DISEASE RELATED TO LATE-ONSET ATTR V30M AMYLOIDOSIS AND WITH CONSEQUENCES FOR PERIPHERAL NERVES

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Late-onset hereditary transthyretin (TTR) of V30M type is usually associated with progressive restrictive cardiomyopathy and amyloid fibrils are characterized by C-terminal TTR fragments. The heart disease is more or less indistinguishable from that in systemic amyloidosis with wild-type TTR, referred to as Senile Systemic Amyloidosis (SSA), which is a disease known to particularly affect elderly men. Symptoms in SSA are usually due to the cardiomyopathy. SSA is, however, systemic and autopsy studies have shown wide-spread vascular deposits in lung, kidney, gastrointestinal tract and other organs. Commonly, carpal tunnel syndrome has been documented in SSA and can also precede cardiac disease. TTR deposits have repeatedly been described in joints and ligaments in conjunction with aging without known systemic disease. There are single case reports on TTR amyloid found in tissues associated with lumbar spinal stenosis. Lumbar spinal stenosis is often treated by surgery at which thickened connective tissue and osteophytes are removed. In this first study we wished to find out whether such tissue contained amyloid of TTR origin. We also determined whether full-length or fragmented TTR was involved. In a series of 23 randomly selected patients (f/m =11/12; mean age 62 y, range 44-86), removed fibrous and cartilaginous tissue contained amyloid in 16 cases. In most of these, at immunohistochemistry (IH), TTR reactivity was absent or not convincing in most cases. However, in 3 of the 23 cases (f 78y, m 76y, m 83y) all amyloid deposits were strongly labeled at IH. Western blot analyses revealed fragmented TTR in all cases with pattern identical to that seen in SSA and late onset ATTR V30M amyloidosis. These findings raise the important question whether lumbar spinal stenosis often is a consequence of deposition of ATTR amyloid. Furthermore, it is possible that problems from lumbar spinal stenosis can be mistaken for symptoms of peripheral neuropathy and patients thereby being deprived of correct treatment. Since our study was performed on anonymized tissue material it is not possible to determine whether a systemic disease was present. Further studies in which analyses of removed tissue are combined with imaging of heart are needed.

SP.02-02 - CLEARANCE OF EXTRACELLULAR MISFOLDED PROTEINS IN SYSTEMIC AMYLOIDOSIS: EXPERIENCE WITH TRANSTHYRETIN

Maria Joao Saraiva
Instituto de Biologia Molecular e Celular (IBMC), Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Portugal

Increasing evidence indicates that accumulation of misfolded proteins in the form of oligomers, protofibrils or amyloid fibrils, and their consequences in triggering intracellular signaling cascades with toxic consequences represent unifying events in many of slowly progressive neurodegenerative disorders. Studies with small compounds or molecules, known to recognize and disrupt amyloidogenic structures, have proven efficient in promoting clearance of protein aggregates in experimental models of systemic and localized forms of amyloidoses. Doxycycline and EGCG were efficient in removing aggregates in pre-clinical studies in a transgenic mouse model for transthyretin (TTR) systemic amyloidosis and represent an opportunity to address mechanisms and key players in deposit removal. Extracellular chaperones, such as clusterin and metalloproteinases play an important role in this process as well as different types of cells, like macrophages and fibroblasts.
SP.1-03 - THE ANTI-AMYLOIDOGENIC EFFECTS OF TRANSTHYRETIN (TTR) IN VIVO AND IN VITRO

Joel N. Buxbaum
The Scripps Research Institute (LaJolla CA, USA)

The amyloidogenic properties of TTR are well known and the mechanisms of tetramer dissociation and monomer misfolding to yield cytotoxic aggregation products have been established to an extent sufficient to bring mechanism based therapies to the clinic. However, it has also become clear over the last decade that the absence of TTR in the mouse is not without consequences, particularly in the presence of a human Alzheimer’s disease gene, in which case the trajectory of appearance of the AD pathology is accelerated, apparently in a gene dose dependent fashion. Consistent with those observations is the demonstration that genetically programmed over-expression of a wild type human TTR gene suppresses both the neuropathologic and behavioral abnormalities usually seen in mice carrying the human AD gene with marked reduction of soluble and insoluble Aβ1-40 and 1-42 deposited in the cerebral cortices of the mice. In a series of studies our laboratory has shown increased synthesis of TTR in the hippocampal and cortical extracts of APP23 AD model mice, TTR mRNA in primary neurons cultured from such mice and binding of TTR to Aβ in the brains of AD model mice and some human AD patients. In vitro, using NMR and isothermal titration calorimetry we have seen that the human TTR tetramer binds Aβ 1-40 monomer with a KD in the micromolar range and a stoichiometry of less than one with the interaction mediated by residues in the T4 binding site of TTR and amino acids 18-21 in the Aβ peptide. TTR binding reduced Aβ oligomer and fibril formation as well as the formation of thioflavine T positive fibrils when pre-incubated with the bacterial amyloids HypF-N (Chiti lab) and Curli (Chapman laboratory). In collaboration with Fabrizio Chiti’s laboratory we showed that addition of TTR to cytotoxic Aβ, IAPP or HypF-N oligomers increased oligomer size and rendered them non-toxic. We found no evidence for disaggregate activity in the recombinant TTR preparations. It thus appears that the human TTR tetramer has the capacity to inhibit amyloid fibril formation by a variety of eukaryotic and prokaryotic amyloidogenic proteins in vitro and at least one human amyloid precursor responsible for neurodegenerative disease in vivo. It appears that these chaperone-like interactions may be sequence or conformation specific and could be related to the structural properties of TTR that are intrinsic to its amyloidgenicity.
Transthyretin familial amyloid polyneuropathy (TTR-FAP) is an inherited amyloidosis that presents as a progressive sensorimotor and autonomic polyneuropathy, which initially involves unmyelinated and small myelinated nerve fibers. Subsequent degeneration of larger nerve fibers results in deep sensory changes, areflexia and muscle weakness. Without treatment, the natural history of TTR-FAP is rapidly progressive course to death within a decade following symptoms onset. Liver transplantation (LT) was proposed as a potential intervention to halt abnormal Val30Met TTR (TTR-FAP) production. More recently, a new molecule (tafamidis) a TTR stabilizer, granted marketing authorization for the treatment of stage 1 TTR-FAP patients, as it has been demonstrated to delay neurological progression. In either case, early affected patients are the best candidates to benefit from the available and potential new treatments, because it is not reasonable to expect that treatments will be able to revert organ lesions due to amyloid deposition. In this context, the existence of neurophysiological markers that could early detect nerve dysfunction is of paramount importance, not only to confirm early diagnosis and monitor disease progression, but also to evaluate treatment efficacy. In most neuropathies, conventional nerve conduction studies (NCS) are the standard method for diagnosis. Nevertheless, NCS have the disadvantage of only assessing large myelinated fibers. In TTR-FAP, only in the later stages, when larger myelinated fibres are affected, NCS can detect the presence of a predominantly axonal sensory-motor neuropathy. At present, in general, detection of small fiber neuropathy is based on epidermal nerve fiber density measurement in skin biopsies, quantitative sensory testing or on autonomic nervous system testing, in particular evaluating sympathetic C-fibers sweat gland innervation. Plantar sympathetic skin responses (SSR) has been showed to be a sensitive method to detect small fibre dysfunction. It is already reduced in carriers suggesting that autonomic C fibers are dysfunctional before clinical presentation, but changes tend to be more severe in early-symptomatic patients. Laser Evoked potentials activate Aδ and C nociceptors with an afferent volley that is conducted along small-myelinated (Aδ) primary sensory neurons, and relayed to spinothalamic neurons and brain. Cortical responses seems also to be a sensitive method, with an abnormal trend in carriers but significantly abnormal in early affected patient. Illustrating the very early phase of disease involvement, the sensitivity was low for the SSR and LEPs , but slightly better for SSR amplitude. A careful neurological and neurophysiological assessment in order to provide early clinical diagnosis should be done.

SP.2-01 - EARLY MARKERS OF THE DISEASE: THE IDEAL MOMENT TO TREAT.

Mathew Maurer
Columbia University, New York, USA - Cardiac Markers

Approaches to target the underlying proposed biologic mechanisms of this ATTR cardiac amyloidosis have been developed which include TTR tetramer (native state) kinetic stabilization by small molecule binding, gene therapy with small interfering RNAs, antisense oligonucleotides and single-stranded oligonucleotides; all promising strategies based on our understanding of the pathogenesis of TTR amyloidosis. However, since these approaches are aimed at preventing further progression of disease by reducing or eliminating subsequent amyloid deposition, early identification of affected subjects is critical. Early identification of ATTR-CM has been a challenge as most patients with ATTR-CM are identified when symptomatic and because the condition is often overlooked and underappreciated most patients do not have early disease. Emerging stratagies for early idenfication of a cardaic phenotype include biomarkers particularly the natruiretic peptides, newer sophisticated echo doppler techniques that employ strain and speckled strain imaging, bone isotopes such as 99mTc-DPD and Tc-Pyp scintigraphy and cardaic MRI using T1 imaging. This seminar will focus on techniques to identify ATTR-CM in the early stages and track the progression of disease, which will be essential for the execution of successful future clinical trials.
Inherited neuropathies, collectively known as Charcot-Marie-Tooth disease (CMT), are a group of genetically and phenotypically heterogeneous peripheral neuropathies associated with mutations or copy number variations in over 70 distinct genes. They can manifest as motor and sensory neuropathies (HMSN), pure motor neuropathies (dHMN), or sensory and autonomic neuropathies (HSAN), depending on the specific gene affected. Autosomal dominant forms are subdivided into demyelinating (CMT1) and axonal (CMT2) forms based on electrophysiological and neuropathologic criteria. X-linked (CMTX) and autosomal recessive (CMT4) forms are also seen. Each type of CMT is subdivided according to the specific genetic cause of the neuropathy. For example, the most common form of CMT1, termed CMT1A, is caused by a duplication of a fragment of chromosome 17 containing the peripheral myelin protein 22-kD (PMP22) gene. Making the distinction between FAP and some of the commonest forms of CMT can be relatively straightforward, especially for the demyelinating types; however, some of the Hereditary Sensory and Autonomic Neuropathies (HSAN) can have an overlapping presentation with FAP, thus requiring careful evaluation to accurately determine the correct diagnosis. Some forms of axonal CMT (type 2) can also be mistaken for FAP and vice-versa, especially in patients with de novo mutations and, therefore, no family history. Phenotype specific panels based on next generation sequencing (NGS) technology are becoming increasingly available and will soon be the most cost effective way to make the differential diagnosis of rare inherited neuropathies. Nonetheless, careful clinical and electrodiagnostic characterization will still be a fundamental step in the diagnostic evaluation of these patients and will continue to help guide molecular diagnosis.

Hereditary transthyretin amyloidosis (ATTR) is characterized by high genetic and phenotypic variability, with differences in disease presentation and progression across different countries. Therapeutic resources are limited. We report longitudinal data of 14 years follow-up of patients affected by ATTR in Italy. One hundred and fifty patients with ATTR amyloidosis were diagnosed and follow-up at the Amyloidosis Research and Treatment Center, Pavia, between July 1999 and September 2013. They were investigated according to a standardized protocol including genetic testing, typing of amyloid deposits (immunohistochemical and/or proteomic assessment) and thorough laboratory and clinical investigation. Patients were offered standard and experimental therapies. Median age of onset was 60 years (range 28-82) and male-to-female ratio was 3:1. A positive history was present in 40% of index cases. The mean delay to diagnosis was 36 ± 25 months and in 35% of cases the disease was initially misdiagnosed and unsuccessfully treated with steroids and immunoglobulins. A predominantly sensory length dependent mixed peripheral neuropathy was the most frequent presentation of the disease (58%), followed by heart failure (24%), diarrhea (9%) and vitreal opacities (3%). Significant weight loss was reported in 50% of cases, and 41% of patients had previously undergone surgery for bilateral carpal tunnel syndrome. Twenty percent of patients already lost walking independency at diagnosis (PND score ≥ 3). Mutations in TTR detected in our cohort included Val30Met (25.5%), Glu89Gln (17.8%), Phe64Leu (12.1%) and Ile68Leu (10.2%) and 23 other mutations with lower frequency in the remaining 34.4%. Private mutations were identified in 9 subjects. Although Italy is considered a non-endemic area, over 50% of patients came from cluster zones in Sicily, Piedmont, Lazio and Emilia. Twenty-two patients (14%) underwent liver or combined liver-heart transplant, forty-five patients (29%) were treated with anti-amyloidogenic drugs including diflunisal, doxycycline-TUDCA, and tafamidis with promising results. Median survival from disease onset was 7.2 years. Mutated TTR amyloidosis shows high genetic and clinical heterogeneity in Italy. A sensory-motor peripheral neuropathy is the commonest presentation; however, one fourth of patients presented with isolated cardiomyopathy. The disease is still largely misdiagnosed. Early diagnosis is essential to improve the care considering the availability of novel promising therapies.
SP.4-02 - FRENCH REFERENCE CENTER FOR FAP AND OTHER RARE PERIPHERAL NEUROPATHIES, NNERF. FRENCH NETWORK FOR FAP, CORNAMYL.

David ADAMS
French Reference Center for FAP and other rare Peripheral Neuropathies (NNERF). French Network for FAP (CORNAMYL) CHU Bicêtre APHP Univ Paris-Sud France

France is a non endemic country for TTR-FAP. First cases were identified in the 1980s in young patients of Portuguese origin. TTR-FAP were described in patients with sporadic presentation of non Portuguese origin in the 1990s. Additional cases of TTR-FAP were progressively identified with the help of nerve biopsy and TTR gene analysis later. According to French database for TTR-FAP, they are characterized by: - a large genetic heterogeneity with up to 37 TTR gene mutations (met30 in 61%; the 3 most frequent ones are Tyr77, Phe77 and Val107) using TTR gene sequencing - varied neurological phenotypes including classical length dependent small fiber polyneuropathy or autonomic polyneuropathy but also all fiber polyneuropathy and new ones including upper limb neuropathy, ataxic neuropathy and motor neuropathy. - absence of positive family history of TTR-FAP in 55% of cases. - late onset (>50 yo) in 75% of cases, and very late onset (>70 yo) in 1 third - delay for diagnosis by 3 to 4 years in sporadic cases. One half of TTR-FAP cases are diagnosed at a stage 2 at first consultation. Diagnosis of TTR-FAP is still difficult in sporadic cases because of: i) misleading presentations mimicking many varieties of peripheral neuropathies; ii) amyloid deposits are inconstantly found in biopsy (sensitivity of labial salivary gland biopsy= 70%; of nerve biopsy=70%; in combination=90%). Reference center work altogether with the 10 French satellite reference centers for neuromuscular diseases (French Network for FAP CORNAMYL) to sensitize french neurologists to the disease and homogeneize the investigations to confirm the diagnosis and the care of patients. The network allowed to identify 4 times more cases of TTR-FAP during the last 5 years and in 79/100 of french geographical departments. This has major implications for proposing early an anti-amyloid therapy and for genetic counseling to families.

SP.4-03 - ATTR: CLINICAL AND DEMOGRAPHIC CHARACTERISATION OF THE SWEDISH ATTR V30M POPULATION

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Northern Sweden comprises one of the well known agglomeration areas of hereditary transthyretin V30M (ATTR V30M) amyloidosis with a very high frequency of gene carriers, but a comparatively low penetrance of the trait. Within the clustering area, a surprisingly variation of the penetrance of the trait has been observed, with a comparatively high penetrance in the Skellefteå area compared to that observed in Piteå and especially the Lycksele areas. In addition, phenotypical differences are also noted for age of onset and clinical presentation, where heart and eye complications are more commonly found in patients from Lycksele and Piteå, compared to the Skellefteå area. The Swedish ATTR V30M population appears to have a common Swedish founder, and transthyretin (TTR)-gene analysis disclosed a unique SNP in the 3’ UTR region that, however, had no functional impact on TTR synthesis. Epigenetic factors, such as mitochondrial function may play a role. The variation in onset and clinical presentation of ATTR V30M amyloidosis appears to be related to differences in amyloid fibril composition, where a mixture of truncated and full length TTR amyloid fibrils predominantly is present in late onset patients, i.e.,those with amyloid cardiomyopathy, whereas patients with full length TTR dominantly are those with an early onset, neuropathy and without amyloid cardiomyopathy. The difference in fibril composition is also related to the outcome of liver transplantation, where patients with a mixture of truncated and full length TTR are those who develop cardiomyopathy, and this fibril composition also appears to be the type of amyloid with the highest turn over rate. The Swedish ATTR V30M amyloid population comprises of both early and late onset patients, and their type of amyloid fibril composition is related to their phenotype and outcome of liver transplantation. The presence of two distinct types of ATTR fibrils could have an impact on the outcome of therapeutic clinical trials, since it suggests that different pathway for amyloid fibril formation may be operating.
SP.4-04 - FAMILIAL AMYLOID POLYNEUROPATHY TTRMET30 IN PORTUGAL: AN OVERVIEW

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Background: Familial Amyloid Polyneuropathy (FAP) is highly prevalent in Portugal. Genetic heterogeneity is increasingly described around the world but Portuguese focus is mainly related to the mutation TTRVal30Met.

Objectives and methods: To give an overview of the past and present the reality of FAP in Portugal. We describe the evolution of patient number and the variability of sex and age related penetrance. We reviewed our register that includes 2567 patients observed between 1939 and September 2013 with molecular diagnosis of TTRVal30Met mutation and a clearly defined age-of-onset. We ascertained sex, age-of-onset and the sex and clinical situation of the transmitting parent, when known. We also reviewed the clinical and therapeutic situation of patients observed during the last year. Results: The mean age of onset (mean ± sd) of all patients is 35.4 ± 10.9; male predominance is marginal (1365 men and 1202 women; M:F = 1.1:1). The mean age of onset is significantly different (p<0.001) between men (33.5 ± 11.1) and women (37.5 ± 10.5) but only for those 2051 patients with a clinically affected parent. In the group of 640 symptomatic patients regularly observed during the last year in our Unit, 546 (85%) had onset <50 years of age. Most patients had a typical clinical onset with sensory and/or autonomic neuropathy but some group had atypical presentations with predominant vitreous opacities, cardiac disease (conduction disturbances and/or cardiomyopathy) or renal disease. 212 patients are receiving Tafamidis, 313 are transplanted, 91 are in an advanced stage without any disease modifying treatment and 24 patients are under early evaluation. Conclusion: In Portugal FAP TTRVal30Met is a disease with variable penetrance and variable clinical expression. The number of patients and the constant follow-up along decades is the basis for the present studies looking for factors that influence variability.

SP.4-05 - FAMILIAL AMYLOIDOTIC POLYNEUROPATHY IN JAPAN: GENOTYPES AND PHENOTYPES

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In Japan, transthyretin (TTR)-related familial amyloidotic polyneuropathy (FAP) was first reported by Araki et al 1968. Since then, we Kumamoto FAP research group have 50-years-history for clinical and basic FAP researches. Recently, in addition to big foci of FAP ATTR Val30Met in Kumamoto and Nagano districts, the third focus of the patients was identified in Ishikawa district. In addition, many sporadic FAP ATTR Val30Met patients with late onset have been reported. 2002, a nationwide survey for hereditary neuropathy was performed, and FAP was the second most disease and about 300 FAP patients were registered in Japan. The number of the patients was unexpectedly small probably because the study relied on a questionnaire. In genotypes of FAP, about 30 different points of mutation in TTR gene have been reported in Japanese patients. The numbers and genotypes of FAP are increasing year by year in Japan. From 2012, we newly established Diagnostic Unit for Amyloidosis, Department of Laboratory Medicine, Kumamoto University Hospital. In addition to conventional diagnostic methods, we newly applied laser micro-dissection and liquid chromatography/ mass spectrometry mass spectrometry (LC/MS MS) system to screen TTR related FAP and other types of amyloidosis. Using this system we could screen a mutation of TTR gene and components of TTR related amyloid fibrils in tissues. From 2012-2013, 292 cases were consulted from hospitals all over Japan, and 33 new FAP patients were confirmed. Of those, ATTR Ala45Asp, and Leu55Pro was the first Japanese cases of FAP and Thr59Arg was first case in the world.
Although ATTR is generally considered a mainly neurological disease, there is much phenotypic heterogeneity. The clinical spectrum varies widely from almost exclusive neurologic involvement within a clearly familial context to apparently sporadic cases with strictly cardiologic presentation. Cardiologists may encounter ATTR in two main clinical situations: 1) patients referred with neurological impairment or previously diagnosed “FAP”; 2) patients with cardiological problems without any apparent sign of neurological disease. If there is a strong suspicion or an existing diagnosis of “FAP”, the cardiologist’s role is to look for signs of cardiac involvement. In such situations, ECG and echocardiography generally provide all the necessary diagnostic information. Study of the longitudinal left ventricular function with tissue-Doppler echocardiography is particularly revealing to recognise very early signs of myocardial involvement. When phenotypic expression of ATTR is exclusively or predominantly cardiac, the situation is far more challenging. Such patients may present for a wide variety of reasons, including heart failure symptoms, arrhythmias, syncope, orthostatic hypotension, or ECG/echocardiographic abnormalities in the absence of symptoms. The problem of differential diagnosis with cardiomyopathies of other aetiology is rather common and a frequent pitfall is misdiagnosis of cardiac amyloidosis as sarcomeric hypertrophic cardiomyopathy. However, a number of particular echocardiographic signs should raise suspicion of amyloidotic aetiology once “hypertrophic phenotype” has been recognised. Recently we assessed the phenotypic and genotypic spectrum of ATTR in a non-endemic, Caucasian area and evaluated prevalence, genetic background and disease profile of cases with an exclusively cardiac phenotype, highlighting possible hints for the differential diagnosis with hypertrophic cardiomyopathy (HCM) and senile systemic amyloidosis (SSA). In this Italian multicenter study, 186 patients with ATTR were characterized at presentation. Thirty patients with SSA and 30 age-gender matched HCM patients were used for comparison. Phenotype was classified as: exclusively cardiac (n=31, 17%), exclusively neurologic (n= 46, 25%), mixed cardiac/neurologic (n=109, 58%). Among the 8 different mutations responsible for an exclusively cardiac phenotype, Ile68Leu was the most frequent. Five patients with an exclusively cardiac phenotype developed mild abnormalities at neurological examination but no symptoms during a 36[14−50] months follow-up. Exclusively cardiac phenotype was characterized by male gender, age > 65 years, heart failure symptoms, symmetric left ventricular (LV) “hypertrophy” and moderately depressed LV ejection fraction. This profile was similar to SSA but relatively distinct from HCM. Compared to patients with a mixed phenotype, patients with an exclusively cardiac phenotype showed a more pronounced cardiac involvement on both echocardiogram and ECG. So, a clinically relevant subset of Caucasian ATTR patients present with an exclusively cardiac phenotype, mimicking HCM or SSA. Echocardiographic and ECG findings are useful to differentiate ATTR from HCM but not from SSA. The role of liver transplantation in these patients is questionable.
Introduction: Progress in our knowledge of phenotypic presentation of TTR-FAP and the use of new diagnostic tools permit a better diagnostic approach. In this presentation we will report on our recent experience in the field.

Population and Method: We reviewed the data of symptomatic TTR-FAP patients investigated from September 2010 to September 2013 in our center. All patients underwent neurological examination, DNA testing, cardiac examination and extensive investigations including ECG, echography and cardiac MRI. Small-fibre tests included laser evoked potentials, sympathetic skin responses, cold and warm detection thresholds and heart-rate variability. Nerve endings were also studied in skin biopsies. Results: Seventy five new symptomatic FAP patients were recruited during that period of time: 25 patients (32%) originated from; 44 patients from France and 6 patients from Africa. All patients from Portugal had a family history, an age at diagnosis at 37 years on average. In patients from France, 27% had no family history; first manifestations after 50 years of age in 85% of them and diagnosis at age 61 years on average. In patients from Portugal 22/25 had the V30M, 3 the V28M mutation. In patients from France, the V30M mutation accounted for 36%, S77T for 29% and I22V for 4% of the mutations. From a neurological point of view all Portuguese patients had a length dependent small fibre polyneuropathy with autonomic manifestations. The phenotype of population from France was more heterogeneous: 70% of the patients had prominent neuropathic manifestations, 20% had isolated cardiac manifestations and 10% mixed cardiac and neuropathic manifestations. Abnormalities in small-fibre tests confirmed the onset of neuropathy in 9 of the 12 pauci-symptomatic carriers, while conventional conduction studies were still normal. Conclusion: Our findings illustrate the high frequency of isolated cardiac manifestations in late onset sporadic forms of TTR-FAP. Small fibre neurophysiological tests are helpful to confirm the onset of clinical neuropathy in follow-up of symptomatic carriers of amyloidogenic mutations before initiating treatment.

Asking the right question in medicine frequently takes us in the right direction. When one suspects TTR-FAP, however, it is not easy to predict which will be the right question to address. Owing to the rarity of TTR-FAP, it is certainly not enough to inquire about a patient’s relatives with amyloidosis. Broader investigations into a patient’s family may provide invaluable information, which can include a history of symptoms such as neuropathy or muscular weakness and wasting, chronic unrelenting diarrhea that leads to severe involuntary weight loss, or sudden cardiac death, in close or distant family members. In some cases, many unconventional causes of death in a family, or evidence of many relatives dying young, are sufficient clues to warrant suspicion. On the other hand, after one person obtains a positive result from genetic testing, other relatives can be tested. However, it is paramount to undertake genetic counseling in order to provide useful information on the benefits of testing, as many communities may continue to perceive TTR-FAP as untreatable. It is our job to reassure our patients that times have changed. There is much that we can offer: from genetic counseling, emotional support, physical therapy, cardiac pacemakers, implantable defibrillators, nutrition plans and, ultimately, pain control. There is now also the possibility of new medical therapies that may delay the progression of the disease. Liver transplantation may also be appropriate for some. When doctors achieve empathy with a patient who is diagnosed with TTR-FAP, and the patient understands the importance of making an early diagnosis, they give up their fear and resentment, and contact estranged members of the family, or may even break conventional barriers in order to discuss unrecognized offspring. Understanding the genetic links of families is difficult because human relationships are complicated. The challenge may seem enormous in large cities where the later generations of immigrants fail to recall their ancestors, and it can often be a bigger problem in small communities where social secrecy is encouraged. This situation can only be resolved with the unlimited patience, respect, understanding and discretion that we provide to our patients and their families. And of course, by asking the right question.

TTR-FAP is one of the most severe hereditary neuropathies in adults. The disease is progressive and disabling, involving both the peripheral and autonomic nervous systems and is usually associated with weight loss and cardiomyopathy. Development of the disease occurs in carriers of the relevant genetic mutation following autosomal transmission. Once developed, TTR-FAP is irreversible and life-threatening, with survival ranging from 7 to 12 years from the time of onset of initial symptoms. From the initial recognition of the disease in the early 1950s until 1990, TTR-FAP was considered to be an early-onset disease found in few endemic areas. Subsequently, through the contribution of more widespread genotyping, it is now recognized as a global disease found in many countries on all continents. Sporadic cases of ValMet30 TTR-FAP were first identified in France in the 1990s, usually presenting as an idiopathic peripheral neuropathy. Age of onset is deemed late in France (75% >50 years old), relative to that seen in the endemic region of Portugal. Cases of TTR-FAP have now been identified in most French geographic regions (79/100), comprising 37 TTR gene variants (ValMet30 TTR represents 50% of these cases) with heterogeneous clinical presentation including three new phenotypes. Structured genetic counseling (GC) of family members of people diagnosed with TTR-FAP is one major factor for achieving an earlier diagnosis in carriers of a genetic mutation at risk of developing the disease. Priority for GC are siblings, as well as children of identified carriers, as supported by work in Portugal that has shown anticipation of TTR-FAP (C Lemos et al; J Neurol Neurosurg Psychiatry In Press). It is important to educate those identified as carriers of a genetic mutation to the early symptoms of the disease. Physicians should perform an initial screening, including clinical tests and questionnaires, neurological examination, nerve conduction studies and cardiac investigations (minimum ECG, ECHO) and then propose a personalized program of periodic visits and check-ups. The earliest neurological signs of active disease are distal sensory loss for thermal modalities. Other initial symptoms are often those of autonomic dysfunction (digestive; sexual impotence), neurogenic pain or distal sensory loss or walking difficulties, and unintended weight loss. In France, a structured approach to rare diseases such as TTR-FAP has been significantly improved through the establishment of a National Reference Center, which now plays a key role in coordinating actions at a national level, as well as through regional satellite centers (Expert Network). This network develops programs for training specialists, offers therapeutic education for patients, and organizes the periodic follow-up, with adequate diagnostic tests, of those individuals identified with a genetic mutation. References: Adams D, et al. Amyloid neuropathies. Curr Opin Neurol 2012;25:564–572. Adams D, et al. Regional difference and similarity of familial amyloidosis with polyneuropathy in France. Amyloid.2012;19(Suppl 1):61–64. Adams D. Recent advances in the treatment of familial amyloid polyneuropathy. Ther Adv Neurol Disord. 2013;6(2):129–139. Hellman U, et al. Heterogeneity of penetrance in familial amyloid polyneuropathy, ATTR Val30Met, in the Swedish population. Amyloid 2008;15:181–186. Lemos C, et al. Overcoming artefact: anticipation in 284 Portuguese kindreds with familial amyloid polyneuropathy (FAP) ATTRV30M. J Neurol Neurosurg Psychiatry. 2013;doi:10.1136/jnnp-2013-305383 [Epub ahead of print].
SP.6-03 - Identifying the early symptoms of TTR-FAP

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SP.8-01 - SUMMARY DATA FROM TAFAMIDIS TREATMENT

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Introduction and Objectives: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a progressive, fatal neurodegenerative disorder. Tafamidis is a small molecule that prevents TTR amyloid formation and has been shown to delay neurologic impairment in a pivotal clinical trial. This presentation describes our experience at Hospital de Santo António in Porto, Portugal, in patients with TTR-FAP who have been treated with commercial tafamidis for ≥1 year, as well as an update of results from an ongoing, open-label trial (Study Fx1A-303) to assess the long-term effects of tafamidis treatment in patients who had completed either treatment arm in an 18-month, double-blind, randomized, placebo-controlled trial (Study Fx-005) and then received tafamidis in a 12-month, open-label extension of the trial (Study Fx-006), with up to 5.5 years of tafamidis exposure. Results: Real-life experience includes NIS-LL and other outcome measures in 78 patients in Porto, Portugal. In Study Fx1A-303, those in the tafamidis-tafamidis arm had numerically smaller increases in NIS-LL scores at each time point. Furthermore, once patients in the placebo- tafamidis arm began treatment with tafamidis, their apparent rate of increase in NIS-LL scores was similar to those in the tafamidis-tafamidis arm. At 54 months, patients in the tafamidis-tafamidis arm had numerically smaller increases in NIS-LL, NIS-LL Muscle Weakness, and NIS-LL Distal–Toe scores than patients in the placebo-tafamidis arm. Conclusions: Longer-term use of tafamidis for the treatment of TTR-FAP appears to be associated with reduced disease progression and less advancement in polyneuropathy impairment. The advantage of the earlier initiation of treatment appears to persist with continued long-term follow-up. Disclosure: These studies were sponsored by FoldRx Pharmaceuticals, which was acquired by Pfizer Inc in October 2010.
SP.8-04 - SUMMARY DATA FROM DIFLUSINAL PHASE III TRIAL

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Introduction: Familial amyloid polyneuropathy (ATTR-FAP) produces progressive peripheral nerve deficits, sensorimotor impairment, and functional disability. Diflunisal, a non-steroidal anti-inflammatory agent, stabilizes transthyretin tetramers and inhibits amyloid fibril formation in vitro. Objective: To determine the effect of diflunisal on polyneuropathy progression in patients with ATTR-FAP. Methods: We conducted an investigator-initiated international, randomized, double-blind, placebo-controlled study at amyloid centers in Umea, Pavia, Matsumoto and Kumamoto, London, Boston, New York, and Rochester, MN from 2006 through 2012. We enrolled 130 ATTR-FAP patients with clinically detectable peripheral or autonomic neuropathy and randomly assigned them in 1:1 fashion to diflunisal 250 mg or placebo twice daily for 2 years. Outcome Measures: Our primary endpoint measured the difference in polyneuropathy progression between study arms using the Neuropathy Impairment Score plus 7 nerve tests (NIS+7). Secondary outcomes included a quality of life questionnaire (Short Form-36 (SF-36)) and modified body mass index (mBMI). Results and Conclusions: We will present our primary and secondary outcomes for subjects completing 1 and 2 years treatment using appropriate statistical methods.
Oral Presentation
OP.1-01 - GENE EXPRESSION PROFILE IN HEREDITARY TRANSTHYRETIN AMYLOIDOSIS: DIFFERENCES IN TARGETED AND SOURCE ORGANS

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Introduction: Hereditary transthyretin amyloidosis (ATTR) is a genetic disease caused by a point mutation in the TTR gene, causing the liver to produce an unstable TTR protein. The most effective treatment has been liver transplantation in order to replace the variant TTR producing liver with one producing only wild-type TTR. ATTR amyloidosis patients’ livers are reused for liver sick patients, i.e., the Domino procedure. However, recent findings have demonstrated that ATTR amyloidosis can develop in the recipients within 7-8 years. The aim of this study was to characterize the genetic profiles of the target- and source organs of ATTR amyloid patients and compare the outcome with those of controls. Methods: Gene expression analysis was used to unravel the genetic profiles of Swedish ATTR V30M patients and controls. Biopsies from adipose tissue, heart and liver were examined. Results and Conclusions: ATTR amyloid patients’ gene expression profile of the main source organ, the liver, differed markedly from that of the controls, whereas the target organs’ gene expression profiles were not markedly altered in the ATTR amyloid patients compared to those of the controls. An impaired ER/protein folding pathway might suggest ER overload due to mutated TTR protein.

OP.1-02 - INVOLVEMENT OF M2 MACROPHAGES IN THE PATHOGENESIS OF FAP AND EFFICACY OF HUMAN IPS CELL-DERIVED MACROPHAGES IN THE TREATMENT

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Introduction: Macrophages are classified into two groups: pro-inflammatory M1 and anti-inflammatory M2 macrophages. In the present study, we hypothesized that tissue-resident macrophages in familial amyloid polyneuropathy (FAP) patients had the qualitative or quantitative abnormalities, and this phenomena accelerated transthyretin (TTR)-derived amyloid deposits. To evaluate this hypothesis, we examined the number and subset of tissue-resident macrophages in amyloid-deposited FAP patients. Furthermore, we investigated the phagocytic function of human iPS cell-derived macrophages (iPS-MPs) against TTR in vitro. Materials and Methods: FAP (n = 15) and control (n = 11) patients were analyzed the number and subset of resident macrophages in the heart tissue by immunohistochemistry. Next, iPS-MPs were cultured in the presence of aggregated wild or mutated TTR in vitro. After 3 days, we examined the phagocytic capacity of iPS-MPs against TTR. Results: Almost of heart tissue-resident macrophages were Iba+ CD206+ M2 macrophages in the both control and FAP patients. However, the number of M2 macrophages in FAP patients was significantly decreased in comparison to control. In vitro, iPS-MPs performed the phagocytic function against the aggregated TTR in a cell-dependent manner. In addition, iPS-MPs were CD163+ CD206+ M2 macrophages. Discussion and conclusion: These results suggest that the decrease of tissue-localized M2 macrophages exacerbate the clearance of TTR-derived amyloid deposits in the involved organs of FAP, leading to the progression of pathological condition in FAP patients. In this regard, the therapy using iPS-MPs may be a promising means for the treatment of FAP.
OP.1-03 - NERVE INJURY IN A MOUSE MODEL OF FAP LEADS TO INCREASED LOCAL EXPRESSION OF TTR AND DECREASED REGENERATION

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Inflammation is a hallmark of several neurodegenerative disorders including Familial Amyloidotic Polyneuropathy (FAP), a hereditary disease associated with extracellular deposition of mutant transthyretin (TTR) ultimately leading to degeneration of cells and tissues. The most common mutation is the V30M and a mouse model has been created carrying this TTR mutation. In this model, nonfibrillar deposition begins at three months-old affecting only the gastrointestinal tract and skin. With this work, our goal was to characterize TTR deposition/expression and the associated inflammatory immune response in V30M model as compared to wild-type (WT) mice, upon nerve injury. Seven days post injury V30M TTR deposits were detected in the sciatic nerve and also in the ipsilateral and contralateral L4, L5 and L6 dorsal root ganglia. Furthermore, we found that in both V30M and WT mice the majority of TTR protein has liver origin, reaching the nerve via the blood stream. Additionally, nerve injury led to TTR expression also by V30M-Schwann cells. In response to nerve injury V30M mice develop a downregulated innate immune response when compared to WT mice. More specifically, we saw decreased expression of chemokines important for the recruitment of immune cells like macrophages and neutrophils, which might contribute for the tissue degenerative process. However, when we compared injured with uninjured nerves within the V30M mice group we saw that injury results in increased TTR expression and upregulation of the inflammatory response. In conclusion, with this work we were able to characterize the expression of TTR both in WT and V30M animals, upon nerve injury, and found that the level of the inflammatory response is associated with the type of TTR being expressed. Moreover, mechanisms behind the observed immune response upon injury should be next investigated to bring to light the observed lack of cellular infiltrate found in nerves of FAP patients.

OP.2-01 - TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY IN BULGARIA

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Transthyretin (TTR) familial amyloid polyneuropathy (FAP) is a rare condition identified worldwide. More then 100 mutations have been reported to date. Genetic diagnosis has been available in Bulgaria since 2010. We have verified 39 families so far. The most common mutation is Glu89Gln, found in 31 unrelated families by family history. Most of them reside in the western part of Bulgaria which appears endemic for this mutation. There are additional three unrelated families with Ser77Phe mutation, coming from one and the same village and another four with Val30Met mutation. Recently another mutation was identified in a single patient – Ser52Pro. The clinical picture is of a typical constellation of progressive sensory and motor polyneuropathy, cardiomyopathy, autonomic and gastrointestinal involvement. The age at onset is relatively late with an average of 52 years for the Glu89Gln mutation, 55,6 for the Ser77Phe mutation, 61,5 for the Val30Met mutation and 44,2 years for the Ser52Pro mutation. The average life expectancy from disease onset is 6 years with a range of 3 to 18 years. We have also genetically identified 23 asymptomatic carriers, most of the under the age of 45. TTR FAP in Bulgaria is a genetically heterogeneous disorder, though with a common mutation identified most probably due to a founder effect.
OP.2-02 - QUANTITATION OF SUDOMOTOR INNERVATION IN SKIN BIOPSIES OF FAMILIAL AMYLOID POLYNEUROPATHY WITH TRANSTHYRETIN ALA97SER MUTATION IN TAIWAN

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Familial amyloid polyneuropathy (FAP) is an autosomal dominant neurodegenerative disease affecting the motor, sensory, and autonomic components of the peripheral nervous system. It is a form of amyloidosis and was first identified in Portugal. In contrast to the most common V30M mutation in the western countries and Japan, we have previously identified TTR A97S as the most important hot spot mutation of FAP in Taiwan (Neurology 2010; 75: 532-538). Autonomic neuropathy is one of the major manifestations of FAP. The symptoms of autonomic neuropathy are diverse with involvement ranging from the cardiovascular system to the sudomotor system. Both preganglionic lesions in central nervous system and postganglionic peripheral neuropathies can cause autonomic symptoms. Most tests for autonomic neuropathy depend on functional assessments. Only limited studies have demonstrated evidence of nerve degeneration due to postganglionic nerve abnormalities. To evaluate postganglionic autonomic nerve pathologically and quantitatively, we have developed a new morphometry-based method to quantify the pathology of sudomotor innervations (sweat gland innervation index, SGII) on skin biopsies. (J Neuropathol Exp Neurol 2011;70:930-8). This tool provides an opportunity to investigate the clinical significance of sweat gland innervations in neuropathy with autonomic dysfunction, i.e. diabetic neuropathy (Diabetes Care 2012;35:612-6). Autonomic dysfunction in FAP is diverse. Sudomotor symptoms are a common component, but the pathology of sudomotor innervation and its clinical significance remained obscured. To investigate the pathology of postganglionic sudomotor nerves, we performed skin biopsy to evaluate SGII in 18 patients (15 males and 3 females aged 61.7 ± 5.8 years) with A97S FAP and autonomic dysfunction. In comparison with age- and gender-matched control subjects, FAP patients had lower SGII in the leg skin (1.89 ± 0.92 vs. 4.79 ± 1.52%, p < 0.001). In FAP patients, the SGII was higher in those with positive sympathetic skin response than those with absent sympathetic skin response at palm (2.41 ± 0.55 vs. 1.25 ± 1.08%, p < 0.020). In addition the SGII was demonstrated evidence of nerve degeneration due to postganglionic nerve abnormalities. To evaluate postganglionic autonomic nerve pathologically and quantitatively, we have developed a new morphometry-based method to quantify the pathology of sudomotor innervations (sweat gland innervation index, SGII) on skin biopsies. (J Neuropathol Exp Neurol 2011;70:930-8). This tool provides an opportunity to investigate the clinical significance of sweat gland innervations in neuropathy with autonomic dysfunction, i.e. diabetic neuropathy (Diabetes Care 2012;35:612-6). Autonomic dysfunction in FAP is diverse. Sudomotor symptoms are a common component, but the pathology of sudomotor innervation and its clinical significance remained obscured. To investigate the pathology of postganglionic sudomotor nerves, we performed skin biopsy to evaluate SGII in 18 patients (15 males and 3 females aged 61.7 ± 5.8 years) with A97S FAP and autonomic dysfunction. In comparison with age- and gender-matched control subjects, FAP patients had lower SGII in the leg skin (1.89 ± 0.92 vs. 4.79 ± 1.52%, p < 0.001). In FAP patients, the SGII was higher in those with positive sympathetic skin response than those with absent sympathetic skin response at palm (2.41 ± 0.55 vs. 1.25 ± 1.08%, p < 0.020). In addition the SGII was also higher in those without orthostatic hypotension than those with the symptoms (2.29 ± 0.60 vs. 1.38 ± 1.04%, p < 0.033). These observations suggested sudomotor denervation is a significant presentation of A97S FAP, and the SGII was associated with certain autonomic symptom and autonomic functional test.

OP.2-03 - INCREASED CIRCULATING PLASMA GHRELIN CONCENTRATIONS IN ATTRV30M AMYLOIDOSIS PATIENTS.

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Background Gastrointestinal (GI) complications are commonly encountered in patients with hereditary transthyretin amyloidosis and unintentional weight loss has been reported in patients before development of symptoms of neuropathy. The pathogenesis of weight loss and GI complications is poorly understood, though disturbances of the enteric nervous system (ENS), including interstitial cells of Cajal and the neuroendocrine cells are generally assumed to play an important role. Ghrelin is a peptide hormone that is mostly released from cells of oxyntic mucosa of the stomach and has been shown to stimulate appetite and gastric emptying. The aim of the present study was to establish a possible role of ghrelin in the pathophysiology of GI-complications in patients with ATTRV30M amyloidosis. Material and Methods Blood samples from twenty patients with ATTRV30M amyloidosis and 18 healthy controls and biopsies from 12 ATTRV30M patients and 12 controls were collected for the study. After an overnight fast, blood samples were drawn from patients and controls, and a gastroduodenal endoscopy was performed. Biopsies were taken from the oxyntic mucosa of the stomach. Ghrelin cell density was determined by computer image analysis after immunohistochemical staining of the tissues. Total ghrelin was detected in plasma by commercially available ELISA Kit. Results The concentration of total ghrelin was significantly higher in ATTRV30M amyloidosis patients compared with that of controls (P = 0.001). There was no statistical difference in the density of ghrelin immunoreactive cells between patients and controls, though the patients tended to have higher density of ghrelin immunoreactive cells. Conclusion The increased plasma concentration of ghrelin in ATTRV30M amyloidosis patients may be related to the loss of appetite and weight often encountered in ATTRV30M amyloid patients, even before gastrointestinal symptoms have emerged.
OP.2.04 - PRE-CLINICAL IDENTIFICATION OF TTR-RELATED AMYLOIDOSIS (SENILE SYSTEMIC AMYLOIDOSIS) BY 99mTc-DPD SCINTIGRAPHY: A COHORT STUDY OF 12,400 SUBJECTS

Simone Longhi 1, Pier Luigi Guidalotti 3, Christian Gagliardi 1, Candida Cristina Quarta 1, Massimiliano Lorenzini 1, Nelson Gentile 3, Ilaria Bartolomei 2, Fabrizio Salvi 2, Claudio Rapezzi 1

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Background: We have previously shown that 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) scintigraphy has a high affinity for TTR-infiltrated myocardium, allowing an accurate diagnosis of both mutant and wild-type cardiac amyloidosis. The potential role of this method as preclinical screening tool has not yet been evaluated. This study aimed to evaluate prevalence and diagnostic implications of incidental myocardial uptake among patients who underwent scintigraphy for oncologic or rheumatologic reasons.

Methods: We retrospectively analysed all DPD scintigraphies performed between 2008 and May 2013 in outpatients referred to our Nuclear Medicine Unit for oncologic or rheumatologic reasons and assessed clinical and instrumental details of patients with incidental myocardial tracer uptake.

Results: Incidental myocardial uptake was detected in 45 subjects (0.36%): 28 males (62%), median age 81 [IQR 77-84]. Prevalence was higher among men and increased progressively with age: age ≤ 60, 0 patients; age 61-70, men 4% and women 2%; age 71-80, men 22% and women 20%; age ≥ 81, men 36% and women 16%. Fourteen of the 45 patients agreed to undergo a cardiological evaluation; 5 also underwent endomyocardial biopsy and genetic analysis was carried out in 7 cases. Abnormal ECG and increased left ventricular wall thickness (LVWT) were detected in all 14 patients (median LVWT 14 [13-15] mm). LV “hypertrophy” was completely unexplained by hypertension or valvular heart disease in 10 cases and was out of proportion in the remaining 4. TTR related myocardial amyloid infiltration was detected in all the 5 biopsied cases. TTR gene analysis was negative in all cases.

Conclusions: DPD scintigraphy appears to be specific for the preclinical diagnosis of senile systemic amyloidosis and can be useful for non-invasive screening of subjects at risk for this disease. The prevalence of positive DPD scintigraphy in our large cohort is in line with the current knowledge on epidemiology of senile systemic amyloidosis.
OP.2-05 - A TEST TO DIAGNOSE AND MEASURE CARDIAC AMYLOID IN AL AND ATTR AMYLOIDOSIS USING NON-CONTRAST CARDIAC MRI.

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Background: Cardiac involvement drives outcome and guides therapy in amyloidosis but, to date, there is no truly quantitative test for cardiac amyloid. This impedes both drug development and optimal clinical management. We have developed a quantitative cardiac MRI test for amyloid, measuring the native myocardial T1 - an intrinsic property of the myocardium (no contrast required). We have developed it in AL amyloid. Here we assess the test in transthyretin amyloidosis (ATTR amyloidosis).

Objectives: We hypothesized that the native myocardial T1 would be elevated in ATTR amyloid; that T1 elevation would track the cardiac amyloid burden as measured by DPD grading; and that T1 elevation would be an early disease marker.

Methods: 3 groups were studied: ATTR amyloid patients (n=85; 70 male; age 73±10); healthy mutations carriers (n=8; 3 male; age 47±6); and AL amyloid patients (n=79; 55 male; age 62±10). These were compared with 52 healthy volunteers and 46 patients with hypertrophic cardiomyopathy (HCM). All underwent T1 mapping. ATTR patients and mutation carriers also underwent cardiac DPD scintigraphy.

Results: T1 was elevated in ATTR patients compared to HCM and normal subjects (1097±43ms vs 1026±64ms vs 967±34ms, both p<0.0001). In established cardiac ATTR amyloidosis, T1 elevation was not as high as in AL amyloidosis (AL 1130±68ms, p=0.01) (Figure 1 and 2). Diagnostic performance was similar for AL and ATTR amyloid (vs HCM: AL AUC 0.84 (95%CI 0.76-0.92); ATTR 0.85 (0.77-0.92) P<0.0001) (Figure 2). T1 correlated with cardiac amyloid burden as determined semi-quantitatively by DPD scintigraphy (p<0.0001). T1 was not elevated in mutation carriers (952±35ms) but was in isolated DPD grade 1 (n=9, 1037±60ms, p=0.001).

Conclusion: Native myocardial T1 mapping detects cardiac ATTR amyloid with similar diagnostic performance and potential for quantitation as AL amyloid, but with lower maximal T1 elevation and appears to be an early marker of disease.

Figure 1: Characteristic examples from CMR scans - CMR end-diastolic cine still (upper panel); ShMOLLI native T1 map (middle) and late gadolinium enhancement (LGE) images (lower) in (left to right) healthy volunteer, HCM, definite AL amyloid and definite ATTR amyloid. Note the markedly elevated myocardial T1 time in the AL cardiac amyloid patient and ATTR patient into the red range of the colour scale (the elevation is higher in AL, i.e. more red) compared to the normal control (green) and the patient with hypertrophic cardiomyopathy.

Figure 2: ROC curve for native T1 – Left pane: Native T1 in healthy volunteers, mutation carriers, HCM, definite AL and definite ATTR. Right panel: Receiver-operating characteristic (ROC) curve for the discrimination of possible or definite cardiac amyloid by native myocardial T1 from HCM.
OP.2-06 - EX VIVO CHARACTERIZATION OF TRANSTHYRETIN CARDIAC AMYLOIDOSIS (TTR-CA) USING AN AMYLOID B SPECIFIC PET IMAGING AGENT. PRELIMINARY DATA

Michel S Slama1,3, N Arlicot1, Rana Ben-Azzouna1, J Vercouillie1, Vincent Algalarrondo1,3, F Capron1, D Guilloteau1, David Adams2, D Le Guludec1,3

Objectives: Familial amyloid polyneuropathy is a hereditary form of amyloidosis due to a mutated variant of transthyretin (TTR) produced by the liver. Cardiac involvement is characterized by extracellular depositions of amyloid β (Aβ) which can lead to heart failure, conduction disorders, and arrhythmias. While very early diagnosis of Transthyretin cardiac amyloidosis (TTR-CA) would have important therapeutic and prognostic impact, there is no specific, sensitive and quantitative tool to document the location and extent of cardiac Aβ in these patients. So we aimed to test ex vivo the usefulness of 18F-AV45, a high specific Aβ PET tracer recently FDA approved in Alzheimer’s disease. Methods: Binding of 18F-AV45 to Aβ was evaluated by autoradiography in myocardial tissue obtained from patients who underwent cardiac transplantation either for TTR-CA, or ischemic heart failure (controls). Frozen heart sections (20μm-thickness, n=2 for each data point) were incubated with 18F-AV45 at concentrations of 1 or 3nM. Nonspecific binding was assessed by incubation of adjacent sections in the presence of an excess (300μM) of cold AV45. After the film was developed, images were scanned and analysed by grey-level semi-quantification process using βvision+ software. Results: 18F-AV45 uptake was significantly higher in TTR-CA myocardial sections when compared to controls (+70%±10%). The intensity of 18F-AV45 binding markedly decreased in sections incubated with unlabeled AV45 (-83%±5%), strongly suggesting Aβ specificity. Conclusions: These preliminary data demonstrate 18F-AV45 capability to bind specifically Aβ in heart tissue, and provide the basis for a clinical trial designed to determine its diagnostic potential in TTR-CA patients.

OP.3-01 - STEADY AMYLOID TURN-OVER AFTER LIVER TRANSPLANTATION IN FAP PATIENTS

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Backgrounds: Previously we reported paradoxical deterioration of cardiac amyloidosis due to wild-type transthyretin (TTR)-derived amyloid deposition and disappearance of abdominal fat-pad amyloid deposition in liver-transplanted FAP patients, suggesting that both wild-type TTR deposition and clearance of deposited amyloid fibrils (amyloid turn-over) may occur in some organs of FAP patients. The aim of this study is to elucidate that this phenomenon can be regularly observed in other organs of liver-transplanted FAP patients. Methods: We histologically and biochemically examined gastric mucosal amyloid before and after liver transplantation (LT) in 6 FAP patients (ATTR V30M). In one FAP patient with V30M who died 15 years after LT, we investigated amyloid fibril protein of myocardium and sciatic nerve as well as stomach obtained at autopsy. The biochemical study was performed by determination of the composition ratio (wild-type vs. variant TTR ratio) of extracted amyloid fibril protein with tandem mass spectrometry. Results: The amount of deposited amyloid was not markedly different before and after liver-transplant. However, the composition ratios of wild-type TTR in gastric mucosal amyloid significantly increased from 20.0% ± 11.4% (before LT) to 43.2 % ± 13.8% (after LT). In the autopsied patient, the composition ratios of wild-type TTR in deposited amyloid were 88% (stomach), 85% (myocardium), and 70% (sciatic nerve), showing high contribution of wild-type TTR to amyloid formation in posttransplantation stage. Conclusion: Our results showed that a significant portion of preexisting amyloid seemed to be reconstructed by wild-type TTR-derived amyloid deposition and clearance of deposited amyloid (steady turn-over) after liver transplantation and this phenomenon can occur in every organ of transplanted FAP patients. It was also confirmed that wild-type TTR plays an important role in the pathogenesis of postoperative amyloid deposition in transplanted FAP patients.
OUTCOME OF LIVER TRANSPLANTATION FOR NON-ATTR V30M: REPORT FROM THE FAP WORLD TRANSPLANT REGISTRY (FAPWTR)

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Background. Liver transplantation (LTx) has been performed for hereditary ATTR amyloidosis since 1990 (1). The outcome of relative large series of transplanted ATTR Val30Met patients have been reported from different centres (2); however, for non-ATTR Val30Met, only a few reports with small number of patients have emerged. The aim of the present study is to report the survival of LTx non-ATTR Val30Met amyloid patients as reported to the FAP world transplant registry (FAPWTR). Methods. Survival data were extracted for all non-ATTR Val30Met patients. Kaplan Mayer plots were constructed for the most prevalent mutations. Results. The total number of patients with a non-ATTR Val30Met mutation in the registry was 249. The largest group consisted of the Ser77Tyr mutation with 38 patients, of which 6 had received both heart and liver grafts. The 10 years survival was 41 and 44% respectively. The transplantations (liver or heart/liver) and survival of Especially the Leu111Met, Val71Ala and Leu58His was encouraging. Conclusions. Large differences for survival were observed between different non ATTR Val30Met mutations, where a good survival was noted for mutations such as Leu111Met, Val71Ala and Leu58His. Presence of a non-ATTR Val30Met mutation is not a contraindication for LTx.

<table>
<thead>
<tr>
<th>TTR mutation</th>
<th>n</th>
<th>Liver (n) / Liver &amp; Heart (n)</th>
<th>Liver / Liver &amp; Heart 10 yr survival (%)</th>
<th>Sex M (%)</th>
<th>Age at tx (yrs)</th>
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<tbody>
<tr>
<td>Ser77Tyr</td>
<td>38</td>
<td>32 / 6</td>
<td>41 / 44</td>
<td>76 %</td>
<td>57.3±5.7</td>
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<tr>
<td>Thr60Ala</td>
<td>23</td>
<td>14 / 9</td>
<td>36 / 55</td>
<td>96 %</td>
<td>59.5±4.9</td>
</tr>
<tr>
<td>Tyr114Cys</td>
<td>15</td>
<td>15 / 0</td>
<td>53 / -</td>
<td>47 %</td>
<td>48.6±6.7</td>
</tr>
<tr>
<td>Leu111Met</td>
<td>12</td>
<td>5 / 7</td>
<td>100 / 71</td>
<td>58 %</td>
<td>48.0±4.7</td>
</tr>
<tr>
<td>Ser50Arg</td>
<td>12</td>
<td>11 / 1</td>
<td>28 / 0</td>
<td>50 %</td>
<td>41.1±6.6</td>
</tr>
<tr>
<td>Val71Ala</td>
<td>11</td>
<td>11 / 0</td>
<td>82 / -</td>
<td>55 %</td>
<td>37.5±10.6</td>
</tr>
<tr>
<td>Leu58His</td>
<td>11</td>
<td>11 / 0</td>
<td>76 / -</td>
<td>73 %</td>
<td>60.3±3.6</td>
</tr>
<tr>
<td>Val30Met</td>
<td>1628</td>
<td>1624* / 4</td>
<td>73 / 100</td>
<td>55 %</td>
<td>59.0±10.2</td>
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Lever transplantation (LT) has been proposed 20 years ago as a treatment of TTR-FAP by suppressing the main source of mutant TTR. The question is still opened concerning the long term (>10 years) ability of LT to stop the progression of the disease. To described the profile of patients with a long term stable polyneuropathy, among the 200 patients who underwent LT (138 Met30 (69%)). Follow-up included walking disability PND score, NIS weakness score, modified Norris Score (MNS), Compound Autonomic Dysfunction Test (CADT), Neurophysiological score, survival. 24/41 patients followed more than 10 y after LT (95% Met30), were considered stable (PND score). The mean follow-up was 12.4 y (10-20). All patients had Met30 TTR mutations, 22/24 (92%) had an early onset (<50 y.o.). Mean duration of the disease at LT was 3 y (1-7). At LT, the mean MNS was 70 (51-75). The mean NIS weakness was 13 (2-46), the mean CADT was 12.9/16, 5/24 had postural hypotension. 92% had stage 1 FAP. Weight loss at LT was <20% in 92%. MNS was stable in 19/24 (85%). Motor NIS was stable in 19/24 but decreased by a mean of 1/year in 5. EMG sensory score were stable or improved in 18 (75%), CADT worsened in 5 pts. Six patients (25%) died from stroke (3), suicide (2), end stage cardiac insufficiency (1). Long term stability of TTR-FAP after LT is frequent in long survivers (59%), especially stage 1 and early onset Val30Met TTR FAP. These patients may die from stroke or suicide.

OP.4-01 - MOLECULAR TWEEZERS TARGETING TRANSTHYRETIN AGGREGATION: THERAPEUTIC APPLICATIONS ON FAMILIAL AMYLOIDOTIC POLYNEUROPATHY

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Familial Amyloidotic Polyneuropathy (FAP) is a fatal neurodegenerative disorder characterised by the extracellular deposition of aggregates and fibrils of mutant forms of Transthyretin (TRT), particularly in the nerves and ganglia of the peripheral nervous system. Though most of the compounds proposed for FAP therapy aim TTR stabilization other approaches target different steps of the process of aggregation or deposition are possible. Recently, a “molecular tweezer” [1], CLR01, has been proposed as a general inhibitor of aggregation and toxicity of several amyloidogenic proteins in vitro [2] and in vivo in mice model of Alzheimer’s disease [3]. CLR01 inhibits amyloidogenic pathway in a process-specific manner by interfering with hydrophobic and electrostatic interactions known to play an important role in the aggregation process. CLR01 has also been shown to inhibit TTR aggregation in vitro. In the present study, we assessed the ability of CLR01 to modulate TTR aggregation and toxicity in cell culture and in an animal model. In cell culture assays, we found that CLR01 inhibited TTR oligomerization in the conditioned medium and alleviated TTR induced neurotoxicity by redirecting TTR aggregation into formation of innocuous assemblies. To determine whether CLR01 was effective in vivo, we tested the compound in mice expressing TTR V30M, a model of familial amyloidotic polyneuropathy (FAP), which recapitulates the main pathological features of the human disease. Immunohistochemical and Western blot analyses showed a significant decrease in TTR burden in the gastrointestinal tract and peripheral nervous system in mice treated with CLR01 with a concomitant reduction in aggregate-induced endoplasmic reticulum (ER) stress response, protein oxidation, and apoptosis. Taken together, our pre-clinical data suggest that CLR01 is a promising lead compound for development of innovative, disease modifying therapy for FAP. References [1] Talbiersky P, Bastkowski F, Klärner FG, Schrader T. J Am Chem Soc. 2008; 130: 9824-8. [2] Sinha S, Lopes DH, Du Z, Pang ES, Shanmugam A, Lomakin V, Tennstaedt A, McDaniel K, Bakshi R, Kuo PY, Ehrmann M, Benedek GB, Loo JA, Klärner FG, Schrader T, Wang C, Bitan G. J Am Chem Soc. 2011; 133:16958-69. [3] Attar A, Ippoliti C, Riccardi E, Maiti P, Li Puma DD, Liu T, Hayes J, Jones MR, Lichti-Kaiser K, Yang F, Gale LD, Tseng CH, Tan M, Xie CW, Straudinger JL, Klärner FG, Schrader T, FrAUTschy SA, Grassi C, Bitan G. Brain. 2012; 135:3735-48.
Familial amyloidotic polyneuropathy is a neurodegenerative disorder characterized by the extracellular deposition of amyloid fibrils composed by mutated transthyretin (TTR) mainly in the peripheral nervous system (PNS). At present, liver transplantation is the only available treatment to halt the progression of clinical symptoms in FAP. Here we propose to establish a new gene therapy approach using AAV vectors to efficiently deliver the trans-suppressor TTR T119M variant to the liver of transgenic V30M mice. Analysis of the GI tract of injected animals revealed a significant reduction in the deposition of TTR non-fibrillar aggregates in as much as 34% in stomach and 30% in colon, as well as to decreased levels of biomarkers associated with TTR deposition, namely the ER stress marker BiP and the ECM protein MMP-9. However, these results were only observed in animals injected at younger ages, with no therapeutic effect observed when TTR deposition is already installed. Altogether our data suggest the possibility to use this gene therapy approach in a prophylactic manner to prevent FAP pathology.

In FAP transgenic mice treatment with doxycycline and tauroursodeoxycholic acid (TUDCA) showed a synergistic effect on removal of TTR deposits and normalization of tissue disease biomarkers. The long-term safety profile of these drugs is well established and favorable. The possible impact of this treatment on transthyretin amyloidosis (ATTR) warrants evaluation in a clinical trial. Methods We designed a phase II, open-label study to evaluate the efficacy, tolerability, safety and pharmacokinetics of orally doxycycline (100 mg BID) and TUDCA (250 mg three times/day) administered continuously for 12 months. Primary endpoint is response rate defined as: < 2 point increase in NIS-LL in patients with neuropathy and less than 30% or < 300 pg/mL increase in serum NT-proBNP concentration in subjects with isolated cardiomyopathy. Additional evaluations include change in quality of life (SF-36), Kumamoto score, nerve conduction studies, echocardiographic parameters and PK analysis. Entry criteria include hereditary or senile ATTR confirmed by histological and genetic evaluation. Patients with progressive neuropathy and/or cardiomyopathy 1 year after liver transplantation are also eligible. Efficacy evaluations are scheduled at entry, 6 and 12 months. Results We enrolled 37 subjects (28 males, median age 68 years, range 43-82). 24 patients have hereditary ATTR (Val30Met in 7 patients). 11 patients have senile systemic amyloidosis and 2 were domino-transplanted. Of the 24 patients with hereditary TTR amyloidosis 4 underwent previous liver or liver/heart transplantation. 10 patients discontinued the treatment before M12 visit (4 due to doxycycline-related GI events, 1 for a maculopapular rash at limbs, 1 for diagnosis of lymphoma, 1 for disease progression and 3 were lost at follow-up). Treatment was well tolerated in all the other patients, except for a mild and reversible skin redness at hands and face. 20 patients completed the 12-month treatment period according to protocol. 11 were evaluable for neurological disease. A stable neuropathy (NIS-LL < 2) was observed in 6/11 patients. PND remained unchanged in all patients. 19 patients were evaluable for heart disease. A stable disease according to NT-proBNP changes was observed in 13/19 patients. BNP was stable in 16/18 patients (not available in one case). Quality of life and nutritional status were maintained. Conclusions Preliminary data indicate that doxycycline and TUDCA have an acceptable safety profile in patients with TTR amyloidosis with a trend for clinical benefit in cardiomyopathy.
Poster Presentation
A - Liver Transplantation

A-01 - DOMINO LIVER TRANSPLANTATION (DLT) AND DE NOVO FAMILIAL AMYLOID POLYNEUROPATHY (FAP) AT HOSPITAL SANTO ANTÓNIO/CENTRO HOSPITALAR DO PORTO (CHP).

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Hospital Santo António, Centro Hospitalar Porto, Portugal

Background. Liver transplant (LT) to treat FAP started in 1990. Five years later DLT using grafts from FAP patients follows to increase the number of grafts available. Assuming the natural course of FAP, early estimation of disease transfer risk was expected to have a minimum delay of 20 years. After 2005 several papers describe cases of de novo FAP, starting 5 to 9 years after DLT. Pathologic and neurophysiologic signs of disease in asymptomatic patients were also reported. Since Portugal has the largest DLT population in the world, we present our results from patients transplanted between 1999 and 2010 and observed between 09/2009-09/2013. Material and methods. One hundred and eighty (180) DLTs were performed between 1999 and 2010. 78 patients have died before we started regular evaluations. We present 77 patients (18 females and 59 males), who completed clinical, electrophysiologic and pathologic [salivary gland biopsy (SGB)] evaluation. Twenty-two (22) patients are scheduled for evaluation and 3 are lost to follow-up. Results. Forty-eight (48) patients were asymptomatic, 29 were symptomatic, albeit only 23 meet criteria for de novo FAP. The 23 FAP symptomatic patients started with sensory and/or autonomic complaints, 5 to 12 years after DLT. Their SGB showed amyloid deposition and neurophysiologic evaluation was abnormal for 21 patients. Out of the 48 asymptomatic patients, 39 SGB showed amyloid deposition and 27 had abnormal neurophysiologic evaluation. Four (4) patients were retransplanted to halt FAP progression, another 4 are now waiting for retransplant and 4 have died along the way. Conclusion. Our results, as described by other centres, confirm that de novo amyloidosis in Portuguese domino recipients manifests earlier than expected when DLT was started and this imposes that more strict selection criteria are implemented.

A-02 - WORSENING OF FAMILIAL AMYLOID POLYNEUROPATHY AFTER LIVER TRANSPLANTATION IN NATIONAL REFERENCE CENTER (NNERF) IN FRANCE : PROFILE OF PATIENTS.

David Adams 1, Zoia Mincheva 1, Teresa Antonini 3, Vincent Algalarondo 2, Cecile Cauquil 1, Pierre Lozeron 1, Denis Castaing 3, Michel Slama 2, Didier Samuel 3
1 Univ Paris Sud, APHP, CHU Bicêtre Neurology Department, 2 Univ Paris Sud, APHP Hepatobiliary Center, 3 Univ Paris Sud, APHP, CHU A Beclere Cardiology Department, French Reference Center for FAP FRANCE (NNERF) French Network for FAP (CORNAMYL)

TTR-FAP are life-threatening and progressive sensorymotor and autonomic polyneuropathy. Twenty years ago, liver transplantation (LT) has been proposed as a treatment as it suppresses the main source of mutant TTR. More than 2000 FAP patients underwent LT until now. Worsening of the polyneuropathy after LT has been seldom reported. To analyze the patients with a clear progression of the polyneuropathy among the 200 patients who underwent LT during the last 20 years in our Center (138 Met30TTR (69%)). Follow up included : modified Norris Score (MNS), Compound Autonomic Dysfunction Test (CADT), NIS, strength (dynamometer), EMG score, cardiac function, survival. Characteristics of 26 patients with worsening of the neuropathy: late onset (>50 y.o.): N=23/26pts (88%), non Met30 TTR variant: 20/26 (77%) including 8 varied variants and 8 with Tyr77. The mean duration of the disease was 3.5 y. (1-6.5). Median CADT was 13 (6-16). Eight of them became bedridden (31%). The mean reduction of MNS was 41% (4 to 80%), of strength was 22%/year. The mean increase of NIS score was 32; 8.84/year (max 15.4). CADT worsened in 15/26 including 11/14 patients who developed orthostatic hypotension. Eleven (42%) patients developed cardiac insufficiency (1 required a heart transplantation). 16/26 (62%) patients died including 7 from endstage cardiac insufficiency, and 4 from endstage disease. In conclusion: a clear progression of polyneuropathy may occur in at least 12% of FAP patients after LT and concerns essentially non Met30 TTR-FAP with a late onset; an in-depth analysis of the factors influencing this course is necessary.
The familial amyloid neuropathy (FAP) is a rare genetic disease, due to endoneurial amyloid deposits made of mutant TTR produced by the liver. Liver transplantation (LT) is the main treatment for metTTR FAP and the use of explanted livers through the «domino» procedure is extensively used due to a constant shortage of liver donors. To assess the place of Nerve conduction studies (NCS) for monitoring the onset of amyloid neuropathy.

We have prospectively periodically evaluated 95 Domino Liver Transplantation recipients (DLT) since 1997, at a mean interval of 1.3 y. Nine patients (7 men/2 women) developed definite Amyloid Neuropathy confirmed by nerve biopsy. 7/9 patients had neuropathy risk factors and 8/9 patients had abnormal neurological examination on the first visit, 1.9 years after DLT. The first NCS, performed 2.5 years after DLT, showed abnormal lower limbs SNAP in 5/9 patients. At the time of symptoms onset (delay after DLT m=6 y. (2-11.6)), 4/9 patients had still normal lower limbs SNAP values, but 3 for them had dropped as compared to the previous NCS (by 9% to 60%). A progressive sensory and motor axonal loss followed the first symptoms, either rapid (no SNAP recorded at 3 years of evolution in spite of normal values 1 y after symptoms onset) or more slowly. Conclusion: Nerve Conduction Studies may be useful in some DLT patients for diagnosis of acquired amyloid neuropathy but require to be periodically repeated and associated with a close clinical follow-up.

Domino transplantation using the liver (DLT) from patient with familial amyloid polyneuropathy (FAP) has been developed to alleviate the graft shortage. 153 consecutive patients received a FAP domino graft (FAP-DLT) between 1997 and 2011. We studied 95 FAP-DLT patients to assess the risk of developing de novo amyloid neuropathy. Systematic and periodic follow-up included neurological evaluations with NCS, a labial salivary gland biopsy (LSGB) to look for amyloid deposits and nerve biopsy in case of evolutive peripheral neuropathy. At first visit, 30/95 (32%) patients had already a peripheral neuropathy (due to diabetes, alcohol, drugs). During follow-up (53 patients with more than 5 years), 13 patients developed amyloid polyneuropathy including 9 with positive endoneurial amyloid deposits on nerve biopsy (5.2-13.1 years from DLT). 26/53 patients (49%) had positive amyloid deposits on systematic LSGB. They had a mean age of 63.8 y. (54-76). De novo amyloid polyneuropathy (DAP) (n=9) mimicked usually inherited FAP with length dependent small fiber polyneuropathy. DAP started after a mean delay of 6.3 y. after DLT (2.0-11.6). Initial manifestations included pain in the feet (n=8/13), paresthesiae of the feet (2), fatigue (2), weakness of hands (1). Eight developed walking difficulty requiring aid in 2. 6/13 patients developed orthostatic hypotension, 3 gastroparesia and 2 diarrhea. 6/13 patients lost weight (6-24 kilos). Three patients underwent a retransplantation from cadaveric donor. Discussion: peripheral neuropathy occurs in many DLT recipients with a variable delay from LT. Conclusion: Long term neurological monitoring of FAP-DLT recipients is required to detect onset of amyloid neuropathy and to decide for retransplantation.
A-05 - TRANSTHYRETIN Y114C-RELATED ANGIITIS IN CEREBRAL AMYLOID ANGIOPATHY AFTER LIVER TRANSPLANTATION

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BACKGROUND: Patients with amyloidogenic transthyretin (TTR, ATTR) Y114C show fatal lobular hemorrhage and rapidly progressive dementia, presenting with cerebral amyloid angiopathy (CAA). Since TTR is produced by the liver, liver transplantation (LTx) reduces the occurrence of fatal lobular hemorrhage. However, mild cognitive impairment progresses slowly even after LTx. We describe an autopsy case of CAA ATTR Y114C after LTx. RESULTS: A patient with CAA ATTR Y114C developed vitreous opacity at the age of 30, underwent LTx at the age of 39, and subsequently showed fluctuating consciousness, slowly progressive cognitive impairment, visual hallucinations, and progressive apraxia. At the age of 49, neuroradiological examination revealed a cerebral microbleed in the cerebellum, and the patient died of apnea 9 months later. Postmortem examination showed TTR amyloid deposition in the cortical and leptomeningeal blood vessels. Additionally, severe amyloid deposition in the leptomeninges and amyloid infiltration to the parenchyma were observed in the brainstem and the spine. Moreover, there were massive amyloid deposits in the cerebral ventricle wall, and ATTR-related cerebral angiitis with infiltration of inflammatory mononuclear cells in a few leptomeningeal blood vessels.

CONCLUSIONS: ATTR-related cerebral angiitis were observed in the patient. The remarkable amyloid deposition seen in the brainstem might have been associated with the apnea, fluctuating consciousness, and visual hallucinations. Continuing amyloid fibril formation from ATTR produced by the choroid plexus might have caused the manifestations after LTx.

A-06 - ORGAN TRANSPLANTATION STRATEGIES IN HEREDITARY TRANSTHYRETIN AMYLOIDOsis (ATTR) LONG-TERM EXPERIENCE AT A SINGLE CENTER WITH COMBINED HEART AND LIVER TRANSPLANT FACILITIES

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Background. Although orthotopic liver transplantation (OLT) and combined heart and liver transplantation (CHLT) are accepted therapeutic strategies in ATTR, Centers with an unrestricted access to both facilities are few and long term results are poorly defined. We report results of our transplantation strategy: OLT in symptomatic stage I neuropathy with no or mild heart involvement, CHLT in non-Val30Met stage I neuropathy and severe cardiomyopathy, including those without hemodynamic impairment. Between 1993 and December 2012, 47 patients underwent organ transplantation (34 OLT and 13 CHLT) and were evaluated for survival and incremental risk factors for mortality. Results. 31 patients were males (66%) with median age of 47 years (range 22-68). Val30Met patients were 15 (32%) and all underwent OLT; the most frequent non-Val30Met mutations were Glu89Gln (n=9, 19%) and Thr49Ala (n=6, 13%). During a median follow-up of 5 years (range 0-20), 19 (40%) patients died (16 OLT, 3 CHLT). Neurologic progression was the cause of death in 6 patients (5 in OLT and 1 CHLT subgroups), severe heart failure in 3 OLT. One-year survival was 77% (71% in OLT and 92% in CHLT). Survival at 5 years was 58% (54% in OLT and 68% in CHLT groups). Cox analysis identified the following independent variables associated with overall mortality: peripheral neuropathy (P=0.03), coexistence of orthostatic hypotension and gastrointestinal symptoms (P=0.02), compromised nutritional status (P=0.03) and long waiting time in list (P=0.04). Conclusions. CHLT improves mid term survival in patients with non-Val30Met mutations, mild neuropathy and definite cardiomyopathy, including those without hemodynamic impairment. However, both OLT and CHLT are suboptimal therapeutic solutions in ATTR since long term efficacy is hampered by disease progression in at least 40% of cases. Pharmacologic strategies against post-transplant wild type transthyretin deposition are needed.
A-07 - REVERSIBILITY OF ACQUIRED AMYLOID POLYNEUROPATHY AFTER LIVER RETRANSPANTATION

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Domino liver transplantation (DLT) has become a common procedure designed to overcome problems with organ supply. However, cases of acquired amyloid neuropathy are increasingly being recognized following this procedure. Until now, only one patient had undergone re-transplantation and follow-up findings were not reported. We describe the case of a 72-year-old patient with partial recovery from acquired amyloid neuropathy following re-transplantation with a deceased donor 7 years after DLT performed for end-stage liver disease. Six and a half years after DLT, he started complaining of burning pain in the feet associated with constipation and weight loss. During the following year, the patient's clinical and electrophysiological parameters deteriorated considerably, with an extension of sensory deficits up to the knees and slight weakness in the small hand muscles, associated with orthostatic hypotension. The diagnosis of post-DLT acquired amyloid polyneuropathy was based on clinical, physiological and anatomo-pathological findings. Because of those findings and moderate renal insufficiency he underwent combined liver-kidney transplantation to halt amyloid progression. Eighteen months after the second LT, his nerve conduction parameters showed a slight increase in both the sensory and motor sum scores when compared to post-transplantation values, but his clinical condition only partially improved after 2.5 years. Four years after the second liver transplant the patient is asymptomatic. In this case we were able to demonstrate the possibility of reversing acquired amyloid polyneuropathy, this needs to be confirmed by further studies.

A-08 - FOLLOW-UP OF TRANSTHYRETIN AMYLOIDOSIS PATIENTS WITH LIVER TRANSPLANTS OR RECEIVING TAFAMIDIS TREATMENT AND PATIENTS WITHOUT TREATMENT.

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1HU CFF - Hospital Universitário Clementino Fraga Filho (Rio de Janeiro, Brazil), 2UHHM - University Hospital Henri Mondor (Créteil, France)

Introduction: We describe the 1- to 4-year follow-up of patients who received tafamidis, had a liver transplant (LT) or received no treatment (NT) within at least 1 year of registry enrollment. Tafamidis is a new treatment for TTR FAP, but its long-term effects are not well documented. Methods: We analyzed the neurologic assessments and quality of life of ATTR-V30M Brazilian and French stage I or II patients for whom baseline and at least 1-year follow-up results were available. The mean follow up for patients on tafamidis was 2.85 ± 0.16 years (n=7), for patients with LT, 2.75±1.38, (n=8) and for patients with NT, 2.21±1.21 (n=7). Evaluation parameters included Karnofsky, Neuropathy impairment score (NIS), and Polyneuropathy Disability (PND). NIS deterioration was considered an increase in two points comparing the first and last assessment. Results: Tafamidis-treated patients were similar in age, disease duration (DD), to LT recipients and NT patients respectively (Age [y-o]: 47.42±16.69, 44±12.68 and 40.28±14.25, DD: 6.85±4.18, 7.25±2.65 and 4.57±1.90 years). Duration of treatment was 3.42±1.13 and 3.5±1.06 years (tafamidis and LT respectively). During follow-up, tafamidis-treated patients and LT recipients showed no deterioration (ND) in PND 85.72%, 87.5%, respectively against 28.57% NT patients. The NIS showed ND 42.86% of tafamidis-treated patients, 75% of LT recipients and 14.29% in NT patients. Conclusions: In this small sample population study, after a mean follow up period of 2.60 years, similar neurological outcomes were observed in both groups compared to NT patients. Long-term Evaluation of Tafamidis treated patients are needed to clarify their outcome compared to LT inATTR FAP.
Background: Liver transplantation is the most current treatment in ATTR amyloidosis. However, some patients still deteriorate thereafter, due to ongoing tissular amyloid deposition (AD) of wild type fibrils. Tafamidis (Vyndaqel®) increases stability of wild type and mutant ATTR tetramers. Therefore it might benefit to such patients, to prevent further AD. So far, data on the safety of Tafamidis in transplanted patients receiving immunosuppressive drugs are lacking. We present a 1 year follow up study on safety and efficacy of Tafamidis in 3 transplanted ATTR-FAP patients.

Patients/Methods: A M/66y-o ATTR-S77T (pt1) and a F/44 y-o ATTRS71A (pt2) had a sensori-motor neuropathy which progressively deteriorated, after LT performed in 2005. A 58 y-o ATTR S77T male (pt3) with mild a sensory neuropathy had heart transplant in 2011 because of terminal cardiomyopathy. He then refused LT. After informed consent, all patients received tafamidis since July 2012. Safety and tolerance were assessed monthly for 1 year. Neurological scores (NIS, PND) neurophysiological examination, BMI and Karnovsky index were recorded at base line and after 1 year. Results At baseline, all patients had normal biological work up. Immunosuppressive drugs included Tacrolimus (Advagraf®) in pt 1, 2, Mycophenolate mofetil (Cellcept®) in pt 2; Mycophenolate sodium (Myfortic®) and cyclosporin (Neoral®) in pt 3. Dosings treatments were stable in pts 1, 2. Patient 3 had an acute rejection 15 after starting Tafamidis. He was therefore switched to Prograf®. Overall, no significant variation of immunosupressor concentrations occurred during tafamidis administration compared to baseline. Evaluation showed a progression of the neuropathy i.e. the NIS increased from 79, 63 and 8 to 101, 69 and 17, respectively in patients 1,2,3. The PND (stage IIIa in pt 1, II in pt2 and I in pt 3) remained stable. BMI increased in pt1 and was stable in pts 2, 3. Karnofsky index remained stable in pts 1, 3, and deteriorated from 80 to 70 in pt2. There were no safety concerns during the follow up. Conclusion Tafamidis was well tolerated in 3 non V30M ATTR transplanted FAP patients. We observed no significant interactions with the relevant immunosuppressive drugs. However, Tafamidis was unable to halt the progression of the neuropathy after 1 year follow up.
A-11 - The FAPWTR experience of combined liver and heart transplantation in patients with hATTR amyloidosis.

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Background: Transthyretin (TTR) systemic amyloidosis disorders can be treated with liver transplantation (Ltx) and the FAPWTR registry receives reports on these patients from all over the world. Most patients need only Ltx, but some mutations also affect the patient’s heart necessitating replacement of both liver and heart. Results: Until 2010-12-31 a total of 53 patients received liver/heart transplants (LHtx) in various combinations, simultaneous liver/heart(n=38), heart+subsequent liver(n=11), liver+subsequent heart(n=1) and combined liver/heart/kidney(n=3). Seventeen different TTR mutations were represented among these patients. In 4 patients information on TTR mutation was missing. More males(n=43) than females(n=10) underwent (LHtx). There was no mean age difference between males and females, 55.0±7.8 years and 53.3±4.6 years, respectively. NonVal30Met patients more often require (LHtx). Most of the (LHtx) were done in USA, while Germany has the largest experience in heart+subsequent Ltx. 34 patients (64%) are still alive with a follow-up time between 4.8 months and 16 years. Survival was similar in patients undergoing simultaneous liver/heart transplantation and heart with subsequent Ltx. Conclusion: NonVal30Met patients are more likely to require LHtx. Survival is comparable to that seen after adult Htx in nonamyloidosis patients. Simultaneous tx appears as safe as sequential.

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<th>TTR</th>
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A-12 - TRANSTHYRETIN AMYLOIDOSIS DUE TO DOMINO LIVER TRANSPLANTATION

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Transmission of systemic transthyretin (TTR) amyloidosis after domino liver transplantation (DLT) from donors with familial amyloid polyneuropathy (FAP) is possible. We report a 57-year old FAP male patient whose complaints started 8 years after DLT. Numbness and burning sensation of feet followed by the hands and erectile dysfunction, orthostatic hypotension, decreased sweating, intestinal dysmotility and diarrhea were prominent. His neurological examination showed distal weakness of all limbs with bilateral steppage gait, stocking and glove type hypoesthesia and hypoalgiesia, diminished vibration sensation and absent tendon reflexes. EMG showed findings consistent with distal sensory motor axonal polyneuropathy accompanied by autonomic involvement. His sural nerve biopsy disclosed severe axon loss with amyloid deposition. He did not have any cardiac, renal or eye involvement due to amyloidosis. He refused to undergo a new liver transplantation and was put under treatment with Tafamidis Meglumine (Vyndaqel). Although estimated time of de novo amyloidosis transfer risk was expected to be minimum 20 years, according to records patients can become symptomatic earlier than expected. We do not yet know the effects of the Tafamidis treatment in this patient.

B - Treatment

B-01 - DISEASE-MODIFYING THERAPY FOR TRANSTHYRETIN AMYLOID CARDIOMYOPATHY

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BACKGROUND: Transthyretin (ATTR) cardiac amyloidosis is a rare but increasingly recognized cause of heart failure with preserved ejection fraction. Recent data suggest that pharmacologic stabilization of the mutant TTR protein may slow the tissue deposition of amyloid fibrils in patients with familial amyloid polyneuropathy. Two TTR-stabilizing agents – the nonsteroidal anti-inflammatory drug diflunisal, and the recently developed small molecule tafamidis – have been proposed as potential disease-modifying therapies for TTR amyloid cardiomyopathy. We report on the use of these agents at our center. METHODS: Of 98 patients with TTR cardiac amyloidosis, 28 (29%) received either diflunisal or tafamidis. Baseline demographic and clinical data were collected for all patients. Univariate and multivariate cox proportional hazards modeling was used evaluate the association between baseline variables, including the use of a disease-modifying agent, and overall survival. RESULTS: Sixteen of 98 patients (16%) received diflunisal and 12 patients (12%) received tafamidis as a disease modifying therapy. In a univariate model, treatment with either agent was associated with a significantly decreased risk of overall mortality (HR 0.37, 95% CI 0.17-0.80, p=0.01). Given the non-randomized nature of the study, subjects who received disease-modifying therapy differed from those who did not (see table). In a multivariate model incorporating other clinically significant variables, disease-modifying therapy was still associated with a lower risk of death (HR 0.62), however this association was no longer statistically significant (95% CI 0.23-1.65, p=0.33). CONCLUSIONS: These data suggest a trend toward survival benefit among patients with TTR cardiac amyloidosis who received disease-modifying therapy with diflunisal or tafamidis. Further study in a randomized controlled trial is warranted to determine the efficacy of these agents.

<table>
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<tr>
<th>Characteristic</th>
<th>Disease-modifying therapy (n = 28)</th>
<th>No disease-modifying therapy (n = 70)</th>
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<td>MAP, mm hg</td>
<td>88 ± 13</td>
<td>82 ± 9.1</td>
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<td>EF, %</td>
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<td>39 ± 18</td>
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<td>CrCl, mL/min</td>
<td>62 ± 24</td>
<td>53 ± 23</td>
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<td>BNP, pg/mL</td>
<td>456 ± 394</td>
<td>913 ± 634</td>
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<td>Troponin I, ng/mL</td>
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<tr>
<td>ATTR wild-type, no. (%)</td>
<td>18 (64)</td>
<td>30 (43)</td>
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<td>ATTR mutant, no. (%)</td>
<td>10 (36)</td>
<td>40 (57)</td>
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B-02 - TRANSTRHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY – DISEASE PROGRESSION OVER 12 MONTHS ON TAFAMIDIS: THE LISBON CENTER EXPERIENCE.

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Background: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is an inherited sensorimotor and autonomic neuropathy. Class II evidence of efficacy lead to European Medicine Agency approval of Tafamidis, in 2011, as a new TTR-FAP treatment. Objectives: Assess disease progression in TTR-FAP patients treated with Tafamidis over 12 months. Methods: We performed a retrospective analysis of the clinical and laboratorial data of patients treated with Tafamidis in the last year at CHLN-Hospital de Santa Maria (Lisbon, Portugal). We accessed the Neuropathy Impairment Score-Lower Limbs (NIS-LL) and Norfolk Quality of Life- Diabetic Neuropathy total score (TQOL) at baseline, 6 and 12 months of therapy. Other clinical and laboratory endpoints considered relevant in this condition were also screened. Repeated measures ANOVA and Friedman tests were performed using a significance level of 0.05. Results: From the 41 patients in treatment, 3 dropout (1 due to hepatotoxicity, 1 for liver transplant and 1 emigrated) and 9 patients had only baseline data. The remaining 29 patients had an average age of 42±11.2 years (16 female; 13 male), with 12 months and 11 with 6 months follow-up. NIS-LL increased 1.14 points from baseline to 6 months (p<0,05). From 6 to 12 months, 18 patients were evaluated and showed a non-significant increase of 0.67 points. No substantial changes of TQOL, Karnofsky index, modified BMI and NT-ProBNP were found over time. Glomerular filtration rate (GFR) and sensory nerve amplitudes of the sural and peroneal nerves decreased overtime (p<0,05). A significant increase in pre-albumin levels was also found. Conclusion: Lack of obvious response to treatment in the first 6 months may not imply true failure of treatment, since a slower clinical progression can be achieved afterwards. Deterioration in GFR and nerve conduction studies indicating subclinical disease activity seems to be present even when no clinical progression is noticed.

B-03 - Safety and efficacy of long-term diflunisal administration in late-onset ATTR Val30Met FAP

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Objectives: To evaluate the safety and efficacy of long-term diflunisal administration in late-onset FAP patients with Val30Met mutation in the transthyretin (TTR) gene (ATTR Val30Met FAP). Methods: Diflunisal was administered orally at 500 mg/day to 25 late-onset ATTR Val30Met FAP patients. The observation period ranged from 3 to 100 months (mean 37.8 ± 29.7 months). Treatment effect was assessed by serial measurements of ulnar and tibial nerve compound muscle action potential (CMAP) amplitudes, cardiothoracic ratio (CTR) on chest X-ray, and intraventricular septum (IVS) thickness on echocardiogram. The historical control group consisted of 27 late-onset ATTR Val30Met FAP patients. Results: Diflunisal-related adverse event, thrombocytopenia, resulted in discontinuation of the study drug in one patient. Nine patients dropped out due to reasons unrelated to diflunisal. Orally administered diflunisal significantly increased serum TTR concentration (p=0.001) and stabilized TTR tetramer structure in each patient. The %decreases of ulnar nerve CMAP amplitude in the diflunisal treatment group and the control group were 10.2% ± 12.1%/year and 30.5% ± 15.2%/year, respectively (p = 0.001). The ratios of patients in whom tibial nerve CMAP became undetectable during the follow-up period were 41.7% (5/12) in the diflunisal treatment group and 100% (10/10) in controls (p = 0.003). Although there was a trend toward lower deterioration rate of CTR and IVS thickness in the diflunisal treatment group than the controls, these differences were not statistically significant. Conclusions: Diflunisal was well-tolerated by most late-onset ATTR Val30Met FAP patients and increased serum TTR concentration and stability. Diflunisal significantly slowed the deterioration rates of ulnar and tibial nerve CMAP compared to the untreated control group.
B-04 - SAFETY, TOLERABILITY AND EFFICACY OF DIFLUNISAL IN LATE-ONSET FAP PATIENTS WITH MODERATE TO SEVERE NEUROPATHY

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FAP treatment with tafamidis is presently limited to stage I. In non-endemic areas like Italy, where the disease is mostly associated with a late-onset phenotype and a broad spectrum of TTR variants, several patients are diagnosed when walking disability already occurred. No drug is licensed for this patients' population. Diflunisal is another candidate TTR stabilizer whose activity is under evaluation in a phase 3 trial. Methods. We assessed the tolerability, safety and efficacy of diflunisal (250 mg BID) in late-onset FAP, including patients with moderate to severe neuropathy. Baseline evaluations included nerve conduction studies, Kumamoto score, polyneuropathy disability score (PND), mBMI, echocardiography and cardiac biomarkers. Monitoring of adverse events was performed every three months. Results. 18 patients, 14 males, median age 71 years (range 57-82), median disease duration 55 months (range 17-105), affected by FAP associated with 6 different mutations (Val30Met, Glu89Gln, Phe64Leu, Gly47Glu, Tyr78Phe, Ile107Phe) were included. Patients were treated for a median of 21 months (range 12-36). Median PND score was 3 (range 1-5), median Kumamoto score was 26 (2-40), median BMI was 879 (range 604-1090). 16 patients presented with heart involvement. Median NT-proBNP was 1124 pg/ml (range 147-5965), cTnI 0.038 ng/ml (range 0.017-0.65), mLVW 14.7 mm (range 13-18). Nutritional status improved during treatment with a mean mBMI change from baseline to 12 months of 58 (SEM 21). Mean change of Kumamoto score was 4 points/year. PND increased by 1 point in 3/18 patients at 12 months. Cardiac progression occurred in 1/16 patients. One patient discontinued at 12 months due to an asymptomatic increase in NT-proBNP and TnI, with unchanged echocardiographic parameters. His cardiac biomarkers improved following discontinuation. A mild increase in serum creatinine was observed in two patients. No GI events were recorded. Treatment with diflunisal appears to be safe and well tolerated in late-onset FAP with moderate to severe neuropathy and cardiomyopathy. Our results support a beneficial effect of this drug on disease progression in stage 2 and 3 FAP patients.

B-05 - Four years of Follow-up of Brazilian Transthyretin Amyloidosis Patients Receiving Tafamidis Treatment

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Introduction: Tafamidis is a new, well-tolerated oral agent, believed to stop or slow the progression of disease, but its long-term effects are not well documented. We describe four years follow-up of five Brazilian patients who received tafamidis. Methods: We analyzed the neurologic assessments, Karnofsky index and body mass index (BMI). All patients had Val30Met mutation and received Tafamidis 20mg daily. First visit occurred 12 months after start date of Tafamidis and last visit 48 months after. We did 6 visits in this period and used neuropathy impairment score (NIS), our clinical evaluation scale and our electromyographic scale to better analyze progression disease. Duration of the disease before the onset of treatment in patients 1,2,and 3, were 1, 3, and 1 year respectively. Two of five patients stopped the drug because one became pregnant and one decided to undergo liver transplantation (LT). Results: Two of the three patients had a stable NIS comparing the first and last visit: Patient 1: NIS: 85 (12M) and 86 (48M) and Patient 2: NIS: 12 (12 and 48M). Patient 3 had an important deterioration on NIS: 12 (12M) and 52,5 in (48M). No patients had deterioration in BMI. During follow-up, all patients lost 10 points in Karnofsky index and had deterioration in our electromyographic scale. All patients had deterioration in our clinical evaluation scale, but patient 3 had a marked worsening. Patient 4 used Tafamidis during one year and went through LT in August 2011. He presented renal failure and chronic graft vs. host disease after LT, but after two years he is stable. No significant side effects were seen in these three patients. Conclusions: It seems that some patients have a better response to the medication than others irrespective of disease duration before treatment. Continuous evaluation, more expressive numbers and knowledge of biological markers of disease aggressiveness will make us better understand this context.
B-06 - THE CLINICAL COURSE OF TTR-FAP UNDER TAFA MIDS MEGLUMINE: SIX PATIENTS FROM TURKEY

Zeliha Matur 1, Murat Mert Atmaca 2, Hacer Durmuş 2, Piraye Oflazer 2, Feza Deymeer 2, Yesim Parman 2
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Transthyretin-related familial amyloid polyneuropathy (TTR-FAP) is an autosomal dominant disorder caused by mutations of the transthyretin (TTR) gene. TafamidisMeglumine (Vyndaqel®) ameliorates TTR-FAP via preventing dissociation of the native TTR tetramer into monomers, which results in the inhibition of TTR amyloid fibril formation. We present six FAP patients (5 males) treated with Tafamidis Meglumine. Mean age was 61 years (range 36 to 72). Three of them were relatives and had the Glu89Gln mutation. They have been receiving Tafamidis for 1.5 years and the progression of the disease stopped in two of them and was very slowed in the remaining one who had the longest disease duration. One patient had the most common Val30Met mutation and the last patient was a domino transplant. Both also benefitted from Tafamidis. Only one patient who had Gly53Glu mutation with already described leptomeningeal and central nervous system involvement did not well under the treatment and became bedridden in six mounts. Decrease in paresthesiae, amelioration of orthostatism and weight gain were also observed in patients who were better with the treatment, whereas diarrhea and urinary tract infections were noted as adverse effects. These findings in this small cohort were compatible with those already reported. Tafamidis slowed disease progression and reduced burden of the disease.

B-08 - LONG-TERM TAFA MIDS TREATMENT IN PATIENTS WITH NON-V30M MUTATIONS: INTERIM ANALYSIS OF THE FX1A-303 STUDY

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Background: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is associated with both V30M and non-V30M mutations of the transthyretin gene. TafamidisMeglumine (Vyndaqel®) ameliorates TTR-FAP via preventing dissociation of the native TTR tetramer into monomers, which results in the inhibition of TTR amyloid fibril formation. We present six FAP patients (5 males) treated with Tafamidis Meglumine. Mean age was 61 years (range 36 to 72). Three of them were relatives and had the Glu89Gln mutation. They have been receiving Tafamidis for 1.5 years and the progression of the disease stopped in two of them and was very slowed in the remaining one who had the longest disease duration. One patient had the most common Val30Met mutation and the last patient was a domino transplant. Both also benefitted from Tafamidis. Only one patient who had Gly53Glu mutation with already described leptomeningeal and central nervous system involvement did not well under the treatment and became bedridden in six mounts. Decrease in paresthesiae, amelioration of orthostatism and weight gain were also observed in patients who were better with the treatment, whereas diarrhea and urinary tract infections were noted as adverse effects. These findings in this small cohort were compatible with those already reported. Tafamidis slowed disease progression and reduced burden of the disease.
C - Cardiology

C-01 - THE MYOCARDIAL CONTRACTION FRACTION IS SUPERIOR TO EJECTION FRACTION IN PREDICTING SURVIVAL IN PATIENTS WITH CARDIAC AMYLOIDOSIS

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Background: Cardiac amyloidosis is an under-recognized cause of diastolic heart failure in which progressive declines in end diastolic volume from amyloid infiltration are accompanied by concomitant declines in stroke volume, such that ejection fraction remains normal or preserved despite progression of disease. The myocardial contraction fraction (MCF) is a novel index of myocardial function that is defined as the ratio of stroke volume (SV) to myocardial volume (MV). We hypothesize that MCF would be superior to EF, the conventional measure of left ventricular function, in predicting survival among patients with cardiac amyloidosis. Methods: Sixty-six subjects (67±12 years; 20% women) with cardiac amyloidosis (34 with light-chain AL amyloid and 32 with transthyretin amyloid) underwent conventional measurements of left ventricular volumes using 2-dimensional echocardiography. Measurements of end-diastolic volume (EDV), end-systolic volume (ESV), and myocardial mass (LV Mass) were determined. Using these parameters, stroke volume (EDV-ESV), ejection fraction ((EDV-ESV/EDV)*100), myocardial volume (LV mass/density of myocardium), and MCF ((SV/MV)*100) were calculated. Cox-proportional hazards modeling was used to determine the association of these measures of chamber function with survival. Results: Over a mean follow-up of 1.86±1.78 years (range 0.03-7.36 years), 37 subjects (56.1%) died. The mean EF of the study population was 51±13%. There was no significant difference in EF between patients who survived the study period and those who died (54±11% vs. 49±14%; P=0.1196) while there were differences in the MCF (35±19% vs. 23±10%, P=.0065). Using Cox proportional hazards modeling, MCF was associated with death (Hazard Ratio=.953, 95% CI 0.932-0.984, p=0.0031) while EF was not (HR=.991, 95% CI 0.968-1.014, p=.4320). In a multivariate model, an MCF Conclusion: These data suggest that MCF, a novel measure of myocardial chamber function, is superior to EF in predicting overall survival outcomes among patients with cardiac amyloidosis. Further use of MCF in the staging of patients with cardiac amyloidosis warrants investigation. Key words: cardiac amyloidosis, ejection fraction, myocardial contraction fraction, survival
C-02 - RELATIONSHIP BETWEEN STRAIN RATE AND MYOCARDIAL UPTAKE OF TECHNETIUM PYROPHOSPHATE (TCE-PYP) IN ATTR CARDIAC AMYLOIDOSIS

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Background: Echocardiographic imaging demonstrates preserved apical wall motion and preserved strain rate which are unique characteristics of cardiac amyloid that can distinguish it from other conditions that result in significantly increased left ventricular wall thickness. We hypothesized that there is a relationship between myocardial strain and amyloid deposition. Methods: 20 subjects with ATTR cardiac amyloid (71±7 years, 18 males, 10 ATTRwt and 10 ATTRmt including 8 V122I and 2 T60A) underwent echocardiographic imaging with color-coded tissue Doppler for derivation of longitudinal strain and 99mTc-PYP SPECT scanning to quantify myocardial uptake based on semi-quantitative scale (0 = significant uptake to 3 = no uptake). A bull’s eye plot demonstrating segmental average strain values was constructed. Differences in myocardial segmental strain by degree of segmental cardiac 99mTc-PYP uptake (indicative of amyloid density) were determined by ANOVA. Results: Myocardial strain values showed preserved apical strain as compared with other segments (see bull’s eye below). There was a significant association of myocardial strain with semi-quantitative 99mTc-PYP myocardial uptake (p=0.0074 by ANOVA). Specifically, segments with minimal 99mTc-PYP uptake showed better strain than those with significant uptake (-15±2% vs. -7.1±5.7%, p =0.008). Conclusion: Myocardial uptake of Tc-PYP indicative of amyloid infiltration was associated with reduced strain.

Average Strain by Segment

Relationship between strain rate (%) with degree of myocardial uptake of Tc-PYP.
C-03 - AMYLOIDOGENIC TRANSTHYRETIN VARIANT His88Arg IN SWEDEN ASSOCIATED WITH LATE ONSET CARDIOMYOPATHY AND MILD NEUROPATHY

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Introduction and case histories. In 2005 the first case of ATTR His88Arg was reported. Four additional cases (3 males and one female) have now been diagnosed in Sweden. Genealogically all five patients have been shown to be related and originate from the same region in Sweden. They all sought medical attention for dyspnea and tiredness. After initial diagnose of cardiomyopathy and cardiac failure, TTR-amyloid deposits were proven in biopsies and the TTR His88Arg mutation identified. Three of the patients had a history of a carpal tunnel syndrome. They all are late onset cases (onset above 50 years of age) and in all but one case the presenting symptom was that of heart failure. One case presented with a progressive neuropathy, and only later developed heart failure. He is currently severely disabled by his polyneuropathy and bound to a wheelchair. One of the patients has died after five years of disease, and his son has been identified as an asymptomatic carrier of the His88Arg mutation. One patient has had heart transplantation and is currently waiting for liver transplantation. One of the remaining 3 patients is considered for heart/liver transplantation, whereas for the other two patients, transplantation is currently not considered an option.

Conclusions. The phenotype of ATTR His88Arg is predominantly that of cardiomyopathy, but peripheral neuropathy can be the presenting and dominating symptom.

C-04 - ECHOCARDIOGRAPHIC FEATURES PRECEDING CARDIAC SYMPTOMS IN TRANSTHYRETIN-RELATED FAMILIAL AMYLOIDOTIC POLYNEUROPATHY: A CASE REPORT

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Context: Transthyretin-related amyloidosis (ATTR) is usually manifested as a neurological disease, but its vast, heterogeneous clinical presentations can at times be recognized through cardiac findings during routine screening. Case Report: A 34-year-old man, carrier of the Val30Met transthyretin mutation, with no comorbidities, and with a walking disability (WD) score of zero, is admitted for routine cardiologic screening. The rest electrocardiogram demonstrates a normal sinus rhythm. The echocardiogram shows a mild asynchronous septal motion with normal left ventricular systolic and diastolic functions as well as normal cardiac chamber sizes and wall thickness. The patient is then investigated with a 24-hour Holter, with sinus rhythm, normal intraventricular conduction with episodes of first-degree atrioventricular block as well as Mobitz I second-degree atrioventricular block. Conclusion: Cardiac involvement in ATTR may be underdiagnosed. In spite of the enormous scientific effort to recognize and clinically follow ATTR, it remains a challenging disease due to its wide range of clinical presentations, including mainly cardiac manifestations. This report is part of a case study of echocardiographic features in ATTR patients being followed at the reference center of Familial Amyloidotic Polyneuropathy (CEPARM), of the Federal University of Rio de Janeiro, Brazil.
C-05 - THE RELATIONSHIP BETWEEN CARDIAC UPTAKE IN 99MTC-DPD SCINTIGRAPHY, HISTOLOGY AND CARDIAC FUNCTION IN ATTR AMYLOIDOSIS

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Background: Recent studies have shown that 99mTc-DPD scintigraphy is useful in identifying patients with ATTR amyloidosis. This scintigraphic cardiac uptake pattern is specific for ATTR amyloidosis but does not detect AL amyloidosis or hypertrophic heart disease and the reason for this uptake is not well understood. Recently, two different types of amyloid fibrils have been detected in ATTR: full length transthyretin fibrils or mixed truncated and full length amyloid fibrils. We aimed to investigate the relationship between 99mTc-DPD scintigraphy cardiac uptake, the amyloid fibril type and echocardiographic measures of left ventricular (LV) function and structure in ATTR amyloidosis.

Methods: We investigated 17 patients with abdominal fat biopsy verified ATTR and used 99mTc-DPD scintigraphy to detect the presence of cardiac isotope uptake. Western blot was utilised to determine the fibril type. Echocardiographic measurements of global LV long axis strain (GLS), LV torsion using speckle tracking, septal thickness (IVS), posterior wall thickness (PW) and stroke volume (SV) were performed. Results: ATTR patients with positive scintigraphic uptake (scint+)(n=10) were older (p
C-06 - THE ECG FEATURES AT PRESENTATION IN PATIENTS WITH ATTRWt, ATTR ASSOCIATED WITH T60A VARIANT AND ATTR ASSOCIATED WITH V122I VARIANT.

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1UCL - National Amyloidosis Centre, University College London, UK (UCL Medical School, Lower 3rd Floor, Rowland Hill St, London, UK, NW3 2PF.)

Background: Transthyretin cardiac amyloidosis (ATTR) is a rare but increasingly recognised cause of heart failure. Referrals to our centre have increased significantly in the last 5 years in patients with wild type transthyretin cardiac amyloidosis (ATTRwt) and with familial amyloid cardiomyopathy. Novel treatments are currently being assessed in phase 2/3 trials in FAC and these drugs may also be applicable to ATTRwt. Early accurate diagnosis is therefore essential. The ECG in cardiac AL amyloidosis characteristically shows small QRS complexes. We sought to determine if features in the ECG at presentation in ATTR can aid diagnosis. Methods and Results The electrocardiogram at presentation was reported for 125 patients with ATTRwt, 39 patients with FAP T60A and 64 patients with ATTR v122I. The results are presented in the table below.

<table>
<thead>
<tr>
<th>Feature</th>
<th>ATTR wt n=125</th>
<th>T60A n=39</th>
<th>V122I n=64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinus rhythm</td>
<td>50 (40%)</td>
<td>25 (64%)</td>
<td>40 (62.5%)</td>
</tr>
<tr>
<td>AF/flutter</td>
<td>57 (45.6%)</td>
<td>3 (7%)</td>
<td>19 (29.7%)</td>
</tr>
<tr>
<td>Paced rhythm</td>
<td>17 (13.6%)</td>
<td>9 (31%)</td>
<td>3 (4.7%)</td>
</tr>
<tr>
<td>PR interval &gt; 0.2s</td>
<td>29/50 (58%)</td>
<td>6/25 (24%)</td>
<td>23/40 (58%)</td>
</tr>
<tr>
<td>IV conduction delay</td>
<td>70 (56%)</td>
<td>25 (64%)</td>
<td>12 (19.7%)</td>
</tr>
<tr>
<td>Positive sokolov index (criteria for LVH)</td>
<td>10 (8%)</td>
<td>3 (8%)</td>
<td>16 (26.2%)</td>
</tr>
<tr>
<td>Limb lead QRS&lt;5mm</td>
<td>46 (36.8%)</td>
<td>7 (18%)</td>
<td>34 (55.7%)</td>
</tr>
<tr>
<td>Pacemaker implant during follow up</td>
<td>12 (9.6%)</td>
<td>5 (12.8%)</td>
<td>4 (6.2%)</td>
</tr>
</tbody>
</table>

In patients with ATTRwt, 45.6% were in atrial fibrillation or atrial flutter at presentation compared 29.7% of V122I patients and 7% of T60A patients. 31% of T60A patients were in a paced rhythm compared to 13.6% and 4.7% of ATTRwt and V122I patients respectively. 26.2% V122I patients met the criteria for left ventricular hypertrophy (LVH) compared with 8% each of ATTRwt and T60A patients. Small QRS complexes were found in 18% T60A patients compared with 36.8% ATTRwt and 55.7% V122I patients. IV conduction delay was found in 56% ATTRwt and 64% T60A patients compared with 19.7% V122I patients. 58% of both ATTRwt and V122I patients in sinus rhythm were also in first degree heart block compared with 24% T60A patients. Conclusion: In ATTRwt patients, the presence of an atrial arrhythmia, first degree heart block and IV conduction delay were prominent findings but LVH criteria was uncommon. In T60A patients, previous pacemaker insertion and IV conduction delay were commonly found but first degree heart block, atrial arrhythmias and LVH criteria were not. In V122I patients, first degree heart block and small QRS complexes in the limb leads were commonly found. The criteria for LVH were found in one third of these patients. Only 4.7% of V122I patients had previously had a pacemaker. Several patients in all 3 groups required subsequent pacemaker insertion during follow up. Physicians should be aware that in these groups of amyloidosis patients, features other than the low QRS complexes, may be more commonly found. In addition, there are considerable differences in the ECG features at presentation between these groups of patients.
C-07 - TWENTY-FOUR HOUR HOLTER ECG MONITORING IN CARDIAC AMYLOIDOSIS

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Background: There have been few studies examining rhythm disturbance in cardiac ATTR amyloidosis, and data on cause of death are scant. Infiltration of the heart confers a poor prognosis in AL amyloidosis and is associated with sudden death. We sought to determine the spectrum and frequency of rhythm disturbances in patients with cATTR amyloidosis and compare those in cardiac AL amyloidosis. Patients and Methods: This prospective, single centre study was performed between May 2010 and September 2013 (censor date). All amyloidosis patients newly referred to the UK National Amyloidosis Centre (NAC) with cardiac involvement were eligible to participate. Subjects underwent a 24 hour Holter ECG study and were asked to record periods of strenuous activity and symptoms during the monitoring period using a diary card. A case report form detailing previous cardiac history, symptoms of syncope, palpitations, chest pain and breathlessness was completed and NYHA class, exercise tolerance and medications were recorded in each case. Electrocardiography, echocardiography and biochemical analysis of serum including troponin T and NT-proBNP was undertaken at baseline in each case and patients were followed until censor or death. Results: 250 patients with cardiac amyloidosis (94 ATTR and 156 AL) were studied between May 2010 and June 2012. During the recruitment period a total of 137 newly diagnosed cardiac ATTR and 192 newly diagnosed cardiac AL patients were assessed at the NAC. A previous history of arrhythmia was present in 53% of ATTR vs 23% of AL patients (P<0.001), but NYHA class and ‘Mayo’ disease stage by cardiac biomarkers did not differ between the groups. Ten percent of ATTR patients had a pacemaker vs only 1% of AL patients at the time of their initial evaluation (P=0.009) and significantly more ATTR than AL patients were taking β-blockers at this timepoint (49% vs 29%; P=0.002). Arrhythmias, including complex ventricular arrhythmias (65% vs 45%), atrial fibrillation (43% vs 18%) and bradycardias (11% vs 6%) were demonstrated in a significantly higher proportion of ATTR than AL patients. Interestingly, there was no correlation between severity of cardiac involvement by ventricular wall thickness or cardiac biomarkers and arrhythmia frequency in ATTR amyloidosis, and nor did conduction disturbance correlate with mortality. Survival was significantly poorer among patients with AL compared to ATTR despite fewer complex ventricular arrhythmias in this patient group (P=0.002). Conclusions: Arrhythmias, including disturbances of rhythm that necessitate pacemaker insertion and complex ventricular tachycardias, are common in patients with ATTR amyloidosis but their influence on patient survival in this patient group remains uncertain.

C-08 - LEFT VENTRICULAR STRUCTURE AND FUNCTION IN TTR-RELATED VERSUS AL CARDIAC AMYLOIDOSIS

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Background. Immunoglobulin light chain (AL)-related cardiac amyloidosis (CA) has a far worse prognosis than either wild type (ATTRwt) or mutant (ATTRm) transthyretin (TTR) CA. Detailed echocardiographic studies have been performed in AL, but not in TTR amyloidosis, and might give insight into this difference. We assessed cardiac structure and function in a large population of patients with CA and compared the profiles of TTR and AL-related disease. Methods. We analyzed 172 patients affected by CA (AL, n=80; ATTRm, n=36; ATTRwt, n=56) with standard echocardiography and two-dimensional speckle tracking imaging (STI)-derived left ventricular (LV) longitudinal (LS), radial (RS) and circumferential strain (CS). Results. Despite an overall preserved LV ejection fraction (56 [47-65]%), LS was severely impaired in CA. Standard parameters of systolic and diastolic function as well as STI worsened as wall thickness increased, while apical LS was preserved irrespective of the etiology of CA and the entity of wall thickening. Compared to ATTRm and AL, ATTRwt was characterized by a greater mean LV wall thickness and a lower ejection fraction. LS was more depressed in ATTRwt and AL (respectively -11[-13,-9]% and -11[-15,-10]%, p=0.17), than in ATTRm (-15[-18,-11]%, p<0.01 vs. AL and ATTRwt). Conclusions. In patients with cardiac amyloidosis, worsening functional parameters correlated with increasing wall thickness regardless of etiology. Despite a statistically greater wall thickness, ATTRwt had a very similar pattern of myocardial dysfunction to AL amyloidosis, including the typical “apical sparing pattern” of regional strain. This paradox suggests an additional mechanism for LV dysfunction in AL amyloidosis, such as previously demonstrated light-chain toxicity.
C-09 - 3,3'-DIPHOSPHONO-1,2-PROPANODICARBOXYLIC ACID (99mTC-DPD) SCINTIGRAPHY IN SYSTEMIC AMYLOIDOSIS IN 321 PATIENTS

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99mTc-DPD scintigraphy is a sensitive method for imaging cardiac transthyretin (ATTR) amyloid. We report our findings of 99mTc-DPD scintigraphy in 321 patients with suspected cardiac amyloidosis assessed at the UK National Amyloidosis Centre. The cohort included: wild type ATTR (ATTRwt) amyloidosis in 94 (29%); ATTR-Val122Ile amyloidosis in 38 (12%); hereditary ATTR (ATTRh) amyloidosis in 46 (14%); primary light chain (AL) amyloidosis in 44 (14%); secondary (AA) amyloidosis in 3 (1%); other types of hereditary amyloidosis in 9 (3%); undetermined types in 2 (0.5%); and 85 (26.5%) patients in whom systemic amyloidosis was ultimately excluded. All 158 patients with ATTR amyloidosis with clinical cardiac involvement had cardiac 99mTc-DPD uptake, with median Perugini grade 2 uptake. Thirteen further ATTR amyloidosis patients without other evidence of cardiac involvement also demonstrated 99mTc-DPD cardiac uptake. 18/35 (51%) of AL patients with cardiac involvement had 99mTc-DPD cardiac uptake, with median grade 1 uptake. Extensive soft tissue uptake was frequently seen in ATTR amyloidosis, especially ATTRwt and ATTR− Val122Ile types suggesting a wider clinical phenotype than previously thought. Diffuse soft tissue uptake appears to account for the modulation in apparent bone signal and identifies muscle as a hitherto unrecognised site that merits investigation as a target organ in ATTR amyloidosis. 99mTc-DPD scintigraphy is emerging as an important investigation in the diagnostic pathway of patients with suspected cardiac amyloidosis but must be interpreted in a broad context to avoid diagnostic errors.

C-10 - SUBCLINICAL NEUROLOGICAL ABNORMALITIES IN SENILE SYSTEMIC AMYLOIDOSIS AND IN HEREDITARY ATTR AMYLOIDOSIS WITH “CARDIOGENIC” MUTATIONS.

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BACKGROUND: Transthyretin (TTR)-related amyloidosis is a highly heterogeneous disease at both phenotypic and genotypic level. Phenotype associated with some TTR mutations (typically Ile122Leu and Ile68Leu) and with wild-type TTR is generally considered as exclusively or dominantly “cardiac”. However, detailed neurologic assessments of these patients are not generally available. This study aimed to evaluate the presence of subclinical neuropathy in these patients at presentation and after mid-term follow-up. METHODS: 34 patients with cardiologic phenotype (19 ATTR-Ile68Leu and 15 SSA, median age 73 [66-79]) were assessed at our Cardiac Amyloidosis center and also received a detailed clinical evaluation by a dedicated neurologist. RESULTS: 18 patients (53%) self-reported neurologic symptoms including: carpal tunnel syndrome (CTS) (8 ATTR-Ile68Leu, 7 SSA), paresthesia (1 ATTR-Ile68Leu, 1 SSA), ipo-anhidrosis (1 ATTR-Ile68Leu), stypsis (1 ATTR-Ile68Leu), restless legs syndrome (1 ATTR-Ile68Leu), walking disability (1 ATTR-Ile68Leu). Among these patients, in 72% of cases (8 ATTR-Ile68Leu and 5 SSA) symptoms related to CTS anticipated diagnosis of amyloidosis up to 10 years. Dedicated neurologic evaluation disclosed abnormal findings in 18 patients overall (9 ATTR-Ile68Leu, 9 SSA), including 4 cases (25%) among patients with no self-reported neurologic symptoms (2 ATTR-Ile68Leu and 2 SSA). The abnormalities included: tactile and/or termodolorific hypo/anesthesia, hypo/apallesthesia and hypo/asthenia. At mid-term follow-up (12 months), no one of these patients showed a progression of baseline pathologic findings. CONCLUSIONS: Although 53% of patients with “cardiac phenotype” present self-reported symptoms or neurologic abnormalities, these problems are mild and seemingly non progressive at a medium follow up. CTS is the most frequent abnormality and can precede cardiac symptoms by months/years. Importantly, in 25% of asymptomatic patients an accurate neurologic examination uncovered subclinical pathological findings.
C-11 - AL AND ATTR CARDIAC AMYLOID ARE DIFFERENT: INSIGHTS FROM CARDIAC MRI T1 MAPPING

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Background: Cardiac involvement drives prognosis in amyloidosis. The new Cardiac MRI technique “ECV mapping” directly quantitates interstitial expansion and hence myocardial amyloid burden. “Native T1” is also elevated in amyloid but reflects both cell and interstitial changes. The combination gives insight into amyloid burden and the myocyte response. Previously we have shown the utility of both techniques in AL amyloidosis. Here, we explore the differences between AL and ATTR types.

Methods: 3 groups were studied: ATTR amyloid patients (n=102; age 72±10); transthyretin mutations carriers (n=8; age 47±6); AL amyloid patients (n=81; age 62±10). These were compared with 52 healthy volunteers and 43 patients with hypertrophic cardiomyopathy (HCM). All underwent T1 mapping and ECV measurement. ATTR patients and mutation carriers also underwent DPD scintigraphy. Results: ECV was massivley elevated in ATTR patients compared to HCM and healthy volunteers (0.58±0.06 ms vs 0.37±0.12 ms vs 0.27±0.03ms, both p>0.0001). In established cardiac ATTR amyloidosis, ECV elevation was higher than AL amyloidosis (AL 0.53±0.07ms, p=0.008) (Figure 1). Conversely, T1 was lower in TTR than AL amyloidosis (Figure 1). Diagnostic performance of ECV was similar for AL and TTR (vs HCM: AL AUC 0.824 (0.745-0.902); TTR AUC 0.805 (95%CI 0.748-0.862); both P<0.0001). ECV tracked cardiac amyloid burden as determined by DPD scintigraphy (figure 2). ECV was not elevated in mutation carriers (0.27±0.02 ms) but was in isolated DPD grade 1 (n=8, 0.37±0.09 ms, p=0.001). Correlations between ECV and other parameters showed specific differences between AL and ATTR (Table 1). Conclusion: ECV detects cardiac ATTR amyloid with similar diagnostic performance and disease tracking to T1. The ECV is higher in TTR – i.e. there is proportionately more amyloid in TTR than AL hearts. However, native T1 is lower in TTR than AL suggesting differences in the myocyte response to amyloid. This is supported by the ECV tracking PR and QRS duration lengthening in TTR, but limb lead voltage falls in AL.

Table 1 - Correlations between ECV, cardiac function, biomarkers, ECG and 6 MWT in ATTR and AL amyloidosis patients.

<table>
<thead>
<tr>
<th></th>
<th>ATTR patients (R)</th>
<th>AL patients (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV structure by MRI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV mass, g/m²</td>
<td>0.695*</td>
<td>0.435*</td>
</tr>
<tr>
<td>LA area, cm²/m²</td>
<td>0.410*</td>
<td>0.258†</td>
</tr>
<tr>
<td>LV systolic function by CMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LV EF, %</td>
<td>-0.523*</td>
<td>0.504*</td>
</tr>
<tr>
<td>SV, ml/m²</td>
<td>-0.428*</td>
<td>0.312†</td>
</tr>
<tr>
<td>LV diastolic function by echo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E/E'</td>
<td>0.512*</td>
<td>0.365*</td>
</tr>
<tr>
<td>E-deceleration time, ms</td>
<td>-0.240 †</td>
<td>-0.252†</td>
</tr>
<tr>
<td>6 minutes walking test</td>
<td>-0.357*</td>
<td>Ns</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP, pmol/L</td>
<td>0.789*</td>
<td>0.670*</td>
</tr>
<tr>
<td>Troponin T, pmol/L</td>
<td>0.681*</td>
<td>0.531*</td>
</tr>
<tr>
<td>ECG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR, ms</td>
<td>0.472*</td>
<td>0.234*</td>
</tr>
<tr>
<td>QRS, ms</td>
<td>0.283*</td>
<td>Ns</td>
</tr>
<tr>
<td>ECG limb lead mean voltage</td>
<td>-0.263 †</td>
<td>-0.242 *</td>
</tr>
</tbody>
</table>

* P< 0.01 level; †P<0.05;

Figure 1. ECV (left panel) and native myocardial T1 (right panel) in healthy volunteers, HCM, definite AL and definite ATTR amyloidosis.

Figure 2. ECV (left panel) and native myocardial T1 (right panel) versus DPD scintigraphy.
C-12 - ASSESSMENT OF RIGHT VENTRICULAR FUNCTION IN DIFFERENTIATING PATIENTS WITH THICKENED SEPTUM DUE TO HEREDITARY TRANSTHYRETIN AMYLOIDOSIS AND HYPERTROPHIC CARDIOMYOPATHY

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Background: Reduced right ventricular (RV) function has been found in both hypertrophic cardiomyopathy (HCM) and light chain (AL) amyloidosis and has also shown to be prognostic in the latter. As there are difficulties in differentiating between HCM and the hereditary transthyretin (ATTR) type of amyloidosis using echocardiography in general practice, we aimed to investigate if RV function could separate these two conditions. Methods: We investigated 33 subjects with abdominal fat biopsy verified ATTR and septal thickness > 12 mm, 20 HCM patients all with septal thickness > 12 mm, and 40 healthy controls. Diastolic RV wall thickness was measured from the subcostal projection. Tricuspid annular plane systolic excursion (TAPSE) was measured from M-mode registrations. Pulsed tissue Doppler echocardiography was used to measure the systolic component (s’) and isovolumetric relaxation time, which was corrected for heart rate (RV IVRTcorr). Finally, we used speckle tracking echocardiography to measure RV free wall deformation. As patients with HCM were younger, this was corrected for in the comparison between ATTR and HCM (three-group ANOVA, with a simple contrast for post-hoc comparisons) Results: RV wall thickness was slightly, but not significantly, increased in ATTR patients compared to HCM patients. RV s’ and TAPSE were not different between the two groups. However, in ATTR group RV IVRTcorr was prolonged (p=0.04) and RV deformation decreased (p=0.007) in comparison to HCM. Conclusion: In ATTR, RV deformation was found to be decreased and RV IVRTcorr prolonged in comparison to HCM. These findings might be of importance in differentiating the conditions in clinical practice. It is however unclear whether these findings depend on differences in the magnitude of hypertrophy or if they reflect pathophysiological differences between the two conditions.

Table 1. Echocardiographic findings in patients with ATTR amyloidosis, hypertrophic cardiomyopathy and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>ATTR</th>
<th>HCM</th>
<th>Controls</th>
<th>ATTR - vs HCM p-value (age corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>33</td>
<td>20</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>66 ± 9</td>
<td>51 ± 13</td>
<td>60 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RV wall thickness, mm</td>
<td>5.5±1.3</td>
<td>4.8±1.0</td>
<td>4.2±0.8</td>
<td>0.07</td>
</tr>
<tr>
<td>TAPSE, mm</td>
<td>22 ± 5</td>
<td>23 ± 4</td>
<td>25 ± 3</td>
<td>0.20</td>
</tr>
<tr>
<td>RV IVRTcorr, ms</td>
<td>66 ± 38</td>
<td>38 ± 40</td>
<td>22 ± 22</td>
<td>0.04</td>
</tr>
<tr>
<td>RV s’, cm/s</td>
<td>11.9±3.5</td>
<td>12.7±2.3</td>
<td>12.3±1.7</td>
<td>0.53</td>
</tr>
<tr>
<td>RV strain, %</td>
<td>-24 ± 6</td>
<td>-30 ± 4</td>
<td>-28 ± 7</td>
<td>0.007</td>
</tr>
</tbody>
</table>

C-13 - DESCRIPTION OF A NOVEL TRANSTHYRETIN VARIANT WITH CARDIAC INVOLVEMENT: A STUDY OF STRUCTURAL PREDICTION

Cinthia Lima 1, Priscila Ferreira 1, Nathalia Varejão 1, Ricardo Santanna 1, Concy Maya Caldeira 2, Franklin D. Rumjane 1, 2, Marcia Waddington Cruz 3, Debra Fouguel 1
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Mutations in the TTR gene are known to destabilize the structure of protein and facilitate the aggregation process causing an amyloidosis which can be characterized by the involvement of peripheral nerves, cardiac function and other disorders. Here we report a patient from Santa Catarina, state in the south region of Brazil, and his family is from Swedish/German origin, with a rare mutation in exon 2 of TTR gene where we have a substitution of a Ala for a Asp at the codon 19, causing a severe compromise of cardiac function characterizing the Familial Amyloidotic Cardiomyopathy (FAC). To predict the stability of this mutant we use bioinformatics tools how FoldX and PDBSum to analyze the thermodynamic stability of this mutant. We predicted that the tetramers of A19D presented a decreased thermodynamic stability, when compared to the WT-TTR, and is more amyloidogenic than V30M tetramers. The PDBSum analyses demonstrated that the strongest interface of TTR (AB) loses two hydrogen bonds and drastic changes in the orientation of some amino acids in C2 axis can facilitate the dissociation process in this interface. Taken together, all the structural changes imposed by the mutation A19D to the tetramers resulted in a destabilized protein that presents alterations in the monomer-monomer and dimer-dimer interface. Indeed the patient that presents this mutation has a severe cardiomyopathy which developed very fast in a short period of time. We describe for the first time in Brazil this mutation.
C-14 - Pre-existing pacemaker implantation at time of diagnosis of ATTRwt is associated with poorer survival.

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Background: Wild type transthyretin cardiac amyloidosis (ATTRwt) is increasingly recognised as cause of heart failure in the older (particularly male) population. Referrals to our centre have increased 3 fold in the last 5 years. Although the prognosis is better than that of cardiac AL amyloidosis, median survival is typically only 3-5 years. We have previously found that patients with a pacemaker in situ at time of diagnosis have worse survival. We sought to determine whether this reflected late presentation or possible causality. Methods and Results 125 patients with biopsy proven ATTRwt seen at the UK National Amyloidosis Centre between 2002 and 2012 were included. Echocardiography findings and pacemaker status at time of diagnosis were analysed. Severity of diastolic dysfunction and left ventricular wall thickness were assessed for each patient. 17 (14%) patients had a pacemaker in situ at the time of diagnosis. 11 (9%) patients subsequently went on to have a pacemaker implanted during follow up. Out of these 28 patients with pacemakers, 13 (46%) died during follow up compared with 34 (35%) of the 97 patients without a pacemaker. Median follow up was 22 months in both groups. Diastolic and systolic function determined by echocardiography at the time of diagnosis showed the following: of the 4 patients with normal diastology (E/E’15), 13 had a pacemaker in situ and 6 subsequently required one. Of those patients with borderline increased left ventricular posterior wall (LVPW) thickness (1.7cm), 6 had a pacemaker in situ and 13 subsequently required one. Conclusion Patients with a pacemaker in situ at time of presentation have a poorer prognosis than other patients. The associated wide range of echocardiography findings suggests that the requirement for pacemakers does not simply reflect more severe disease, raising the possibility that device implantation may itself have an adverse effect on outcome. Right ventricular pacing is known to be associated with decreased survival in other types of heart failure. The role of cardiac resynchronisation therapy should be explored to determine if it is of benefit in ATTRwt patients.

C-15 - PREVALENCE, INCIDENCE, DETERMINANTS AND PROGNOSTIC ROLE OF ATRIAL FIBRILLATION IN AMYLOIDOTIC CARDIOMYOPATHIES

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Purpose. The study was aimed to evaluate prevalence, incidence, risk factors for atrial fibrillation (AF) and prognostic significance of AF in the three main etiological subgroups of Amyloidotic Cardiomyopathy (AC): light-chain (AL), hereditary transthyretin-related (mATTR) and non-mutant transthyretin-related (wtATTR). Methods. We studied 262 patients with AC (123 AL, 94 mATTR, 45 wtTTR) and assessed clinical and instrumental details at presentation. Results. Prevalence of AF at first evaluation was 14.5% overall: 8.9% in AL, 10.6% in mATTR and 37.8% in wtTTR. During a median follow up of 2.5 (IQR 0.18–5.8) years, 11 other patients developed AF (2.1% person-years). At univariate analysis, age (OR 1.10, 95% CI 1.04–1.15), NYHA class III-IV (OR 3.96, 95% CI 1.53–10.28), wtATTR etiology (OR 3.05, 95% CI 1.04–8.95), left ventricular ejection fraction (LVEF) (OR 0.96, 95% CI 0.93–0.99), left atrial (LA) diameter (OR 1.11, 95% CI 1.04–1.19) right atrial pressure (RAP) (OR 1.14, 95% CI 1.05–1.24) and pulmonary capillary wedge pressure (PCWP) (OR 1.08, 95% CI 1.02–1.15) were associated (p<0.01) with the risk of AF. At multivariate analysis however, only age (OR 1.14, 95% CI 1.06–1.22), LVEF (OR 0.95, 95% CI 0.90–0.99) and RAP (OR 1.15, 95% CI 1.02–1.30) remained associated as independent variables. Left ventricular wall thickness was not associated with AF in any of the three etiological subgroups. Although mortality was not increased, survival free from heart failure was reduced among patients with AF. Conclusions. Prevalence of AF at presentation was 15%, with a maximum value of 40% in ATTRwt. Incidence was 2.1% person-years. Heart failure, age, LVEF, LA diameter and mean RAP were the main risk factors for the development of AF (RAP more important than LA diameter). AF is an important determinant of heart failure even though it does not appear to influence mortality.
C-16 - CARDIAC STRUCTURE AND FUNCTION AND OUTCOME OF AFRICAN-AMERICANS CARRYING THE AMYLOIDOGENIC V122I TRANSTHYRETIN MUTATION: THE ARIC (ATHEROSCLEROSIS RISK IN COMMUNITIES) STUDY

Candida Cristina Quarta 1,2, Joel N. Buxbaum 3, Rodney H. Falk 4, Dalane Kitzmann 5, Amil M. Shah 1, Thomas H. Mosley 6, Ervin R. Fox 7, Kenneth R. Butler 6, Scott D. Solomon 1

1 Cardiovascular Division, Brigham and Women’s Hospital (Boston, MA), 2 Cardiology, DIMES, Alma Mater University of Bologna, Italy (Bologna, Italy), 3 The Scripps Research Institute (La Jolla, CA), 4 Harvard Vanguard Medical Associate (Boston, MA.), 5 Cardiology and Geriatrics, Wake forest School of Medicine (Winston-Salem, NC), 6 Department of Medicine-Geriatrics, University of Mississippi (Jackson, MS), 7 Cardiovascular Disease Division, University of Mississippi (Jackson, MS)

Background. As many as 3-4% of African Americans carry an amyloidogenic TTR mutation, V122I, typically characterized by a late-onset restrictive amyloid cardiomyopathy and congestive heart failure (HF). Before the age of 65 the allele has no discernible impact on cardiac function or mortality. We aimed to determine the clinical significance of the V122I transthyretin (TTR) mutation in African-Americans older than 65 years. Methods. We compared the clinical profiles, mortality and incident HF rates of 115 TTR mutation carriers in relation to 3593 non-carriers previously identified in the Atherosclerosis Risk in Communities (ARIC) study. Cardiac structure, function, and occurrence of features of cardiac amyloidosis were assessed in 45 carriers and 1113 non-carriers who underwent echocardiography during visit 5 (2011-2013), when they were older than 65 years. Results. There were no major differences between carriers and non-carriers in clinical profiles, mortality or frequency of congestive HF. Although carriers did not display more clinical and echocardiographic findings consistent with cardiac amyloidosis than non-carriers, carriers had slightly larger left ventricular size and reduced diastolic function and higher values of NT-proBNP (140 [60-424] vs. 93 [45-189] pg/mL, p=0.02). Also, despite similar ejection fraction, systolic function assessed by tissue Doppler and strain imaging were slightly worse in carriers. After a mean follow-up of 18±6 years, adjusting for age and gender, the presence of the V122I TTR mutation did not increase the risk of all-cause mortality (HR 0.97, 95% CI 0.69-1.35, p=0.85) or incident HF (HR 1.37, 95% CI 0.92-2.0, p=0.12). Conclusions. Despite the high prevalence of the TTR V122I allele in African-Americans, the penetrance of the disease over the age of 65 years was low. Contrary to what previously suggested, the presence of the V122I TTR mutation did not impact the frequencies of mortality or incident HF.

<table>
<thead>
<tr>
<th></th>
<th>TTR V122I non carriers (n=1113)</th>
<th>TTR V122I carriers (n=45)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>75 ± 5</td>
<td>74 ± 4</td>
<td>0.15</td>
</tr>
<tr>
<td>Male, n [%]</td>
<td>390 (35)</td>
<td>3 (20)</td>
<td>0.037</td>
</tr>
<tr>
<td>Hypertension, n/N [%]</td>
<td>820/1075 (76)</td>
<td>25/43 (67)</td>
<td>0.18</td>
</tr>
<tr>
<td>Smoking, n/N [%]</td>
<td>79/1076 (7)</td>
<td>3/43 (7)</td>
<td>0.98</td>
</tr>
<tr>
<td>Diabetes, n/N [%]</td>
<td>405/1069 (38)</td>
<td>16/44 (35)</td>
<td>0.37</td>
</tr>
<tr>
<td>Total cholesterol, n/N [%]</td>
<td>650/1067 (61)</td>
<td>21/43 (49)</td>
<td>0.1</td>
</tr>
<tr>
<td>Interventricular septum thickness, cm</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Posterior wall thickness, cm</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.78</td>
</tr>
<tr>
<td>Left ventricular diastolic diameter, cm</td>
<td>4.3 ± 0.5</td>
<td>4.4 ± 0.6</td>
<td>0.33</td>
</tr>
<tr>
<td>Left ventricular systolic diameter, cm</td>
<td>2.6 ± 0.5</td>
<td>2.8 ± 0.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>64 ± 7</td>
<td>63 ± 11</td>
<td>0.15</td>
</tr>
<tr>
<td>Left atrial volume index, ml/m²</td>
<td>25 ± 9</td>
<td>27 ± 9</td>
<td>0.42</td>
</tr>
<tr>
<td>E/E' ratio</td>
<td>10 ± 4</td>
<td>11 ± 5</td>
<td>0.05</td>
</tr>
<tr>
<td>s' wave, cm/s</td>
<td>7.2 ± 1.6</td>
<td>5.6 ± 1.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Left ventricular longitudinal strain, %</td>
<td>-17 ± 3 (n=580)</td>
<td>-16 ± 3 (n=41)</td>
<td>0.05</td>
</tr>
<tr>
<td>Left ventricular radial strain, %</td>
<td>25 ± 8 (n=580)</td>
<td>22 ± 7 (n=41)</td>
<td>0.05</td>
</tr>
<tr>
<td>Left ventricular circumferential strain, %</td>
<td>-26 ± 5 (n=465)</td>
<td>-25 ± 7 (n=41)</td>
<td>0.44</td>
</tr>
</tbody>
</table>
C-17 - THE ETIOLOGY OF AMYLOIDOSIS INFLUENCES THE PATHOPHYSIOLOGY AND OUTCOME OF HEART FAILURE IN AMYLOIDOID CARDIOMYOPATHY

Candida Cristina Quarta 1, Simone Longhi 1, Christian Gagliardi 1, Agnese Milandri 1, Nelson Gentile 1, Lisa Manuzzi 1, Ilaria Bartolomei 1, Fabrizio Salvi 1, Claudio Rapezzi 1
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Background. Irrespectively of the specific etiology of cardiac amyloidosis (CA), heart failure (HF) has been traditionally attributed to diastolic dysfunction. Risk factors and role of the different amyloid subtypes of CA in the pathophysiology of HF remain undefined. Methods. We analyzed the profile and outcome of patients who presented at our Centre in 1990-2011 with advanced HF (NYHA III-IV) due to the three main etiologies of CA: light-chain (AL), mutant (ATTRm) and wild type transthyretin (ATTRwt) amyloidosis. Results. Ninety-nine of 273 (36%) patients diagnosed with CA at our Center presented with advanced HF and showed severe symmetric LV wall thickening (higher values in ATTRm), non-dilated LV and preserved EF (lower values in ATTRwt). Hemodynamically, elevated filling pressures on both cardiac sides were present in all etiologies. Histologically, AL showed small vessels involvement in 89% of cases; ATTRwt showed inflammatory infiltrates in 1/3 of cases. Survival at 2 years was 39% for AL, 80% for ATTRm, 89% for ATTRwt. Freedom from MACE was 23% for AL, 56% for ATTRm, 41% for ATTRwt. ATTRm and ATTRwt were favourable predictors of survival; RFP and creatinine were negatively associated with overall survival and MACE, respectively. Conclusions. In AC, both pathophysiology of HF and outcome are influenced by the etiology of amyloidosis. Despite shorter disease duration and lesser LV wall thickening, AL amyloidosis shows the worst outcome probably due to a combination of interstitial infiltration and light chains cardiotoxicity.

C-18 - LATE ONSET CARDIOMYOPATHY DUE TO TRANSTHYRETIN ILE68LEU MUTATION: A CARDIOGENIC VARIANT OF FAMILIAL AMYLOIDOSIS POTENTIALLY MIMICKING SARCOMERIC HYPERTROPHIC CARDIOMYOPATHY

Candida Cristina Quarta 1, Agnese Milandri 1, Simone Longhi 1, Francesco Cappelli 2, Stefano Perlini 3, Laura Obici 3, Ilaria Bartolomei 1, Fabrizio Salvi 1, Giampaolo Merlini 3
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Background. In hereditary transthyretin (TTR) amyloidosis (ATTR), cardiac involvement usually occurs in association with neurologic signs. A few variants responsible for isolated cardiomyopathy have been identified in well-defined populations (V122I in African-Americans, L111M in Denmark). We describe a TTR variant, the I68L, responsible for an exclusively cardiac phenotype in Caucasian patients. Methods. We retrospectively analyzed baseline clinical, ECG, echocardiographic characteristics and outcome of I68L-related ATTR patients diagnosed in 1990-2012 at three large Italian Centers. Results. Of 190 patients diagnosed with ATTR during the study period, 34 (18%) carried the I68L mutation: 31 (91%) were men, age was 69 [64-72] years. All patients were from a large area in the Central-Northern part of Italy (around 20000 Km2) and belonged to 31 unrelated families. Haplotype analysis (performed in twenty subjects) showed a shared haplotype in all. All patients sought medical attention due to cardiac symptoms, with marked heart failure in 44% of cases. Echocardiographically, they showed symmetric left ventricular (LV) "hypertrophy" (septal thickness=17±3mm, posterior wall=16±3mm), with normal LV cavity size (diastolic LV diameter=49±9mm), enlarged left atrium (47±7mm), slightly reduced LV ejection fraction (47±12%). Low QRS voltage was present in 35% of cases. Carpal tunnel syndrome was the only extracardiac manifestation (35%). Five patients had subclinical sensory-neuropathy, not responsible for seeking medical attention. During a follow-up of 23[11-43]months, 12 patients (35%) died due to progression of heart failure; no patients developed major neurologic symptoms. Conclusions. In addition to Val122Ile and Leu111Met, TTR Ile68Leu mutation represents a "cardiogenic" variant of ATTR, responsible for an exclusively cardiac phenotype in this heterogeneous disease. Ile68Leu is endemic in Northern Italy and could have been inherited from a common ancestor. Awareness of this variant is essential to avoid misdiagnosing ATTR cardiac amyloidosis with other causes of LV hypertrophy, such as hypertrophic cardiomyopathy, in this relatively elderly population.
C-20 - MOLECULAR IMAGING OF CARDIAC AMYLOIDOSIS WITH F18 FLORBETAPIR PET/CT AND Tc99m PYROPHOSPHATE SPECT/CT

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Purpose: To test the ability of molecular imaging agents F18 Florbetapir and Tc99m PYP to identify and differentiate AL and TTR cardiac amyloidosis. Methods: Four patients with TTR cardiac amyloidosis and four patients with AL cardiac amyloidosis were selected prospectively and compared with control patients. All patients were required to have biopsy proven presence or absence of amyloid. Patients were imaged with dynamic F18 Florbetapir PET/CT and 3 hour Tc99m PYP SPECT/CT. Results: Florbetapir PET/CT showed more activity in both AL and TTR laden myocardium than in myocardium of control patients. PYP SPECT/CT showed more activity in TTR laden myocardium than in AL laden or control myocardium. Florbetapir PET/CT showed activity in non-cardiac tissue in AL patients as well, consistent with the known pattern of AL deposition. Conclusions: Florbetapir PET/CT and PYP SPECT/CT may help identify and differentiate the two most common forms of cardiac amyloid.

C-21 - EVOLUTION OF ECHOCARDIOGRAPHIC PARAMETERS IN TTR FAP PATIENTS TREATED WITH TAFAMIDIS

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BACKGROUND: Tafamidis has shown promising effects on neurological symptoms in TTR FAP, but little is known regarding its effects on cardiac infiltration. We performed a longitudinal survey of the main cardiac echo parameters in TTR FAP patients treated by Tafamidis. METHODS: The study carried out at the French National Reference of FAP (CRMR-NNERF), included 22 patients with genetically proven symptomatic TTR FAP (68% Val Met 30 mutation , mean age 61[26-87] years) enrolled between February and September 2012. A transthoracic echocardiography was performed on each patient before and after 6 months and 1 year of treatment. RESULTS: At baseline, 11/22 patients had LV septal wall thickness greater than 12 mm. IVS: interventricular septum; LVEDD: left ventricle end diastolic diameter; PW: posterior wall; LA: left atrium; LVEF: left ventricular ejection fraction; DT: deceleration time of the E wave; TDI: tissue Doppler imaging. TAPSE: tricuspid annular plane systolic excursion; PAPs: pulmonary artery systolic pressure. CONCLUSION : There was no significant change of echocardiographic parameters in patients with TTR FAP treated with TAFAMIDIS over a 1 year period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>6 months</th>
<th>1 year</th>
<th>P ANOVA</th>
<th>P (ref vs.6m)</th>
<th>P (ref vs 1y)</th>
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<tr>
<td>N</td>
<td>22</td>
<td>22</td>
<td>17</td>
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<tr>
<td>IVS (mm)</td>
<td>13±5</td>
<td>14±4</td>
<td>13±3</td>
<td>0.61</td>
<td>0.89</td>
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<td>LVEDD (mm)</td>
<td>46±5</td>
<td>46±5</td>
<td>48±5</td>
<td>0.71</td>
<td>0.91</td>
<td>0.52</td>
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<td>PW (mm)</td>
<td>12±4</td>
<td>12±4</td>
<td>12±3</td>
<td>0.35</td>
<td>0.89</td>
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<td>LA area (cm²)</td>
<td>18±6</td>
<td>21±6</td>
<td>21±6</td>
<td>0.39</td>
<td>0.54</td>
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<td>LVEF (%)</td>
<td>63±8</td>
<td>61±6</td>
<td>64±6</td>
<td>0.39</td>
<td>0.82</td>
<td>0.29</td>
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<td><strong>Pulsed Doppler</strong></td>
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<tr>
<td>E wave (cm/s)</td>
<td>74±14</td>
<td>77±19</td>
<td>74±18</td>
<td>0.90</td>
<td>0.88</td>
<td>0.76</td>
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<td>DT msec</td>
<td>242±71</td>
<td>221±52</td>
<td>231±46</td>
<td>0.99</td>
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<td>A wave (cm/s)</td>
<td>77±27</td>
<td>80±29</td>
<td>80±24</td>
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<td>E/A ratio</td>
<td>1.2±0.9</td>
<td>1.1±0.5</td>
<td>1.1±0.6</td>
<td>0.77</td>
<td>0.51</td>
<td>0.57</td>
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<tr>
<td><strong>LV TDI</strong></td>
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<tr>
<td>S wave (cm/s)</td>
<td>7.2±3.1</td>
<td>7.4±3.0</td>
<td>7.6±2.8</td>
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<td>Ea wave (cm/s)</td>
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<td>7.7±3.7</td>
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<td>E/Ea ratio</td>
<td>11±6</td>
<td>12±7</td>
<td>11±6</td>
<td>0.77</td>
<td>0.51</td>
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<td><strong>RV TDI</strong></td>
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<td>TAPSE (mm)</td>
<td>23±4</td>
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<td>22±4</td>
<td>0.34</td>
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<td>S wave (cm/s)</td>
<td>12.6±2.8</td>
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<td>0.78</td>
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<td>PAPs (mmHg)</td>
<td>33±10</td>
<td>36±8</td>
<td>37±14</td>
<td>0.24</td>
<td>0.58</td>
<td>0.25</td>
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C-22 - NON-INVASIVE RISK STRATIFICATION IN PATIENTS WITH TRANSTHYRETIN-RELATED AMYLOIDOSIS

Arnt V. Kristen1, Katrin Scherer2, Sebastian Buss1, Fabian aus dem Siepen1, Sabine Haufe5, Ralf Bauer1, Katrin Hinderhofer3, Evangelos Giannitsis5, Stefan Hardt1, Uwe Haberkorn1, Hugo A. Katus1, Henning Steen1
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Introduction: Transthyretin amyloidosis (ATTR) is characterized by heterogeneous phenotypes, including polyneuropathy and cardiomyopathy, the latter representing an important predictor for outcome. However, data on risk stratification is lacking. We therefore evaluated the impact of non-invasive imaging tools on risk assessment in ATTR. Methods: 70 patients with ATTR were evaluated by echocardiography, cardiac biomarkers, 99mTc-DPD scintigraphy, and magnetic resonance imaging (n=30). Echocardiographic findings and plasma levels of biomarkers were correlated with scintigraphic whole body as well as heart tracer retention. Univariate and multivariate analysis were performed to obtain non-invasive predictors of outcome. Results: WBR of 99mTc-DPD was 79.6 [20.8] % and HR was 6.4 [3.2] %, finally resulting in heart-to-body ratio of 7.8 [3.9] %. WBR, HR, and heart-to-body ratio correlated well with morphological parameters (interventricular septum thickness, LV hypertrophy index) as well as with plasma levels of cardiac biomarkers (troponin T, NT-proBNP). An inverse correlation of WBR, HR, and heart-to-body ratio was observed with functional parameters of amyloid burden (MASV, MAPSE, TAPSE) as well as renal function (table 1). A significant correlation of late gadolinium late enhancement and WBR (r=0.506; p=0.0071) and heart-to-body ratio (8.6 [0.3] vs. 7.0 [0.4]; p<0.05) in SSA. No difference was observed regarding scintigraphy parameters in the subgroup of SSA as compared with TTR-FAC revealed higher HR (7.0 [0.3] vs. 5.8 [0.4]; p<0.01) and heart-to-body ratio (8.6 [0.3] vs. 7.0 [0.4]; p<0.05) in SSA. No difference was observed regarding WBR (8.0 [0.9] vs. 7.4 [0.3]; n.s.). In total, 26 of 70 patients died during median follow-up of 31.0 [38.4] months. By ROC analysis best cut-off values predicting overall survival were 81.6% for WBR, 6.9% for HR, and 7.7% for heart-to-whole-body retention. A significant correlation of WBR, HR, and heart-to-body ratio was observed with functional parameters of amyloid burden (troponin T, NT-proBNP) and heart retention as predictors of outcome; however, by multivariate analysis troponin T remained the only independent predictor of survival (p=0.0332; HR=10.469; 95% CI=1.206-90.902). Conclusions: Quantitative analysis of late gadolinium enhancement correlated with troponin T (r=0.613; p<0.0001) and NT-proBNP (r=0.620; p=0.0006). Comparison of correlation of WBR, HR, and heart-to-body ratio was observed. Late gadolinium enhancement correlated with troponin T (r=0.613; p=0.0001) and NT-proBNP (r=0.620; p=0.0006). Comparison of scintigraphy parameters in the subgroup of SSA as compared with TTR-FAC revealed higher HR (7.0 [0.3] vs. 5.8 [0.4]; p<0.01) and heart-to-body ratio (8.6 [0.3] vs. 7.0 [0.4]; p<0.05) in SSA. No difference was observed regarding WBR (8.0 [0.9] vs. 7.4 [0.3]; n.s.). In total, 26 of 70 patients died during median follow-up of 31.0 [38.4] months. By ROC analysis best cut-off values predicting overall survival were 81.6% for WBR, 6.9% for HR, and 7.7% for heart-to-whole-body ratio, respectively. Univariate Cox regression revealed atrial fibrillation, NT-proBNP, troponin T, eGFR, MAPSE, LV hypertrophy index, and heart retention as predictors of outcome; however, by multivariate analysis troponin T remained as the only independent predictor of survival (HR 16.088, p<0.0001) of the whole patient cohort. Cox regression analysis of the subgroup of patients assessed by cardiac MRI revealed troponin T as the only predictor of survival (p=0.0332; HR=10.469; 95% CI=1.206-90.902). Conclusions: Quantitative analysis of tracer retention is capable of characterizing severity of cardiac involvement in ATTR; however, it was not indicative for outcome. By multivariate analysis troponin T remained the only independent predictor of survival. The potential role of cardiac magnetic resonance imaging needs to be established in a larger patient cohort.

Table 1: Correlation of scintigraphy and clinical parameters

<table>
<thead>
<tr>
<th></th>
<th>whole body retention</th>
<th>heart retention</th>
<th>heart to whole body retention</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>IVS</td>
<td>0.440</td>
<td>0.0002</td>
<td>0.533</td>
</tr>
<tr>
<td>LV hypertrophy index</td>
<td>0.425</td>
<td>0.0003</td>
<td>0.455</td>
</tr>
<tr>
<td>troponin T</td>
<td>0.357</td>
<td>0.0032</td>
<td>0.549</td>
</tr>
<tr>
<td>logNT-proBNP</td>
<td>0.634</td>
<td>&lt;0.0001</td>
<td>0.660</td>
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<tr>
<td>MASV</td>
<td>0.364</td>
<td>0.0021</td>
<td>0.526</td>
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<tr>
<td>MAPSE</td>
<td>0.250</td>
<td>0.0379</td>
<td>0.470</td>
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<tr>
<td>TAPSE</td>
<td>0.302</td>
<td>0.0125</td>
<td>0.326</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.525</td>
<td>&lt;0.0001</td>
<td>0.478</td>
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IVS thickness of interventricular septum; LV left ventricular; MAPSE mitral annular plane systolic excursion; TAPSE tricuspid annular plane systolic excursion; eGFR estimated glomerular filtration rate
C-23 - FREQUENCY OF CARDIOVASCULAR INVOLVEMENT IN HEREDITARY TRANSTHYRETIN-RELATED AMYLOIDOSIS IN BRAZILIAN PATIENTS

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1UFRJ - Federal University of Rio de Janeiro (Cidade Universitária - Ilha do Fundão)

Background: Hereditary transthyretin-related amyloidosis is a rare disease diagnosed worldwide, including Brazil. Exact frequency of cardiovascular involvement in Brazilian patients is unknown. This study had the intention of detecting the frequency of this involvement and correlating it with the walking disability. Methods: In a Brazilian national reference centre, the "Centro de Estudos em Paramiloidose Antônio Rodrigues de Mello", 48 patients were evaluated (ten of them already transplanted). They were submitted to clinical cardiological evaluation, electrocardiogram (ECG), echocardiogram (ECO) and Holter. All of them were classified according to the modified polyneuropathy disability (PND) score. They were shared in three groups: 1- Those with PND (or walking disability) zero, 2- Those with walking disability ≥ I without liver transplantation and 3- Those with walking disability ≥ I with liver transplantation. Correspondence analysis was the statistical method applied in order to investigate the associations between those groups and the various possible combinations of altered cardiovascular exams. Result: Patients with walking disability zero had a greater correspondence with all the exams normal. Patients with walking disability ≥ I with or without liver transplantation had a greater correspondence with all exams altered. The electrocardiogram was abnormal in the groups with more severe neurological involvement. Conclusions: This study seems to indicate a relationship between severity of neurological involvement and presence of cardiovascular involvement. Electrocardiogram and Holter seems to best discriminate between asymptomatic carriers of the mutation and patients with the disease.

Table 1: Frequencies of cardiovascular altered exams in the different groups. 0 means that the exam is normal, 1 means that it is altered.
**C-24 - PROGNOSTIC VALUE OF RIGHT VENTRICULAR SYSTOLIC FUNCTION IN CARDIAC AMYLOIDOSIS ACCORDING TO ITS ETIOLOGY**

Diane Bodez 1, Julien Ternacle 1, Stéphane Rappeneau 1, Aurélia Lamine 1, Aziz Guellich 1, Soulef Guendouz 1, Jean-Luc Dubois-Randé 1, Luc Hittinger 1, Violaine Planté-Bordeneuve 1, Thibaud Damy 1

1 AmN - Amyloidosis Mondor Network (Créteil, France)

**Background.** Right ventricular (RV) systolic echocardiographic parameters are routinely used in chronic heart failure to identify patients with bad prognosis. There is a lack of data in the specific context of cardiac amyloidosis.

**Methods** Among a cohort of 229 patients consecutively referred to our French amyloidosis network for a diagnosis work-up, we identified 107 patients, by a standard two-dimensional echocardiography, with increased interventricular septal (IVS) thickness ≥12mm and all RV systolic function echocardiographic parameters measurable (tricuspid annular plane systolic excursion (TAPSE), lateral tricuspid annulus peak systolic wave (S'p) measured by tissue Doppler imaging and global and regional RV longitudinal strain (LS') of whom 62 had cardiac amyloidosis (CA) with a proven biopsy and 45 had a negative biopsy (controls). **Results.** The mean±SD age, of the 107 selected patients, was 68.8±13.7 years, 76% were male, mean left ventricular ejection fraction was 54±14%, and mean IVS thickness was 14.9±5mm. There was no difference between the two groups. Origin of amyloidosis was transthyretin-related hereditary (ATTR, n=26), senile (wild type TTR, n=16) or linked to immunoglobulin light chain (AL, n=20). CA patients had significant lower TAPSE (16±5mm vs 20±6mm, p=0.001) and S'p (8.8±3.3 vs 10.4±4.1, p=0.04) than controls. There was no difference in global or segmental RVLS between CA and controls. Among CA, TAPSE and S'p were correlated with all RVLS values (p<0.01 for all). Finally, among patients with CA during one year of follow-up, TAPSE, S'p, were significantly lower in patients presenting outcome (death, cardiogenic shock or acute cardiac decompensation), compared to those who did not, whereas global and segmental RVLS were not. **Conclusions.** Standard two-dimensional RV systolic echocardiographic parameters are impaired in cardiac amyloidosis and more relevant than RVLS to identify patients at higher risk of outcome.

**C-25 - CLINICAL, ELECTROCARDIOGRAPHIC AND ECHOCARDIOGRAPHIC SIGNS ASSOCIATED WITH INCREASED SEPTAL THICKNESS IN TRANSTHYRETIN AMYLOIDOSIS**

Thibaud Damy 1, Mathew S. Maurer 2, Claudio Rapezzi 3, Violaine Planté-Bordeneuve 1, Onur Karayal 4, Rajiv Mundayat 4, O B Suhr 5, Rodney Falk 6, Arnt V Kristen 7 on behalf the THAOS investigators

1Amyloidosis Network, Department of Cardiology, all at CHU Henri Mondor, and INSERM U955 and clinical investigation center 006, and DHU ATV all at Creteil, France. 2Center for Advanced Cardiac Care, Columbia University Medical Center, New York, NY, USA. 3Institute of Cardiology, University of Bologna and S. Orsola-Malpighi Hospital, Bologna, Italy. 4Pfizer Inc., New York, NY, USA. 5Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden. 6Cardiac Amyloidosis Program, Brigham and Women’s Hospital, Boston, MA, USA. 7Amyloidosis Center, Department of Cardiology, Heidelberg University, Heidelberg, Germany.

**Background:** Signs that could guide cardiologists to suspect cardiac transthyretin amyloidosis (ATTR) in patients with echocardiographic increase in interventricular septal thickness (IVST) are lacking. **Aim:** Identify clinical, electrocardiographic and echocardiographic signs associated with increased IVST measured by echocardiography in ATTR. **Methods:** Analysis of the patients with baseline echocardiography included in the Transthyretin Amyloidosis Outcomes Survey (THAOS) registry (N=1744). Patients were firstly divided to 4 IVST classes according to the American Society of Echocardiography classification adapted to gender (normal, mild, moderate, severe) and secondly in two combined IVST classes (normal-mild and moderate-severe). **Results:** 459 patients were included, of whom 379 had a TTR mutation (m-TTR) and 90 had Wild-Type TTR (WT-TTR). 72% were men. The median (IQR) age was 61(44,72) years. Non-Val30Met and WT-TTR were frequent in moderate (38% and 51%) and severe (51% and 32%) IVST. Median IVST in moderate and severe classes were 15mm(14,16) and 20mm(18,22). In these two classes, combined, the prevalence of patients with age above 55 years was 84%, 81% were men, 85% had blood pressure <140mmHg and 70% had RV hypertrophy (≥7mm). Up to 65% of these patients had cardiac sparkling. Systolic dysfunction (LVEF<50%), transmittal flow restrictive pattern and low voltage were less frequent and observed respectively in 47%, 17% and 31%. **Conclusion:** In patients with increased IVST especially older men with normal systolic blood pressure, RV hypertrophy and sparkling should alert practitioners to the possibility of ATTR. Absence of restrictive pattern and low voltage should not rule out the suspicion.
C-26 - DIAGNOSIS OF CARDIAC AMYLOIDOSIS USING LATE ENHANCED CARDIAC MDCT

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1Amyloidosis network, Henri Mondor University Hospital, Creteil, France ; 2Department of Cardiology, Henri Mondor University Hospital, Creteil, France ; 3Department of Radiology, Henri Mondor University Hospital, Creteil, France ; 4Department of Cardiology, Henri Mondor University Hospital, Creteil, France

Objectives: Evaluate diagnostic value of late-enhanced Multi-Detector Computed Tomography (MDCT) acquisition in cardiac amyloidosis. Methods: Fifteen consecutive patients with amyloidosis (10 Transthyretin Familial amyloidosis and 5 AL amyloidosis) and evidence of cardiac involvement on 1.5 T MRI, and 12 control subjects, were included in this prospective study. All subjects were scanned with a 128-slice MDCT. The MDCT protocol included a prospective triggering (100 kV; 500 mAs) and a delayed images acquisition performed 5 minutes after injection of 1.5 mg/Kg of contrast medium. Myocardial volume (mL) and mean myocardial density (HU) of left ventricle (LV) were calculated from manual contouring of LV boundaries using a dedicated software. Region-of-interests (ROIs) were placed in the left ventricular cavity and in the air outside the patient in order to calculate blood density (UH), myocardial signal-to-noise ratio (SNRmyocardium), blood SNR (SNRblood) and contrast-to-noise ratio (CNR) between myocardium and blood within left ventricular cavity (CNRmyocardium-blood). Results: Myocardial density and SNRmyocardium were significantly (P<0.05) higher in patients with cardiac amyloidosis than in control subjects (117 ± 38 vs. 81 ± 16, and 6.55 ± 2.8 vs. 4.88 ± 1.8, respectively). SNRblood did not exhibit significant difference between amyloidosis patients and control subjects (8.10 ± 3.1 and 7.69 ± 3.0, respectively; P=0.7). CNRmyocardium-blood of amyloidosis patients was significantly lower than this of control subjects (1.55 ± 0.9 and 2.82 ± 1.3; P<0.05). Volume of LV trended to be higher in patients with cardiac amyloidosis (67 ± 46 mL) than in control patients (50 ± 28 mL) but the difference was not statistically significant (P=0.28). Conclusion: Late-enhanced MDCT can detect abnormal myocardial enhancement in patients with cardiac amyloidosis, in comparison to control subjects. This technique could prove useful in case of contraindications for an MRI in patients cardiac amyloidosis who may require permanent pacing.

C-27 - DIAGNOSTIC AND PRONOSTIC VALUE OF T2-WEIGHTED MRI IMAGING IN CARDIAC AMYLOIDOSIS

JF Deux1, A Rahmouni1,2, F Legou1,2, J Mayer1,2, T Damy1,3, V Planté-Bordeneuve1,4
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Objectives: Evaluate diagnostic and prognostic values of T2 imaging in patients with amyloidosis. Methods: Seventy-three consecutive patients with amyloidosis (50 with Transthyretin Familial amyloidosis (TTR) and 23 with AL amyloidosis) and suspicion of cardiac involvement were explored with a 1.5 T cardiac MR imaging. MR protocol included cine imaging, Short Tau Inversion Recovery (STIR) T2-weighted and late gadolinium enhancement (LGE) images. Myocardium T2 signal intensity (SI), T2 myocardial ratio (= myocardial SI/ skeletal muscle SI) and LGE myocardium SI were calculated for all subjects. LGE images were also scored on a four-point scale from 1 (no enhancement) to 4 (diffuse myocardial enhancement). Major Adverse Cardiovascular Events (MACE) were noticed for amyloidosis patients. A control group of 41 patients with normal cardiac MRI was used as control. Results: Median follow-up was 17 months. Mean left ventricular (LV) mass was significantly higher in amyloidosis than in control patients (92 ± 30 vs. 75 ± 25 g/m²; P<0.05). LV ejection fraction were in the same range between amyloidosis and control patients (51 ± 15 vs. 51 ± 12 %. P=NS). Mean myocardium T2 SI and T2 myocardial ratio were significantly (P<0.05) lower in amyloidosis than in control patients (151 ± 43 vs 177 ± 35, and 1.28 ± 0.3 vs 1.41 ± 0.2, respectively). AL amyloidosis patients exhibited significantly (P<0.05) lower myocardium T2 SI and T2 myocardial ratio than TTR amyloidosis patients (137 ± 35 vs. 156 ± 44, and 1.13 ± 0.2 vs. 1.34 ± 0.3). A negative correlation was detected between T2 and LGE myocardium SIs for amyloidosis patients (r=0.28; P<0.05). Patients with a LGE score >2 had significantly (P<0.001) lower myocardium T2 SI and T2 myocardial ratio than the remaining amyloidosis patients (129 ± 38 vs. 168 ± 38, and 1.16 ± 0.3 and 1.39 ± 0.3). Amyloidosis patients with MACE exhibited a lower myocardial T2 SI than those without MACE (134 ± 42 vs. 156 ± 41; P<0.05) in the follow-up. Conclusion: Cardiac amyloidosis is associated with low SI on T2-weighted images. Myocardium T2 SI and T2 myocardial ratio were significantly lower in AL than in TTR amyloidosis patients, and were correlated to intensity of enhancement on LGE images. A low T2 myocardium SI was associated with higher MACE in the follow-up of amyloidosis patients.
C-28 - POSTERIOR LONGITUDINAL STRAIN BY SPECKLE TRACKING ECHOCARDIOGRAPHY FOR DETECTING CARDIAC AMYLOIDOSIS

All at Amyloidosis Mondor - Amyloidosis Mondor Network (Créteil, France)

Objective—Evaluate the accuracy of longitudinal strain (LS) in basal posterior segment for early detection of cardiac involvement in amyloidosis. Methods—Among a cohort of 162 patients with proven amyloidosis on biopsy, we selected 55 patients without evidence of cardiac involvement on standard two-dimensional echocardiography: interventricular septum thickness (IVST) <12mm, and preserved left ventricular ejection fraction (LVEF>50%) and diastolic function. All patients had a comprehensive echocardiography with a global and regional (17 segments) quantification of LS by speckle tracking. Forty-three of these patients had cardiac magnetic resonance imaging (cMRI). Cardiac amyloidosis was defined by a typical late gadolinium enhancement (LGE) on cMRI in absence of valvular or coronary artery disease. Results—The mean age of the 55 patients included was 62±16 years. 50% were male. Amyloidosis was related to light-chain amyloidosis in 16, wild-type transthyretin in 5 and hereditary transthyretin in 34. The mean echocardiographic IVST was 9±1.6mm. Cardiac amyloidosis on cMRI (cMRI-CA) was observed in 37% (n=16) of the 43 patients. Patients with cMRI-CA had no difference in LVEF and diastolic function (E/A and E/e’ ratio) compared to patients without typical LGE. Interestingly, global LS was impaired only in patients with cMRI-CA (-14±3 vs. -18±2%, p<0.001). This loss of myocardial deformation in cMRI-CA patients was more marked in basal (-11±6%) than in median (-13±4%) or apical segments (-16±4%), and the lowest value of regional LS was observed in the basal posterior segment (-9±10%). Moreover, basal posterior segment LS had the best accuracy for detecting typical LGE on cMRI (AUC=0.85, p=0.002 95% CI (0.06-1.15)). Conclusions—Longitudinal strain in the basal posterior segment may help to detect cardiac amyloidosis in patients with proven biopsy but without usual 2D echocardiography abnormalities. Key Words: Strain • Speckle tracking • amyloidosis • cardiac magnetic resonance imaging

C-29 - Prevalence and severity of sleep apnoea syndromes in cardiac amyloidosis patients.

Aurélia Lamine, Ala Noroc, Diane Bodez, Laurent Boyer, Stéphane Rappeneau, Dionyssis Pongas, Claire Marie Tissot, Soulef Guendouz, Serge Adnot, Violaine Planté-Bordeneuve, Thibaud Damy.

Background: Cardiac diseases are associated with a high prevalence of sleep apnoea syndrome (SAS) particularly in heart failure. Two types of SAS are known: central or obstructive. Heart failure can occur in patients with primary systemic amyloidosis (AL), senile systemic amyloidosis (SSA), and Transthyretin-Related Amyloidosis (TTR). There is no data about prevalence and severity of sleep disordered breathing in cardiac amyloidosis. Aims: Assess the prevalence and severity of SAS in cardiac amyloidosis. Methods: Patients prospectively referred in our cardiology department for cardiac amyloidosis underwent polygraphy to diagnose sleep apnoea syndrome (SAS) between 2010 and 2012. SAS was defined as an apnoea-hypopnoea index greater or equal to 5 events/h. Results: Thirty five patients were included, of whom 15 had AL, 9 FAP and 11 SSA. Mean age, body mass index, NTproBNP, and left ventricular ejection fraction, of the overall cohort were respectively 72 ± 12 years, 24 ± 4 kg/m², 5642 ± 7812 and 48 ± 13% and. The prevalence of SAS was 86%. 29% of syndromes were classified as central and 57% as obstructive. The mean apnoea hypopnoea index was 22 ± 14 events/h and was superior to 30 events/h in 11 patients. SSA were significantly older but NTproBNP and LVEF were not different between the three type of amyloidosis. Apnoea hypopnoea index was more elevated in SSA and FAP than in AL (p=0.01). Conclusion: The prevalence of sleep-disordered breathing is high in cardiac amyloidosis population, with most syndromes having an obstructive pattern. Effect of SAS treatment should be investigated in this population.
C-30 - POSTERIOR LONGITUDINAL STRAIN BY SPECKLE TRACKING ECHOCARDIOGRAPHY, A MARKER OF CARDIAC AMYLOIDOsis EXTENSION?

all at Amyloidosis Mondor Network, Créteil, France

Objective—Evaluate the relation between longitudinal strain (LS) in basal posterior wall and late gadolinium enhancement (LGE) in patients with cardiac amyloidosis. Methods—Among a cohort of 162 patients with proven amyloidosis on biopsy, we selected 97 patients with cardiac involvement defined by a mean interventricular septum thickness (IVST) ≥ 12mm in the absence of hypertension or by a typical LGE on cardiac magnetic resonance imaging (cMRI). All patients had a comprehensive two-dimensional echocardiography with a global and regional quantification of LS in 17 segments. Basal posterior wall LS was defined as the mean LS of inferior, posterior and lateral basal segments. Cardiac MRI was performed in 72% of patients and LGE was compared with regional speckle tracking analysis. Results—Overall, 97 patients with cardiac involvement were enrolled, of whom 30 had light-chain amyloidosis, 21 had wild-type transthyretin amyloidosis and 46 had hereditary transthyretin amyloidosis. Their mean age, IVST and LVEF were respectively 73±14 years, 15±3.5mm and 55±14%. Diastolic function (mean E/e’ ratio 16±8) and global longitudinal strain (mean -11±4%) were similarly impaired in the three type of amyloidosis. All patients with a positive LGE on cMRI (n=64, 93%) had basal posterior wall involvement with decreased LS (-6±6% vs. -17±2%, P=0.003) and low LVEF (p=0.01). Moreover, a fair correlation was observed between basal posterior wall LS and LVEF (r=-0.5, P<0.001), and the number of segments with LGE (r=0.56, p<0.001). Conclusions—Longitudinal strain in basal posterior wall may be used to appreciate the severity of cardiac amyloidosis involvement.

Key Words: Strain • Speckle tracking • amyloidosis • cardiac magnetic resonance imaging

D - Basic Research

D-01 - INVOLVEMENT OF TRANSCRIPTION FACTOR NFAT IN ALZHEIMER’S DISEASE.

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Alzheimer’s disease (AD) is a neurodegenerative disease and the primary cause of dementia in elderly. It is characterized by a progressive loss of cognitive function and it’s main symptom is the loss of recent memory. The responsible factor for the clinical manifestation of the disease is the progressive loss of sinapses and neurons, especially in the hippocampus region. An importante feature of AD is the presence of amyloid plaques, found in the extracellular region of patients’ brains, composed mostly of the peptide beta-amyloid (Aβ). Exposed neurons to Aβ display higher levels of intracellular calcium, which could lead to the activation calcineurin A (CnA). CnA is a Ca2+/calmodulin dependent phosphatase and one of it’s main targets is the transcription factor NFAT. Inhibition of NFAT leads to a prevention of both spine loss and dendritic simplification, even though it is not know which genes would have been regulated. The primary objective of the project is to define the molecular mechanisms of gene control by the transcription factor NFAT that may be involved in the AD. We already demonstrated that mice primary neuron culture treated with Aβ oligomers display NFAT translocation to the nucleus that can be completely reversed by ciclosporin A (CsA), an inhibitory drug of the NFAT activation pathway, sugesting an activation of this transcription factor. We also have evidence that these primary cultures treated with Aβ peptides have a smaller amount of dendritic spines and pre-synaptic terminals (in relation to the post-synaptic terminals), suggesting a decrease of synapses, which is also reversed by CsA. Based on these results, we can assume that Aβ peptides are promoting a decrease of spines and synapses through activation of the calcineurin/NFAT pathway. We now seek to investigated genes that may be regulated by NFAT and are involved in the process of neurodegeneration in AD.
D-02 - IDENTIFICATION OF DIFFERENT TTR MUTATIONS IN BRAZILIAN PATIENTS DIAGNOSED WITH FAMILIAL AMYLOIDOTIC POLYNEUROPATHY.

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Familial amyloid polyneuropathy (FAP) is an autosomal dominant polyneuropathy of adult onset which used to lead to death within 10 years on average after the first symptoms. More than 100 mutations were described as causing PAF. In Brazil until now the only one mutation described was V30M. However since the diagnosis of the disease was implemented in Brazil we found 5 mutations not described. The aim of this work is to characterize these mutation in Brazilian population. Initial patient data collection includes information on family and medical history, physical and laboratory exam results, and patient reported outcomes. The prediction of stability of these mutations using a bioinformatics tool called FoldX showed us that the G53E is highly amyloidogenic. Studies estimate the frequency of each mutation showed us that the V30M is the most frequent followed by I107V, V122I, A19D and T119M respectively. These date are important due to the lack of information regarding FAP characterization in Brazilian population.

D-03 - DO AMYLOID FIBRIL COMPOSITION AFFECT THE DEVELOPMENT OF CARDIAC ARRHYTHMIA IN PATIENTS WITH FAMILIAL AMYLOIDOTIC POLYNEUROPATHY?

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Background: Transthyretin (TTR) amyloidosis (ATTR) is a systemic amyloid disease presenting symptoms from various organs such as the heart, the gastrointestinal tract and the autonomic nervous system. Cardiac arrhythmias are common and approximately 25% of patients need permanent pacemaker treatment. The amyloid fibrils consist of two different structures: full-length TTR (type B); and a mixture of full-length and truncated TTR (type A). Type A is the most common composition and is associated with late onset patients with signs of cardiomyopathy. Type B is more frequent in early onset patients without cardiomyopathy. This retrospective study analysed the relation between amyloid fibril composition and cardiac arrhythmias in 24-hours Holter-ECG recordings in Swedish TTR amyloidosis patients. Methods/Patients: Holter-ECG:s from the first available examination were analysed in 104 patients: 49 type B patients (31 male, mean age 54 years), and 55 type A patients (42 male, mean age 67 years). Forty-five patients had performed follow-up Holter-ECG:s, where 32 patients were re-evaluated after liver transplantation. Results: At baseline, cardiac arrhythmia was found in 27% of type B patients, and in 41% of type A patients, respectively (P=0.006). There were no differences between patient groups and type of cardiac arrhythmia (P=0.43). In the follow-up group, the proportion of patients with cardiac arrhythmia increased from 36 % to 54% in type B patients, and from 65 % to 76 % in type A patients (P=0.12). At follow-up, 11 (24%) patients were pacemaker treated: 6 type B and 5 type A patients, respectively. In conclusion, our data displays differences at baseline examination between the two types of amyloid fibril composition regarding cardiac arrhythmia, and that both types appears to develop severe cardiac arrhythmia during the course of the disease.
D-04 - FRAGMENTATION OF AMYLOID-FORMING TRANSTHYRETIN IN CULTURED CELLS AND A TEST TUBE

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Background and Objective. Fragmentation of transthyretin (TTR) at positions 46-52 were reportedly found in amyloid deposits in familial amyloid neuropathy (FAP) and senile systemic amyloidosis (SSA). Recently, several studies also reported that the fragmentation of TTR in amyloid deposits were related to the age of onset of FAP. However, the mechanism and pathological roles of the TTR fragmentation in FAP and SSA remain to be determined. To elucidate the mechanism involved in TTR fragmentation, we investigated how TTR fragments were formed in cultured cells and a test tube. Materials and Methods. Recombinat V30M and wild-type TTRs were employed in this study. Several kinds of cultured cells were incubated for 24 hours with soluble TTR or TTR aggregates formed in vitro by pretreating in an acidic buffer. After incubation, cell lysates were examined by SDS-PAGE and amino-acid sequence analysis to detect TTR fragments. To investigate roles of trypsin on fragmentation of TTR, we also analyzed cultured cells with and without pretreatment of siRNA for trypsin. In addition, to analyze direct effects of trypsin on TTR fragmentation, soluble TTR and TTR aggregates were incubated with trypsin in a test tube. Results. TTR fragments were detected in lysates of neuron and astrocyte cell lines which were incubated with TTR aggregates, but not in those which were incubated with soluble TTR. Amino-acid sequence analysis revealed that cleavages of TTR were made at C-terminal of positions K15, K48, and K80, which suggested that trypsin might cleave TTR aggregates. After knocking down of trypsin using siRNA, the TTR fragmention decreased in the cells. Similar cleavages of TTR were detected from TTR aggregates directly incubated with trypsin in a test tube, but were not detected from soluble TTR incubated with trypsin. Conclusion. Trypsin may play an important role on TTR fragmentation in amyloid deposits.

D-05 - ROLE OF FIBROBLASTS IN THE PATHOGENESIS OF FAMILIAL AMYLOIDOTIC POLYNEUROPATHY

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Background: The ECM microenvironment is thought to have a key role in amyloid fibril formation in TTR-related amyloidosis because prefibrillar aggregate and fibrillar forms of TTR exist in the ECM generated from blood-soluble TTR. Regression of amyloid deposits in FAP patients who undergo liver transplantation to remove the main source of mutant TTR suggests the existence of mechanisms for the clearance of TTR deposits from the ECM. Fibroblasts have a central role in the maintenance of ECM, but their precise role in the pathogenesis of transthyretin-related FAP is largely unknown. Materials and Methods: To investigate the function of fibroblasts in the endocytosis and degradation of aggregated TTR, we performed in vitro studies with a fibroblast cell line and in vivo study using an experimental mouse model with subcutaneous injection of TTR and a transgenic mouse model. We also analyzed the role of fibroblasts using sections from FAP patients. Results: In vitro studies with the fibroblast cell line revealed that fibroblasts endocytosed and degraded aggregated TTR. Subcutaneous injection of soluble and aggregated TTR into WT mice showed internalization and clearance over time by both fibroblasts and macrophages. Immunohistochemical studies of skin biopsies from V30M patients, asymptomatic carriers, recipients of domino FAP livers as well as mice transgenic for human V30M showed intracellular TTR immunoreactivity in fibroblasts and macrophages that increased with clinical status and with age in the transgenic mice. Conclusions: Fibroblasts endocytose and degrade TTR aggregates. The function or dysfunction of TTR clearance by fibroblasts may have important implications for the development, progression, and regression of TTR deposition in the ECM.
D-06 - PROTEOMIC ANALYSIS OF AMYLOID DEPOSITS IN SUSPECTED TRANSTHYRETIN AMYLOIDOSIS

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Background: Accurate identification of amyloid type is critical in every case of amyloidosis since therapy is type-specific. At the UK National Amyloidosis Centre (NAC), biopsy specimens are routinely stained with Congo red (CR) and a panel of antibodies to determine the amyloid fibril protein. In ~20-25% cases however, immunohistochemistry (IHC) fails to definitively confirm the amyloid type. Laser capture microdissection (LMD) of amyloid deposits and proteomic analysis by mass spectrometry is a powerful tool for identifying proteins from formalin-fixed, paraffin embedded tissues. We sought to determine the role of proteomics in the diagnostic evaluation of patients with suspected ATTR amyloidosis.

Methods: Between January 2012 and September 2013, all cardiac, selected gastrointestinal and nerve, and certain other tissue biopsy specimens, received as part of routine clinical practice at the NAC, were stained in the usual way with CR and IHC but, in addition, processed for proteomics. Proteomics was performed on the Velos platform and analysed using MASCOT software. Blind interpretation was by two independent experts. Results: A total of 66 biopsy specimens were analysed (36 endomyocardial, 26 gastrointestinal, 2 neural, and 2 other). Of 36 cardiac biopsies, 4 did not contain amyloid by CR, corroborated in each by absence of amyloid signature on proteomics. The amyloid fibril protein was identified with certainty or a high degree of probability in 23 of 32 (72%) remaining cases, including 10/11 cases in which IHC had not been diagnostic. There was 100% concordance between IHC and proteomics whenever the probability of fibril protein identification by proteomic analysis was determined to be high. ATTR amyloid was present in cardiac biopsies in one third of patients. Amyloid was identified by CR staining in each of 26 gastrointestinal biopsies but IHC failed to confirm amyloid fibril type in 19/26 (73%) cases. The fibril protein was identified with certainty or a high degree of probability by proteomic analysis in 19/26 (73%) of these cases, including 13/19 (68%) of those that could not be typed by IHC, but failed to identify the fibril protein in 7 cases. Importantly, ATTR amyloid was identified by proteomics in 4 cases in which the IHC did not stain with antibodies against ATTR; in all 4 cases the clinical picture was entirely consistent with ATTR amyloidosis. One neural biopsy contained amyloid by CR staining that did not stain immunospecifically by IHC; the other did not show amyloid by CR staining. Presence/absence of amyloid was corroborated in each case by proteomic analysis but the amyloidotic specimen could not be typed by proteomics due to presence of multiple amyloid fibril proteins. ATTR amyloid was unexpectedly identified by proteomics in one bone marrow and one pleural biopsy specimen, corroborated in one case by IHC and in the other by the clinical phenotype.

Conclusions: Proteomic analysis of 66 specimens from a variety of tissues resulted in a definitive diagnosis in 47 (71%) cases, including ATTR amyloidosis in more than one quarter. Proteomics is complimentary to CR staining and IHC, and does not obviate the need for the latter in cases of suspected amyloidosis.

D-07 - EFFECT OF AGE AND SEX DIFFERENCES ON TRANSTHYRETIN AMYLOID FORMATION IN FAP

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Background: Age and sex differences are closely related to the onset of senile systemic amyloidosis (SSA) caused by wild-type (WT) transthyretin (TTR). However, the effects of these differences on the amyloid formation mechanism in familial amyloidotic polyneuropathy (FAP) caused by variant TTR, have remained unclear. To elucidate age and sex differences in FAP, we investigated biochemical characteristics of amyloid deposits in different tissue sites of FAP by proteomic analysis. Methods: We used shotgun liquid chromatography/tandem mass spectrometry to analyze the proportions of variant and WT TTR in amyloid deposits in different tissues, such as cardiac, renal, and nerve tissues, from 23 autopsied FAP cases. Results and Conclusions: The analysis revealed a highly significant correlation between the proportion of WT TTR and age at autopsy in cardiac tissues, whereas the analysis indicated no correlation in peripheral nerve and renal tissues. In addition, we demonstrated age-related significantly increased WT TTR deposits, but not variant TTR deposits, in cardiac tissues of male patients. Taken together, these data suggest that both age and sex differences affect cardiac amyloid formation, mainly derived from WT TTR, in FAP.
D-08 - AMYLOID DEPOSITS IN THE KNEE JOINT MENISCI

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Introduction
In several ligaments and tendons, amyloid deposits were found especially in elderly people and were mostly derived from wild-type (WT) transthyretin (TTR). Recently, amyloid deposits were also frequently found in the knee joint menisci, but two different proteins, TTR and apolipoprotein AI (Apo AI), have been reported as amyloid precursor proteins. The aim of this study was to elucidate frequency and pathological difference in those two kinds of amyloidosis in the knee joint.

Materials and Methods
We employed 51 osteoarthritis (OA) patients. Meniscus samples were subjected to Congo-red staining and immunohistochemical stainings using anti-TTR and Apo AI antibodies. We also investigated amyloid precursor proteins by means of Western blotting and LS-MS/MS. In five cases with TTR amyloid deposits, we performed genetic testing and mass spectrometric analysis for TTR.

Results
We found amyloid deposits in all menisci specimens. Amyloid deposits were derived from TTR, Apo AI, and both of them, in 17 (33.3%), 10 (19.6%), and 7 (13.7%) of 51 patients, respectively. All of 5 patients with TTR amyloid deposits had WT TTR. Age of the patients with TTR amyloid deposits was higher than that with Apo AI amyloid deposits (79.3 ± 5.8 vs 75.6 ± 5.6 years, p < 0.05). We also found positive correlation between frequency of TTR amyloid deposits and age in OA patients. Conclusions: Amyloid deposits derived from TTR may be more frequent than those derived from Apo AI in the menisci of OA patients. Aging of the patients may affect TTR amyloid deposits in the knee joint of those patients.

D-09 - BIO-DISTRIBUTION EXPERIMENTS OF MICE USING A NEW AMYLOID IMAGING PROBE 125I-EISB FOR CLINICAL USE

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We performed bio-distribution experiments in mice to examine in vivo behavior of 125I-EISB and find an affinity of the compound to $\beta$-amyloid plaques in amyloid deposition mice. Zero point one ml of 125I-EISB was injected by tail vein to normal and amyloid deposition mice. After 30 minutes, each organ tissues brain, liver, blood, and others are taken out and the weight and radioactivity of the organs are measured and obtained %Dose/ g. A section of brain was exposed on autoradiographs for a week. With the normal mouse, 125I-EISB was accumulated mainly to liver(40.1%) and kidney(10.1%). With the amyloid deposition mouse, 125I-EISB was observed an high brain uptake in autoradiograph. 125I-EISB was a promising new radio-compound for the detection of the amyloid plaques for clinical use.
D-10 - CHARACTERIZATION OF FAMILIAL AMYLOIDOTIC POLYNEUROPATHY-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

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Introduction: Induced pluripotent stem (iPS) cells have an unlimited replicative ability and the potential to differentiate into most of cell types. iPS cells has allowed development of patient-specific pluripotent cells and provided valuable experimental platforms as useful models of human diseases. In this study, in view of our need for a novel useful tool in elucidating the molecular pathogenesis of familial amyloidotic polyneuropathy (FAP), we established and characterized the heterozygous FAP-specific iPS cells. Materials and Methods: To induce the reprogramming of somatic cells into iPS cells, Sendai virus (SeV) was used to transduce the four reprogramming factors (OCT3/4, SOX2, KLF4, and c-Myc) into the dermal fibroblasts obtained from heterozygotic FAP V30M patients. Fibroblast growth factors and bone morphogenetic proteins were used to differentiate FAP-specific iPS cells into hepatocytes. Results: We successfully generated heterozygous FAP-specific iPS cells by introducing four reprogramming factors (Oct3/4, Sox2, Klf4, and c-Myc). FAP-specific iPS cells had the potential to differentiate into hepatocyte-like cells, a major TTR-producing cell, and indeed expressed both ATTR Val30Met and wild-type TTR protein. Conclusion: FAP-specific iPS cells demonstrated the possibility of serving as a model that will contribute to understanding the pathogenesis of FAP and development of FAP treatments.

D-11 - IDENTIFICATION OF TRANSTHYRETIN IN WHITE BLOOD CELL IN FAMILIAL AMYLOID POLYNEUROPATHY PATIENTS

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Introduction Transthyretin (TTR) is synthesized in the liver, eyes, pancreas and the choroid plexus. However, some articles have recently reported that other some tissues also expressed mRNA or protein for TTR. In the present study, we investigated the expression of mRNA and protein for TTR in leukocytes, and made a comparison of that between familial amyloid polyneuropathy (FAP) patients and healthy control subjects. Materials and Methods Polymorphonuclear neutrophil leukocytes (PMNL) and peripheral blood mononuclear cells (PBMC) from 5 healthy volunteers and 5 FAP patients were isolated from heparinized blood by density gradient centrifugation. The expression of TTR mRNA in PMNL and PBMC was analyzed by RT-PCR. The expression of TTR protein was investigated by western blotting and immunohistochemistry. Results We found that the TTR mRNA was expressed in both PMNL and PBMC between the two groups. Furthermore, western blotting showed the existence of TTR protein. On the other hand, the frequencies of TTR-positive cells in PMNL and PBMC of FAP patients were significantly decreased in comparison to healthy volunteers. Discussion and Conclusion We identified the expression of TTR mRNA in both PMNL and PBMC. Although we also confirmed the existence of TTR protein, it was unclear whether leukocytes actually produced TTR protein. On the other hand, FAP patients showed the decrease of the existence of TTR protein in leukocytes, and this result may be involved in pathological processes of FAP.
D-12 - WHAT DOES THE TTR LOCUS TELLS US ABOUT THE VARIATION IN AGE-AT-ONSET IN FAMILIAL AMYLOID POLYNEUROPATHY?

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Introduction: Familial amyloid polyneuropathy (FAPATTRV30M) is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. A wider variability in age-at-onset (AO) has been uncovered, including among Portuguese patients [17-82 yrs]. However, early (AOObjectives: Therefore, our aim is to identify genetic modifiers closely linked to the TTR locus that may in part explain the observed AO variability.

Methods: Haplotype analysis is underway, using intragenic SNPs for extended haplotypes. Tagging SNPs were selected in the TTR locus and SNP genotyping is being performed by SNaPshot, using a multiplex approach. Furthermore, we are currently searching for rare variants in the TTR locus by PCR amplification of the four exons and intron-exon boundaries, followed by bi-directional direct automated DNA sequencing. Results: We found one haplotype, which is the most frequent in our Portuguese sample. Importantly, we also found, in late-onset patients a more frequent haplotype, than in early-onset group, suggesting a possible protective effect in these patients. Conclusions: To determine whether genetic variability in TTR locus modulates and contributes to the variability in age-at-onset, we will continue to assess the TTR haplotypes in around 60 Portuguese FAP families with ATTRV30M mutation and we expect to find some variants or regions that may confer protection (late-onset patients or aged asymptomatic carriers). These variants, once lost, may result in earlier-onset in the next generation. These findings may have important clinical implications.

D-13 - STRUCTURE-FUNCTION RELATIONSHIPS IN APOLIPROTEIN A-I MUTANTS ASSOCIATED WITH AMYLOIDOSIS

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Amyloidosis induced by Human apolipoprotein A-I (apoA-I) represent a broad range of clinical manifestations, depending on the protein variant which is involved. Senile non hereditary amyloidosis, is characterized by deposits of protein with the wild type sequence (WT) in atherosclerotic plaques, thus opening the hypothesis that a chronic inflammatory micro environment could elicit protein misfolding and or loss of function. But in addition, more than fifteen single point mutants of this protein are associated with amyloidosis in patients, affecting different organs and with different severity. In order to get insight into the mechanism inducing protein misfolding, we constructed three natural amyloidogenic variants (Arg173Pro, Gly26Arg and Lys107-0), and compared their behavior with WT. The three mutants are less stable than WT at physiological pH, and show a non cooperative unfolding. However only Arg173Pro and Lys107-0 have a stronger tendency to aggregate under this condition. Arg173Pro shows also higher tendency to bind to heparin under mild acidic pH and in the presence of small amounts of dodecyl sodium sulfate. Instead Gly26Arg but not Lys107-0 enhances macrophages activation, which could be a clue mechanism to perpetuate a pro-inflammatory micro environment. Results indicate that subtle structural changes are required to induce protein pathogenicity, however this is not only related to protein instability but to the specific activation of cellular pathways that could enhance their toxicity under specific conditions as well. Authors acknowledge support from UNLP (M158), ANPCT (PICT 2008-2106), and CONICET (PIP PIP 112-200801-00953 and 112 201101-00648
D-14 - STUDY OF APOE POLYMORPHISMS AS POSSIBLE MODIFIERS OF AGE-AT-ONSET IN FAMILIAL AMYLOID POLYNEUROPATHY (FAP ATTR V30M)

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Familial amyloid polyneuropathy (FAPATTRV30M) is an autosomal dominant disease, due to a point mutation in the TTR gene. Remarkable differences in mean age-at-onset (AO) have been described in different clusters, including within Portuguese population. Among Portuguese families, FAP shows a wide variation in AO (17-82 yrs) and asymptomatic carriers aged 95 can be found; this variation is also often observed between generations. Few studies have been published aiming to disentangle possible genetic modifiers involved in AO variability, however mechanisms involved in anticipation in FAP remain unclear. Our aim was to assess if ApoE gene has a modifier effect in AO variation in a group of FAPATTRV30M Portuguese families. Several studies described that ApoE genotype influence AO of some neurodegenerative diseases such as Alzheimer’s disease and the rate of progression in others such as multiple sclerosis. We collected a sample of 71 FAP families with 139 patients. Variants screening was performed by PCR amplification of all coding and flanking regions, followed by direct automatic DNA sequencing of the ApoE gene. Results are being analyzed with the SeqScape v.2.6 software. In a preliminary analysis of 77 patients we found some frequent polymorphisms: rs405509 in the 5’UTR region, rs769449 in intron 2 and rs429358 and rs7412 in exon 4. These last two variants are non-synonymous missense mutations that define the common allelic variants of ApoE, known as ApoE-ε2, ApoE-ε3, and ApoE-ε4. The statistical analysis revealed significant differences in mean AO when we compared patients with ε2/ε3 and ε3/ε3 genotypes against ε2/ε4, ε3/ε4 and ε4/ε4 genotypes (p=0.004), showing that patients with at least one ε4 allele present an early mean AO. Therefore, the ApoE gene may have a modifier effect in AO variation in our group of FAPATTRV30M patients studied, which may have an important impact in genetic counselling.

D-15 - DISSECTING THE MECHANISM OF TOXICITY TRIGGERED BY AGGREGATES OF TRANSTHYRETIN

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The Transthyretin (TTR) related amyloidoses are neurodegenerative disorders characterized by the extracellular deposition of TTR fibrils in several tissues. The TTR is involved in the Familial Amyloidotic Polineuropathy (FAP), Familial Amyloidotic Cardiomyopathy (FAC) and oculoleptomeningeal amyloidosis (OA). FAP is the most common form of hereditary systemic amyloidosis and all these diseases are an autosomal dominant condition, usually caused by mutation in the gene for plasma TTR. The aggregates are deposited in particularly in the peripheral nervous system, kidney, thyroid, gastrointestinal tract and cardiovascular system in the case of FAC. The cellular effects of TTR deposition on neuronal function in FAP remain however to be elucidated. In the present study we evaluate which is the most toxic species in the aggregation pathway of TTR and the cytotoxic mechanism activated by them. Our data showed that the most toxic specie formed in the aggregation pathway of TTR are the oligomers with molecular weight of approximately 100 kDa. The fibers are not toxic for any cell culture. The viability assay also showed that these aggregates are specifically toxic to neural and kidney lineages. The oligomers of TTR activate caspase 3 and caspase 7 in primary retinal neurons indicating the induction of apoptosis in the presence of these aggregates. All together these dates demonstrate that oligomers of TTR and not the fibers are the most toxic species and have specific toxicity as occurs in vivo. Moreover these aggregates induce cell death through apoptotic mechanisms.
D-16 - HEPARIN MODULATION OF PRION SEEDING ACTIVITY

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INTRODUCTION. The conversion of PrP into scrapie PrP is the central event of prion diseases (TSEs). Such conversion and propagation is seeded or templated by a polymerization mechanism. Some authors have suggested that glycosaminoglycans (GAGs) directly convert PrP into a protease resistant form, while others have proposed that these molecules have a protective activity. Our group recently reported that low molecular weight heparin (LMWHep) does not induce recombinant mouse prion protein (rPrP23-231) conversion, protecting rPrP23-231 from RNA-induced aggregation (1). MATERIAL AND METHODS: Real-time quaking-induced conversion (RT-QuIC) is an assay in which disease-associated PrP initiates a rapid conformational transition in recombinant PrP, resulting in the formation of amyloid fibrils that can be monitored in real time using the dye thioflavin T. We used rPrP from mouse and hamster as substrate (23-231 and 90-231), and TSE-associated forms were from mouse and hamster brain homogenates (RML and 263K strain respectively). LMWHep was used in order to determine the effect of this GAG on PrP fibrillization. RESULTS AND DISCUSSION: In the present work, we show that LMWHep delays and decrease fibril formation. It also inhibits fibrilization depending on the seed used. There is no effect when rPrP 90-231 is used, or with high salt concentration. Moreover, it is effective when added at the lag phase of the polymerization process. When a soluble LMWHep-rPrP complex is added to the reaction, no fibrils are detected. On the contrary, the addition of a LMWHep-rPrP aggregated complex results in conversion.

CONCLUSIONS: Through electrostatic interactions, LMWHep interaction with PrP N-terminal domain, modulates PrP fibrilization. It affects the nucleation processes and the formation of oligomers, the first step of fibrilization. Our findings may explain the protective effect of these molecules in different models.

D-17 - THE ENDOPLASMIC RETICULUM-ORIENTED DRUG DEVELOPMENT FOR TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY

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Familial amyloid polyneuropathy (FAP) is one of the hereditary amyloidoses caused by a point mutation in the human plasma protein, transthyretin (TTR). Amyloid fibrils derived from TTR variants accumulate in peripheral nerves and visceral organs. TTR variants are easily dissociated from tetramer to monomer because of having low energetic stability of their tetrameric structure in comparison with wild-type (WT) TTR. Previously, we demonstrated that endoplasmic reticulum (ER) quality control system and ER associated degradation (ERAD) of TTR are pathological determinants of FAP. The monomeric mutation introduced in the vast majority of TTR mutants that are normally secreted resulted in the ER retention and efficient degradation of these monomeric mutants by ERAD. Therefore, inhibition of TTR tetramerization in ER lumen and suppression of TTR variants secretion could be a promising therapeutic strategy for FAP. Here, we employed structure-based virtual screening, and we identified some candidate compounds with a potential as destabilizer of intracellular TTR tetramer. The selected compounds were assayed using HEK293 cells stably expressing the most common variant, V30M TTR. Our data indicated that some of these compounds may inhibit extracellular secretion of V30M TTR, suggesting a possible use for preventing TTR amyloid pathogenesis.
D-18 - INVOLVEMENT OF THE TRANSCRIPTION FACTOR NFAT IN PARKINSON’S DISEASE: CONTRIBUTION TO INFLAMMATION AND APOPTOSIS.

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Parkinson’s disease (PD) is a neurodegenerative disorder that is caused by the death of midbrain dopaminergic neurons and affects nearly 5 million individuals worldwide. There are a large number of studies suggesting that the mechanism which leads to neuronal loss in PD consists by an abnormal accumulation of a protein known as α-synuclein and subsequent formation of intracellular protein aggregates called Lewy bodies. Studies have shown that α-synuclein aggregates alter membrane fluidity and increase calcium (Ca+2) influx, rise levels of intracellular calcium lead to the activation of calcineurin phosphatase (CnA). Although, the main calcineurin target are Nuclear Factor of Activated T-Cells (NFAT), its contribution to the PD is very poorly understood. NFAT proteins directly regulate the expression of genes involved in the control of cell death by apoptosis, as well as genes involved in the inflammatory process. Apoptosis and inflammation are known to be key events in neurodegeneration, which is triggered upon a remarkable increase in intracellular Ca+2. Therefore, the main goal of the present project is to evaluate the involvement of NFAT in the neurodegenerative process induced by aggregates of α-synuclein. Our initial results show that only oligomers of α-synuclein were able to mediate increase in LDH release in primary cultures of dopaminergic neurons and this effect was partially reversed when these cultures are pretreated with cyclosporin, an inhibitor of calcineurin. In addition we examined the involvement of monomers, oligomers and fibers in the synaptic events. We found that only oligomers and fibers showed to be capable of causing a decrease in Synapsin I, a protein involved in pre-synaptic events. These results suggest that the NFAT may have a possible role in the process of neurodegeneration mediated by α-synuclein aggregates.

D-19 - AMYLOID FIBRILS TRIGGER THE RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS), CAUSING FIBRIL FRAGMENTATION BY NET-ASSOCIATED ELASTASE*

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The accumulation of amyloid fibrils is a feature of amyloid diseases, where cell toxicity is due to soluble oligomeric species that precede fibril formation or are formed by fibril fragmentation, but the mechanism(s) of fragmentation still unclear. Neutrophil-derived elastase and histones were found in amyloid deposits from patients with different systemic amyloidoses. Neutrophil extracellular traps (NETs) are key players in a death mechanism in which neutrophils release DNA traps decorated with proteins such as elastase and histones to entangle pathogens. Herein, we asked whether NETs are triggered by amyloid fibrils, reasoning that since proteases are present in NETs, protease digestion of amyloid may generate soluble, cytotoxic species. We show that amyloid fibrils from three different sources (α-synuclein, Sup35 and transthyretin) induced NADPH oxidase-dependent NETs in vitro from human neutrophils. Surprisingly, NET-associated elastase digested amyloid fibrils into short species that were cytotoxic for BHK-21 and HepG2 cells. In tissue sections from patients with primary amyloidosis we also observed the co-localization of NETs with amyloid deposits as well as with oligomers, which are probably derived from elastase-induced fibril degradation (amyloidolysis). These data reveal that release of NETs, so far described to be elicited by pathogens, can also be triggered by amyloid fibrils. Moreover, the involvement of NETs in amyloidoses might be crucial for the production of toxic species derived from fibril fragmentation.
D-20 - THE EFFECT OF POTENTIAL ANTI-PARKINSONIAN COMPOUNDS ON THE TOXICITY OF A-SYNUCLEIN AGGREGATES

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Parkinson’s Disease (PD) is the second most common neurodegenerative disorder in human, being only less prevalent than Alzheimer’s Disease. It is mainly characterized by loss of the dopaminergic neurons in the substantia nigra (SN) and presence of the intracellular proteinaceous inclusions called Lewy bodies (LBs). The LBs are mainly composed by amyloid aggregates of α-synuclein (α-sin), a protein abundantly expressed in brain and located in the presynaptic terminals. PD is clinically defined by four cardinal signals (resting tremor, bradykinesia, rigidity and postural instability). The role of the α-synuclein in degeneration observed in PD was highlighted after the identification of three mutants (A53T, A30P and E46K) involved in the earlier and inherited forms of this disease. However, the molecular mechanisms for which aggregation contributes to neurodegeneration and signaling pathways affected are not know. Recently, some compounds are studies as strategies to prevent and treat amyloids diseases as PD, in attempt to inhibit or modulate the protein aggregation process. One of the compounds tested in this work is CLR01, known as molecular tweezer that binds specifically to lysine residues (Lys) proteins. The Lys are involving both hydrophobic and electrostatic interactions, with combined to start the aggregation process and induced formation of toxic oligomeric species. α-sin has 15 Lys residues in its sequence and these are possible acting as nucleation seeds. These results suggest that CLR01 is able to inhibit its amyloid fiber formation and neurotoxicity in vitro and in vivo. The aim of our study is to evaluate the effect of inhibiting compounds as CLR01 in amyloid protein aggregation, synaptic integrity and cytotoxicity induced by oligomers wild type and mutant α-synuclein. For this, cytoxicity assays were conducted by the release of lactate dehydrogenase (LDH) and immunostaining for synapsin and PSD-95, pre- and post-synaptic proteins, respectively. Inaddition, high performance liquid chromatography on a reverse phase column is carried out to evaluate the levels of dopamine in primary cultures of mesencephalic dopaminergic neurons. Our initial results suggest that CLR01 is able to reduce the neurotoxicity induced by oligomers of α-syn A30P mutant maintaining the integrity of the synaptic neurons.

D-21 - DISSOCIATION AND REASSOCIATION OF L55P TTR FIBRILS

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There are several human neurodegenerative diseases caused by extracellular protein deposition in the form of ordered structures known as amyloid fibrils. Transthyretin (TTR) is a tetrameric protein composed of identical 127-residue subunits having predominantly a β-sheet structure that belongs to this group. Here we subject the amyloid fibrils of L55P TTR obtained at acid conditions (pH 4.4) to HHP (2.9 kbar) at pH 7.0 or 6.5. As revealed by the experimental procedures, the extent of dissociation and re-association depends on the pH. At pH 7.0, fibrils are completely dissociated by pressure and after decompression no sign of aggregation is observed. On the other hand, when the fibrils were submitted to 2.9 kbar at pH 6.5, we observed an incomplete dissociation of fibrils. After the return to atmospheric pressure, L55P TTR forms new aggregates at pH 6.5, where pressurized native protein is stable and soluble. Furthermore, when native tetramers of L55P TTR at pH 6.5 is submitted to 2.9 kbar in the presence of fibrils, we observed that these tetramers are incorporated into aggregates, as determined by quantification of aggregates and fluorescence experiments with L55P TTR labeled with acrylodan (L55P TTR-acry). These results suggest that the native tetramer of L55P TTR is incorporated into fibril structure, even at pH 6.5. Then, we conclude that the species obtained from fibrils after pressure treatment accelerated the fibril growth, acting as seed.
E - Clinic

E-01 - FACTORS INFLUENCING TTR-FAP MEAN TIME FOR DIAGNOSIS

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Background: Currently, with different treatment options (Tx) available, the mean time for diagnosis (MTD) becomes an even more determinant factor. In order to reduce the MTD is crucial to find out the factors that influence it the most. Purpose: To evaluate in a cohort of FAP patients the MTD and to determine the influence of family history (FH), age of onset (AO) and available Tx in the MTD. Methods: We performed a retrospective study selecting from a clinical database all FAP patients followed in Unidade Clínica de Paramiloidose (Porto, Portugal), diagnosed after 1985 with well-established date of disease onset and diagnosis. Liver transplant became available at Porto in 1996. Statistical analysis was made using SPSS. Results: We identified 1685 patients fulfilling the inclusion criteria (844 male; 841 female) belonging to 529 families. Mean AO was 36.64±11.64years. The MTD was 2.02±2.35years. In 1322 patients there was a known FH, 1211 first degree with MTD of 1.21±1.35years, 111 second degree with MTD 3.32±2.48years. In the group without confirmed FH but with previous descriptions in the family the MTD was 4.13±2.48years and for the ones without any FH was 4.87±3.27years. All statistically significant. Analyzing MTD according to AO the only significant difference was found in the group with known FH, respectively 1.18±1.3years and 1.63±1.9years (p=0.02) for those with AO below or above 50yrs. Comparing MTD according to available Tx the only statistical difference was in the group with known FH, respectively 1.71±1.6years and 0.96±1.1years (p=0.001) for those diagnosed between 1985-1996 and after 1996. Discussion: According to the results, the existence of family history is a critical factor to determine the MTD. In contrast to what would be expected, the AO and the Tx were only determinant when the hole population was considered and in the cases with known first degree family history.

E-02 - CENTRAL NERVOUS SYSTEM MANIFESTATIONS IN LONG SURVIVAL FAP TTR V30M PATIENTS

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Background: Central nervous system manifestations have been rarely reported in familial amyloid polyneuropathy (FAP) TTR V30M patients. Ocular involvement (glaucoma, vitreous opacities), seizures, dementia and stroke are frequent manifestations of oculoleptomeningeal amyloidosis variants in other mutations. Liver transplant prolongs significantly life expectancy in these patients, changing the natural disease progression. Objective: describe the incidence of central nervous system manifestations of oculoleptomeningeal amyloidosis variants in other mutations. Liver transplant prolongs significantly life expectancy in these patients, changing the natural disease progression. Objective: to evaluate in a cohort of FAP patients the MTD and to determine the influence of family history (FH), age of onset (AO) and available Tx in the MTD. Methods: Patients were consecutively evaluated for the presence of central nervous system dysfunction, namely seizures, migraine, TIA/stroke events and cognitive decline. Those with CNS manifestations performed transcranial doppler and carotid ultrasound, echocardiogram, EEG, CT scan, electroencephalography (EEG) and neuropsychological tests. Results: Among 33 long-term transplanted TTR-FAP V30M patients, with more than 15 years (19.7±8.0) disease duration, seven patients (21,2%) have CNS manifestations: 6 with transient left hemisphere neurological deficit; 3 with tonic-clonic epileptic seizures, 1 (3%) have symptoms of hemiplegic migraine, all 7 patients showed some cognitive decline. No significantly differences was found in age, at first symptom or disease duration between patients with CNS manifestations and without it. All patients have pacemaker due to conduction abnormalities (2nd degree AV block), no arrhythmias were detected on EKG. No abnormalities were found in Echocardiogram or on transcranial doppler and carotid ultrasound, Brain CT Scan was normal in all patients except one were a small left frontal ischemic lesion was detected. Brain MRI without gadolinium done in two patients showed no abnormalities. A focal slow activity over the fronto-temporal was a consistent finding in 4 of 7 patients, in EEG. Two patients had also a diffuse slowing of the background activity and in one an electroencephalographic seizure was recorded. From the neuropsychological assessments done in 3 of these patients, all have in common a marked deficit on divided attention, and, in lesser extent, all present some degree of decline in initiative and learning ability, suggesting a dysexecutive (frontal) profile. Conclusion: Although manifestations of oculoleptomeningeal amyloidosis had been thought to be rare in FAP TTR V30M, with the long survival given by the liver transplantation, more advance stages of disease are seen and CNS manifestations become a problem to deal in the disease approach.

85
E-03 - THAOS: GASTROINTESTINAL MANIFESTATIONS IN TRANSTHYRETIN AMYLOIDOSIS – COMMON COMPLICATIONS OF A RARE DISEASE

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Background: Transthyretin amyloid (ATTR) amyloidosis is a systemic disorder caused by amyloid deposits formed by misfolded transthyretin (TTR) monomers. There are two main forms: hereditary and wild-type ATTR amyloidosis, the former associated with TTR gene mutations. There are several disease manifestations; however, gastrointestinal complications are common, at least in the hereditary form. The aim of this study was to thoroughly explore the prevalence and distribution of the gastrointestinal manifestations in ATTR amyloidosis and to evaluate their impact on the patients’ nutritional status and health-related quality of life (HRQoL).

Methods: The Transthyretin Amyloidosis Outcomes Survey (THAOS) is the first global, multicenter, longitudinal, observational survey that collects data on patients with ATTR amyloidosis and the registry is sponsored by Pfizer Inc. This study presents baseline data from patients enrolled in THAOS as of June 2013. The modified body mass index (mBMI), in which BMI is multiplied with s-albumin, was used to assess the nutritional status and the EQ-5D Index was used to assess HRQoL. Data from 1579 patients with hereditary ATTR amyloidosis and 160 patients with wild-type ATTR amyloidosis were analyzed. Sixty-three percent of those with the hereditary form and 15% of those with the wild-type form reported gastrointestinal symptoms at enrollment. Unintentional weight loss and early satiety were the most frequent symptoms, reported by 32% and 26% of those with TTR gene mutations, respectively. For patients with cardiac phenotypes, the prevalence of gastrointestinal manifestations was not evidently higher than for the general population. Early-onset patients (< 0.001). Upper gastrointestinal symptoms and disease duration were significant negative predictors of mBMI (p = 0.003 and p < 0.001, respectively). Upper and lower gastrointestinal symptoms, age at onset and disease duration were all negatively associated with the EQ-5D Index Score (p < 0.001 for all), whereas a positive association was found for liver transplant at enrollment (p = 0.002). Conclusions: Gastrointestinal symptoms were common in patients with hereditary ATTR amyloidosis and had a negative impact on their HRQoL. However, patients with wild-type ATTR amyloidosis or TTR gene mutations associated with cardiac phenotypes did not show an increased prevalence of gastrointestinal disturbances. Upper gastrointestinal symptoms and duration of disease were significant negative predictors of the patients’ nutritional status.

E-04 - OUTCOME OF GASTRIC EMPTYING AND GASTROINTESTINAL SYMPTOMS AFTER LIVER TRANSPLANTATION FOR HEREDITARY TRANSTHYRETIN AMYLOIDOSIS

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Background: Gastrointestinal complications are common in hereditary transthyretin amyloid (ATTR) amyloidosis and are important for the patients’ morbidity and mortality. A liver transplantation (LTx) has, in selected patients, been proven to halt the progression of the disease and some patients even report symptom improvement after LTx. However, most studies show unchanged or progressing symptoms after LTx. The aim of the present study was to explore the short- and long-term outcome of gastric emptying, gastrointestinal symptoms and nutritional status after LTx for hereditary ATTR amyloidosis. Methods: Patients with hereditary ATTR amyloidosis who had been evaluated at our centre and undergone LTx as of January 2012 were included in the study. Gastric emptying rates were measured according to the method employed in the Swedish multicenter study of gastric emptying. The patients’ nutritional status was assessed with the modified body mass index (mBMI), in which BMI is multiplied by s-albumin to compensate for edema. A validated ten-point rating scale and the well-documented Gastrointestinal Symptoms Rating Scale (GSRS) were used to assess gastrointestinal symptoms. All data analyzed, except for questionnaire data, were from routine investigations performed before LTx and for the evaluation of disease progression. Results: One-hundred fifteen patients were available for the study and all but three carried the TTR V30M-mutation. The long-term follow-up questionnaires were sent to the 92 patients (80%) who were still alive and Swedish residents at the time of the study. Fifty-two patients (68%) had completed all three sets of questionnaires, in median 0.72 years before LTx and 1.91 and 8.35 years after LTx, respectively, and they were included in subsequent analyses. A significant increase in total gastrointestinal symptoms scores was found over time (median score 7 vs. 9 vs. 12, p < 0.001). Gastric emptying rates were evaluated in median 0.66 years before and 1.91 and 4.53 years after LTx, respectively. No significant change in gastric emptying rates was found at the short-term (mean T50 138 vs. 149 min, p = 0.225) or long-term follow-up (mean T50 147 vs. 141 min, p = 0.702). The nutritional status was assessed in median 0.66 years before LTx and 1.89 and 4.62 years after LTx, respectively, and no significant change in mBMI was found (median mBMI 971 vs. 946 vs. 910, p = 0.966). Conclusions: Liver transplantation did not completely halt the progression of gastrointestinal symptoms in patients with hereditary ATTR amyloidosis. However, their gastrointestinal symptoms scores were generally low and their gastric emptying rates and nutritional status were maintained.
Familial amyloid polyneuropathy (FAP) due to transthyretin (TTR) mutations is a progressive neurodegenerative disorders affecting the motor, sensory, and autonomic components of the peripheral nervous system. We have previously identified TTR A97S as the most important hot spot mutation of FAP in Taiwan (Neurology 2010; 75: 532-538) in contrast to the most common V30M mutation in the western countries and Japan. One of the major manifestations of FAP is the early development of small fiber symptoms and signs, such as neuropathic pain and reduced nociceptive sensations. To evaluate small fiber neuropathy pathologically and quantitatively, we and other groups have established skin biopsy with quantification of intraepidermal nerve fiber (IENF) density as a pathologic marker of small fiber neuropathy, which is also prevalent in diabetes (Diabetes Care 2010; 33: 2654-2659) and uremia (Arch Neurol 2011; 68: 200-206). This technique offers an approach to investigate the pathology of neurological deficits in addition to nerve conduction studies, which only access larger fiber deficits. FAP patients frequently show sensory symptoms and neuropathic pain with length-dependent distribution, i.e. starting from toes and feet and progression to feet, legs, palms, and forearms later on. To investigate the pathology of this phenomenon, we analyzed nerve morphometry of sural nerves and IENF density of the leg skin of our FAP patients with TTR A97S. In comparison with control subjects, FAP patients had lower IENF density in the leg skin (0.8833 ± 0.2546 vs. 7.800 ± 4.700 fibers/mm, p < 0.001) and myelinated fiber density in the sural nerve (1086 ± 190.3 vs. 7595 ± 859.1 fibers/mm2, p < 0.001). The degree of nerve degeneration was more obvious in the skin compared with that in the sural nerve. These observations provide integrated assessments of small fiber neuropathy and the spatial pattern of nerve degeneration in FAP.
Background There is a paucity of data on the natural history of transthyretin (ATTR) amyloidosis. The imminent prospect of disease-modifying therapeutics has identified an urgent need to carefully define the natural history of this predominant cardiac and neuropathic disease as well as its impact on quality of life (QoL) in order to inform the design of future clinical trials. We report preliminary data from a comprehensive, systematic model of clinical care. Methods We have developed a specialist clinical service for patients with ATTR amyloidosis. Routine comprehensive annual assessments included a detailed clinical evaluation (physical examination, lying and standing blood pressure, ECOG performance status, NYHA class and 6 minute walk test (6MWT)); serological investigations including cardiac biomarkers; cardiac investigations (ECG, echocardiography, DPD scintigraphy and cardiac MRI using a novel method of extracellular volume (ECV) mapping to measure the myocardial amyloid burden), neurological investigations (neuropathy impairment score (NIS), Coutinho and PND scores, electrophysiological tests including electromyography (EMG), sympathetic skin response and RR interval testing); and general and disease-specific health surveys (SF36, KCCQ and Norfolk QOL-DN). Results Since May 2013, 45 patients have each undergone a first comprehensive clinical assessment. Twenty-seven (60%) patients had senile systemic amyloidosis (SSA) and the remainder had variant transthyretin-associated amyloidosis (8 with V122I and 10 with other non-V30M variants). Selected clinical findings are presented in Table 1. General health-related (QoL), as measured by the SF-36v2 questionnaire was poor across the cohort. Mean scores are shown in Figure 1. Conclusions This protocolised model of clinical care will enable the natural history and clinical outcome of ATTR amyloidosis to be carefully and systematically determined. It will provide a framework within which the cardiac and neuropathic effects of senile systemic amyloidosis and the different variant ATTR amyloidoses can be compared and provide, for the first time, a detailed prospective assessment of quality of life in patients with ATTR amyloidosis. This will be invaluable in order to design meaningful clinical trials to test novel therapeutic agents.
E-07 - A NOVEL FAMILIAL AMYLOIDOTIC POLYNEUROPATHY (FAP) ASSOCIATED WITH THE TRANSTHYRETIN (TTR) VARIANT THREONINE59ARGININE (THT59ARG)

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The proband was a 52-year-old Japanese man who had dyspnea, pitting edema in his legs and cardiac arrhythmias. Three years after onset, increased left ventricular wall thickness was detected. Histochemical analyses, using Congo red and anti-TTR-antibody, demonstrated massive TTR amyloid deposition on myocardial biopsy. Genetic analysis of TTR revealed a C to G transition in codon 59 of exon 3 causing a TTR mutation Thr59Arg which was not reported previously. Thus, we concluded that the cause was FAP associated with amyloidogenic TTR Thr59Arg (FAP ATTR Thr59Arg). Sensory disturbance was not shown. The results of nerve conduction studies were all normal at that time, but peripheral vasoconstrictor responses to sympathetic activation following deep inspiration were abnormally low on a laser-Doppler flowmetry test in his finger-tip. Other typical FAP manifestations were not observed. His family history of FAP was not revealed. The distribution of amyloid deposition, which may be heavy in the heart, reflects the clinical features of the disease. Another case of FAP causing a TTR mutation in codon 59 has been reported as cardiac amyloidosis type. Although reasons for the phenomenon are not well understood, this fact may provide additional impetus to examine the mechanism of TTR amyloid formation.

E-08 - NOVEL PHENOTYPE OF FAMILIAL AMYLOIDOTIC POLYNEUROPATHY WITH A RARE TRANSTHYRETIN VARIANT: ATTR ALA45ASP

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Of pathologic transthyretin (TTR) mutations, ATTR Val30Met is the most common and the only mutation found in large foci of familial amyloidotic polyneuropathy (FAP) patients; its characteristics include an autonomic, sensory dominant polyneuropathy. Over 120 different points of single or double mutations, or a deletion in the TTR gene, have so far been reported. Here, we present a rare case with FAP ATTR Ala45Asp associated with novel symptoms. This mutation has been reported in only one American family and the details of the associated clinical symptoms were not well documented. The proband is a 48-year-old Japanese man. He presented with diarrhea as the initial manifestation at age 46, followed by orthostatic hypotension, sexual impotence, painful sensory disturbance, dysgeusia, macroglossia, muscle weakness, and decreased body mass. Neurological examination revealed muscle atrophy and weakness predominantly in the proximal extremities. The tendon reflex could be elicited at the ankle, but not at the knee. Distal-dominant dissociated sensory disturbance and autonomic dysfunction were observed. EMG showed an axonal degeneration-type polyneuropathy. Echocardiographic examination disclosed a cardiomyopathy with thickened and sparkling ventricular wall. Holter ECG was normal, without atrioventricular conduction disturbances. Renal dysfunction, eye manifestation, and cerebral amyloid angiopathy were not detected. Biopsy from the biceps muscle and tongue muscle showed massive deposition of TTR-derived amyloid at the perimysium. Genetic analysis revealed the rare mutation: ATTR Ala45Asp. In addition to the distal dominant sensory disturbance and severe autonomic failure, the phenotype was characterized by proximal dominant muscle weakness, macroglossia, and cardiomyopathy. This may have resulted from massive amyloid deposition in the striated muscular tissue in the pathogenesis of this mutation.
E-09 - TRANSTHYRETIN FAMILIAL AMYLOID NEUROPATHY : STUDY OF EPIDERMAL NERVE FIBER DENSITY IN SYMPTOMATIC PATIENTS

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Aim of the study was to assess punch skin biopsies (PSB) in transthyretin familial amyloid neuropathy (TTR-FAP) patients. We performed distal (D) and proximal (P) lower limb PSB in 47 patients, with symptomatic neuropathy and genetic proven (TTR-FAP). Age ranged between 24 and 81 yo. There was 13 varied TTR mutations (Val30Met mutation 47%). Patients underwent LSGB (40), nerve (15) and/or other tissues biopsy (10). 3 mm PSB were done 10 cm above the lateral malleolus and 20 cm below the anterior iliac spine under local anesthesia. Amyelinic skin fiber density was calculated on bright-field PGP-immunofluorescence along EFNS guidelines (J PNS 2010; 15:79-92). Congo red staining was done on each skin biopsy to detect amyloid deposition. Delay between the first symptom and the PSB ranged from 0.9 to 10.35 year. Decrease to complete loss of epidermal fibers (EF) was observed in all patients; only 1 patient had normal density at the proximal level. EF had disappeared at the 2 levels in 6 pts , only at the lower level in 7. Reduced density was present at the lower level except 5 patients with more EF at the distal level than in the proximal. Congo positive amyloid deposits were present in 31 patients, on both levels in 16. In conclusion: dramatic decrease of epidermal fibers and amyloid deposits were detected in the majority of the patients with symptomatic FAP. This technique may be useful for the follow up of the patient under treatment, future clinical trials or to detect early the neuropathy.

E-10 - PREDOMINANTLY UPPER LIMB NEUROPATHY (UL0-FAP) AS A FREQUENT PHENOTYPE IN FAMILIAL AMYLOID POLYNEUROPATHY (FAP) : THE FRENCH REFERENCE CENTER EXPERIENCE

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In non endemic areas, diagnosis of TTR-FAP is difficult and usually delayed by 3 to 5 years after first symptoms. We describe a new phenotype of FAP with Upper Limb Onset (UL0-FAP). UL0-FAP was first described by Rukavina in 1956 et al and exceptionally later. 28/119 patients enrolled in the database of NNERF between 2008 and 2012 had an UL0-FAP. We retrospectively reviewed their clinical, genetic, neurophysiologic and histopathologic characteristics. There were 23 men, mean age at onset: 62.6 y.o.(41-84). Mean time to diagnosis was 4.1 y.o.(0.5-21.7). Nine different TTR gene mutations were detected (Val30Met in 12(43%)). The first UL symptoms were purely sensory in 23 patients, without any troncular topography. The mean NIS-UL at diagnosis was 24.6 (SD 14.5), mean NIS LL (25.7(SD 12)). Lower limb sensory symptoms occurred 2.6 years (0.5-10) after UL symptoms. Autonomic symptoms occurred in 23 pts (orthostatic hypotension in 12), at a mean delay of 2.3 y after UL onset. The nerve conduction studies disclosed a multifocal sensorymotor axonal neuropathy with a more severe SNAPs amplitude decrease in median nerves than in ulnar or sural nerves. Endoneurial isolated or perivascular amyloid deposits were found in 19/22 nerve biopsies. There were epineurial amyloid deposits, mainly in the vessel walls in 7/11 radialis nerve biopsies. Misleading diagnosis were CTS (11), idiopathic polyneuropathy (9), paraneoplastic neuropathy(4),CIDP (3), MND (2), spinal arthrosis (2). In France, FAP with upper limb onset are common. This phenotype could be due to an epineurial vessels occlusion by amyloid deposits, which may mimic vasculitic neuropathies.
E-11 - CHARACTERISTICS OF FRENCH TTR-FAP AND THE IMPACT OF THE CREATION OF FRENCH REFERENCE CENTER AND NETWORK FOR FAP

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TTR-FAP are progressive, disabling irreversible and life-threatening neuropathies due to a point mutation of TTR gene with autosomal dominant transmission. 428 FAP patients were subsequently evaluated since 1986 in the Department of Neurology (272 M, 156 W). Ethnical origin: 55.6% French, 35.8% Portuguese, 8.6% other. 36 TTR mutations identified: Met30-61%, Tyr77-12%, Phe77-7%, Val107-TTR-5%. 19 variants TTR were found in single case (1986-2012). The French Reference Center for FAP was certified by the Health Ministry in 2005 and a national Network for FAP-CORNAMYL was built in 2010 including 10 regional centers. Aims: to describe the population of 130 consecutive cases referred to the French Center (2008-12). We noticed an increase of: 1)new TTR-FAP cases (39 (2012) vs 13 (2008));2)genetic mutations by 7;3)12 new geographical departments with TTR-FAP reaching 79/100. We identified 3 new phenotypes: All-Fiber SM-PNP (13%), Upper Limbs NP (22%), Ataxic NP (14%). The mean age was 59 y (range:22-89), with a late onset (≥ 50 y) in 69% (26% > 70 y). The mean PND score at referral 2.6 (1-5). In French origin population (60%): 16 TTR variants, a late onset (90%), a rare family history of FAP (44%). LSGB was contributive for diagnosis in 63/92 (68%), nerve biopsy in 16/22 pts (73%). Conclusion : The labeling of a national reference center and of a national network for FAP allowed to identify many new cases of FAP, TTR gene mutations and phenotypes in the major part of the country (79%).

E-12 - SUDOMOTOR FUNCTION ASSESSMENT BY SUDOSCAN IN FAP PATIENTS: THE EXPERIENCE OF THE FRENCH REFERENCE CENTER FOR FAP

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Despite its frequency, autonomic neuropathy is often poorly investigated. Sympathetic C-fibers innervating sweat glands can be impaired early and measurement of sweat function has been suggested for early assessment of autonomic dysfunction in particular in diabetic polyneuropathy. SUDOSCAN is a quick, non-invasive and quantitative method to assess sudomotor function, based on an electrochemical reaction between sweat chloride and stainless-steel electrodes. The device measures the Electrochemical Sweat Conductance (ESC) of the hands and feet expressed in microSiemens (µS). ESC > 60 µS were considered as normal. The aim of this study is to assess the use of Sudoscan in exploration of Familial Amyloidosis with polyneuropathy (FAP). Methods: Patients with FAP, 80 patients (36 women/44 men), 53 patients with Met30TTR mutation (36 symptomatic/17 asymptomatic), 27 patients without Met30TTR mutation (21 symptomatic/6 asymptomatic). Sudoscan measurement: hands and feet. Results: In FAP patients with or without Met30TTR mutation, hands and feet ESC are dramatically decreased in symptomatic patients as compared to asymptomatic patients. In symptomatic patients, conductances are much lower in patients with orthostatic hypotension. It seems that there is link between time after Domino Liver transplantation and feet ESC. In the 13 subjects Domino Liver Transplant recipients, correlation between feet ESC and time after transplantation was 0.59 (p=0.003); Conclusions: Measure of sweat function with SUDOSCAN is a rapid and simple method which shows frequently abnormalities in FAP; its place in the management of patients with symptomatic TTR-FAP and those at risks for Amyloid Polyneuropathy should be assessed in larger cohorts.
E-13 - NERVE CONDUCTION STUDY PROFILE ACCORDING TO TTR MUTATION AND PHENOTYPE IN A NON ENDEMIC AREA OF TTR-FAP: AN EXPLANATION FOR MISLEADING DIAGNOSIS.

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Familial amyloid polyneuropathies (FAP) are initially small fibre neuropathies that evolve towards severe sensory motor neuropathies (SMN). Most often nerve conduction studies (NCS) disclose a severe axonal SMN but can be normal at the early stages. Atypical demyelinating electrophysiological profiles have also been reported. We compared initial NCS of 56 consecutive FAP French patients to those from PortMet30 Portuguese (PortMet30) and CIDP. 77 consecutive FAP patients had the 3 most common mutations in France (Late onset Met 30, Tyr 77, and Val 107) and varied phenotypes. - Our cohort presented a significantly much more severe axonal loss than PortMet30 as reflected by the sensory (ulnar+sural) and motor (ulnar+peroneal) sum score. -26/56(42%) of FAP patients fulfilled EFNS/PNS 2010 criteria for demyelination. Definite demyelinating profile was more common in Val107 (61%) than in Met30 (45%) and Tyr77 (32%) groups and in patients with large fibre or multifocal presentation (66% each). - Compared to CIDP, motor axonal loss was more important in patients with the Tyr77 mutation. F waves and distal latencies were often significantly more abnormal in FAP than in CIDP. The mean motor conduction velocities (MCV) did not vary significantly from CIDP patients. - Sensory conduction velocity was significantly lower than in the CIDP group. Our cohort of FAP patients, present a peculiar pattern of NCS abnormalities associating important axonal loss and prominent demyelinating abnormalities in sensory nerves and in the proximal and distal segments of motor nerves. This pattern can help to suspect FAP in patients with an unusual large fibre or multifocal clinical presentation.

E-14 - FREQUENCY OF THE TRANSTHYRETIN VAL30MET MUTATION IN THE NORTHERN SWEDISH POPULATION

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By genotyping a large number of samples from the Northern Sweden Health and Disease Study cohort, a carrier frequency could be determined for the Skellefteå and Lyckeå populations. A previous study of the amyloidogenic transthyretin mutation TTRV30M in Northern Sweden’s endemic area has shown a large variation in carrier frequency and penetrance of the trait within the area. However, the estimations have been based on a small sample size within the different regions in the area and therefore, the wide variation in TTRV30M carrier frequency observed between the Lyckeå and Skellefteå populations are uncertain. Based on a total of 3460 samples, the estimated overall carrier frequency in the two regions was 1.82% with a carrier frequency in the Skellefteå and Lyckeå population of 1.63% and 2.02%, respectively. Thus, the previously reported extremely high frequency in the Lyckeå region compared to that of the Skellefteå region could not be substantiated. However, it does not change the previous finding of a surprisingly higher carrier frequency in the population from endemic area of Northern Sweden compared to that reported from endemic areas in Portugal.
Transthyretin-related familial amyloidotic polyneuropathy (TTR-FAP) is a life-threatening systemic disease. Despite over 100 different TTR gene mutations have been reported to date, the Val30Met-TTR is considered the most frequent variant. Different early and late-onset FAP phenotypes have been described related to Met30 mutation. We report the clinical features of two non-related patients from Argentina sharing the same TTR-FAP Met30 mutation highlighting the differences among the early and late onset phenotypes. Patient 1: a 31-year-old man from endemic area consulted for erectile/ejaculatory failure and urinary urgency. Few months later, he referred symmetrical burning sensation on his feet. Pinprick and temperature sense were reduced in stockings distribution. Orthostatic hypotension was observed. Electrophysiological studies demonstrated a length-dependent symmetric sensory-motor axonal polyneuropathy. Sural nerve biopsy failed to show amyloid deposits. Genetic studies confirmed heterozygous Val30Met mutation. The patient is waiting for liver transplantation. Patient 2: a 71-year-old female from a non-endemic area complained of numbness and pain in her extremities. History of hypothyroidism and left carpal tunnel syndrome was referred. Due to prior diagnosis of CIDP she received immunomodulatory treatment with no clinical response. Examination showed decreased sensation to pinprick and temperature over hands and feet. Vibration and joint position sense were absent at the toes. Muscular strength (MRC): 4/5 on dorsiflexion of the feet; 4+/5 on wrist flexion/extension. Absent reflexes in lower limbs. Electrophysiological studies demonstrated a length-dependent axonal sensory-motor polyneuropathy. Sural nerve biopsy was unable to demonstrate amyloid deposits. All lip, abdominal fat and colon biopsies revealed amyloid deposits. DNA analysis confirmed heterozygous Val30Met TTR mutation. After 3 years of follow up, the patient is wheelchair-bound suffering of pain crises, autonomic dysfunction and swallowing difficulties. Liver transplantation was not considered. Tafamidis is not still available in Argentina. Conclusion: unlike the onset of severe autonomic dysfunction seen in our young patient, the older one showed a disabling motor compromise and late autonomic manifestations. These findings confirm the contrasting initial manifestations when comparing early and late onset FAP sharing the same transthyretin gene mutation. TTR FAP should be considered in patients with polyneuropathy of unclear origin, despite negative nerve biopsy and absence of family background.
E-16 - EVALUATING THE QUALITY OF LIFE AND BURDEN OF ILLNESS IN AN ULTRA-RARE DISEASE IN THE U.S.: TRANSTHYRETIN FAMILIAL AMYLOID POLYNEUROPATHY (TTR-FAP) PATIENTS & CAREGIVERS

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Objectives: Transthyretin familial amyloid polyneuropathy (TTR-FAP), a progressive, degenerative ultra-rare genetic disease, causes extensive neurological and functional impairment, requiring substantial caregiver support. This study evaluated the burden of disease on patients’ and caregivers’ mood symptoms, functional status, health-related quality of life (HRQoL), and caregiver burden. Methods: An online survey including the SF-12, Hospital Anxiety & Depression Scale (HADS), Kansas City Cardiomyopathy Questionnaire (KCCQ), Norfolk Quality of Life—Diabetic Neuropathy (Norfolk QOL-DN), and caregiver questions recruited patients and caregivers through two U.S.-based patient advocacy groups. Interim descriptive statistics were summarized separately for those with symptoms of TTR-FAP only, those with TTR-FAP and cardiomyopathy (TTR-FAP+CM), and caregivers with and without disease. Subgroup analyses were done for those who had undergone liver transplantation (LT). Results: A total of 38 patients and 16 caregivers completed the survey as of September 2013. Ages for patients ranged from 37-74 years and 36-72 years for caregivers with most between 50-60 years. Patients were mostly male and caregivers were mostly female. The majority of caregivers were spouses/partners and were the primary caregiver for the patient; 50% had TTR amyloidosis. Caregiving duration ranged from 0.1 to 9.1 years. The majority of patients relied on walking aids for ambulation. Median disease duration ranged from 8 months to 15 years. Less than half (42.1%) had undergone LT. Non-V30M mutations predominated. Patients with TTR-FAP+CM reported more impairment on the Norfolk. TTR-FAP patients who had not undergone LT reported the highest current pain levels. Scores on the SF-12 subscales were generally lower than age-matched population norms, some markedly so, but were fairly comparable across LT groups. TTR-FAP+CM patients who had undergone LT had greater impairment on the KCCQ compared to those without LT (29.6 vs. 42.4). Caregivers reported moderate levels of fatigue and overall life satisfaction. Higher rates of clinically significant depression and anxiety were seen for caregivers. Patients with LT had higher rates of depression, but lower anxiety. Conclusions: TTR amyloidosis is associated with substantial disruption in HRQoL for patients and impaired mental health for caregivers. Many caregivers face the added burden of also having TTR amyloidosis. The pattern of impairment was variable by LT status. Disclosure: This study was sponsored by Pfizer Inc.
E-17 - THE NEUROPHYSIOLOGICAL PHENOTYPE OF AH-TTR AMILOIDOSES

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Partially supported by FAMY association The classical neurological presentation of HA-TTR is that of a sensorimotor progressive axonal polyneuropathy with dysautonomia. Misdiagnosis of the disease is not uncommon, and a wrong interpretation of neurophysiological data is one of the reasons for delayed diagnosis of HA-TTR. We describe the neurophysiological patterns of presentation of somatic polyneuropathy and of dysautonomia in a large cohort of HA-TTR patients. 137 patients with TTR-related amyloidosis and their affected relatives have been referred to our Centre from 1980 up to 2013. At onset 15/137 (10.9%) patients presented a mild reduction of sensorimotor conduction velocities, but the neurophysiological pattern did never fulfill the criteria for CIDP. 6/137 (4.3%) patients presented lower motor neuron-like onset, with progressive motor symptoms preceding onset of sensory and autonomic disturbances. The EMG data fill all criteria for motor neuron disease diagnosis, apart from mild involvement of sensory conduction velocities. At follow up all patients developed a sensorimotor axonal polyneuropathy. In some patients abnormal Sympathetic Skin Response (SSR) and cardiovascular tests preceded the clinical onset of the somatic neuropathy and of orthostatic hypotension by many months. Axonal sensorimotor polyneuropathy with dysautonomia is not the only pattern of neurophysiological presentation of HA-TTR. "Demyelinating" sensorymotor neuropathy or motor neuron-like presentation are also possible in early stages of the disease. In amyloidotic cardiomyopathy, amyloid deposition is highly heterogeneous and different patterns of infiltration are identifiable with distinctly different clinical outcomes (Rapezzi 2006, 2012). Likewise, the neurophysiological heterogeneity of TTR neuropathy may reflect variability of the amount and distribution of amyloid deposits in the skin, nerves, nerve roots and central nervous system. Further studies are needed to correlate neurophysiological phenotypes and histological patterns in HA-TTR. Rapezzi C. et al. “Phenotypic and genotypic heterogeneity in transthyretin-related cardiac amyloidosis: towards tailoring of therapeutic strategies? Amyloid 2006; 13(3): 143-53. Leone O. et al. New pathological insights into cardiac amyloidosis: implications for non-invasive diagnosis. Amyloid 2012, 19(2): 99-105

E-18 - FAMILIAL AMYLOID POLYNEUROPATHY PRESENTING AS ATYPICAL LOWER MOTOR NEURON DISEASE: A CASE REPORT

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Rare diseases are a tough problem for medical community. More important is to make an early diagnosis of a treatable disease such as familial amyloid polyneuropathy (FAP) due to mutations of the transthyretin (TTR) gene. The aim of this work is to report a series of six patients with amyloid polyneuropathy characterized by a lower motor neuron-like onset. The unusual presentation of the disease was mistaken for untreatable diseases such as amyotrophic lateral sclerosis (ALS), so that the definite diagnosis of FAP was delayed of many years. We suggest the utility to search for TTR mutations in all subjects presenting with atypical lower motor neuron disease.
E-19 - DETECTION OF EARLY ABNORMALITIES IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY BY QUANTITATIVE SENSORY TESTING

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Introduction. The small fiber neuropathies alter myelinated Aδ fibers and non-myelinated type C fibers, involved in autonomic functions, nociception, thermal perception and sweating. Genetic mutation TTR-Val30Met is the etiologic factor in the Portuguese variant of Familial Amyloid Polineuropathy, compromising earlier the small nerve fibers. Conventional neurophysiological studies are unable to detect those abnormalities, delaying specific treatments. The main goal of the present study was to evaluate the Quantitative Sensory Testing –QST, as an early diagnostic tool, identifying abnormalities in patients with the genetic mutationVal30Met in transthyretin (TTR) gene, followed at Santa María Hospital, Lisbon, Portugal. Patients were classified in 3 groups according to symptoms and neurological examination. Thresholds for cold perception, heat-pain and vibration were assessed in the different groups, compared to healthy controls. Results 33 cases and 18 controls, divided in asymptomatic (24.2%), symptomatic with normal neurological examination (42.4%) and symptomatic with abnormal neurological examination (33.3%). There were no differences between asymptomatic patients and healthy controls. The abnormalities on cold threshold detection and intermediate severity of heat-pain response (HP5), can occur before changes in the neurological examination. In patients with abnormal neurological examination differences include the stimulus-response slope (HP 5-0,5) between intermediate severity (HP 5.0) and onset perception of pain with heat (HP 0.5). Conclusions Cold, heat pain 5,0 and stimulus response slope (HP 5-0,5) thresholds are the most useful parameters showing differences between symptomatic and asymptomatic patients, including those without neurological abnormalities. The Quantitative Sensory Testing showed to be a useful tool in the detection of earlier abnormalities in patients with TTR Val30Met mutation.

E-20 - TRANSTHYRETIN-RELATED FAMILIAL AMYLOIDOTIC POLYNEUROPATHY: DESCRIPTION OF SIX UNRELATED PATIENTS FROM AN ARGENTINEAN HOSPITAL

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INTRODUCTION AND OBJECTIVES: TTR-FAP has been reported throughout the world, particularly in Portugal, Sweden and Japan. Focus of the disease in Portuguese descendent has been described in Argentina but clinical data are still scarce. The objective was to describe the clinical and genetic findings in patients with TTR-FAP evaluated in an Argentinean Hospital. METHODS: We retrospectively evaluated clinical and genetic data of all TTR-FAP patients registered in our institution. All patients had presence of amyloid deposits in tissue biopsy and genetic confirmation by PCR amplification technique. RESULTS: We collected six unrelated patients (4 male and 2 female) with TTR-FAP. Five patients had the Val30Met mutation and one had Pro36Ala mutation. The mean age of onset for symptomatic disease was 36 years (range 26 ± 53). One patient had late onset (>50 years of age) of symptoms. The clinical onset was a length-dependent sensory neuropathy in all patients. Autonomic dysfunction was manifested in all cases. One patient had cardiac involvement. Two patients had history of Argentinean ancestry. Portuguese family origin was detected in two patients. One patient had Peruvian ancestry and the remaining patient did not know the accurate origin of their family. Three patients had undergone liver transplantation. Of these, one died in the postoperative period. The other two patients remain clinically stable. CONCLUSIONS: TTR-FAP is a progressive and fatal disease that exists in the Argentinean population and is clinically and genetically variable. Sensory-motor and autonomic neuropathy are predominant but cardiac involvement is rare in our series.
E-21 - TRANSTHYRETIN AMYLOIDOSIS OUTCOMES SURVEY (THAOS): CLINICAL PRESENTATION OF SYMPTOMS ACROSS THE DISEASE COURSE FOR MAJOR GENOTYPE GROUPS

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Objective: To map the clinical presentation of transthyretin amyloidosis (ATTR) symptoms over time for different genotypes using data from the Transthyretin Amyloidosis Outcomes Survey (THAOS). Background: The natural history of ATTR is poorly defined and its characterization is complicated by the extreme variability in penetrance and expression of different symptoms. The global, observational THAOS patient registry offers a unique dataset to delineate the occurrence of ATTR symptoms for the various genotypes. As of June 2013, a total of 1744 subjects were enrolled in THAOS representing 64 different TTR genotypes. Methods: Symptomatic patients without disease-modifying treatment were categorized into one of three genotypes: wild-type, Val30Met, and non-Val30Met mutation. Val30Met patients were further divided into those with early onset (2 to 4, >4 to 6, >6 to 8, >8 to 10, and >10 years, and the percentage of patients experiencing a given symptom at the last visit was calculated. Results: The overall pattern of the clinical presentation of sensory, autonomic, or motor neurological symptoms across different disease duration categories was similar for early and late onset Val30Met subgroups. In both subgroups, sensory and autonomic neuropathy affected high proportions of patients irrespective of duration while motor neuropathy steadily increased from 70% as disease duration increased. The percentages of late onset patients with cardiac disorders consistently exceeded those of the early onset cohort. Virtually all wild-type patients reported cardiac disorders, with heart failure occurring in ≥80% in all disease duration categories. About half of the wild-type cohort also experienced symptoms attributed to autonomic neuropathy regardless of disease duration. Symptoms of sensory neuropathy were also reported in all disease duration categories albeit with lower frequency in short disease duration groups. Data from the heterogeneous non-Val30Met cohort show more variable patterns of symptom presentation. Conclusions: The current observations enhance the emerging picture of disease history. The clinical presentation of neurologic symptoms is largely similar in early and late onset Val30Met patients and appears more frequent amongst wild-type patients than anticipated from reports of an exclusively cardiac phenotype. Future analyses promise to further improve our understanding of ATTR disease progression. Disclosure: Data presented in this abstract are derived from the THAOS registry, which is sponsored by Pfizer Inc.

E-22 - COMBINED SKIN BIOPSY AND NEUROPHYSIOLOGICAL STUDY IN TTR-AMYLOIDOSIS ALLOWS EARLY DETECTION OF SMALL FIBER NEUROPATHY

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Introduction Small-fiber neuropathy (SFN) is the most frequent and early manifestation of transthyretin familial amyloid polyneuropathy (TTR-FAP). In this study, we evaluated the value of intraepidermal nerve fibers density (IENFD) quantification by skin biopsy and neurophysiological investigation of small nerve fibers to detect SFN in TTR-FAP. Method We evaluated 7 patients with clinical symptoms of polyneuropathy (SM/2F; 40-75 y-o) and 5 carriers (SM; 30-63 y-o). They presented 5 types of pathogenic ATTR-variants: V30M (n=8), V28M (n=1), S77T (n=1), S77P (n=1) and T49I (n=1). Skin biopsies were performed at thigh (proximal) and leg (distal); IENFD was measured after immunofluorescence staining of PGP9.5 in nerve terminals. Lower limit of normal values were 12.8 f/mm at thigh and 7.6 at leg (Devigili et al, 2008). Congo red staining was performed to detect amyloid deposits. Neurophysiological investigation including laser evoked potentials (LEP), quantitative sensory testing (QST), sympathetic skin response (SSR) and heart-rate variability (HRV). Results In the 7 patients with overt neuropathy, skin biopsy evidenced SFN, with proximal IENFD (mean±SD) at 4.3±3.9 f/mm, and distal IENFD at 2.3±1.6 f/mm. Neurophysiological investigation showed abnormal LEP (n=5), QST(n=5), SSR (n=6), and HRV (n=7). In the 5 carriers, proximal IFND was decreased in 5/5 at 7.1±4.3 f/mm, and distal IENFD in 4/5 at 3.8±1.9 f/mm. Neurophysiological investigation showed abnormal LEP (n=4), QST(n=1), SSR (n=2), and HRV (n=4). Finally, congo stain disclosed amyloid deposits in 5/7 patients and 2/5 carriers in skin biopsy. Conclusion This pilot study showed that a combined approach based on IENFD quantification and a battery of neurophysiological tests are appropriate tools for evaluation of SFN in TTR-FAP. Such a combined approach may detect early stage of SFN in this context and therefore identify potential candidates for innovative therapeutic strategies. In addition, skin biopsy can evidence amyloid deposits associated with TTR-FAP.
E-23 - NEW DEVELOPMENT OF PENETRANCE ESTIMATES IN TRANSTHYRETIN FAMILIAL AMYLOID NEUROPATHY (ATTR) USING A NON PARAMETRIC APPROACH

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ATTR is the most severe inherited neuropathy of adulthood, with autosomal dominant transmission. Onset ranges from early twenties to late seventies depending on the geographic origin. In previous work, we estimated penetrance i.e. the risk for a carrier to become affected as a function of age in ATTR families from different geographic origin using a parametric method. Ascertainment bias was corrected by exclusion of the proband. This approach allowed unravel components that influence the time of onset like gender, sex of the transmitting parent, ATTR variant. However, the method was limited by the choice of a Weibull distribution that lacks the flexibility to estimate the true penetrance function. We present a non-parametric method to assess the penetrance, with a Kaplan-Meier estimate for familial data. Correction for ascertainment bias is done as before. This method is also able to accommodate covariates as sex, parent of origin, etc, through a Cox model. We illustrate this new approach on 158 ATTR families including 33 Portuguese, 77 Swedish and 48 French kindred, and investigate the effects of sex and parent-of-origin in these families. We compare our results with the results obtained by the parametric method. In most cases, the parametric method seems to overestimate the penetrance function. At age 70 years, the estimation of the cumulative risk with the parametric method for French, Portuguese and Swedish families are 74.5%, 93.6% and 62.5%, respectively while the non-parametric approach estimates this risk at 71.1%, 86.3% and 50.4%. Moreover, the non-parametric approach shows a difference on the penetrance depending on parent-of-origin, as shown previously with the parametric method. This new non-parametric approach for penetrance estimation in ATTR is more accurate and will help to refine our knowledge on underlying factors that modify phenotypic expression.

E-24 - NEUROLOGIC INVOLVEMENT IN VAL-122 ILE FAMILIAL AMYLOIDOSIS

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Objective: Describe new phenotype in familial amyloid cardiomyopathy. Introduction: Late onset cardiomyopathy with heart failure is the phenotype of familial amyloidosis, which occurs in African Americans (AA) in the United States at a rate 4 times that of whites after the age of 60. 3.9% of AA are heterozygous for the amyloidogenic allele where isoleucine substitutes for valine at position 122 (Val- 122-Ile) of the serum carrier protein transthyretin (TTR). We report on our observed neurologic changes in addition to the cardiomyopathy. Setting: Academic, tertiary care referral centers. Methods: case series, retrospective review. Results: Six patients with the TTR 122 Ile gene mutation had amyloid staining on myocardial biopsies. All had symptoms of peripheral neuropathy. Causes of a peripheral neuropathy other than amyloid were excluded. All six patients had axonal predominant peripheral neuropathy, confirmed by NCV and EMG. One patient had a superimposed painful (narcotic requiring), lumbar radiculopathy, by EMG, without corresponding structural MRI abnormalities. One patient had multiple entrapment neuropathies (bilateral wrists and elbows), as well as, a prominent small fiber neuropathy documented by reduced intraepidermal nerve fiber density and autonomic nerve abnormalities with reduced sweat gland nerve fiber density. Conclusion: Val-122 Ile patients who are known to have amyloid cardiomyopathy may also have a peripheral neuropathy possibly due to amyloid deposition in the nerve. Clinical symptoms of radiculopathy confirmed by EMG may be explained by dural amyloid infiltration. Prospective studies of this phenotype are warranted to confirm these findings and to determine whether the peripheral neuropathy occurs earlier than the cardiomyopathy.
E-25 - PROFILE OF CLINICAL AUTONOMIC DYSFUNCTION IN V30M TRANSTHYRETIN PERIPHERAL NEUROPATHY

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Objective: To characterize the clinical autonomic dysfunction in transthyretin (TTR) amyloid neuropathy.

Background: Hereditary amyloidosis due to TTR gene mutations manifests with somatic and autonomic neuropathy. The autonomic dysfunction has a major impact in the quality of life on some patients. In this series of patients we assessed the prevalence and type of clinical autonomic dysfunction. Design /Methods: Patients with amyloid neuropathy and TTR gen mutations were questioned about symptoms of autonomic dysfunction including the following domains: vasomotor, sudomotor, upper and lower gastrointestinal, urinary and sexual. The proportion of patients with clinical autonomic dysfunction was analyzed for associations with nerve conduction abnormalities and sensory-motor disability. Results: Seventeen subjects with V30M mutation in the TTR gene and evidence of peripheral neuropathy on clinical and ancillary examinations were evaluated. Eight (47%) presented episodic sweating, 1 (5%) heat intolerante, 5 (29%) orthostatic hypotension, 7 (41%) gastrointestinal symptoms, 3 (17%) urinary symptoms and 3 (60% of men) erectile dysfunction. Overall, 68% of patients with TTR-FAP presented some kind of autonomic dysfunction. There was an association between the presence of clinical autonomic dysfunction and nerve conduction abnormalities and degree of sensori-motor disability as assessed by the mPND scale. Conclusions: Symptomatic autonomic dysfunction is frequent in our population of TTR-FAP patients. In particular, we found a high incidence of episodic sweating. Episodic sweating and other autonomic symptoms correlate with sensor-motor disability.
Author’s Index
Adam, C.; E-09, E-10
Adams, D.; A-02, A-03, A-04, A-07, E-09, E-10, E-11, E-12, E-13, OP.2-06, OP.3-03, SP.4-02, SP.6-03
Adnot, S.; C-29
Aguirre, A.; E-20
Akagami, T.; E-07
Alagalarurondo, V.; A-02
Alarcon, F.; E-23
Alfonsi, E.; B-04
Alagalarurondo, V.; OP.2-06
Almeida, A.P.; D-21
Almeida, M.R.; OP.4-01
Alonso, I.; D-12, D-14
Alvarez, J.; B-01
Alvarez, V.C.; E-15
Alves, C.; E-01
Alves, M.C.; A-01
Alves-Ferreira, M.; D-14
Alvir, J.; E-16
Amer, Y.B.; E-22
Anan, I.; E-03, E-04, OP.2-03
Anavekar, N.S.; C-20
Ando, T.; E-07
Ando, Y.; A-05, D-04, D-05, D-07, D-08, D-10, D-11, E-07, E-08, SP.4-05, OP.1-02
Andrade, C.; D-02
Andrade, C.; D-15
Andujar, I.; D-13
Antonini, T.; A-02, A-03, A-04, OP.3-03
Antonini, T.M.; A-07
Arlicot, N.; OP.2-06
Askew, J.W.; C-20
Atmaca, M.A.; B-06, A-12
Authier, F.J.; E-22
Ayache, S.S.; E-22
Azevedo, E.; KN-01
Azevedo, E.P.C.; D-19
Azevedo,E; D-20

Bangova, A.; E-06
Banyperasad, S.M.; C-11, OP.2-05
Barroso, F.A.; E-25, E-15, SP.6-02
Bartolomei, I.; C-08, C-10, C-15, C-17, C-18, OP.2-04
Batista, A.R.; OP.4-02
Bauer, R.; C-22
Beaudonnet, G.; E-12
Ben-Azzooua, R.; OP.2-06
Benson, M.D.; D-04, KN-05
Bentes, C.; E-02
Berardi, S.; A-06
Berenshtein, A.C.; C-04, C-23
Berg, S.; SP.1-01
Berk, J.; SP.8-04
Bettini, M.; E-20
Bitan, G.; D-20, OP.4-01
Björn, P.; C-12
Bodez, D.; C-24, C-28, C-29, C-30
Bokhari, S.; C-02
Boyer, L.; C-29
Braga, C.; D-18, D-20, KN-01
Braga, C.A.; D-19
Braga, C.A.C.A.; D-21
Brannagan III, T.H.; E-24
Burniston, M.; C-09
Buss, S.; C-22
Butler, K.R.; C-16
Buxbaum, J.N.; C-16, SP.1-03

Caldeira, C.M.; C-13, D-02
Calvet, J.Y.; E-12
Camdessanche, J.P.; E-11
Campos, R.; OL.1
Cappelli, F.; C-18
Capron, F.; OP.2-06
Catarini, S.; B-04, SP.4-01
Castaing, D.; A-02, OP.3-03
Castano, A.; C-02
Castro, J.; B-02, E-19
Catherine, L.; A-07
Caughey, B.; D-16
Cauquil, C.; A-02, A-03, A-04, E-09, E-10, E-11, E-12, E-13, OP.3-03
Cavalla, F.; C-06, C-14
Cederquist, K.; E-14
Chao, C.C.; E-05, OP.2-02
Chapman, J.; KN-02
Chaves, M.; E-20
Chiò, A.; E-18
Chosa, K.; D-17
Cicchetti, M.; E-16
Coelho, T.; A-01, D-12, D-14, E-01, E-21, KN-02, SP.4-04, SP.8-01
Collins, L.M.; C-07
Conceicao, I.; B-02, E-02, E-19, SP.2-01
Cornamyl, F.; E-10, E-11
Cortese, A.; B-04, OP.4-03, SP.4-01
Cristiano, E.; E-20
Cruz, M.W.; A-08, B-05, C-04, C-13, C-23, D-02, E-21, KN-06

D

Da Silva, A.M.; E-01
Da Silva, P.S.F.; D-21
Da Silva, R.V.B.; A-08
Damy, T.; A-10, C-24, C-25, C-26, C-27, C-28, C-29, C-30
Daniel, J.; A-01
David, A.; C-21
De Freitas, J.A; D-18
De Oliveira, R.S.; D-21
De Tayrac, M.; OP.1-01
Deux, J.F.; C-26, C-27, C-28, C-30
Deymeer, F.; B-06
Díaz, A.; E-19
Didier, S.; A-07
Dinanian, S.; C-21
Dispenzieri, A.; C-20
Divoto, P.; E-24
Dodet, P.; E-13
Doki, T.; E-08
Domingos, J.; E-01
Dominique, L.G.; C-21
Drachman, D.; E-24
Duarte, M.T.; C-04, C-23
Dubois-Randé, J.L.; C-24, C-28, C-30
Dungu, J.; C-06
Durmush, H.; B-06
Durrbach, A.; A-07

E

Era, T.; D-10
Ercolani, G.; A-06
Ericzon, B.G.; A-11, KN-09, OP.1-01, OP.3-02
Escher, S.A.; C-03

F

Falk, R.H.; C-08, C-16, C-25
Fernandes, L; D-20
Ferreira, J.; E-02
Ferreira, M.A.; D-12
Ferreira, N.; OP.4-01
Ferreira, P.; C-13, D-02
Ferreira, P.S.; D-15
Ferreira, S.T.; D-01, KN-01
Filho, W.B.C.; C-23
Foguel, D.; C-13, D-01, D-02, D-15, D-18, D-19, D-20, D-21, KN-01
Fontana, M.; C-06, C-07, C-11, E-06, OP.2-05
Fox, E.R.; C-16
Franco, A.; E-02
Franques, J.; E-11
Freitas, V.; E-02
Frikke-Schmidt, R.; OL-1
Fusaki, N.; D-10

G

Gagliardi, C.; C-08, C-10, C-15, C-17, OP.2-04
Gallelli, I.; C-15
Genin, E.; OP.1-01
Gentile, N.; C-10, C-17, OP.2-04
Gianni, D.; OP.4-02
Giannitsis, E.; C-22
Gilbertson, J.A.; D-06
Gillmore, J.D.; C-06, C-07, C-09, C-11, C-14, D-06, E-06, OP.2-05
Gleeson, S.; E-16
Gobbi, M.; OP.4-03
Goncalves, N.P.; D-05, OP.1-03
González-Duarte, A.; SP.6-01
<table>
<thead>
<tr>
<th>Name</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gospodinova, M.</td>
<td>OP.2-01</td>
</tr>
<tr>
<td>Grigioni, F.</td>
<td>A-06</td>
</tr>
<tr>
<td>Grisoni, M.L.</td>
<td>E-12</td>
</tr>
<tr>
<td>Grogan, M.</td>
<td>C-20</td>
</tr>
<tr>
<td>Guellich, A.</td>
<td>C-24, C-28, C-30</td>
</tr>
<tr>
<td>Guendouz, S.</td>
<td>A-10, C-24, C-29</td>
</tr>
<tr>
<td>Guergueltcheva, V.</td>
<td>OP.2-01</td>
</tr>
<tr>
<td>Guidalotti, P.L.</td>
<td>OP.2-04</td>
</tr>
<tr>
<td>Guilloteau, D.</td>
<td>OP.2-06</td>
</tr>
<tr>
<td>Guimarães, A.</td>
<td>A-01</td>
</tr>
<tr>
<td>Guimarães, A.C.</td>
<td>D-21</td>
</tr>
<tr>
<td>Guimarães-Costa, A.B.</td>
<td>D-19</td>
</tr>
<tr>
<td>Gustavsson, S.</td>
<td>C-05, C-12</td>
</tr>
<tr>
<td>Haberkorn, U.</td>
<td>C-22</td>
</tr>
<tr>
<td>Hall, M.L.</td>
<td>C-09</td>
</tr>
<tr>
<td>Hammarström, P.</td>
<td>OL-1</td>
</tr>
<tr>
<td>Hardt, S.</td>
<td>C-22</td>
</tr>
<tr>
<td>Hasegawa, K.</td>
<td>D-11</td>
</tr>
<tr>
<td>Haufe, S.</td>
<td>C-22</td>
</tr>
<tr>
<td>Hawkins, P.N.</td>
<td>C-06, C-07, C-09, C-11, D-06, E-06, OP.2-05</td>
</tr>
<tr>
<td>Hellman, H.</td>
<td>E-23</td>
</tr>
<tr>
<td>Hellman, U.</td>
<td>C-03</td>
</tr>
<tr>
<td>Helmke, S.</td>
<td>B-01, C-01, C-02</td>
</tr>
<tr>
<td>Hinderhofer, K.</td>
<td>C-22</td>
</tr>
<tr>
<td>Hirotani, E-7</td>
<td></td>
</tr>
<tr>
<td>Hittinger, L.</td>
<td>C-24, C-28, C-30</td>
</tr>
<tr>
<td>Hörnsten, R.</td>
<td>D-03</td>
</tr>
<tr>
<td>Hornstrup, L.S.</td>
<td>OL-1</td>
</tr>
<tr>
<td>Hsie, S.T.</td>
<td>E-05, OP.2-02</td>
</tr>
<tr>
<td>Hulin, A.</td>
<td>A-10</td>
</tr>
<tr>
<td>Hutt, D.F.</td>
<td>C-09, C-11, E-06, OP.2-05</td>
</tr>
<tr>
<td>Ihse, E.</td>
<td>D-03</td>
</tr>
<tr>
<td>Ikeda, S.</td>
<td>B-03, OP.3-01, KN.7</td>
</tr>
<tr>
<td>Ikeda, T.</td>
<td>D-11, OP.1-02</td>
</tr>
<tr>
<td>Iliescu, I.</td>
<td>A-03</td>
</tr>
<tr>
<td>Isono, K.</td>
<td>D-10</td>
</tr>
<tr>
<td>Ito, T.</td>
<td>D-11</td>
</tr>
<tr>
<td>Jiang, X.</td>
<td>KN-02</td>
</tr>
<tr>
<td>Johnson, G.B.</td>
<td>C-20</td>
</tr>
<tr>
<td>Jonasson, J.</td>
<td>E-14</td>
</tr>
<tr>
<td>Jono, H.</td>
<td>D-10</td>
</tr>
<tr>
<td>Judge, D.</td>
<td>E-24</td>
</tr>
<tr>
<td>Judge, D.P.</td>
<td>B-08</td>
</tr>
<tr>
<td>Kai, H.</td>
<td>D-17</td>
</tr>
<tr>
<td>Kametani, F.</td>
<td>OP.3-01</td>
</tr>
<tr>
<td>Karam, V.</td>
<td>A-04</td>
</tr>
<tr>
<td>Karayal, O.</td>
<td>B-08, C-25, E-03, E-21</td>
</tr>
<tr>
<td>Karling, P.</td>
<td>E-03, E-04</td>
</tr>
<tr>
<td>Karlsson, M.</td>
<td>D-03</td>
</tr>
<tr>
<td>Katus, H.A.</td>
<td>C-22</td>
</tr>
<tr>
<td>Kelly, J.N.</td>
<td>D-19, KN-02</td>
</tr>
<tr>
<td>Kemp, B.J.</td>
<td>C-20</td>
</tr>
<tr>
<td>Khella, S.</td>
<td>E-24</td>
</tr>
<tr>
<td>Kirov, A.</td>
<td>OP.2-01</td>
</tr>
<tr>
<td>Kitagawa, K.</td>
<td>D-07</td>
</tr>
<tr>
<td>Kitagawa, K.</td>
<td>D-11</td>
</tr>
<tr>
<td>Kitzmann, D.</td>
<td>C-16</td>
</tr>
<tr>
<td>Kluve-Beckerman, B.</td>
<td>D-04, KN-05</td>
</tr>
<tr>
<td>Koike, H.</td>
<td>B-03, D-07</td>
</tr>
<tr>
<td>Komohara, Y.</td>
<td>OP.1-02</td>
</tr>
<tr>
<td>Koyama, J.</td>
<td>B-03</td>
</tr>
<tr>
<td>Kristen, A.</td>
<td>C-22, E-21</td>
</tr>
<tr>
<td>Kristen, A.V.</td>
<td>C-25</td>
</tr>
<tr>
<td>Kruger, J.</td>
<td>C-08</td>
</tr>
<tr>
<td>Kubis, N.</td>
<td>E-13</td>
</tr>
<tr>
<td>Kume, S.</td>
<td>D-10</td>
</tr>
<tr>
<td>Labeyrie, C.</td>
<td>E-12</td>
</tr>
<tr>
<td>Lachmann, H.</td>
<td>C-06, C-14</td>
</tr>
<tr>
<td>Lachmann, H.J.</td>
<td>C-07, D-06</td>
</tr>
<tr>
<td>Lacour, A.</td>
<td>E-11</td>
</tr>
<tr>
<td>Lacroix, C.</td>
<td>A-04, E-09, E-10, E-12</td>
</tr>
<tr>
<td>Lamine, A.</td>
<td>C-24, C-29</td>
</tr>
<tr>
<td>Lane, T.</td>
<td>C-07, C-11, C-14, E-06, OP.2-05</td>
</tr>
<tr>
<td>Larsson, M.</td>
<td>A-11, OP.3-02</td>
</tr>
</tbody>
</table>
Lavatelli, F.; SP.4-01
Le Guludec, D.; OP.2-06
Ledo, H.; D-01
Ledo, J.H.; KN-01
Lefaucheur, J.P.; A-10, E-22
Legou, P.; C-27
Lemos, C.; D-14
Lemos, L.; D-12
Lenderking, W.; E-16
Liepnieks, J.; D-04
Liepnieks, J.J.; KN-05
Lim, P.; C-28, C-30
Lima, C.; C-13, D-02
Lindqvist, P.; C-05
Lindqvist, P.; C-12
Loftus, J.; E-16
Longhi, S.; A-06, C-08, C-10, C-15, C-17, C-18, OP.2-04
Lorenzini, M.; OP.2-04
Lozzeron, P.; A-02, A-03, A-04, E-10, E-13, OP.3-03
Lozza, A.; B-04, OP.4-03
Lucchetti, J.; OP.4-03
Ludvine, E.; C-21
Lundgren, H.; C-03

Maeda, Y.; A-05
Maleszewski, J.J.; C-20
Mandel, F.S.; B-08
Manuzzi, L.; C-10, C-17
Marciano, S.; E-20
Mariani, L.L.; E-13
Mastroroberto, M.; A-06
Matur, Z.; B-06, A-12
Maurer, M.S.; B-01, C-01, C-02, C-25, E-24, SP.2-02
Mayer, J.; C-26, C-27, C-28, C-30
Mazia, C.G.; E-15
Merlini, G.; B-04, B-08, C-18, OP.4-03, SP.4-01
Michel, S.; C-21
Mihalache, C.I.; C-26
Milandri, A.; C-08, C-10, C-15, C-17, C-18
Mincheva, Z.; A-02, A-03, A-04, E-09, E-10, E-11, E-12, E-13, OP.3-03
Miranda, M.; D-20
Misumi, Y.; D-11, E-07, OP.1-02

Mizutani, M.; D-04, D-17
Monin, J.L.; C-28
Monteiro, C.; E-21, KN-02
Moon, J.C.; C-11, OP.2-05
Moreira, I.; E-01
Mörner, S.; C-12
Mörner, S.; C-05
Mosley, T.H.; C-16
Mullan, B.P.; C-20
Mundayat, R.; C-25, E-03, E-21
Murphy, B.; E-16

N

N Rendell; D-06
Nishimura, Y.; D-11
Norgren, N.; OP.1-01
Novais, M.; KN-02
Novis, S.C.S.; A-08, B-05
Nucifora, E.; E-20
Nyström, H.; OP.1-01

O

Obayashi, K.; A-05, D-07, D-08, E-07, E-08
Obici, L.; B-04, B-08, C-18, OP.4-03, SP.4-01
Oflazer, P.; B-06
Ogawa, C.; D-11, OP.1-02
Ogli, Y.; D-07, E-07
Ohya, Y.; D-10
Okumura, K.; A-05
Oliveira, L.T.; D-15
Olsson, M.; E-14
Olsson, O.; OP.1-01
Orrell, R.W.; E-06
Oshima, T.; A-05, D-08

105
Page, J.; C-09
Palhano, F.L.; D-19, KN-01
Palladini, G.; SP.4-01
Parman, F.Y.; A-12, B-06
Pastorelli, F.; E-17, E-18
Patel, K.; C-06, C-14
Pedrosa, R.C.; C-04, C-23
Perdry, H.; E-23
Pereira, B.B.; C-23
Pereira, P.; B-02
Pereira-Henriques, A.; OP.4-01
Perlini, S.; B-04, C-18, OP.4-03, SP.4-01
Pessegueiro, H.; A-01
Philip, H.; C-14
Pierre, L.; A-07
Pilato, E.; A-06
Pinna, A.D.; A-06
Pinney, J.; C-14
Pinney, J.H.; C-07
Pires, M.; A-01
Pitman, M.; B-01
Planté-Bordeneuve, V.; A-08, A-10, C-24, B-08, C-25, C-26, C-27, C-28, C-29, C-30, E-22, E-23, OP.1-01
Plasmati, R.; E-17, E-18
Polydefkis, M.; E-24
Pongas, D.; C-29
Quarta, C.C.; C-08, C-10, C-15, C-17, C-16, C-18, OP.2-04
Queiróz, M.C.; C-04
Queiroz, M.C.C.; C-23
Quigley, A.M.; C-09
Rahmouni, A.; C-26, C-27, C-28, C-30
Ramella, N.A.; D-13
Rannigan, L.; C-07
Rapezzi, C.; A-06, C-10, C-15, C-17, C-25, E-17, E-18, OP.2-04, SP.5-01
Rappeneau, S.; A-10, C-24, C-28, C-29, C-30
Rapley, I.; KN-02
Requiao, R.D.; D-01
Rimoldi, O.J.; D-13
Ríos, J.L.; D-13
Robbs, B.K; D-01, D-18
Rodrigues, C.; E-01
Romão, L.; D-20
Rosas, G.; B-08
Rosu, S.A.; D-13
Rugiero, M.; E-20
Rumjanek, F.D.; C-13, D-02
Said, G.; KN-03
Sakaguti, M.; D-08
Salhi, H.; E-22
Salmona, M.; OP.4-03
Salutio, V.L.; E-15
Salvi, F.; C-10, C-15, C-17, C-18, E-17, E-18, OP.2-04
Sama, C.; A-06
Samuel, D.; A-02, A-03, A-04, OP.3-03
Santanna, R.; C-13, D-02
Santos, D.; D-12, D-14
Saporta, M.A.C.; SP.3-03
Sarafkov, S.; OP.2-01
Saraiva, E.; KN-01
Saraiva, E.M.; D-19
Saraiva, M.J.; D-05, OP.1-03, OP.4-01, OP.4-02, OP.4-03, SP.1-02
Sato, T.; D-17
Sayed, R.H.; D-06
Scherer, K.; C-22
Schellina, G.R.; D-13
Schmidt, H.; B-08
Sekijima, Y.; B-03, OP.3-01
Sena-Esteves, M.; OP.4-02
Senju, S.; D-11, OP.1-02
Sequeiros, J.; D-12, D-14
Sforzini, C.; SP.4-01
Shaffer, S.; E-16
Shah, A.M.; C-16
Shinriki, S.; E-07
Shiraki, N.; D-10
Shuto, T.; D-17
Siepen, F.A.D.; C-22
Signate, A.; E-11
Silva, A.; A-01
Silva, J.L.; D-16, D-21
Slama, M.S.; A-02, A-07, OP.3-0, OP.2-06
Sobue, G.; B-03, D-07
Solomon, S.D.; C-08, C-16
Soumyia, B.; C-21
Sousa, A.; D-12, D-14
Steen, H.; C-22
Stewart, M.; B-08, E-21
Stewart, V.M.; E-16
Suarez, M.C.; D-12, D-14
Suenaga, G.; D-07, D-11, OP.1-02
Suhr, O.B.; A-11, C-03, C-05, C-12, C-25, D-03, E-03, E-04, E-14, E-23, OP.1-01, SP.1-01, SP.4-03, OP.3-02
Suico, M.A.; D-17
Sundström, T.; E-04

T

Taipa, R.; A-01
Tasaki, M.; D-07, D-08, D-11, E-07, OP.1-02
Taylor, G.W.; D-06
Teixeira-Coelho, M.; OP.1-03
Tendler, A.; C-01
Ternacle, J.; C-24, C-28, C-30
Theaudin, M.; A-04, E-09, E-10, E-11, E-12, E-13, OP.3-03
Tidswell, T.; E-06
Tissot, C.M.; C-29
Todorov, T.; OP.2-01
Todorova, A.; OP.2-01
Tojo, K.; B-03
Torezan, G.S.; D-19
Tournev, I.; OP.2-01
Tricerri, M.A.; D-13
Tsuchiya-Suzuki, A.; OP.3-01
Tsukimoto, S.; D-09
Tybjærg-Hansen, A.; OL-1

U - V

Ueda, M.; D-04, D-07, D-08, D-10, D-11, E-07, E-08, OP.1-02
Uraizee, I.; C-08
Valdrez, K.; A-01
Varejão, N.; C-13
Vasconcelos, R.; B-05
Vercouillie, J.; OP.2-06
Verga, L.; SP.4-01
Vibert, E.; A-07
Vieira, T.C.R.G.; D-16
Vilas-Boas, M.; A-01
Vincent, A.; C-21

W

Watanabe, T.; E-07
Wechalekar, A.D.; C-06, C-09, C-11, C-14, D-06, OP.2-05
Westermark, P.; C-05, D-03; SP.1-01
Westermark, G.T.; SP.1-01
Whelan, C.J.; C-06, C-07, C-09, C-11, C-14, E-06, OP.2-05
Wiklund, U.; C-12
Wiklund, U.; C-05, D-03
Wilczek, H.E.; A-11, OP.3-02
Wixner, J.; E-03, E-04, OP.2-03
Wood, M.R.; E-06

Y - Z

Yalo, B.; E-10
Yamakawa, R.; D-17
Yamashita, S.; A-05
Yamashita, T.; A-05, E-08
Yanagisawa, A.; D-08
Yazaki, M.; OP.3-01
Yokoyama, T.; D-17
Zeldenrust, S.R.; C-20
Zoia, M.; A-07
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E que se a vida é azul, a Pfizer Brasil é cada vez mais verde, amarela, azul e branca.