Abstracts from the First European Meeting for ATTR Amyloidosis for Doctors and Patients

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Introduction

David Adams¹, Philip Hawkins², Hartmut Schmidt³
¹Department of Neurology, NNERF, Bicêtre Hospital, Assistance Publique - Hôpitaux de Paris, Paris-Sud University, Le Kremlin-Bicêtre, France; ²National Amyloidosis Centre, Division of Medicine, University College London, London, UK; ³University Hospital of Muenster, Muenster, Germany

ATTR amyloidosis comprises a group of rare multisystem diseases including non-hereditary wild type ATTR amyloidosis (also known as senile cardiac amyloidosis, senile systemic amyloidosis), Familial Amyloid Polyneuropathy (FAP) and Familial Amyloid Cardiomyopathy (FAC). FAP was first described in the 1950s in Portugal presenting as autosomal dominant disease, whereas wild type ATTR amyloidosis was recognized in the 1980s as a non-hereditary form of restrictive cardiomyopathy in older patients. ATTR amyloidoses are global diseases that are now being identified in most countries. They are progressive and life-threatening, and diagnosis is usually delayed.

The First European meeting for ATTR amyloidosis for doctors and patients (Fig. 1) will inaugurate a new era in relations between patients, between doctors, and between doctors and patients from many European countries with the ultimate aim of improving diagnosis, treatment and care of this serious disease. Advocacy and Education experts from international institutions including EURORDIS (the Voice of Rare Disease Patients in Europe), ARC (Amyloidosis Research Centre), and the ISA (International Society of Amyloidosis) will be present. Since it is the first event of this nature, we propose three interconnected meetings over the course of two days. On Day One, Patients and Doctors will each have their own meeting; on Day Two, Patients and Doctors will share a common session.

During the meeting for specialists, six keynote lectures and 59 abstracts will be presented, comprising 11 oral communications and 48 posters. Final data from two major phase 3 clinical trials for TTR-FAP will be presented. Eleven national Patients’ Organizations will participate. Attendees will include specialists predominantly from Europe but also from USA, South America and Asia.

Fig. 1. Conference poster
TTR-FAP is a dominantly inherited systemic disease caused by over 100 different pathogenic mutations in the transthyretin (TTR) gene. The classic phenotype of TTR-FAP is characterised by a sensory motor neuropathy with varying degrees of autonomic and cardiac involve-
ment. Since the original description of TTR-FAP Met 30 in Portugal, there have been multiple epidemiological and genotype / phenotype studies published. These studies highlight a number of issues including the phenotypic heterogeneity seen even with the same mutation with early onset and late onset cases, the high incidence of certain mutations in individual ethnic groups e.g. TTR Met 30 in Portugal, TTR Ala 60 in Ireland and the UK and the variety of mutations that can be seen in individual countries e.g. UK, France, USA. There is also increasing recognition that TTR amyloidosis is a systemic disease with reports of involvement of almost every organ including the cen-
tral nervous system, muscle, lungs, kidneys etc. Neuropathy is increas-
ingly described even in mutations originally though just to cause a cardiomyopathy such as TTR Ile 122 and amyloidosis due to wild type TTR.

The development of successful novel therapies has raised a number of important and urgent challenges. These include the importance of early diagnosis particularly in non endemic areas and with the less common TTR mutations and the need to increase awareness of the risk of misdiagnosis especially by neurologists and cardiologists. A further challenge is both the need to define the optimum time to start therapy in an individual patient and the optimum follow up protocol to monitor therapies. Finally the new genetic therapies will not be cheap and as in many other genetic diseases, a major challenge will be to make these ther-
apy available in a cost effective way internationally.

TTR-FAP is a rare autosomal dominant disorder caused by mutations of the TTR gene with variable penetration. More than 100 different mutations of TTR have been identified worldwide, but the first-described Val30Met mutation remains the most common. The prev-
alence of different mutations varies according to ethnicity and geo-
graphic region. As a rare disease, the European prevalence of amyloidosis (including secondary amyloidosis) was estimated at 47/100000 in 2014. In particular regions of Portugal and Sweden where TTR-FAP is endemic, disease prevalence ranges from 1 in 1000 to 1 in 10 000 people. Smaller endemic foci have also been identified in Cyprus and Majorca. According to data derived from the ATTReuNET questionnaire, Portugal has the highest number of diagnosed, symp-
tomatic cases (~2000) and more than 500 diagnosed asymptomatic carriers of the disease. The gene carrier frequency for Val30Met in the northern parts of Sweden has been recently estimated at 2% . However, the penetrance is low and the onset is late. These facts lead to a lower prevalence of the disease.

Beyond these endemic regions, the incidence of TTR-FAP is much lower in nonendemic regions. France, Italy, and Germany have the most confirmed cases of TTR-FAP. A prevalence of 8.8/1 000 000 has been reported in Sicily. TTR-FAP cases from non-endemic regions are mainly sporadic or scattered as well as genetically and clinically het
erogenous. In endemic regions (Portugal, Cyprus, and Majorca), TTR-FAP often has a younger age of onset, starts before the age 40 with a progressive sensory-motor and autonomic neuropathy, leading to cachexia and eventually death. Frequently, cardiac, renal, and ocular involvement accompany the clinical picture.

In nonendemic areas, the onset is late, after the age of 50 years. A male predominance for the late-onset TTR-FAP has been observed. Although rare, the neuropathy tends to show different clinical charac-
teristics such as upper limb onset, motor neuropathy, and ataxic fea-
tures. Typically, sensory and motor neuropathy symptoms are usually associated with mild autonomic symptoms.

TTR-FAP is a rare, yet devastating, systemic disorder with predomin-
ant neurologic involvement, with genotypic/phenotypic and epi-
demiological variability.

References
Parman Y, Adams D, Obici L et al. Sixty years of transthyretin familial amyloid polyneuropathy (TTR-FAP) in Europe: where are we now? A European network approach to defining the epidemiology and management patterns for TTR-FAP. Curr Opin Neurol. 2016 Feb;29 Suppl 1:S3-13.
Adams D. Hereditary and acquired amyloid neuropathies. J Neurol 2001; 248:647 – 657.
Adams D, Lozeron P, Lacroix C. Amyloid neuropathies. Curr Opin Neurol 2012; 25:564 – 572.
Orphanet Report Series – Rare Disease Collection. Prevalence of rare diseases: bibliographic data. Orphanet. http://www.orpha.net/orphacom/cahiers/docs/Gb/Prevalence_of_rare_diseases_by_alphabetical_list.pdf.
Ando Y, Coelho T, Berk JL, et al. Guideline of transthyretin-related hereditary amyloidosis for clinicians. Orphanet J Rare Dis 2013; 8:31.
Suhr OB, Lindqvist P, Olofsson BO, et al. Myocardial hypertrophy and & function are related to age at onset in familial amyloidotic polyneuropathy. Amyloid 2006; 13:154 – 159.
Adams D, Suhr OB, Hund E et al., First European consensus for diagnosis, management, and treatment of transthyretin familial amyloid polyneuropathy. Curr Opin Neurol. 2016 Feb;29 Suppl 1:S14-26.

Multimodal imaging of cardiac amyloidosis in aTTR
Michel Slama (prmslama@gmail.com)
NNERF (National Reference Centre for Rare Disease), Paris, France
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Cardiac amyloidosis (CA) is the main cause of mortality in many muta-
tion types of aTTR, and therefore is of major prognostic value. CA is ini-
tially latent, and remains underdiagnosed and undertreated. The-
oretically, the definitive diagnosis should be established by the dem-
onstration of Congo red staining in cardiac biopsy specimens. How-
ever, it cannot reasonably be proposed as an early diagnostic tool, considering its invasive character and limited value due to sam-
pling bias, if alternate reliable noninvasive techniques are available, in addition to genetic testing and non-cardiac positive biopsy. This fuelled the search for multimodal imaging (ECG, echocardiography, MRI, scintigraphy) to assess CA.

The diagnosis is easy in the typical forms of CA, with severe Heart Failure with Preserved Ejection fraction (HPEF), microvoltage and/or conduction disorders on the ECG, myocardial thickening and abnor-
mal strain on echocardiography, cardiac uptake of bone scintigraphy tracers, late gadolinium enhancement (LGE) and T1 mapping with extracellular volume fraction measurement with MRI, and markedly elevated cardiac markers (BNP and troponin). This is a situation where cardiac imaging is at its best, each technique does not add much to the other. Bone scintigraphy is the most specific of aTTR CA, and all have a powerful prognostic value. This is also a situation with no validated therapeutic options.

The diagnosis is more difficult for the early stages of CA, where a dis-
ease modifying treatment can possibly translate into an improved prognosis. With the recent refinement of MRI, routine detection of discrete global and regional longitudinal strain abnormalities on echocardiography, cardiac uptake of “bone” tracers (99m Tc-DPD, HMIDP), or PYP by scintigraphy, it is possible to precisely detect CA. As proposed by Gillmore et al, cardiac aTTR can be defined using a multimodality imaging approach as the combination of symptoms
Unmet need for ATTR amyloidosis therapy?

Ole B Suhr (ole.Suhr@umu.se)
Department of Medicine, Umeå University, Umeå, Sweden

Current available treatment modalities

Even though liver transplantation (LTX) since the last 27 years has improved survival for hereditary transthyretin (TTR) amyloid (ATTRm) amyloidosis patients. However subsequent analysis has shown that it is a limited group of patients that is helped by the procedure, foremost early onset ATTR Val30Met amyloidosis patients that is transplanted early after onset of disease. It has also become apparent that ATTR cardiomyopathy and arrhythmia generally are unaffected by the procedure.

Medical treatments with TTR tetramer stabilisers have showed beneficial outcome on neuropathy in two controlled trials. For Tafamidis, the outcome is depending on age at onset of the disease, type of mutation and also on an early start of treatment after disease onset to reach an acceptable outcome. For DiFunisal, the data are more limited, but for both therapies, a progression of the disease are observed in many individuals, and the efficacy on cardiomyopathy has not been proven.

Lately, differences in amyloid fibril composition has been noted, where occurrence of fragmented TTR can be related the phenotype of the ATTR Val30Met mutation and also to the outcome of Ltx. It relationship with response to TTR stabilisers has not been evaluated.

Medical treatments at the horizon

We hope that the outcome of two controlled clinical trials, both aiming at profoundly suppress TTR synthesis by either interfering RNA or by antisense oligonucleotides, will be available at this meeting. They both profoundly reduce TTR (both mutant and wild type) concentration in the circulation and should thereby halt ATTR formation. From available data from open-label studies, RNA interference appears to be effective for preventing progression of neuropathy irrespective of mutation and patient’s age at disease onset. Both gene-silencing trials evaluate the efficacy of the drugs on neuropathy, thus, ATTR cardiomyopathy are not primarily targeted in the trials.

However, none of the treatments available or under investigation will have an impact on complications caused by local TTRm synthesis within the brain or eye.

Conclusions

Substantial progress in the treatment of ATTR amyloidosis has been made since 1990, but many problems remain to be solved before we have met the “Unmet need for ATTR amyloidosis therapy”
characterise the cohort of patients with ATTR-CM under the care of the UK National Amyloidosis Centre (NAC).

Materials and methods
We conducted a mixed retrospective and prospective study of patients with ATTR-CM, examining characteristics at diagnosis and at 6 to 12 month follow-up, including assessment of functional status and quality of life (QoL).

Results
Nine hundred ninety-seven patients with ATTR-CM attended the NAC between July 2005 and July 2017. 862 were male (86%). Median age at the diagnostic visit (case 1) was 77 (interquartile range, IQR, 71-82) years. 681 patients (68%) were wild-type (ATTRwt), 316 (32%) hereditary (ATTRv). The commonest variants in the ATTRv population were V122I (n=201; 64% of the ATTRv cohort), T60A (96; 30%) and S77Y (14%; 4%). Within the ATTRwt cohort, 640 (94%) were male, whilst in the ATTRv cohort, 222 (70%) were male. Median age of ATTRwt patients at diagnosis was 79 (73-83), compared with 73 (67-79) in ATTRv patients (p<0.0001). Patients with the V122I variant, although similar in age to the ATTRwt patients, at case 1, at 77 (72-80), appeared to be functionally worse at baseline: 28% of V122I patients were too unwell to perform the baseline six-minute walk test (6MWT) compared with 15% of ATTRwt; the median distance walked by those able was 241 (147-349) metres by V122I versus 340 (230-414) metres by ATTRwt. Analysis of SF-36 data revealed that V122I patients had worse limitation of physical and social function, greater symptom burden and poorer overall QoL than ATTRwt at case 1. Survival differed between the cohorts: 57 months from baseline in ATTRwt versus 43 in ATTRv (p=0.0047) and 31 in V122I (p<0.0001). Baseline factors prognostic of survival included physical impairment, cardiac biomarkers and renal function.

Conclusions
Patients with ATTR-CM are already significantly functionally and socially impaired by the time they are diagnosed, indicating substantial diagnostic delay. Patients with ATTR-CM associated with the V122I variant appear to have the most severe phenotype at diagnosis, and poorer outcomes. In this era of promising novel therapies for ATTR amyloidosis, population screening studies may be useful to establish the diagnosis earlier in the disease course.

O2
A new staging system for cardiac ATTR amyloidosis
Julian D. Gillmore1, Thibaud Damy2, Marianna Fontana1, Matthew Hutchinson1, Helen J. Lachmann1, Ana Martinez Naharo1, Candida Quarta1, Tamer Rezk1, Carol J. Whelan1, Esther Gonzalez-Lopez1, Thirusha Lane1, Janet A. Gilbertson1, Dorota Rowczenio1, Aviva Petrie1, Matthew Hutchinson1, Helen J. Lachmann1, Ana Martinez Naharo1, Candida Quarta1, Tamer Rezk1, Carol J. Whelan1, Esther Gonzalez-Lopez1, Thirusha Lane1, Janet A. Gilbertson1, Dorota Rowczenio1, Aviva Petrie1, Matthew Hutchinson1, Helen J. Lachmann1, Ana Martinez Naharo1, Candida Quarta1, Tamer Rezk1, Carol J. Whelan1, Esther Gonzalez-Lopez1, Thirusha Lane1, Janet A. Gilbertson1, Dorota Rowczenio1, Aviva Petrie1, Matthew Hutchinson1, Helen J. Lachmann1, Ana Martinez Naharo1, Candida Quarta1, Tamer Rezk1, Carol J. Whelan1, Esther Gonzalez-Lopez1, Thirusha Lane1, Janet A. Gilbertson1, Dorota Rowczenio1, Aviva Petrie1, Matthew Hutchinson1, Helen J. Lachmann1, Ana Martinez Naharo1, Candida Quarta1, Tamer Rezk1, Carol J. Whelan1, Esther Gonzalez-Lopez1, Thirusha Lane1, Janet A. Gilbertson1, Dorota Rowczenio1, Aviva Petrie1

Background
Cardiac transthyretin (ATTR) amyloidosis is an increasingly recognised, progressive and fatal cardiomyopathy, the natural history of which remains unclear. We sought to establish and validate a new prognostic staging system applicable to patients with both wild-type (ATTRwt) and hereditary variant (ATTRv) ATTR amyloid cardiomyopathy.

Materials and methods
Eight hundred and sixty-nine patients with cardiac ATTR amyloidosis (553 with ATTRwt and 316 with ATTRv) attending the UK National Amyloidosis Centre, were stratified into 3 disease stages at baseline on the basis of cut points in two universally measured biomarkers, NT-proBNP and estimated GFR (eGFR). Stage I was defined as NT-proBNP ≤3000 ng/L and eGFR ≥45 ml/min, Stage III was NT-proBNP >3000 ng/L and eGFR <45 ml/min; the remainder were Stage II. The staging system was validated in a cohort of 318 patients with cardiac ATTR amyloidosis from France.

Results
Median survival among 393 (45%) Stage I patients was 69.2 months, 334 (38%) Stage II patients was 46.7 months, and 142 (16%) Stage III patients was 24.1 months (p<0.0001). Median survival among 393 (45%) Stage I patients was 69.2 months, 334 (38%) Stage II patients was 46.7 months, and 142 (16%) Stage III patients was 24.1 months (p<0.0001). After adjusting for age, compared to Stage I the hazard ratio (HR) for death for Stage II was 2.05 (CI: 1.54-2.72, p<0.001) and for Stage III was 3.80 (CI: 2.73-5.28, p<0.001). HRs and statistical significance were little altered by TTR genotype, and were maintained in the validation cohort.

Conclusions
This simple, universally applicable staging system stratifies patients with both ATTRwt and ATTRv amyloid cardiomyopathy into prognostic categories. It will be of value in the design of forthcoming clinical trials of novel amyloid-specific therapies.

O3
Transthyretin familial amyloid polyneuropathy (ATTR V30M) in Cyprus: an updated epidemiological, clinical and genetic study
Savanna Andreou1, Elena Panayiotou1, Kyriaki Michailidou2, Savanna Andreou1, Elena Panayiotou1, Kyriaki Michailidou2

Background
ATTR V30M is a lethal autosomal dominant sensorimotor and autonomic neuropathy caused by amyloid deposition. Amyloid consists of aggregated misfolded TTR monomers with the V30M mutation. ATTR V30M neuropathy has been described in many endemic foci such as Portugal, Sweden, Japan and Cyprus. The varied age of onset in patients with ATTR V30M varies in different foci and the mechanism behind it is still unknown. The modifying role of the complement protein C1q has recently been identified as a possible modifier. The current study aims to provide an updated epidemiological status of ATTR V30M in Cyprus and investigate the modifying effect of the C1q component on the patients’ age of onset.

Materials and methods
Demographic data were collected from the patients’ files at the Cyprus Institute of Neurology and Genetics, where all Cypriot ATTR V30M patients are diagnosed by clinical and genetic testing. DNA was collected and a candidate gene approach was performed for 21 C1q tagging SNPs: 19 SNPs were assessed by allelic discrimination using real-time PCR and 2 SNPs using conventional PCR/sequencing protocol.

Results
Eighty-two patients (deceased included) have been diagnosed with ATTR V30M in Cyprus so far, with the mean age of onset being 44.5±15. On the 31st of December 2015, the prevalence was 6.61/100,000, while the mean incidence between 2003 and 2015 was 0.32/100,000. Anticipation analysis indicated that the disease’s onset is on average 9.57 years earlier in offspring than in manifesting parents. SNP analysis of 80 manifesting carriers revealed rs672693 (A>G) with the most significant modifying effect on age of onset. When analyzing only the manifesting carriers, the most significant modifying effect comes from rs665691 (G>C) with minor allele frequency 0.38 and hazard risk 1.54 (CI: 1.17-2.03).
Background

TTR amyloidosis was first identified in Northern Portugal, where it was found to be associated with a Val30Met mutation of the TTR gene. Therefore, this tandem is considered endemic to this region. More than 120 different mutations were identified in the whole world. In the last ten years there is a big advance in identification of the disease in Central and Eastern Europe. The disease was found in Bulgaria, Turkey, Cyprus, Poland, Romania, Israel, Slovenia, Macedonia, Kosovo. The materials and methods

A survey was performed among reference centers for ATTR amyloidosis in central and eastern Europe countries to detect TTR-FAP cases.

Results

In Bulgaria, 112 TTR-FAP patients and 94 asymptomatic carriers from 80 affected families were identified with the following mutations: Gly6Ser – 9 families; Val30Met – 8 families; Ser37Gln – 6 families, Ser52Pro – 1 family and Gly47Glu – 2 Roma (Gypsy) families. In Turkey 28 TTR FAP patients were diagnosed – in Istanbul and 9 – in Ankara. Val30Met – 11 patients. The following mutations were found: Glu89Gln – 5 patients; Gly47Glu – 4 patients, Gly53Glu – 3 patients; Thr49Ser – 2 patients; Glu54Gly – 1 patient. In Cyprus 50 patients and 140 asymptomatic carriers were identified. All of them have Val30Met mutation. In Poland the following TTR FAP mutations were found: V71A – 9 family members over four generations diagnosed with TTR-FAP; D38V – 1 patient; F33L – 1 patient and 1 carrier; Val30Met – 1 patient and 2 carriers; p.Glu81Lys mutation – one family. In Slovenia nine TTR FAP patients from 4 different families with four different rare mutations were found: Val122Ala, Val130Ala, Ile107Phe and Asp38Asn. In Romania four TTR-FAP cases were diagnosed.

Conclusions

There is a significant genetic and clinical heterogeneity of TTR FAP. Val30Met mutation is found in many countries in Central and Eastern Europe and it causes TTR FAP with late onset. Glu89Gln is specific for regional Balkan-Mediterranean mutation, identified in Turkey, Bulgaria, Macedonia, Kosovo, Italy. Second regional Balkan-Mediterranean mutation, identified in Italy, Greece, Bulgaria. In Bulgaria the mutation was identified in Gypsies.

04

TTR FAP in Central and Eastern Europe

Ivailo Tournev,1 Yesim Parman,2 Sevim Erdem-Ozdamar,3 Marta Lipovska4, Janez Zidar5, Menachem Sadeh6, Daniel Coru7, Djiana Plaseska − Karanfiltska1, Enver Bogdanov1, Olga Zinovyeva1, Mariana Gospodinova1,8 Stjepko Sarafov9, Expert TTR FAP Center, UMBAL Aleksandrovska; Department of Neurology, Medical University, Sofia, Bulgaria; 2Neurology Department, Istanbul Medical Faculty, Istanbul University, Istanbul, Turkey; 3Neurology Department University Neuromuscular Disease Research Laboratory, School of Medicine, Ankara, Turkey; 4Department of Neurology, Medical University of Warsaw, Warsaw, Poland; 5Institute of Clinical Neurophysiology, University Medical Centre Ljubljana, Ljubljana, Slovenia; 6Department of Neurology, Edith Wolfson Medical Center, Holon, Israel; 7Department of Hematology, University of Medicine “Carol Davila”, Bucharest, Romania; 8Research Centre for Genetic Engineering and Biotechnology “Georgi D. Efremov”, within the Macedonian Academy of Sciences and Arts – Skopje, Macedonia; 9Department of Neurology, Kazan State Medical University, Kazan, Russian Federation; 10Department of nervous diseases and neurosurgery, IM. Sechenov First Moscow State Medical University, Moscow, Russian Federation; 11Medical Institute of Ministry of Inferior, Sofia, Bulgaria

Correspondence: IvailoTournev (itournev@emhpf.org)

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05

Mass spectrometry and immunohistochemistry are complementary techniques for typing of cardiac amyloid

Tamer Rezk, Janet Gilbenson, Nigel Rendell, Graham Taylor, Patrizia Mangione, Diana Canetti, Vittorio Bellotti, Philip Hawkins, Julian Gillmore

UCL Division of Medicine. National Amyloidosis Centre, London, UK

Correspondence: Tamer Rezk (t.rezk@ucl.ac.uk)

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Background

Cardiac amyloidosis is a progressive and fatal condition the clinical management of which depends upon identifying the correct amyloid fibril precursor protein. Systemic chemotherapy is the treatment for cardiac AL amyloidosis but should be avoided in patients with cardiac ATTR amyloidosis, up to 20% of whom have a monoclonal gammopathy which is incidental to their amyloid. Proteomic analysis of amyloid can be used to determine the fibril protein when immunohistochemistry (IHC) is non diagnostic. We describe our single centre experience of proteomics in endomyocardial biopsies (EMB).

Materials and methods

EMBs from 59 patients with suspected cardiac amyloid were stained with Congo red at the UK National Amyloidosis Centre (NAC) by the method of Puchtel et al along with a panel of monospecific antibodies against known amyloid fibril proteins, including TTR and immunoglobulin light chains. Proteomic analysis, on the Velos platform, was performed on laser micro-dissected tissue. Mass spectrometry data files were analysed using Mascot software.

Results

Fifty-six samples were Congo Red (CR) positive and 3 CR negative. Amyloid was confirmed on proteomics by presence of all 3 signature proteins (SAP, APOE and APOA4) in 44/56 (79%) and 2 signature proteins in 11/56 (20%); in 1 (2%) case proteomics failed to support CR staining, likely due to scantly available tissue. The proteomic ‘amyloid signature’ was not present in any of the 3 CR negative samples. 47/56 (84%) CR positive biopsies were definitively typed by IHC. However, there was no immunospecific staining (NISS) in 8/47 cases and in the remaining case, the tissue was too scanty for IHC. Proteomic analysis concurred with definitive IHC typing of amyloid in 44/47 (94%) cases but failed to support IHC results in 3/47 cases. Among 8 cases in which there was NISS by IHC, 3 were identified as AL (2 kappa, 1 lambda), 3 were ATTR, 1 was AApoA4 and one was not conclusively typed by proteomic analysis.

Conclusions

Proteomic analysis and Congo red/immunohistochemical staining of EMBs are complementary techniques for identifying and typing amyloid in EMBs. Both are associated with occasional false negative results but used together, presence and type of amyloid can be definitively determined in >96% of EMBs.

06

T1, extracellular volume and rest myocardial blood flow mapping: a multiparametric mapping approach in ATTR cardiac amyloidosis

Ana Martinez-Naharro, Daniel Knight, Tushar Kotecha, Roshin Francis, Tamer Rezk, Cristina Quarta, Esther Gonzalez, Richa Manwani, Carol Whelan, Helen Lachman, Ashutosh Wechalekar, Julian Gillmore, Philip Hawkins, Marianna Fontana

National Amyloidosis Centre, UCL, London, UK

Correspondence: Ana Martinez-Naharro (a.naharro@ucl.ac.uk)

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Background

Cardiac involvement is the main driver of outcome in systemic amyloidosis, but the relationship between amyloid deposits and cellular injury is not well understood. The simple explanation of physical, mechanical replacement of parenchymal tissue seems insufficient, and preliminary studies support the hypothesis that
myocardial hypoperfusion could contribute to cell damage in amyloidosis. The aim of this study was: 1) To assess feasibility of fully automated pixel-wise rest myocardial blood flow (MBF) mapping in cardiac amyloidosis during routine clinical scans; 2) To assess the prevalence of myocardial hypoperfusion and correlation with amyloid deposits and disease severity.

Materials and methods

Patients with cardiac ATTR (n=28) amyloidosis and healthy volunteers (n=16) were recruited. All subjects underwent CMR at 1.5T (Siemens) with standard SSFP cine imaging, Phase Sensitive Inversion Recovery Reconstruction Late Gadolinium Enhancement (PSIR-LGE), T1 mapping, Extracellular Volume (ECV) mapping and rest MBF mapping.

Results

The pixel-wise MBF maps for all slices were generated automatically in all patients within 2.5 minutes after image acquisition. Myocardial perfusion was globally reduced in patients with cardiac amyloidosis compared to healthy volunteers (0.53±0.12ml/min/g vs 0.85±0.09ml/min/g, p<0.05). Myocardial perfusion inversely correlated with amyloid burden measured as extracellular volume fraction (r = 0.57, p<0.01) and with the transmurality of LGE (no LGE 0.84±0.19ml/min/g, subendocardial LGE 0.56±0.10ml/min/g and transmural LGE 0.51±0.14ml/min/g, p<0.01).

Conclusions

Myocardial perfusion can be measured in cardiac amyloidosis during routine clinical scans with fully automated MBF mapping. Myocardial hypoperfusion at rest is highly prevalent in subjects with cardiac amyloidosis, and correlates with the degree of amyloid infiltration and disease severity.

O7

Rethinking heart failure in TTR amyloidosis
Rodney Falk, Avinander Singh
Cardiac Amyloidosis Program, Brigham and Women’s Hospital, Boston, MA, USA
Correspondence: Rodney Falk (rfalk@bwh.harvard.edu)
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Background

The mechanism of heart failure in TTR amyloidosis is complex, and not fully understood. Epidemiological data from patients with V122I indicate that this variant is associated with an increased risk of heart failure (HF), even though the typical phenotype of cardiac amyloid is rare. This suggests that the presence of a modest amount of amyloid, insufficient to cause significant LV thickening, may result in HF, perhaps requiring perhaps a “second hit”, such as hypertension.

It is well-recognized that patients with ATTRm neuropathy undergoing liver transplantation may have post-transplant progressive cardiac disease, presumably due to ongoing ATTRwt deposition in the heart, triggered by a seeding phenomenon. Whether seeding plays a role in other amyloid patients is unknown.

Materials and methods

We present four illustrative cases clinical cases, unrelated to liver transplantation for ATTRm, with unusual manifestations, that illustrate the two aspects of the disease noted above. These cases cast a broader light on heart failure mechanisms in this increasingly-recognized disease.

Results

Case 1: TTRwt on cardiac biopsy with an amyloidogenic TTRm -Error in pathological diagnosis or another explanation?

Case 2: A Thickening heart two decades after AL “cure” for AL nephropathy -

Case 3: HF with normal wall thickness and HFREF - amyloid or not?

Case 4: AL amyloidosis in an African man destined for HF despite hemodialysis cure?

Conclusions

These patients highlight the importance of thinking outside the box when evaluating patients with potential cardiac amyloidosis and the potential role of a seeding phenomenon.

08

Safety and efficacy of inotersen in patients with hereditary transthyretin amyloidosis with polyneuropathy (hATTR-PN)
Merrill Benson 1,2, María Waddington-Cruz 1, Annabel Wang 1, Michael Polydefkis 1, Violaine Plante-Bordeneuve 1, John Berk 1, Fabio Barroso 1, David Adams 1, Peter Dyck 1, Bernhard Brunnagel 1, Carol Whelan 1, Giampaolo Merlino 2, Morton Scheinberg 1, Brian Drachman 2, Stephen Heitner 2, Isabel Conceição 3, Hartmut Schmidt 4, Giuseppe Vita 1, Josep Maria Campistol 5, Josep Gamez, Edward Gane 6, Peter Gorevic 7, Acary Olivieria 8, Brett Monia 9, Steven Hughes 10, Jesse Kwoh 11, Bradley Mc Evoy 11, Brenda Baker 12, Andrew Shenker 13, Helen Mills, Rito Bergmann 14, Elizabeth Ackermann 15, Marie Gertz 16, Teresa Coelho 17

1Indiana University School of Medicine, Indianapolis, IN, USA; 2Federal University of Rio de Janeiro University Hospital, Rio de Janeiro, Brazil; 3University of California, Irvine, CA, USA; 4Johns Hopkins University, Baltimore, MD, USA; 5CHU Henri Mondor, Creteil, France; 6Boston University, Boston, MA, USA; 7FLENI, Ciudad Autónoma De Buenos Aires, Argentina; 8CHU Bicêtre, Universite Paris-Sud, Paris, France; 9Mayo Clinic, Rochester, MN, USA; 10Columbia University Medical Center, New York, NY, USA; 11UCL, National Amyloidosis Centre, London, UK; 12Centro Amiloidosi Fondazione IRCCS, Pavia, Italy; 13Associação de Assistência a Criança Deficiencia, Sao Paulo, Brazil; 14University of Pennsylvania, Philadelphia, PA, USA; 15Oregon Health and Science University, Portland, OR, USA; 16CHLN - Hospital de Santa Maria, Lisbon, Portugal; 17Universitätsklinikum Münster, Münster, Germany; 18A.O.U. Policlinico G.Martino, University of Messina, Messina, Italy; 19Hospital Clinic, Barcelona, Spain; 20Hospital Universitari Vall D’ Hebron, Barcelona, Spain; 21Auckland City Hospital, Auckland, New Zealand; 22Mount Sinai Medical Center, New York, NY, USA; 23Universidade Federal de Sao Paulo, Sao Paulo, Brazil; 24Inonis Pharmaceuticals, Inc, Carlbad, CA, USA; 25GlaxoSmithKline, Philadelphia, PA, USA; 26GlaxoSmithKline, London, UK; 27Centro Hospitalar do Porto, Porto, Portugal

Correspondence: Merrill Benson (mdbenson@iupui.edu)
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Background

Transthyretin (TTR) is a liver-derived protein that functions to transport vitamin A and thyroxin to peripheral tissues. In patients with hereditary TTR amyloid polyneuropathy (hATTR-PN), point mutations in the TTR gene encode single amino acid substitutions that induce deposition of amyloid in various organs including peripheral and autonomic nerves. Amyloid deposition eventually leads to multi-organ failure, with life expectancy ~10 years from symptom onset. Despite the use of liver transplantation and small molecule TTR stabilizers, there remains a high unmet medical need for new therapies to treat all forms of ATTR. Inotersen is a generation 2+ antisense oligonucleotide (ASO) inhibitor of TTR protein production.

Materials and Methods

A randomized, double-blind, placebo controlled phase 3 study (NEURO-TTR, NCT01737398) of inotersen in patients with hATTR-PN was conducted at 24 sites worldwide (USA 48%, Europe 35%, South-America 17%). The trial design included two co-primary endpoints: change from baseline in the composite modified Neuropathy Impairment Score +7 (mNIS+7) and patient reported Norfolk Quality of Life Diabetic Neuropathy (Norfolk QoL-DN) score. Eligible patients were adults who had Stage I (ambulant) or Stage II (ambulant with assistance) disease characterized by an NIS score of 10 to 130 at screening, documented TTR amyloidosis with polyneuropathy (hATTR-PN) point mutations in the TTR gene, presence of amyloid deposits and disease severity.
patients completed the 15-month treatment period. Inotersen-treated patients achieved statistically significant benefit compared to placebo for mNIS+7 (p<0.0001) and Norfolk QoL-DN (p=0.0006). Key safety findings were thrombocytopenia and renal dysfunction in some patients. More than 95% who completed treatment have participated in the open-label extension study.

Conclusions

Inotersen demonstrated significant benefit on both primary endpoints of neurological disease progression and quality of life in patients with hATTR amyloidosis in the Phase 3 NEURO-TTR study.

O9

Patisiran, an investigational RNAi therapeutic for patients with hereditary transthyretin-mediated (hATTR) amyloidosis with polyneuropathy: results from the phase 3 apoP study

David Adams1, Alejandra Gonzalez-Duarte1, William O’riordan3, Jared Gollob13, Ole Suhr14

1National Reference Center for FAP, CHU Bicêtre, APHP, Le Kremlin-Bicêtre, France; 2National Institute of Medical Sciences and Nutrition - Salvador Zubiran, Mexico City, Mexico; 3eStudy Site, La Mesa, CA, USA; 4National Taiwan University Hospital, Taipei, Taiwan, Republic of China; 5Kumamoto University Hospital, Kumamoto, Japan; 6Heidelberg University Hospital, Heidelberg, Germany; 7University Multi-profile Hospital for Active Treatment, Sofia, Bulgaria; 8University Hospital Muenster, Muenster, Germany; 9Hospital de Santo António, Centro Hospitalar do Porto, Porto, Portugal; 10Amyloid Treatment and Research Program, Boston University, Boston, MA, USA; 11Taipei Veterans General Hospital, Taipei, Taiwan, Republic of China; 12Mayo Clinic Hospital, Rochester, MN, USA; 13Aynaylam Pharmaceuticals, Cambridge, MA, USA; 14Umeå University Hospital, Umeå, Sweden

Correspondence: David Adams (david.adams@aphp.fr)

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):O9

Background

hATTR amyloidosis is a multisystemic, rapidly progressive, life-threatening disease caused by a mutation in the TTR gene, resulting in deposition of amyloid fibrils in multiple organs. Heterogeneous clinical presentation of hATTR amyloidosis includes sensory, motor and autonomic neuropathy, as well as cardiac involvement, resulting in significant morbidity and mortality. Patisiran, an RNAi investigational agent, uses a naturally occurring pathway to target TTR mRNA in the liver. Previous Phase 2 OLE study data with patisiran in patients with hATTR amyloidosis with polyneuropathy showed that chronic dosing over 24 months was generally well-tolerated and resulted in a >80% sustained reduction of serum TTR and improvement of the mNIS+7 neuropathy impairment score. The Phase 3 APOLLO study aimed to further evaluate the efficacy and safety of patisiran in patients with hATTR amyloidosis with polyneuropathy.

Methods

Multi-center, international, randomized (2:1), double-blind, placebo-controlled study of patisiran 0.3mg/kg or placebo IV q3W in adult patients with hATTR amyloidosis with polyneuropathy (NCT01960348). Symptomatic patients with a neurological impairment score (NIS) of 5-130 were eligible. Select exclusion criteria: patients with prior liver transplant, PND score IV, and NYHA Class >2. Primary endpoint was a change from baseline at 18 months in the mNIS+7 neuropathy impairment score. Secondary endpoints included assessment of quality of life (Norfolk QoL-DN), motor strength (NIS), disability (R-ODS), gait speed (10-MWT), nutritional status (mBMI) and autonomic function (COMPASS-31).

Results

From Dec 2013 to Jan 2016, 225 patients were enrolled at 44 sites in 19 countries (EU: 51%; N. America: 21%; Asia-Pac: 20% and LatAm: 8%). Baseline demographics include median age: 62 years (range: 24-82); males: 74%; V30M: 42%; non-V30M: 57% (including 49 different TTR genotypes); and previous TTR tetramer stabilizer use: 53%. Measures of baseline disease severity included FAP Stage 1: 47%, FAP Stage 2: 52% and mean baseline mNIS+7: 78.6 (range: 80-165.0). Echocardiographic evidence of cardiac involvement was noted in 54% of patients.

Conclusions

APOLLO is the largest, controlled study of patients with hATTR amyloidosis with polyneuropathy and includes patients with a wide range of TTR genotypes and neuropathy severity, including >50% with cardiac involvement. Efficacy and safety results from this Phase 3 study will be presented.

O11

Proposal for a minimal neuropathy assessment protocol for ATTR amyloidosis in routine clinical practise

Mary M. Reilly1, Matilde Laura1, Andrea Cortese1, Michael Shy2, Davide Pareyson2

1UCL Institute of Neurology, Queen Square, London, UK; 2University of Iowa, Iowa City, IA, USA; 3IRCCS Foundation, CBesta Neurological Institute, Milan, Italy

Correspondence: Mary M. Reilly (m.reilly@ucl.ac.uk)

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):O11

Background

Hereditary transthyretin (ATTR) amyloidosis is a dominantly inherited systemic disease caused by over 100 different pathogenic mutations in the transthyretin (TTR) gene. The multisystem involvement, the phenotypic heterogeneity and the variable disease course make the clinical evaluation of the disease and of its progression difficult. This is particularly true of the neuropathy. Although detailed protocols of the neuropathy have been developed for use in a clinical trial setting, there is no standardised agreed protocol for the neuropathy assessment in routine clinical practise. Many ATTR patients first present to a neurologist and early diagnosis is increasingly important with the advent of disease modifying treatments. Once the diagnosis is established it will become important to standardise the neuropathy evaluation so that decisions on the optimal time to initiate treatments can be made. In many countries it may be some time before a patient can be seen in a specialised amyloid centre so having accurate retrospective neuropathy assessments to help inform treatment decisions will be critical. This protocol needs to be simple enough to be performed in any neurological clinic.

Materials and methods

Based on current clinical practice, a literature review and our experience we propose the following minimal assessment protocol: Neurorpathy Impairment Scale (NIS) and NIS-Lower Limb subscale (NIS-LL), Kumamoto scale, FAP stage, Norfolk Quality of life scale, FAP-R-ODS and the weighted Charcot-Marie-Tooth Neuropathy or examination score (CMTNSv2, CMTESTV2).

Conclusions

We would like to discuss this protocol and agree an international standardised protocol going forward.

Poster presentations

Topic: Genotype, phenotype

P1

Genetic diagnosis in ATTR amyloidosis; a single UK centre 26 year experience

Dorota Rówczenio, Janet Gilbertson, Marianna Fontana, Ashutosh Wechalekar, Carol Whelan, Ana Martinez-Naharro, Candida Quarta, Tamer Rezk, Esther Gonzalez-Lopez, Philip Hawkins, Julian Gilmore, Helen Lachmann National Amyloidosis Centre, UCL Meical School, London, UK

Correspondence: Dorota Rówczenio (drowczenio@ucl.ac.uk)

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P1

Background

ATTR amyloidosis is caused by a deposition of either wild-type (ATTRwt) or variant (ATTRv) TTR fibrils. More than 120 amyloidogenic TTR mutations have been described and typically these are rare, reported only in isolated kindreds. ATTRv can be associated with a range of organ involvement,
particularly neuropathy and cardiomyopathy, whereas ATTRwt presents with restrictive cardiomyopathy in the elderly.

**Materials and Methods:**
This retrospective study includes all patients referred between 1991 and 2017 for assessment of known or suspected amyloidosis, who underwent TTR gene sequencing as part of their routine work up. Additional diagnostic procedures included immunohistochemistry, proteomics, echocardiography, CMR, DDx and SAP scintigraphy.

**Results:**
TTR gene was sequenced in 4457 patients, mutations were found in 724 (16%). The most prevalent variants were: V122I (40%); T60A (26%); V30M (17%). Less common mutations found in 125 cases (17%) included: S77Y (n=16 cases); G47E (8); G47V (7); V20 (7); E89K (6); A97S (5); F33V, I68L and I107V (5 each); E89Q (4); I107F (4); G53E/A, V71A, S77F, A81V and H90D (3 each). 27 variants were found in ≤2 individuals. 16 patients were homozygous; 13 had V122I (all Afro-Caribbean); and single cases for V30M, T60A and D38V (Greek, Irish and Ghanaian ancestry respectively).

557 patients (94% male) were diagnosed with ATTRwt. The protective TTR substitution T119M was found in 11 subjects (allele frequency 0.12%). None had ATTR amyloidosis; 8 had AL lambda, 1 AL kappa, and in 2 there was no evidence of amyloid. 5 patients (all Afro-Caribbean) diagnosed with AL amyloidosis (4 lambda, 1 kappa) carried V122I. 4 of these patients died of cardiac amyloidosis between the ages of 46 and 55 years.

**Conclusions:**
Improvements in diagnostics resulted in an increase in referrals with ATTR type amyloid, now accounting for 23.7% of new cases seen in the last 2 years of whom 69.3% were TTRwt. Of our TTRwt cohort V122I was the commonest variant, almost always found in patients of African ancestry; T60A was the second commonest identified largely in patients of Irish ancestry; whilst V30M was found in a heterogeneous group originating from Cyprus, Greece, Portugal, Sweden, UK and Spain. Our findings of TTRwt in 16% of screened patients highlights the need for genetics in the routine evaluation of suspected ATTR amyloidosis but the incidental finding of variants in cases of AL amyloidosis shows the importance of interpreting genetics in context.

**P3**
Clinical-genetic correlations in Bulgarian TTR–FAP cohort – ten years observations
Stayko Sarafov1, Marianna Gospodinova2, Andrey Kirov3, Tihomir Todorov4, Teodora Chamova1, Albena Todorova5, Ivaylo Tournev1
1Expert TTR FAP Center, UMBAL Aleksandrovska, Sofia, Bulgaria; 2Medical Institute of Ministry of Inferior, Sofia, Bulgaria; 3IMDL Genome Centre Bulgaria, Sofia, Bulgaria; 4Genetic Medico-Diagnostic Laboratory “Genica”, Department of Medical Chemical and Biochemistry, Medical University Sofia, Sofia, Bulgaria
**Correspondence:** Stayko Sarafov (stayko_sarafov@abv.bg)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P3

**Background**
To present clinical-genetic correlations in Bulgarian TTR-FAP cohort according to: patient’s ethnicity, individual mutation, clinical phenotype, positive familial history, average age of onset and range, average survival and range, male/female ratio.

**Materials and methods**
Glu89Gly in 64 families, Val30Met in 9, Ser77Phe in 7, Gly47Glu in 2 gipsy families, Ser52Pro in 1. Salting out DNA extraction from whole blood samples from our patients. Sanger sequencing of targeted part (usually including exons 2 and 3) of TTR gene.

**Results**
Glu89Gly: mean age of onset - 52.7 years for both sexes, m/f ratio 1:2; mixed phenotype: onset with PNP+CTS in a ~2/3, cardiac onset in a ~1/3, GI onset in ~7-10%. In a 4/5 of the cases the onset is after the age of 45-50. M/F survival is the same. Familial history (+) in 85%. Ser77Phe: clinically mixed phenotype similar to Glu89Gly, average age of onset - 57.6 years. Mostly men are affected. Familial history (+) in 75%.

Val30Met: highest average of onset ~ 66 years, not rare after age of 70. Phenotype at the onset is usually Polyneuropathy. Mostly men are affected. Familial history is uncertain, (+) in no more than 25%, usually presented as “sporadic”.

Ser52Pro – one female, age of onset 44.2 years; mixed phenotype PNP onset with early kidney involvement. Gly47Gly (in gipsies) – mean age of onset 35.3 years, mixed phenotype with GI onset, early kidney involvement. The average survival for all mutations is 8.3; range 7.9-9.4 except for Gly47Glu – 5 years.

**Conclusions**
The different mutations expresses less or more mixed phenotype at the onset. The average age and rage of onset are connected. Age of onset differs – earliest in Glu47Gly and latest in Val30Met. Family history appears to be inversely related to the age of onset. The average survival is lowest in Glu47Gly and the same for the other mutations. Males appear to have more severe phenotype and were affected more than females except for Glu89Gln - preliminary data. Val30Met is “rare”, due to a “specific” clinical phenotype possible explanation for a small number of patients.

**P4**
Transthyretin amyloidosis in Slovenia
Janez Zidar (janez.zidar@kclj.si)
Institute of Clinical Neurophysiology, Ljubljana, Slovenia
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P4

**Background**
Two years ago we reported on two families with 4 members having transthyretin amyloidosis (TTR-A) and one sporadic patient...
Late-onset transthyretin familial amyloid polyneuropathy: characterization of Brazilian subjects from the THAOS registry

Márcia Waddington-Cruz1, Amanda Berensztejn1, Marcus V. Pinto1, Rajiv Mundayat*

1Federal University of Rio de Janeiro, National Amyloidosis Referral Center; 2Pfizer Inc., New York, NY, USA

Correspondence: Márcia Waddington-Cruz (mwaddingtoncruz@gmail.com)

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P6

Background: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a severe small-fiber predominant polyneuropathy, typically emerging in patients’ third or fourth decade. More recently, a late-onset form of TTR-FAP involving both large and small sensory fibers and more severe motor and cardiac involvement has been recognized. Little is known about the condition in many countries, including Brazil, despite increasing numbers of late-onset FAP patients being diagnosed. To improve characterization of late-onset TTR-FAP by comparing demographic and clinical characteristics of patients in Brazil with late-onset and early-onset TTR-FAP.

Materials and Methods: Demographic and clinical data at the time of enrolment for Brazilian subjects with symptomatic Val30Met TTR-FAP were extracted from the ongoing, multinational, longitudinal, observational Transthyretin Amyloidosis Outcomes Survey (THAOS; cut-off date: January 30, 2017). Subjects were divided into those with symptomatic onset at age <50 years (early-onset), and at age ≥50 years (late-onset).

Results: A total of 162 subjects with TTR-FAP were enrolled in Brazil: 148 had the Val30Met mutation and, of these, 96 were symptomatic. Late-onset subjects (n=25, 26.0%) had a longer time to diagnosis (mean 5.1 years compared to 2.8 years) and were more likely to be misdiagnosed (68% of the cases compared to 26.8%) than early-onset subjects. Clinically, subjects with late-onset tended to have more severe neurological impairment and more frequent cardiac involvement, as shown by NIS (mean Neurology Composite Score of 101 compared to 70) and cardiac measures (ECG abnormalities in 88.9 % in contrast to 59.4 %) (Echocardiogram IVS thickness >12 mm in 69.2% of the cases in contrast to none of the early onset group).

Conclusions: The late-onset form of TTR-FAP is not unusual in Brazil, tending to be more difficult to diagnose and presenting with a more severe phenotype. Increased characterization may assist earlier recognition and improve patient outcomes.

P7

Amyloidosis research consortium cardiac amyloidosis survey: results from patients with ATTR amyloidosis and their caregivers

Isabelle Lousada1, Mathew Maurer2, Melissa Warner1, Spencer Guthrie3, Kristen Hsu1, Martha Grogan4
1Amyloidosis Research Consortium, Newton, MA, USA; 2Columbia University - New York, NY, USA; 3Biopharma Strategic Consulting, Seattle, WA, USA; 4Mayo Clinic, Rochester, MN, USA

Correspondence: lsmedicine@arci.org

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P7

Background: Cardiac amyloidosis is a severe disease that can lead to cardiac dysfunction and death. Both hereditary transthyretin (hATTR) amyloidosis and wild-type transthyretin (wtATTR) amyloidosis may result in cardiac amyloidosis. Initial symptoms are often nonspecific, causing delays in diagnosis until the disease is advanced. To understand delays and errors in the diagnostic pathway for ATTR cardiac amyloidosis.

Materials and methods: The Amyloidosis Research Consortium (ARC) developed an online survey, which was distributed to patient lists of ARC, the Amyloidosis Foundation, and Amyloidosis Support Groups in January 2017. The survey was designed for all forms of amyloidosis, but is limited to ATTR cardiac amyloidosis.

Results: In this subanalysis, 139 responders (76 patients, 63 caregivers) completed the survey for ATTR amyloidosis (88 hATTR, 51 wtATTR). Initial reported symptoms were most commonly shortness of breath, fatigue, and dizziness (Table 2). A majority of hATTR (52%) and wtATTR patients (65%) were diagnosed with carpal tunnel before their diagnosis of amyloidosis. Most wtATTR patients had solely cardiac involvement (53%), while nearly all hATTR patients had involvement of ≥1 other organ (94%), most commonly nerves (74%) (Table 1). Caregivers reported that 35% of wtATTR patients were diagnosed in <12 months from the start of symptoms, while this was true in only 14% of hATTR patients. 17% of all respondents reported visiting 5 different

P6

Late-onset transthyretin familial amyloid polyneuropathy: characterization of Brazilian subjects from the THAOS registry

Márcia Waddington-Cruz1, Amanda Berensztejn1, Marcus V. Pinto1, Rajiv Mundayat*

1Federal University of Rio de Janeiro, National Amyloidosis Referral Center; 2Pfizer Inc., New York, NY, USA

Correspondence: Márcia Waddington-Cruz (mwaddingtoncruz@gmail.com)

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P6

Background: Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a severe small-fiber predominant polyneuropathy, typically emerging in patients’ third or fourth decade. More recently, a late-onset form of TTR-FAP involving both large and small sensory fibers and more severe motor and cardiac involvement has been characterized in patients with late-onset TTR-FAP by comparing demographic and clinical characteristics of patients in Brazil with late-onset and early-onset TTR-FAP.

Materials and Methods: Demographic and clinical data at the time of enrolment for Brazilian subjects with symptomatic Val30Met TTR-FAP were extracted from the ongoing, multinational, longitudinal, observational Transthyretin Amyloidosis Outcomes Survey (THAOS; cut-off date: January 30, 2017). Subjects were divided into those with symptomatic onset at age <50 years (early-onset), and at age ≥50 years (late-onset).

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Conclusions: The late-onset form of TTR-FAP is not unusual in Brazil, tending to be more difficult to diagnose and presenting with a more severe phenotype. Increased characterization may assist earlier recognition and improve patient outcomes.

P7

Amyloidosis research consortium cardiac amyloidosis survey: results from patients with ATTR amyloidosis and their caregivers

Isabelle Lousada1, Mathew Maurer2, Melissa Warner1, Spencer Guthrie3, Kristen Hsu1, Martha Grogan4
1Amyloidosis Research Consortium, Newton, MA, USA; 2Columbia University - New York, NY, USA; 3Biopharma Strategic Consulting, Seattle, WA, USA; 4Mayo Clinic, Rochester, MN, USA

Correspondence: lsmedicine@arci.org

Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P7

Background: Cardiac amyloidosis is a severe disease that can lead to cardiac dysfunction and death. Both hereditary transthyretin (hATTR) amyloidosis and wild-type transthyretin (wtATTR) amyloidosis may result in cardiac amyloidosis. Initial symptoms are often nonspecific, causing delays in diagnosis until the disease is advanced. To understand delays and errors in the diagnostic pathway for ATTR cardiac amyloidosis.

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physicians before receiving the correct diagnosis (Table 1). 57% of hATTR and 39% of wtATTR patients received a misdiagnosis; of those 76% and 75%, respectively, received treatment for the misdiagnosed condition. While cardiologists were found to correctly diagnose the most patients (35% in hATTR, 78% in wtATTR), they were also found to miss the most (26% in hATTR, 31% in wtATTR) (Table 1).

Conclusions
This represents the first survey compiling caregiver and patient experiences with cardiac ATTR amyloidosis. Alignment of caregiver with patient responses validates our patient-directed research. hATTR and wtATTR patients experience some differences in disease presentation and diagnosis; however, similarities in the diagnostic journey are present. Patients frequently receive misdiagnoses and often receive incorrect treatment for the misdiagnosed condition. Disease awareness and understanding of common presenting symptoms, like carpal tunnel, is vital, especially among those to whom patients are initially referred.

Table 1 (abstract P7). Hereditary ATTR (hATTR) vs. Wild-type TTR (wtATTR) survey responses, % (n) •

| What organs are involved besides the heart? * |     |     |     |   |   |
|---------------------------------------------|-----|-----|-----|---|---|
| Kidney                                      | 18% (16) | 74% (65) | 25% (22) | 45% (40) | 11% (10) |
| Nerves                                      |     |     |     |   |   |
| Liver                                       |     |     |     |   |   |
| GI                                          |     |     |     |   |   |
| Skin                                        |     |     |     |   |   |
| hATTR                                       | 11% (10) | 31% (27) | 16% (14) | 6% (5) | 2% (2) |
| wtATTR                                      | 30% (27) | 8% (4) | 8% (4) | 2% (1) | 0% (0) |

| Number of organs involved besides the heart | 0 | 1 | 2 | 3 | 4 | ≥5 |
|---------------------------------------------|---|---|---|---|---|----|
| hATTR                                       | 11% (10) | 31% (27) | 16% (14) | 6% (5) | 2% (2) |
| wtATTR                                      | 18% (15) | 16% (8) | 8% (8) | 2% (1) | 0% (0) |

| What diagnostic procedures were done? * |     |     |     |     |     |
|----------------------------------------|-----|-----|-----|-----|-----|
| Heart biopsy                           | 35% (31) | 59% (52) | 14% (12) | 7% (6) | 23% (20) |
| Fat pad biopsy                         |     |     |     |   |   |
| Rectal biopsy                          |     |     |     |   |   |
| Kidney biopsy                          |     |     |     |   |   |
| MRI                                     |     |     |     |   |   |
| hATTR                                   | 13% (11) | 13% (11) | 16% (14) | 23% (20) |
| wtATTR                                  | 16% (14) | 8% (8) | 8% (8) | 2% (1) | 0% (0) |

| How many different doctors were seen before diagnosis? | 1 | 2 | 3 | 4 | 5 or more |
|-------------------------------------------------------|---|---|---|---|----------|
| hATTR                                                 | 23% (20) | 13% (11) | 13% (11) | 16% (14) | 23% (20) |
| wtATTR                                                | 18% (15) | 16% (8) | 8% (8) | 2% (1) | 0% (0) |

| What type of physician made the diagnosis? |     |     |     |     |     |
|------------------------------------------|-----|-----|-----|-----|-----|
| Cardiologist                             | 35% (31) | 5% (4) | 6% (5) | 17% (15) | 2% (2) |
| Hematologist                             |     |     |     |   |   |
| Internist                                |     |     |     |   |   |
| Neurologist                              |     |     |     |   |   |
| Nephrologist                             |     |     |     |   |   |
| hATTR                                    | 10% (9) | 5% (4) | 8% (7) | 16% (14) |
| wtATTR                                   |     |     |     |   |   |

| What type of doctor made a misdiagnosis? |     |     |     |     |     |
|-----------------------------------------|-----|-----|-----|-----|-----|
| Cardiologist                            | 26% (23) | 3% (3) | 10% (9) | 5% (4) | 16% (14) |
| Hematologist                            |     |     |     |   |   |
| Internist                               |     |     |     |   |   |
| Neurologist                             |     |     |     |   |   |
| Nephrologist                            |     |     |     |   |   |
| GP                                      |     |     |     |   |   |
| hATTR                                   | 31% (26) | 2% (2) | 6% (5) | 2% (1) | 6% (3) |
| wtATTR                                  |     |     |     |   |   |

Table 2 (abstract P7), Patient vs. caregiver survey responses, % (n) *

| What organs are involved besides the heart? * |     |     |     |   |   |
|---------------------------------------------|-----|-----|-----|---|---|
| Kidney                                      | 11% (8) | 50% (38) | 17% (13) | 26% (20) | 5% (4) |
| Nerves                                      |     |     |     |   |   |
| Liver                                       |     |     |     |   |   |
| GI                                          |     |     |     |   |   |
| Skin                                        |     |     |     |   |   |
| Patient                                    | 11% (8) | 50% (38) | 17% (13) | 26% (20) | 5% (4) |
| Caregiver                                  | 28% (18) | 27% (17) | 37% (23) | 16% (10) | 16% (10) |

| What diagnostic procedures were done? * |     |     |     |     |     |
|----------------------------------------|-----|-----|-----|-----|-----|
| Heart biopsy                           | 57% (36) | 27% (17) | 37% (23) | 16% (10) | 16% (10) |
| Fat pad biopsy                         |     |     |     |   |   |
| Rectal biopsy                          |     |     |     |   |   |
| Kidney biopsy                          |     |     |     |   |   |
| MRI                                     |     |     |     |   |   |
| Patient                                 | 57% (36) | 27% (17) | 37% (23) | 16% (10) | 16% (10) |
| Caregiver                               | 43% (27) | 10% (6) | 8% (5) | 8% (5) | 35% (22) |

| What were the presenting symptoms? * |     |     |     |     |     |
|-------------------------------------|-----|-----|-----|-----|-----|
| Shortness of breath                 | 54% (41) | 21% (19) | 24% (18) | 21% (16) | 25% (19) |
| Fatigue                             |     |     |     |   |   |
| Edema                                |     |     |     |   |   |
| Dizziness                            |     |     |     |   |   |
| Fainting                             |     |     |     |   |   |
| Neurupathy                           |     |     |     |   |   |
| Patient                              | 54% (41) | 21% (19) | 24% (18) | 21% (16) | 25% (19) |
| Caregiver                            | 43% (27) | 22% (14) | 21% (11) | 11% (7) | 32% (20) |

Topic: Phenotypic Variability

P8
Phenotypic variability of TTR Val122Ile mutation: a Caucasian patient with axonal neuropathy and normal heart after two years follow up
Claudia Stancanelli1, Luca Gentile2, Gianluca Di Bella2, Fabio Minuto3, Massimo Russo4, Giuseppe Vta2, Anna Mazzeo2
1Biomedical Department of Internal Medicine and Specialist, University of Palermo, Palermo, Italy; 2Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; 3Department of Biomedical and Dental Sciences and of Morphological and Functional Images, University of Messina, Messina, Italy; 4Nemo Sud Clinical Centre, AOU Policlinico, Messina, Italy
Correspondence: Claudia Stancanelli (claudia.stancanelli09@gmail.com) Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P8

Background
Val122Ile is one of more than 100 mutations in transthyretin gene (TTR) that is associated with transthyretin-related hereditary amyloidosis (ATTR) with a prevalence as high as 3.9% in Afro-Americans. It has, as main clinical expression, a hypertrophic restrictive cardiomyopathy with mild or no neurological symptoms. A prospective study performed in an American urban population showed only two of 453 DNA samples from Caucasian newborns being positive for Val122Ile (0.44%). Significant ethnic differences
are present in the TTR gene, and some differences may affect its function. In particular, the non-coding variants potentially associated with regulatory function showed a significant diversity between African and non-African individuals.

**Case report**

We report the first Caucasian patient carrying Val122Ile mutation with an axonal neuropathy and no cardiac involvement. The patient came to our attention 2 years ago complaining of numbness in the lower limbs and poor balance. Family history was negative for neuro muscular diseases. He had been diagnosed with a normal pressure hydrocephalus at age 69. At the first evaluation, his neurological examination showed deep tendon areflexia, hypoesthesia, and hypopallesthesia on hands and feet. Neurophysiological study showed a significant reduction of compound motor and sensory action potentials (SNAP) on the peroneal and ulnar nerve and absent sural SNAP indicating an axonal neuropathy. After 1 year, we reviewed the patient clinically because of a rapid worsening of gait, so that he required a stick to walk. He underwent a sural nerve biopsy that showed loss of axonal fibers, negative at Congo Red staining. Since our region Sicily is endemic for late-onset FAP due to Phe64Leu mutation, we performed TTR genetic analysis. Surprisingly, the patient carried Val122Ile mutation. In two years follow-up ECG, cardiac ultrasound and MR remained normal and (99m)Tc-DPD scintigraphy did not detect any heart uptake after 1 and 2 years.

**Conclusions**

Our report confirms the heterogeneity in the genotype–phenotype correlation of ATTR, suggesting that other factors may interact with disease causing mutations. The absence of cardiac involvement suggests the possible underestimation of Val122Ile mutation in non-African population. In addition, with the recent discovery of gene modifiers and the “cis-regulatory” hypothesis in Val30Met, we have now greater needs for TTR genetic analysis than in the past as well as more uncertainties in family genetic counseling.

**Consent to publish**

Written informed consent was obtained from the patients involved in this study.

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**P9**

The c.337-18 G>C mutation in the transthyretin gene is amyloidogenic

Sami Khella, Patricia Divito, Brian Drachman
University of Pennsylvania, Philadelphia, PA, USA

**Correspondence:** Sami Khella (sami.khella@mail.med.upenn.edu)

**Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P9**

**Background**

To describe a previously unreported amyloidogenic autonomic neuropathy in a patient with a single point polymorphism (SNP) in the transthyretin (TTR) gene. Hereditary amyloid neuropathy is most often due to a SNP in the TTR gene. There have been a number of variants of unknown significance (VUS) in that gene. Most recently, a report of patients with combined autonomic and small fiber neuropathy had an association with non-amyloidogenic c.337-18 G>C mutation in the TTR gene.*

**Case report**

Case presentation from an amyloid referral practice in a large university. 74 year old African American man with progressive severe orthostatic hypotension and mild sensory motor neuropathy; both have been present 2 years. He had a history of long standing well controlled diabetes mellitus (recent HbA1C 7.2%) and a remote history of non-hodgkins lymphoma (2008). A fat pad aspirate revealed apple green birefringent material on congo red staining. Neither a skin nor an open fat biopsy showed similar findings. Genetic testing revealed c.337-18 G>C mutation in the TTR gene.

**Conclusions**

We describe a patient with a c.337-18 G>C mutation, a severe progressive autonomic and somatic neuropathy and evidence of amyloidosis. It is possible that this previously considered VUS affects the TTR gene function and results in amyloidosis.

**Consent to publish**

Written informed consent was obtained from the patients involved in this study.

*Levine, TD; Bland RJ: Incidence of Nonamyloidogenic Mutations in the Transthyretin Gene in Patients with Autonomic and Small Fiber Neuropathy. Muscle Nerve 14 JUN 2017, DOI: 10.1002/mus.25701*
P12

Proteinuric renal phenotype in the Mallorca cohort of hereditary transthyretin amyloidosis patients

Asunción Ferrer-Nadal1, Mercedes Usón2, Tomás Ripoll3, Hernán Andreu4, Manuel Raya-Cruz5, Eugenia Cisneros-Barroso6, Juan Buades5
1Nephrology Department, Hospital Son Llàtzer, Palma De Mallorca, Spain; 2Neurology Department. Hospital Son Llàtzer, Palma De Mallorca, Spain; 3Cardiology Department. Hospital Son Llàtzer, Palma De Mallorca, Spain; 4Gastroenterology Department. Hospital Son Llàtzer, Palma De Mallorca, Spain; 5Internal Medicine Department. Hospital Son Llàtzer, Palma De Mallorca, Spain

Correspondence: Asunción Ferrer-Nadal (asfnadal@yahoo.com)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P12

Background

Kidney disease has been reported in patients with Transthyretin-Hereditary Amyloidosis (TTRHa) with different mutations over the last years. Portuguese group has published the largest series of patients with Val30Met mutation. In Portugal the most frequent type of renal manifestation is proteinuria with moderate or severe kidney disease. They identified one third of patients who developed proteinuria and 10% progressing to End Stage Renal Disease (ESRD). However, in Mallorca we observed 2 different phenotypes of renal disease in patients with Val30Met mutation. One, more common that is non-proteinuric and the other much less frequent proteinuric. Both types of phenotypes developed ESRD with need of renal replacement therapy (RRT) by different mechanisms.

Materials and methods:

Observational study of proteinuric patients in Mallorca Cohort with TTRHa (V30M).

Results

We present 7 patients (4,6%) with proteinuria more than 1 g/24h, median 2,5 g/24h (IQR 1,8-3,4). Mean age at the onset was 60,3 years (SD 8,7). 57% were female. 4 patients developed ESRD with need of RRT. The mean duration of renal replacement therapy was 1,9 years, with a 33% survival at two year treatment. 3 patients died at the mean age of 66,7 years (SD 5). 57% of patients had received a liver transplant and one liver-kidney transplant.

Conclusions

We have observed few patients with severe proteinuria (> 1g/24h), lower than other groups. It can be explained in part because of the high prevalence of patients with liver transplantation with anticalcineurin inhibitors drugs and high use of other common antiproteinuric therapies such as Angiotensin Converting Enzyme (ACE) inhibitors.

References

1. Lobato L. Portuguese-type amyloidosis (transthyretin amyloidosis, ATTR V30M). J Nephrol. 2003 May-Jun;16(3):438-42.
2. End-stage renal disease and dialysis in hereditary amyloidosis TTR V30M: presentation, survival and prognostic factors. L Lobato, I Beirão, M Silva, I Fonseca, J Queiôs, G Rocha, A Morais Sarmento, A Soua, J Sequeiros. Amyloid 2004; Vol. 11: 27-37.
3. Lobato L, Rocha A. Transthyretin amyloidosis and the kidney. Clin J Am Soc Nephrol. 2012 Aug;7(8):1337-46.

P13

Ocular manifestations in S77T transthyretin-related familial amyloid polynue ropathy

Roxane Bunod1, Cécile Caquili2, Halima Bourenane2, Emmanuel Barreau1, Marc Labetouille1, David Adams1, Antoine Rousseau1
1Department of Ophthalmology, NNERF, Bicêtre Hospital, APHP. PHU Vision & Handicaps, Paris-Sud University, Le Kremlín-Bicêtre, France; 2Department of Neurology, NNERF, Bicêtre Hospital, Assistance Publique - Hôpitaux de Paris, Paris-Sud University, Le Kremlín-Bicêtre, France

Correspondence: Roxane Bunod
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P13

Background

Transthyretin-related familial amyloid polyneuropathies (TTR-FAP) are associated with ocular manifestations including dry eye, amyloid deposits in the anterior chamber, secondary glaucoma and vitreous amyloid deposits and retinal angiopathy, which have been extensively described for V30M mutation. S77T is the second most frequent transthyretin amyloidogenic mutation in France after V30M and is associated with a severe neurologic and cardiac phenotype. However, ocular manifestations of this particular form of TTR-FAP have been scarcely described.

Materials and methods

This monocentric observational study was conducted at the French national reference center for TTR-FAP. Genetically confirmed S77T-TTR-FAP patients had complete neurologic and ophthalmologic evaluation. Sensorimotor polyneuropathy (SPN) was staged using the Polyneuropathy Disability (PND) score. Ophthalmological examination included best corrected visual acuity (BCVA), Schirmer test, intraocular pressure (IOP), slit lamp photographs, fundus examination with retinography. Medical and surgical treatments were analyzed for each patient.

Results

Eighteen S77T-TTR-FAP patients (11 males and 7 females), aged 31-71 years, mean (58.2±11.1 years), all originating from France, were included. Symptomatic patients (N=15), had a mean PND of 1.9±1.3, and a delay between first symptoms and ophthalmologic evaluation of 5.6±4.4 years. None of them presented with amyloid deposits in the anterior chamber, secondary glaucoma nor vitreous amyloid deposits. However, conjunctival lymphangiectasia were present in both eyes in 9 patients (50%) and associated with more severe neurologic disease (PND = 2.5±1.0 vs 1.2±1.2; p<0.05). Retinal ischemic angiopathy was found in one patient (5%). Dry eye was found in one third of patients.

Conclusions

Our results suggest that, unlike in V30M-TTR-FAP, anterior chamber deposits, secondary glaucoma and vitreous deposits do not occur in S77T-TTR-FAP. However, S77T-TTR-FAP is associated with conjunctival lymphangiectasia, dry eye and amyloid angiopathy. Conjunctival lymphangiectasia occur in patients with severe neurologic disease and were not described in TTR-FAP associated with other TTR mutations, suggesting a genotype-phenotype correlation in TTR-FAP ocular manifestations, with conjunctival lymphangiectasia being a specific feature of S77T-TTR-FAP.

Topic: Wild Type Ttr Amyloidosis

P14

The frequency of wild-type transthyretin amyloidosis in Russia according to the results of an autopsy study

Anzhelika Poliakova1, Evgeny Sernennin2, Mariya Sitnikova2, Karapat Avagyan3, Roman Grozov2, Svetlana Pyko2, Alexander Krutikov2, Viktoriya Davydova2, Tatinit Bezhanshivili2, Karina Khmelnitskaya2, Mihail Shavlakoski2, Dmitrii Kozhevski2, Alexandra Gudkova2
1Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russian Federation; 2Almazov Federal Medical Research Centre, St. Petersburg, Russian Federation; 3St. Petersburg Electrotechnical University "LETI", St. Petersburg, Russian Federation; 4Federal State Budgetary Scientific Institution «Institute of Experimental Medicine», St. Petersburg, Russian Federation

Correspondence: Anzhelika Poliakova (lica.polyakova@mail.ru)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P14

Background

Life-time diagnostics of wild type transthyretin amyloidosis (ATTR wt) is practically absent. At the same time, ATTR wt is an underestimated cause of morbidity and mortality, especially in the older age group. To study the frequency of detection, demographic parameters and morpho-functional features of TTR wt in patients with chronic heart failure (CHF) I-IV functional classes and left ventricular hypertrophy ≥ 15 mm according to autopsy results.

References

1. Lobato L. Portuguese-type amyloidosis (transthyretin amyloidosis, ATTR V30M). J Nephrol. 2003 May-Jun;16(3):438-42.
2. End-stage renal disease and dialysis in hereditary amyloidosis TTR V30M: presentation, survival and prognostic factors. L Lobato, I Beirão, M Silva, I Fonseca, J Queiôs, G Rocha, A Morais Sarmento, A Soua, J Sequeiros. Amyloid 2004; Vol. 11: 27-37.
3. Lobato L, Rocha A. Transthyretin amyloidosis and the kidney. Clin J Am Soc Nephrol. 2012 Aug;7(8):1337-46.
Materials and methods
A retrospective analysis of the postmortem investigations of the pa-
tients of the cardiological department (n = 141) with the leading syn-
drome of CHF was carried out. The age was ≥ 69 years, men - 19%,
women - 81%. All formalin-fixed paraffin blocks were stained with
Congo red and viewed under polarized light. Immunohistochemical
analysis was also performed using antibodies to AA-amyloid, trans-
thyretin, prealbumin, kappa and lambda-light chains.

Results
Amyloid deposits were detected in old age and in long-livers, the
average age was 91.25 ± 9.67 years, mainly in women due to the
lower life expectancy of men. In patients with CHF of a different
functional classes, associated with left ventricular hypertrophy (LVH),
amyloid deposits occur in almost every fourth deceased (in 21% of
cases) according to autopsy data. The amount of amyloid deposits in
the myocardium were mostly small (56% of observations had amyloid
deposits - (+) and 27% - (++), a significant amount of amyloid was detected in 17% of cases (+++ - 7% and ++++ - 10 %). The presence of amyloid deposits did not significantly affect
on the indices of myocardial hypertrophy, such as the thickness of
the interventricular septum, left ventricle posterior wall and left
ventricular mass index. In the presented cases, a focal amyloid
deposits in the myocardium was observed which is typical for
TTR wt, with that in 97% of cases amyloid deposits located in
the interstitial area around the cardiomyocytes, and in 3% - exclusively
around the vessels.

Conclusions
ATR wt was detected in every fourth patient in a cohort of patients
of old age and long-livers, predominantly in women (83%), and was not
diagnosed during life. Typical morphological manifestations of ATR wt
are focal amyloid deposits, located mainly in myocardial interstitium.

P16
Machine learning predicts mortality better than biomarker staging
in wild-type transthyretin cardiac amyloidosis
Avinander Singh, Tara Mirto, Rodney Falk
Brigham & Women’s Hospital, Boston, MA, USA

Correspondence: Avinander Singh (asingh11@bwh.harvard.edu)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P16

Background.
A biomarker-based staging system has recently been described
for prognosis in wild-type transthyretin cardiac amyloidosis (ATTRwt). We validated this staging system in consecutive ATRRwt patients and compared its predictive accuracy to machine learning
algorithms.

Materials and methods.
Clinical data was extracted from medical records of patients diag-
nosed with ATRRwt over the past 5 years. Diagnosis was based on
technetium pyrophosphate scanning, or histopathological analysis of
cardiomyocyte specimens. Data on death was collected from medical
records as well as social security death index. The following machine
learning algorithms were tested — Random Forest learner, AdaBoost,
Naive Bayes, Stochastic Gradient Descent (SGD). The model was built
on a random sampling of 80% data, and tested on the remaining
20%, over 100 iterations. For comparison, a recently described stagi-
ging system was used, which allots one point each for NT-proBNP
>3000 pg/mL and troponin- T > 0.05 ng/mL, which results in the stages
1(0 points), 2 & 3 (2 points). The Area under the Curve (AUC)
and the classification accuracy (proportion of outcome correctly pre-
dicted) were compared.

Results
Among 197 individuals with ATRRwt (mean age 76 ± 6 years, 6
women) 86 were in Stage 1 (44%), 66 in Stage 2 (33%) and 45 in
Stage 3 (23%). There were 59 deaths (30%), with a median survival of
5.1 years. The staging system had an AUC of 0.70 (95% CI 0.62-0.77).
All machine learning approaches performed better than clinical
staging, except for AdaBoost (AUC ~ 0.71) (Table 1). Naive Bayes had
the highest AUC (0.86), whereas SGD had the highest classification
accuracy (0.83).

Conclusion
Biomarker based staging is valid and has moderate accuracy for pre-
dicting mortality in patients with ATTRwt cardiac amyloidosis. How-
ever, machine learning algorithms can provide superior predictions
compared with biomarker staging.

Table 1 (abstract P16). Predictive Accuracy of Machine Learning
Approaches for Mortality in ATTRwt Cardiac Amyloidosis

| Algorithm       | AUC      | Classification Accuracy |
|-----------------|----------|-------------------------|
| Random Forest   | 0.84     | 79%                     |
| Naive Bayes     | 0.86     | 77%                     |
| AdaBoost        | 0.71     | 74%                     |
| Stochastic Gradient Descent | 0.80 | 83%                     |
| Clinical Staging| 0.70     | 73%                     |

P17
Characterization of wild-type transthyretin amyloidosis among
women: preliminary results from an international multicenter
study
Cristina C. Quarta1, Anna L. Tinuper2, Esther Gonzalez-Lopez1,
Thirasha Lane1, Mathew Maurer3, Carol J. Whelan1, Arnt Kristen4,
Rodney H. Falk5, Thibaud Damy6, Pablo Garcia-Pavia4, Giampaolo Merlini6,
Claudio Raperzi2, Julian D. Gillmore1, Philip N. Hawkins1
1National Amyloidosis Centre, Division of Medicine, University College
London, London, UK; 2Cardiology, Department of Experimental
Diagnostic and Specialty Medicine (DIMES), Alma Mater Studiorum,
University of Bologna, Bologna, Italy; 3Center for Advanced Cardiac Care,
Columbia University College of Physicians and Surgeons, New York, NY,
USA; 4Amyloidosis Center, Department of Cardiology, Heidelberg
University, Heidelberg, Germany; 5Cardiac Amyloidosis Program, Division
of Cardiology, Department of Medicine, Heart & Vascular Center,
Brigham and Women’s Hospital, Harvard Medical School, Boston, MA,
USA; 6Centre de Reference National, Amyloses Cardiaques, UPEC, Créteil,
France; 7Heart Failure and Inherited Cardiac Diseases Unit, Department of
Cardiology, Hospital Universitario Puerta de Hierro Majadahonda,
Madrid, Spain; 8Amyloidosis Research and Treatment Center, Fondazione
IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy

Correspondence: Cristina C. Quarta (ccquarta@gmail.com)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P17

Background
Over 90% of patients with wild-type transthyretin amyloidosis (ATTRwt)
are reported to be male. This gender disproportion remains unex-
plained and little is known about the characteristics of female patients
affected by ATRRwt. We aimed to to assess the clinical and instrumental
findings of women with ATRRwt.

Materials and methods
We conducted a multicenter study of female patients diagnosed with
ATTRwt at 8 international Amyloid Centres (London, Boston, New
York, Paris, Pavia, Madrid, Heidelberg and Bologna).

Results
In 2005-2016, 97 women were diagnosed with ATTRwt. Exertional
dyspnea was the main presenting symptom, with 75 patients (77%)
showing NYHA class ≥2. A history of carpal tunnel syndrome was re-
ported in 45 (46%) patients. Electrocardiographically, low QRS voltage was present in 16% of
cases, I degree ativoventricular block in 17%, atrial fibrillation in 35%;
left or right bundle branch block in 25%. Of the 62 patients with
available scintigraphic data (either DPD or PYP or HMDP), 57 (92%)
showed an intense cardiac uptake, with a visual score of 2 and 3 in
28 (45%) and 29 (47%) of cases, respectively. Echocardiographically, patients showed a severe symmetric increase of
the left ventricular (LV) wall thickness with no LV dilatation (LV
diad-dia stolic diameter 40±6mm), preserved LV ejection fraction (54
±12%) and diastolic dysfunction (E/E’ > 17±2). Longitudinal systolic
function was impaired (S' 0.06 [0.04-0.07]/m/s; global longitudinal strain -11±6%). Table 1 shows the main findings of the 97 female ATTRwt patients compared to 598 male patients diagnosed in the same period. Compared to men, women showed a better overall survival (Log-rank p=0.02).

**Conclusions**

This is the largest series of female ATTRwt patients ever studied. Compared to men, female patients were older, suggesting that women may get the disease slightly later in life, which could only partly explain the different incidence between men and women. Also, female patients showed less functional cardiac impairment and better survival. Our findings suggest the need of larger prospective studies involving more referral centers across the world in order to assess the real impact of gender and age on the incidence and pathophysiology of ATTRwt.

| Table 1 (abstract P17). Main characteristics of female vs male patients with ATTRwt |
|--------------------------------------|-----------------|
| Age, yrs                             | Female (n=97)   | Male (n=598) |
|                                      | 79±8            | 77±7          |
| NT-proBNP, ng/L                      | 3271 [1778-6151]ng/L | 3087 [1624-5461] ng/L |
| LV wall thickness, mm                | 16±3            | 16±3          |
| E/E                                 | 17±7            | 19±2          |
| LV ejection fraction, %              | 54±12           | 47±13         |
| Global longitudinal strain, %       | -11±6           | -9±6          |

*p < 0.05

**Topic: Clinical Biology In ATTR Amyloidosis**

**P18**

**Serum transthyretin levels are significantly lower in Val122Ile cardiomyopathy compared with wild-type transthyretin cardiomyopathy**

Avinainder Singh, Kevin Alexander, Rodney Falk
Brigham & Women’s Hospital, Boston, MA, USA

**Correspondence:** Avinainder Singh (assingh11@bwh.harvard.edu)
*Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P18*

**Background**

Recent data suggest that serum levels of biomarkers, such as prealbumin/transthyretin (TTR) levels or retinol binding protein-4, may assist in differentiating patients with Val122Ile amyloid cardiomyopathy from non-amyloid heart failure. Our objective was to assess whether TTR levels can help differentiate patients with Val122Ile cardiomyopathy from wild-type ATTR (ATTRwt) cardiomyopathy.

**Materials and methods**

Data from the Brigham & Women’s Hospital Cardiac Amyloidosis Program Database were retrospectively analyzed, querying patients with ATTR cardiomyopathy who had a serum TTR level measured at the time of diagnosis or initial visit. Diagnosis of amyloidosis was based on consensus criteria. The Val122Ile mutation was identified by genetic testing or mass spectrometry analysis of endomyocardial biopsy specimens. Patients with mutations other than Val122Ile were excluded. Receiver operator characteristic curves were constructed to identify optimal cut-offs. Cox proportional hazards modeling was performed to assess the prognostic impact of serum TTR concentration.

**Results**

The cohort consisted of 78 patients (mean age 74±6 years, 4 females), of whom 26 (33%) had Val122Ile cardiomyopathy. Serum TTR levels were significantly lower in patients with Val122Ile compared with ATTRwt (14.9±1.5 vs. 20.4±0.8; p=0.001). A serum TTR value less than 19.25 mg/dL had a sensitivity of 77% and specificity of 60% for Val122Ile cardiomyopathy (area under the curve=0.68). Low serum TTR was a predictor of mortality (HR 0.93, p=0.02). This effect was attenuated but remained significant after adjusting for NT-proBNP and troponin-T concentrations (HR 0.94, p=0.04). This association was stronger in those with ATTRwt (HR 0.93, p=0.06) compared to Val122Ile ATTR (HR 0.95, p=0.23).

**Conclusions**

Serum TTR levels are significantly lower in patients with Val122Ile ATTR than ATTRwt. Among patients with ATTRwt, a lower TTR level is associated with a worse prognosis. Mechanisms for a low serum TTR concentration in ATTRwt may include poor nutritional status or decreased TTR stability.

**References**

Avrantis, Marios, et al. "Identification of Transthyretin Cardiac Amyloidosis Using Serum Retinol-Binding Protein 4 and a Clinical Prediction Model." *JAMA Cardiology* 2.3 (2017): 305-313.

**P22**

**Immortalization of primary cells derived from attr patients**

Paula Ballmaier, Christoph Niemitz, Sarah Guttmann, Sara Reinartz Groba, Andree Zibert, Hartmut Schmidt
Klinik für Transplantationsmedizin, Universitätsklinikum Münster, Münster, Germany

**Correspondence:** Paula Ballmaier
(paulajohanna.ballmaier@ukmuenster.de)
*Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P22*

**Background**

The penetrance, onset, symptoms, and prognosis differ between ATTR patients, even for the same mutant. Patient-specific mechanisms of amyloid fibril formation and clearance have been proposed to affect penetrance. Amyloid deposits preferentially occur in the extracellular matrix (ECM), largely produced by stromal cells. The impact of primary stromal cells derived from different TTR-FAP patients (e.g. presymptomatic vs. overt disease) for accumulation/internalization of TTR is not known. Urine-derived cells (UCs) are an easily attainable source of primary, fibroblast-like cells. The aim of this study is to establish immortalized primary cells from ATTR patients for the study of patient-specific amyloid deposits.

**Materials and Methods**

Retroviral transduction of UCs was performed using various gene combinations of transgenes. Cells were transduced with either hTERT/p53, CyclinD1/CDK4(R24C), and HPV16E6E7 or combinations thereof. The influence of gene transfer was assessed by determination of cell proliferation, mRNA expression (qRT-PCR) and protein expression (e.g. flow cytometry, immunofluorescence).

**Results**

Untreated or GFP transduced UCs underwent senescence after 5-10 days as determined by senescence-associated beta-galactosidase assay. In contrast, UCs could be cultured for several months (presently > 130 days) after HPV16E6E7 gene transfer (n=3). UCs which were also transduced with CyclinD1/CDK4(R24C) showed accelerated cell growth as compared to other combinations and single HPV16E6E7 expression. High expression levels of KRT7, FN1, SLC2A1, CD29 and CD105 and CD90 expression. High expression levels of KRT7, FN1, SLC2A1, CD29 and CD44 where observed in the cells, whereas CD105 and CD90 expression was almost absent indicating epithelial and fibroblast cell marker expression. Immortalization did not affect marker epithelial/fibroblast marker expression as observed by immunofluorescence and qRTPCR analysis. However, cell cycle regulator p21 was significantly downregulated after immortalization.

**Conclusion**

Our data indicate that immortalization of urine-derived cells is an excellent tool to generate primary ATTR fibroblast-like cell lines that are highly valuable for the molecular understanding of TTR ECM deposition using a patient-specific analysis.
P23
Mechano-enzymatic mechanism of transthyretin amyloidogenesis: long distance effects of optimally effective inhibitors
Vittorio Bellotti1, Patrizia P. Mangione1, Guglielmo Verona1, Alessandra Corazza2, Diana Canetti1, Julian D. Gillmore2, Phillip N. Hawkins3, Graham W. Taylor1, Mark B. Pepsi1
1Wolfson Drug Discovery Unit, Centre for Amyloidosis and Acute Phase Proteins, UCL, London, UK; 2Department of Medical and Biological Sciences, University of Udine, Udine, Italy; 3National Amyloidosis Centre, UCL and Royal Free Hospital, London, UK
Correspondence: Vittorio Bellotti (v.bellotti@ucl.ac.uk)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P23

Background
We have recently identified a new mechano-enzymatic pathway of transthyretin (TTR) amyloid fibrillogenesis, under physiological conditions, which is catalyzed by a selective proteolytic cleavage of the loop interconnecting the strands C and D. This model is consistent with the presence of the corresponding large TTR fragment in most of natural amyloid fibrils, particularly in cardiac amyloid deposits.

Materials and methods
We have used a range of physico-chemical techniques to monitor the effects of prototypic ligands bound by TTR on the structural dynamics, kinetics of proteolytic cleavage and fibrillogenesis of TTR.

Results
We observed that occupancy of the two symmetrical binding sites by known TTR stabilizers can modulate the susceptibility of the protein to the amyloidogenic proteolytic cleavage.

Conclusions
The most potent inhibition of mechano-enzymatic fibrillogenesis is achieved by ligands that simultaneously occupy both the binding sites and, uniquely by our family of bivalent ligands, also the central channel present at the dimer-dimer interface.

P24
Serum protein electrophoresis (spe): a review in hereditary transthyretin amyloidosis
Juan Buades, Manuel Raya-Cruz, Cristina Gallego-Lezaun, Asunción Ferrar-Nadal, Mercedes Uson, Antoni Figueroa, Cristina Descals, Joan Carlos Montala, Tomas Ripoll, Juana Nuñez, Francisco Vega, Maria Ángeles Alonso, Hernán Andreu, Mateu Antonia, Eugenia Cisneros-Barcos, Hospital Son Llàtzer, Palma De Mallorca, Spain
Correspondence: Juan Buades (doctorjuanbuades@gmail.com)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P24

Background
Proteins are made up of amino acids chains linked by peptide bonds. Proteins have in their structure carboxyl and amine groups that confer negative or positive charge to the proteins depending on the number of free acidic and basic amino acids, ternary and quaternary structure and pH and ionic strength of the media.

Proteins can be separated when exposed to an electric current because the speed of the movement depends on the charge of the protein and its strength of the electric field.

Materials and methods
Electrophoresis is a method of separating proteins based on their physical properties. Serum is placed on a specific medium, and a charge is applied. The net charge (positive or negative) and the size and shape of the protein commonly are used in differentiating various serum proteins.

Results
We have analyzed a cohort of 23 patients of AhTTR with the Val30-Met mutation. We have found that 13 of 23 patients had serum protein electrophoresis alterations. Among them, 5 had a SPE image suggestive of chronic inflammation, 4 had acute inflammation profile, 3 a monoclonal band in the γ region and 1 showed hypogammaglobulinemia.

Conclusions
Classical immunofixation using IgG, IgM, IgA, kappa and lambda light chains antibodies is indicated to characterize the monoclonal band in the γ region. However, immunofixation failed to identify the composition of the band in 2 of the 3 patients.

Topic: New Tools In ATTR Amyloidosis

P25
Diagnostic accuracy of 99mTc-DPD scintigraphy for detecting ATTR cardiac amyloid deposits
David F. Hutt1, Simona F. Grigore1, Joanne Page1, Maria Burniston2, Ann M. Quigley3, Daniel Knight1, Ana Martinez-Naharro1, Ashutosh D. Wechalekar1, Helen J. Lachmann1, Candida C. Quarta1, Tamer Hezk1, Richa Manwani1, Shameem Mahmood1, Sajitha Sachchithanantham1, Taryn Youngstein1, Carol J. Whelan1, Thiirsha Lane1, Janet A. Gilbertson1, Dorota Rowczeno1, Julian D. Gillmore1, Marianna Fontana1, Philip N. Hawkins1
1National Amyloidosis Centre, Division of Medicine, UCL, London, UK; 2Nuclear Medicine Department, Barts Health NHS Trust, London, UK; 3Nuclear Medicine Department, Royal Free London NHS Foundation Trust, London, UK
Correspondence: David F. Hutt (d.hutt@ucl.ac.uk)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P25

Background
A number of authors have suggested that bone scintigraphy with planar quantitation is able to distinguish between cardiac AL and cardiac ATTR amyloidosis. We sought to investigate the diagnostic accuracy of 99mTc-Tc-DPD scintigraphy for detecting cardiac ATTR amyloid in a large population of patients with endomyocardial biopsy-proven cardiac amyloidosis.

Methods
All patients with endomyocardial biopsy-proven cardiac amyloid who underwent 99mTc-DPD scintigraphy were included in the analysis. Delayed whole body scan images were graded as Perugini 0-3. Planar quantitation was performed on all scans using both a heart to contralateral (H/CL), and heart retention to whole body region (HR/WBR) ratio, as previously reported.

Results
Two hundred sixty-two patients were included in the analysis, 201 with ATTR (wild-type in 136), 55 with AL, four with AApoA4 and two with AApoA1 cardiac amyloid. 99mTc-DPD scans were positive in 200/201 patients with ATTR and 33/55 (60%) patients with AL amyloid (21 grade 1 uptake, eight grade 2 and four with grade 3). Both patients with AApoA1 amyloid had a grade 1 scan and none of those with AApoA4 amyloid demonstrated cardiac tracer uptake. A positive (Perugini grade 1-3) 99mTc-DPD scan on its own was 99.5% sensitive but only 43% specific for diagnosing cardiac ATTR amyloid. A 99mTc-DPD scan with ≥ grade 2 uptake was 96% sensitive and 80% specific for ATTR amyloid, similar to that reported for pyrophosphate (PYP) scintigraphy by Bokhari and colleagues (97% sensitivity, 83% specificity), whilst Cappelli et al reported 93% sensitivity and 100% specificity using hydroxymethylene diphosphonate (HMDP). ROC analyses of H/CL and HR/WBR methods of planar quantitation demonstrated an AUC for both of 0.962 (p<0.001) for differentiating cardiac ATTR from non-ATTR using 99mTc-DPD. A H/CL ratio cut-off of 2.06 was 91% sensitive and 93% specific whilst a HR/WBR ratio cut-off of 3.07 yielded 92% diagnostic sensitivity and specificity for cardiac ATTR amyloid.

Conclusions
Bone scintigraphy with DPD, like PYP and HMDP, is extremely sensitive for diagnosing cardiac ATTR amyloid. Although the visual score (Perugini grade) and planar quantitation can lead to improved specificity, they should not be relied upon, in isolation, to differentiate between ATTR and non-ATTR forms of cardiac amyloidosis.
Background
Cardiac transthyretin amyloidosis (ATTR amyloidosis) is an increasingly recognised cause of heart failure. Cardiovascular magnetic resonance (CMR) with late gadolinium enhancement (LGE) and T1 mapping is emerging as a reference standard for diagnosis and characterisation of cardiac amyloid.

Materials and methods
We used CMR with extracelluar volume fraction (ECV) measurement to characterise cardiac involvement in relation to outcome in ATTR amyloidosis. Subjects comprised 263 patients with cardiac ATTR amyloidosis corroborated by grade 2-3 99mTc-DPD cardiac uptake. 17 with suspected cardiac ATTR amyloidosis (grade 1 99mTc-DPD) and 12 asymptomatic individuals with amyloidogenic transthyretin (TTR) mutations. Fifty patients with cardiac ATTR amyloidosis acted as disease controls.

Results
In contrast to AL amyloidosis, asymmetric septal hypertrophy was present in 79% of ATTR patients (70% sigmoid septum and 30% reverse septal curvature), whilst symmetric left ventricular hypertrophy (LVH) was present in only 18%: 3% of patients has no LVH. In patients with cardiac amyloidosis, the pattern of LGE was always typical for amyloidosis (29% subendocardial, 71% transmural) including right ventricular LGE (96%). 65 patients died during follow-up (19±14 months). ECV independently correlated with mortality and remained independent after adjustment for age, N-terminal pro-brain natriuretic peptide, ejection fraction, E/E' and left ventricular mass index (hazard ratio, 1.164; 95% confidence interval, 1.066-1.271; p<0.01).

Conclusions
Asymmetric hypertrophy, traditionally associated with hypertrophic cardiomyopathy, is the commonest pattern of ventricular remodelling in ATTR amyloidosis. LGE imaging is typical in all patients with cardiac ATTR amyloidosis. ECV correlates with amyloid burden and provides incremental information on outcome even after adjustment for known prognostic factors.

Materials and methods
We aimed to assess the ability of native T1 to 1) diagnose cardiac amyloidosis and 2) stratify prognosis. 134 wild-type ATTR (ATTRwt) (122 males, age 76±7 years), 81 mutant-type (ATTRm) (60 males, age 69±11 years) and 12 mutation carriers (4 males, age 47±10 years) were compared to 44 HCM patients. All subjects underwent CMR with standard SSFP-cine imaging and T1 mapping. ATTR patients underwent Tc-DPD scintigraphy, the current diagnostic imaging reference standard for ATTR.

Results
Native T1 was elevated in ATTR compared to HCM (p<0.001) (mean T1: in ATTRwt 1092 ± 51 ms, in ATTRm 1086 ± 67 ms, in HCM 1026 ± 64 ms). No significant difference between native T1 was found between ATTRwt and ATTRm. Native T1 diagnostic performance was similar for ATTRwt and ATTRm (AUC 0.865). Native T1 tracked amyloid burden (p < 0.001) measured by DPD scintigraphy. During follow-up, 95 deaths occurred: 55 ATTRwt, 40 ATTRm. Native T1 was predictive of death (HR 1.225; 95% confidence interval, 1.010-1.486; p<0.05).

Conclusions
CMR-determined native myocardial T1 has excellent diagnostic accuracy for identification of ATTR cardiac amyloidosis, tracks DPD-determined amyloid burden well and correlates with prognosis.

Background
99mTechnetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) is a bone tracer used for imaging cardiac amyloid deposits. However the kinetics of its uptake and clearance in amyloid deposits and other body tissues is not well understood, and therefore it is unclear how quantitative measures would be affected by the choice of measurement time point, given that different imaging protocols have developed at various centres.

A commonly used measure is the heart to contralateral ratio (H/CL) on planar imaging. This measure aims to cancel out the effect of overlying bone and soft tissue in the heart region by mirroring the region of interest (ROI) across the body and it might be hypothesised that it would be minimally sensitive to changes in measurement time points. This work seeks to test this hypothesis.

Materials and methods
Nineteen patients under investigation for cardiac amyloidosis underwent static imaging over the thorax at multiple timepoints up to 4 hours post-injection in addition to their routine whole body scan at 3 hours. H/CL was calculated for each timepoint and the range in H/CL between 1 and 4 hours post-injection was calculated for each patient.

Results
Patients were grouped by Perugini grade and an average patient range in H/CL was calculated for each group: grade 0 = 0.21 (n=4); grade 1 = 0.23 (n=6); grade 2 = 0.45 (n=5); grade 3 = 0.14 (n=4). In the grade 0, 1 and 3 patients there was a slight downward trend in H/CL over time, mirroring a trend noted in PYP patients by Castane et al [Castano, A et. al. Multicenter Study of Planar Technetium 99m Pyrophosphate Cardiac Imaging Predicting Survival for Patients With ATTR Cardiac Amyloidosis. JAMA Cardiol. 2016;1(8):880-889]. However an upward trend with a greater range in H/CL was seen in grade 2 patients.

Background
Heart failure caused by transthyretin amyloidosis (ATTR) is underdiagnosed and has an overlapping clinical phenotype with hypertrophic cardiomyopathy (HCM). Native myocardial T1 mapping by CMR is useful for diagnosis in cardiac amyloidosis. We investigated the diagnostic and prognostic value of T1 mapping in the largest ATTR population studied so far as well as patients with HCM.
Conclusions
While uptake of 99mTc-DPD in different tissues has previously been demonstrated to be dependent on the timepoint at which it is measured, H/CL seems to remain relatively constant between 1 and 4 hours post-injection, although this variation can be significant for grade 2 patients. These differences need to be considered when combining results from different centres.

P29
The relationship between 99mTc-DPD uptake and amyloid fibril composition in hereditary cardiac TTR amyloidosis: is the Glu92Lys variant an exception to the rule?
Laura Obici1, Stefano Perlini1, Robanta Mussinelli1, Elisa Arbusiti1, Masayoshi Tasaki3, Francesca Lavatelli1, Simona Casarin1, Ambra Raimondi1, Giampaolo Merlino2
1Amyloidosis Research and Treatment Centre, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; 2Clinica Medica II, Fondazione IRCCS Policlinico San Matteo and Department of Internal Medicine, University of Pavia, Pavia, Italy; 3Centre for Inherited Cardiovascular Diseases, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

P30
Comparison of immunohistochemistry and proteomic analysis for identification and typing of amyloid in different histological tissues
Janet A. Gilbertson, Tamer Rezk, Nigel B. Rendell, Graham W. Taylor, Patrizia Mangione, Diana Canetti, Vittorio Bellotti, Philip Hawkins, Julian Gillmore
National Amyloidosis Centre, Division of Medicine, Royal Free Campus, UCL Medical School, London, UK
Correspondence: Janet A. Gilbertson (j.gilbertson@ucl.ac.uk)

Background:
Accurate identification of the amyloid fibril protein is essential. Immunohistochemistry (IHC) fails to type amyloid in up to 30% cases. Proteomic analysis of amyloidotic material by mass spectrometry is reported to be a powerful tool for identifying the amyloid fibril protein in fixed tissue sections. We report proteomic findings in patients referred to the UK National Amyloidosis Centre.

Materials and methods:
Two hundred seventy-one biopsies from 30 different tissue types were evaluated by Congo red (CR) staining, IHC and proteomic analysis. Proteomic findings and IHC were interpreted by two experienced operators, blind to any clinical details.

Results:
Of the 272 biopsies, 251 contained amyloid (CR+) and 21 did not (CR-). Presence of amyloid was supported by at least 2 amyloid signature proteins (SP: SAP, apoa-A IV or apoE), in the proteome of 225/251 (90%) CR+ biopsies (181/251 contained all 3 SPs, 44/251 had 2 of 3 SPs. The ‘amyloid signature’ was not identified in the proteome of 26/251 (10%) CR+ samples, with 12 (4%) containing only one SP and 14 (6%) no SPs. Absence of the ‘amyloid signature’ supported CR- staining in 20/21 (95%) samples, but the proteome of 1/21 CR- case revealed AL (kappa sub-type) amyloid on the basis of presence of all 3 SPs and kappa light chain. In 125/251 (50%) CR+ samples, the amyloid subtype was definitively established by IHC, and supported in 114/125 (91%) cases by proteomic analysis. In 1/125 (9%) samples, results of IHC and proteomics differed for the following reasons: no amyloid SPs by proteomics (n=2/11), uncertain fibril protein by proteomics (n=8/11), different fibril protein (lambda by IHC, kappa by proteomics) (n=1/11). Of 126/251 (50%) CR+ samples with amyloid of indeterminate type by IHC, the amyloid sub-type was determined in 103/126 (82%) cases. The remaining 23 cases were not definitively typed by either IHC or proteomic analysis, 7/126 (6%) containing no SPs by proteomics, and 16/126 (12%) yielding uncertain results by proteomics.

Conclusions:
Proteomic analysis revealed false negative and false positive results for amyloid in 10% and 5% of cases respectively. IHC concurred with proteomic analyses in determining the amyloid subtype in 91% of cases. The amyloid subtype was successfully determined in over 80% of samples in which IHC was inconclusive. Proteomic analysis and IHC are complimentary techniques for diagnosis and typing of amyloid and should be interpreted in the context of the overall clinical picture.

P32
A novel serum microrna signature to screen ATTR
M’hammed Aguennouz1, Anna Mazzeo1, Claudia Stancanelli2, Francesca Polito1, Marco Ragusa1, Roberto Aringo1, Luca Gentile1, Anna Maria Cirinni1, Rosa Maria Di Giorgio1, Giuseppe Vita1
1Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; 2Biomedical Department of Internal Medicine and Specialist, University of Palermo, Palermo, Italy
Correspondence: M’hammed Aguennouz (aguennoz@unime.it)

Consent to publish
Written informed consent was obtained from the patients involved in this study.

P30
Comparison of immunohistochemistry and proteomic analysis for identification and typing of amyloid in different histological tissues
Janet A. Gilbertson, Tamer Rezk, Nigel B. Rendell, Graham W. Taylor, Patrizia Mangione, Diana Canetti, Vittorio Bellotti, Philip Hawkins, Julian Gillmore
National Amyloidosis Centre, Division of Medicine, Royal Free Campus, UCL Medical School, London, UK
Correspondence: Janet A. Gilbertson (j.gilbertson@ucl.ac.uk)

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Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):165
Background
Translhyretin amyloidosis (ATTR), is the most common form of genetic amyloidosis. It is a progressive devastating disease presenting with a heterogeneous spectrum of manifestations, thus diagnosis is often overlooked. Given that ATTR is now susceptible of treatment and early diagnosis provides for better prognosis, potential biomarkers are needed. Circulating microRNAs (miRNAs) have been described as promising diagnostic biomarkers in many chronic and degenerative neurological disorders. miRNAs show an advantage as biomarkers, being exceptionally stable among blood macromolecules, as they have been reported to be transported in blood on exosomes, high-density lipoproteins, and on complexes with proteins, protecting them from degradation. To evaluate the expression of miRNAs in sera from symptomatic and asymptomatic ATTR patients from South Italy, carrying three endemic mutations, and to identify a miRNA cluster as potential biomarker predictive of disease’s progression.

Materials and methods:
A quantitative reverse transcription polymerase chain reaction (qRT-PCR) of an array panel containing approximately 836 human miRNAs.

Results and conclusions:
Bioinformatics tools showed an involvement of autophagy and apoptotic pathways in ATTR patients vs asymptomatic and pathological controls (other genetic neuropathies), suggesting to use miRNAs as potential biomarkers of disease.

Conclusion
We report the case of a female patient with ATTR amyloid deposition in the inferior nasal conchival vessels, was performed incidentally by a nonspecific inflammatory polyp. Small focal deposits of amyloid TTR were observed on deep thick walled vessels. This location could be a suitable biopsy site.

Consent to publish
Written informed consent was obtained from the patients involved in this study.
non-Val30Met mutation. Recent evidence suggests that the overall prognosis of Val30Met patients is better than that of non-Val30Met patients.

Materials and methods

We therefore conducted a post hoc analysis comparing data from Val30Met patients who participated in the 18-month registration trial of tafamidis, a selective kinetic stabilizer of TTR shown to delay neurologic progression and the only medicine approved for the treatment of TTR-FAP, with data from a 12-month, open-label trial of tafamidis that included non-Val30Met patients. The data was comprised of three groups: tafamidis-treated Val30Met (n=64); tafamidis-treated non-Val30Met (n=21); and placebo-treated Val30Met (n=61). The severity and progression of neurologic impairment was evaluated using the Neupathy Impairment Score-Lower Limbs (NIS-LL) scale, and a mixed-effects model for repeated measures (MMRM) was used to compare the efficacy of tafamidis versus placebo.

Results

The baseline characteristics of the three groups showed that non-Val30Met patients were older, had a longer duration of symptoms, and had a higher level of baseline neurologic impairment compared with the Val30Met patients. Following 12 months of treatment, baseline-adjusted changes in NIS-LL were similar in the 2 tafamidis-treated groups (mean ± standard error: 1.60 ± 0.78 and 1.62 ± 1.43 in the Val30Met and non-Val30Met groups, respectively), but was greater in the Val30Met placebo-treated group (4.72 ± 0.77; p=0.0055 and p=0.00592 versus the Val30Met and non-Val30Met tafamidis-treated groups, respectively). The MMRM analysis predicted similar changes in NIS-LL for the Val30Met and non-Val30Met tafamidis-treated groups across a range of baseline NIS-LL scores, and the predicted changes for both these groups were consistently smaller than those predicted for the placebo-treated group. The MMRM also predicted that the extent of disease progression increased as baseline NIS-LL increased.

Conclusions

This post hoc MMRM analysis demonstrated that tafamidis delayed neurologic progression of TTR-FAP to a similar extent in patients with a Val30Met or non-Val30Met TTR mutation. Sponsored by Pfizer Inc. ClinicalTrials.gov identifiers: NCT00409175; NCT00791492; NCT00925002.

P39

Influence of baseline neurologic severity on disease progression and the associated disease-modifying effects of tafamidis in transthyretin familial amyloid polyneuropathy

Leslie Armass1, Huihua Li1, Balarama Gundapapeni1, Jeffrey Schwartz2, Denis Kechhane2

1Pfizer, New York, NY, USA; 2InVentiv Health Inc. Burlington, MA, USA

Correspondence: Leslie Armass (leslie.armass@pfizer.com)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P39

Background

A number of factors can influence disease progression in transthyretin familial amyloid polyneuropathy (TTR-FAP), a rare, progressive, and fatal hereditary amyloidosis.

Materials and methods

This analysis utilized longitudinal data from 5.5 years of exposure in the clinical trials of tafamidis, a selective TTR stabilizer, to evaluate the impact of baseline neurologic severity on disease progression in the Val30Met group. A linear mixed-effects model was constructed using data from the intent-to-treat Val30Met patients in the tafamidis registration trial and its 2 consecutive open-label extensions. The second extension is ongoing but an interim analysis involving a cleaned and locked database has been conducted (cut-off: December 31, 2014). Groups in the current analysis consisted of patients who received tafamidis during the registration and open-label studies (T-T group), or who received placebo during the registration trial and tafamidis during the open-label extensions (P-T group). This analysis focused on the first 18 months of treatment and disease progression as measured by the Neupathy Impairment Score-Lower Limbs (NIS-LL).

Results

The T-T (n=64) and P-T (n=61) groups included approximately equal numbers of men and women (mean age: 39 years) with early stage neurologic disease at baseline (mean NIS-LL ± standard deviation: 8.4 ± 11.4 and 11.4 ± 13.5, respectively). The slopes (rates of change) for NIS-LL from baseline to Month 18 were different across baseline NIS-LL scores (p<0.0001): patients with a lower baseline NIS-LL showed less progression than those with a higher baseline NIS-LL. Neurologic progression in the T-T group was less than in the P-T group across all levels of baseline NIS-LL (p=0.0004), and the degree of separation increased with longer durations of treatment. Similar results were seen in the NIS-LL muscle weakness subscale. These findings illustrate that neurologic progression depends strongly on baseline neurologic severity. The disease-modifying effect of tafamidis treatment relative to placebo was seen across the range of baseline levels of neurologic severity and treatment durations. The differences in the slopes of disease progression across groups support an increasing clinical benefit from tafamidis treatment over time.

Conclusions

Overall, this analysis supports the value of tafamidis treatment in patients with TTR-FAP. Sponsored by Pfizer Inc. ClinicalTrials.gov identifiers: NCT00409175; NCT00791492; NCT00925002.
Results
APOLLO enrolled 225 patients with hATTR amyloidosis with polyneuropathy. Among these, 119 patients were previously treated with tafamidis (62%), diflunisal (33%), or diflunisal/doxycycline combination (5%). Mean age: 61 yrs (range 27-83); males: 76%; V30M: 45%; non-V30M: 55%. Disease severity measures, mean: NIS: 58 points (6-142); mean KPS: 71 (60-100); PND I: 23%; PND II: 34%; PND IIIa: 29%; PND IIIb: 15%; NYHA Class 1, 2: 50% each. Physician-reported reasons captured at time of enrollment for patient discontinuation of therapies prior to APOLLO enrollment: clinical study enrollment (72%), disease progression (20%), safety (1%), or other (7%).

Conclusions
APOLLO, the largest, controlled study of patients with hATTR amyloidosis with polyneuropathy, includes patients with a wide range of TTR genotypes and neuropathy severity. Of patients previously treated with TTR stabilizers/disrupters, >90% discontinued treatment for APOLLO eligibility or disease progression on therapy. Data highlight significant unmet need in patients with hATTR amyloidosis with polyneuropathy.

Table 1 (abstract P41). Characteristics of Wait List and Liver Transplant Cohort

| Characteristic & Clinical Outcomes | Cohort at listing time (n=225) | Cohort at time of removal for OLT (n=170) |
|-----------------------------------|-------------------------------|-------------------------------------------|
| Mean age (SD) years               | 53.4 (10.7)                  | 53.6 (10.7)                               |
| Males                             | 73.8%                        | 72.4%                                     |
| Mean mHb (SD) kg/m² x g/L          | 1032.3 (239.2)               | 1007.1 (261.3)                            |
| Mean Scr (SD) mg/dL               | 1.3 (2.4)                    | 1.0 (0.4)                                 |
| Medical condition between listing and OLT |                              |                                           |
| ICU                               | 6.5%                         |                                           |
| Hospitalized not in ICU           | 3.5%                         |                                           |
| Not hospitalized                  | 90%                          |                                           |

Background
Familial amyloid polyneuropathy (FAP) is an autosomal dominant disease caused by mutations in transthyretin (TTR) gene. Val30Met is the most common variant. Although rare worldwide, there are descriptions of some endemic foci, such as in Majorca (Spain). The TTR Val30Met mutation presents classically with peripheral sensory neuropathy and progresses to autonomic and motor neuropathy, with occurrence of cardiac conduction abnormalities late in the disease progression. Orthotopic liver transplantation (OLT) is considered the best treatment. The abnormal TTR protein is synthesized in 95% in the liver, but also in retina and choroid plexus. Thus, it could be some worsening in spite of OLT. There are few published data about cardiac disease in post-OLT FAP patients.

The aim of this study was to investigate the occurrence and development of heart symptoms, arrhythmias, conduction abnormalities, and myocardial involvement in Majorca TTR Val30Met FAP patients who underwent OLT.

Materials and methods
Retrospective observational study selecting those patients who underwent OLT and comparing cardiac involvement prior to OLT and after follow up.

Results
The cohort comprised 132 FAP patients (69 males and 63 females). 54(41%) had received an OLT. Thirty-two(60%) were men. The mean age was 42.3±12.6 years at diagnosis. The time to inclusion on the transplant waiting list was 29.56 months. During follow up after OLT (14.4 years) several patients showed disease progression referred to neuropathy, nephropathy (more related to immunosuppression drugs) and cardiopathy (26.4% preOLT vs 61.5% postOLT), specially rhythm disturbances (9 vs 38.5%), atrium-ventricular block (AVB) (9 vs 35%), heart failure (HF) (2 vs 10%), increasing in left ventricular hypertrophy (LVH) (10 vs 13 mm) and diastolic dysfunction (13 vs 53%). Pacemaker were implanted in 31 patients (58.5%), but 21 (39.6%) were prophylactic pre-OLT (an ancient and controversial indication). 14 patients died (26.4%), the majority (10) FAP-related. In some cases a fatal arrhythmia was the probably cause of death. Mortality correlated with neurological worsening after OLT (p 0.025), nephropathy (p 0.008), any cardiac progression (p 0.04) and HF (p 0.014) after OLT.

Conclusions
There is a disease progression in FAP patients in spite of OLT, especially after long follow up, related specially to neurological and cardiac involvement. New rhythm abnormalities, AVB, HF and LVH appear after OLT, and confer a bad prognosis.
P43
Cardiomyopathy and peripheral polyneuropathy progression in patients with hereditary transthyretin-related amyloidosis associated with Glu89Gln mutation treated with tafamidis
Marianna Gospodinova1, Stayko Sarafov1, Teodora Chernova1, Andrey Kirov1, Tihomir Todorov1, Albena Todorova1, Ivailo Tournev1, Ivailo Tournev1, Stefan Denchev1
1Medical Institute, Ministry of Interior, Clinic of Cardiology, Sofia, Bulgaria; 2University Aleksandrovsk Hospital, Clinic of Neurology, Medical University, Sofia, Bulgaria; 3Department of Chemistry and Biochemistry, Medical University, Medicodiagnostic Lab. Genika, Sofia, Bulgaria; 4New Bulgarian University, Department of Cognitive Science and Psychology, Sofia, Bulgaria
Correspondence: Marianna Gospodinova (margyy2009@yahoo.com)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P43

Background
Patients with hereditary transthyretin-related amyloidosis (ATTR) with Glu89Gln mutation are characterized by mixed phenotype – cardiac and neurological. To evaluate the progression of cardiac involvement in patients with coexisting peripheral polyneuropathy, treated with Tafamidis.

Materials and methods
Twenty-seven patients (13 males) at mean age 57±7 years with ATTR were evaluated by clinical exam and Echocardiography. The patients have been diagnosed with Glu89Gln mutation. Tafamidis was initiated after the first assessment. The patients were followed for 23 (12-36) months on average.

Results
At the time of first evaluation, cardiac and neurological involvement was found in all the patients. All had signs and symptoms of peripheral polyneuropathy defined as stage 1, NYHA class 3 was found in 3 patients. At the second assessment, all patients remained in first neurological stage. A progression to NYHA class 3 was observed in 6 patients. The echocardiographic measurements revealed significant increase in left ventricular (LV) posterior wall thickness (p<0.02). Significant changes were found in some left ventricular (LV) diastolic function parameters (e’ septal < 0.01, e’ lateral < 0.005, a’ septal <0.03, a’ lateral <0.006, E/e’ septal < 0.002, E/e’ lateral < 0.0008, left atrium < 0.0005), but not in LV systolic function (EF, s wave, Global Longitudinal Strain – p > 0.05). No significant change was found in right ventricular wall thickness.

Conclusions
The evaluated cohort is characterized by more advanced cardiac than neurological involvement at baseline. The follow up revealed that all the patients remained in first neurological stage, but some progression in NYHA class was observed. Worsening of LV diastolic function without significant reduction in systolic function was found. Initiation of any treatment at an early stage of heart involvement may be beneficial.

P44
Long-term treatment of ATTR with tafamidis: the Sicilian experience
Luca Gentile1, Claudia Stancanelli1,2, Massimo Russo1, Giuseppe Vita1 and Anna Mazzoc2
1Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy; 2Biomedical Department of Internal Medicine and Specialistic, University of Palermo, Palermo, Italy; 3Nemo Sud Clinical Centre, AOU Policlinico, Messina, Italy
Correspondence: Luca Gentile (lucagentile84@yahoo.it)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P44

Background
Tafamidis is a transthyretin (TTR) stabilizer able to prevent TTR tetramer dissociation. There have been a few encouraging studies on Tafamidis efficacy in early-onset inherited transthyretin amyloidosis (ATTR) due to Val30Met mutation. However, less is known about its efficacy in later disease stages and in non-Val30Met mutations.

Materials and methods
Here we report the experience on long term Tafamidis treatment in a ATTR population referring to our tertiary care center in Sicily, an endemic area. We studied 21 patients carrying non-Met30 mutations (Glu89Gln: n 11; Phe64Leu: n 8; Thr49Ala: 2), who had undergone Tafamidis treatment from 1 to 6 years. We evaluated modifications of BMI, NIS, FAP stage, NYHA class and CADT.

Results
Our results confirmed the long term well tolerability of Tafamidis, the effects on body weight preservation and the lower progression of neuropathy in a subgroup of patients.

Conclusions
Neuropathy and cardiomyopathy progressed in a significant proportion of patients despite treatment, mainly in subjects with poor baseline status.

P45
Long-term, open-label clinical experience with patisiran, an investigational RNAi therapeutic for patients with hereditary transthyretin-mediated (hATTR) amyloidosis with polyneuropathy
Angela M. Partisano1, John L. Berk2, David Adams3, Ole Suhr4, Isabel Conceição5, Marcia Waddington Cruz6, Hartmut Schmidt7, Juan Buades8, Josep M. Campistol8, Jean-Yves Pouget9,10, Michael Polydefkis11, Marianne Sweeter12, Jihong Chen1, Jared Gollob1, Teresa Coelho12
1Alynlam Pharmaceuticals, Cambridge, MA, USA; 2Amyloidosis Center, Boston University, Boston, MA, USA; 3National Reference Center for FAP, CHU Bicêtre, APHP, Le Kremlin-Bicêtre, France; 4Umeå University, Umeå, Sweden; 5Centro Hospitalar Lisboa Norte-Hospital de Santa Maria, Lisbon, Portugal; 6Hospital Universitario Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, 7University of Münster, Münster, Germany; 8Hospital Son Llatzer, Palma De Mallorca, Spain; 9Hospital Clinic, University of Barcelona, Barcelona, Spain; 10Hospital de La Timone, Marseille, France; 11Johns Hopkins University, Baltimore, MD, USA; 12Hospital de Santo António, Centro Hospitalar do Porto, Porto, Portugal
Correspondence: Angela M. Partisano (apartisano@alnylam.com)
Orphanet Journal of Rare Diseases 2017, 12(Suppl 1):P45

Background
hATTR amyloidosis is a multisystemic, rapidly progressive, life-threatening disease caused by a mutation in the TTR gene, resulting in deposition of amyloid fibrils in multiple organs. Heterogeneous clinical presentation of hATTR amyloidosis includes sensory, motor and autonomic neuropathy, as well as cardiac involvement, resulting in significant morbidity and mortality. Patisiran, an investigational RNAi therapeutic targeting TTR mRNA, previously reported sustained mean reduction of serum TTR. The objective of this abstract is to describe the long-term safety and efficacy of patisiran in patients with hATTR amyloidosis with polyneuropathy.

Materials and methods
Patients with hATTR amyloidosis with polyneuropathy originally dosed on the Phase 2 patisiran study were eligible to roll over onto a Phase 2 OLE study and continue receiving patisiran 0.3mg/kg IV q3W for up to a total of 24 months (NCT01961921). Primary endpoint was safety; secondary objectives included: effects on neurologic impairment (mNIS+7, NIS), QoL, mBMI, mobility, grip strength, autonomic symptoms, and serum TTR levels. Following completion of the study, patients were eligible to continue treatment on a global OLE study (NCT02510261).

Results
Phase 2 OLE study included 27 patients; median age 64 years (range: 29-77). Patisiran given for 24 months was generally well tolerated; 7 patients experienced SAEs unrelated to study drug, including 1 patient with fatal gastrointestinal cancer. Another unrelated death (myocardial infarction) occurred after completing dosing, but prior to the final visit. Flushing (22.2%) and infusion-related reactions (22.2%) were the most common related AEs; all were mild in severity and did not result in discontinuations. Sustained mean serum TTR lowering of ~80% was achieved for >24 months (mean maximal TTR lowering: 93%) and resulted in an improvement in neuropathy with a mean
7.0-point decrease in mNIS+7 ($n=26$). A total of 25 patients subsequently enrolled onto the global OLE study.

**Conclusions**

Long-term (24 month) administration of patisiran was generally well tolerated, resulted in robust and sustained serum TTR lowering, and supported the therapeutic hypothesis that TTR lowering can potentially halt or improve neuropathy progression in patients with hATTR amyloidosis with polyneuropathy. Safety and mNIS+7 data from patients treated for another 12 months (total of 36 months) on the global OLE study will be presented.

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**P46**

**Treatment of ATTR cardiomyopathy with a TTR specific antisense oligonucleotide**

Merrill D. Benson1, Noel R. Dasgupta2

1Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA; 2Division of Cardiology, Indiana University School of Medicine, Indianapolis, IN, USA

**Correspondence:** Merrill D. Benson (mdbenson@iupui.edu)

**Orphanet Journal of Rare Diseases** 2017, 12(Suppl 1)

**Background**

Cardiomyopathy is a major manifestation for patients with ATTR amyloidosis, both hereditary (hATTR) and wild-type (wtATTR). A recent phase 3 study of a TTR specific antisense oligonucleotide (Inotersen) has shown significant therapeutic effect in patients with hATTR polyneuropathy (hATTR-PN). Approximately 62% of patients enrolled in this study had concomitant hATTR cardiomyopathy (hATTR-CM), however wtATTR patients and hATTR-CM patients with insufficient or no neuropathy were not eligible.

**Materials and methods**

In a phase 2 open label study we have now treated 19 patients with ATTR cardiomyopathy (8 hATTR-CM, 11 wtATTR) with Inotersen for 12 months and of these 11 (hATTR-CM, 3 wtATTR) have been treated for 2 years.

**Results**

Safety profile has been good and the drug well tolerated. Cardiomyopathy parameters determined by transthoracic ECHO and MRI have remained stable in most subjects. Functional cardiac parameters (6MWT, NYHA class, LV strain, BNP) have also been consistent in most cases with lack of progression of cardiomyopathy.

**Conclusions**

In general, the results of this phase 2 study have been favorable and support pursuit of a phase 3 study in patients with ATTR cardiomyopathy.

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**Decreased S100A8/A9 in V30M related familial amyloid polyneuropathy: a possible pathway in misregulation of Schwann cells**

João Moreira1, Nádia P. Gonçalves2, Margarida Saraiva1, Maria João Saraiva1

11IS - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; 2Danish Research Institute of Translational Neuroscience DANDRITE, Nordic-EMBL Partnership,Department of Biomedicine, Aarhus University, Aarhus, Denmark

**Correspondence:** João Moreira (joao.moreira@ibmc.up.pt)

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**Background**

Familial amyloidotic polyneuropathy (FAP) is an autosomal dominant neurodegenerative disorder with extracellular deposition of mutant transthyretin (TTR) aggregates and fibrils, particularly in nerves and ganglia of the peripheral nervous system (PNS). The most common TTR mutation is a substitution of a Methionine for a Valine at position 30 (TTR V30M) that predisposes TTR to form aggregates and fibrils. V30M human FAP nerve biopsies display increased cytokine production, but intriguingly no immune inflammatory cellular infiltrate is observed around TTR aggregates, which contributes to disease aggravation [1]. Moreover, in response to nerve injury, a V30M transgenic mice model display a downregulated innate immune response when compared to wild type (WT) mice [2]. Schwann cells are the nerve sentinel cells and have the ability to regulate the PNS immune response by secreting cytokines and chemokines thus having a central role for nerve repair. In nerves of FAP patients, Schwann cells are impaired in their ability to express chemokines, which are important drivers of tissue regeneration [3].

**Results**

In this study, we showed that the expression of S100A8/A9 molecules, known potent initiators of the immune response in stimulated Schwann cells of injured peripheral nerves, is downregulated in primary Schwann cells incubated with aggregated protein as compared to WT TTR. In line with this, we found that S100A8/A9 mRNA levels are highly decreased in V30M mice, as compared with WT controls. By ELISA, S100A8 and S100A9 protein levels were found downregulated in plasma samples from V30M FAP patients.

**Conclusions**

The presence of V30M TTR impacts S100 expression and appears to impair the immune activation of Schwann cells in V30M nerves. This may be linked to the diminished immune cellular activation and infiltration observed in FAP nerves, contributing in this way for the neuronal dysfunction present in the disease.

**References**

1. Nyhlin, N., et al., Advanced glycation end product in familial amyloidotic polyneuropathy (FAP). J Intern Med, 2000. 247(4): p. 485-92.
2. Goncalves, N.P., M. Teixeira-Coelho, and M.J. Saraiva, The inflammatory response to sciatric nerve injury in a familial amyloidotic polyneuropathy mouse model. Exp Neurol, 2014. 257: p. 76-87.
3. Sousa, M.M., et al., Familial amyloid polyneuropathy: receptor for advanced glycation end products-dependent triggering of neuronal inflammatory and apoptotic pathways. J Neurosci, 2001. 21(19): p. 7576-86.

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**P48**

**Real-life iluvien (fluocinolone acetonide) case study: rapid drying of the macula edema and improved vision within 1 year after therapy initiation in ATTRV30M retinal angiopathy**

João Beirão, Bernardete Pessoa, Maria-João Meneres, Pedro Meneres

Centro Hospitalar do Porto, Hospital Santo António, Porto, Portugal

**Correspondence:** João Beirão (brandaobeirao@gmail.com)

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**Background**

A case showing sustained structural and functional responses 1 year after a single treatment with ILUVIEN (0.2 µg/day fluocinolone acetonide, FAc) despite suboptimal responses to anti-VEGFs treatment.

**Case report**

A liver transplanted ATTRV30M 48-year-old female patient with amyloid retinal angiopathy macular edema was first diagnosed in October 2015 and had a baseline visual acuity (VA) of 0.1 (Snellen chart) in the left eye. Central foveal thickness (CFT) was 502 microns. She was previous submitted to vitrectomy for vitreous amyloidosis and an Ahmed valvule implantation for glaucoma. The patient was treated with 5 intravitreal injections of bevacizumab and by May 2016, VA and CFT were largely unchanged (0.1 and 511 microns). An implant releasing FAc at a dosage of 0.2 µg/day (ILUVIEN) was administered in May 2016, and the optical coherence tomography indicated that the macula was dryer after 7 days (CFT was below 300 microns). This was sustained at 6 and 12 months after the treatment. VA improved to 0.4 within 14 days, and this was maintained after 12 months. Throughout the duration of this study, the intraocular pressure was 10 mmHg, and no glaucoma medication was administered.

**Conclusions**

In real-life practice, this ATTRV30M vitrectomized and Ahmed valvule implanted patient showed a suboptimal response to multiple intravitreal injections of bevacizumab. When subsequently treated with a single injection of ILUVIEN, there were large and rapid improvements in VA and CFT that were maintained for the following 1 year.

**Consent to publish**

Written informed consent was obtained from the patients involved in this study.

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