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INVITED SPEAKER PRESENTATIONS

I3

Which assessment for the carriers: the cardiac view

Arnt V Kristen
Amylodosis Center, Department of Cardiology, University of Heidelberg, Germany

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I3

Cardiac manifestation is common in transthyretin-related (ATTR) amyloidosis. However, the age of onset of first symptoms ranges widely. Moreover, it differs between the individual mutations.

Potential treatment options to disrupt amyloid fibrils are lacking and cardiac manifestation is associated with poor prognosis. Thus, early identification of patients at risk for development of systemic ATTR amyloidosis is crucial to avoid deterioration of organ function and maintain quality of life. Predictive molecular genetic testing of family members is the initial step to identify a patient at risk for hereditary ATTR amyloidosis. However, incomplete penetrance and high variability of age of onset of disease complicates the diagnostic strategy. Thus, regular screening is required for early identification of the disease. However, the question arises what diagnostic tools should be used for assessment of asymptomatic carriers of a TTR gene variant.

Although endomyocardial biopsy has the highest sensitivity and specificity for detection of cardiac amyloidosis it is not capable for longitudinal evaluation in asymptomatic carriers. Non-invasive strategies are required to allow frequent testings with time intervals ranging between 6 and 24 months. ECG, echocardiography, cardiac magnetic resonance imaging, skeletal scintigraphy, and cardiac biomarkers are potential tools for assessment. In general, routine assessment should be based on ECG and echocardiography. Precise screening of ECG for any abnormalities including conduction disturbances, QRS or T-wave abnormalities is mandatory as abnormal findings are present in almost all patients with cardiac ATTR amyloidosis. Echocardiography assessment should include evaluation of diastolic function, longitudinal impairment, as well as speckle tracking. Cardiac MRI has become the gold standard for evaluation of cardiac morphology and function. Moreover, application of gadolinium allows characterization of the myocardium by a characteristic pattern of late gadolinium enhancement in cardiac amyloidosis. Despite high resolution images and excellent reproducibility, this technique is time consuming, cost intensive, and ultimately ira availability is limited to large centers. Recently, native cardiac T1 mapping has been reported to abnormal early during the clinical course.

Cardiac retention of bone tracers has been reported to be specific for detection of ATTR amyloid. However, the clinical use for early identification remains debatable. Finally, longitudinal assessment is limited due to radioactivity. Cardiac biomarkers, including brain-natriuretic peptides and troponin T are sensitive indicators of myocardial stress and injury and appear to be an easy and useful tools for work-up of carriers.

Due to lack of potential treatment options to disrupt amyloid fibrils the main goal in carriers of transthyretin variants is to dectect manifest amyloid disease as early as possible. From the cardiological point of view carriers of TTR variants should routinely be assessed by ECG, echocardiography with speckle tracking, and cardiac biomarkers. If available cardiac MRI with mapping techniques appears to be of additional benefit.

I4

When to decide to enroll a TTR-FAP patient in a Clinical Trial?

Juan Buades
Hospital Clínico San Carlos, Madrid, Spain

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I4

Background: The Transthyretin-Familial Amyloid Polyneuropathy (TTR-FAP) is a disease caused by deposition of mutant transthyretin (TTR), produced approximately 95% in the liver and the rest in the plexus choroideus and retina. In 1990 the first TTR-FAP patient liver transplant was performed. The liver transplant, which suppresses TTR synthesis, was the only treatment available to modify this disease until 2011, when the European Medicines Agency approved Tafamidis, a TTR stabilizer, for stage I patients (Val30Met and non-Val30Met). To improve upon the pre-existing therapy of liver transplants and given the results of 68% of Tafamidis respondents, other disease-modifying therapeutic approaches were developed: Silencers, Stabilizers, Degraders and Reabsorption agents. In light of these multiple agents clinical trials are the key to improve TTR-FAP treatment.

Objectives: The aim of the analysis is to determine eligibility criteria and optimal timing of patient's enrollment in a clinical trial.

Methods: We reviewed the literature using the online database PUBMED. Standard search strategies were applied using the following MESH terms alone or in combination: amyloidosis, transthyretin, liver transplant, tafamidis and clinical trial. Evaluation of the bibliographies was done in order to select relevant articles. Additionally, clinical experience achieved by lead investigators and our own experience was applied in this analysis.

Results: Nowadays siRNA (Patisaran®), Antisense Oligonucleotides, Diflunisal and Doxycicline-TUDCA are under clinical trial investigation. Election criteria definition is essential to enroll a patient in one of those clinical trials. Based on the bibliography, inclusion criteria protocols of each clinical trial and our own experience the following criteria have been developed:

1. Stage I to Stage IIIB can be recruited into a clinical trial at any time.
2. Stage I non-respondents to Tafamidis after 12 months treatment who show progression of the disease indicated by.
    - Worsening of ambulation (increase of PND score by one point).
    - Onset of orthostatic hypotension or impotence.

• Progress of cardiomyopathy with worsening of stage of cardiac insufficiency NYHA by 1 point or of cardiac conduction disorders.

3. It is expected than in the short future OLT patients who underwent illness progression would be enrolled in a clinical trial.

Conclusions: Disease staging, tafamidis response and OLT limitations are the main factors to be considered before recruiting a patient for a clinical trial.

I5

Preimplantation genetic diagnosis for TTR-FAP in Portugal

Filipa Carvalho[1,2]
[1]Department of Genetics, Faculty of Medicine, University of Porto, Portugal; [2]Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I5

Background: Portuguese Familial Amyloidotic Polyneurophaty (FAP) (OMIM #105210) is a rare systemic amyloidosis characterized by a progressive, autonomic and sensory-motor neuropathy. FAP is an autosomal dominant disorder caused by mutations in the transthyretin (*TTR*) gene. Preimplantation genetic diagnosis (PGD) is currently one of the options available for couples at-risk to avoid disease transmission. The review the PGD cycles for TTR-FAP performed in the last 15 years in Portugal will be presented.

Methods: Embryos were biopsed at day 3 of development and diagnosis was initially performed using fluorescent PCR primers designed to amplify the p.Val50Met in exon 2 of the *TTR* gene and is currently done by multiplex PCR for the mutation detection and polymorphic markers.

Results: Two hundred and thirty-five clinical cycles were performed in 118 couples (74 with paternal and 44 with maternal mutation), with a mean number of 2 cycles per couple and a mean maternal age of 31.4 years old. The mean number of MII injected oocytes per cycle was 8.7. Fertilization and cleavage rates were 74.5% and 98.2%, respectively. Eighty-nine percent of embryos were biopsied (mean number = 5.8) and amplification was obtained in 94% with a ratio of normal *versus* mutated embryos of 1:1,3 ($p < 0.0001$). Interestingly, if only the cycles of paternal transmission were considered the ratio of normal *versus* mutated embryos was 1:1,03 (p=0,7018) whereas if the cycles of maternal transmission were analyzed the ratio of normal *versus* mutated embryos was 1:1,77 (p<0,0001). One hundred and fifty-eight cycles had embryo transfer (mean embryo number = 1.7) leading to 57 biochemical (36%) and 50 clinical pregnancies (32%) (45 term pregnancies, 3 ongoing pregnancies and 2 miscarriages). Three pregnancies correspond to frozen embryos transfers. Thirty-seven term pregnancies were singleton (mean gestational age = 38.4W; average weight at birth = 3093g), 7 were twins (mean gestational age = 34.5W; average weight at birth = 2310g) and one was a triplet pregnancy (two embryos transfer) (gestational age = 34W; average weight at birth = 1700g).

Conclusion: PGD for Portuguese type TTR-FAP is a well established procedure allowing the birth of unaffected children with a take-home baby rate similar to the one described in the literature.

I6

TTR-FAP: liver transplant vs oral medication. How and when

Teresa Coelho
Unidade Corino de Andrade, Centro Hospitalar do Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I6

Liver transplant was introduced as a treatment for Familial Amyloid Polyneuropathy (FAP) in 1990 and since then it was demonstrated that the best results are seen with early onset patients with predominant neurologic involvement (typically Val30Met patients), at the first years of symptomatic disease. Complete stabilization of the neuropathy is seen in a significant proportion of patients. However the treatment introduced mortality and morbidity due to the surgical procedure itself, variable from center to center.

Tafamidis, a transthyretin (TTR) stabilizer, administered orally, was approved in Europe in 2011 to treat neurologic involvement of stage 1 patients with any TTR mutation. We have limited long term data on the efficacy of this treatment and after starting treatment we need at least 6 to 12 months to evaluate the impact of the drug on disease progression. This means that choosing Tafamidis first, may significantly delay the option for liver transplant, in case of oral treatment failure, impacting the results of surgery. The choice between these options is not easy because we have not yet enough comparable data.

Most patients would prefer to avoid or to delay the need for an aggressive treatment, hoping to be part of the group of good responders to oral medication. How much should doctors point to a given option?

Oral treatment should be prescribed as soon as patients develop the first symptoms and signs of neuropathy. Patients diagnosed with advanced stage 1 disease with high probability of being refused for liver transplant in one year should be sent directly to surgery. If the waiting time is less than one year the benefit of receiving Tafamidis while waiting must be weighted.

Patients with more advanced neurologic disease and/or important cardiac involvement should be considered for clinical trials or for other drug treatments.

I7

Which assessment for the carriers ? The point of view of the neurologist

Isabel Conceicão
Department of Neurosciences, Centro Hospitalar Lisboa Norte - Hospital de Santa Maria and Clinical and Translational Physiology Unit, Physiology Institute, Faculty of Medicine, IMM, Lisbon, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I7

Transthyretin-related familial amyloid polyneuropathy (TTR-FAP) is a rare, autosomal-dominant, adult-onset, systemic disease caused by mutations in the transthyretin (TTR) gene that lead the TTR protein to misfold and deposit as insoluble amyloid fibrils in peripheral and autonomic nerves, the heart, and other organs. [1,2].

Initial clinical symptoms may appear between the second and ninth decade of life and, without treatment, TTR-FAP leads to death on average within 10 years of symptom onset[1,2].

The disease can be difficult to recognize due to extreme phenotypic heterogeneity and nonspecific clinical symptoms.

Regular clinical surveillance for detection of first signs and symptoms of TTR-FAP in carriers should focus on clinical, neurophysiological and cardiological approaches.

Neurological involvement should be assessed by a careful clinical history looking for positive, negative sensory and motor signs and symptoms as well autonomic complaints. The use of validated clinical scales (NIS, Utah, CAD, Compass 31) and a complete small and large fibers neurophysiological evaluation should be done every year although frequency of visits should be determined on a case-by-case basis depending on clinical. Asymtomatic carriers should be evaluated once an year and symptomatic carriers under needs a closer follow-up every 6 months.

Detection of such early sign and symptoms in asymptomatic carriers establishes a diagnosis of TTR-FAP, and anti-amyloid treatment should be promptly initiated. In symptomatic patients under therapeutics a regular follow-up allows a longitudinal evaluation to reflect the maintenance of therapeutic efficacy.

References

1. Ando Y, *et al*: *Orphanet J Rare Dis* 2013, **8**:31.
2. Plante-Bordeneuve V: *J Neurol* 2014, **261(6)**:1227-33.

I8

ATTR-FAP: liver transplantation vs oral medication, how and when

Bo Goran Erikzon
Center for Surgical Sciences, Karolinska Institutet, Karolinska University Hospital Huddinge, 141 86 Stockholm, Sweden
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I8

Background: Liver transplantation (LTx), introduced in 1990, has served as the only available treatment with capacity to halt the progress of disease in transthyretin amyloidosis. For the most common variant, Val30Met, the effect of a new liver is well known leading to stabilization in the majority of patients. However, not all patients are helped by

transplantation. Progress of cardiac amyloidosis is not uncommon necessitating both liver and heart transplantation (LTx/HTx). The effect of LTx is less well studied in patients with non-Val30Met mutations, and outcome has generally been inferior to that seen in patients with the Val30Met mutation. Large variations in survival, not only between different mutations but also between mutations with similar phenotypes, have been noted and it is clear that each mutation needs to be considered individually. Some mutations have similar long-term survival as the Val30Met, while LTx is not to be recommended for other mutations. Several novel pharmaco-therapeutical approaches have emerged over the last years and may provide a more attractive and less invasive treatment for this patient population.

Methods: Data concerning outcome after LTx for ATTR amyloidosis was extracted from the FAPWTR registry. Survival rates were analyzed by the Kaplan-Meier method and Log-Rank test.

Results: In total, 58 different mutations were treated by LTx alone or by LTX/HTx. Data from more than 2000 patients were accumulated from 77 collaborating liver transplant centers. Overall, 20-year survival after LTx was 55.3%. Modified Body Mass Index, early onset of the disease, disease duration before transplantation and Val30Met versus non Val30Met mutations were independent significant survival factors. Cardiovascular death was markedly more common than that observed in patients undergoing LTx for end stage liver disease. There has been a significant drop in the annual number of transplants over the last years following the introduction of pharmacotherapy in Europe. A careful evaluation regarding the effect of new promising pharmacotherapies in relation to LTx is of the outmost importance. There is a risk, that patients not responding to pharmacotherapy may be exposed to a less favorable surgical outcome because LTx is delayed. Furthermore, it is unknown if patients not responding to LTx will respond better to alternative treatment and vice versa.

Conclusion: Long-term survival after LTx for many TTR variants is excellent. Several new promising pharmacological treatments are under evaluation. In order to determine the most optimal use, these pharmaco-therapeutical approaches must be compared to the existing surgical therapy. Perhaps the best treatment for some patients will be a combination of pharmacotherapy and surgery.

I9

Ophtalmologic changes in transthyretin familial amyloid polyneuropathy (ATTR-FAP)

Natália Ferreira
Ophthalmology Department, Centro Hospitalar do Porto, Hospital Santo António (HSA), Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I9

Background: Familial amyloid polyneuropathy (FAP) is an inherited disorder with autosomal dominant transmission and multiple phenotypes, characterized by systemic accumulation of amyloid fibrils. The most common type of FAP is related to a mutant transthyretin (TTR). TTR is mainly synthesized in the liver, but few amount of TTR is produced in the eye, namely in retinal pigment epithelium, which explains the continuous intra-ocular amyloid deposition observed in patients submitted to liver transplantation. The incidence of ophthalmic manifestations related to FAP depends on mutation involved, geographical area of patient and time of evolution of the disease. More than 100 mutations of TTR have been described but the most frequent in Portugal is TTR Val30Met. Even the same population with the same mutation can present significant clinical variability. TTR Met30Val FAP patients have different phenotypes according to their age at onset of the disease.

Methods: Dry eye, abnormal conjunctival vessels, pupillary abnormalities, vitreous opacities and glaucoma are common ocular changes associated to FAP. All are described as well as their etiology and incidence. Clinical cases with demographic data, TTR mutation involved, age at beginning of disease, period of evolution of disease, previous liver transplant or medical treatment, specific ophthalmologic alterations related to FAP and previous ocular surgeries are presented.

Results: The most specific ocular manifestations of ATTR-FAP are deposits on lens anterior capsule and pupillary border, scalloped pupil and vitreous amyloidosis and the most severe one is glaucoma.

Amyloid deposits on anterior lens surface are central, disciform opacities with more dense border. Amyloid deposits on pupillary border are irregularities of white membranous material. Scalloped pupil, an irregular outlines and fringed edges of pupil, is pathognomonic of ATTR-FAP.

Peculiar vitreous opacities are the most common specific change of late onset TTR Met30Val population. There are four types of amyloid vitreous opacities: *pseudopodia lentis*, fibrils, spherical opacities and pre-vascular opacities. *Pseudopodia lentis* and typical fibrils, since numerous and dense, are also pathognomonic.

Dry eye is a common ocular change in FAP but a *non* specific. Signs of *keratoconjunctivitis sicca* like diminution of Break Up Time and *punctata* epitheliopathy are frequent and complications like corneal neovascularization or opacity and neurotrophic corneal ulcer are unusual.

Abnormal conjunctival vessels are a *non* specific modification of shape of vessels.

Pupillary reflex changes, light pupillary hiporreactivity or redilatation lag without loss of light reflex response, can be observed.

Glaucoma related to ATTR-FAP is an agressive secondary open angle glaucoma. Frequently, surgery is required (trabeculectomy or valve implants). Glaucoma leads to blindness if left untreated.

Amyloid retinal microangiopathy with peripheral retinal ischemia is a rare manifestation. It can be observed in advanced stage of disease.

Conclusion: Frequently, the severity of ocular symptoms does not correlate with systemic symptoms, particularly in late onset disease. Vitreous opacities can be the first manifestation of the disease in older patients. Ocular manifestations are common in FAP TTR Val30Met patients and might be potentially severe with visual impairment like vitreous opacities and glaucoma. The most frequent specific alterations observed in early onset cases are signs related to dry eye and in late onset cases, vitreous opacities.

Vitrectomy is frequently required to remove amyloid in vitreous cavity to regain vision. In isolated cases with later onset and milder symptoms of the disease, vitreous opacities are frequently the first symptom. Ophthalmologist has an important role in follow-up of FAP patients to accurately treat sight-threatening manifestations and to diagnosis new cases, particularly in late onset TTR Met30Val.

I10

Imaging cardiac ATTR amyloid

Philip N Hawkins
National Amyloidosis Centre, UCL, London, UK
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I10

The heart is the principal site of involvement in non-hereditary ATTR amyloidosis and a major driver of treatment options and prognosis in hereditary forms of the disease. The goals of cardiac imaging in amyloidosis are to aid diagnosis, provide prognostic information, track disease progression and evaluate response to therapy.

Echocardiography has long been the mainstay of cardiac evaluation in amyloidosis, particularly that of diastolic dysfunction, but its precision is limited and both inter and intra-observer variability constrain its capacity to detect changes. The recent refinement of myocardial strain imaging provides more reproducible and more sensitive evaluation of systolic impairment, which may be overlooked in amyloidosis, and the longitudinal strain pattern of relative apical sparing strongly supports diagnosis of amyloidosis.

Two investigations that are transforming our understanding of cardiac amyloidosis are bone scintigraphy and cardiovascular magnetic resonance (CMR).

CMR with gadolinium contrast has proved to be invaluable for identification of cardiac amyloid, showing characteristic patterns of global subendocardial and transmural late gadolinium enhancement associated with abnormal myocardial and blood pool kinetics. The recent refinement of phase-sensitive inversion recovery sequence is highly sensitive and specific, producing findings that are virtually pathognomonic for amyloid. CMR is inferior to echocardiography for evaluating diastolic function but can assess the heart's structure and systolic function with greater accuracy and precision. A key advantage of CMR is its unique ability to give insight about tissue composition through myocardial tissue characterization. T1 mapping studies can quantify the massive expansion of the extracellular space caused by amyloid deposition as well as evaluating the myocyte response to it, i.e. associated hypertrophy or cell loss. The ability to independently track changes in amyloid load and myocardial cell mass will

be invaluable in assessing new therapies. One significant limitation of CMR is its incompatibility with most implanted pacemakers and ICDs, an issue that may be addressed by the development of dynamic equilibrium CT, in which gated five minute contrast-enhanced scans have shown excellent potential for diagnosis and quantification of cardiac amyloid.
It has been known for decades that bone scintigraphy tracers can localize to cardiac amyloid deposits, but the enormous potential of this tool has only been fully realized in recent years. Studies have confirmed that ^{99m}Tc-3, 3-diphosphono-1, 2-propanodicarboxylic acid (DPD) localizes to the heart in patients with cardiac ATTR amyloidosis with incredible sensitivity and high specificity. The mechanism remains unknown, but virtually all patients with clinically significant cardiac ATTR amyloidosis show Grade 2 or greater localization to the heart using the simple Perugini Grade 1-3 scoring system. Indeed, scans in apparently healthy individuals who have undergone predictive genetic testing for hereditary ATTR amyloidosis have shown that Grade 1 DPD uptake occurs before symptoms or abnormalities on echo or CMR have developed, reflecting the earliest evidence of cardiac ATTR amyloid. DPD scintigraphy is not completely specific for ATTR amyloid in that positive scans occur in a small proportion of patients with (usually very advanced) cardiac AL amyloidosis and some other very rare types, but Grade 2 or 3 cardiac uptake in the presence of a consistent echo and/or CMR and in the absence of a monoclonal gammopathy is in practice sufficient to make a non-invasive diagnosis of cardiac ATTR amyloidosis.

I11

Minimal assessment of index cases: the point of view of the neurologist

Ernst Hund
University Heidelberg, Germany
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I11

The first and most important step is to consider TTR-FAP as a diagnosis upon thorough patient history and clinical examination. The further diagnostic process includes the following assessments: clinical examination for polyneuropathic and autonomic signs including temperature and pain sensitivity in the feet. In addition, nerve conduction studies are essential to document large fiber neuropathy. Further on, the patient should be checked for signs of cardiomyopathy and cardiac conduction disorders, since cardiac disease is common in TTR-FAP. Although there will be a characteristic pattern of findings with these examninations, they cannot prove the diagnosis of TTR-FAP. Further investigations include histopathology with demonstration of amyloid by Congo red staining, immunhistochemistry to show that amyloid is composed of TTR, and genetic testing to reveal the specific mutation underlying the disorder.

I12

The landscape of treatment of chronic kidney disease in hereditary ATTR amyloidosis

Luisa Lobato[1,2]
[1]Department of Nephrology and Unidade Corino de Andrade, Hospital de Santo António, Centro Hospitalar do Porto - CHP, 4099-001 Porto, Portugal; [2]Multidisciplinary Unit for Biomedical Research - UMIB, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, 4050-313 Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I12

Hereditary transthyretin amyloidosis (ATTR) is an autosomal dominant disease, caused by mutations in the *TTR* gene, the most prevalent being V30M. Although each TTR variant has a different involvement, peripheral neuropathy and cardiomyopathy are predominant. Kidney deposits are well recognized since the original description of the disease as well as a renal phenotype is also likely. Kidney involvement with proteinuria and progressive renal failure is a major cause of morbidity and mortality in ATTR amyloidosis. However, kidney impairment is not as common as in AA or AL amyloidosis. Epidemiological studies from Portugal and Sweden reveal that approximately one third of the patients display varying degrees of albuminuria and renal dysfunction. Dialysis improves the prognosis and survival, but morbidity and mortality are higher than in other populations given this treatment.
The low level of proteinuria or slight renal impairment does not suppose a so heavy glomerular and vascular deposition of amyloid as in ATTR, particularly in V30M mutation. Moreover, severity of renal amyloid deposition did not consistently parallel that of myelinated nerve fiber loss, with dissociation between kidney and neurological involvement. These are pitfalls that motive troublesome criteria for therapy in ATTR nephropathy. Twenty five years ago, liver transplantation (LT) was introduced as a treatment of ATTR, since this suppresses the production of circulating mutant TTR and theoretically stops the amyloid formation and disease progression, but uncertainties remain about its role on kidney deposits. Renal dysfunction pre LT and acute renal injury post LT are risk factors for chronic renal disease development after LT.
The approach for stage 4 or 5 kidney disease remains the combined or sequential liver-kidney transplantation in eligible patients. However, in the majority of patients, hemodialysis is the unique option even in the presence of a well-functioning liver graft. Tafamidis, a TTR stabilizing drug, was described as having a benefit effect on albuminuria and renal function. Non-steroidal anti-inflammatory drugs showed efficacy stabilizing ATTR in early forms of amyloid neuropathy. However, studies with diflunisal noted decline in eGFR even with exclusion of patients with significant renal impairment.
RNA interference (RNAi) is an endogenous cellular mechanism for controlling gene expression with application to ATTR neuropathy. The therapeutic value in kidney disease with successfully silenced intraglomerular genes in mouse models was proved, raising the possibility of a future role for renal ATTR amyloidosis. Antisense oligonucleotides are also under clinical trials; renal epithelial cells efficiently take up oligonucleotides without apparent degradation, rendering the kidney an excellent target for site-directed antisense therapy, but also a site of antisense toxicity. Other several natural products that inhibit TTR amyloid fibril formation where progressively investigated to stop neuropathy, but the specificity for renal disease was never evaluated. Until now, doubts remain about the role of new therapies in nephropathy, if there are preferential indications for a specific one and if dialysis patients should be included in a particular treatment.

I13

Do we need to demonstrate Amyloid in tissue?

Laurent Magy
Service et Laboratoire de Neurologie, Centre de Référence "Neuropathies Périphériques Rares", CHU Limoges, France
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I13

Background: Famililal amyloid polyneuropathy due to mutations in the Transthyretin gene (TTR FAP) is a constantly disabling condition leading to death from cardiac or renal failure, or cachexia linked to end-stage neuropathy with autonomic involvement. It has an autosomal dominant inheritance and according to the literature, the phenotypic presentation has a bimodal age of onset with early onset cases occurring before 50 years of age and so-called late onset cases occurring thereafter. To date, therapeutic options are proposed to people who carry a TTR mutation and have evidence of organ involvement. Additional histopathologic demonstration of amyloid deposits in a tissue sample is often considered mandatory to start treatment, although this relies more on professional agreement than on medical or scientific evidence.
Methods: We reviewed the literature (PubMed Search and personal files) on TTR FAP with an emphasis on the requirements for amyloid deposit demonstration for its diagnosis in individuals with or without previous evidence of a mutation in the TTR gene. Additionally, we looked for available evidence for amyloid deposit demonstration in TTR mutation carriers before initiating treatment. Finally, we reviewed the diagnostic tools that are available to detect any meaningful change in the peripheral (autonomic and somatic) nervous system in patients at risk for peripheral neuropathy, especially in TTR mutation carriers.
Results: The diagnosis of TTR FAP in patients with an apparently sporadic peripheral neuropathy is said to require the presence of a mutation in the TTR gene AND evidence of amyloid deposit in at least one of the following tissues: labial salivary gland, abdominal fat aspirate, gastro-intestinal tract and (sural) nerve. However, we found surprisingly low scientific evidence to support the mandatory demonstration of amyloid in any tissue in individual with peripheral neuropathy AND TTR mutation after excluding any other possible cause of neuropathy. This issue is very important in the genetic era because we are increasingly aware of "asymptomatic" TTR mutation carriers and there is still no consensus on

the adequate follow-up for these patients (in terms of timing and investigations tools).
We found a wide variety of available tools to detect somatic and autonomic peripheral neuropathy in TTR mutation carriers. Based on the available literature, some proposals may be done in order to diagnose as early as possible peripheral neuropathy in these individuals.
Conclusion: Available literature shows that the need for demonstration of amyloid deposit in any tissue in patients with peripheral neuropathy and a mutation in the TTR gene is more a dogma than an evidence-based requirement. Although search for amyloid deposits should be conducted when amyloid neuropathy is suspected, we think people who carry a TTR FAP mutation and have reasonable evidence of peripheral neuropathy should not be denied access to effective therapy, even if no demonstration of amyloid deposit can be done.

I14

Emerging CNS involvement in FAP-TTR long survival patients

Luisa Maia
Neurology Department, Hospital Santo António – CHP, 4099-001 Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I14

Background: Transthyretin-related amyloidosis (ATTR) are the most common hereditary forms of amyloidosis and Familial Amyloid Polyneuropathy (FAP) with the TTR V30M mutation, also designated as ATTR-V30M, is the most frequent form. Up until recently, despite TTR deposition in the CNS of FAP patients at a meningeal-vascular location, clinically relevant presentations were rarely recognized.
Methods: We evaluated a series of consecutive liver transplanted patients (ATTR-V30M and non-ATTR) for the presence and type of focal neurological episodes due to CNS dysfunction (FNEs). We characterized brain neuroimaging (CT scan) in patients presenting FNEs and transthyretin amyloid deposition in the brains of ATTR-V30M autopsied patients.
Results: We found that CNS clinical involvement occurs in FAP patients that underwent liver transplantation (LT). Patients exhibited positive and negative FNEs clinically similar to the recently described "amyloid spells" characteristic of Aβ related cerebral amyloid angiopathy (CAA) patients. Longer disease duration, male gender and renal dysfunction were associated with the presence of FNEs in FAP patients after LT. In post-mortem brain analysis, FAP patients exhibit prominent CAA associated to progressive TTR meningeal-vascular deposition, that progressed from the meninges and its vessels towards meningo-cortical vessels and the superficial brain parenchyma, as disease duration increased. Neuroimaging findings (lobar brain hemorrhage, localized subarachnoid hemorrhage and white matter damage) also support that TTR related CAA contributes to this new clinical phenotype.
Conclusions: Both the clinic presentation and the topography of the neuropathological abnormalities of FAP patients with this new CNS phenotype overlaps to what is known to occur in sporadic Aβ-associated CAA. Moreover, neuroimaging findings in such patients suggest that CAA may be a leading player, hinting that these two pathologies could also share devastating consequences (brain hemorrhage, localized subarachnoid hemorrhage and, eventually, cognitive decline). Given the hemorrhagic risk of these vasculopathies, our results have implications in the treatment and clinical follow-up of FAP patients. In addition, sensitive imaging methods (e.g. MRI) need to be implemented in the study of ATTR patients presenting FNEs. Present and future disease modifying therapies should consider CNS TTR deposition as a target.

I15

Biopsy experience in a FAP endemic area

Manuel Melo Pires
Neuropathology Unit, Hospital Santo António / Centro Hospitalar do Porto, Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I15

Background: Over a thousand nerve, skin and salivary gland biopsies were performed in FAP patients during the last forty years in our centre. We will try to demonstrate the different biopsy approaches over the years and confirm that biopsy of different types of tissue are useful not just in the diagnosis but also in the study of the pathogenesis of the disease.
Methods: Retrospective analysis of our database since 1973 till 2014 showed 253 nerve biopsies, 441 skin and 738 salivary gland biopsies. Some of these were combined biopsies, nerve and skin or muscle nerve and skin. Except for the salivary gland biopsies they were all performed by one of our neuropathologists.
Results: Until 1988, nerve and skin biopsies were commonly performed for diagnostic purposes, after that with genetic testing being available all tissue biopsies declined in number and were only needed in difficult diagnostic cases or when genetic testing was not available. Nerve biopsy became relevant again with the appearance of atypical clinical presentations or late onset or sporadic patients where the clinical diagnosis of FAP had not been considered. Nerve biopsy was also performed to establish the diagnosis of "de novo" amyloid neuropathy in recipients of domino liver transplants from FAP individuals, who started showing symptoms of neuropathy, and to rule out other causes of neuropathy.
Since 2005, salivary gland biopsy has become our method of choice to demonstrate amyloid and is as effective as nerve or skin biopsies.
Conclusions: During the last forty years biopsies of different tissues were used to demonstrate amyloid. Nerve biopsy is still a useful tool not just for diagnostic purposes in problematic cases but also to understand the pathogenesis of the disease. Salivary gland biopsy is now our favoured method as it is a minimally invasive procedure and amyloid is demonstrated in very early stages of the disease. Biopsy could also be a tool to monitor treatment with the new drugs available.

I16

New tools to diagnose and follow FAC patients: biomarkers

Giampaolo Merlini
Amyloidosis Research and Treatment Center, University Hospital Policlinico San Matteo, Pavia, Italy
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In the last decade, the availability of the cardiac biomarkers N terminal pro-natriuretic peptide type-B (NT-proBNP) and troponins has radically changed the approach to diagnosis, staging, and response assessment of cardiac amyloidosis. Despite similar appearance at standard imaging, the main types of amyloidosis involving the heart are characterized by different rates of progression and different outcomes. In general, patients with ATTRm amyloidosis have lower concentrations of cardiac biomarkers and usually a better prognosis than subjects suffering from AL amyloidosis. Nevertheless, the clinical manifestations of ATTRm amyloidosis are heterogeneous. Different amyloidogenic TTR mutations give rise to different cardiac phenotypes, ranging from exclusively neuropathic diseases, through mixed phenotypes, to mutation characterized by severe cardiac dysfunction. For instance, one of the mutations associated with cardiac involvement in ATTRm amyloidosis, Ile68Leu, presents with high NT-proBNP concentrations, comparable to those observed in patients with cardiac AL amyloidosis. In our series, survival of patients with Ile68Leu ATTRm is not different from that of cardiac AL patients. Thus, it is possible that NT-proBNP represents a viable marker for early diagnosis of cardiac involvement in ATTR patients with "aggressive" mutations. This marker could be considered in the screening of carriers of these mutations. Despite the lack of systematic studies of cardiac biomarkers in ATTR amyloidosis, the results of several small published series and the analysis of the Pavia patient population, indicate that NT-proBNP and cardiac troponins have prognostic value in this disease, correlating to amyloid burden and adding to known prognostic factors. The availability of several novel therapeutic options for ATTRm, require objective means for the assessment of treatment efficacy in routine clinical practice and in clinical trials. Early results from the Pavia series indicate that NT-proBNP progression portends a poor prognosis, and cardiac biomarkers will probably find their place in the evaluation of response to therapy in ATTRm amyloidosis. Cardiac biomarkers will most likely become increasingly useful in the management of patients with ATTRm amyloidosis in the near future. However, studies of biomarkers in this disease are hindered by small numbers and disease heterogeneity. Prospective studies and international collaboration are warranted to define the role of cardiac biomarkers in the diagnosis and follow-up of cardiac ATTRm amyloidosis.

I17

New therapeutic perspectives – amyloid removal

Mark Pepys
FRS, Wolfson Drug Discovery Unit & NHS National Amyloidosis Centre, Centre for Amyloidosis & Acute Phase Proteins, University College London & Royal Free Hospital, London, UK
E-mail: m.pepys@ucl.ac.uk
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Background: In systemic amyloidosis, disease is caused by extracellular accumulation of amyloid fibrils which, unlike other interstitial debris, are not cleared and which disrupt tissue structure and function. Direct removal of amyloid deposits is required to preserve and possibly restore tissue and organ function. We are targeting serum amyloid P component (SAP) for this purpose. SAP normal plasma protein is a normal plasma protein which binds to all amyloid fibrils and is thus always present in all human amyloid deposits. Administration of hexanoyl bis(D–proline) (CPHPC) swiftly depletes circulating SAP but leaves some SAP in amyloid deposits as an amyloid-specific antigen target. In human SAP transgenic mice with systemic AA amyloidosis, CPHPC treatment, to deplete human SAP from the plasma, followed by a single dose of anti-human SAP antibodies produced swift, almost complete, clearance of visceral amyloid (Bodin *et al*. *Nature*, 2010;468:93-7). In a mouse SAA transgenic systemic amyloidosis model, which uniquely includes cardiac amyloid, a second dose of anti–SAP antibody, after the first dose had eliminated massive liver and spleen deposits, significantly removed amyloid from the heart (Simons *et al*. *Proc Natl Acad Sci USA*. 2013;110:16115-20). Amyloid clearance by anti-SAP antibody required classical complement pathway activation and macrophages. Amyloid destruction was mediated by multinucleated giant cells (MGCs), formed by macrophage fusion. MGCs have abundant surface membrane ruffles, enabling the engulfment of very large complement opsonised amyloid targets which were then swiftly destroyed within phagolysosomes. Amyloid clearance was maximal by 14 days. No ill effects were detected. After licensing this new treatment in February 2009, GlaxoSmithKline (GSK) fully humanised our optimal mouse monoclonal anti-SAP antibody and prepared for the first in human clinical study that started in June 2013. We have lately reported that a single dose of humanized monoclonal anti-SAP antibody, following depletion of circulating SAP by CPHPC, substantially reduced the amyloid load, especially from the liver, in patients with systemic AL, AA and AApoAI amyloidosis, (Richards *et al*, *New Engl J Med*, July 15 2015; DOI: 10.1056/NEJMoa1504942).
Methods: GSK's phase I study of the obligate therapeutic partnership of CPHPC and anti–SAP antibody in patients with systemic amyloidosis is ongoing (http://www.clinicaltrials.gov/ct2/show/NCT01777243?term=amyloid+gsk&rank=2). The first patients with ATTR are about to be treated.
Results: Efficacy in amyloid removal requires a sufficient dose of antibody and is associated with a transient early acute phase response of CRP and SAA, transient early mild neutrophilia and then notable depletion of plasma C3 and a less marked fall in C4 and CH50 (Richards *et al*, *New Engl J Med*, July 15 2015). Amyloid removal has not been associated with detectable additional organ dysfunction. On the contrary, abnormal liver function tests improved following clearance of hepatic amyloid and the treatment has so far been generally well tolerated (Richards *et al*, *New Engl J Med*, July 15 2015).
Conclusions: Treatment with CPHPC and anti–SAP antibody promotes amyloid removal in all types of systemic amyloidosis tested so far. Efficacy in ATTR, in which hepatic amyloid is never present and the main organs involved are the nerves and heart, will be of considerable interest.
The Phase I study of CPHPC and anti-SAP antibody is funded by GlaxoSmithKline.

I18

Cardiologic Phenotypes and Natural History of FAC

Claudio Rapezzi
Department of Experimental, Diagnostic and Specialty Medicine, Alma Mater-University of Bologna, Bologna, Italy
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Fifty years ago, the focus on the Val30Met type of the disease, in which neurologic manifestations predominate, led to the widespread notion that hereditary transthyretin-related amyloidosis (ATTR) was essentially a neurologic disease. It is now clear that ATTR is extremely heterogeneous on both genotypic and phenotypic grounds. The clinical spectrum of the disease ranges from an almost exclusive neurologic involvement to strictly cardiac manifestations. This heterogeneity is linked to several factors including specific transthyretin mutations, geographic distribution and endemic vs. non-endemic aggregation type. The existence of exclusively or predominantly cardiac phenotypes makes the recognition of the disease very challenging since it can mimic other more common causes of left ventricular "hypertrophy". Assessment of such patients should include an active search for possible red flags that can indicate the correct final diagnosis. More in general the clinician must be aware that:

- Cardiac amyloidosis (CA) should be suspected in any patient with heart failure, unexplained increased LV wall thickness and non-increased end systolic left ventricular volume.
- In a patient with an initial diagnosis of hypertrophic cardiomyopathy (HCM), look for the infiltrative phenotype hidden beneath the hypertrophic one!.
- A distinctive sign of CA is the abnormal ratio between left ventricular thickness and QRS voltages rather than low QRS voltages, alone. The absence of low QRS voltages doesn't rule out a CA if the context is otherwise fitting and up to 20% of subjects with CA can have electrocardiographic evidence of left ventricular hypertrophy.
- In an elderly man with unexplained concentric left ventricular hypertrophy, especially in the absence of hypertension, always consider the possibility of wtTTR CA!.
- Amyloidotic cardiomyopathy in an elderly patient with monoclonal gammopathy is not necessarily related to AL: consider the possibility of wTTR + MGUS.
- Longitudinal LV function can be severely depressed despite a normal LVEF and the myocardial contraction fraction is often low suggesting reduced global myocardial shortening.
- Myocardial deformation is reduced in amyloidotic cardiomiopathy but the apex is generally spared.
- In CA, Gadolinium distribution at MRI is heterogeneous: subendocardial late gadolinium enhancement (LGE) is not the only diagnostic pattern and the absence of LGE does not exclude CA.
- Bilateral carpal tunnel syndrome in a man with HCM-like phenotype on echo is highly suggestive of TTR-related CA.

The natural history of ATTR is only partially known, due to the rarity of the disease and its high genotypic heterogeneity. Patients with ATTR cardiomyopathy have a much more indolent course than those with AL etiology. Progression of neurological disease and heart failure are the leading causes of death. As in AL amyloidosis, in ATTR the presence of cardiac involvement is an incremental risk factor for overall and cardiovascular events. Consequently, non-Val30Met mutations, in which cardiac involvement is more frequent and severe, appear to be associated with a particularly poor prognosis, even after successful OLT . Very little is known about the underlying cause and the incidence of sudden death, which has been reported to be associated with the occurrence of advanced atrioventricular blocks, ventricular tachycardia/fibrillation, or, probably more frequently, electromechanical dissociation.

I19

TTR amyloidosis: a scientific journey since Andrade

Maria João Saraiva[1,2]
[1]Instituto de Inovação e Investigação em Saúde (I3S), Universidade do Porto, Porto, Portugal; [2]Unidade de Neurobiologia Molecular, IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal
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Mário Corino de Andrade described in 1952 in the journal "Brain" the first form of an hereditary amyloidosis, Famlial Amyloidotic Polyneuropathy, FAP - affecting the peripheral nervous system, also known as Andrade´s

disease. In 1939 Andrade observed different patients complaining of loss of sensitivity to temperature and pain. These symptoms affected other families in the area, namely fisherman who complained of loss of sensitivity in their feet when touching the ropes of their small fishing boats. Andrade with the extraordinary intuition that all collaborators admired, realized the clinical symptoms deviated from what he had seen until then.

Andrade suspected he was in the presence of a rare hereditary disease. To understand in depth what he considered new, he asked the collaboration of experts in different fields, like genetics, pathology, confirming the genetic nature of the disease and the presence of systemic amyloid deposits, particularly in the peripheral nervous system. Since this seminal work, the explosion of molecular biology tools gave us today perspectives for therapies.

Some of the concepts and hypotheses put forward then with basic equipment and techniques available in the last century evolved tremendously giving us molecular and cellular in-depth knowledge; Andrade´s vision is still updated but need urgent clarification if we want to move towards urgent efficiency therapies of the disease. This lecture will exemplify one case where we still stand behind a black box despite access to modern know-how.

I20

Guidelines for genetic counselling in ATTR amyloidosis

Jorge Sequeiros
IBMC – i3S and ICBAS, Univ. Porto, Porto, Portugal
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Definition: Genetic counselling is "a process of communication that deals with the occurrence, or risk of occurrence, of a (possibly) genetic disorder in the family" (ASHG, 1975; EuroGentest guidelines). It involves an attempt, by appropriately trained persons, to help patients and their families to (1) understand the medical facts of the disease; (2) appreciate the contribution of heredity and risks of recurrence in relatives; (3) understand the consultands' options to deal with those risks, including all their reproductive options; (4) use this genetic information in a personally meaningful way that promotes health, minimizes psychological distress and increases personal control; (5) choose the course of action that seems appropriate to them and act in accordance with that decision; and (6) make the best possible adjustment to the disease or the genetic risks (modified by EuroGentest Unit 3 from Frazer, AJHG 1974; Biesecker & Peters, AJMG 2001; Harper, 2012).

Guidelines: Genetic counselling must always begin with the establishment or documentation of a clinical and genetic diagnosis in a proband, followed by counselling of relatives at risk. In the context of presymptomatic testing (PST), i.e., in healthy relatives at high-risk for a late-onset monogenic disease with high penetrance, as ATTR amyloidosis, a protocol of pre- and post-test genetic counselling needs to be offered; and be accompanied by psychosocial evaluation and support, their purpose being to assess motivations for testing, explore decision-making processes, test coping mechanisms, predict risk of adverse emotional reactions, prepare psychosocial support, identify values and family dynamics and reinforce social support networks. Family's experience (late vs. early-onset) is critical. PST should begin with relatives at higher risk (cascade testing). Minors should not be tested, as they might lose their autonomy and suffer discrimination. In the context of diagnostic testing, neurological symptoms must be evident, so that the genetic laboratory does not perform an inadvertent PST. In the context of prenatal diagnosis (PND) or of preimplantation genetic diagnosis (PGD), both parents must be involved in the counselling protocol, one of them must be affected or a presymptomatic carrier, with a TTR mutation previously identified in the family; in addition, for PND, there must be a clear motivation for termination of pregnancy if the foetus is a mutation carrier.

Conclusions: Availability of therapies and ongoing clinical trials may be a (false) motivation for increased adherence to PST; however, this is not relevant for treatment (will change when preventive measures or presymptomatic treatment become possible). ATTR amyloidosis, though potentially treatable, is still currently incurable (mutation is always present and heritable). Genetic counselling is always mandatory.

I21

Minimal assessment of the index case: the point of view of the cardiologist

Michel Slama
Hôpital Antoine-Béclère, Clamart, France
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**I21

TTR familial amyloidosis is a systemic disease, with a frequent cardiac involvement.

For clinical reasons – related to its prolonged latency - and historical reasons – as the first cases where diagnosed by neurologists, in young patients who died from neurological disease before cardiac complications occurred- familial cardiac amyloidosis (TTR-FAC) has been and still remains underestimated. It is known to be responsible of 40% of mid-term deaths after liver transplantation. TTR-FAC appears irreversible; it can progress after liver transplantation, and is associated with a poor prognosis. Hence the heart deserves a special attention when taking in charge patients with familial amyloidosis, especially since there are several new promising therapeutic options.

When following patients with a TTR mutation, the occurrence of a cardiac disease can be anticipated considering gene mutation (opposing the so called cardiac vs neurologic mutations), family history, and age, but as it can be observed in all types of mutations, with various frequencies, it can never be ruled out beforehand. The initial workup of those patients should take into account:

- Cardiac infiltration, responsible for restrictive cardiomyopathy, detected by clinical examination, assessment of cardiac capacity with a 6 mn walk test, ECG (microvoltage), echocardiography with typical thickened sparkling myocardium, decrease of longitudinal systolic deformation (strain), and biomarkers (NT pro BNP, troponin). Early detection of amyloid deposits can be obtained by MRI (T1 mapping) and bisphosphonate scintigraphy. Cardiac catheterization and myocardial biopsy are rarely mandatory.
- Conduction abnormalities can be responsible of syncope by atrioventricular block. A simple ECG assessment is a good start, and any abnormality (any degree of AV block, bundle branch block) should prompt electrophysiological study. Prophylactic pacemaker implantation is widely used; in our experience it is necessary in about 1/3 of patients during the course of the disease. Malignant arrhythmias (ventricular tachycardia or fibrillation) appear uncommon.
- Cardiac sympathetic and parasympathetic denervation, as assessed by cardiac variability (Holter), atropine challenge, and MIBG scintigraphic imaging, occurs very early, and has a very strong prognostic value.

The initial cardiac assessment of patients with TTR amyloidosis should not be limited to the evaluation of obvious cardiac damage, which is irreversible and can lead to cardiac transplantation in a limited number of patients. All our efforts must be directed to achieve a very early diagnosis of TTR-FAC, which should be taken into account to start the treatment as early as possible.

In the era of SiRNA therapy for this rare and deadly disease, the "minimal" cardiac assessment should be performed in specialized reference centers, devoting their efforts to research for very early and accurate diagnosis and development of new therapies, and should by no means be minimal.

I22

Heart transplantation in hereditary ATTR amyloidosis

M Slama
Hôpital Antoine-Béclère, Clamart, France
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Systemic amyloidosis related to mutation of TTR gene can be complicated with Familial Amyloid Cardiomyopathy (TTR-FAC), a severe and life threatening form of heart failure with preserved ejection fraction, with a poor prognosis. To date there is no proven effective specific treatment against TTR-FAC, and the usual treatments of chronic heart failure are

either ineffective or contra indicated, except for diuretics. Progression of the disease results in reduction of physical capacity, repeated hospitalizations, increase in diuretics dose, and ultimately in refractory heart failure.
Cardiac transplantation has been performed in TTR-FAC since 2003, and reported in 40 patients in the literature with acceptable results in highly selected patients. The results of combined heart and liver transplantation appear to be similar or even better than for isolated heart transplantation, due to a "tolerance" phenomenon, with less cardiac rejection and less graft coronary artery disease.
We performed heart transplantation for TTR-FAC in 10 pts (9 men; mean age 59 years range 49 to 70), NYHA class II to IV, isolated in 6 and combined with a liver transplantation in 4. Patients had received a pacemaker (n=3) or a defibrillator (n=3) during their preoperative course. Mutations of patients were TYR77 in 5, SER24 in 1, GLU62 in 1, MET30 in 1. In hospital mortality was 40%.
Patients can be considered as good candidates to heart transplantation for TTR-FAC if they have symptomatic heart failure persisting under optimal medical therapy, disregarding their ejection fraction, normal pulmonary resistance, no major comorbidities, an acceptable physical and psychological condition, and limited neurologic involvement (ability to engage in a post-operative rehabilitation program is mandatory).
In the future, indications of combined heart and liver transplantations will have to be weighed against isolated heart transplantation in association with specific medical treatments (Tafamidis, or SiRNA).

I23

Treatment of gastrointestinal complication in transthyretin amyloidosis. A single centre's experience

Ole B Suhr
Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden
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Background: Gastrointestinal complications have a substantial impact on ATTR amyloidosis patients' survival and quality of life.
The disturbances are generally attributed to motility disturbances caused by autonomic denervation, but the pathogenesis is poorly defined. The most common symptoms are those of early satiety, nausea and vomiting from the upper gastrointestinal (GI) tract, and constipation, constipation alternating with diarrhoea, or continuous diarrhoea from the lower. Faecal incontinence is common in later stages of the disease and has a devastating impact on the patient's quality of life.
Evaluation and treatment: Upper endoscopy and scintigraphic visualisation of gastric emptying can diagnose gastric retention often before the patients develop symptoms. For treatment, motilin agonists, such as erythromycin can be used, and though it often increases gastric emptying its symptomatic efficacy is limited. Symptomatic relief can be achieved with dopamine 2 receptor antagonists such as metoclopramide, which, however, has little impact on gastric emptying. Gastric pacing by a gastric pacemaker is effective for symptom relief in diabetes mellitus induced gasroparesis, but with only limited efficacy on gastric emptying.
Constipation is a common symptom, and osmotic active preparations (polyethylene glycol) and picosulfate are often effective. Alternating diarrhoea and constipation are often induced by small bowel contamination caused by stagnant content in the small intestine. Small intestine culture or more convenient the hydrogen breath test can disclose the condition, and antibiotics such as tetracycline and/or metronidazole are generally effective, and repeated short courses of treatment can be prescribed when needed.
The onset of more continuous diarrhoea is often related to malabsorption, especially of fat and bile acids. Various tests can diagnose the conditions, such as the ^{75}Se-homocholicacid-taurine (SeHCAT) test, which diagnose bile acid malabsorption, a condition that often accompany fat malabsorption. Bile acid sequestrates and fat restricted diet, with the help of a dietician to ensure sufficient nutritional intake should be tried. Octreotide, a somatostatin analogue has also been reported to be effective. When treatment fails, and the patient has devastating faecal incontinence, a sigmoid stoma can help the patient to gain control of his/hers bowel movements.
It is important to give the patients adequate supplementation with fat-soluble vitamins and calcium to avoid osteoporosis, and B12 vitamin supplementation may be needed.
Symptoms of adrenal insufficiency can be difficult to distinguish from GI symptoms caused by ATTR amyloidosis. Cortisol supplementation can have a dramatic effect on the patient's symptoms, including those of orthostatic hypotension.

I24

Therapeutic education programme in TTR-FAP

Marie Théaudin[1,2,3*], Cécile Cauquil[1,2], Teresa Antonini[1,4], Vincent Algalarrondo[1,5,6], Céline Labeyrie[1,2,6], Sophie Aycaguer[7], Marie Kubezyk[1,2], Colombe Lemoine[1,2], Géraldine Nonnez[1,2], Agnès Morier[1,2], Nadia Kouchi[1,2], Amandine Darras[1,2], Nathalie Coste[1,2], Mireille Clément[8], David Adams[1,2,3,6]
[1]French Reference Centre for Familial Amyloid Polyneuropathy, NNerf, Neurology Department, Hôpital Bicêtre, Assistance Publique Hôpitaux de Paris (APHP), HUPS, Le Kremlin-Bicêtre, France; [2]Hôpital Bicêtre, APHP, Neurology Department, APHP, HUPS, Villejuif, France; [3]INSERM UMR788, Université Paris Sud, Paris, France; [4]Hôpital Paul Brousse, Centre Hepato-Biliaire, APHP, HUPS, Villejuif, France; [5]Hôpital Antoine Béclère, Cardiology Department, APHP, HUPS, Clamart, France; [6]Université Paris Sud, Paris, France; [7]EDU-santé, Vanves Cedex, France; [8]Association Française contre l'Amylose, Aix en Provence Cedex 3, France
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Background: The French National Reference centre for Transthyretin-related amyloidosis (ATTR) was accredited in 2005. One of its 10 lines of action is to inform and educate patients about their disease to improve their care and reduce morbidities. We thus decided to elaborate a therapeutic patient education (TPE) programme, starting with patients' needs assessment. We will describe our needs assessment and discuss our experience in TPE for ATTR patients.
Methods: A qualitative research study was conducted with one-to-one semi-structured interviews of selected individuals. Recorded interviews were analysed to identify the skills that patients need to acquire. A TPE programme was elaborated on the basis of these findings.
Results: Analysis of the interviews showed that interviewees had a good knowledge of the disease and its symptoms but they had difficulties explaining the disease mechanism and did not have an adequate knowledge of the available treatment options, although they knew that liver transplant might halt progression of the disease. ATTR amyloidosis appeared to have a major negative impact on the patient's physical and mental well-being. Patients feared loss of autonomy and having to require assistance from their relatives and spouses. All interviewees were keen to participate in a TPE programme. Based on this needs assessment, we identified seven skills that patients need to acquire and several pedagogical goals to be achieved during the education programme. An interdisciplinary team then elaborated a complete TPE programme and its educative tools. The programme includes 2 individual sessions (initial educational diagnosis assessment and final assessment of the skills acquired during the programme) and 7 collective sessions. First TPE sessions started in October 2014. To date, 8 patients living close to Paris attended 4 collective TPE sessions. After the sessions, all patients stated they were very satisfied with the quality of the education provided, the educational tools, the timing of the sessions. They all stated the collective sessions met their expectations.
Conclusion: Elaboration of a TPE programme for ATTR amyloidosis required to obtain useful information from the patients themselves, and their relatives, concerning their perception of their disease. This needs' assessment constituted the basis for designing the first TPE programme, to our knowledge, for ATTR amyloidosis. Patients who attended the first TPE sessions were very satisfied and felt that the sessions fully met their educational expectations. Further TPE sessions are scheduled within the end of 2015 and we plan to organize TPE sessions in other French cities where ATTR amyloidosis patients are followed.

I25

Do we need to demonstrate amyloid in tissue for hereditary ATTR amyloidosis ?

Per Westermark
Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
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Amyloidosis is by definition deposition of aggregates of proteins in a characteristic β-pleated sheet fibrillar conformation. Amyloid is recognized by its histological appearance particularly after some specific stainings among which Congo red most commonly is used. A green birefringence is the hallmark of all kinds of amyloid. There is a number of emerging diagnostic techniques based on labelling with antibodies, other proteins and some other ligands that are at least to some degree specific for amyloid and may be used for *in vivo* detection of amyloid. With some exceptions, such techniques are still not sensitive enough to detect small deposits. Biopsy stained with Congo red and examined in a polarization microscope is necessary presently.

Hereditary ATTR amyloidosis constitutes a heterogeneous disease group with varying organ consequences, not only from peripheral nerves and heart. Amyloid staining properties vary and Congo red affinity and birefringence may be very weak, particularly in amyloid consisting of TTR fragments which is common (1). Therefore is amyloid sometimes difficult to recognize also in biopsies.

Given the varying clinical manifestation of ATTR amyloidosis it is my opinion that a histological proof should be obtained if possible. There may be exceptions sometimes depending on local conditions. In endemic areas with one specific *TTR* mutation, high penetrance and a relative monomorphous presentation of ATTR amyloidosis, diagnosis may sometimes be safe without a histological proof. This may be the case in some areas of Portugal. In most parts of the world, however, cases of systemic amyloidosis are usually scattered and a histological proof should be recommended.

The site of biopsy may be discussed. We perform subcutaneous adipose tissue biopsies (surgical or by punch technique) with good results. Fine needle biopsy is not recommended since with this technique very little connective tissue is obtained, which is the site of ATTR amyloid containing TTR fragments (2). In other centers, labial salivary gland biopsies are preferred. Gastrointestinal tract is another option. A substantial advantage with biopsy techniques is that the biochemical type can be determined directly.

A question to discuss is whether indirect techniques such as DPD scintigraphy, SAP scintigraphy or PET with various ligands are safe enough for a definite amyloid diagnosis. Also alternatives to Congo red are under development but not yet validated enough for clinical use.

References

1. Ihse E, Rapezzi C, Merlini G, Ando Y, Suhr OB, Ikeda S, *et al*: **Amyloid fibrils containing fragmented ATTR may be the standard fibril composition in ATTR amyloidosis.** *Amyloid* 2013, **20**:142-50.
2. Westermark P: **Amyloid diagnosis, subcutaneous adipose tissue, immunohistochemistry and mass spectrometry.** *Amyloid* 2011, **18**:175-6.

ORAL PRESENTATIONS

O1

TTR-FAP: a single-center experience in Sicily, an Italian endemic area

Anna Mazzeo[1*], Massimo Russo[2], Gianluca Di Bella[3], Fabio Minutoli[4], Claudia Stancenelli[1], Luca Gentile[1], Antonio Toscano[1], Giuseppe Vita[1]
[1]UOC Neurologia e MNM - Universita' di Messina - Messina, Department of Neurosciences, University of Messina - AOU G. Martino - Messina, 98100, Messina, Italy; [2]NEMO SUD Center for Neuromuscular Disorders-, AOU G. Martino - Messina, 98100, Messina, Italy; [3]Department of Clinical and Experimental Medicine, University of Messina, AOU G. Martino - Messina, 98100, Messina, Italy; [4]Department of Biomedical Sciences and Morphological and Functional Images, University of, AOU G. Martino - Messina, 98100, Messina, Italy
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O1

Background: Familial amyloid polyneuropathy related to transthyretin gene (TTR-FAP) is a life-threatening disease transmitted as an autosomal dominant trait. Val30Met mutation accounts for the majority of the patients with large endemic foci especially in Portugal, Sweden and Japan. However, more than one hundred other mutations have been described worldwide. A great phenotypic variability among patients with late- and early-onset has been reported.

Objective: To present a detailed report of TTR-FAP patients diagnosed in our tertiary neuromuscular center, in a 20-year period.

Methods: Clinical informations were gathered through the database of our center.

Results: The study involved 76 individuals carrying a TTR-FAP mutation. Three phenotypes were identified, each corresponding to a different TTR variant, homogeneous within and heterogeneous between each other: i) Glu89Gln mutation, characterised by 5th to 6th decade onset, neuropathy as presenting symptoms, early heart dysfunction, cardiomyopathy as major cause of mortality followed by dysautonomia and cachexia; ii) Phe64Leu mutation, marked by familiarity reported in one-half of cases, late onset, severe peripheral neuropathy, moderate dysautonomia and mild cardiomyopathy, death for wasting syndrome; iii) Thr49Ala mutation, distinguished by onset in the 5th decade, autonomic disturbances as inaugural symptoms which may remain isolated for many years, moderate polyneuropathy, cachexia as major cause of mortality followed by cardiomyopathy.

Conclusions: This survey highlighted a prevalence of 8.8/1,000,000 in Sicily Island. Good knowledge of the natural history of the disease according to different TTR mutations allow clinicians to optimise multiprofessional care for patients and to offer carriers a personalized follow-up to reveal first signs of the disease.

O2

Epidemiology of Familial Amyloid Polyneuropathy in Bulgaria

Stayko Sarafov[*], Mariana Gospodinova, Velina Guergueltcheva Velina, Andrei Kirov, Chamova Teodora, Tihomir Todorov, Alebena Todorova, Ivailo Tournev
Sofia, Bulgaria
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O2

Background: TTR FAP was diagnosed in 2008 for first time in Bulgaria. Molecular analysis is available since 2010.

Methods: Four mutations have been found in the country so far: Glu89Gln, Ser77Phe, Val30Met, Ser52Pro. Glu89Gln is the most frequent mutation affecting 48 different families. Selective genetic screening program is performed in the affected families. A total of 261 individuals belonging to affected families were examined. All individuals who took part in the screening program signed an informed consent for a voluntarily participation in the program. TTR-FAP mutations were found in 130 individuals: Glu89Gln – 107; Val30Met – 15; Ser77Phe – 5; Ser52Pro – 2; compound heterozygous carrier of two mutations: Glu89Gln and Val30Met – 1.

Results: Most of the FAP families with Glu89Gln mutation are concentrated in two districts of the South-West part of the country on the boundary with Macedonia. We accept this mutation as typical for the Balkan – Mediterranean region with possible founder effect connecting patients with Glu89Gln from neighbouring countries. Ser77Ple is presented in 5 families originating from only one village. In a historical view we do not exclude inserting of this mutation in our population century ago. Val30Met is relatively rare till now in Bulgaria. Most of the patients originate from small focus placed in the south-east part near the border with Greece.

Conclusion: Every one of the mutations presents with specific clinical phenotype as: average age of onset, disease course / survival, male / female distribution, first symptoms – polyneuropathy, cardiac, gastro-intestinal or other involvement, sporadic or familial. The clinical phenotype and the place of origin are helpful for preliminary information for the expected mutation.

O3

Unravelling the epidemiology of late-onset and asymptomatic carriers of FAP ATTR V30M in a Portuguese population

Carolina Lemos[1*], Teresa Coelho[2], Miguel Alves-Ferreira[1], Diana Santos[1], Jorge Sequeiros[1], Alda Sousa[1]
[1]IBMC and ICBAS, UnIGENe, 4150-180, Porto, Portugal; [2]Centro Hospitalar do Porto, Unidade Corino de Andrade, 4050 - 345, Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O3

Background: Familial Amyloid Polyneuropathy (FAP ATTRV30M) is an AD systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene. Although in Portugal the disease has been characterized by its early

onset (lower than 40yrs), a wider age-at-onset (AO) variability has been uncovered. The mean AO is 35.3, but more and more late-onset (higher than 50yrs) cases are being ascertained, often matched with older asymptomatic parents. Our aim now was to look into late-onset cases and aged-asymptomatic carriers in order to unravel familial aggregation of late-onset and to characterize these families regarding their epidemiology.

Methods: From the largest registry worldwide with 2754 patients (678 families), we analyzed a group of 326 late-onset cases (133 families) regarding gender and also their transmitting parent. Additionally we analyzed 222 asymptomatic carriers on regular follow-up, aged above 40 at last observation and their first-degree relatives, belonging to 122 families. We performed a descriptive analysis and used the Student's t-test for comparisons between groups.

Results: Age-at-onset was 60.03 for men and 59.25 for women (NS), as opposed to the general sample where women had a later onset (37.6) than men (33.4). Familial aggregation of late-onset cases is apparent, with some families having up to 11 late-onset cases. Out of 678 probands, ~40% had no affected parent at time of diagnosis, this figure being 86% (115/133) among late-onset probands. These parents had died with no signs of the disease mostly at old-age. No one had an affected parent with early-onset of the disease.

For asymptomatic carriers, age-at-last-observation varies between 40 and 49 for 103 subjects and was above 50 for 119 of them. Mean age-at-last-observation was 54.06 (SD: 12.2; range: 40-89) and no gender differences were found. We were able to identify 92 transmitting-parents (59 fathers, 33 mothers) with know AO. Their mean AO was 56.91 (SD: 12.8; range: 25-80) and no differences in AO were found between parent's gender. Also, we found a mean AO close to 40 years for siblings of these asymptomatic carriers (mean: 39.91; SD: 8.89; range: 24-65).

Conclusions: While most of FAP probands had one affected parent (as expected in an AD disease), a significant number has a late-onset and no affected parent at time of diagnosis. We confirmed familial aggregation of late-onset cases. We also found that for late-onset cases no gender differences are observed. This shows that some families are protected from the severe manifestations of FAP. Due to these different clinical aspects of FAP in late-onset patients it is crucial to explore mechanisms that can be related with aging and protective factors that can lead to new therapeutic strategies.

O4

The hidden story behind gender differences in familial amyloid polyneuropathy (FAP) ATTRV30M

Diana Santos[1*], Teresa Coelho[2], Miguel Alves-Ferreira[1], Jorge Sequeiros[1], Isabel Alonso[1], Manuela Grazina[3], Alda Sousa[1], Carolina Lemos[1]
[1]IBMC and ICBAS, UnIGENe, 4150, Porto, Portugal; [2]Unidade Corino de Andrade, Unidade Corino de Andrade, 4050 - 345, Porto, Potugal; [3]CNC - FMUC, LBG, 3000-354, Porto, Portugal

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O4

Background: Familial amyloid polyneuropathy (FAP ATTRV30M) is an autosomal dominant systemic amyloidosis, due to a point mutation in the transthyretin (TTR) gene (chr18q12.1). The most frequent one, V30M is associated with several clusters. Among Portuguese families, FAP shows a wide variation in in age-at-onset (AO) [19-82 yrs] and this variability is also apparent between generations. Also, significant differences in AO regarding gender are known in Portuguese series, where women were found to have a later-onset than men. Moreover, mother-son pairs showed larger anticipation (> 10 yrs) while the father-daughter pairs only showed residual anticipation. Therefore, to unravel these gender-related differences in AO, we studied three candidate-genes (AR, HSD17B1 and BGN) linked to sex-steroid hormones or X-linked as genetic modifiers of AO. We also evaluated if mitochondrial DNA (mtDNA) copy number is associated with AO.

Methods: We analysed a DNA sample of 318 Portuguese patients (106 families) corresponding to 152 males and 166 females. Additionally, asymptomatic carriers and non-carriers were also included in the study. Polymorphisms in candidate genes were genotyped by several standard techniques and mtDNA copy number was assessed using appropriate software for analysis.

Results: Our patients' sample shows a mean AO of around 39 years, but mean AO in males (37.28) is lower than in females (40.52), as already described in the literature. Moreover, we found some polymorphisms significantly associated with AO variation. For the AR gene, in the male group, three polymorphisms were associated with an early AO, while in the female group, four were associated with both an early and later AO. Regarding parental transmission in this gene, for rs5919392, we found that e affected mothers transmitted the T allele more often than expected (which is associated with an early-onset). For HSD17B1 gene, we did not find any significant results. Concerning BGN gene, in the male group no significant results were found associated with AO but, in the female group, one polymorphism was associated with a later AO. Regarding mtDNA copy number, there are significant gender differences when we compared controls and patients groups. Patients present an mtDNA copy number higher than controls. We also found significant differences in the female group when we compared late and early patients.

Conclusions: This study revealed for the first time the contribution of the AR and BGN genes as AO modifiers both in males and females. Moreover, it was important to show that mtDNA copy number is associated with FAP. Therefore, we showed that FAP expresses differently in males and females. These results are significant to improve clinical management, with important implications in genetic counselling and therapeutic strategies.

O5

Clinical, epidemiological, genetic, and electrophysiological characteristics of transthyretin familial amyloid polyneuropathies in Israel

Menachem Sadeh[1*], Avi Livneh[2], Michael Arad[3], Alexander Lossos[4]
[1]Wolfson Medical Center, Department of Neurology, 58100, Holon, Israel; [2]Sheba Medical Center, Department of Medicine F, 52621, Ramat Gan, Israel; [3]Sheba Medical Center, Institute of Cardiology, 52621, Ramat Gan, Israel; [4]Hadassah Medical Center, Department of Neurology, 91120, Jerusalem, Israel

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O5

Background: Only a few patients and families with transthyretin associated familial amyloid polyneuropathy (TTR-FAP) have been described by different authors in Israel. The objective of this study was to elucidate the natural history, clinical manifestations, electrophysiological features, ethnic origin and genetic findings of all the known patients with TTR-FAP in Israel.

Methods: We reviewed the medical records of all the patients that have been reported and those who have not yet been described. We retrospectively assessed the major clinical, laboratory and genetic findings of the patients.

Results: Seventeen patients were studied. All were Jews. Eleven were of Yemenite descent, harboring the ser77tyr mutation. Of these, seven belonged to a large 3-generation family, and each of the other four to different unrelated families. Three patients were Ashkenazi; one carried the val30met mutation, another the phe33leu mutation, and the third had two mutations on one allele: phe33Ile and gly6ser. Two patients were of Iranian origin showing val32ala mutation, and one of Tunisian origin showing the val30met mutation. Onset in most patients was in the sixth decade, presenting with sensory loss of the lower and upper limbs. About half of the patients experienced at onset pain, autonomic nervous system manifestations and demonstrated evidence of amyloid cardiomyopathy. One patient of Yemenite descent presented with amyloid cardiomyopathy without neuropathic features. Nerve conduction studies showed sensorimotor axonal neuropathy in all. Sural nerve biopsies were obtained in eight patients; two biopsies did not reveal amyloid deposit. The average course was rapid, and most patients died within 4-7 years. The cause of death was intestinal malabsorption and cardiomyopathy. Two patients of Yemenite origin underwent liver transplantation, which slowed down the disease progression.

Conclusion: TTR-FAP exists in the Israeli population and is disproportionately common among Yemenite Jews. The worldwide common mutation val30met is rare. The presence of other mutations may explain the relatively rapid course of the disease in the Israeli patients.

O6

Familial amyloidotic polyneuropathy in Crete, Greece

Minas Tzagournissakis[1*], Cleanthe Spanaki[1], Georgios Amoiridis[1], Demetrios Samonakis[2], Andreas Plaitakis[1], Panayiotis Mitsias[1]
[1]University Hospital of Herakion, Neurology, 71500, Heraklion, Greece; [2]University Hospital of Herakion, Gastroenterology, 71500, Heraklion, Greece

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O6

Background: Familial amyloidotic polyneuropathy (FAP) has been related to more than 100 transthyretin (TTR) gene mutations. The disease has been reported among different ethnic groups including some kindreds of Greek origin. Here we report the clinical and molecular data, as well as treatment outcome of all Cretan patients with FAP seen at the University Hospital of Heraklion-Crete.
Methods: Seventeen patients (8 men and 9 women), members of 6 unrelated families originating from 4 different foci on the island were studied. All had a positive family history for polyneuropathy. Extended pedigrees spanning 5 generations were constructed. All patients underwent thorough clinical and laboratory investigation including rectal and/or nerve biopsy as well as molecular analysis.
Results: The mean age of disease onset was 30 years (range: 27 to 43). All patients presented with paresthesias, temperature loss and progressive weakness at the lower extremities, urinary difficulties, diarrhea, postural dizziness and weight loss. The upper extremities were involved later during the disease progression. Neurological examination revealed loss of pain and temperature sensation in a glove and stocking distribution and distal weakness. All but two exhibited orthostatic hypotension. Four patients presented with carpal tunnel syndrome. Although cardiac arrhythmia was a common symptom to most patients, heart failure developed in 3 patients during the late phase of the disease. One patient presented with chronic kidney disease for which she was treated with hemodialysis. Electromyographic examination revealed evidence of denervation in the muscles of the lower limbs. Conduction velocities were slightly below the normal range. Rectal and/or sural nerve biopsy revealed the presence of amyloid deposit. Molecular analysis showed that all patients were heterozygotes for the TTR Met30 mutation. Eleven patients underwent orthotopic liver transplantation (OLTx) from 1993 to 2013. Eight of them showed remarkable improvement especially of their autonomic symptoms and muscle strength. They gained weight and their paresthesias also subsided. Of the operated patients, two died of post-operative complications, one of intracerebral hemorrhage and one of unrelated cause.
Conclusion: FAP that occurs on the island of Crete is due to Met30 mutation. Haplotype analysis that is in progress may help to elucidate the origin of this mutation in relation to other populations. Our results regarding the liver transplantation corroborate those of other groups suggesting that this is the most effective treatment currently available for FAP.

O7

The phenotypical expression of an European inherited TTR amyloidosis in Brazil

Márcia Waddington Cruz[1*], Débora Foguel[2], Amanda Berensztejn[3], Roberto Pedrosa[3], Priscila Ferreira da Silva[2]
[1]Federal University of Rio de Janeiro, CEPARM. Neurology, 21941913, Rio de Janeiro, Brazil; [2]Federal University of Rio de Janeiro, CEPARM. Instituto de Bioquímica Médica, 21941913, Rio de Janeiro, Brazil; [3]Federal University of Rio de Janeiro, CEPARM. Cardiology, 21941913, Rio de Janeiro, Brazil
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Background: Brazil is a country of Portuguese colonization with massive numbers of immigrants and Portuguese descendants (25 millions). At least half of all Brazilian Y chromosomes are from Portuguese origin. Nevertheless, this population suffered miscegenation with native Indians and African descendants. African descendants Brazilians have 48% of non-African genes, probably from Portuguese ascendance. In a previous work from our center a common haplotype was demonstrated in Portuguese and Brazilian patients from 22 families and the calculation of the most recent common ancestor in 13 families demonstrated that it has occurred at 26 past generations about 650 years ago hence before the time of Brazil's discovery (1500) [1]. The objective of this work is to characterize demographic and clinical aspects of Brazilian patients presenting with ATTR in light of the clear Portuguese origin of the cases in a background of miscegenation and heterogeneity of the population.
Methods: Baseline clinical and demographics aspects of Brazilian patients included into THAOS (Transthyretin Amyloidosis Outcomes Survey) patient registry were extracted from 2007 to January 2015 in a total of 148 patients (68 women and 80 men).
Results: Val30MetTTR mutation was found in 91.9% of the cases. Other mutations included Ile107Val, Val122Ile, Ala19Asp and Gly53Glu. The mean age at onset of disease was 37 years for men and 35 years for women. Mean time from onset of symptoms to diagnosis was 5.9 years (median 3.3 years). 93.9% informed a family history with more than 90% of Portuguese origin and 69% with aspects from Caucasian ethnicity. In 23% of the cases, the diagnosis in family members was based on clinical suspicion only. Amyloid deposit was found in 80% of the biopsies performed (34% salivary gland, 38% nerve and 3.7 cardiac). Misdiagnosis was noticed in 27.4% of the cases (26% of Val30Met mutations and 37.5% of non Val30 Met mutations cases) with CIDP being the most common. 25% of the patients took more than 1 year to have their correct diagnosis. From 117 symptomatic patients 79.5% presented with motor neuropathy (78% Val30 Met and 87.5% non-Val30Met); 85.5% presented with sensory neuropathy (85% Val30 Met and 87.5% non-Val30Met); 93% presented with autonomic neuropathy (93.6% Val30 Met and 87.5% non-Val30Met); 80.3% presented with gastrointestinal complaints. Unintentional weight loss was present in 50% of the cases. 80.6% of early onset cases presented with a motor neuropathy and 78.3% of late onset. Corresponding numbers for sensory neuropathy were 87% and 82.6%; and 94.6% and 87% for autonomic neuropathy. Cardiac disorders were noted in 35.9% of the cases (33.9% Val30 Met and 62.5% non-Val30Met 33.9%) (NYHA 1 or > in 3% of patients with early onset and 17.4% of late onset). ECG abnormalities were found in 56% of the cases (71% being conduction abnormalities). Left ventricular septum > 10 mm was seen in only 25% of the cases.
Conclusion: The population was mostly characterized by early onset TTR Val30Met neuropathic phenotype presentation, with several cases also featuring some degree of cardiac disease, very similar to cases from endemic regions of Portugal even after several generations from the original immigration and a very important mixed racial population.

Reference

1. Cruz MW: **Regional differences and similarities of familial amyloidotic polyneuropathy (FAP) presentation in Brazil.** *Amyloid* 2012, **19(Suppl 1)**:65-67.

O8

DISCOVERY: a study examining the prevalence of transthyretin mutations in subjects suspected of having cardiac amyloidosis

Olakunle Akinboboye[1*], Karthik Ananthasubramaniam[2], Amir Malik[3], Alberta Warner[4], Verena Karsten[5], Thibaud Damy[6], Herman A Taylor[7], Mathew S Maurer[8]
[1]Queens Heart Institute, Medicine, 11422, Rosedale, USA; [2]Henry Ford Hospital, Heart and Vascular Institute, 48202, Detroit, USA; [3]Heart Center of North Texas, Cardiology, 76104, Fort Worth, USA; [4]Los Angeles Veterans Affairs Health Care System, Echo lab and HF service, 90073, Los Angeles, USA; [5]Alnylam Pharmaceuticals, Clinical Development, 02142, Cambridge, USA; [6]APHP, Cardiology and Amyloidosis Mondor Network, 94000, Creteil, France; [7]Morehouse School of Medicine, Cardiovascular Research Institute, 30310, Atlanta, USA; [8]Columbia University Medical Center, Medicine, 10032, New Yok, USA
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O8

Background: Cardiac amyloidosis (CA) is caused by extracellular myocardial deposition of either immunoglobulin light-chain (AL) or transthyretin (ATTR) fibrils. Two forms of ATTR CA cause life-threatening cardiomyopathy: an inherited form arising from misfolding of mutated ATTR (familial amyloid cardiomyopathy [FAC]) and a sporadic form caused by wild-type ATTR (senile systemic amyloidosis [SSA]). More than half of over 100 reported ATTR mutations are associated with FAC. The most common mutation in the US is Val122Ile found in 3-4% of African Americans (AA). FAC can be difficult to recognize clinically and is likely under diagnosed. The DISCOVERY study aims to determine the prevalence of TTR mutations and FAC diagnosis in a cohort of patients (pts) with clinical features suggestive of CA.
Methods: This is a prospective, multi-center study in adults with two or more of the following eligibility criteria: heart failure signs and symptoms, intraventricular septal thickness (IVS) of >12 mm, LV diastolic dysfunction, low voltage ECG, or history of carpal tunnel disease (CTD). DNA from blood samples is used for sequencing of the TTR gene coding regions by

a central lab. Assessments in pts with TTR mutations include cardiac biomarkers, echocardiogram and optional abdominal fat pat aspiration and 6-minute walk test. Descriptive statistics will be utilized.

Results: As of May 2015, 146 pts have been enrolled. At baseline, the mean (Std) age of pts is 64 (13) yrs, 61% are men and 66% are AA. A total of 14 (10%) pts had a Val122Ile mutation and 1 pt had a novel mutation Arg103His. The Gly6Ser polymorphism was found in 8 (5%) pts. The Val122Ile cohort consisted of 40% males with a mean (StD) age of 66 (16). Heart failure signs and symptoms and IVS > 12 mm was reported in 71% and 79% of Val122Ile pts respectively. The majority of pts had NYHA class II (56%) and III (22%) heart failure, 21% had low voltage ECGs and 14% had CTD.

Conclusions: These preliminary data suggest that approximately 10% of pts with clinical and/or radiologic findings suggestive of cardiac amyloidosis have a pathogenic TTR mutation which could potentially lead to a diagnosis of FAC. Additional data on clinical features and tissue diagnosis of FAC in these pts will be presented.

O9

Wild-type transthyretin amyloidosis in female patients

Arnt V Kristen[1*], Ralf Bauer[1], Fabian aus dem Siepen[1], Christoph Kimmich[2], Katrin Hinderhofer[3], Christoph Röcken[4], Hugo A Katus[1]
[1]Department of Cardiology, University of Heidelberg, 69120, Heidelberg, Germany; [2]Department of Hematology, University of Heidelberg, 69120, Heidelberg, Germany; [3]Institute of Human Genetics, University of Heidelberg, 69120, Heidelberg, Germany; [4]Institute of Pathology, University of Kiel, 24105, Kiel, Germany

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O9

Background: Wild-type TTR amyloidosis (wt-ATTR) is a common aging phenomenon in the elderly population. It is claimed to affect exclusively males. Female gender was assumed as a protective factor. Clinical data on wt-ATTR in female gender is lacking. This single center analysis reported on gender differences of clinical variables in wt-ATTR.

Methods: Patient records of 207 consecutive patients with wt-ATTR were analyzed for clinical variables obtained during the initial assessment at Heidelberg Amyloidosis Center, including electrocardiography, echocardiography, and laboratory results. All variables were compared between male and female gender. Finally, predictors of survival (onset of first symptoms to death) were evaluated.

Results: Comparison of clinical findings between males and females affected by wt-ATTR amyloidosis are shown in table 1. Female patients with wt-ATTR did not differ from male patients regarding demographic or clinical parameters except for modified body mass index (1140±184 vs. 1029±154, $p<0.05$), glomerular filtration rate (66±23 vs. 85±31 ml/min*m^2*1.73; $p<0.05$), NYHA class (2.4±0.7 vs. 2.9±0.3; $p<0.01$) and PQ interval (211±50 ms vs. 170±26; $p<0.01$). Interestingly, both groups especially did not differ in age at onset of symptoms, but longer delay between start of symptoms and diagnosis of wt-ATTR in females was observed when compared to male patients with wt-ATTR.

In total, 6 deaths (35%) occurred in females and 45 deaths (24%) in males. No gender differences were observed regarding mean survival (females 54±35 month, males 56±107 months). By multivariate analysis independent predictors of mortality in the whole cohort were use of diuretics (HR 8.657, 95%CI 1.160-64.17; p=0.035) and hs-TnT (HR 1.009, 95%CI 1.004-1.015; p=0.001).

In total, 6 deaths (35%) occurred in females and 45 deaths (24%) in males. No gender differences were observed regarding mean survival (females 54±35 month, males 56±107 months). By multivariate analysis independent predictors of mortality in the whole cohort were use of diuretics (HR 8.657, 95%CI 1.160-64.17; p=0.035) and hs-TnT (HR 1.009, 95%CI 1.004-1.015; p=0.001).

Conclusion: Although wt-ATTR is claimed to be a disease of male gender there is a considerable number of females affected with cardiac manifestation of wt-ATTR. According to this first report on clinical characteristics of a relatively well sized cohort of females no gender-specific differences regarding clinical characteristics and median survival were observed, except for modified body mass index and PQ interval as well as higher glomerular filtration rate and NYHA class in female patients. Moreover, use of diuretics and hs-TnT appeared to be predictors of mortality.

O10

Analysis of disease progression in patients with transthyretin cardiac amyloidosis

Julian D Gilmore[1*], Mathew S Maurer[2], John Vest[3], Verena Karsten[3], Christine Powell[3], Andrew Strahs[3], Jared Gollob[3], Philip N Hawkins[1]
[1]University College London Medical School, National Amyloidosis Center, NW32PF, London, UK; [2]NewYork-Presbyterian/Columbia, Clinical Cardiovascular Research Lab for the Elderly, 10034, New York, NY, USA; [3]Alnylam Pharmacuetical, Clinical Development, 02142, Cambridge, MA, USA

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O10

Background: In transthyretin (TTR) cardiac amyloidosis, myocardial deposition of liver-derived TTR fibrils results in heart failure and death. Wild-type TTR (ATTRwt) amyloidosis is an acquired disease, whereas familial amyloidotic cardiomyopathy (FAC) is a hereditary form of the disease caused by mutations in the TTR gene resulting in the deposition of both mutant and wild-type TTR. Published data on disease progression in TTR cardiac amyloidosis is limited. To explore this further, we analyzed natural history data in ATTRwt amyloidosis patients to characterize disease progression in this population and compared this to our previously reported data in FAC.

Methods: Demographic and overall survival (OS) data from patients with ATTRwt amyloidosis (ATTRwt OS cohort) was collected retrospectively from two centers. Six-minute walk distance (6MWD) data from patients with ATTRwt amyloidosis (ATTRwt 6MWD cohort) was collected prospectively from a single center. Data from ATTRwt amyloidosis patients was compared descriptively with our previously reported results from FAC patients collected at the same centers.

Results: The ATTRwt OS cohort (N=255, median age 78 years, 47% NYHA class II, 22% NYHA class III) had a median survival (95% CI) from time of first visit of 31.6 months (23.6, 41.0). The ATTRwt 6MWD cohort (N=153, median age 77 years, 65% NYHA class II, 25% NYHA class III), had a mean baseline 6MWD (+/- SEM) of 313m (10). In these ATTRwt amyloidosis patients, the mean change from baseline in 6MWD (+/- SEM) at 6 (N=125), 12 (N=88) and 18 (N=55) months was -30m (7), -59m (13) and -89m (16) respectively. In our previously reported data in FAC patients (N=137, median age 72 yrs, 40% NYHA class II, 43% NYHA class III), median survival (95% CI) was 34.1 months (28.6, 38.5). For a subset of FAC patients in whom 6MWD data was available (N=39, median age 76 yrs, 59% NYHA class II, 38% NYHA class III), mean baseline 6MWD (+/- SEM) was 281m (20), and mean change from baseline in 6MWD (+/- SEM) was -36m (23), -106m (24) and -140m (39) at 6 (N=32), 12 (N=27) and 18 (N=16) months respectively.

Conclusion: Survival is limited in both ATTRwt amyloidosis and FAC. Patients with both ATTRwt amyloidosis and FAC demonstrate a decline in functional status over an 18 month time period.

O11

Vasculopathy in transthyretin Val30Met familial amyloid polyneuropathy

Haruki Koike*, Shohei Ikeda, Mie Takahashi, Yuichi Kawagashira, Masahiro Iijima, Masahisa Katsuno, Gen Sobue
Nagoya University Graduate School of Medicine, Neurology, 466-8550, Nagoya, Japan

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O11

Background: Transthyretin (TTR) Val30Met-associated familial amyloid polyneuropathy (FAP ATTR Val30Met) is the most common form of FAP and has become prevalent in areas other than conventional endemic foci. The clinicopathological features of FAP ATTR Val30Met are known to vary between endemic foci and non-endemic areas in Japan. Characteristic features of early-onset cases from Japanese endemic foci include the presence of sensory dissociation and marked autonomic dysfunction associated with a predominant loss of small-diameter myelinated and unmyelinated nerve fibers. These characteristics are uncommon in late-onset cases from non-endemic areas.

Methods: We examined sural nerve biopsy specimens from 42 patients with FAP ATTR Val30Met using electron microscopy, particularly focusing on the morphology of nerve microvascular endothelial cells. This study

included 5 early-onset cases from endemic foci (3 men and 2 women) and 37 late-onset cases from non-endemic areas (33 men and 4 women).
Results: Greater amyloid deposition was observed in early-onset cases than in late-onset cases, whereas reduced nerve fiber density was more conspicuous in late-onset cases than in early-onset cases. Retraction of the processes of Schwann cells associated with amyloid fibrils was observed, particularly in early-onset cases. In addition, basement and cytoplasmic membranes of Schwann cells associated with amyloid fibrils were indistinct, indicating that direct invasion of amyloid to Schwann cells had resulted in predominantly small-fiber axonal loss characteristic of early-onset cases. In late-onset cases, nerve microvascular endothelial cell morphology was frequently found to be abnormal with loss of tight junctions and fenestrations.
Conclusion: These findings suggest that, in addition to direct invasion of amyloid to Schwann cells, the disruption of the blood–nerve barriers contributes to the pathogenesis of neuropathy in FAP.

O12

Neurophysiological pitfalls in TTR-FAP Val30Met

Isabel Conceição*, Cristiana Silva, Mariana Costa, José Castro
Centro Hospitalar Lisboa Norte - Hospital de Santa Maria, Department of Neurosciences, 1649-028, Lisbon, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O12

Introduction: The neurological hallmark of TTR-FAP is a length-dependent axonal neuropathy that initially involves the unmyelinated and small myelinated nerve fibers that mediate pain and temperature sensation, causing sensory disturbances that typically start in lower limbs. Subsequent degeneration of larger myelinated fibers results in large fiber sensory deficit and muscle weakness.
The disease can be difficult to recognize due to extreme phenotypic heterogeneity and nonspecific clinical symptoms even within the same mutation.
In TTR-FAP related to Val30Met mutation, different neuropathy phenotypes have been reported mainly in patients from non endemic areas as well in late onset cases.
Case report: We described 3 TTR-FAP Val30Met early onset cases from endemic areas with a different neuropathy phenotype that can be easily misdiagnosed as a different entity. One case presented as a bilateral carpal tunnel syndrome without neuropathy; another with a demyelinating neuropathy with predominant upper limb involvement and other with a demyelinating neuropathy with conduction blocks mimicking a CIDP.
Conclusion: TTR-FAP is frequently misdiagnosed, e.g. as idiopathic polyneuropathy or chronic inflammatory demyelinating polyneuropathy, and may be greatly under diagnosed. However, early accurate diagnosis of TTR-FAP is crucial for effective disease control.

O13

Misdiagnoses of transthyretin amyloidosis: a clinical and electrodiagnostic study

Andrea Cortese[1*], Alessandro Lozza[1], Elisa Vegezzi[1], Enrico Alfonsi[1], Arrigo Moglia[1], Giampaolo Merlini[2], Laura Obici[2]
[1]C. Mondino National Neurological Institute, Department of Neurological Sciences, 27100, Pavia, Italy; [2]Amyloidosis Research and Treatment Center, Department of Molecular Medicine, Fondazione Istituto Di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo and University of Pavia, 27100, Pavia, Italy
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O13

Background: Misdiagnosis of ATTR and late diagnosis may be detrimental hampering adequate management and delaying therapy onset. Objective of the present study was to investigate in a large single-centre cohort of genetically–confirmed ATTR patients the prevalence, type and causes of misdiagnoses. Given the high frequency of cases erroneously diagnosed as having chronic inflammatory demyelinating polyneuropathy (CIDP), we investigated the electrodiagnostic (EDx) features which can help distinguish ATTR from CIDP.
Methods: Retrospective study design. Review of clinical notes and EDx studies of ATTR patients referred to Amyloid Research and Treatment Centre and C. Mondino National Neurological Institute (Pavia) between 1999 and 2013. EDx of thirty-five patients diagnosed with CIDP were used as control for comparison.
Results: Out of 150 patients with ATTR 51(32%) were initially misdiagnosed including 30(59%) CIDP and 11(22%) lumbar spinal stenosis. Eleven (22%) patients underwent spine surgery and 38(74%) were treated with immunotherapies. Patients misdiagnosed had a significant longer delay before diagnosis of 47±3.7 months vs 34±2.7 months (p= 0.01). Lack of family history and onset after 56 years were significantly associated with misdiagnosis($p<0.01$). Out of 30 patients misdiagnosed as having CIDP, 17 had original EDx available for review. Six (35%) had definite and 3(17%) possible CIDP according to EFNS criteria, while 8(47%) did not show demyelinating features. Eleven(37%) had a negative tissue biopsy and 4/5 (80%) had raised proteins in cerebrospinal fluid (CSF). We next compared EDx of 53 ATTR with EDx from 35 matched CIDP patients. Conduction slowing and prolongation of distal motor latencies were less prominent in ATTR vs CIDP while conduction blocs were almost invariably absent in ATTR. Conversely, in ATTR motor nerves were more often not excitable both at upper and lower limbs.
Conclusions: ATTR was misdiagnosed in 1/3 of cases, particularly in patients with late onset and without family history. CIDP was the most common alternative diagnosis, which was supported by EFNS EDx criteria for demyelinating neuropathy in half of them. However conduction slowing is less prominent in ATTR while severe axonal loss is the major EDx feature. DNA testing for TTR should be performed in patients with progressive axonal or mixed axonal-demyelinating peripheral neuropathy, who do not respond to immunotherapies, regardless the lack of family history and a late onset. Raised proteins in CSF and a negative biopsy do not rule out the diagnosis of ATTR.

O14

Quantitative MR-neurographic parameters can determine and specify nerve injury in amyloid related polyneuropathy

Jennifer Kollmer[1*], Ernst Hund[2], Stefan Schönland[3], Ute Hegenbart[3], Christoph Kimmich[3], Arnt Kristen[4], Martin Bendszus[1], Mirko Pham[1]
[1]University of Heidelberg, Department of Neuroradiology, 69120, Heidelberg, Germany; [2]University of Heidelberg, Department of Neurology, 69120, Heidelberg, Germany; [3]University of Heidelberg, Medical Department V (Amyloidosis Center), 69120, Heidelberg, Germany; [4]University of Heidelberg, Medical Department III (Amyloidosis Center), 69120, Heidelberg, Germany
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O14

Background: Hereditary transthyretin-familial-amyloid-polyneuropathy (TTR-FAP) usually manifests with a rapidly progressive, distally-symmetric polyneuropathy [Plante-Bordeneuve, V. and G. Said, Lancet Neurol, 2011; Hund et al, Neurology 2001]. Recently, we were able to show that nerve-injury in TTR-FAP is detectable in-vivo by applying high-resolution MR-Neurography [Kollmer et al, Brain 2015]. The aim of the current study is to further quantify nerve-lesions at thigh-level where nerve-injury has been shown to be strongest, and to determine the ability of two quantitative parameters to clearly differentiate between symptomatic TTR-FAP, asymptomatic gene-carriers and healthy volunteers.
Methods: 20 patients with confirmed mutations in the TTR-gene (13 with symptomatic TTR-FAP, 7 asymptomatic gene-carriers), and 40 age/gender-matched healthy volunteers were prospectively included and classified according to neurological and electrophysiological findings. MR-Neurography with high structural resolution was performed on a 3T-MR-scanner (Magnetom/TIM-TRIO/Siemens):1) T2-TSE-fs (TR/TE 5970/55ms, voxel-size 0.4x0.3x3.5mm^3); 2) Dual-echo-TSE-fs (TR 5210ms, TE1/TE2 12/73ms, voxel-size 0.4x0.3x3.5 mm^3).
Manual voxel-vise segmentation of the sciatic/tibial/common-peroneal nerve with subsequent fully-automatic classification as nerve-lesion-voxels was performed on each axial imaging slice (280/subject). The apparent-T2-relaxation-time (T2app) and proton-spin-density as distinct and quantifiable parameters that measure microstructural nerve-tissue-composition in-vivo [Heiland et al, Neurosci Lett. 2002] were then calculated for all nerve-lesion-voxels.
Results: One-way-ANOVA and post-hoc comparisons showed that proton-spin-density was highest in symptomatic TTR-FAP (549.97±35.78), decreased significantly in asymptomatic gene-carriers (406.09±28.22; $p=0.002$), and further decreased significantly in controls (286.56±10.04;

p<0.0001 vs. symptomatic TTR-FAP and vs. asymptomatic gene-carriers (p=0.004).
Post-hoc comparisons showed that T2app was significantly increased only in symptomatic TTR-FAP (103.92ms±6.4) vs. asymptomatic gene-carriers (79.14ms±1.8; p=0.012) and vs. controls (84.08ms±2.54; p=0.003), but not between asymptomatic gene-carriers and controls (p=0.783).
Conclusion: For the first time, we were able to prove that alterations of the evaluated quantitative markers were highly specific: Asymptomatic carrier-status and symptomatic disease were both closely associated with a strong increase of proton-spin-density, while a significant increase of the T2-relaxation-time was found only in symptomatic TTR-FAP, but not in asymptomatic carriers. These findings suggest that proton-spin-density is more sensitive for the detection of early or even subclinical nerve-lesions, while T2app may serve to specifically differentiate increasing disease severity in already symptomatic TTR-FAP.

O15

Positron Emission Tomography (PET) utilizing Pittsburgh compound B (PIB) detects amyloid heart deposits in hereditary transthyretin amyloidosis (ATTR)

Björn Pilebro[1*], Per Lindqvist[2], Sandra Gustafsson[2], Per Westermark[3], Torbjörn Sundström[4], Gunnar Antoni[5], Ole Suhr[1], Jens Sörensen[5]
[1]Umeå University, Dep of Public Health and Clinical Medicine, 90185, Umeå, Sweden; [2]Umeå University, Dep of surgical and preoperative Sciences, 901 85, Umeå, Sweden; [3]Uppsala University, Dep of Immunology, Genetics and PAtihology, 75185, Uppsala, Sweden; [4]Umeå University, Dep of Radiation Sciences, 90185, Umeå, Sweden; [5]Uppsala University, Deep of Radiology, Oncology and Radiation Sciences, 75185, Uppsala, Sweden
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Background: Severe cardiac involvement in hereditary V30M transthyretin (TTR) amyloidosis (ATTRV30M) has been linked to amyloid containing fragmented TTR, fibril type A and "early onset" mainly neurological disease to fibrils containing only full length TTR, type B. A number of different modalities to diagnose for cardiac imaging amyloid deposition in the heart have been developed, but so far, none have been proven to specifically identify cardiac amyloid deposits in ATTR. Positron emission tomography (PET) using the tracer Pittsburgh compound B (11C-PIB) has been shown to identify cardiac amyloidosis in a small pilot. The aim of this study was to evaluate the accuracy of PIB-PET identify cardiac amyloidosis in all patients with ATTR.
Method: Ten patients with ATTRV30M, five with each type of amyloid fibril composition, selected on criteria of having no or mild cardiac involvement, underwent 11C-PIB PET. The results were compared to results from 99Tc-DPD scintigraphy and echocardiography.
Results: All patients had pathological 11C-PIB uptake but the pattern of uptake differed between the two groups. Patients with type B fibrils had significantly higher 11C-PIB retentions index (RI) than those with type A fibrils. Inversely, all patients with type A and none with type B fibrils had pathological 99Tc-DPD uptake. No significant differences in echocardiographic measurements were observed.
Conclusion: 11C-PIB PET identifies presence of ATTR amyloid in all patients and strengthens the argument that the fragmentation of TTR is central in the pathogenesis of ATTR cardiomyopathy. The higher 11C-PIB RI seen in patients with type B fibrils could be explained by better microcirculation or amyloid deposition pattern in the heart tissue. This phenomenon severely limits the usefulness of the method for quantifying amyloid burden.

O16

DPD Scintigraphy for diagnosis of amyloidosis in 1191 patients– a single centre experience

David Hutt[*], Helen Mcphillips, Stephanie Mcknight, Julian Gillmore, Carol Whelan, Helen Lachmann, Ashutosh Wechalekar, Philip Hawkins
University College London, National Amyloidosis Centre, NW3 2PF, London, UK
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Background: 99mTechnetium labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) scintigraphy has been re-purposed in recent years as a sensitive non-invasive method for imaging transthyretin (ATTR) cardiac amyloid. We have routinely performed DPD scans since June 2010 at the National Amyloidosis Centre in London, and review here our findings in a large cohort of mostly British patients.
Methods: Following administration of 99mTc-DPD, patients underwent whole body planar imaging three hours after injection followed by a cardiac SPECT-CT (single photon emission computed tomography with a low-dose, non-contrast CT scan). As of December 2014 we had performed 1454 DPD scans on a total of 1191 patients, which formed the basis of our cohort for analysis.
Results: The most frequent amyloid types seen were light chain (AL) (n=277), wild-type ATTR (n=372) and hereditary ATTR (n=237). Of those AL patients with a positive scan (n=75), 24% had grade two or higher uptake compared to wild-type ATTR in which 95% of positive scans were grade two or three. A total of 22 different TTR variants were identified, most commonly Val122Ile (43%), Thr60Ala (25%) and Val30Met (16%); cardiac uptake at clinical presentation of patients with TTR Val122Ile and Thr60Ala were grade two or higher in 99% and 98% of cases respectively. Conversely, of the 16 TTR Val30Met patients with positive scans, four were grade one and the rest grade two. Interestingly, nine patients with the TTR Ser77Tyr mutation were studied, and despite a median left ventricular wall thickness of 15.5mm (range 13.5 – 20.0 mm), cardiac DPD tracer uptake was less than patients with other mutations who had an apparently similar amount of amyloid. Cardiac amyloidosis was ultimately excluded in 256 patients, in none of whom was there any cardiac uptake of DPD. Two patients with cardiac DPD uptake were found to have both AL and ATTR amyloid fibrils on cardiac biopsy.
Positive scans were obtained in three patients in whom cardiac biopsies were negative for amyloid. DPD scintigraphy was also performed on four patients who had received domino liver transplants from donors with familial amyloid polyneuropathy (one with Val30Met mutation). Cardiac uptake was seen in one patient (Phe33Val) who, eight years post transplantation, has now progressed to develop cardiac, peripheral and autonomic disease.
Extra-cardiac uptake of DPD was also noted, including soft tissue/skeletal muscle uptake in ATTR and AL patients, in some cases virtually obscuring cardiac tracer uptake. Lymph node and uptake in some cases of localized AL amyloidosis were also seen.
Conclusion: DPD scintigraphy has proved to be a remarkably sensitive tool for the diagnosis and exclusion of clinically significant ATTR cardiac amyloidosis. Extra-cardiac uptake of DPD in amyloid deposits is an interesting phenomenon which warrants further investigation.

O17

Multi-modality imaging in cardiac ATTR amyloidosis: agreement between echocardiography, MRI and DPD-scintigraphy

Ludivine Eliahou[1*], Renata Chequer[2], Phalla Ou[3], Vincent Algalarrondo[4], Teresa Antonini[5], Michel Slama[6], Dominique Le Guludec[7], François Rouzet[8]
[1]Hopital Antoine Béclère, Cardiologie, French Referent Center for Rare Diseases for FAP (Familial Amyloid Polyneuropathy) (NNERF), 92140, Clamart, France; [2]Hopital Bichat-Claude Bernard, Médecine Nucléaire, 75018, Paris, France; [3]Hopital Bichat-Claude Bernard, Radiologie, 75018, Paris, France; [4]Hopital Antoine Béclère, Cardiologie, French Referent Center for Rare Diseases for FAP (Familial Amyloid Polyneuropathy), 92140, Clamart, France; [5]Hopital Paul Brousse, Centre Hépato-Biliaire, 94800, Villejuif, France; [6]Hopital Antoine Béclère, Cardiologie,French Referent Center for Rare Diseases for FAP (Familial Amyloid Polyneuropathy), 92140, Clamart, France; [7]Hopital Bichat-Claude Bernard, Médecine Nucléaire, 75018, Paris, France; [8]Hopital Bichat-Claude Bernard, Médecine Nucléaire, 75018, Paris, France
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Background: Three main imaging techniques are commonly used to identify cardiac transthyretin (ATTR) amyloidosis: echocardiography, MRI and DPD scintigraphy. Each one provides specific diagnostic and prognostic informations but also has its specific limitations. We sought to evaluate a multimodality imaging strategy to diagnose cardiac amyloidosis in ATTR.
Methods: Seventy seven consecutive patients with multimodality imaging evaluation (echocardiography, 1.5T MRI and 99mTc-DPD scintigraphy) to diagnose cardiac amyloidosis were identified from the database of the French National Reference Center for Amyloidosis. Patients with pacemaker or severe renal failure did not undergo cardiac

MRI and were analyzed on the basis of the echocardiography and scintigraphy (n=17). Three groups were compared: patients with positive agreement to diagnose cardiac ATTR (PA-ATTR group), patients with positive agreement to exclude cardiac ATTR (PA-normal) and patients with negative agreement (NA).
Results: The mean age was 52 years [44-70]; 59% were male. Transthyretin mutations were Val30Met in 67%, other in 21%, and 12% had acquired ATTR from previous domino liver transplantation; 30 patients had a positive echocardiography, 37 positive MRI and 36 positive DPD scintigraphy. Positive imaging agreement was encountered in 50/77 patients (65%: 30 PA-ATTR and 20 PA-normal). Negative agreement was observed in 27/77 patients (35%). Compared with PA-ATTR patients, NA patients were younger (68 [64-72] years vs. 46 [41-64], had lower BNP levels (149 [94-248] pg/ml vs. 40 [25-102],) and thinner interventricular septum (17 [14-20] mm vs. 12 [10-14]), all p values <0.0001). The two main causes for negative agreement between techniques was the sole positivity of the MRI (n=10) and the sole negativity of the DPD scintigraphy(n=6). Compared with PA-ATTR patients, patients with a sole MRI positivity were younger (41 [39-44] years vs. 68 [64-72] p<0.001), more frequently women (80% vs.26% p=0,002), had thinner interventricular septum (9 [8-11] mm vs.17 [14-20], p<0.0001), had lower BNP levels (26 [24-36] vs 149 [92-249] p<0.0001) and had less diffuse late gadolinium enhancement pattern (10% vs. 66% patients; p<0.0001). As compared with PA-ATTR patients, patients with a sole negativity of the DPD scintigraphy had acquired ATTR from domino liver transplantation in all but one case (83% vs. 6% patients; p=0.02).
Conclusions: In transthyretin amyloidosis, the agreement between echocardiography, cardiac MRI and DPD scintigraphy to diagnose cardiac amyloidosis was observed in 65% of patients. Patients without agreement between these three techniques had distinct patterns of cardiac involvement: sole positivity of the MRI was encountered in patients in the early stages of ATTR; patients with acquired ATTR due to domino liver transplantation often had negative DPD scintigraphy.

O18

ATTR Amyloidosis: development of cardiac symptoms during 6 years of follow up in different ATTR-variants

Sebastiaan Klaassen[1*], Jasper Tromp[1], Bouke Hazenberg[2], Dirk-Jan Van Veldhuisen[1], Maarten Van Den Berg[1], Peter Van Der Meer[1]
[1]University Medical Center Groningen, Department of Cardiology, 9713AV, Groningen, The Netherlands; [2]University Medical Center Groningen, Department of Rheumatology and Clinical Immunology, 9713AV, Groningen, The Netherlands
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Background: At least 100 mutations in the TTR gene are known to cause ATTR amyloidosis. Limited information is available with regard to the progression of cardiac involvement in different amyloidogenic TTR mutations. This study investigates clinical characteristics and cardiac involvement of some ATTR-associated genotypes in the Netherlands.
Methods: Clinical data and information with regard to survival was collected for all ATTR patients between 1994 and 2014 in the University Medical Centre Groningen, which is the Dutch national centre of expertise. In total 114 consecutive patients carrying 10 different TTR mutations were admitted. Patients were divided into different groups based on their mutation. Only mutations present in more than 5 patients were included for further analysis. The TTR mutations studied were Val71Ala (n=9), Tyr114Cys (n=21) and Val30Met (n=31). For each mutation clinical and demographical characteristics, laboratory measurements and echocardiography data were collected at baseline and follow-up. Baseline was determined as time of diagnosis based on histological confirmation of amyloid, end of follow-up was defined as the last complete measurement available. The primary endpoint was the development of heart failure (HF) at follow-up, defined as NT-proBNP plasma levels above 125 ng/L.
Results: Patients were predominantly male (59%). Mean follow-up was 6 + 4 years and did not differ among the mutations (P=0.144). Patients with the Val71Ala and Tyr114Cys genotypes were younger at diagnosis compared to the Val30Met genotype (44 and 50 vs.55 years respectively; P=0.057). Most patients presented with neurological symptoms (79%) and most of the remaining patients presented because of familial amyloidosis (10 %). At baseline the NT-proBNP levels were raised in Val71Ala (11%), in Tyr114Cys (14%) and inVal30Met (35%). NT-proBNP levels did not differ among the mutations; median 20 (18-28), 97 (64-288) and 170 (112-365) ng/L (P= 0.096). At follow-up, NT-proBNP levels of only Val71Ala and Val30Met increased further, median increase 206 (44-221) and 194 (89-706) ng/L (P=0.043 & P<0.001). Echocardiography in patients with Val30Met showed an increase in thickness of septal wall and posterior wall, median 2 (0-4) and 3 (0-3) mm as compared to the other mutations (p=0.005 & p<0.001). The 5-year survival rates among mutations were similar (78%, 81% vs. 81%, P=0.978).
Conclusions: During follow-up, levels of NT-proBNP in both Val71Ala and Val30Met patients increased significantly, whereas the posterior wall thickness in Val30Met also increased. These results signify that despite initial presentation with neurological symptoms, cardiac involvement is already present and progressive in some of the TTR mutations.

O19

Ocular manifestations of transthyretin-related familial amyloid polyneuropathy

Antoine Rousseau[1*], Emmanuel Barreau[1], Julia Meney[1], Zoia Mincheva[2], Cécile Cauquil[2], Marie Théaudin[2], Marc Labetoulle[1], David Adams[2]
[1]French Reference Center for FAP (NNERF), Bicêtre Hospital, Ophthalmology, 94275, Le Kremlin-Bicêtre, France; [2]French Reference Center for FAP (NNERF), Bicêtre Hospital, Neurology, 94275, Le Kremlin-Bicêtre, France
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Background: Ocular manifestations of transthyretin-related familial amyloid polyneuropathy (TTR-FAP) mainly include keratoconjunctivitis sicca, secondary glaucoma and vitreous deposits. Because liver transplantation (LT) and symptomatic treatments greatly improve life expectancy of patients, ocular involvement is becoming a more frequent challenge to address. We aimed at studying the prevalence and the clinical characteristics of ocular manifestations of TTR-FAP.
Methods: This prospective monocentric observational study was conducted at the French national reference center for TTR-FAP. Genetically confirmed TTR-FAP patients had a complete neurologic and ophthalmologic evaluation. Sensorimotor polyneuropathy (SPN) was staged with the Polyneuropathy Disability (PND) score, vegetative neuropathy was staged with the Compound Autonomic Dysfunction Test (CADT). Ophthalmological examination included best corrected visual acuity (BCVA), Schirmer test, intraocular pressure (IOP), slit lamp photographs, gonioscopy, fundus examination with retinography. Medical and surgical treatments were analyzed for all patients.
Results: One hundred and three patients (60 males and 43 females), aged 26-83 years, (mean 55.8±14.1 years), were included. Mean delay between first symptoms and inclusion was 7.0±5.4 years. Patients of Portuguese origin accounted for 41% (N=42). Val30Met mutation was present in 69 patients (67%). Ocular Hypertension (OHT) and glaucoma occurred in 17 patients (16.5%), were associated with amyloid deposits in the anterior chamber in 76.5% of cases and were more frequent among Val30Met carriers (p<0.05). Amyloid vitreous deposits were present in 21 patients (20.3%). Lacrimal hyposecretion (Schirmer < 5 mm/5 minutes) was found in at least one eye in 38.9% of patients. A BCVA of 20/200 or worse in one eye was present in 12 patients (11.6%) and was caused by secondary glaucoma in 58.3% of cases. OHT/glaucoma and vitreous amyloid deposits were significantly more frequent in patients with autonomic neuropathy, long duration of disease (>5 years) and history of liver transplantation (p<0.05). Anterior chamber amyloid deposits were more frequent in patients with severe SPN (PND ≥ 2, p<0.05).
Conclusions: Ocular manifestations of TTR-FAP are common. Vision-threatening manifestations of TTR-FAP are more frequent in patients with a long duration of disease and a history of liver transplantation. Secondary glaucoma is more frequent in Val30Met patients and is the main cause of severe irreversible visual impairment. Ophthalmological testing of TTR-FAP patients should be planned on a regular basis in order to detect and treat ocular manifestations as early as possible.

O20

Phase 2 open-label extention (OLE) study of patisiran, an investigational siRNA agent for familial amyloidotic polyneuropathy (FAP)

David Adams[1*], Ole B Suhr[2], Isabel Conceicao[3], Marcia Waddington-Cruz[4], Hartmut Schmidt[5], Juan Buades[6], Josep Campistol[7], Teresa Coehlo[8]
[1]CHU Bicêtre, APHP, French Reference Centre For FAP (NNERF), 94275, LE KREMLIN-BICETRE, France; [2]Umea University, Department of Public Health and Clinical Medicine, SE-901 85, Umea, Sweden; [3]Centro Hospitalar Lisboa Norte-Hospital de Santa Maria, Department of Neurology, 1649-028, Lisbon, Portugal; [4]Hospital Universitario Clementino Fraga Filho, UFRJ, CEP21941-913, Rio de Janeiro, Brazil; [5]The University Hospital of Münster, Department of Transplantation, 48149, Münster, Germany; [6]Hospital Son Llatzer, Servicio de Medicina Interna, 7198, Palma de Mallorca, Spain; [7]Hospital Clinic Barcelona, Instituto Clinic de Nefrologia y Urologia (ICNU), 8036, Barcelona, Spain; [8]Hospital de Santo Antonio, Unidade Clinica de Paramiloidose, 4099-001, Porto, Portugal
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Background: Familial amyloidotic polyneuropathy (FAP) is a progressive and fatal, autosomal dominant disease caused by deposition of mutant and wild-type transthyretin (TTR). Patisiran is an investigational, systemically administered lipid nanoparticle (LNP) formulation of a small interfering RNA (siRNA) targeting wild-type and mutant TTR. This formulation delivers the siRNA predominantly to the liver, thereby inhibiting synthesis of TTR at the primary site of production. A recently completed multi-center, multi-dose Phase 2 trial of patisiran in FAP patients (N=29) showed >80% sustained mean knockdown of serum TTR when administered at a dose of 0.3 mg/kg every 3 weeks with a generally favorable safety profile (Suhr O, ISA 2014).
Methods: A Phase 2 open-label extension (OLE) study of patisiran in patients with FAP who participated in the aforementioned trial, was initiated in October 2013. The primary objective of the study is to evaluate the safety and tolerability of 0.3 mg/kg patisiran administered intravenously once every 3 weeks for up to 2 years. Secondary objectives include assessment of patisiran's effect on serum TTR levels, as well as evaluation every 6 months of its impact on clinical measures, including the mNIS+7 composite neurologic impairment score and quality of life (QOL).
Results: Twenty-seven patients were enrolled; median age 64 years (range: 29-77 years). Chronic dosing with patisiran has been generally well tolerated. Three patients experienced serious adverse events unrelated to study drug. Flushing and infusion-related reactions were observed in 22.2% and 18.5% of the patients, respectively; these were mild in severity, and did not result in any discontinuations. Sustained mean serum TTR lowering of approximately 80% was achieved, with further mean nadir of up to 88% between doses for approximately 16 months. Stabilization of quality of life (QOL) measures was observed. Among the 20 evaluable patients at the time of data cutoff, neuropathy impairment scores were stable through 12 months with a mean change in mNIS+7 and NIS of -2.5 and 0.4 points, respectively; this compares favorably to the 10-18 point increase in neurologic impairment scores estimated at 12 months from prior FAP studies in a patient population with similar baseline NIS.
Conclusion: Data from this Phase 2 OLE study demonstrate that 12-months of patisiran administration was well-tolerated, resulted in sustained mean serum TTR lowering, and has the potential to halt neuropathy progression. As of March 2015, dosing continues for all patients; 18-month results will be presented.

O21

Phase 2, open-label extension (OLE) study of revusiran, an investigational RNAi therapeutic for the treatment of patients with transthyretin cardiac amyloidosis

Julian D Gillmore[1*], Rodney H Falk[2], Mathew S Maurer[3], Mazen Hanna[4], Verena Karsten[5], John Vest[5], Jared Gollob[5], Philip N Hawkins[1]
[1]University College London Medical School, 1. National Amyloidosis Center, NW32PF, London, UK; [2]Brigham and Women's Hospital, 2. Amyloidosis Program, 02115, Boston, MA, USA; [3]NewYork-Presbyterian/Columbia, Clinical Cardiovascular Research Lab for the, 10034, New York, NY, USA; [4]Cleveland Clinic, 4. Heart and Vascular Institute, 44106, Cleveland, OH, USA; [5]5. Alnylam Pharmaceuticals, Clinical Development, 02142, Cambridge, MA, USA
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Background: In transthyretin (TTR) cardiac amyloidosis, myocardial deposition and accumulation of liver-derived TTR fibrils results in heart failure and death. The hereditary form of the disease is caused by mutations in the TTR gene and presents as familial amyloidotic cardiomyopathy (FAC), whereas senile systemic amyloidosis (SSA) is an acquired disease caused by wild-type TTR. There are currently no approved therapies available for ATTR cardiac amyloidosis. Revusiran is a liver-directed subcutaneously administered investigational RNA interference therapeutic comprised of a GalNAc-siRNA conjugate targeting both mutant and wild-type TTR mRNA. A Ph 2 study of revusiran in 26 patients with ATTR cardiac amyloidosis, in which revusiran was generally well tolerated and associated with > 85% sustained knockdown of serum TTR, was recently completed. A Ph 2 open label extension (OLE) of revusiran, available to all patients who participated in the Ph 2 trial, was initiated in November 2014.
Methods: The primary objective of the OLE is to evaluate the long-term safety of 500 mg revusiran administered as 5 daily doses followed by weekly dosing for 2 years. Data on adverse events, laboratory assessments and ECG is collected. Secondary and tertiary objectives include serial assessments of pharmacodynamics, clinical outcomes including 6-Minute walk test, mortality, hospitalization, cardiac magnetic resonance imaging, 99mTechnetium scan, cardiac biomarkers and patient-reported QoL.
Results: Patients who completed the Ph 2 trial include 12 patients with SSA and 14 patients with FAC (7 T60A, 5 V122I, 2 other). At the beginning of the Ph 2 study baseline data for these 26 patients included: mean age, 68 years; mean 6-minute walk distance, 408 meters; mean NT-proBNP, 3435 pg/mL; troponin I and T 0.13 and 0.045 ng/mL, respectively. The majority of patients had mild or moderate heart failure with NYHA class II (81%) and III (12%). All patients presented with intraventricular septal thickness (IVS) of > 15 mm (mean IVS 19 mm).
Conclusions: As of June 8th 2015, 25 patients have been enrolled in the OLE. Interim 6-month data on safety, PD and clinical outcomes will be presented.

O22

The ISIS-TTRRx-CS2 phase 3 study in patients with familial amyloid polyneuropathy: Baseline results of the first 100 patients for the NIS, NIS+7 and mNIS+7 using different methods of scoring: identification of consistencies and key differences

Rito Bergemann[1*], Elizabeth Ackermann[2], Brett Monia[2], Andrew Shenker[3], Peter Dyck[4]
[1]GSK, VEO Rare Diseases, Brentford, TW8 9GS, UK; [2]Isis Pharmaceuticals, CD, Carlsbad, CA 92010, USA; [3]GSK, Rare Disease Unit, King of Prussia, PA 19406, USA; [4]Mayo Clinic, Department of Neurology, Rochester, MN 55905, USA
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Background: Familial amyloid polyneuropathy (FAP) is a devastating disease with continuous progression over time leading to complete disability, bedridden status, and ultimately death within 10 to 15 years from symptom onset. In order to document disease progression, it is necessary to have valid instruments which allow assessment of the severity of the sensory, motor and autonomic neuropathy components of the disease. Over the last decades, multiple related instruments have been used and modifications developed to measure disease progression in FAP patients in clinical trial settings. These include the Neuropathy Impairment Score (NIS), originally called Neuropathy Disability Score, the Neuropathy Impairment Score for the Lower Limbs (NIS-LL), the Neuropathy Impairment Score +7 (NIS+7) and the modified NIS +7 (mNIS +7). The NIS scores were first used to quantitate neuropathic impairment in a series of therapeutic trials in chronic inflammatory demyelinating polyradiculoneuropathy and were also used as primary endpoints in clinical trials for FAP in the completed diflunisal (NIS+7) and tafamidis (NIS-LL) Phase 3 trials. Building on experience from these trials, modifications to the NIS+7 were developed (Suanprasert et al, 2014, J Neurol Sci) and are currently being applied in the ongoing ISIS-TTRRx-CS2 (mNIS+7 Isis) and patisiran (mNIS+7 ALN) Phase 3 trials. Even though the

mNIS+7 Isis and mNIS+7 ALN scores are very similar, they are not identical. The details in the assessment and scoring for the various versions of the NIS based scores are described and their scoring methods applied to baseline data collected on the first 100 patients in the ISIS-TTRRx-CS2 trial to better understand and illustrate the key differences.
Methods: The demographics and neuropathic status of the first 100 patients enrolled in the ISIS-TTRRx-CS2 trial were analysed. At baseline, all assessments needed to calculate NIS, NIS-LL, NIS+7 and mNIS+7 Isis were obtained in duplicate. All assessments required to calculate the mNIS+7 ALN were also obtained except for postural blood pressure, which accounts for < 1% of the mNIS+7 ALN score. Data are presented using descriptive statistics.
Results: The most common mutation was Val30Met followed by Thr60Ala. The mean age was 62 yrs (range 27-81) and 76% were male. The differences in the NIS based scales and impact of the changes in relative weights of the sub-scores were investigated. The results will be presented for each score and scoring method and will include the baseline data for NIS (median 41; range 3 - 106), NIS+7 (median 57; range 10 – 120) and mNIS+7 Isis (median 74; range 10 - 163).
Conclusion: As interest in developing improved therapeutics for FAP continues to grow and as more physicians are recognizing and following FAP patients in clinical practice, it is important that the differences between the various instruments used to measure disease progression are acknowledged, compared and understood.

O23

Diflunisal therapy for cardiac ATTR amyloidosis: a longitudinal, prospective, single centre study

Candida C Quarta*, Sevda Ozer, Carol J Whelan, Marianna Fontana, Dorota M Rowczenio, Janet A Gilbertson, Philip N Hawkins, Julian D Gillmore
Royal Free Hospital, Division of Medicine, University College of London, National Amyloidosis Centre, NW3 2PF, London, UK
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**O23

Background: The transthyretin (TTR) stabilizer diflunisal has been shown to reduce the rate of progression of neurological manifestations in patients with hereditary ATTR amyloidosis. However, data on the effect of diflunisal on cardiac structure and function in amyloidotic hearts are lacking. We report the echocardiographic profiles and cardiac biomarkers of patients with either mutant (M) or wild-type (WT) TTR-related cardiac amyloidosis (ATTR-CA) who received treatment with diflunisal compared to matched untreated patients.
Methods: We included in the analysis patients with clearly defined ATTR-CA, who received diflunisal for at least 24 months. For comparison, we included a group of patients with similar age, left ventricular (LV) wall thickness and renal function who were not treated with diflunisal. Patients with coexistent rhythm abnormalities (e.g. atrial fibrillation) or pacemaker implantation were excluded. All subjects underwent a standardized comprehensive protocol of evaluations and follow-up.
Results: We identified 18 patients aged 70 [67-73] who received diflunisal for at least 24 months (9 WT; 9 M: 7 T60A, 1 S77Y, 1 G47R). For comparison we included 17 untreated patients, aged 70 [68-75] (p=0.43) (14 WT; 3 M: 2 T60A, 1 V122I). At baseline, treated and untreated patients did not show significant differences in terms of LV wall thickness (16.6±2 vs. 16.9±2 mm, p=0.45), LV ejection fraction (53±9 vs. 48±10 %, p=0.1), and global longitudinal strain (GLS, -11.4±4 vs. -10.9±4%, p=0.74). NT-proBNP (log transformed) was 5.9±0.9 and 6.2±0.7ng/L in the treated and untreated group respectively (p=0.41). Estimated glomerular filtration rate (eGFR) was mildly reduced in both treated and untreated patients (69±2 vs. 63±20 ml/min, p=0.3).
Over 42 [34-64] months, LV wall thickness remained stable and comparable in both groups (p=0.76). LV ejection fraction worsened within the untreated group (p=0.03), but remained stable in the treated one (p=0.2), although there was no significant difference in rate of decline between the groups (p=0.24). GLS worsened in the untreated group (from -10.9±4 to -8.7±3 %, p=0.03) but not in the treated one (from -11.4±4 to -11.2±3 %, p=0.88; generalized estimating equation p=0.02). NT-proBNP increased in both the untreated group (from 6.2±0.7 to 6.9±0.6 ng/L, p<0.001) and (less) in the treated one (from 5.9±0.9 to 6.2±0.7 ng/L, p=0.02; generalized estimating equation p<0.01), in the absence of a significant decrease of eGFR over time, both between groups (generalized estimating equation p=0.29) and within each group (from 69±17 to 65±20 ml/min in the treated group, p=0.19; from 63±20 to 55±22 ml/min in the untreated group, p=0.07).
Conclusion: The use of sensitive markers of change in cardiac function, such as GLS and NT-proBNP, suggests that diflunisal may slow or halt disease progression in mutant and wild-type ATTR-CA. These findings encourage further systematic study of diflunisal in ATTR-CA.

O24

Diflunisal in late-onset FAP patients with moderate to severe neuropathy

Laura Obici[1*], Andrea Cortese[2], Stefano Perlini[3], Alessandro Lozza[4], Simona Casarini[1], Enrico Alfonsi[4], Giampaolo Merlini[1]
[1]Fondazione IRCCS Policlinico San Matteo, Amyloidosis Research and Treatment Center, 27100, Pavia, Italy; [2]Fondazione IRCCS Istituto Neurologico Mondino, General Neurology, 27100, Pavia, Italy; [3]Fondazione IRCCS Policlinico San Matteo, Clinica Medica II, 27100, Pavia, Italy; [4]Fondazione IRCCS Istituto Neurologico Mondino, Neurophysiopathology Unit, 27100, Pavia, Italy
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Background: TTR stabilizers have proved effective in slowing neurological progression in FAP. However, wider experience outside trials is required to further establish their safety profile and clinical benefit in the general FAP population.
Objective: We evaluated the safety and efficacy of diflunisal (250 mg BID) in late-onset FAP patients with moderate to severe neuropathy and cardiomyopathy treated for at least 24 months.
Methods: Evaluations included Kumamoto score, polyneuropathy disability score (PND), mBMI, echocardiography and cardiac biomarkers. Adverse events were monitored every three months. Response was evaluated every 12 months.
Results: 24 patients (20 males) affected by FAP associated with 7 different mutations were treated for a median of 24 months (range 12-60). Median age at baseline was 69 years (range 57-82), disease duration 43 months (range 17-90), PND score IIIA (range I-IV), Kumamoto score 25 (2-39), BMI 890 (range 604-1458). 21 patients presented with heart involvement. Median NT-proBNP was 728 pg/ml (range 141-5965), cTnI 0.04 ng/ml (range 0.029-0.65), mLVW 14.2 mm (range 12.5-17.5).
PND increased by 1 point from baseline in 8/18 patients. mBMI remained stable during treatment. Mean change in Kumamoto score was 2.9/year (95% CI -0.3 to 4.8). Cardiac progression occurred only in 2/21 patients. One patient discontinued due to renal failure and three presented with a mild increase in serum creatinine. One patient discontinued after 3 years of treatment due to asymptomatic TnI increase that improved following discontinuation. No GI events were recorded.
Conclusion: Our results are consistent with the reported beneficial effect of this drug on neurological progression and suggest a favourable impact also on cardiac disease. Potential renal and cardiac toxicity deserves close monitoring.

O25

Familial Amyloid Polyneuropathy treatment with Tafamidis – evaluation of one- and two-year treatment in Porto, Portugal

Teresa Coelho*, Ana Martins da Silva, Cristina Alves, Márcio Cardoso, Cecília Monteiro, Isabel Fonseca, Carla Rodrigues, Vanessa Costa
Centro Hospitalar do Porto, Corino de Andrade Unit, 4099-001, Porto, Portugal
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Introduction: Transthyretin (TTR) related Familial Amyloid Polyneuropathy presents as a severe sensory, motor and autonomic neuropathy. Tafamidis, an oral drug that stabilizes TTR preventing amyloid deposition, was recently introduced in Europe to delay neuropathy progression in ambulatory patients.
Objectives: To present Tafamidis efficacy and safety data after 12 and 24M treatment in patients from Porto, Portugal.
Methods: Patients were evaluated at baseline, 6, 12 and 24M. Adverse events and body mass index were registered. Renal, thyroid, and liver functions were screened. Neuropathy impairment score (NIS), the Norfolk

Quality of life (QoL) – diabetic neuropathy total score (Norfolk), this last only at baseline, 12 and 24M. Patients were classified as responders (NIS change across 12 and 24M<2) or non-responders (if greater).
Paired samples t test and ANOVA with repeated measures were used.
Results: 163 patients (92 males), with a mean age of 41.04 ± 11.68 years [26-80] and a mean duration of disease of 29.66 ± 17.48 months [4-90], completed a 12M evaluation. Body mass index remained stable throughout these 12M (3.13 vs. 3.14, p<0.008).
Mean NIS score decreased from baseline to 12M (2.35 vs. 2.34, p<0.694, ns) and Norfolk score improved between baseline and 12M (3.03 vs. 2.74, p<0.000).
Responders (n=112, 68,7%) showed a significant NIS-score decrease between baseline and 12M (2.24 vs. 2.05, p<0.000). Non-responders showed a significant increase across one year (2.56 vs. 2.88, p<0.000). Nonetheless, even in this group there was a Norfolk decreased in the same period (3.27 vs. 3.06, p<0.020).
The group that completed a 24M evaluation consisted of 104 patients (56 males), with a mean age of 40.04 ± 10.14 years [26-76] and a mean duration of disease of 32.03 ± 17.97 months [4-77]. Once again, body mass index remained stable throughout 24M (3.12 vs. 3.13, p<0.414, ns).
Mean NIS score increased from baseline to 24M (2.35 vs. 2.45, p<0.079, ns) and Norfolk score changed between baseline and 24M (3.10 vs. 2.85, p<0.001).
Responders (n=60, 57.7%) presented a significant NIS score decrease between baseline and 24M (2.13 vs. 1.97, p<0.002), while non-responders showed a significant increase across two years (2.63 vs. 3.04, p<0.000). On the other hand, non-responders' Norfolk decreased in the same timespan (3.22 vs. 3.06, p<0.029).
No safety problems were detected including, renal, thyroid and liver functions.
Conclusion: Tafamidis stabilized 69% of patients treated for one year and 57% of patients treated for two years. Even patients classified as non-responders according to NIS score showed a good response both on QoL and BMI. No major safety issues were detected.

O26

Quality of life in ATTR amyloidosis

Thirusha Lane*, Alica Bangova, Marianna Fontana, David F Hutt, Svetla G Strehina, Carol J Whelan, Philip N Hawkins, Julian D Gillmore
University College London, National Amyloidosis Centre, NW3 2PF, London, UK

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Background: Quality of life (QoL) is of paramount importance in chronic diseases. There is anecdotal evidence on the poor QoL of patients with ATTR amyloidosis, but few or no prospective data. In an era of development of disease-modifying drugs, it has become extremely important to quantify their effects on QoL.
Methods: As part of a protocolised model of care involving comprehensive annual clinical evaluation (including DPD scintigraphy, echocardiography, ECG, neurological testing, cardiac magnetic resonance imaging (CMR), 6-minute walk test and blood tests), patients completed the KCCQ and SF-36 questionnaires. The KCCQ quantifies physical function (PF), symptoms, social function (SF), and QoL in cardiomyopathy. The SF-36 is a general health survey which measures functional health and well-being. Individual domains in both questionnaires are scored out of a maximum of 100, with scores closer to 100 representing lower disease burden.
Results: Over a 2½ year period, 179 KCCQs were completed by 137 patients – 74 wild-type (WT), 22 V122I, 21 T60A, 4 V30M and 10 with various other mutations. 5 patients were asymptomatic V30M carriers and 1 was a domino transplant recipient, also asymptomatic. Patients with a phenotype of cardiac-isolated disease (WT and V122I) showed significant limitation of PF (scores out of 100, 54 and 46 respectively) and SF (58 and 32), greater symptom burden (64 and 50) and poorer overall QoL (59 and 41). 38 patients repeated the questionnaire over a one or two year period, WT patients making up 25 of the 38. 60% of WT patients showed significant worsening in PF, SF and QoL over the 1-2 year follow-up period. Over the same time period, 167 SF-36 health surveys were completed by 132 patients, including 33 who repeated the assessment at least annually. Impairment of PF and SF and general health (GH) in the cardiac-isolated cohort were comparable with that indicated by the KCCQ, with the V122I patients scoring worse than the WT group, and with similar deterioration over the follow-up period. However, the SF-36 revealed poor scores in nearly all domains in patients with a neuropathy-dominant phenotype, in some domains comparable with that of the V122I group - PF scores: WT 39, V122I 34, neuropathy-dominant 34; SF scores: 65, 53, 55; Bodily pain: 61, 49, 51; GH: 45, 35, 37; Vitality: 43, 41, 44 respectively. Grade of DPD uptake, extracellular volume on CMR, and 6-minute walk test distance all reflected physical disease burden as measured by both questionnaires.
Conclusion: Patients with ATTR amyloidosis have significant impairment over several areas of health and holistic well-being, resulting in generally poor QoL. Those with the V122I variant appeared to show the greatest disease burden in all domains, but patients with neuropathic phenotypes also exhibited severe limitation. QoL deteriorated markedly over 1-2 years follow-up. The effect of the various new treatments in development is eagerly awaited.

O27

Psychopathological dimensions in familial amyloid polyneuropathy patients

Alice Lopes[1*], Alexandra Sousa[1], Isabel Fonseca[1], Margarida Branco[1], Carla Rodrigues[1], Paula Freitas[2], Teresa Coelho[1]
[1]Centro Hospitalar do Porto, Corino de Andrade Unit, 4099-001, Porto, Portugal; [2]Universidade do Porto, Instituto de Ciências Biomédicas Abel Salazar, 4050-313, Porto, Portugal

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Introduction: There are very few studies about psychopathology in familial amyloid polyneuropathy patients or in asymptomatic carriers. In our clinical experience in a psychiatric and psychological consultation, we mostly see patients suffering from depression and anxiety symptoms and emotional distress related to some specific, emotionally charged moments caused by the disease.
We wanted to evaluate psychopathological dimensions in the population that attends external consultation at Corino de Andrade Unit.
Methods: The sample was composed by 211 subjects (in 110, the disease had already begun, 82 were asymptomatic carriers, and 19 had no established diagnosis); 84 were men and 127 were female. Mean ages: carriers 33.9±9.8yr, patients 37.8±8.1yr, and for subjects that had no established diagnosis, 40.9±14.0 yr. Most subjects were married or lived with a partner (67.1%) and most of them were still working; 33% were retired from work or on a sick leave.
A sociodemographic questionnaire and The Brief Symptom Inventory – BSI(Derogatis,1982, Canavarro,1999, 2007) were applied to these patients.
BSI is an inventory that evaluates 9 psychopathological dimensions (somatization, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism) and includes a global symptom index (GSI).
Statistical analysis was applied (descriptive analysis, Mann-Whitney and Wilcoxon, Spearman tests).
Results: Considering Global Symptom Index (GSI), 32.7 % of total subjects were above media for general population. When sub groups were evaluated, 25.6% of symptomatic carriers,26.3 % of subjects without established diagnosis and 39.1% of patients were above media.
All dimensions of BSI were significantly higher in the group of patients when compared with that of the carriers, with the exception of obsession-compulsion, phobic anxiety and interpersonal sensitivity. The global symptom index was significantly higher in patients (p=0.001).
When we considered differences between gender, women who were asymptomatic carriers had statistically significant more phobic anxiety (p=0.01) and almost significant interpersonal sensitivity, anxiety and depression.
In the group of patients, almost all dimensions scored significantly higher for women, with the exception of somatization (p=0.065).
In the group of patients, all dimensions and GSI (rho=0.33, p=0.002) had positive correlations with years of disease, except for interpersonal sensitivity.

In patients, only the hostility dimension had positive correlation (rho=-0.234, p=0.031).
Conclusions: A high number of FAP patients have psychopathological symptoms and also asymptomatic carriers, have scores above those for general population, in a significant number.
The group of patients are at higher risk for most of the psychopathological dimensions.
Sick women are more vulnerable to psychological distress, and as time goes by, patients may have more psychic problems.

O28

Transthyretin familial amyloid polyneuropathy impact on health-related quality of life

Mónica Inês[1*], Teresa Coelho[2], Isabel Conceição[3], Lara Ferreira[4], Mamede de Carvalho[1], João Costa[1]
[1]Instituto de Medicina Molecular - Service : Unidade de Fisiologia Clínica e Translacional, 1649-028 Lisboa – Portugal; [2]Centro Hospitalar do Porto - Service : Unidade Corino de Andrade, 4000-436 Porto – Portugal; [3]Centro Hospitalar Lisboa Norte - Service : Serviço de Neurologia, 1649-035 Lisboa – Portugal; [4]Universidade do Algarve - Service : ESGHT, 8005-139 Faro – Portugal
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Background: Transthyretin Familial Amyloid Polyneuropathy (TTR-FAP) is a rare, progressive, debilitating and life-threatening neurodegenerative disease. The purpose of this study was to assess the health-related quality of life (HRQoL) impairment of TTR-FAP disease versus Portuguese general population and to identify individual patient characteristics - such as disease stage – that affects their HRQoL. Literature on TTR-FAP patients HRQoL is scarce at worldwide level and no evidence for Portugal has been published.
Methods: HRQoL was measured using the validated EuroQoL five dimensions three levels (EQ5D-3L) questionnaire being the index score (utility) calculated trough the Portuguese scoring algorithm. The Portuguese general population reference set (n = 1500) was pooled with TTR-FAP patients specific data extracted from Transthyretin Amyloidosis Outcomes Survey (THAOS) registry. Ordinary Least Squares regression for utility was set to test if being asymptomatic carrier caused HRQoL impairment, conditional in other individual characteristics. Generalized linear models (GLM) were specified for disutility in order to disentangle individual patient characteristics that affect quality of life and quantify the impact. Demographic variables include gender and age. Clinical variables include disease onset (early/late), polyneuropathy disability (PND) score, liver transplant and pharmacologic treatment. Akaike information criteria were used to select the most adequate statistical model.
Results: In a scale from -0.50 to 1.00 the average utility score was 0.76 (0.25) for general population, 0.823 (0.24) for TTR-FAP asymptomatic carriers (n=525) and 0.50 (0.37) for symptomatic TTR-FAP patients (n = 566). Ordinary Least Squares regression indicated no significant statistical effect on utility for being a TTR-FAP asymptomatic carrier versus general population (p-value 0.54). The GLM was used to detect a significant statistical effect for gender, age and being symptomatic TTR-FAP patient versus general population. Average women aged 44 years and symptomatic TTR-FAP patient, has average 40% impairment on utility versus women aged 44 years from general population. Within TTR-FAP population, individual patient characteristics such as gender, age, disease onset (early/late), polyneuropathy disability (PND) score, liver transplant and pharmacologic treatment were tested for significant statistical effect (p-value <0.005).
Conclusion: The preference-based utility measures used in this study adequately quantify the large impact of TTR-FAP disease on patient's health-related quality of life and allow discriminating across different TTR-FAP clinical stages, interventions and demographic characteristics. Assuming that these values represent the patients' preferences and the utility associated with their health state, the results presented in this study may be used in future health technologies cost-utility studies.

POSTER PRESENTATIONS

P1

Novel conformation-specific monoclonal antibodies against amyloidogenic forms of transthyretin

Jeffrey N Higaki[1*], Avi Chakrabartty[2], Natalie J Galant[3], Bradley Hammerson[1], Ronald Torres[1], Joshua Salmans[1], Robin Barbour[1], Gene G Kinney[1]
[1]Prothena Biosciences Inc, N/A, 94080, South San Francisco, USA; [2]University of Toronto, Department of Medical Biophysics, M5G 2C1, Toronto, Canada; [3]University of Toronto, Princess Margaret Cancer Centre, University Health Network, Department of Medical Biophysics, M5G 2C1, Toronto, Canada
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Background: Amyloidoses are a progressive, systemic disease caused by the accumulation in tissues of misfolded proteins that induces multiorgan dysfunction. The most common hereditary form is transthyretin amyloidosis (ATTR), caused by the accumulation of transthyretin protein (TTR). There are no approved pharmacologic therapies for ATTR in the United States, and liver transplantation is the only disease-modifying treatment. TTR, a redundant thyroxin transport protein, comprises four single-chain monomers assembled into a tetrameric complex in its native state. During the amyloidogenic process, the tetramer dissociates into monomeric subunits that then undergo conformational change, making them more prone to aggregation and fibril formation. Comparison of the crystal structure of tetrameric TTR and the monomeric TTR identified a region that is inaccessible in the tetramer but exposed upon monomer dissociation. By targeting this site with a monoclonal antibody (mAb), it might be possible to prevent TTR monomers from assembling into fibrils without influencing the function of the native tetramer. The objective of this study was to produce mAbs targeting this exposed epitope of monomeric TTR and to (a) demonstrate conformational specificity toward misfolded versus native forms of TTR and (b) determine whether they are able to recognize TTR deposits in diseased tissue.
Methods: Mice were immunized with a multiple antigenic peptide comprising the target sequence identified in the structural analysis of TTR. Clones were screened for reactivity against misfolded TTR fibrils and counter-screened against native tetrameric TTR. Selected mAbs were characterized by sandwich ELISA, SPR, and Western blot. Immunohistochemistry was performed in combination with Congo red and thioflavin-T staining to demonstrate specificity to TTR-amyloid in ATTR patient-derived tissue sections.
Results: Four mAbs were identified that bind to the target epitope on monomeric and nonnative conformations of TTR. These mAbs bound nonnative forms of TTR (KD values 7.7-18.6 nM) but, importantly, did not recognize native tetrameric TTR. These mAbs also recognized TTR deposits in a variety of ATTR heart tissues. They did not recognize control heart tissue (normal or AL amyloidosis) or the native tetrameric TTR present in human liver tissue.
Conclusions: Conformation-specific mAbs immunoreact with an amyloidogenic epitope of TTR but not with native tetrameric TTR. These mAbs specifically recognize TTR deposits in ATTR heart tissue, not in control tissue (normal and AL amyloidosis). These novel mAbs may be useful in preventing deposition and/or enhancing clearance of TTR amyloid in ATTR patients.

P2

Diflunisal compassive use in transthyretin familial amyloidotic polyneuropathy (TTR-FAP): report of the first Spanish experience

Sebastián E Azorín Contesse[1*], Christopher Cabib[2], Josep M Campistol[1]
[1]Hospital Clínic de Barcelona, Amyloidosis and Monoclonal Gammopathies' Unit (UDAM); Nephrology and Transplant Unit (SNiTR), 08036, Barcelona, Spain; [2]Hospital Clínic de Barcelona, Electromyography, Motor Control and Neuropathic Pain Unit, Neurology Service, 08036, Barcelona, Spain
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Background: Diflunisal is a well known FDA-registered commonly used NSAID therapy in the USA since the 1970's. In Europe, the drug has been seldom authorised on a national basis with the next safety update report scheduled for 2025 (http://www.ema.europa.eu/ema/). Spain is one such

country where commercial use has not been authorised, likely because of concerns on liver hypersensitivity and availability of other NSAIDs. Interestingly, recent advances have shown a potential beneficial effect in transthyretin (TTR) hereditary amyloidosis, as evidenced by encouraging data from the diflunisal trial consortium (Berk et al. JAMA. 2013;310 (24):2658-2667) where quality of life, neuropathy impairment scores and nutritional status showed significant, though modest, better results in patients randomised to receive diflunisal instead of placebo.

Methods: We aimed at describing the first off-label (compassive) use of diflunisal in a small cohort of 10 patients affected by variable degrees of TTR-FAP in our centre. A protocol for off-label use Diflunisal was introduced and accepted early in 2014 by the amyloidosis and monoclonal gammopathies' unit (UDAM) at Hospital Clínic de Barcelona. Inclusion criteriae consisted of any symptomatic hereditary TTR-amyloidosis patient with progression of FAP either (i) unfit or unwilling to receive either liver transplantation (LT)/Vyndaqel® as per on-label indication or to enter an ongoing clinical trial (e.g. RNA silencing), (ii) already under on-label treatments (Vyndaqel®, liver transplantation (LT)) or (iii) any Domino-LT (DLT) recipient with de novo signs/symptoms of polyneuropathy with biopsy proven culprit ATTR deposits. Exclusion criteriae consisted of a known previous adverse reaction to Diflunisal, estimated GFR <60mL/min/1.73m2, concommittant lithium therapy, renin-angiotensin-aldosterone system antagonists, being already under investigational drug or tafamidis, Karnofsky score <40 and unwillingness to comply to follow-up. Basal demographic and clinical characteristics were collected. Follow-up was performed every 2 months with particular attention to disease progression "red flag" signs (polyneuropathy, dysautonomy, ECG) and incidence of adverse events.

Results: A total of 10 patients were included. After a median follow-up of 8 months, diflunisal showed overall improvement of neuropathic pain and quality of life as well as stabilisation of disease stage. Mean initial eGFR was 83 and did not change significantly. Three cases presented transient acute renal failure that recovered once diflunisal dose was lowered, without a detrimental effect on TTR-FAP.

Conclusion: This is the first Spanish report of diflunisal off-label use in the setting of hereditary TTR-FAP. Diflunisal seems a relatively safe and effective option for patients with TTR-FAP in progression and who are not candidates for other therapies. Particular attention must be paid to renal function as dose adaptation may be warranted.

P3

The role of complement in ATTR amyloidosis: a new therapeutic avenue?

Elena Panayiotou*, Eleni Fella, Rebecca Papacharalambous, Stavros Malas, Theodore Kyriakides
Cyprus Insitute of Neurology and Genetics, Non-profit Private Academic, Medical Center, 2370, Nicosia, Cyprus
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Background: Familial Amyloidotic Polyneuropathy Type I is a lethal autosomal dominant sensorimotor and autonomic neuropathy due to deposition of amyloid fibrils composed of aberrant transthyretin (TTR) protein (ATTR neuropathy). A substitution of valine for methionine at position 30 of the protein is the commonest mutation. ATTRMet30 neuropathy exhibits a great degree of variability, both in the age of onset as well as penetrance among different populations. The penetrance in Cyprus is 28% compared to 2% and 80% in north Sweden and Portugal respectively. Genetic and epigenetic factors have been implicated and although we have previously demonstrated a correlation of complement C1q polymorphisms with age of onset among the Cypriot population, the exact mechanisms remain undetermined. The complement cascade, as a whole, has long been investigated for its association with inflammation and macromolecule aggregate clean-up. In the mouse model of Alzheimer disease, C1q has been shown to modulate beta-amyloid induced complement activation and neuronal loss. C1q has also been shown to be neuroprotective against toxic concentrations of serum amyloid P and to modulate phagocytosis of soluble pre-amyloid aggregates. Thus C1q appears to be strong candidate for being a modifier in the phenotype of ATTRMet30 neuropathy.

Methods: A transgenic mouse model of ATTRMet30 was cross bred with a C1q knockout strain in order to produce a complement deficient ATTRMet30 strain. In addition, the C5a receptor inhibitor PMX53 was administered to the original ATTRMet30 mouse model. Conventional and real-time PCR were carried out to characterize all mice, Thioflavin S, immunocytochemistry and immunoblotting were utilized to assess amyloid and a number of molecular markers of apoptosis, oxidative stress and endoplasmic reticulum stress.

Results: Amyloid deposition was increased by over 30% by C1q ablation and by over 600% by PMX53 administration. A parallel increase was also recorded in apoptotic and pathogenic markers such as MMP9, BiP (GRP78), Fas and Caspase-3.

Conclusion: Whereas the exact role of complement in FAP has not yet been fully elucidated, it is likely that localized activation of certain complement components may contribute to the successful removal of amyloid. Complement manipulation can perhaps be potentially exploited therapeutically as a generic therapy in amyloidosis.

P4

Positive real-world effectiveness of tafamidis for delaying disease progression in transthyretin familial amyloid polyneuropathy

Michelle Stewart*, Denis Keohane, Sarah Short, Jose Alvir, Moh-Lim Ong, Rajiv Mundayat
Pfizer Inc., New York, NY, USA
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Background: Tafamidis (Vyndaqel) was approved by the EMA in 2011 and is emerging as the standard of care for transthyretin familial amyloid polyneuropathy (TTR-FAP) in clinical settings. Efficacy was demonstrated in the clinical trials, yet little is known about its real-world effectiveness. A global disease registry, the Transthyretin Amyloidosis Outcomes Survey (THAOS), collects data on both treated and untreated patients from real-world settings. Ethics committee approval was obtained prior to patient enrolment.

Objective: To demonstrate the real-world effectiveness of tafamidis.

Methods: THAOS registry data were used to match 258 treated patients to untreated controls in a 1:4 non-randomized retrospective cohort study. Genetic mutation, birth region, and propensity scores derived from clinical status variables were used in matching. Descriptive statistics were calculated. Treatment effects were tested by repeated measures analyses with appropriate covariates (age, gender, disease duration, propensity score, and baseline values).

Results: The matched sample was predominantly Val30Met (93%) with roughly equal gender ratio (52% male) and an average age of 41.4 years. Less disease progression was seen in the tafamidis treated group over 24 months on neurological and quality of life endpoints. The neurologic endpoints with statistically significance favoring tafamidis include the derived NIS-LL and the Neurologic Composite Score including sub-scores. The Norfolk TQoL Score was also statistically significant favoring tafamidis treatment. No significant differences were found for the modified BMI or the Karnofsky Performance Status Index.

Conclusion: Tafamidis treatment resulted in less neurological progression. The results extend the efficacy observed in the clinical trials to real-world clinical settings.

P5

Tissue remodeling after RNAi-mediated knockdown of TTR in a Familial Amyloidotic Polyneuropathy mouse model

Nádia Pereira Gonçalves[1*], Paula Gonçalves[1], Miguel Ventosa[2], Ana Varela Coelho[2], Maria João Saraiva[1]
[1]Instituto de Inovação e Investigação em Saúde (I3S), Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Molecular Neurobiology Unit, 4150-180, Porto, Portugal; [2]Instituto de Tecnologia Química e Biológica António Xavier (ITQB), Universidade Nova de Lisboa, Mass Spectrometry, 2781-901, Oeiras, Portugal
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Background: Transthyretin (TTR) deposition in the peripheral nervous system (PNS) is the hallmark of Familial Amyloidotic Polyneuropathy (FAP). Mice expressing human TTR with the V30M mutation in a heterozygous heat shock factor 1 (Hsf-1) background show extensive TTR deposits in PNS and gastrointestinal tract, as well as extracellular matrix

(ECM) remodeling, similar to those seen in human FAP patients. Currently, liver transplantation is the only available treatment to halt the progression of clinical symptoms in FAP. Due to the limitations of this procedure, it is of utmost importance to develop alternative therapeutic strategies. In this regard, an RNAi therapeutic targeting TTR for the treatment of FAP is currently in Phase 3 clinical development. An ongoing phase 2 clinical trial in FAP patients demonstrated promising results as a mean plasma TTR reduction of 80%, sustained for over nine months, led to a decrease in neuropathy progression compared to historical data.

Methods: To dissect molecular changes occurring in tissues upon RNAi-mediated knockdown of TTR, we treated both chronically and acutely the Hsf/V30M FAP mouse model, in different stages of TTR deposition and analyzed histopathological and biochemical changes in the most affected organs.

Results: Our data show that inhibition of TTR expression by the liver prevent and reverse TTR deposition in PNS and GI tract. In addition, this treatment resulted in ECM remodeling with decreased levels of matrix metalloproteinase-2 (MMP-2) expression and MMP-9 activity in dorsal root ganglia. Importantly, MMP-2 protein levels were found down regulated in plasma samples from older mice treated with RNAi while animals treated with Tafamidis, Anakinra or Doxycycline/TUDCA showed no difference, suggesting that ECM remodeling with decreased MMP-2 might be a specific effect of RNAi.

Conclusion: Collectively, our data show that silencing TTR liver synthesis in vivo can modulate TTR-induced pathology in the PNS.

P6

Monitoring safety and effectiveness of Tafamidis in transthyretin amyloidosis in Italy: a 3-year longitudinal multicenter study in a non-endemic area

Andrea Cortese[1*], Giuseppe Vita[2], Laura Obici[3], Marco Sabatelli[4], Gian Maria Fabrizi[5], Angelo Schenone[6], Giampaolo Merlini[3], Davide Pareyson[7]
[1]C. Mondino National Neurological Institute, General Neurology, 27100, Pavia, Italy; [2]University of Messina and NEMO SUD Center for Neuromuscular Disorders, Fondazione Aurora Onlus, Department of Neurosciences, 98121, Messina, Italy; [3]Amyloidosis Research and Treatment Center, Department of Molecular Medicine, Fondazione Istituto Di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo and University of Pavia, 27100, Pavia, Italy; [4]Institute of Neurology, Catholic University of Sacred Heart, Department of Geriatrics, Neurosciences and Orthopedics, 00118, Roma, Italy; [5]University of Verona, Department of Neurological, Neuropsychological, Morphological and Motor Sciences, 37010, Verona, Italy; [6]University Federico II of Naples, Department of Neurosciences, Reproductive and Odontostomatological Sciences, 80121, Napoli, Italy; [7]IRCCS Foundation, C. Besta Neurological Institute, Department of Neurolog, 20124, Milano, Italy
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Background: Tafamidis is a transthyretin (TTR) stabilizer able to prevent mutated TTR tetramer dissociation into amyloidogenic monomers. There have been a few encouraging studies on safety and long-term efficacy of Tafamidis in early-onset Val30Met TTR-familial amyloid polyneuropathy (TTR-FAP) patients. However, less is known about its efficacy in later stages of the disease and in non-Val30Met mutations.

Methods: Multi-center observational study on symptomatic TTR-FAP patients prescribed to receive tafamidis. We followed up patients according to a standardized protocol including general medical, cardiological and neurological assessments at baseline and every 6 months up to 3 years.

Results: 61 (42 males) patients were recruited. Only 28% of enrolled subjects had the common Val30Met mutation, mean age of onset was remarkably late (59 years) and 18% was in an advanced disease stage at study entry. Tafamidis proved safe and well-tolerated. One third of patients did not show significant progression along 36 months, independently from mutation type and disease stage. Neurological function worsened particularly in the first 6 months but slowed significantly thereafter. Fifteen percent of patients showed cardiac disease progression and 30% new onset of cardiomyopathy. A higher mBMI at baseline was associated with better preservation on neurological function.

Conclusions: Neuropathy and cardiomyopathy progressed in a significant proportion of patients despite treatment. However, the worsening of neurological function slowed after the first 6 months and also subjects with more advanced neuropathy, as well as patients with non-Val30Met mutation, benefited from Tafamidis treatment. Body weight preservation is an important favorable prognostic factor.

P7

Treatment of transthyretin (TTR) amyloid cardiomyopathy with an antisense oligonucleotide inhibitor of TTR synthesis

Merrill D Benson[1*], Elizabeth J Ackermann[2], Brett P Monia[2]
[1]Indiana University School of Medicine, Pathology and Laboratory Medicine, 46202, Indianapolis, USA; [2]ISIS Pharmaceuticals, Inc., Management, 92010, Carlsbad, USA
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Background: While transthyretin (TTR) amyloidosis is usually characterized by peripheral and autonomic neuropathy, a majority of patients also have evidence of restrictive cardiomyopathy. In addition, amyloid cardiomyopathy may occur in the elderly without the presence of a mutant form of TTR. Both the hereditary and wild-type TTR amyloidosis are characterized by progressive restrictive cardiomyopathy. The disease usually progresses over a five to ten year period from time of diagnosis to demise from congestive heart failure.

At present there are ongoing pharmaceutical studies to suppress the synthesis of TTR by the liver using antisense oligonucleotides or siRNA. Both types of agents have been shown to be effective in lowering blood levels of TTR but efficacy measured by inhibition of progression of disease has not yet been established. The present study is an investigator sponsored Phase-2 study to determine the safety and tolerability of an ISIS generation 2.0 antisense oligonucleotide in patients with moderate to advanced TTR cardiomyopathy.

Methods: Subjects are admitted to the study with biopsy proven transthyretin amyloidosis (ATTR) and a left ventricular (LV) wall thickness of ≥ 1.3 cm. Safety parameters are observed over a 24-month period. Echocardiography is conducted at baseline and at 6-month intervals. Cardiac MRI is obtained at baseline and 12-month intervals. ISIS-TTRRx 300 mg is administered weekly by subcutaneous injection.

Results: To date, ten patients have been admitted to the study. Five patients have completed greater than 6-months on drug. TTR plasma levels have fallen steadily with reduction up to 88% and a mean suppression of 78% by 39-weeks. No serious adverse events have occurred.

Conclusion: ISIS-TTRRx appears to be well tolerated by patients with ATTR cardiomyopathy. Efficacy evaluation awaits longitudinal studies determining stability of LV mass.

P8

A phase 3 clinical trial with ISIS-TTRRx, a 2nd-generation antisense oligonucleotide targeting transthyretin (TTR), for the treatment of TTR amyloid cardiomyopathy

Helen Millns[1*], Rito Bergemann[2], Elizabeth Ackermann[3], Brett Monia[3], Mary Ann Lukas[4]
[1]GlaxoSmithKline, Clinical Statistics, SG1 2NY, Hertfordshire, UK; [2]GlaxoSmithKline, Rare Diseases, TW8 9GS, Middlesex, UK; [3]Isis Pharmaceuticals, Clinical Development, 92010, Carlsbad, USA; [4]GlaxoSmithKline, Clinical Development, 19112, Philadelphia, USA
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Background: Transthyretin (TTR) amyloidosis is a progressive and fatal systemic disorder caused by misfolded TTR monomers that cumulatively deposit in the heart, peripheral nerves and other organ systems. TTR Amyloidosis-associated cardiomyopathy (ATTR-CM), caused by TTR amyloid infiltration of the myocardium and conduction system, results in a restrictive cardiomyopathy associated with atrial arrhythmias, progressive heart failure with preserved ejection fraction in early phases, and leads to reduced life expectancy. ISIS-TTRRx is a first-in-class, 2nd-generation antisense oligonucleotide designed to produce substantial reductions in the levels of both mutant and wild type TTR produced from the liver.

Methods: We plan to conduct a Phase 3 randomized, double-blind, placebo-controlled, global study designed to evaluate the efficacy and

safety of ISIS-TTRRx in patients with ATTR-CM. The study will evaluate the differences observed between patients treated with placebo and with ISIS-TTRRx over 24 months evaluating several key endpoints. Serum biomarkers including circulating TTR levels, NT-pro-BNP and troponin, imaging endpoints including global longitudinal strain by echocardiography, clinical outcomes including mortality, transplants, cardiovascular hospitalization and arrhythmic events, and clinical status including New York Heart Association (NYHA) class, and the patient-reported instrument Kansas City Cardiomyopathy Questionnaire (KCCQ) will be evaluated. The trial is designed to enroll approximately 400 ATTR-CM patients with documented senile systemic amyloidosis (SSA) or documented familial amyloid cardiomyopathy (FAC), including theVal122Ile mutation and other TTR mutations, in addition to biopsy-proven amyloid deposits. Patients will be randomized 2:1, ISIS-TTRRx: placebo, with ISIS-TTRRx administered subcutaneously at 300 mg every other day for five days, then weekly for 24 months. The study will have 85% power to detect a difference between treatment groups in the final primary endpoint, with a significance level of $p < 0.05$. All patients completing the Phase 3 study will be eligible to enroll in a Phase 3 open-label extension study or otherwise receive access to ISIS-TTRRx. An academic Steering Committee will oversee trial conduct, an independent Clinical Event Committee will adjudicate study outcome endpoints, and an independent Data Safety Monitoring Committee will periodically review and evaluate accumulated study data for participant safety and, when appropriate, efficacy, and make recommendations to the sponsor and Steering Committee concerning the continuation, modification, or termination of the trial.

Results and conclusions: Results of the trial are anticipated in 2019. The trial will establish the role of therapy with ISIS-TTRRx. in this patient population for whom effective treatments to alter the progression of the disease are needed.

P9

SOM0226, a repositioned compound for the treatment of TTR amyloidosis

Núria Reig[1*], Salvador Ventura[2], Maria Salvadó[3], Josep Gámez[3], Raúl Insa[1]
[1]SOM Biotech, R&D, 08028, Barcelona, Spain; [2]Universitat Autònoma de Barcelona, Institute of Biotechnology and Biomedicine, 08193, Bellaterra, Spain; [3]Hospital Universitari Vall d'Hebron, Neurology, 08035, Barcelona, Spain

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Background: Transthyretin amyloidosis is a protein aggregation disorder caused by deposition of transthyretin, a tetrameric plasma protein in charge of the transport of thyroxin and retinol in plasma and cerebrospinal fluid. Kinetic stabilization of the TTR tetramer has proved to be a valid therapeutic strategy to prevent the release of unstable TTR monomers and their aggregation into oligomers and amyloid fibrils. SOM0226 is tolcapone, a repositioned compound with a newly identified activity as a potent TTR stabilizer and TTR fibril disruptor.

Methods: A proof of concept Phase IIa clinical trial has been conducted in diagnosed Familial Amyloid Polyneuropathy (FAP) patients, asymptomatic carriers and healthy volunteers with the objective to determine whether treatment with SOM0226 stabilizes plasmatic TTR. The trial has been conducted at the Hospital Universitari Vall d'Hebron (Barcelona) and has involved 6 healthy individuals and 15 asymptomatic carriers with mutations in the TTR gene and FAP patients at different stages of disease progression. It was an interventional open label study organized in two phases separated by a washout period of 6 weeks. During the first phase, patients were administered a single dose of SOM0226 (200mg) and blood was collected at different times for determination of drug levels and TTR stabilization activity, which was the primary efficacy endpoint of the study. The second phase was similar but involved multiple doses of 100mg of SOM0226: one every 4 hours up to a total of 3 doses. TTR stabilization was measured in plasma samples using an immunoturbidity method after urea denaturation and crosslinking with glutaraldehyde. Safety endpoints were also determined at baseline and at the end of each phase.

Results: Treatment with a single oral dose of 200mg or three doses of 100mg SOM0226 induce a clear and robust stabilization of plasmatic TTR in all patients studied, allowing the protection of 100% of TTR present in the plasma samples. This activity is maximal after 2 hours of dosing and clearly correlates with drug levels in plasma. The studied administration regime has demonstrated to be safe, with no adverse events related to the investigational product observed. Moreover, significant clinical or analytical hepatotoxicity has not appeared in any patient.

Conclusion: SOM0226 is capable of stabilizing all TTR present in plasma samples in all patients studied, supporting further development of SOM0226 for the treatment of TTR amyloidosis. As a repositioned drug, SOM0226 (tolcapone) can bypass much of the early cost and time needed to bring a drug to market and bears the potential to become the most potent TTR stabilizer in the market to prevent the progression of the different forms of TTR amyloidosis.

P10

TUDCA as an autophagic modulator of ATTR V30M Amyloidosis

Cristina Teixeira[*], Maria João Saraiva
I3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto; IBMC - Instituto de Biologia Molecular e Celular, Molecular Neurobiology, 4150-180, Porto, Portugal

Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**P10

Background: Different compounds have been studied for the treatment and/or symptoms amelioration of Familial Amyloidotic Polyneuropathy (FAP). We previously showed that Tauroursodeoxycholic acid (TUDCA), a biliary acid with anti-oxidant properties known to reduce non-fibrillar TTR aggregates is also capable of diminishing ER stress by its action on Bip in young transgenic Val30Met mice. This compound has already proved to be neuroprotective in several studies using genetic animal models of Huntington's and Parkinson's disease although the mechanisms underlying its neuroprotection action are still unknown.

Other cellular mechanisms are being explored for possible actions of TUDCA, with special highlight to autophagy, a cellular mechanism that involves the delivery of large protein aggregates, defective organelles and other cellular debris to lysosomes for degradation that has lately been linked to several degenerative diseases where it appears to be impaired.

The objective of our work is to investigate whether improvements previously observed in TUDCA-treated Val30Met mice involves autophagy.

Methods: For this study, nine months old mice bearing the human TTR Val30Met mutation, in a TTR null background-hTTR Met30 were used. Animals were treated with TUDCA (500 mg/kg/day) in the drinking water, for a 3 month period after which the animals were sacrificed and organs from gastrointestinal tract were collected. An age-matched control group of animals was maintained in the same conditions, drinking regular tap water.

Immunohistochemistry analyses were performed in order to evaluate the expression levels of an autophagic marker, p62, a protein naturally degraded in the final steps of the autophagic flux and that typically accumulates when autophagy is impaired.

Results: Our preliminary results point to a significant reduction in p62 accumulation in colon samples of TUDCA treated mice.

Conclusion: TUDCA is a promising compound that has already been proved to significantly reduce TTR toxic aggregates in vivo which points to its capability of modulating TTR aggregation by cellular responses, such as by oxidative stress, ER stress and apoptosis. Our results indicate that this modulation involves the autophagic machinery where it seems to enhance/restablish the autophagic flux that in turn may be directly involved in the pathological cascade in FAP, thus TUDCA contributes to the clearance of Val30Met TTR aggregates.

P11

Tafamidis reduces disease progression in patients with transthyretin familial amyloid polyneuropathy: supportive post-hoc analyses of a pivotal trial

Denis Keohane[1*], Jeffrey Schwartz[2], Balarama Gundapaneni[3], Michelle Stewart[4], Leslie Amass[5]
[1]Pfizer, Global Innovative Products, NY 10017, New York, USA; [2]Pfizer, Global Innovative Products-Statistics, CT 06340, Groton, USA; [3]inVentiv Health, Statistician, MA 01803, Burlington, USA; [4]Pfizer, Specialty Care Medicines Development Group, CT 06340, Groton, USA; [5]Pfizer, Global Innovative Pharma Business, PA 19426, Collegeville, USA

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Background: Safety and efficacy of once-daily 20 mg tafamidis, a transthyretin (TTR) stabilizer, was evaluated in an 18-month, multicentre, randomized, double-blind, placebo-controlled study in 128 patients with early symptomatic V30M TTR familial amyloid polyneuropathy (TTR-FAP). In the intent-to-treat population, a responder analysis for Neuropathy Impairment Score-Lower Limb (NIS-LL) (co-primary with Norfolk Quality of Life-Diabetic Neuropathy) favoured tafamidis (P=0.07). A pre-specified, key secondary analysis of change from baseline to Month 18 in NIS-LL continuous scores was significant (P=0.04). Placebo-corrected point estimates for 5 pre-specified and validated measures of disease progression also favoured tafamidis and were directionally consistent. Additional post-hoc analyses supporting tafamidis for delaying progression of TTR-FAP are reported here.
Methods: Change from baseline in NIS-LL over time was analysed with the addition of the baseline values as a covariate in a repeated measures model. A sensitivity multiple imputation analysis with imputed values based on assigned treatment group was also performed. Additionally, change in NIS-LL+ summated 7 (neurophysiological function composite endpoint) over time was assessed.
Results: When adjusted for baseline NIS-LL disease severity, statistical significance in change from baseline to Month 18 NIS-LL was retained. The magnitude of separation between placebo and tafamidis was consistent across the full range of baseline values (least squares mean difference = 2.7 points; 95% confidence interval [CI]: 0.1, 5.2; P<0.05). Treatment effect estimates from the multiple imputation analysis, although reduced, were similar to those from the analysis of change from baseline to Month 18, and remained significant. With each batch run representing the results combined from 1000 multiply imputed data sets, the difference in NIS-LL change from baseline to Month 18 for tafamidis versus placebo was -2.787 (standard error [SE]: 1.345; 95% CI: -5.423, -0.151; P=0.04) for Batch Run 1; for Batch Run 2, the difference was -2.815 (SE: 1.351; 95% CI: -5.464, -0.166; P=0.04); and for Batch Run 3, the difference was -2.798 (SE: 1.347; 95% CI: -5.438, -0.157; P=0.04). For NIS-LL + summated 7, at Month 6, the mean change (standard deviation) was 1.8 (4.82) for the tafamidis group and 4.0 (7.12) for the placebo group (P=0.053); while significant differences between treatment groups were observed at Months 12 (1.6 [5.27] vs 7.8 [8.87], P=0.00009) and 18 (3.4 [5.95] vs 8.8 [10.44], P=0.0043).
Conclusions: The beneficial effects of tafamidis in delaying neurological impairment in TTR-FAP are further supported by these post-hoc analyses.
Note: Note: First presented at the 1st Congress of the European Academy of Neurology, Berlin, Germany, June 2015.

P12

Early intervention with tafamidis provides long-term benefit in delaying neurological progression in patients with transthyretin familial amyloid polyneuropathy

Marcia Waddington-Cruz[1*], Leslie Amass[2], Denis Keohane[3], Jeffrey Schwartz[4], Balarama Gundapaneni[5], Hui Hua Li[2]
[1]Hospital Universitario Clementino, Neuromuscular Diseases Unit, 21941-913, Rio de Janeiro, Brazil; [2]Pfizer, Pfizer, PA 19426, Collegeville, USA; [3]Pfizer, Pfizer, NY 10017, New York, USA; [4]Pfizer, Pfizer, CT 06340, Groton, USA; [5]InVentiv Health, Statistics, MA 01803, Burlington, USA
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Background: Tafamidis is a transthyretin (TTR) stabilizer approved to delay neurological progression associated with stage 1 TTR familial amyloid polyneuropathy (FAP). A placebo-controlled, randomized 18-month registration trial allowed for continued evaluation of patients receiving tafamidis (20 mg oral once-daily) through an ongoing open label extension study. The effectiveness of tafamidis for delaying long-term neurological progression relative to baseline levels of neuropathy impairment at the start of treatment has not been reported previously.
Methods: This analysis describes the trajectory of the disease progression over 5.5 years or more for 71 patients with the V30M mutation and stage 1 TTR-FAP who received tafamidis either at the start of the registration trial or after the switch from placebo following 18 months of study participation. All V30M patients treated with tafamidis in the original trial or its extension and for whom follow-up data were available were included. The impact of tafamidis on neurological progression over time was evaluated using the Neuropathy Impairment Scale for lower limbs (NIS-LL). Baseline NIS-LL at active treatment start was defined as the last measurement before the first active dose of tafamidis.
Results: Patients (30 male, 41 female) were primarily Caucasian (91.5%) with a mean (standard deviation [SD]) age of disease onset of 35.7 (11.31) years. Very low baseline disease severity, defined as NIS-LL ≤10 at treatment start (baseline mean NIS-LL [SD] = 4.1 [3.1]), was associated with a minimal level of disease progression over time. Mean change in NIS-LL from baseline (SD) over 5.5 years of treatment was: 0.6 (3.2) at 1 year, 1.3 (3.7) at 2 years, 2.3 (6.0) at 3 years, 1.7 (4.8) at 4 years and 5.3 (10.1) at 5.5 years. Additional categorization of patients according to baseline NIS-LL of 0-5 and 5-10 revealed a similar pattern of reduced disease trajectory. For 3 patients, there was evidence after 3-5 years of treatment of an increase in the NIS-LL score to 30 or more.
Conclusion: Early intervention with tafamidis provides long-term benefit in delaying neurological progression associated with TTR-FAP. These data underscore the need to intervene as early as possible with symptomatic TTR-FAP patients.
Note: First presented at the Peripheral Nerve Society Congress, Montreal, Canada, June 2015.

P13

Neuroprotection of Anakinra on peripheral nerve neurodegeneration in single and combination protocols with TTR siRNA in a transgenic mouse model for human V30M transthyretin

Maria João Saraiva[*], Paula Gonçalves, Diana Martins, Nádia Pereira Gonçalves
Instituto de Inovação e Investigação em Saúde (I3S), Instituto de Biologia Molecular e Celular (IBMC), Universidade do Porto, Molecular Neurobiology Unit, 4150-180, Porto, Portugal
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Background: We previously showed the properties of interleukin-1β antagonist Anakinra on unmyelinated nerve fibers protection in a transgenic mouse model for human V30M transthyretin. In these studies, Anakinra decreased, among other markers, nerve levels of IL-1β, NF-κB and activated-caspase 3, associated with a decrease in TTR-non fibrillar deposition.
In the present work, we compared the efficacy of other compounds under human therapeutical trials for Familial Amyloidotic Polyneuropathy (FAP) in the same animal model, having 5 months of age.
Methods: V30M transgenic mice were treated daily with subcutaneous injections of Anakinra at 25 mg/kg over 6 weeks. Age-matched controls were injected with phosphate buffer saline (PBS). TTR siRNA, at a concentration of 1 mg/kg was injected in the tail vein for 4 weeks. One intravenous injection was performed per week and animals were euthanized 48 h after the last injection. Untreated age-matched controls received vehicle intravenously. Anakinra plus TTR siRNA combination therapy was performed using the same therapeutical design. In addition, other V30M mice group was treated with Tafamidis meglumine with 3 subcutaneous injections weekly, over 6 weeks. Controls received meglumine alone. Finally, combination strategy with Doxycycline/TUDCA was also achieved in V30M mice. Animals were treated with 8 mg/kg Doxycycline daily in the drinking water and received intraperitoneal injections of 500 mg/kg TUDCA twice a week for 4 weeks. Controls were injected with intraperitoneal PBS.
After mice sacrifice, nerves were collected into a 0.1 M sodium cacodylate solution containing 1.25% glutaraldehyde and 4% paraformaldehyde for posterior fiber counting. Nerves from animals treated with Anakinra, TTR siRNA or both agents combined were also analyzed by immunohistochemistry for inflammatory and apoptotic markers, namely NF-κB transcription factor, IL-1β and FAS death receptor.
Results: In contrast with Anakinra treatment alone, no differences in both myelinated and unmyelinated fiber density as compared with vehicle were found for the other tested compounds. However, combined Anakinra and siRNA administration resulted in increased density of unmyelinated fibers as compared to controls. Efficiency of Anakinra as a neuroprotective molecule was corroborated in sciatic nerve analyses of NF-κB, FAS death receptor and IL-1β in animals treated with a combination of Anakinra and siRNA, since these markers were found downregulated in animals receiving this combined therapy. Mice treatment with single protocols of siRNA,

Tafamidis or Doxycycline/TUDCA, did not change levels of the selected biomarkers.
Conclusion: Anakinra should be considered for its potential in single and/or combination protocols for FAP studies.

P16

Familial amyloidotic polyneuropathy associated with the transthyretin CYS 114 gene in a Russian pair of monozygotic twins

Igor Srokov Igor[1*], Galina Diukova[1], Aleksandr Pogromov[1], Maria Kovalchuk[2], Eduard Generozov[3]
[1]First Moscow Medical University named after I.M. Sechenov, Department of neurological disorders, 119991, Moscow, Russia; [2]University Medical Center, Department of neuro-muscular disorders, 3584 CX, Utrecht, Netherlands; [3]Institute of physical and chemical medicine, Department of genetics, 119435, Moscow, Russia
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Background: Discordant course of the disease in monozygotic (MZ) twins is known to be characteristic for familial amyloidotic polyneuropathy (FAP). Existing cases of FAP in MZ twins refer to amyloidosis due to mutant transthyretin (TTR) Val30Met gene. We present a case of a pair of MZ twins associated with a substitution of tyrosine to cysteine at position 114 in the TTR gene in a Russian kindered. Until now FAP due to mutant TTR Cys 114 has only been described in one Japanese and in one Dutch family. Though in none of them MZ twins were present.
Materials: Complete laboratory and instrumental investigation of both of the Russian MZ twins was performed. Detailed life history was evaluated in each of the brothers and compared with other known cases of FAP in MZ twins.
Results: One of the twins had a prominent clinical picture of FAP and visceral amyloidosis, starting around the age of 45 years. In the mean time the other brother was still clinically healthy at the age of 50. DNA confirmed identical mutation of TTR gene in both brothers. Amyloid depositions were found to be similar in the intestines, but not in other locations. Both patients lived in same district and had similar educational background. Though the patient with a prominent clinical picture of FAP experienced vaccination agravated by side effects as well as appendicitis agravated by severe peritonitis in his twenties.
Conclusion: Charactristic feature of FAP in known pairs of MZ twins is the discordance in the disease course with a prominent manifestation in one of the twins, and delayed disease onset and/or only slight presentation in the other. Genetical and non-genetical factors, or their combination, were supposed to be contributing. Non-genetical mechanisms of the phenotypic variability of FAP could consist of influences on the mutant gene expression during twinning process or along the life. In Russian pair of MZ twins different life-course events could determine clinical presentation of the disease.

P17

Hereditary transthyretine amyloidosis in Slovenia

Janez Zidar
University Medical Centre Ljubljana, Institute of Clinical Neurophysiology, 1525, Ljubljana, Slovenia
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The prevalence of transthyretin (TTR) amyloidosis in Europe is supposed to be less than one per 100,000 individuals. Slovenia is a rather small country with approximately two million inhabitants and we know of two families with 4 patients with this disease. The first patient (family A) was diagnosed in 2008 when also one of the asymptomatic family members who agreed to molecular genetic testing was found to be a carrier. Other family members up to now disagreed with clinical, laboratory and molecular genetic testing. Patients from family B were diagnosed in 2014. Their siblings were found not to be carriers.
The index case from family A was diagnosed at the age of 54. The TTR gene mutation was characterised as Val122Ala. First symptoms were due to dysautonomia and appeared 4 years earlier in the form of abdominal colic associated with vomiting and orthostatic hypotension. He also had symptoms and signs of length-dependent axonal polyneuropathy and hypertrophic restrictive cardiomyopathy. He died the day after liver transplantation.
The disease of the family's B index case started approximately one and a half year before the diagnosis with pain in his feet, orthostatic hypotension, sexual impotence, diarrhoea alternating with constipation, and weight loss. Later on he also noticed weakness in his lower limbs but is still able to walk unaided. The Val30Ala mutation was found. Except to polyneuropathy no other organs are affected. He is treated with tafamidis. His sister is asymptomatic but is having electrophysiological signs of polyneuropathy and cardiac vagal denervation. She recently also started taking tafamidis.
With increasing awareness of the transthyretin amyloidosis, especially amongst neurologists and cardiologists in our country, we expect to find some more patients in the near future, particularly among members of the affected families. Two other unrelated patients are currently undergoing testing, one of them with transthyretin amyloid tissue deposits.

P18

Does the course of Val122Ile differ from SSA, or is selection bias a factor?

Hallie Geller*, Tara Mirto, Rodney Falk
Brigham and Women's Hospital, Cardiac Amyloidosis Program, 02115, Boston, MA, USA
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Hypothesis: There are conflicting data regarding survival in Val122Ile versus SSA cardiac amyloidosis, suggesting either better survival in SSA or similar survival between the groups. Since survival is often calculated from time of diagnosis, a delayed diagnosis in Val122Ile from symptom onset would bias survival toward SSA. We therefore sought to determine whether patients with symptomatic SSA and Val122Ile seen at our Program showed different patterns of referral, and whether one group had a longer time to diagnosis than the other.
Methods: Records of all 30 Val122Ile patients with cardiac involvement seen over an 8 year period were compared to records of 34 randomly selected patients with cardiac SSA. We estimated onset of cardiac symptoms likely due to amyloidosis from detailed history review and determined definitive diagnosis based on biopsy or (for Val122Ile) either biopsy or typical echocardiogram with positive genetic testing. Referral patterns were also determined, specifically whether patients were from within our home state or referred from out-of-state. Death was determined by national database of deaths, medical records or on-line obituary search if patient had been lost to follow-up.
Results: Mean age at diagnosis did not differ between groups: Val122Ile =71.4 yr, SSA =73.9 yr.
There was a wide range of symptom duration between symptom onset and diagnosis, ranging from weeks to 9 years in both groups, with a slightly longer duration of symptoms in the SSA group than in Val122Ile (32.4 v 21.6 months p=ns). Actuarial survival from diagnosis did not differ between groups, being 33 months in Val122Ile and 36 months in SSA, but there was a a strong trend to better survival from symptom onset in SSA (66 months in SSA v 51 months in Val122Ile). Referral pattern differed significantly between the 2 groups, with 63% of Val122Ile patients referred from in-state compared to only 34% of SSA ($p<0.05$).
Conclusion: The higher rate of out-of-state referrals for SSA may suggest either an increased awareness of the disease locally, or possibly a socioeconomic bias in favor of SSA patients. The trend toward earlier diagnosis from symptom onset in Val122Ile patients may affect analysis of survival comparison with SSA if date of diagnosis is used in calculating survival, but the overall median survival in both groups, whether from diagnosis or symptom onset remains poor, and underscores the pressing need for disease-modifying drug trials in both groups.

P19

Preliminary assessment of neuropathy progression in patients with hereditary ATTR amyloidosis after orthotopic liver transplantation (OLT)

David Adams[1*], Juan Buades[2], Ole Suhr[3], Laura Obici[4], Teresa Coelho[5]
[1]Centre Paris-Sud, APHP, Hopital de Bicetre and Centre de Reference National des Neuropathies Amyloides Familiales (NNERF), Service de Neurologie, 94275, LE KREMLIN-BICETRE, France; [2]Hospital Son Llatzer,

Servicio de Medicina Interna, 7198, Palma de Mallorca, Spain; [3]Umea University, Department of Public Health and Clinical Medicine, SE-90185, Umea, Sweden; [4]Fondazione IRCSS Policlinico,) Amyloid Research and Treatment Centre, 27100, Pavia, Italy; [5]Hospital de Santo Antonio, Unidade Clinica de Paramiloidose, 4099-001, Porto, Portugal
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Background and objectives: Familial amyloidotic polyneuropathy (FAP) is a fatal, autosomal dominant disease caused by abnormal tissue deposition of mutant and wild-type transthyretin (TTR) in peripheral nerves, gastrointestinal tract, and heart. Since the liver is the primary source of WT and mutant TTR, orthotopic liver transplantation (OLT) has been the standard of care in select patients. Despite undergoing OLT, some patients experience progression of their underlying disease. There is evidence to suggest that disease progression after OLT is caused by ongoing deposition of WT TTR protein produced by the transplanted liver. In order to better understand the natural history of patients receiving FAP-OLT, we performed a retrospective assessment of time to polyneuropathy disability (PND) score progression.
Methods: We characterized neuropathy severity with the (PND) score. Descriptive statistics including proportions of FAP patients who progress by at least one PND stage after OLT and median time to PND stage progression were assessed in available FAP-OLT patients in Sweden, Portugal, Spain, Italy and France from 1991-2015. The Italian cohort only included patients that had progressed, while patients from other countries included all-comers (progressors and non-progressors).
Results: A total of 362 FAP-OLT patients were preliminarily assessed from: Portugal, 67; Spain, 59; Sweden, 29; France, 200; Italy, 7. Patients from Portugal, Spain, and Sweden all had the Val30Met (V30M) TTR variant, whereas 69% and 14% of patients from France and Italy had the V30M TTR variant, respectively. Among all-comers with a pre-OLT PND score (n=322/355), 61% underwent transplantation at PND stage of 0 or I, 26% at stage II, 12% at stage IIIa or IIIb, and <1% at stage IV. Within the Italian cohort, 43% underwent transplantation at stage I, 43% at stage II and 14% at stage IIIb. Following OLT, 33% of all-comers (n=107/322) experienced a PND progression; the percent of patients who progressed varied by country (Sweden, 31%; Portugal, 21%; Spain, 37%; France, 37%). Overall, median time from transplantation to PND progression, was 2.9 years (range: 2.5-4.5 years); median time to progression within the individual countries included: Portugal, 4.5 yrs; Spain, 2.6 yrs; Sweden, 4.0 yrs; France, 2.5 yrs; Italy, 3.0 yrs.
Conclusions: These preliminary data suggest that despite receiving an OLT, disease progression may not be halted in some patients with FAP. Based on this multi-national population of FAP-OLT patients with both V30M and non-V30M TTR genotypes, clinically meaningful neuropathy progression was observed in a median of 2.9 years after OLT. These findings suggest the need to carefully monitor FAP-OLT patients for signs of progression.

P20

FAP in India: a first genetically proven case

Daniel Pan[1*], Jérôme Bouligand[2], Anne Guiochon-Mantel[2], David Adams[3]
[1]Imperial College School of Medicine, Undergraduate student, SW7 2AZ, London, UK; [2]Publique Hôpitaux de Paris, Génétique moléculaire, Pharmacogénétique et Hormonologie, INSERM U693, Paris, France; [3]Publique Hôpitaux de Paris, French Reference Center for FAP (NNERF), INSERM U1191, Paris, France
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A 28 year old male with a background of abdominal tuberculosis living in Tekhand slum district of Delhi presented with two and half year history of recurrent loose stools following tingling sensation in the upper and lower limbs, as well as weakness in the lower limbs. He also reports erectile dysfunction and postural hypotension. He had lost weight : kilos. Upon examination the patient has lost all sensory perception in the lower limbs up to the upper thigh with severe wasting and dull lower limb reflexes. There was pes cavus and a high stepping gait.
Investigations revealed persistent circumferential thickening in the abdomen on CT. Sural nerve biopsy showed inflammation with demyelination and amyloidosis. There was a loss of parasympathic/ sympathetic reactivity. Bone marrow biopsy revealed amyloid deposits compatible with secondary AA with increase in anti-LKM antibodies. Endoscopy revealed increased inflammation in the lamina propia. Familial amyloid polyneuropathy was suspected, and TTR Sanger sequencing revealed detection of a missense mutation of Val30Ala at a heterozygous state.
Transthyretin familial amyloid polyneuropathy (TTR-FAP) is a rare systemic disease due to endoneurial amyloid deposit, and is one of the most severe disabling hereditary peripheral neuropathies in adults. It has been described clinicopathologically in 1952 by Corino Andrade in north of Portugal and genetically in 1984 by Maria Joao Saraiva. TTR-FAP has long been considered an endemic disease in Portugal, Sweden and Japan, although sporadic cases have also been reported in Europe initially in France and the UK. There is genetic heterogeneity in TTR-FAP, with Val30Met being the most frequent genotype in the world. Val30Ala is a rare mutation, which have previously been described in the Chinese population.
In this manuscript, we document to our knowledge the first genetically proven case of FAP in India, a populated country of more than 1.2 billion inhabitants, and a detailed description of the patient case, as well as family investigations are provided. The patient is awaiting for anti-amyloid treatment.
Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient /parent/guardian/ relative of the patient.

P21

Epidemiology of transthyretin familial amyloid polyneuropathy in Portugal

Mónica Inês[1*], Teresa Coelho[2], Isabel Conceição[3], Filipa Duarte-Ramos[4], Mamede de Carvalho[1], João Costa[5]
[1]Instituto de Medicina Molecular, Unidade de Fisiologia Clínica e Translacional, 1649-028, Lisboa, Portugal; [2]Centro Hospitalar do Porto, Unidade Corino de Andrade, 4000-436, Porto, Portugal; [3]Centro Hospitalar Lisboa Norte, Serviço de Neurologia, 1649-035, Lisboa, Portugal; [4]Faculdade de Farmácia da Universidade de Lisboa, Departamento de Sócio Farmácia, 1649-003, Lisboa, Portugal; [5]Instituto de Medicina Molecular, Unidade de Farmacologia Clínica, 1649-028, Lisboa, Portugal
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Background: Transthyretin Familial Amyloid Polyneuropathy (TTR-FAP) is a rare, progressive, debilitating and life-threatening neurodegenerative disease. TTR-FAP is a rare disease worldwide. In Europe a disease is defined as rare when it affects less than 1 in 2000 inhabitants. Portugal has the largest cluster worldwide nonetheless recent Portuguese epidemiologic data is lacking. The purpose of this study is to estimate TTR-FAP prevalence in Portugal.
Methods: In Portugal, TTR-FAP patient's medicines utilization is fully funded by National Health Service since 2001. Since March 2013 Portuguese electronic prescription system became more generalized, allowing central monitoring and validation of medicines prescription and dispensing. TTR-FAP anonymized patient's prescription data was requested to Administração Central do Sistema Saúde (ACSS). For each prescription the database has information regarding the local where the medicines were dispensed. Hence, for each patient, the most frequent municipality was identified and used as a proxy for residence. Portuguese total population by municipality was obtained from the official source - Instituto Nacional de Estatística. Prevalence was reported for mainland country and by municipality as number of cases per 2000 inhabitants.
Results: Trough year 2014, a total of 70286 electronic dispensing acts were detected in ACSS database, unravelling a total of 2013 distinctive TTR-FAP patients in Portugal. A prevalence of 0,41 per 2000 inhabitants was estimated for TTR-FAP in Portugal. The disease is currently spread across 160 of the 278 Portuguese municipalities and in 19 of them affects more than 1 per 2000 inhabitants. The municipalities with higher TTR-FAP prevalence are: Póvoa de Varzim, Pampilhosa da Serra, Seia, Esposende, Vila do Conde, Figueira da Foz, Boticas and Barcelos.
Conclusion: We can estimate that TTR-FAP disease has a current prevalence of 0,41 per 2000 inhabitants in Portugal and it is now disseminated across the country being Póvoa de Varzim historically and still the most impacted municipality with 3,95 cases per 2000 inhabitants, nearly ten times higher than overall country prevalence.

P22

Patient experience with hereditary and senile systemic amyloidoses: a survey from the Amyloidosis Research Consortium

Isabelle Lousada[1*], Raymond L Comenzo[2], Heather Landau[3], Spencer Guthrie[4], Giampaolo Merlini[5]

[1]Amyloidosis Research Consortium, Inc., N/A, 01773, Lincoln, USA; [2]Tufts Medical Center, N/A, 02111, Boston, USA; [3]Memorial Sloan-Kettering Cancer Center, N/A, 10065, New York, USA; [4]Prothena Biosciences Inc, N/A, 94080, South San Francisco, USA; [5]Fondazione IRCCS Policlinico San Matteo, and University of Pavia, N/A, 27100, Pavia, Italy

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Background: Amyloidosis is caused by the accumulation of misfolded proteins, resulting in dysfunction of vital organs (eg, heart, kidneys, nervous system). Diagnosis and access to appropriate therapy pose significant challenges, and there is a paucity of literature depicting the patient (pt) experience. We conducted a survey to identify the challenges in establishing a diagnosis of amyloidosis and to gain insight into the pt experience.

Methods: Pts with any type of systemic amyloidosis and their family members and caregivers were invited to participate in an anonymous online survey through email and social media channels of the Amyloidosis Foundation and an amyloidosis awareness group on Facebook. Caregivers and family members could answer on behalf of pts. The 16-question survey was developed by the authors and was available to participants online from January 29 to February 5, 2015. Here we present survey results focusing on pts with hereditary and senile systemic amyloidosis (SSA).

Results: In total, 533 persons completed the survey (pts, 58%; family members, 34%; caregivers, 8%). Overall, most pts were female (62%) and between 50 and 69 years of age (62%) (average age at diagnosis, 57 years). Most pts (72%) had light chain (AL) amyloidosis. Hereditary forms of amyloidosis included transthyretin (TTR)-related amyloidosis (ATTR; n=37) and hereditary non-TTR amyloidosis (n=18); SSA/wild-type ATTR was reported by 13 patients. 57%, 33%, and 77% of pts with ATTR, non-TTR, and wild-type ATTR were male, and average age at diagnosis was 54, 55, and 71 years, respectively. The most frequently involved organs were heart and nervous system in pts with ATTR, kidney and nervous system in pts with non-TTR, and heart in pts with wild-type ATTR. In pts with ATTR, non-TTR, and wild-type ATTR, the correct diagnosis was made within 6 months in 35%, 22%, and 46% of pts, respectively. For pts with ATTR, the correct diagnosis was most commonly made by a cardiologist (24%), neurologist (22%), or primary care physician (11%). For those with non-TTR, the correct diagnosis was most commonly made by a hematologist (22%), nephrologist (17%), or specialist at an amyloidosis center (17%). Pts with wild-type ATTR most commonly received the diagnosis from cardiologists (62%). Across groups, less than one-third of pts knew how to enroll in a clinical trial, but half would consider participating if more informed.

Conclusions: A correct diagnosis of hereditary or wild-type ATTR amyloidosis often requires numerous physician visits to different medical specialists and often occurs when disease is advanced. Responses obtained in this survey highlight the challenges experienced by pts with these rare diseases. These data may identify opportunities to educate pts and physicians in order to expedite diagnosis, facilitate appropriate disease management and access to clinical trials, and ultimately improve pt survival.

P23

Glu89Gln transthyretin-related amyloidosis in Italy and Bulgaria: does geographic area influence phenotype beyond the shared mutation?

Christian Gagliardi[1*], Mariana Gospodinova[2], Simone Longhi[1], Agnese Milandri[1], Mario Cinelli[1], Ivailo Tournev[3], Fabrizio Salvi[4], Claudio Rapezzi[1]

[1]Diagnostic and Specialty Medicine – DIMES, Alma Mater Studiorum, University of Bologna, Cardiology, 40138, Bologna, Italy; [2]University Hospital Alexandrovska, Clinic of Cardiology, 1000, Sofia, Bulgaria; [3]University Hospital Alexandrovska, Clinic of Neurology, 1000, Sofia, Bulgaria; [4]Bellaria Hospital, Neurology, 40100, Bologna, Italy

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Background: Glu89Gln transthyretin (TTR) variant is a well-known cause of systemic amyloidosis with a cardiologic, neurologic or mixed phenotype. Even though Glu89Gln transthyretin (TTR) variant has been described worldwide, it remains unknown whether geographical area influences the phenotypic expression of the disease (as happens with the Val30Met mutation, which is known to manifest with different phenotypes in different geographical contexts). We hypothesized that significant phenotypic differences exist between patients with Glu89Gln-related amyloidosis according to the specific geographic origin.

Methods: We retrospectively analysed and compared the clinical, electrocardiographic and echocardiographic findings of 64 patients with Glu89Gln TTR-related amyloidosis from Italy and Bulgaria.

Results: Despite a similar age at diagnosis of the disease (55 [52-60] years) patients in Bulgarian cohort have a mixed phenotype. A more severe left ventricular wall thickening was present in Bulgarian cohort compared to Italian one (17 [16-18] mm vs 15 [13-16], p=0.009) with a normal ejection fraction in all cases.

Conclusion: This is the largest series so far of patients with Glu89Gln TTR-related amyloidosis systematically analysed. Overall, disease onset is late with a mixed phenotypic expression. Despite a similar age at onset and a predominance of neurological routes, Bulgarian patients showed a more pronounced cardiomyopathy.

P24

Val50Ala variant of familial amyloid neuropathy – a rare case in the Czech Republic

Tomas Pika[1*], Pavla Latalova[2], Helena Hulkova[3], Patrik Flodr[2], Vladimir Mejzlik[4], Vlastimil Scudla[1]

[1]Faculty of Medicine and Dentistry, Palacky University Olomouc and Olomouc University Hospital, Department of Hemato-oncology, 77900, Olomouc, Czech Republic; [2]Faculty of Medicine and Dentistry, Palacky University Olomouc and Olomouc University Hospital, Department of Clinical and Molecular Pathology, 77900, Olomouc, Czech Republic; [3]General University Hospital Prague, Institute of Inherited Metabolic Disorders, 12808, Prague, Czech Republic; [4]Centre of Cardiovascular Surgery and Transplantation Brno, Centre of Cardiovascular Surgery and Transplantation Brno, 656 91, Brno, Czech Republic

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Background: Hereditary amyloidosis represents approximately 4% of the total cases of amyloidoses. The most frequent familial type is caused by deposition of mutated transthyretin (TTR, prealbumin). So far it has been identified more than 100 mutations in the transthyretin gene and type of causal mutation is also characterized by a clinical picture of the disease. The most common variant is a neuropathic disease. Characteristic feature is an endemic occurrence with very low incidence in the Central Europe countries.

Methods: The aim of this communication is to present case of a patient with an inherited form of the TTR amyloidosis which is extremely rare in the Czech Republic.

Results: 25-year-old patient was examined for a history of two years lasting and gradually progressing paresthesias of the lower limbs associated with instability, reduced muscle strength, dysesthesias and peroneal gait. In the last year, also present with intermittent dyspepsia, loss of weight was 10 kg (BMI 16.3, BMI 717.2). On the basis of clinical and EMG finding he was treated as CIDP with i.v. pulses of methylprednisolone, followed by maintenance therapy with prednisone and azathioprin. Afterwards the dyspepsia developed. Gastric endoscopy with biopsy revealed massive deposits of amyloid. Family history clarify amyloidosis with neurological impairment in the patient's mother. Immunohistochemistry confirmed strong positivity of transthyretin in amyloid masses. The sequencing of TTR gene confirmed mutation c.149T⊠C with the effect on the coding sequence Val50Ala in heterozygous status. The patient was then registered on the waiting list for OLT. Due to rapid progression of neuropathic involvement despite conservative therapy, therapy with Vyndaqel cps. (Tafamidis meglutime, Pfizer) was initiated at a dose of 20mg per day. Therapy led to a halt of further progression of neuropathic disability, improvement of nutrition and overall improvement of the condition of the patient. 19 months after diagnosis, OLT was performed with good postoperative course. As complications, the early cortico-sensitive rejection episode and bronchopneumonia developed. Currently, the patient is intensively rehabilitating with gradual regression of neuropathic symptoms and improvement of nutritional status (BMI 17.9, mBMI 841).

Conclusions: Although the Czech Republic is not endemic country for the incidence of familial TTR amyloidosis, it is important to consider this clinical entity, especially in the case of family history. Correct differential diagnosis belongs to the fundamental aspects of care for these patients.
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Consent to publish: Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient /parent/guardian/ relative of the patient.

P25

Transthyretin-related Familial Amyloid Polyneuropathy (TTR-FAP) caused by a very rare, de novo mutation in a Polish patient

Marta Lipowska[1*], Dorota Rowczenio[2], Janet Gilbertson[2], Philip N Hawkins[2], Agnieszka Ptasinska-Perkowska[3], Hanna Drac[1]
[1]Medical University of Warsaw, Deparment of Neurology, 02-097, Warsaw, Poland; [2]UCL Division of Medicine, National Amyloidosis Centre, NW3 2PF, London, UK; [3]Medical University of Warsaw, 3. Transplantation Institute, 02-006, Warsaw, Poland
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Introduction: TTR-FAP is rarely diagnosed in Poland – thus far only a few patients of Polish origin have been diagnosed with this type of amyloidosis. We present a case of TTR-FAP in a Polish patient in whom we discovered a rare TTR mutation.
Materials and methods: 56 years old man presented with a 5 -year history of progressive polyneuropathy. His symptoms started with numbness and paresthesia in the feet followed by weakness. Three years after similar symptoms spread into his hands. Subsequently he developed autonomic symptoms: diarrhea, neurogenic bladder with mild urine retention and impotence. Due to progressive neuropathy his movements were limited such that he required rollator for walking after four years of symptoms. Excessive weight loss resulted in cachexia and BMI of 17,5. His family history was negative.
The patient underwent detailed clinical evaluation, since his symptoms were higly suggestive of FAP his blood sample and fat biopsy were sent to the National Amyloidosis Centre (NAC) in London for genetic and histopathological studies.
Results: Neurography revealed diffuse sensory and motor axonopathy. Cardiomyopathy was reported on MR and ultrasounds, but it was clinically mild. Vitreous opacities were found, with no clinical impact.
Genetic analysis demonstrated that the patient was heterozygous for p. Asp58Val (D38V) TTR mutation. DNA isolated from his parents and brother was negative, indicating de novo mutation. Amyloid deposits were identified on fat biopsy by pathognomonic green birefringence when stained with Congo red and viewed under crossed polarised light. The amyloid stained specifically with antibodies to transthyretin.
Conclusions: We report a patient with a very rare TTR p.Asp58Val mutation who presented with typical clinical picture of late-onset TTR-FAP with predominant polyneuropathy. Due to advanced stage of disease the patient had no indications for tafamidis or liver transplantation. This variant has previously been identified in a Ghanaian male who presented at similar age with predominant polyneuropathy (Lachmann, 2002). In our patient this mutation has occurred de novo, which is uncommon in TTR-FAP.
Consent to publish: Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient /parent/guardian/ relative of the patient.

P27

Teachings from the French database of TTR familial amyloidotic polyneuropathy (TTR-FAP): large genetic and phenotypic heterogeneity, usefulness of TTR gene testing

David Adams[1*], Cecile Cauquil[1], Clovis Adam[2], Celie Labeyrie[1], Guillemette Beaudonnet[4], Anne Mantel[3], Marie Theaudin[1], network Cornamyl[5]
[1]Chu Bicetre, Neurology (NNERF), 94270, Le Kremlin Bicêtre, France; [2]Chu Bicetre, Pathology, 94270, Le Kremlin Bicêtre, France; [3]Chu Bicetre, Molecular Biology, 94270, Le Kremlin Bicetre, France; [4]Chu Bicetre, Neurophysiology, 94270, Le Kremlin Bicêtre, France; [5]French Network For Fap, 94270, Le Kremlin Bicetre, France
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Introduction: TTR-FAP is progressive, disabling, irreversible and life-threatening neuropathy due to a point mutation of TTR gene with autosomal dominant transmission. France is a non endemic European country. To study the impact of labeling French reference center for FAP (NNERF) and building of a national network CORNAMYL.
Methods: In 1986-2014 period, 482 FAP patients were registered in NNERF's database. All carried amyloidogenic TTR gene mutations and Congo positive amyloid deposit (CPAD). To report genotypic and phenotypic varieties of FAP in France in 2008-2014 period and the sensitivity of the tools for diagnosis.
Results: In 2008-2014 period: 180 new TTR-FAP cases were identified, in 14 additionnal geographical departments (total 81/100), with 9 further TTR gene mutations (total 41).
Mean age was 60 (22-89), a late onset (> or = 50 y) in 69%. Sex ratio: 2.16. Positive family history of FAP 55%. Portuguese origin 18.3%. Diagnosis of FAP was delayed by 2.93y (0.2-13.5) after first symptoms; 69% had walking difficulties including 39% requiring aid.
Five phenotypes were identified: Small Fiber Polyneuropathy (PNP) (43%), All-Fiber SensoryMotor-PNP (25%), Upper Limbs neuropathy (NP) (17%), Ataxic NP (14%), Motor NP (2%). CPAD after nerve biopsy in 19/26pts (73%), Labial Salivary Gland Biopsy (LSGB) in 91/128 pts (71%); 76% required multiple biopsies.
Conclusions: The French network for TTR-FAP allows to identify new TTR-FAP cases in most of geographical departments with varied phenotypes. The larger use of TTR gene analysis in idiopathic aggressive polyneuropathy cases will help to accelerate diagnosis of TTR-FAP.

P28

Five novel TTR variants: associated phenotypes and structural consequences

Dorota Rowczenio[*], Helen Lachmann, Ashutosh Wechalekar, Janet Gilbertson, Marianna Fontana, Carol Whelan, Julian Gillmore, Philip Hawkins
National Amyloidosis Centre, University College London, NW3 2PF, London, UK
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Objective: Transthyretin (ATTR) amyloidosis is the commonest form of hereditary amyloidosis. More than 120 amyloidogenic TTR mutations have been described and with few exceptions, these are rare, reported only in isolated kindreds. We describe here the clinical characteristics and disease course in 11 patients with novel TTR mutations. The structural consequence of replacing the wild-type amino acid with variant was examined in silico using PyMOL, an online molecular visualization tool.
Methods: 11 individuals with features raising the possibility of ATTR amyloidosis were referred to the UK NAC for clinical and laboratory investigation.
Results: Median (range) age at onset of symptoms was 58 (44-68) years; median age at diagnosis was 61 (46-74) years. Three patients presented with progressively worsening shortness of breath and chest tightness on exertion, six suffered with gradually progressive neuropathy, one was hospitalised with heart failure and oedema whilst one was asymptomatic but had a family history of FAP. Amyloid deposits were identified in biopsies in all cases by Congo red staining and were confirmed to be of ATTR type by immunohistochemistry. Genetic analysis revealed five novel variants: p.E74L (E54L), p.E74Q (E54Q), p.A101V (A81V), p.H110D (H90D) and p.I127F (I107F). The cohort was followed for a median (range) of 4 (1-7) years after referral during which six patients died of cardiac amyloidosis. Median (range) age at death was 66 (60-80) years, and median (range) time from onset of symptoms to death was 8 (4-16) years.
In silico analyses showed small structural changes associated with each of the novel TTR variants. Thus we speculate that these molecular modifications may have contributed to their amyloidogenic properties. Furthermore, we identified the substitutions resulting in the p.E74L/Q (E54L/Q) to likely have a particularly deleterious impact on the TTR molecule. Multiple sequence alignment revealed the native p.E74 (E54) amino acid to be evolutionary highly conserved with no alternation of this residue among other species, indicating that it must be structurally

and functionally important. Indeed, the side chain of the p.E74 (E54) forms hydrogen bonds with thyroxine, thus plays vital role in its transport. Replacement of this negatively charged, polar glutamic acid with the hydrophobic leucine residue may abolish this interaction.
Conclusions: We describe the clinical phenotypes and disease course in 11 patients with novel TTR mutations. Each variant was found in at least two unrelated individuals who had similar clinical features, which make it highly unlikely to be an incidental finding. Immunohistochemistry confirmed that amyloid fibrils were composed of TTR, which in conjunction with the characteristic clinical features of ATTR amyloidosis manifesting with cardiomyopathy and neuropathy, indicates that these novel TTR mutations were indeed the cause of ATTR amyloidosis in our patients.

P29

Transthyretin familial amyloid polyneuropathy (TTR-FAP) in Mallorca: a comparison between late- and early-onset disease

Manuel Raya-Cruz[1*], Juan Buades-Reines[1], Cristina Gallego-Lezaun[1], Ignacio Ferullo[1], Tomas Ripoll-Vera[2], Mercedes Uson-Martín[3], Hernan Andreu-Serra[4]
[1]Son Llàtzer Hospital, Internal Medicine, 07198, Palma de Mallorca, Spain; [2]Son Llàtzer Hospital, Cardiology, 07198, Palma de Mallorca, Spain; [3]Son Llàtzer Hospital, Neurology, 07198, Palma de Mallorca, Spain; [4]Son Llàtzer Hospital, Digestive, 07198, Palma de Mallorca, Spain
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Background: The age of onset (AO) of familial transthyretin-mediated amyloidosis with polyneuropathy (TTR-FAP) is known to vary between populations, with differing characteristics reported according to AO in endemic/non-endemic foci.
Methods: This was a retrospective study of patients with early AO (<50 years) and late AO (≥50 years) TTR-FAP at our community center in Mallorca. Data were collected on patient demographics, clinical disease manifestation, and physical symptoms.
Results: A total of 95 patients were analyzed, with mean follow-up of 9.39 years from diagnosis. The early AO group included 53 patients (33 male) and the late AO group included 42 patients (21 male). Neurologic involvement was the most common initial symptom, although was significantly more frequent in the late AO versus early AO group (p=0.015). Autonomic involvement was observed in 26% of patients in the early AO group, but was rarely observed in the late AO group (5%). During follow-up, cardiologic symptoms, renal involvement, and ophthalmologic symptoms were significantly more common in the late AO group (p<0.05).
Conclusions: This retrospective study demonstrates the variation in disease presentation and progression according to AO of TTR-FAP at our Mallorcan center. These data will inform diagnosis and monitoring of disease, and guide effective treatment choices.

P30

Clinical and laboratory test in patients with familial amyloid polyneuropathy: differences between symptomatic patients and asymptomatic carriers

Manuel Raya-Cruz[1*], Juan Buades-Reines[1], Cristina Gallego-Lezaun[1], Ignacio Ferullo[1], Tomas Ripoll-Vera[2], Mercedes Uson-Martín[3], Hernan Andreu-Serra[4]
[1]Son Llátzer Hospital, Internal Medicine, 07198, Palma de Mallorca, Spain; [2]Son Llátzer Hospital, Cardiology, 07198, Palma de Mallorca, Spain; [3]Son Llátzer Hospital, Neurology, 07198, Palma de Mallorca, Spain; [4]Son Llátzer Hospital, Digestive, 07198, Palma de Mallorca, Spain
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Background: Transthyretin-associated Familial Amyloid Polyneuropathy (TTR-FAP) is a frequent disease in our community caused by the deposit of abnormal transthyretin on the tissues, mainly on the nerves. The clinical affectation and laboratory test alterations depend on the clinical stage and the moment of disease diagnosis.
Methods: A cross-sectional, observational study was performed. Medical records and laboratory test information of 20 patients: 10 symptomatic patients and 10 asymptomatic carriers.
Results: Out of a total of 20 patients: 14 women (70 %) with a median age of 47.5 years. All of asymptomatic carriers were diagnosed for family history and 90 % of the symptomatic patients had neurologic impairment demonstrated with pathological electroneurography (NC) (p=0.016). The symptomatic patients had higher variability of blood pressure both systolic (p=0.016) and diastolic (p=0.045) and of heart rate (p<0.005). Regarding laboratory test alterations this patients presented a decrease of free T4 (p<0.005) and an increase of cystatine C (p=0.046). As for the comparison by age-at-onset in 9 (45 %) cases the diagnosis was late-onset and 11 (55 %) early-onset. Mean age was 38.55 vs 61.56 years (p<0.005). The 63.6 % of the patients less than 50 years old were diagnosed for family history and all were asymptomatic, 62.5% of them had normal NC and 9.1 % were in clinical stage II. The late-onset in comparison with the other group had a decrease of total proteins (p=0.008) and an increase of Blood urea nitrogen (p<0.005) and cystatine C (p=0.04).
Conclusions: Symptomatic patients were diagnosed by the presence of neurologic symptoms by pathological NC, postural hypotension, and laboratory abnormalities of kidney and thyroid function. As to the comparison of age-at-onset, the early-onset has greater family history, minor number of affected organs, low neurological involvement and mild symptoms.

P31

Neurophysiological pitfalls in TTR-FAP Val30Met

Isabel Conceição*, Cristiana Silva, Mariana Costa, José Castro
Centro Hospitalar Lisboa Norte - Hospital de Santa Maria, Department of Neurosciences, 1649-028, Lisbon, Portugal
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Introduction: The neurological hallmark of TTR-FAP is a length-dependent axonal neuropathy that initially involves the unmyelinated and small myelinated nerve fibers that mediate pain and temperature sensation, causing sensory disturbances that typically start in lower limbs. Subsequent degeneration of larger myelinated fibers results in large fiber sensory deficit and muscle weakness.
The disease can be difficult to recognize due to extreme phenotypic heterogeneity and nonspecific clinical symptoms even within the same mutation.
In TTR-FAP related to Val30Met mutation, different neuropathy phenotypes have been reported mainly in patients from non endemic areas as well in late onset cases.
Case report: We described 3 TTR-FAP Val30Met early onset cases from endemic areas with a different neuropathy phenotype that can be easily misdiagnosed as a different entity. One case presented as a bilateral carpal tunnel syndrome without neuropathy; another with a demyelinating neuropathy with predominant upper limb involvement and other with a demyelinating neuropathy with conduction blocks mimicking a CIDP.
Conclusion: TTR-FAP is frequently misdiagnosed, e.g. as idiopathic polyneuropathy or chronic inflammatory demyelinating polyneuropathy, and may be greatly under diagnosed. However, early accurate diagnosis of TTR-FAP is crucial for effective disease control.

P32

Prevalence, risk factors and correlation with cardiac involvement of carpal tunnel syndrome in amyloidosis

Agnese Milandri[1*], Simone Longhi[1], Christian Gagliardi[1], Mario Cinelli[1], Serena Foffi[1], Ilaria Bartolomei[2], Fabrizio Salvi[2], Claudio Rapezzi[1]
[1]Diagnostic and Specialty Medicine – DIMES, Alma Mater Studiorum, University of Bologna, Cardiology, 40138, Bologna, Italy; [2]Bellaria Hospital, Neurology, 40100, Bologna, Italy
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Background: Carpal tunnel syndrome (CTS) is one of the most common clinical manifestations of TTR-related amyloidosis, both hereditary (ATTR), and wild type (senile systemic amyloidosis, SSA) and often precedes cardiac symptoms. The exact prevalence of CTS in light-chain amyloidosis (AL), ATTR and SSA is not known. We therefore aimed to establish prevalence, risk factors and possible association with cardiac involvement in patients with TTR-related and AL amyloidosis.

Methods: We retrospectively analyzed clinical and instrumental (ECG and echocardographic) findings of 260 patients with TTR-related, and 175 with AL amyloidosis evaluated at our Centre between 1990 and September 2013.
Results: Prevalence was 35% in TTR-related amyloidosis (35% in ATTR and 32% in SSA) and 8% in patients with AL ($p< 0.001$). Among TTR patients, CTS was more frequently associated with cardiac involvement (76% vs. 42%; $p<0.0001$) as reflected by the presence of pathological ECG and echocardiogram. Moreover, CTS manifested 9 years before the onset of cardiac symptoms. Among patients with cardiomyopathy with/without CTS there were no significant clinical/instrumental differences. At univariate analysis male gender and genotype were not associated with CTS.
Conclusion: CTS is specifically associated with TTR-related (but not AL) amyloidosis independently from patient gender. In TTR-related amyloidosis, CTS is more frequently associated with cardiac involvement, even though patients with cardiomyopathy with/without CTS have a comparable clinical/instrumental profile. CTS precedes cardiac symptoms onset by 9 years and this awareness is important for an early diagnosis of amyloidotic cardiomyopathy.

P33

Genotypic and phenotypic presentation of Glu89Gln mutation in Turkey

Hacer Durmus[1*], Zeliha Matur[2], Murat Mert Atmaca[1], Mehves Poda[3], Arman Cakar[1], Piraye Serdaroglu-Oflazer[1], Feza Deymeer[1], Yesim Parman[1]
[1]Istanbul University, Istanbul Medical Faculty, Neurology Department, 34093, Istanbul, Turkey; [2]Istanbul Bilim University, Medical Faculty, Neurology Department, 34394, Istanbul, Turkey; [3]Istanbul University, Institute for Experimental Medical Research, Department of Genetics, 34093, Istanbul, Turkey
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Background: Transthyretin-related familial amyloid polyneuropathy (TTR-FAP) is an autosomal dominant disorder caused by mutations of the transthyretin (TTR) gene. More than 100 different mutations of the transthyretin gene are identified worldwide, but still the first described Val30Met is the most common one. The mutant amyloidogenic transthyretin protein causes systemic accumulation of amyloid fibrils that results in organ dysfunction and death. TTR-associated FAP is a progressive and fatal disease if left untreated and should be considered in the differential diagnosis of any patient with a progressive polyneuropathy, especially with an accompanying autonomic involvement.
Patients and methods: We studied clinical, electrophysiological, histopathological, and genetic characteristics in five patients from two unrelated families with Glu89Gln mutation in TTR gene. Genetic testing was performed upon written informed consent to all patients according to the Declaration of Helsinki.
Results: Mean age of onset was 51.8±9.14 (ranges 37-52) years. Three patients (two male) with Glu89Gly mutation had carpal tunnel syndrome (CTS) as presenting symptom. Two of them had asymmetric parasthesias in feet at presentation. Index patient had vocal cord involvement and spinal stenosis due to amyloid accumulation, which are rare manifestations of TTR-FAP. Vocal cord paralysis caused severe obstructive sleep apnea in this patient; he was treated with BIPAP successfully. All patients, except one patient in very early stage of disease had cardiomyopathy and autonomic involvement. One patient underwent intestinal biopsy due to severe diarrhea and biopsy was compatible with intestinal amyloidosis. Two patients died during follow-up at age 60 and 57 due to the systemic involvement. Patients were followed-up under Tafamidis meglumine treatment.
Conclusion: Our cases suggest that Glu89Gln mutation could present with bilateral CTS and cause some atypical manifestations such as asymmetrical onset, vocal cord involvement and spinal stenosis.

P34

Usefulness of combining electrocardiogram and echocardiography findings and brain natriuretic peptide in early detection of cardiac amyloidosis in subjects with transthyretin gene mutation

Gianluca Di Bella[1*], Fabio Minutoli[2], Anna Mazzeo[3], Matteo Cssale[1], Claudia Stancanelli[3], Scipione Carerj[1], Sergio Baldari[2], Giuseppe Vita[3]
[1]University of Messina, Clinical and experimental medicine department, 98100, Messina, Italy; [2]University of Messina, Department of Biomedical Sciences and of Morphologic and Functional Images, 98100, Messina, Italy; [3]University of Messina, Department of Neurosciences, 98100, Messina, Italy
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Early non-invasive identification of cardiac amyloidosis (CA) is of growing clinical importance. Low voltage on electrocardiogram (ECG), increased left ventricular (LV) septal thickness (ST) and global longitudinal strain (GLS) on echocardiography, and elevated brain natriuretic peptides (BNP) are used as surrogates of CA. Thirty-five patients (50 ± 14 years, 22 females) underwent an ECG to analyze low-voltage QRS (<15 mV) pathological Q-waves, poor R-wave progression, ST-T abnormalities - and left bundle branch block. An ECG was considered abnormal if at least one ECG alteration was present. Echocardiography was used to analyze LVST, E/E' and GLS. All participants also had BNP blood testing. 99mTc-DPD scintigraphy assumed as a reference method showed CA in 18 patients (51%, CA group) and no accumulation in 17 patients (no CA group). In descending order of accuracy, LVST >14 mm, E/E' >6.6, GLS <14.1, BNP >129 pg/ml, and an overall abnormal ECG showed good capability to distinguish patients with and without CA. All these parameters were predictors of CA in univariate analysis while low-voltage QRS showed the worst performance. LVST >14 mm ($p = 0.002$) was the best independent predictor of CA, achieving sensitivity of 78% and accuracy of 89%. However, a LVST >14 mm ($p = 0.005$) plus an abnormal ECG ($p = 0.03$) show together a higher sensitivity, equal to 89%, in identifying CA. An integrated evaluation of ECG and echocardiography is a sensitive and low-cost technical approach to identify CA in patients with transthyretin gene mutation.

P35

TTR sequencing should be considered ahead of hypertrophic cardiomyopathy in Afro-Americans

Jean Herlé Raphalen[1*], Mathieu Kerneis[2], Xavier Waintraub[2], Riadh Cheikh-Khelifa[2], Pierre Fouret[3], Philippe Cluzel[4], Zahir Amoura[1], Fleur Cohen Aubart[1]
[1]Pitié-Salpêtrière Hospital, Internal Medicine Department, e3m Institute, French National Reference Center for Rare Systemic diseases, 75013, Paris, France; [2]Pitié-Salpêtrière Hospital, Cardiology Department, 75013, Paris, France; [3]Pitié-Salpêtrière Hospital, Anatomo-pathology Department, 75 013, Paris, France; [4]Pitié-Salpêtrière Hospital, Cardiovascular and Interventional Imaging Department, 75 013, Paris, France
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Background: Amyloid cardiomyopathy is a polymorphic condition with heterogeneous prognosis. Whereas AL amyloid cardiomyopathy is the most frequent type of amyloid cardiopathy, transthyretin (TTR) amyloidosis is often under diagnosed. TTR gene sequencing may be easily performed although usually used after a histological proof of amyloidosis is obtained. We conducted a prospective study to evaluate the interest of TTR gene sequencing in hypertrophic cardiomyopathy with suspicion of amyloidosis before obtaining a histological proof of amyloidosis.
Methods: All patients referred for hypertrophic cardiomyopathy between January 2014 and April 2015 with suspicion of amyloidosis on echocardiography or cardiac MRI were screened with light chain dosage, protein electrophoresis and C-reactive protein dosage. When these exams were normal, they were included in the study and underwent TTR gene sequencing.
Results: Eight patients were included in the study, 7 men and 1 woman, median age at the time of inclusion 74 years. All patients had clinical signs of congestive heart failure with elevated NT-proBNP levels. No familial history was noted. Among them, 4 patients, all from carribean origin, were diagnosed as having hereditary TTR cardiac amyloidosis by sequencing of the TTR gene. These 4 patients had hypertrophic cardiomyopathy with altered diastolic and systolic function, diagnosed respectively 7, 3, 2 years and six months before. Previous exams had eliminated ischemic and valvular cardiomyopathy. Echocardiographic study revealed increase of left ventricular wall thickness predominant on septum wall with shiny appearance. LV ejection fraction was between 35 and 50 %. Two out the 4 patients underwent cardiac MRI which showed diffuse delayed gadolinium enhancement suggestive of amyloidosis. As all the patients included in the study, these 4 patients did not have

monoclonal gammapathy and light chain dosage was normal. Transthyretin sequencing revealed the presence of a missense mutation Val122Ile (c.424 G>A) in these 4 patients. One of the patients underwent a cardiac transplantation and pathological examination confirmed amyloid cardiopathy. Only 1 other patient had a confirmation of amyloid deposit after an extensive work-up (patient #8, see below).
Among the 8 patients, 8 had minor salivary gland biopsy which revealed presence of amyloidosis deposits in only 1 (patient #8, with a Val122Ile TTR mutation), 3 had abdominal fat aspiration (normal in all), 2 underwent rectal mucosal biopsy (normal in all). Among the 4 patients who did not have a TTR mutation, 3 underwent a endomyocardial biopsy to confirm the amyloid nature of the cardiopathy which was positive in all 3. Final diagnosis was AA amyloidosis for one, senile TTR for one and non-typed amyloidosis for the last one.
Conclusion: TTR sequencing is a specific, non-invasive way to diagnose TTR amyloid cardiomyopathy and should be considered after exclusion of alternative causes of hypertrophic cardiopathy.

P36

Delayed small bowel octreotide response in patients with hereditary transthyretin amyloidosis

Wixner Jonas[1*], Ole Suhr[1], Pontus Karling[1], Greger Lindberg[2]
[1]Umeå University, Dept of Public Health and Clinical Medicine, 90187, Umeå, Sweden; [2]Karolinska University Hospital Huddinge, Dept of Medicine, 14186, Stockholm, Sweden
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Background: Gastrointestinal (GI) complications such as constipation, diarrhea and gastroparesis are common in hereditary transthyretin (ATTR) amyloidosis. The mechanisms behind these disturbances have not been fully elucidated and the patients' small bowel function remains largely unexplored. The aim of the present study was to compare the small bowel motility in patients with hereditary ATTR amyloidosis with that in non-amyloidosis controls.
Methods: Ambulatory 24-hour small bowel manometries were performed at Karolinska University Hospital, Huddinge, Sweden. Jejunal recording sites and standardized meals were used during the tests. The somatostatin analogue octreotide (50 μg subcutaneously) was used for inducing fasting motility two hours after the last test meal (breakfast). Patients with hereditary ATTR amyloidosis undergoing evaluation for liver transplantation were consecutively selected for manometry (n = 19), and for each patient three age and gender matched controls (n = 57) with functional GI disorders were selected for comparison. Patients with an age at onset of 50 years or more were defined as late-onset cases. Non-parametrical tests were used for all statistical analyses.
Results: The patients' median age at examination was 52.8 (30.8-66.5) years and the median duration of symptomatic disease was 2.3 (0.5-9.7) years. A majority (89%) of the patients carried the V30M mutation, 58% had GI symptoms and 84% had a PND score of I. Small bowel manometry was judged to be normal in 42% of the patients and 74% of the controls ($p = 0.01$). Patients displayed significantly more phase III migrating motor complexes during day-time than the controls (in median 4 vs. 2, $p < 0.01$), and had a delayed response to octreotide (in median 5.0 min vs. 3.8 min, $p = 0.02$). Low-amplitude complexes were more common in patients than in controls (16% vs. 4%), however, this difference did not reach statistical significance ($p = 0.10$). Among the patients, late-onset cases showed a longer delay in octreotide response (in median 5.4 vs. 4.3 min, $p = 0.03$), but no major difference related to gender, presence of GI symptoms, PND score or TTR mutation was found for any of the variables.
Conclusions: Patients with early-stage hereditary ATTR amyloidosis only showed minor abnormalities in their small bowel motility. The main finding was a delayed response to octreotide injection, which may reflect an autonomic neuropathy and changes in the neuroendocrine system of the gut, including a depletion of interstitial cells of Cajal and a reduction of endocrine cells. Surprisingly, late-onset cases had a longer delay in octreotide response, however, this might be an age-related finding.

P37

What to do when the neuropathy worsens after successful heart and liver transplantation in a Glu89Lys Transthyretin Amyloidosis?

Thierry Kuntzer*, Francois Ochsner
Lausanne University Hospital CHUV, Service of Neurology, Department of Clinical Neurosciences, 1011, Lausanne, Switzerland
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Background: Our patient presented dramatically with a previously unrecognized FAP complicated by heart failure requiring heart transplantation at the age of 49 years and liver transplantation at the age of 51 years. Direct DNA sequencing of the TTR gene showed a heterozygous Glu89Lys mutation in the proband and her daughter (published in Transplantation 2011).
Methods: Longitudinal follow-up with clinical scores with ancillary testings.
Results: After her double transplantation, the patient reported over the 8 subsequent years slowly progressive increasing pain and loss of sensation in the feet, slow bowel habit, and delayed urine flow. On examination, we found orthostatic hypotension, sensory loss, muscle weakness, and mild atrophy in the distal lower extremities with reduced Achilles tendon reflexes. Nerve conduction studies revealed a mild decrease of amplitude of motor and absent sensory action potentials and normal velocities except for bilateral slowing within the carpal tunnels. The sympathetic skin response and Sudoscan responses to electrical stimuli was normal in the palms but not in the soles. No complications were seen, such as acute rejection, portal vein thrombosis, or infectious diseases resulting from administration of immunosuppressive drugs.
Conclusions: As already described, some late-stage patients continue to show FAP progression even after liver transplantation, and longstanding disease is correlated with increased morbidity related to continuing amyloid fibril formation.

P38

[18F]FDDNP performed better than [18F] Florbetapir to distinguish transthyretin cardiac amyloidosis (TTR-CA) patients from healthy controls: an ex vivo study

Anne-Claire Dupont[1*], Denis Guilloteau[3], Rana Ben-Azzouna[2], Rudy Bidault[3], Vincent Algalarrondo[4], Michel Slama[4], Nicolas Arlicot[1], Dominique Le Guludec[2]
[1]CHRU TOURS, Nuclear medicine, 37000, Tours, France; [2]INSERM, U698, 78000, Paris, France; [3]INSERM, U930, 37000, Tours, France; [4]AP-HP, Nuclear medicine, 78000, Paris, France
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Background: TTR-CA is characterized by extracellular depositions of amyloid β (Aβ) which can lead to arrhythmias, heart failure and even sudden death. While early diagnosis of TTR-CA has important therapeutic and prognostic impact, there is no sensitive and quantitative tool to document the location and extent of cardiac Aβ in these patients. So we aimed to test and compare the usefulness for TTR-CA early diagnosis of both [18F]florbetapir and [18F]FDDNP, 2 PET tracers validated for brain detection of Aβ
Methods: Binding of both radiopharmaceuticals to Aβ was evaluated in myocardial tissue from patients who underwent cardiac transplantation either for TTR-CA, or ischemic heart failure as control. Heart sections were incubated with [18F]florbetapir or [18F]FDDNP at concentration of 3nM. Nonspecific binding was assessed by incubation of adjacent sections in the presence of an excess of cold ligand. Autoradiograms were treated with a grey-level analysis method. Regions of interest were delimited and the modal grey value were determined.
Results: [18F]FDDNP uptake in TTR-CA myocardial sections (nP=6) was significantly higher (+86%) compared to controls (nT=3) whereas no significant difference was observed with [18F]florbetapir (nP=4 and nT=2, +32%, p=0.13). The mean ratio (specific binding patient/specific binding controls) were 11.9±2.0 for [18F]FDDNP and 1.5±0.1 for [18F]florbetapir (comparison : p=0.01). Nevertheless, the intensity of both radiotracers

binding strongly decreased in sections with unlabeled ligand (-74%, and -83 respectively), suggesting Aβ specificity.
Conclusion: [18F]FDDNP and [18F]florbetapir, are able to bind ex vivo specifically to Aβ in heart tissue. The largely improved ratio of specific binding (patient/controls) of [18F]FDDNP, compared to [18F]florbetapir, strongly suggests its better sensibility and then diagnostic potential to discriminate in vivo ATTR patients from healthy subjects.

P39

Characterization of conformation-specific, human-derived monoclonal antibodies against TTR aggregates with potential for diagnostic and therapeutic use

Aubin Michalon[1*], Benoit Combaluzier[1], Evita Varela[1], Andreas Hagenbuch[1], Ole Suhr[2], Maria Saraiva[3], Jan Grimm[1]
[1]Neurimmune, 8952, Schlieren, Switzerland; [2]Umeå University, 90187, Umeå, Sweden; [3]IBMC, 4150, Porto, Portugal
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**P39

Misfolding and aggregation of transthyretin (TTR) is the basic pathophysiological mechanism of hereditary and wild type TTR amyloid (ATTR) amyloidosis. Polyneuropathy and/or cardiomyopathy with heart failure dominates the clinical presentation of the disease. Conformational changes of the TTR protein structure produce toxic intermediates that introduce cell death and ultimately loss of organ function. Reliable and early diagnosis of ATTR amyloidosis are, however, difficult to obtain based on the patients' clinical presentation, since different types of neuropathies or cardiomyopathies often exhibit similar clinical findings, especially early after onset of disease.
Since misfolded TTR aggregates constitutes an early phase of amyloid formation, availability of a diagnostic tests able to detect misfolded TTR aggregates either biochemically or by imaging techniques would facilitate a correct and early diagnosis of ATTR amyloidosis.
By comprehensive genetic sequence analyses of the human immune repertoire for TTR-specific memory B-cells, we cloned and recombinantly expressed human-derived monoclonal antibodies, which specifically bind with high affinity and selectivity to misfolded TTR but not physiological TTR tetramers. These antibodies specifically detect ATTR deposits in ATTR amyloidosis patients' biopsies and TTR aggregates in corresponding tissues from TTR transgenic mouse-models. The selectivity of the human antibodies for misfolded TTR is driven by their targeting of cryptic or conformational epitopes which are exposed in misfolded and/or aggregated wild type as well as mutant TTR.
These conformation-specific, human-derived monoclonal antibodies are promising candidates for diagnostic use, and for the development of disease-modifying therapies for TTR amyloidosis by targeting and facilitating the removal of disease-causing TTR aggregates.

P40

MR-Neurography of the sural nerve in patients with hereditary amyloidosis

Jennifer Kollmer[1*], Markus Weiler[2], Jan Purrucker[2], Ute Hegenbart[3], Stefan Schönland[3], Christoph Kimmich[3], Martin Bendszus[1], Mirko Pham[1]
[1]University of Heidelberg, Department of Neuroradiology, 69120, Heidelberg, Germany; [2]University of Heidelberg, Department of Neurology, 69120, Heidelberg, Germany; [3]University of Heidelberg, Medical Department V (Amyloidosis Center), 69120, Heidelberg, Germany
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Background: Sural nerve biopsies are often performed in order to detect the underlying disease in patients suffering from unclear polyneuropathic symptoms. In transthyretin familial amyloid-polyneuropathy (TTR-FAP) the diagnostic value of invasive sural nerve biopsies is controversially discussed as it often lacks to detect amyloid deposits [Simmons et al, J Neurol Sci 1993]. As we recently reported, amyloid related nerve-injury in TTR-FAP can be unambiguously determined in large-caliber nerves (sciatic, tibial and common peroneal nerve) by applying high-resolution MR-Neurography (MRN) [Kollmer et al, Brain 2015]. However, the diagnostic yield of MRN of the small-caliber sural nerve, representing the target nerve specimen for biopsies, is still unclear and was subject to this investigation.
Methods: We prospectively enrolled 25 patients with manifest TTR-FAP, 10 asymptomatic gene-carriers with confirmed mutations in the TTR-gene, and 40 age/gender-matched healthy volunteers. Besides detailed neurological and electrophysiological examinations in all patients, a sural nerve biopsy was obtained in 12/25 manifest TTR-FAP patients. All participants underwent the following high-resolution MRN protocol (3Tesla/Magnetom/TIM-TRIO/Siemens):1) axial 2D-T2-TSE-fs (TR/TE 5970/55ms, voxel-size 0.4x0.3x3.5mm^3); 2) axial 2D-dual-echo-TSE-fs (TR 5210ms, TE1/TE2 12/73ms, voxel-size 0.4x0.3x3.5 mm^3).
On each axial imaging slice the sural nerve was identified and manually segmented. After signal-normalization (histogram-based, comparison with control population), nerve-voxels were statistically classified as nerve-lesion-voxels by operator-independent, threshold-based segmentation. The apparent-T2-relaxation-time and proton-spin-density were calculated for all nerve-lesion-voxels.
Results: Sural nerve lesion-voxels were found to be significantly higher in manifest TTR-FAP vs. controls ($p<0.0001$), in asymptomatic gene-carriers vs. controls ($p<0.0001$) and in manifest TTR-FAP vs. asymptomatic carriers ($p=0.0035$). Wilcoxon rank-sum-test revealed with high statistical significance that proton-spin-density was higher in severely affected TTR-FAP patients ($p<0.0001$), in moderate TTR-FAP ($p<0.0001$) and also in asymptomatic gene-carriers ($p=0.0003$) compared to healthy controls. The apparent-T2-relaxation-time was significantly increased in symptomatic TTR-FAP ($p<0.05$) but not in asymptomatic gene-carriers ($p=0.4286$) compared to controls.
Conclusion: MRN of the sural nerve is a new, non-invasive and highly sensitive diagnostic tool, which can clearly differentiate between symptomatic TTR-FAP, asymptomatic gene-carrier status and healthy controls by evaluating nerve-lesion-voxels and proton-spin-density. Additional analyzes of the apparent-T2-relaxation-time can further confirm symptomatic disease. Results of this evaluation may have a strong impact for a better diagnostic interpretation of negative sural nerve biopsies.

P41

Disphosphonates cardiac uptake in familial amyloid neuropathy: Comparison between DPD and HMDP

Hamza Regaieg[1*], Renata De Paola Chequer[1], Rana Ben Azzouna[1], Vincent Algalarrondo[2], Besma Mahida[1], Michel Slama[2], Dominique Le Guludec[1], François Rouzet[1]
[1]Bichat-Claude Bernard university hospital, Department of nuclear medicine, 75018, Paris, France; [2]Antoine Béclère university hospital, Department of cardiology (NNERF), 92140, Clamart, France
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Background: Familial amyloid polyneuropathy (FAP) is a severe hereditary disease, due to production by the liver of a genetic variant transthyretin (TTR) resulting in tissue amyloid deposits. Cardiac involvement is of major prognostic value. Diphosphonate scintigraphy has been proposed as a diagnostic tool for TTR-related cardiac amyloidosis, but there is no consensus on the optimal radiopharmaceutical. Consequently, we compared the cardiac uptake of two 99mTc-labelled tracers: diphosphono-propanedicarboxylic acid (DPD) and hydroxymethylene diphosphonate (HMDP) in patients with TTR-FAP.
Methods: 122 consecutive patients with TTR-FAP were prospectively included and received randomly DPD or HMDP. Acquisitions (whole-body (WB) and chest SPECT) were performed 3 hours after intravenous injection of the tracer. Quantification of myocardial uptake on WB acquisitions was performed by use of the ratio between the geometric mean of either total or average counts of a region of interest (ROI) drawn over the heart area and the WB total or average counts (H/WBtotal or H/WBaverage). Quantification on SPECT acquisitions was performed by the ratio between 3D isocount volume of interest generated over the myocardium and a standard volume in the right lung (H/L). Quantification of soft tissues uptake was performed by use of ratio between average counts of a ROI drawn over the lumbar spine and a ROI drawn over soft tissues of the lower limb (B/ST) on the WB acquisition.
Results: The DPD and HMDP groups of patients had similar age (62±15 vs. 59±14 years respectively; p=0.3), sex (males: 67% vs. 58% respectively; p=0.4), TTR mutation (Val30Met: 70% vs. 80% respectively; p=0.3) and

activity of the tracer (DPD: 713±86 MBq vs. HMDP: 709±124 MBq; p=0.9). Quantitative parameters derived from whole body acquisition were significantly greater with DPD compared to HMDP (H/WBtotal: 3.8±2.7 vs. 2.4±2.1 respectively; p=0.002 and H/WBaverage: 5.2±2.2 vs. 4.3±1.3 respectively; p=0.01) as well as H/L derived from SPECT acquisition (3.9±3.7 vs. 2.0±1.9 respectively; p=0.001). The uptake by soft tissues was also greater in the DPD compared to HMDP group (B/ST: 3.6±2.0 vs. 5.7±2.9 respectively; p<0.0001).

Conclusion: The present study shows that in patients with TTR FAP, the uptake of DPD in heart and other soft tissues is superior to that of HMDP. This suggests that DPD should be prioritized for initial assessment of patients suspected of cardiac involvement of TTR-related amyloidosis. Further study is required to assess whether this difference impacts the diagnostic performance and whether DPD is more accurate for the assessment of therapy response.

P42

The diagnostic accuracy of Sudoscan in TTR-FAP

Jose Castro[2*], Isabel Conceicao[2], Isabel Castro[1], Mamede de Carvalho[2]
[1]Centro Hospitalar Lisboa Norte - Hospital de Santa Maria, Department of Neurosciences, 1600, Lisboa, Portugal; [2]Instituto de Medicina Molecular, Faculty of Medicine, Translational and Clinical Physiology Unit, 1600, Lisboa, Portugal
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Introduction: Small-fibre length-dependent sensory-motor and autonomic neuropathy is the hallmark of TTR-FAP.

SUDOSCAN was recently introduced as a quick and non-invasive method that measures electrochemical skin conductance (ESC) of palmar and plantar surfaces, through reverse iontophoresis. It has been described as a promising diagnostic tool in distal symmetric polyneuropathies, such as diabetic small fibre neuropathy.

Objective: To evaluate the diagnostic accuracy of Sudoscan in patients with TTR-FAP.

Methods: Forty stage I TTR-FAP patients were compared with 70 TTR-FAP asymptomatic carriers and 37 healthy controls, matched for age, gender and body-mass index. Inclusion criteria for TTR-FAP patients included normal sural nerve sensory action potential amplitude and plantar sympathetic skin response (SSR). Patients with diabetes were excluded. All subjects were assessed with Sudoscan in hands and feet, bilaterally.

Results: Feet ESC was significantly reduced in Stage I patients compared with asymptomatic carriers and controls (57.8 ± 24.3 vs 76.5 ± 7.8 and 79.7 ± 5.1; p < 0.000). Hands ESC did not show significant difference between groups.

Receiver operating characteristic curve analysis revealed an area under the curve of 0.80 for the plantar ESC.

A significant correlation was found between plantar and Sural nerve action potential amplitude (0.320; p < 0.001).

Conclusion: Sudoscan seems to be a promising diagnostic tool in TTR-FAP patients with normal conventional nerve conduction studies and preserved plantar SSR. However, its predictive value is unknown.

P43

Comparison of MIBG and Diphosphonate scintigraphy in cardiac involvement of aTTR-FAP

Renata Chequer[1*], Ludivine Eliahou[2], Hamza Regaieg[1], Vincent Algalarrondo[2], Sylvie Dinanian[2], Dominique Le Guludec[1], Michel S Slama[2], Francois Rouzet[1]
[1]Bichat Hospital, Nuclear Medicine, 75018, Paris, France; [2]Antoine Béclère Hospital, Cardiology and French Reference Center for Rare Diseases for FAP (Familial Amyloid Polyneuropathy), 92140, Clamart, France
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Background: In familial aTTR amyloid polyneuropathy (FAP) cardiac involvement is of major prognostic value. Two approaches using radionuclide imaging proved relevant in the assessment of aTTR-related cardiac amyloidosis: detection of amyloid deposits with disphosphonates (DPD) and of sympathetic denervation with MIBG. The study aimed to compare the respective value of both approaches in patients with aTTR-FAP with suspected cardiac involvement.

Methods: We prospectively included 76 consecutive patients identified from the database of the French National Reference Center for Amyloidosis with genetically proven aTTR-FAP (males 62%, Val30Met: 42%, domino-liver transplantation 13%, symptomatic 57%, interventricular septum (IVS)≥12 mm: 52%; left ventricular ejection fraction: 63±10%). They underwent both MIBG and DPD scintigraphy in a delay <3 months. For DPD SPECT, acquisitions were performed 3 hours after tracer injection. Cardiac uptake was visually scored as present or absent and quantified by the ratio between 3D isocount volume of interest generated over the myocardium and a standard volume in lung (H/L). For MIBG, heart-to-mediastinum ratio (HMR) was calculated on planar acquisitions performed 4 h after tracer injection. Cardiac MIBG was classified as normal, mildly, moderately, or severely abnormal.

Results: The delay between DPD and MIBG 6±12 days. DPD was abnormal in 30 patients (39%) and MIBG in 50 patients (66%; p=0.002). When MIBG was normal (n=26), BS was normal except for 1 patient. When MIBG was abnormal (n=50), BS was normal in 21 patients (42%). The uptake of DPD increased with the denervation score (normal: 0.6±0.2; mild: 0.6±0.4, moderate: 3.4±3.3; severe: 4.5±3.7; p<0.001 between normal and moderate/severe). In patients with a previous domino liver transplantation (n=10), the overall pattern was similar. In asymptomatic patients (n=31), all those with a normal MIBG (n=17) had a normal DPD; MIBG was abnormal in 45% (n=14), 50% had a normal DPD. In addition, HMR was greater (1.8±0.3 vs. 1.6±0.4; p=0.008) and H/L was lower (1.7±2.3 vs. 3.2±3.5; p=0.04) compared to symptomatic patients.

Conclusion: In patients with suspected cardiac involvement of aTTR-FAP, MIBG was abnormal more frequently than DPD. In particular, DPD abnormalities are delayed compared to MIBG since it was abnormal only when denervation was moderate or severe. In the group of asymptomatic patients, MIBG was abnormal in 45% of patients, and only half of those with cardiac denervation had a positive BS. This suggests that innervation abnormalities as seen with MIBG are more frequent and earlier than significant amyloid deposits as seen with Diphosphonates.

P44

Cardiac extracellular volume quantified with T1 mapping techniques reflects degree of cardiac and neurological involvement in Hereditary Transthyretin Amyloidosis

Esther González-López[1*], María Gallego-Delgado[1], Francisco Muñoz-Beamud[2], Juan Buades[3], Lucía Galán[4], Jose Luis Muñoz Blanco[5], Jesús González-Mirelis[1], Pablo García-Pavía[1]
[1]Hospital Puerta de Hierro, Cardiology, 28222, Majadahonda, Spain; [2]Hospital Juan Ramón Jiménez, Internal Medicine, 21005, Huelva, Spain; [3]Hospital Sont Llàzer, Internal Medicine, 07198, Palma de Mallorca, Spain; [4]Hospital Clínico San Carlos, Neurology, 28040, Madrid, Spain; [5]Hospital Gregorio Marañón, Neurology, 28007, Madrid, Spain
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Background: Amyloidotic cardiomyopathy (AC) in Hereditary Transthyretin Amyloidosis (ATTR) determines prognosis and treatment options. Cardiac Magnetic Resonance (CMR) has shown its utility in the diagnosis and characterization of AC. Moreover, CMR T1 mapping techniques are useful to assess myocardial extracellular volume (ECV) fraction in AC.

We hypothesized that ECV allows identification of AC in ATTR patients and that there is a correlation between cardiac ECV and the degree of neurological impairment caused by TTR amyloid extracardiac deposits.

Methods: 31 genetic proven ATTR patients at different stages of the disease (19 males; mean age 49±12 years; 26 with Val30Met mutation) underwent a T1 mapping CMR study and a neurological evaluation with NIS-LL score (sensitive, motor and reflex examination), Norfolk-QOL questionnaire (symptoms and quality of life) and Karnofsky index (general health status). AC was defined by positive 99mTc-DPD scintigraphy (uptake grade>2) or left ventricular hypertrophy >12mm. with typical gadolinium kinetics/enhancement of amyloidosis at CMR in the absence of DPD-scan (9 patients).

Results: 5 patients had AC (all of them determined by scintigraphy). Mean ECV was increased in patients with AC (0.490±0.131 vs. 0.289±0.035; P=0.026). ECV correlated with age (R=0.467; P=0.008), NTproBNP (RS=0.846; P<0.001), maximum wall thickness (R=0.621; P<0.001), left ventricular mass index (R=0.685; P<0.001), left ventricular

ejection fraction (R=-0.378;P=0.036), NIS-LL (RS=0.604; P=0.001), Norfolk-QOL (RS=0.529; P=0.003) and Karnofsky (RS=-0.517; P=0.004). A cut-off value of ECV=0.357 calculated by ROC curve, was diagnostic of AC with 100% sensibility and specificity (P<0.001). ECV and NTproBNP values were the only cardiac parameters that significantly correlated with neurological scores.
Conclusions: ECV quantification by CMR allows identification of AC in ATTR and correlates with the degree of neurological impairment. This non-invasive technique could be a useful tool for early diagnosis and to track cardiac and extracardiac amyloid disease.

P45

Coexistence of degenerative aortic stenosis and wild type transthyretin-related cardiac amyloidosis: a potentially dangerous association that can be non-invasively identified

Simone Longhi[1*], Agnese Milandri[1], Christian Gagliardi[1], Massimiliano Lorenzini[1], Francesco Saia[1], Ornella Leone[2], Pier Luigi Guidalotti[3], Claudio Rapezzi[1]
[1]Department of Experimental, Diagnostic and Specialty Medicine – DIMES, Alma Mater Studiorum, University of Bologna, Cardiology, 40138, Bologna, Italy; [2]Department of Pathology, Sant'Orsola-Malpighi Hospital, Pathology, 40138, Bologna, Italy; [3]Department of Experimental, Diagnostic and Specialty Medicine – DIMES, Alma Mater Studiorum, University of Bologna, Nuclear Medicine Unit, 40138, Bologna, Italy
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Background: Degenerative aortic stenosis (AS) and wild type transthyretin (TTR)-related cardiac amyloidosis (wt-ATTR) share a common demographic and clinical profile. It has been recently suggested that the coexistence of wt-ATTR could negatively influence the outcome of elderly patients with aortic stenosis undergoing transcatheter aortic valve replacement (TAVR). TTR-related cardiac amyloidosis can be accurately identified by technetium-99m-3, 3-diphosphono-1, 2 propanodicarboxylic acid (99mTc-DPD) scintigraphy. We decided to investigate the coexistence of cardiac amyloidosis in elderly patients with aortic stenosis referred for aortic valve replacement (TAVR or surgery).
Methods: Since October 2014 we prospectively evaluated with 99mTc-DPD scintigraphy all patients diagnosed with degenerative AS and one or more of the following: paradoxical low flow-low gradient severe AS, QRS voltage-left ventricular (LV) wall thickness mismatch, echocardiographic findings suggestive of myocardial infiltrative disease (increased thickness of atrio-ventricular valves or interatrial septum or right ventricular free wall, pericardial effusion, granular sparkling of ventricular myocardium). Cases with intense myocardial tracer uptake underwent endomyocardial biopsy (EMB).
Results: Five out of 42 patients underwent 99mTc-DPD scintigraphy that showed strong myocardial uptake in all. EMB demonstrated TTR-related amyloid infiltration in all cases. Genetic analysis excluded TTR gene mutations; so wt-ATTR was diagnosed. Median age was 88 (range 86-91), 3/5 were males. Two had a history of carpal tunnel syndrome and all were symptomatic for exertional dyspnoea (NYHA class III-IV). At echocardiography mean LV wall thickness was 18±2 mm, LV ejection fraction was 54±10% (38%-64%). Functional aortic valve area was between 0.4 and 0.9 cm2;one case had a low flow-low gradient and reduced LV ejection fraction (38%); maximum aortic gradient in the other 4 cases was 59±30 mmHg. Atrio-ventricular valve thickening was present in all, and mild pericardial effusion was present in 3 cases. Tissue Doppler S wave was reduced in all cases. QRS voltage was normal in one and increased in 4 patients.
Conclusion: Coexistence of degenerative AS and wt-ATTR cardiac amyloidosis (a potentially dangerous condition in patients undergoing AVR or TAVR) can be suspected by clinical and echocardiographic elements and effectively diagnosed by 99mTc-DPD scintigraphy.

P46

MALDI spectrometry for salivary samples analysis : a new tool for TTR amyloidosis diagnosis

Julie Seguier[1*], Claude Villard[2], Laurie Anne Maysou[3], Gilbert Habib[3], Annie Verschueren[4], Pauilne Belenotti[1], Daniel Lafitte[2], Jacques Serratrice[5]
[1]CHU Timone, Médecine Interne, 1/2, Marseille, France; [2]INSERM UMR 911, Plateforme protéomique PIT2, 2, Marseille, France; [3]CHU Timone, Service de Cardiologie, 3, Marseille, France; [4]CHU Timone, Service de neurologie, 4, Marseille, France; [5]Hopitaux Universitaires de Genève, Service de Médecine Interne Générale, 5, Genève, Suisse
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Background: Amyloidosis suffers from a lack of accurate diagnosis tools. It results from a wrong folding of specific proteins and their identification is essential for proper medical care. Today most patients cases are identified thanks to immunohistochemistry analysis after surgery or biopsy on the defective tissues. The aim of our study is to show that diagnosis and typing of TTR amyloidosis can be immediately and rapidly achieved on formaline fixed and paraffin embedded biopsy samples using MALDI spectrometry, and also on salivary samples.
Methods: Four fresh or formalin fixed and paraffin embedded Myocardial and salivary glands samples were analyzed. A specific de-waxing protocol using trypsic digestion and antigen retrieval was used for paraffin embedded samples. After CHCA matrix deposit, MALDI mass spectromerty acquisition was performed using MALDI-TOF, MALDI-TOF-TOF et MALDI QIT-TOF. bottom approaches using Electrospray mass spectrometry on high resolution orbitrap instruments was also performed on salivary samples.
Results: On tissue samples the m/z ratio peak was 1366.78. Its MS/MS analysis allows to obtain m/z 1348.70, 1192.59, 1045.54, 946.46 ions, in other words, the following 4 ions coming from the 22-34 TTR-peptide GSPAINVAVHFR. On salivary samples the m/z ratio was 15991 matching with transthyretin. After MS/MS fragmentation, m/z 1047.5113 and 1051.5232 ions were identified corresponding respectively to Wild Type TTR and mutated THR 49ILE.
Conclusions: TTR is responsible for amyloid deposits. Formalin fixed and paraffin embedded samples can be ex post analyzed after a specific de-waxing protocol by MALDI mass spectroscopy. Mutated TTR can also be identified on salivary samples. Such an approach has to be evaluated in further studies.

P47

Quantitative comparison between amyloid deposition detected by 99mTc-diphosphonate imaging and myocardial deformation evaluated by strain echocardiography in transthyretin related cardiac amyloidosis

Gianluca Di Bella[1*], Fabio Minutoli[2], Anna Mazzeo[3], Claudia Stancanelli[3], Luca Gentile[3], Sergio Baldari[2], Scipione Carerj[1], Giuseppe Vita[3]
[1]University of Messina, Clinical and experimental medicine department, 98100, Messina, Italy; [2]University of Messina, Department of Biomedical Sciences and of Morphologic and Functional Images, 98100, Messina, Italy; [3]University of Messina, Department of Neurosciences, 98100, Messina, Italy
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Aims: The aim of our study is to assess the effect of amyloid deposition on myocardial function.
Methods and results: 28 patients with transthyretin mutation and a group of 14 controls underwent echocardiography to quantify left ventricular (LV) dimensions and function, and global (G) longitudinal (L), radial (R) and circumferential (C) strain (S). 99mTc-3, 3-diphosphono-1, 2-propanodicarboxylic-acid-scintigraphy (99mTc-DPD) was used to quantify cardiac amyloidosis (CA). 99mTc-DPD revealed accumulation in 14 of 28 patients (CA-group) and no accumulation (no CA-group) in 14 patients. Cardiac accumulation was mild-moderate in 5 (Mild-Moderate CA-group) and severe in 9 patients (Severe CA-group). Severe CA-group showed higher values of LV septal thickness (LVST), posterior wall thickness and E/E' ratio than the no CA-group and the control group (adj. p<0.05). Ejection fraction was similar among groups (p=0.65). GLS was lower (p=<0.001) in severe CA-group (-12.2 ± 4.5) respect to no CA-group (-19.3 ± 3.0) and to the control group (-20.9 ± 2.5). On the contrary, GCS and GRS were lower (p=<0.05) in mild-moderate CA-group (-10.8 ± 4.1 and 9.5 ± 5.7, respectively) respect to the severe CA-group (-18.9 ± 5.1 and 23.9 ± 6.3 respectively), no CA-group (-19.2 ± 4.1 and 28.4 ± 10.2 respectively) and the control group (-23.9 ± 4.4 and 29.9 ± 8.7 respectively). A correlation was found between the scintigraphic heart retention (HR) index and LVST (ρ=0.72; p<0.001) and E/E' (ρ=0.46; p=0.03). An inverse tendency was observed between HR and GLS (ρ= −0.40; p=0.06).
Conclusions: 99mTc-DPD HR is well correlated with LVST, diastolic and longitudinal systolic dysfunction.

P48

Posterior longitudinal strain by speckle tracking echocardiography, marker of cardiac amyloidosis?

Julien Ternacle*, Jean-François Deux, Diane Bodez, Aziz Guellich, Alain Rahmouni, Jean-Luc Dubois-Randé, Violaine Plante-Bordeneuve, Thibaud Damy
CHU H Mondor, Amyloidosis Mondor Creteil, 94000, Créteil, France
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**P48

Background: Cardiac amyloidosis (CA) is a condition of poor prognosis. The three major forms of amyloidosis are light chain (AL), hereditary transthyretin (M-TTR), and wild-type transthyretin (WT-TTR). Two-dimensional (2D) echocardiography measurement of longitudinal strain (LS) has been reported to be useful in the diagnosis of CA. Regional distribution of LS in CA and its diagnostic value in detecting early left-ventricular systolic dysfunction is unclear.
Objectives: To compare left ventricular LS evaluated by 2D echo-cardiography with cardiac magnetic resonance imaging (MRI) in CA.
Methods: Patients with cardiac amyloidosis were included prospectively. Inclusion criteria were age >18 years, diagnosis of amyloidosis with cardiac involvement defined by an interventricular septum wall thickness (IVST) above 12 mm. For each of the 17 left-ventricular segments in the American Heart Association model, we evaluated LS and late gadolinium enhancement (LGE) by MRI.
Results: Among the 162 patients with amyloidosis, 97 had CA and were included in the study; 30 had AL, 46 m-TTR, and 21 WT-TTR. Mean LS was -11±4% and was similarly impaired in the three types of amyloidosis. 69 patients had cardiac MRI of whom 64 (93%) had positive LGE. The number of segments with LGE was similar across the three CA types. All the 69 patients had basal posterior wall involvement as reflected by decreased LS (-6±6%). Both LS and amyloid deposits showed a basal-to-apical gradient. A significant correlation was found between basal posterior wall LS and the number of segments with LGE ($r=0.56$, $p<0.001$).
Conclusions: Basal-to-apical LS abnormalities are similar across CA types. Basal posterior wall LS may be used to appreciate the severity of cardiac amyloidosis.

P49

Usefulness of 99mTc-HMDP scintigraphy for the etiologic diagnosis and prognosis of cardiac amyloidosis

Arnault Galat*, Jean Rosso, Aziz Guellich, Axel Van Der Gucht, Jean-Luc Dubois-Randé, Violaine Plante-Bordeneuve, Emmanuel Itti, Thibaud Damy
CHU H Mondor, Amyloidosis Mondor Creteil, 94000, Créteil, France
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**P49

Background: Amyloidosis is characterized by extracellular deposits of insoluble proteins that cause tissue damage. The three main types are monoclonal light chain (AL), wild-type transthyretin (wt-TTR), and mutated transthyretin (m-TTR) amyloidosis. Cardiac amyloidosis (CA) raises diagnostic challenges.
Objective: To assess the diagnostic accuracy of 99mTc-HMDP-scintigraphy for typing CA, differentiating CA from non-amyloid left ventricle hypertrophy (LVH), and predicting outcomes.
Methods: 121 patients with suspected CA underwent 99mTc-HMDP-scintigraphy in addition to standard investigations.
Results: CA was diagnosed in all AL (n=14) and wt-TTR (n=21). Among m-TTR (n=34), 26 had CA, 4 neuropathy without CA and 4 were asymptomatic carriers. Of the 52 patients with non-amyloid heart disease, 37 had LVH and served as controls. 99mTc-HMDP cardiac uptake occurred in all wt-TTR, in m-TTR with CA except two, and in one AL. A visual score ≥2 was 100% specific for diagnosing TTR-CA. Among TTR-CA, heart-to-skull retention (HR/SR) correlated with CA severity (LVEF and NT-proBNP). Median follow-up was 111 days (50;343). In a multivariate Cox model including clinical, echocardiographic, and scintigraphic variables, NYHA III-IV and HR/SR>1.94 predicted acute heart failure and/or death.
Conclusions: 99mTc-HMDP-scintigraphy allows differentiating transthyretin from AL-CA and CA from other LVHs and also provides prognostic information.

P50

Diagnostic value of fat aspirates for amyloidosis in 950 patients

JA Gilbertson*, NA Botcher, D Rowczenio, C Whelan, HJ Lachmann, A Wechalekar, JD Gillmore, PN Hawkins
National Amyloidosis Centre, UCL Division of Medicine, NW3 2PF, London, UK
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Background: Aspiration of abdominal subcutaneous fat is a simple outpatient procedure that is well established in management of patients with amyloidosis and is particularly useful when investigating the cause of organ dysfunction and more specifically in the context of a suspicion of amyloidosis. At the National Amyloidosis Centre (NAC), fat aspirates are routinely obtained in patients whom amyloid has not been previously confirmed. To date we have performed 950 fat aspirates to seek the presence of amyloid and identify the respective amyloid fibril protein. Thirty percent (290 cases) of the total aspirates sampled were found to contain amyloid. It was possible to conclusively type the fibril protein using immunohistochemistry (IHC) in 188 of these cases, thereby thwarting the need for a more invasive diagnostic biopsy or further diagnostic tests.
Method: Abdominal fat tissue is aspirated and smears are prepared on to glass slides for Congo red staining. The remainder of the fat tissue aspirated is briefly fixed in formalin, double embedded in agar and then into a paraffin block (FFPE) for routine histology and IHC. Once amyloid has been confirmed IHC is performed using a panel of monospecific antibodies against known amyloid-forming proteins in an attempt to identify the amyloid fibril. Interpretation of all stained slides is carried out by two experienced people with and without crossed polarizing filters.
Results: We found 97% concordance between the smear and the block results. Two percent of the smears gave a negative result for amyloid when the corresponding FFPE was positive, whereas 1% of the smears where positive when the FFPE was negative. Of the samples taken 60% did not contain any amyloid and 10% gave insufficient material for interpretation. Among the amyloidotic samples, IHC identified 25% as ATTR, 2% of other types (AA, AapoA1 and ALYS), 42% AL amyloid and 31% gave no immuno specific staining.
Conclusion: Aspiration of abdominal subcutaneous fat is a valuable method in diagnosing amyloid, in 30% of cases preventing the need for a more invasive biopsy. There was a difference of 3% between the smear and the FFPE indicating that all representative tissue taken must be analysed to give a correct diagnosis. Only 42% of patients with negative samples went on to have a further tissue biopsy, over half of these samples did not contain any evidence of amyloid. Ten percent of the fat aspirates gave insufficient adipose material for interpretation, comprising mainly of blood. Immunohistochemical identification of the amyloid fibril protein was proven in 188 cases, in 31% of the cases the precise amyloid type was only determined after genetic sequencing. TTR by IHC was identified in 25% of the cases and among these patients, 62% were later found by genetic sequencing to have a variant, with V122I (p.V142I) being most common. Of the fat aspirates that did not demonstrate amyloid, only 6% of the patients were found to have a TTR variant.

P51

Spotting senile systemic amyloidosis: why we miss it

Marianna Fontana[1*], Cristina C Quarta[1], Janet Gilbertson[1], Thirusha Lane[1], Carol J Whelan[1], Julian D Gillmore[1], Philip N Hawkins[1], James C Moon[2]
[1]National Amyloidosis Centre, UCL, NW3 2QG, London, UK; [2]Barts Heart Centre, Barts Hospital, E2 9JX, London, UK
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Objectives: To analyze the geometric pattern of cardiac hypertrophy in patients with wild-type transthyretin amyloidosis (wtATTR, formerly called senile amyloid) using cardiovascular magnetic resonance (CMR) and to analyze the diagnostic implications.

Background: wtATTR amyloidosis is an under-diagnosed and underappreciated cause of heart failure. CMR adds value diagnostically for morphological phenotype and tissue characterization.
Methods: At a national referral centre, over 4 years, 87 consecutive recruited patients wtATTR amyloidosis underwent CMR. The diagnosis was confirmed by histological proof of amyloid (62%), exclusion of TTR mutations (100%) and characteristic features including bone tracer scanning (DPD grade 2-3 in 100%). CMR had precipitated the referrals in 71% of the cohort. Standard long and short axis cines derived the presence and distribution of LVH, relative wall thickness (RTW), inversion of the septal curvature and LV remodeling patterns were determined.
Results: There were 82 patients with wt ATTR amyloidosis (82 males (94%), age 75 ± 7 years). Patients with wtATTR amyloidosis had increased LV mass index, EF at the lower limit of normal range and markedly reduced indexes of longitudinal function. LV mass was always large compared to cavity size, as expected. However – there was far more asymmetric hypertrophy than expected – 61% of patients had the septum >1.5x thicker than the posterior wall. Inversion of the septal curvature was found in 34% of patients, features typically associated with hypertrophic cardiomyopathy. Tissue characterization with LGE was typical of amyloidosis in 100% of cases (transmural LGE in 73%, subendocardial in 27%).
Conclusions: CMR is a major source of diagnosis of wtATTR. The majority of patients with wtATTR amyloidosis have a pattern of hypertrophy traditionally thought associated with hypertrophic cardiomyopathy rather than amyloid with asymmetric septal hypertrophy and reverse septal curvature.

P52

Parenteral nutrition improves nutritional status, autonomic symptoms and QoL in patients with TTR-FAP

Massimo Russo[1*], Gianluca Vita[1], Claudia Stancanelli[2], Giuseppe Vita[3], Anna Mazzeo[3], Sonia Messina[1]
[1]Nemo Clinical Center Messina, University of Messina, 98125, Messina, Italy; [2]Biomedical Department of Internal and Specialistic Medicine, University of Palermo, 90100, Palermo, Italy; [3]Department of Neuroscience, University of Messina, 98125, Messina, Italy
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Background: Transthyretin related familial amyloidotic polyneuropathy (TTR-FAP) is an inherited form of amyloidosis, leading to death in about 10 years in most cases for cardiac failure or wasting syndrome. Previous study showed that modified body mass index (mBMI) was related to time before death, duration of gastrointestinal disturbances, malabsorption and functional capacity. Futhermore, outcome after liver tranplantation was greater in patients with an mBMI over 600.
Patients: We report two TTR-FAP patients, carrying respectively the Thr49Ala and the Glu89Gln mutations, in whom nutritional status worsened despite diet modification, hypercaloric supplement and two important therapeutic approaches such as liver transplant and tafamidis meglumine.
In both case, at a late stage of the disease, a peripherally inserted central catheter (PICC) was placed and the parenteral nutrition started.
Results: The parenteral nutrition added to oral nutrition allowed to improve their nutritional status and clinical conditions as documented by increase of body weight and mBMI. Moreover, we recorded reduction of autonomic symptoms including postural hypotension, nausea and diarrhoea and amelioration of quality of life.
Conclusion: Our experience suggests that parenteral nutrition administered by PICC may be useful in reducing complications and disabilities in TTR-FAP patients, even when all dietary adjustments have been ineffective. Reasonably, the improvement in nutritional status may prolong survival in TTR-FAP patients.

P53

Mass spectrometry analysis of transthyretin (TTR) post-translational modifications (PTMs) in hereditary ATTR: a case-control Spanish experience

Marta Vilà-Rico[1*], Sebastián Azorín Contesse[2], José E Barcena Llona[3], Ricardo Rojas-García[4], Fernando Martínez Valle[5], Antoni Planas[1], Francesc Canals[6], Josep M Campistol[2]
[1]Institut Químic de Sarrià, Universitat Ramon Llull, Laboratory of Biochemistry, 08017, Barcelona, Spain; [2]Hospital clínic de Barcelona, Amyloidosis and monoclonal gammopathies' unit (UDAM) - Nephrology and transplant unit (SNiTR), 08036, Barcelona, Spain; [3]Cruces university hospital, Multiple sclerosis and demyelinating diseases unit, Neurology service, Department of neurosciences, 48903, Biscay, Spain; [4]Hospital de la Santa Creu i Sant Pau, Neuromuscular disease unit, Neurology service, 08025, Barcelona, Spain; [5]Vall d'Hebron University Hospital, Internal medicine department I, 08035, Barcelona, Spain; [6]Vall d'Hebron Institute of Oncology, Proteomics laboratory, 08035, Barcelona, Spain
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**P53

Background: Transthyretin (TTR) is an amyloidogenic tetrameric protein, present in human plasma, associated with several familial amyloidoses. Variability of TTR is not only due to point mutations in the encoding gene but also to post-translational modifications (PTMs) at Cys10, being the most common PTMs the S-sulfonation, S-glycinylcysteinylation, S-cysteinylation and S-glutathionylation. It is thought that PTMs at Cys10 may play an important biological role in the onset and pathological process of the amyloidosis. Recently we reported the development of a methodology for quantification of PTMs in serum samples, as well as for the determination of serum TTR levels, from healthy (wt-TTR) and ATTR V30M individuals which involves an enrichment step by immunoprecipitation followed by mass spectrometry analysis of (i) the intact TTR protein and (ii) targeted LC-MS analysis of peptides carrying the PTMs of interest (M Vilà-Rico et al. Analysis of post-translational modifications in human transthyretin associated with familial amyloidotic polyneuropathy by targeted LC-MS and intact protein MS. Journal of Proteomics (2015) in press). Analysis of serum samples by the combination of the two methods affords complementary information on the relative and absolute amounts of the selected TTR PTM forms.
Methods: We aimed at describing the applicability of our mass spectrometry methodology among healthy controls and V30M-TTR patients (cases) at different disease stages followed at our institution and other three spanish health facilities. Inclusion criteriae for cases consisted of a positive genetic testing for V30M-TTR status and a signed ethics' committee approved informed consent. Mass spectrometric analysis was performed as detailed in our previouis work.
Results: A total of 50 healthy controls and 29 patients were included after signing the informed consent. Demographic and clinical characteristics were gathered and correlated to the proteomic analysis profile according to our validated methodology. Significant differences were found for total TTR as well as for specific TTR Cys-10 PTMs between the different V30M ATTR stages as well as between controls and symptomatic patients.
Conclusion: Quantification of wt:V30M TTR ratio and quantification of Cys-10 PTM.
Isoforms is a feasible, reproducible and robust method by intact TTR and targeted LC-MS in the TTR V30M population. Significant differences for wt:V30M TTR ratio as well as for some specific PTMs have been found in a small cohort of V30M-TTR patients at different ATTR stages. These results need to be confirmed in a bigger cohort of patients with ATTR.

P54

Cardiac involvement and clinical follow up of patients with hereditary transthyretin related amyloidosis associated with Glu89Gln mutation

Mariana Gospodinova[1*], Stayko Sarafov[2], Velina Guergueltcheva[3], Andrey Kirov[4], Teodora Chamova[2], Albena Todorova[4], Ivailo Tournev[2], Stefan Denchev[1]
[1]Medical Institute of Ministry of Interior, Clinic of Cardiology, 1606, Sofia, Bulgaria; [2]University Hospital Alexandrovska, Clinic of Neurology, 1431, Sofia, Bulgaria; [3]University Hospital Sofiamed, Neurology Department, 1528, Sofia, Bulgaria; [4]Genetic lab, Genika, 1113, Sofia, Bulgaria
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Background: Cardiac involvement is common in hereditary transthyretin – related amyloidosis (ATTR), but there is a significant phenotypic heterogeneity depending on the mutation.
Patients and methods: We evaluated forty consecutive ATTR patients with Glu89Gln mutation, focusing on cardiac involvement - 18 male, 22 female at a mean age of 57.6±6,7 years. A clinical examination, 12–channel ECG, conventional 2D, Doppler and tissue Doppler echocardiography were performed. The patients were followed for 36 months in the range from 2 to 78 months.

Results: Median age of symptoms development was 52, 3±6, 4 years. Cardiac onset was found in 5 (12,5%) patients. Cardiomyopathy and peripheral polyneuropathy were evident at diagnosis in all patients. Echocardiography revealed a significant increase in wall thickness of both left and right ventricles (septum – 18,6±3,4 mm; posterior wall – 17,5±2,5 mm; RV free wall – 8,4±2,0 mm). Varying degrees of LV diastolic dysfunction were found – Grade 1 in 11 (27,5%) patients, Grade 2 in 12 (30%) and Grade 3 in 17 (42,5%) patients. A reduced LV ejection fraction was found in 9 (22,5%) patients. A common finding were significantly reduced mitral annular systolic velocities (s´septum-5,4±2,0 cm/s, s lat.-5,7 ±1,9 cm/s), registered in all the evaluated patients, pointing to an impaired LV longitudinal systolic function. The systolic myocardial velocities of the tricuspid annulus and TAPSE values were reduced respectively 6,9±2,1cm/s and 12,8±3 mm in 14 of the patients (35%). Pericardial effusion was found in 13 (32,5%) patients.
Pathological ECG was present in 35(87,5%) of the evaluated patients. Atrial fibrillation was registered in 4 (10%) patients, A-V block first degree in 8 (20%), low voltage in 15 (37,5%), left bundle branch block in 3 (7,5%), left anterior fascicular block in 9 (22,5%), pathological Q wave in 14 (35%), right bundle branch block in 2 (5%), and pace-maker rhythm in 2 (5%). Rhythm and conduction disturbances on ECG were found in 24 patients (60%).
The following events occurred during the follow-up period: two deaths (5,4%) (one patient due to ischemic stroke; and another due to heart failure). Two other patients suffered from ischemic strokes. 24-hour Holter ECG revealed short periods of atrial fibrillation and an oral anticoagulant was initiated. A sinus pause > 3 s was observed in one of the patients and a permanent pace-maker was implanted. Four new cases (10%) with symptomatic heart failure, requiring diuretic treatment were observed. In 15 patients a worsening of the symptoms from the peripheral neuropathy were found.
Conclusion: Our study confirms that ATTR associated with the Glu89Gln mutation has a mixed phenotype – neurological and cardiac and an unfavorable prognosis. Our findings imply that patients and carriers of Glu89Gln require close multidisciplinary (both cardiological and neurological) follow-up in order to initiate treatment in time.

P55

Identification of a new variant of TTR involved in familial amyloid cardiomyopathy (FAC) in Brazil: from the patient to the protein

Priscila Ferreira[1*], Carolina Andrade[1], Ricardo Santanna[1], Natalia Varejão[1], Salvado Ventura[2], Fernando Palhano[1], Marcia Cruz[3], Debora Foguel[1]
[1]Universidade Federal do Rio de Janeiro, Medical Biochemistry, 21941902, Rio de Janeiro, Brazil; [2]Universitat Autònoma de Barcelona, Bioquímica i Biologia Molecular, Barcelona, Spain; [3]Hospital Universitário Clementino Fraga Filho, Neurologia, Rio de Janeiro, Brazil
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Background: In Brazil, the most prevalent cases of TTR-related amyloidoses is the V30M variant due the Portuguese colonization. Our group has stablished a center for molecular diagnostic of FAP. Since then, we have sequenced almost one hundred patients form the University Hospital and their relatives. Recently, we identified a patient with a severe cardiomyopathy. This patient has a German ancestry and the sequence of his TTR gene revealed the presence of a new mutation, namely A19D. This patient presented heart failure and was classified by the NIHA as IV. We have also identified a patient, 66-years old, from a family with African ancestry, which bears the typical V122I mutation. This patient presented carpal tunnel syndrome and two years later developed heart failure that progressed to NYHA III. The main goal of the present work is to characterize the Brazilian population with FAC by combining bioinformatics and biophysical studies.
Methods: We built a model for A19D by using FoldX (http://foldx.crg.es/) with the original WT-TTR structure as deposited in the PDB under code 1F41. The toxicity of amyloid aggregates composed of A19D and V122I were evaluated by using cell viability assay in primary culture of murine cardiomyocytes and fibroblasts as well as N2a cell line. Results: Initially we used the bioinformatics tool FoldX to predict the thermodynamic stability of the new mutant A19D. Our predictions have shown that the insertion of mutation caused a decrease in the thermodynamic stability of the protein and cause an electrostatic clash in the region of thyroxine channel that could facilitate their dissociation. A19D was purified heterologously and biophysical studies demonstrated that this mutant is a dimer and not a tetramer as wild type structure. The crystallographic structure of A19D is identical of wild type TTR. Thermodynamic studies with A19D indicated that it has a lower stability than the wild-type protein and other mutants. This new mutant has a faster aggregation kinetics forming amyloid fibers in two hours as shown by images. Amyloid aggregates of A19D and V122I were incubated with primary culture of cardiomyocytes and fibroblasts from murine heart and also in N2a cell line. The viability assay showed that the oligomers of A19D and V122I are toxic for cardiomyocytes and neuroblastoma cells and interestingly fibroblasts also suffer injury in the presence of these aggregates.
Conclusions: The recent consolidation of TTR diagnosis in our University Hospital led to the identification of a rare, new variant of TTR in Brazil, namely, A19D, as well as the common V122I variant. A19D presented a marginal thermodynamic stability as inferred by bioinformatics and by biophysical studies with the purified protein. A19D showed to be dimer in solution. The viability assay shows that toxic mechanism displayed by this new mutant can be directly correlated with the aggressiveness observed in the disease developed by the patient.

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Life paths of familial amiloidotic polyneuropathy patients: a descriptive study

Alice Lopes[1*], Alexandra Sousa[1], Isabel Fonseca[1], Margarida Branco[1], Carla Rodrigues[1], Paula Freitas[2], Teresa Coelho[1]
[1]Centro Hospitalar do Porto, Corino de Andrade Unit, 4099-001, Porto, Portugal; [2]Universidade do Porto, Instituto de Ciências Biomédicas Abel Salazar, 4050-313, Porto, Portugal
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Background: Very few studies describe the relations between biographic events, socio-familiar characteristics, and disease during the lifetime of familial amyloid polyneuropathy (FAP) patients.
Methods: A common social demographic questionnaire and a questionnaire about family history/personal disease and biographic events were applied to 211 subjects (in 110, the disease had already begun, 82 were asymptomatic carriers, and 19 had no established diagnosis), attending external consultation at Corino de Andrade Unit in Centro Hospitalar do Porto.
A descriptive analysis and frequencies were obtained.
Results: 84 of all subjects were men and 127 were female. Mean ages: carriers 33.9 ± 9.8yr, patients 37.8 ± 8.1, and for subjects that had no established diagnosis 40.9 ± 14.0 yr. Most subjects were married or lived with a partner (67.1%); 61.5% had children (mean, 4 children).
Most subjects (96.3%) had contacted the disease before having their diagnosis, most of them through parents (35.7%) but also through uncles, grandparents, other family members; the affected parent was the mother in 53.8% of the cases and the father in 43.3% of the patients (the remaining did not know who the affected parent was; one patient inherited the disease from both parents); 71.8% were deceased. Most living parents had symptoms (74.4%).
Age at time of the affected parent's death: most of the subjects were older than 25 yr (43%); the remaining were under 10 yr (9.9%); between 10 and 14 yr (15.5%); and between 15 and 24 yr.
Age at time of the parent's disease onset: most of the subjects were under 10 yr (30.5%); the remaining were between 10 and 14 yr (14.4%); between 15 and 24 yr (27.0%); and 17.2% were older than 25 yr.
When asked about whether and how their parent's disease had brought changes into their lives, 37.2% of the subjects said yes, namely through residence, psychological and familial questions.
Most subjects (53.3%) had been their parent´s caregivers.
About 7.5 % of all patients had been raised through childhood and youth by others, not by their parents.
Some subjects (8.4%) refused to acknowledge their own genetic test's result for more than 1 year.
Discussion and conclusions: Parent´s death and the presence of an early-onset disease is a constant in FAP patients´ lives and this may be an

important distress factor, eventually making them more vulnerable to psychological distress and psychiatric disease.
During childhood, youth, and as young adults, a great number of these patients were obliged to become caregivers and this implied a change of roles in the family.
These results point to a very important psychosocial charge that FAP imposes to patients throughout their lives, since childhood and youth.

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Axon reflex-mediated vasodilation is reduced in proportion to disease severity in familial amyloid polyneuropathy

Thierry Kuntzer[1*], Irène Calero-Romero[2], Bernard Waeber[2], Francois Feihl[2]
[1]Lausanne University Hospital CHUV, Department of Clinical Neurosciences, 1011, Lausanne, Switzerland; [2]Lausanne University Hospital CHUV, Division of Clinical Pathophysiology, 1011, Lausanne, Switzerland
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Background: Axon reflex-mediated neurogenic cutaneous vasodilatation in response to histamine reflects C-fibre dysfunction. We aimed to evaluate the distribution of the vascular flare area measured by laser doppler imaging ("LDIflare area") in familial amyloid polyneuropathy (FAP) and in healthy volunteers.
Methods: Twenty-one subjects were contacted to participate in this study. Eleven FAP patients and 10 normal volunteers were recruited for this hospital's ethics committee approved study. Confirmed genetically FAP patients were recruited from our registry, and were prospectively re-examined to determine the frequency of neuropathic motor, sensory, and autonomic symptoms and findings, to measure nerve conduction parameters, and to assess thermal testing performed using a Medoc TSA-II thermal analyzer. LDIflare areas were induced by iontophoresis (at 100uA for 1 min) of histamine at the forearm and at the leg.
Results: Six patients had a FAP of variable severity, one had a generalized analgesia secondary to leprosy (used as positive control). Four patients had to be excluded from the analysis as it was not possible to quantify neuropathy severity (asymptomatic carriers). All but one had a Val30Met mutated TTR gene. The median neurological impairment score of the lower limbs (NIS_LL) was 9.3 (0 to 27). Half of the patients had reduced or absent sural nerve potential. The warmth detection thresholds in the feet were higher in patients group (ctrl=37.6°C, 35.3 to 38.3: patients=42.2°C, 38.2 to 48.8: $p<0.015$), indicating small fiber impairment.
Compared to controls, patients had lower LDIflare areas in the legs (ctrl=13.0, 9.2 to 19.8cm2: patients=5.8, 2.7 to 11.4cm2) and in the forearms (ctrl=21.7, 14.3 to 26.1cm2: patients=12.4, 4.2 to 20.6 cm2). However, the differences were statistically different for the legs only ($p=0.015$) ($p=0.089$ for the arms). There was an excellent correlation between the degree of LDIflare area and NIS_LL (Spearman's rank correlation for forearm; $r = -0.71$, $p<0.074$: for leg; $r = -0.84$, $p<0.019$).
Conclusion: Our study underscores that in FAP patients, the amount of LDIflare area is reduced in proportion to disease severity.

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Patients with hereditary ATTR amyloidosis experience an increasing burden of illness as the disease progresses

David Adams[1*], Oved Amitay[2], Teresa Coelho[3]
[1]Univ Paris-Sud, APHP, Hopital de Bicetre, Service de Neurologie and Centre de Reference des Neuropathies Amyloides Familiales (NNERF), INSERM U1195, Le Kremlin-Bicetre, France; [2]Alnylam Pharmaceuticals, Commercial, 02142, Cambridge, USA; [3]Hospital de Santo Antonio, Unidade Clinica de Paramiloidose, 4099001, Porto, Portugal
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Background: Hereditary ATTR amyloidosis with polyneuropathy, also known as Familial Amyloidotic Polyneuropathy (FAP), is a rare, inherited, progressively debilitating disease with a high unmet medical need. The purpose of this analysis is to assess the impact of this disease on healthcare resource utilization, quality of life, employment status, and activities of daily living (ADLs).
Methods: A Phase 2 open-label extension study of patisiran in FAP patients was utilized to collect patient-reported outcomes, including EQ-5D, Rasch-built Overall Disability Scale (R-ODS), and a healthcare resource utilization questionnaire.
Results: The study included 27 patients, 18 males and 9 females, 29-77 years of age. Baseline data are presented for 14 patients with a Polyneuropathy Disability (PND) Score I and 13 patients with a PND Score II or greater. Characterized by FAP Stage, 24 patients are FAP Stage 1 and 3 patients are FAP Stage 2. Two patients (PND Score II or greater) reported a total of six hospitalizations due to FAP in the past 12 months, each for 3 or more nights in duration. Mean EQ-5D scores were 0.82 (PND Score I) and 0.74 (PND Score II or greater). Patients reported their perceived health status on the EQ-VAS with mean scores of 75 (PND Score I) and 60 (PND Score II or greater). Ten patients (8/10 PND Score II or greater) reported they cannot work because of FAP (mean 61 years of age). Patients also reported inability to perform various ADLs. Most commonly, 77% of patients with PND Score II or greater cannot stand for hours (14% in PND Score I) and 69% cannot run (21% in PND Score I).
Conclusions: FAP patients experience considerable burden of illness early in the course of disease and this burden increases with disease progression. The factors described will be influential in the development of a comprehensive FAP cost-consequence analysis. Additional parameters may also be needed to fully capture the totality of disease burden.

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Cardiomyopathy and peripheral polyneuropathy severity in patients with Glu89Gln mutation at the time of diagnosis

Mariana Gospodinova[1*], Stayko Sarafov[2], Velina Guergueltcheva[3], Andrey Kirov[4], Teodora Chamova[2], Albena Todorova[4], Ivailo Tournev[2], Stefan Denchev[1]
[1]Medical Institute of Ministry of Interior, Clinic of Cardiology, 1606, Sofia, Bulgaria; [2]University Hospital Alexandrovska, Clinic of Neurology, 1431, Sofia, Bulgaria; [3]University Hospital Sofiamed, Neurology Department, 1528, Sofia, Bulgaria; [4]Genetic lab, Genika, 1113, Sofia, Bulgaria
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Background: Hereditary transthyretin – related amyloidosis (ATTR), associated with Glu89Gln mutation is characterized by mixed phenotype – cardiac and neurological and an unfavorable prognosis.
Patients and methods: We evaluated forty consecutive ATTR patients with Glu89Gln mutation, assessing cardiac and peripheral polyneuropathy involvement - 18 male, 22 female at a mean age of 57.6±6,7 years. A clinical examination, 12–channel ECG, conventional 2D, Doppler and tissue Doppler echocardiography were performed. A comprehensive clinical neurological assessment was performed, defining the stage of neurological disability according to Familial Amyloidotic Polyneuropathy scale. The routine neurological assessment consisted of evaluating the reflexes, sensation (touch pressure, pin-prick, vibration, joint position) and muscle weakness.
Results: Median age of symptoms development was 52, 3±6, 4 years. In 17 (42,5%) patients the disease started with carpal tunnel syndrome. Sixteen patients (40%) had sensory-motor symptoms at presentation. The first symptoms of the disease were cardiac in 5 (12,5%). Two (5%) patients exhibited gastrointestinal symptoms first. Median (range) delay from symptom onset to diagnosis was 62 (5–149) months. Cardiomyopathy and peripheral polyneuropathy were evident at diagnosis in all patients. Symptoms from the autonomous nervous system were found in 26 (65%) patients. The kidney and liver tests were normal in all patients.
Echocardiography revealed an infiltrative cardiomyopathy with varying degrees of LV diastolic dysfunction – Grade 1 in 11 (27,5%) patients, Grade 2 in 12 (30%) and Grade 3 in 17 (42,5%) patients. A reduced LV ejection fraction was found in 9 (22,5%) of the patients, all with severe diastolic dysfunction. At the time of diagnosis 24 (60%) patients were in the 1st neurological stage, 5 (12,5%) in the 2nd stage and 11 (27,5%) in the 3rd stage.
Conclusion: Despite the fact, that most of the patients presented with neurological symptoms, either from the peripheral polyneuropathy or carpal tunnel syndrome, we found more patients with severe heart involvement than with 3rd stage of the polyneuropathy at the time of diagnosis. Our findings imply that the patients with Glu89Gln mutation have a prolonged period of asymptomatic heart involvement and the

symptoms are further concealed by the development of neuropathy, which impairs functional class assessment. An earlier identification of the cardiomyopathy is needed through close follow up of the patients and the mutation carriers.

P60

Comparison and identification of early clinical, biological and echocardiographic prognostic markers in cardiac amyloidosis

Thibaud Damy[1*], Arnaud Jaccard[2], Aziz Guellich[1], David Lavergne[2], François Deux Jean[1], Jehan Dupuis[1], Valérie Frenkel[1], Dania Mohty[2]
[1]CHU H Mondor, Amyloidosis Mondor Creteil, 94000, Créteil, France; [2]CHU Limoges, AL Amyloidosis Referral Center, 87000, Limoges, France
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Background: The early prognosis of amyloidosis is known to depend heavily on cardiac function and may be improved by identifying patients at highest risk for adverse cardiac events. We looked for early predictors of mortality in patients with cardiac AL amyloidosis, hereditary transthyretin amyloidosis (m-TTR), or senile transthyretin amyloidosis (WT-TTR).
Method: Prospective observational study of 198 patients seen at two French university centers.
Results: NYHA class was III-IV in 31% of patients. Median (25th-75th percentile) values were 69 (60-76) years for age, 3027 (673-7155) pg•mL-1 for NT-proBNP, and 60% (48-66) for left ventricular ejection fraction. Interventricular septal thickness was greater in the m-TTR and WT-TTR groups than in the AL group (P<0.0001). NT-proBNP correlated with IVST (R=0.34; P=0.0001). The 6-month mortality rate was 24% (42 patients). The AL group had higher values for both NT-proBNP (P=0.0001) and 6-month mortality (P=0.0001). By multivariate analysis, independent predictors of 6-month mortality were higher NT-proBNP (Q4), NYHA class (III-IV), lower cardiac output (<4 L.min-1), and pericardial effusion.
Conclusions: NYHA, NT-proBNP, cardiac output, and pericardial effusion were independent predictors of mortality in cardiac disease due to any of the three amyloidosis types. NT-proBNP values were highest in AL amyloidosis.

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Retinal and choroidal vascular abnormalities in TTR-FAP

Antoine Rousseau[1*], Emmanuel Barreau[2], Céline Terrada[3], Lydie Bonin[1], Pierre-Raphaêl Rothschild[4], Marie Théaudin[4], David Adams[4], Marc Labetoulle[4]
[1]French Reference Center for FAP (NNERF), Bicêtre Hospital, Ophthalmology, 94275, Le Kremlin-Bicêtre, France; [2]Reference Centre for Rare Vascular Diseases of the Central Nervous System and the Retina, Lariboisière Hospital, Ophthalmology, 75010, Paris, France; [3]Cochin Hospital, Ophthalmology, 75014, Paris, France; [4]French Reference Center for FAP (NNERF), Bicêtre Hospital, Neurology, 94275, Le Kremlin-Bicêtre, France
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Background: Retinal amyloid angiopathy is considered to be a rare ocular manifestation of TTR-FAP. Choroidal vascular abnormalities have been described in isolated case reports. The purpose of this study was to describe fluorescein and indocyanine green angiographic findings of retinal and choroidal vascular abnormalities in a series of TTR-FAP patients.
Methods: This monocentric observational study was conducted at the French National Reference Center for TTR-FAP. Genetically confirmed TTR-FAP patients with suspected retinal and/or choroidal vascular abnormalities underwent fluorescein and indocyanine green angiography. Sensorimotor polyneuropathy (SPN) was staged with the Polyneuropathy Disability (PND) score, vegetative neuropathy was staged with the Compound Autonomic Dysfunction Test (CADT). Medical and surgical treatments were analyzed for all patients.
Results: Twelve patients (8 males and 4 females), aged 47-82 years (mean 55.8±14.1 years) were included. Mean delay between first symptoms and inclusion was 9.6±5.3 years. Val30Met mutation was present in 10/12 patients. Bilateral retinal ischemic vasculopathy was present in 8/12 patients and included retinal hemorrhages, microaneurysms, venous and arteriolar segmental staining. Retinal ischemia lead to preretinal neovascularization in one patient and to neovascular glaucoma in 5 eyes of 3 patients. Treatments included panretinal photocoagulation (10 eyes, 5 patients), intravitreal anti-VEGF injections (4 eyes, 2 patients) and trabeculectomy (both eyes of one patient). Vitreous amyloid deposits were present in 7/8 cases of retinal amyloid angiopathy. Typical bilateral amyloid choroidal vasculopathy was found in 9/12 patients. It consisted in late and diffuse staining of the arterial choroidal vasculature, and was associated with vitreous amyloid deposits in 7/9 cases. Anterior chamber amyloid deposits were present in 2/8 patients with retinal amyloid angiopathy and in 3/9 patients with choroidal amyloid angiopathy. All the studied patients had a PND score ≥ 1 and 10/12 patients had dysautonomic symptoms.
Conclusions: Retinal amyloid angiopathy can be considered as a severe form of ischemic retinopathy. Clinical significance of choroidal amyloid angiopathy remains unknown. Further studies are warranted to determine the potential systemic counterparts of ocular amyloid angiopathies.

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Is 99mTc-diphosphonate uptake the earliest sign of cardiac amyloidosis development in asymptomatic Glu89Gln transthyretin gene mutation carriers?

Fabio Minutoli[1*], Anna Mazzeo[2], Gianluca Di Bella[3], Giuseppe Vita[2], Sergio Baldari[1]
[1]University of Messina, Department of Biomedical Sciences and of Morphologic and Functional Images, 98125, Messina, Italy; [2]University of Messina, Department of Neurosciences, 98125, Messina, Italy; [3]University of Messina, Clinical and Experimental Medicine Department, 98125, Messina, Italy
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Background: Presenting symptoms in patients with Glu89Gln transthyretin (TTR) gene mutation are related to peripheral and autonomic nervous system damage; nevertheless, Glu89Gln TTR gene mutation is responsible for early and severe cardiac involvement (which significantly worsens the prognosis). Early diagnosis of cardiac involvement in subjects with TTR gene mutation can significantly affect patient therapy.
We compared 99mTc-3, 3-diphosphono-1, 2-propanodicarboxylic acid (DPD) imaging with electrocardiography (ECG), echocardiography, biomarkers dosage (N-terminal pro–B-type natriuretic peptide (NT-proBNP) and troponin-I) and magnetic resonance (MR) imaging with late gadolinium enhancement (LGE) in order to determine the most sensitive technique in early detection of cardiac amyloid deposition in subjects with Glu89Gln TTR gene mutation.
Methods: Seven asymptomatic subjects (3M and 4F; mean age, 42 years) with Glu89Gln TTR gene mutation and normal interventricular septum (IVS) thickness and NT-proBNP level underwent three 99mTc-DPD scans (at baseline and two and four years later) and were followed-up for 5-8 years by clinical examination, ECG, echocardiography and cardiac biomarkers dosage. Baseline MR imaging with LGE was also available.
Scintigraphic images were analyzed visually (grade 0, no abnormal localization of the radiotracer; grade 1, myocardial radiotracer uptake lower than bone uptake; and grade 2, myocardial radiotracer uptake higher than bone uptake) and semiquantitatively.
Results: Three patients showed no myocardial accumulation in all 99mTc-DPD scans; increased IVS thickness occurring four years after the last 99mTc-DPD scan was the only abnormal finding in these patients. In two patients, 99mTc-DPD scan revealed grade 2 radiotracer uptake; baseline MR imaging showed focal LGE in both patients. In these patients, mean left ventricle (LV) wall thickness >12 mm occurred within 3 years; NT-proBNP reached the current diagnostic level for cardiac amyloidosis in only one patient, six years after the positive scan. Two patients had negative baseline 99mTc-DPD scan and cardiac uptake in the following scans. Increased mean LV wall thickness was found three years after positive scintigraphy; NT-proBNP increased later in one patient. ECG abnormalities appeared some years after a positive 99mTc-DPD scan had occurred.
Conclusion: Cardiac uptake of 99mTc-DPD precede clinical, instrumental and laboratory signs of amyloidosis; it may represent the earliest sign of cardiac amyloidosis development in subjects with Glu89Gln TTR gene mutation preceding of some years fulfillment of current diagnostic criteria for cardiac amyloidosis.

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Specific ophtalmologic changes in late onset familial amyloid polyneuropathy (FAP) portuguese patients

Natalia Ferreira[1*], David Dias[1], Teresa Coelho[2]
[1]Ophthalmology Department - Centro Hospitalar do Porto – Hospital Santo António (HSA), Porto, Portugal; [2]Neurology Department – Centro Hospitalar do Porto – HSA - Unidade Corino de Andrade, Porto, Portugal
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Purpose: Report ocular manifestations in late onset familial amyloid polyneuropathy (FAP) patients.
Methods: Retrospective observational consecutive case series of 20 late onset FAP patients. Demographic data, TTR mutation involved, age at beginning of disease, period of evolution of disease, liver transplant or medical treatment, ophthalmological alterations and previous ocular surgeries were evaluated.
Results: Thirteen patients were female. The mean onset age was 58 years and average evolution time of the disease was 5, 6 years. All patients were TTR Met30 and 2 patients were compound heterozygous TTR met30 met119. Four patients had been submitted to liver transplant and nine were on Tafamidis treatment. Amyloid deposits on anterior lens surface were observed in 15 eyes (37,5%), scalloped pupil in 8 eyes (20%) and vitreous opacities in 23 eyes (57,5%). Nine had underwent vitrectomy. Glaucoma was present in 13 eyes and 4 have been submitted to surgery.
Conclusion: Ocular manifestations are common in late onset FAP patients. Vitreous opacities were the most frequent specific alteration. Ophthalmologist has an important role in follow-up of FAP patients to accurately treat sight-threatening manifestations.

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Specific ocular changes in TTR Met30-FAP after liver transplantation

Natalia Ferreira[1*], David Dias[1], Carmen Vilas-Boas[2], Isabel Fonseca[2], Teresa Coelho[3]
[1]Ophthalmology Department - Centro Hospitalar do Porto – Hospital Santo António (HSA), Porto, Portugal; [2]Centro Hospitalar do Porto, Unidade Corino de Andrade, Porto, Portugal; [3]Neurology Department – Centro Hospitalar do Porto – HSA, Unidade Corino de Andrade, Porto, Portugal
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Purpose: To report ocular manifestations in Portuguese TTR Met30 FAP patients submitted to liver transplant (LT).
Methods: Retrospective observational consecutive case series of 78 LT recipients, observed by one ophthalmologist (FN) between January 2007 and July 2015. Demographic data, age at beginning of disease, period of evolution of disease, ophthalmological alterations and ocular surgeries (glaucoma surgery and vitrectomy) were evaluated. Patients were divided in three groups according to date of LT: less than 5 years (group 1); between 5 and 10 years (group 2) and more than 10 years (group 3).
Results: Thirty-six patients were male. Twelve, 32 and 34 patients belong to group 1, 2 and 3 respectively. The mean age of patients was 41 years (group 1), 44 years (group 2) and 50 years (group 3). Symptoms and signs of dry eye were present in 33% (group1), 56% (group 2) and 62% (group 3) of patients. Amyloid deposits on pupillary border were observed in 16%, 27% and 41% of eyes according to each group. Scalloped pupil was present in 16%, 25% and 36% of cases, respectively. Amyloid vitreous opacities could be seen in 33%, 40% and 59% of eyes. Vitrectomy and glaucoma surgery was performed in 19 and 24 eyes of group 3, respectively.
Conclusion: The prevalence of specific ocular changes increase with time after LT. The need of vitrectomy or glaucoma surgery is common 10 years after LT. Regular ophthalmological evaluation of FAP patients submitted to LT is important to accurately treat sight-threatening manifestations.

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Management of stage 1 TTR FAP: French experience

David Adams*, Marie Théaudin, Pierre Lozeron, Clovis Adam, Guillemette Beaudonnet, Céline Labeyrie, Catherine Lacroix, Cécile Cauquil
CHU Bicêtre, APHP, French Reference Center for FAP (NNERF), Univ Paris Sud, INSERM U1191, Le Kremlin Bicêtre, Paris, France
Orphanet Journal of Rare Diseases 2015, **10(Suppl 1):**P65

Introduction: Transthyretin Familial Amyloid Polyneuropathy (TTR-FAP) is a worldwide autosomal dominant disease due to point mutation of TTR gene. The main endemic area is Portugal, associated with V30M variant and an early onset (EO) (mean age 30 yo). Phenotype is a progressive length dependent small fiber polyneuropathy with autonomic dysfunction and cardiac conduction disorders; the median survival is 12 years. France is a prototypic non endemic country, characterized by sporadic cases in 50%, a late onset (LO>50 yo) in 75%, a genetic heterogeneity (41 variants ; V30M in 55%). Liver transplantation (LT) is the gold standard treatment allows to stop progression of the neuropathy in EO V30M patients and to prolonge significantly the survival. Tafamidis, a TTR kinetic stabilizer, received marketing authorization in Europe for stage 1 FAP (walking unaided) allowing to slow progression of the neuropathy. Aims of the study was to assess the place of tafamidis in the management of TTR-FAP.
Methods: In a population of 131 patients diagnosed during period (2008-2012). Mean age : 59.3 yo SD 15.9. Stage 1 (60%) stage 2 (36%) stage 3 (4%). Mutation : V30M: 59%, non V30M variants n=18, Late onset (LO): 73.3%. mean NIS : 40.65 SD 28.6. In Stage 1 mean NIS=27.32 SD 20. Locomotion (PND score) : 71% with Walking difficulties. Patients were followed periodically (every 6 months) in consultation assessing by a questionnaire : new manifestations (sensory complaints, walking difficulties, autonomic dysfunction (digestive (gastroparesia, diarrhea), erectile dysfunction)). And on examination looking for orthostatic dysfunction.
Results: Among the 78 patients of stage 1, 56 accessed to tafamidis (73% V30MTTR), 16 underwent LT, 6 were lost of follow-up. The follow-up ranged from 0.5-4.5 years. Fourteen patients (25%) remained stable for > 2 years under tafamidis (max 4.5 years) including 8/15 with NIS<10, 80% V30MTTR; 7/15 EO. Twenty three/56 pts (41%) worsened with increased or onset walking disability (n=14); daily diarrhea (5) with anal incontinence (3); extensive painful and sensory neuropathy (n=2), hand weakness (n=1), onset of erectile dysfunction (n=1). Worsening occurred during the first year in 65%, between 1 to 2.5 years in 35%. Most of them had a NIS ≥10 (87%), 69% V30MTTR; 48% LO. Among the 23 patients who worsened, 12 were enrolled in clinical trials with TTR gene silencing ; 11 underwent LT.
Conclusions: Treatment of stage 1 TTR-FAP by tafamidis requires a close and long term monitoring of patients including a detailed questionnaire on walking disability, autonomic dysfunction, extension of pain and sensory loss. In case of significant progression in these items, a switch to clinical trials or LT should be done.

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The rehabilitation in the management of Transthyretin Familial Amyloid Polyneuropathy

Agnès Morier[1*], Alyssa De Sousa[2], Colombe Lemoine[1], Cecile Cauquil[1], Marie Théaudin[1], David Adams[1], Hervé Chanut[2]
[1]Centre de Références Neuropathies Rares (NNERF), service de Neurologie Adulte, Univ Paris Sud, CHU Bicêtre, 78 rue du Général Leclerc 94275 Le Kremlin Bicêtre, Paris, France; [2]Equipe de rééducation du Centre de Références Neuropathies Rares (NNERF), CHU Bicêtre, 78 rue du Général Leclerc 94275 Le Kremlin Bicêtre, Paris, France
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Introduction: The rehabilitation is part of a drug free therapy management of peripheral polyneuropathy. The Transthyretin Familial Amyloid Polyneuropathy this illness entails deficiencies that do impact on the day to day physical comfort and everyday life of patients. They have motor function and sensory consequences.
Methods: To be able to offer a well-adapted rehabilitation program, the rehabilitation therapists have put into place assessments in order to

estimate the different clinical manifestations described by the patients. The pain, the paresthesia and strength deficiency will be evaluated in this way, and their evolution followed up, thanks to comparative test. We have been looking for tools to be able to evaluate the clinical manifestations and their evolution.

Results: For that purpose, we have chosen comparative tests that allow to measure quantitative and qualitative results. The pain and the tiredness are evaluated with visual analogue scales, the strength with muscular testing and dynamometer, the functional aspect is tested with an evaluation of: the standing upright; the handicap situation and the 6 minute walking test. The assessments' have improved in recent years with more precise tools.

Conclusion: It is essential to stay as close as possible to the felt effects of the patients to deal with unbiased variations and put into place an adapted rehabilitation program.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Morier *et al.*: The rehabilitation in the management of Transthyretin Familial Amyloid Polyneuropathy. *Orphanet Journal of Rare Diseases* 2015, 10(Suppl 1):P66