MEETING ABSTRACTS

Proceedings of the 1st French-Italian meeting on laminopathies and other nuclear envelope-related diseases

Marseille, France. 15-16 January 2015

Published: 11 November 2015

These abstracts are available online at http://www.ojrd.com/supplements/10/S2

INTRODUCTION

An overview of new translational, clinical and therapeutic perspectives in laminopathies and other nuclear envelope-related diseases

Introduction: Defects of the nuclear envelope are now recognized as a vast group of heterogeneous rare inherited diseases. The first reported nuclear envelope-related disease, has been the X-linked form of the Emery-Dreifuss muscular dystrophy (EDMD1, OMIM#181350). The same gene, LMNA, was then found mutated in a large spectrum of disorders, now called Laminopathies, affecting the skeletal and cardiac striated muscles, the peripheral nerves, the adipose tissue or leading to segmental premature ageing syndromes. These discoveries have shed light on the nuclear envelope, and mutations in genes encoding other nuclear envelope proteins were regularly reported in cascade during the last 15 years. The French network on ‘EDMD & other nuclear envelope related diseases’ directed by Drs. Gisèle Bonne, Rabah Ben Yaou and France Leturcq (Paris), organizes annual meetings since it has been created in 2000. The Italian Network for Laminopathies, directed by Dr Giovanna Lattanzi (Bologna) and established in 2009, convene meetings twice a year. On January, 15-16, 2015, for the first time, the two networks held a joint meeting in Marseille at La Timone Adults’ Hospital: The 1st French-Italian meeting on laminopathies & other nuclear envelope-related diseases. This meeting was organized by Dr. Annachiara De Sandre-Giovannoli and Pr. Nicolas Lévy and the directors of the French and Italian networks. The meeting aimed to provide an update of recently acquired knowledge on: i) preclinical researches, ii) clinical researches, iii) patient registry and databases and iv) clinical trials in some of these rare diseases. The meeting also provided an understanding of the current state of the art on laminopathies and other nuclear envelope related diseases across France, Italy and the Iberian Peninsula and an opportunity to exchange ideas to improve patients’ healthcare organization in the future in a larger European/international context. The meeting has gathered 108 participants during two days. The first day was dedicated to communications among professionals involved in diagnosis, research and treatment of laminopathies and related diseases, and open to industrial partners, and the second day was dedicated to communications and view exchanges among professionals and patients’ families, aiming to inform them of the state of the art concerning their disease in terms of research and treatment. Among the invited speakers, Dr. Carlos Lopez-Otin, a leading scientist in the field of Progeria research, Dr. Raoul Hennekam, an expert clinician in the diagnosis and follow up of progeroid laminopathies and lipodystrophies, Dr. David Araujo-Vilar, the coordinator of the European consortium on lipodystrophies. These two days have been rich of view exchanges and informative for professionals and patients’ families, aiming to further develop novel as well as already established fruitful collaborations. It is planned not only to organize a second joint meeting between the French and Italian networks, but also to more widely open these meetings to other European colleagues, since already for this first edition, the Iberian community was largely represented. No doubt that this first edition will be the first one of a long series, since nuclear envelope proteins and their related diseases are extremely diverse and in continuous evolution.

Acknowledgments: We thank the patients, their families and the patient associations: the Associazione Alessandra Proietti onlus, the Associazione Italiana Distrofia Muscolare di Emery Dreifuss (AIDMED Onlus), the Associazione Italiana Progeria Sammy Basso (AlProSaB Onlus), the Progeria Family Circle for their participation. We thank “MCO congrès” for organizing the logistics of the meeting. This meeting was made possible thanks to the financial supports of the Association Française contre les Myopathies (AFM), Associazione Italiana Progeria Sammy Basso (AlProSaB onlus), the Progeria Family Circle for their participation. We thank “MCO congrès” for organizing the logistics of the meeting.

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1. PRECLINICAL RESEARCH ON LAMINOPATHIES

01 Molecular mechanisms of normal and pathological aging
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O1

We have recently defined nine molecular and cellular hallmarks that represent common denominators of aging in different organisms. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

On the other hand, parallel studies of our laboratory on accelerated aging syndromes, including Hutchinson-Gilford Progeria Syndrome (HGPS) and Nestor-Guillermo Progeria Syndrome (NGPS), have provided relevant information about these hallmarks of aging. HGPS is caused by a point mutation in the LMNA gene that yields a truncated form of prelamin A called progerin, which is also produced during normal aging. Over the last years, the generation of mouse models of HGPS and other progeroid laminopathies has shed light on the molecular alterations functionally involved in these diseases. Thus, knock-out mice deficient in Zmpste24 metalloprotease implicated in prelamin A maturation, mosaic mice containing Zmpste24-deficient and Zmpste24-proficient cells, and knock-in mice carrying the human HGPS mutation which causes progerin accumulation, have allowed us to demonstrate that progeroid laminopathies result from the combined action of both cell-autonomous and systemic factors. Accordingly, we have shown that nuclear envelope defects causative of these complex diseases lead to alterations in stem cell functionality, epigenetic abnormalities, perturbations in cell senescence pathways, metabolic changes and chronic activation of inflammatory responses. We have also demonstrated that the genetic or pharmacological blockade of these altered pathways prevents the development of many age-associated features of these progeroid mice and extends their longevity. On this basis, we have developed therapeutic strategies for progeroid laminopathies which are now in clinical trials coordinated by Pr. Nicolas Lévy for the treatment of HGPS patients. These findings illustrate the importance of mouse models for designing therapeutic strategies to treat rare and dramatic progeroid syndromes as well as for improving our knowledge of the universal and complex process of human aging.

1.1 IPS CELLS AND OTHER IN VITRO MODELS OF LAMINOPATHIES

02 Pluripotent stem cells for pathological modelling of Hutchinson-Gilford Progeria Syndrome (HGPS) and drug discovery
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O2

Progeria, also known as HGPS, is a rare, fatal genetic disease characterized by an appearance of accelerated aging in children. This syndrome is typically caused by mutations in codon 608 (p.G608G) of the LMNA leading to the production of a mutated form of lamin A precursor called progerin. In HGPS, progerin accumulates in cells causing progressive molecular defects including nuclear shape abnormalities, chromatin disorganization, DNA damages and delay in cell proliferation. Although two clinical trials have recently produced promising results, as well as in vitro and in vivo, there is currently no cure for HGPS patients. In collaboration with the teams of Dr Nicolas Lévy (UMR_S 910) and Dr Lino Ferreira (University of Coimbra), we have addressed this challenge by developing two high throughput screenings using the unique self-renewal and pluripotency properties induced pluripotent stem cells (iPS cells). Accordingly, these studies revealed the potential therapeutic effect of two new classes of compounds rescuing both nuclear shape abnormalities and defects of differentiation through on one hand, an inhibition of the prenylation process and on the other hand, a decrease of progerin expression.

03 Development of a SMCs model from HGPS-iPS and proofs of principle
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O3

HGPS is a rare, progressive aging disease in children that leads to premature death. Vascular smooth muscle cells (SMCs) are the most affected cells in HGPS patients, although the reason for such sensitivity remains poorly understood. Induced pluripotent stem cells (iPSCs) offer an unlimited source of SMCs to study the disease. iPSCs are also an important tool to study the molecular mechanisms of the disease from a developmental point of view. In this work, we study the reasons of HGPS-SMCs vulnerability using iPSCs obtained from HGPS fibroblast patients. We have evaluated the differentiation profile of HGPS-iPSCs and normal iPSCs into SMCs. We showed that HGPS-iPSC SMCs shared similar features observed on progerin-expressing cells. We have identified and characterize drugs that prevent SMC loss. Our findings open new opportunities for the treatment of HGPS disease and diseases related to vascular ageing.

04 3D culture system of muscle precursor cell to reveal mechanosensing defects in nuclear envelope related disorders
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O4

Mutations in the Lnk family of the Nucleoskeleton and Cytoskeleton (LINC)-complex associated proteins, including lamins and nesprins cause human muscular dystrophies but disease mechanisms still remain to be elucidated. We aim to determine whether human muscular dystrophies resulting from mutations in A-type lamin and nesprin1 affected the capacity of myoblasts to sense the stiffness of the extracellular matrix. Human myoblasts with various mutations in the A-type lamin encoded by LMNA (LMNA), nesprin1 mutant (SYNE1), and control (WT) myoblasts were cultured in 3D soft matrix (1-10 kPa) or on 2D conventional glass (~ 10 kPa) surfaces. Focal adhesion (vinculin), actin cytoskeleton, and YAP signaling pathway, a particularly important regulator of the mechano-response, were investigated. On 2D hard surface, there was no obvious difference in actin cytoskeleton and focal adhesion between WT, LMNA and SYNE1 myoblasts. In contrast, LMNA and SYNE1 myoblasts cultured in soft matrix exhibited enlarged focal adhesions and stress fibers compared with WT. Cytoplasmic translocation of YAP observed in WT in response to reduced stiffness matrix was absent in LMNA and SYNE1 cells, suggesting a permanent activation of YAP in mutant cells. In conclusion, our data indicate that cell culture matrix stiffness is critical to reveal mechanosensing defects in dystrophic muscle cells.

05 Prelamin accumulation in primary endothelial cells induces premature senescence and activation
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O5

Defects in lamin A maturation result in premature aging syndromes and severe atherosclerosis as observed in Hutchinson-Gilford Progeria Syndrome. In age-related atherosclerosis, several features of cells senescence have been characterized in endothelial cells lamin A...
alterations. We propose a cellular model to study lamin A-related senescence in primary endothelial cells. In this model, lamin A defects were induced by protease inhibitor (PI: Atazaravir) treatment during 48h on normal cells issued from placenta (human umbilical vein (HUVEC) or cord blood (ECFC)). We showed that PI treatment led to the accumulation of farnesylated prelamin A and induced nuclear shape abnormalities and premature senescence in both HUVEC and ECFC. ICAM-1-dependent activation was present and monocytes adhesion was increased in HUVEC whereas ability to generate microvascular network in matrigel was decreased for ECFC. The effects of PI treatment on nuclei shape were reversed when cells were PI-treated in combination with Pravastatin and Zoledronate in both mature and progenitor endothelial cells. Reversion also was demonstrated with 2 antisenses-dinucleotides targeted toward lamin A specific splice sites. This study confirms that PI treatment reproduces premature senescence due to lamin A maturation defects in primary endothelial cells after a 2 days exposure. The cells used were extracted from full term and healthy neonates i.e. from individuals of age 0. This allows us to consider that other senescence pathways were not activated and that the observed alterations were specific of prelamin A accumulation. This model constitutes a valuable tool to test different approaches aimed at reversing specifically lamin A-related cells senescence.

1.2. IN VIVO MODELS OF LAMINOPATHIES

06
Hypothalamic involvement in premature aging laminopathies
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Caloric restriction (CR), the reduced intake of calories without malnutrition, extends lifespan of many organisms, from yeast to mammals, and delays the progression of age-related diseases. Evidence show that hypothalamus is a crucial brain region for the progress of whole-body aging [1] and the beneficial effects induced by CR are regulated by nutrient-sensing neurons located in the hypothalamus [2]. Although CR’s beneficial effects in delaying human aging are promising, its application for long periods is very difficult to maintain and not feasible to apply to fragile children with progeria. To overcome this problem, the induction of protective endogenous mechanisms, or pharmacological agents, could theoretically be used to mimic the beneficial effects of CR without its discomfort. Our group showed that hypothalamic of Zmpste24/- mouse has lower levels of Neuropeptide Y, compared to wild-type animals. Moreover, they showed that targeting the Neuropeptide Y system in hypothalamus, as a CR mimetic strategy, delays or reverts some ageing features of Zmpste24/- mice. Further studies are needed to confirm this innovative approach and if it could be translational to progeria children.

References

07
Investigation of pathomechanisms of ventricular arrhythmias in cardiac laminopathies
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):07

Mutations in LMNA are responsible for an aggressive form of dilated cardiomyopathy due to a high rate of malignant ventricular arrhythmias. Inter-cellular communication is essential for proper cardiac function. Mechanical and electrical activities must synchronize so that the work of individual cardiomyocytes transforms into the pumping function of the heart. This well-coordinated excitation-contraction coupling of the heart relies on an efficient inter-cellular communication, which is under the regulation of the intercalated discs. We focused on the understanding of the molecular mechanisms of components of intercalated disc re-localization in pathological context. For this, we investigated disease mechanisms and identify novel therapeutic targets, using an integrated series of models in cultured cells, mice and humans. Positive results will break new ground for future work towards developing novel treatment for malignant arrhythmias.

1.3 NOVEL THERAPEUTIC APPROACHES, PROOFS OF PRINCIPLE IN LAMINOPATHIES

08
New therapeutic approaches to HGPS based on progerin inhibition
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Hutchinson-Gilford Progeria Syndrome (HGPS) is caused by a de novo heterozygous mutation on LMNA gene that leads to accumulation of progerin, a mutant form of prelamin A. HGPS skin fibroblasts are characterized by multiple nuclear defects: nuclear shape abnormalities, chromatin structure alterations, increased DNA damage and cell cycle alterations. Retinoic acid may modulate LMNA gene transcription, due to the presence of a retinoic acid responsive element (L-RARE) in the LMNA promoter. Based on this knowledge, we investigated if all trans retinoic acid (ATRA) could lower progerin levels in HGPS fibroblasts. We also evaluated the effects of a combined treatment with rapamycin, a drug known to promote autophagy and reduce both farnesylated prelamin A and progerin amount. We demonstrate a surprising effect of ATRA to repress Lamin A/C gene transcription and we show that the combined treatment with ATRA and rapamycin has a synergistic effect: it dramatically lowers progerin levels, restores both heterochromatin organization and nuclear shape, reduces DNA damage markers and improves cell viability. These promising results could open the way to a new therapeutic approach for HGPS.

09
Efficient progerin clearance through autophagy induction and SRSF-1 downregulation in Hutchinson-Gilford Progeria Syndrome
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Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare premature and accelerated aging disease caused by a de novo point mutation in LMNA encoding A-type lamin. Progerin, a truncated and toxic form of prelamin A, accumulates in HGPS cells nuclei and is a hallmark of the disease. We show that progerin is sequestered, together with other proteins (lamins B1/B2, emerin), into abnormally shaped nuclear organelles, identified as novel biomarkers in Progeria. We identified a novel compound that led to effective progerin degradation and clearance from patients’ fibroblasts. This compound induces progerin nucleocytoplasmic translocation, and progerin degradation through macroautophagy. It also strongly reduces progerin production through caspase-linked cleavage of SRSF-1 controlling prelamin A mRNA splicing. In vivo, upon treatment with the compound, progerin expression decreases in skeletal muscle of Lmna–/– mice. Altogether,
we demonstrate increased progerin clearance based on the dual action of a novel compound and shed light on a novel promising class of molecules towards a therapy for Progeria and related diseases.

O10 Impairment of Lamin A/C-Polycomb crosstalk as a possible epigenetic cause of Emery-Dreifuss Muscular Dystrophy (EDMD)
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Traditionally, studies on EDMD have focused on genetic changes affecting molecules involved in the development of pathology. However, emerging findings indicate that a single genetic mutation can be accompanied by a range of different phenotypes, suggesting a contribution of the epigenetic background to the disease progression. This is in line with recent works showing that changes in chromatin architecture are peculiar of several laminopathies [1,2]. Despite much effort has been done to understand the regulation of the complex networks of gene expression that govern muscle differentiation and that is affected in EDMD, little is known about the epigenetic players and molecular mechanisms involved in pathogenesis and progression. Key epigenetic regulators of chromatin architecture are Polycomb group (PcG) of proteins, epigenetic transcriptional repressors of genes primarily involved in differentiation and development [3]. In particular, during myogenesis, modulation of Ezh2 levels, the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2) ensures the correct muscle differentiation [4]. In the nucleus, PcG proteins form microscopically visible foci and high-through-put data together with microscopy analysis revealed that their targets are organized in chromatin loops [5,6]. We have shown that Lamin A/C sustains PcG foci influencing PcG nuclear compartmentalization and modulating their repressive functions. During myogenesis, Lamin A/C depletion leads to an altered timing of muscle differentiation due to the aberrant expression of PcG-regulated genes.

References

O11 Gene Therapy for LMNA-related Congenital Muscular Dystrophy (L-CMD) by Trans-SPlicing
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O11

LMNA-related Congenital Muscular Dystrophy (L-CMD) is a rare genetic disorder characterized by the onset of selective axial weakness and wasting in the first year of life with limited motor achievements, associated with multiple severe contractures and frequent respiratory failure requiring early ventilatory support. We identified heterozygous de novo mutations in LMNA, encoding lamins A/C, as responsible for this sub-group of CMD in which no therapeutic treatment is available [1]. Lamins A/C are nuclear envelope proteins, ubiquitously expressed in all post mitotic cells, which play essential roles in the nucleus structure and in the regulation of gene expression. We generated the first Knock-In mouse model of L-CMD (KI-LmnaΔEx9,10) reproducing a LMNA mutation identified in L-CMD patients. Homozygous mice die within the first 3 weeks of life from striated muscles maturation delay and severe metabolic defects [2]. Heterozygous mice develop an isolated dilated cardiomyopathy and die by one year of age [3]. We aim to assess the possibility of LMNA-mRNA repair by spliceosome-mediated RNA trans-splicing (SMaRT) as a potential therapeutic approach for L-CMD. This gene therapy strategy will allow inhibition of mutated LMNA transcript expression for the benefit of corresponding wild type transcripts. We developed 5’-RNA pre-trans-splicing molecules (PTM) capable of repairing the murine LMNA transcripts. Efficiency of these PTM was assessed in vitro in C2C12 cells and in vivo using Adeno-Associated Virus (AAV) transduction in tibialis anterior of WT mice. We will now determine the ability of the best PTM to restore normal muscular phenotype, in vitro in Ki myoblasts/myotubes and in vivo after injection of AAV-PTM vectors in new born homozygous and adult heterozygous mice. Histological and metabolic parameters will be monitored to evaluate the degree of phenotype rescue.

References

1.4 NOVEL BIOMARKERS IN LAMINOPATHIES

O12 Chromatin dynamics and in vitro biomarkers in laminopathies: an overview
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Chromatin regulation in eukaryotes occurs through complex and interconnected mechanisms that ensure heterochromatin maintenance and compartmentalization of chromosome domains, genome stability, chromatin conformational changes before and after mitosis, gene silencing and transcriptional activation and chromatin remodeling at specific promoters. We refer to these events as a whole using the term “chromatin dynamics.” Chromatin dynamics involves a number of protein families including epigenetic enzymes, DNA repair factors, heterochromatin proteins, transcription factors and transcriptional regulators. Although laminas have been involved in almost all the processes that regulate chromatin dynamics [1], three main functions link lamins to chromatin regulation: recruitment of the DNA damage response proteins [2], transcription factor binding [3,4] and modulation and maintenance of heterochromatin domains [5]. Our preliminary data have shown that lamin A/C plays a major role in anchorage of epigenetic enzymes in nuclei and loss of lamin A/C- histone deacetylase (HDAC) binding, as occurs in Hutchinson-Gilford Progeria (HGPS) cells, affects enzyme activity and histone acetylation. These results may explain our previously published data [6] showing that the heterochromatin defects...
of HGPS cells can be rescued by combined inhibition of prelamin A farnesylation and HDAC activity and pave the way to new therapeutic perspectives. Moreover, altered lamin A/C-HDAC interaction and histone acetylation patterns can be explored as potential biomarkers for laminopathies.

References

O13
SREBP1 (Sterol regulatory element binding protein 1), transcription factor that regulates hundreds of genes involved in lipid metabolism and adipocyte differentiation, is a direct partner of A-type lamins. We show that i) in vitro, the tail regions of prelamin A, lamin A and lamin C bind a polypeptide of SREBP1 and ii) within cells, interactions between wild-type A-type lamins and SREBP1 occur mainly at the nuclear periphery but also within the nucleoplasm. While A-type lamin R482W mutation is responsible for Dunnigan type familial partial lipodystrophy (FPLD2), we show that both overexpression of LMNA p.R482W in primary human preadipocytes and endogenous expression of A-type lamins p.R482W in FPLD2 patient fibroblasts, reduce A-type lamins-SREBP1 in situ interactions and upregulates a large number of SREBP1 target genes [1]. As this LMNA mutant was previously shown to inhibit adipogenic differentiation, we propose that deregulation of SREBP1 by mutated A-type lamins constitutes one underlying mechanism of the physiopathology of FPLD2.

Reference

O14
Altered cytokine profiles in laminopathic patients
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Prelamin A accumulation is known to dysregulate the NF-kB signaling cascade, causing a secretion of high levels of proinflammatory cytokines, which in turn might contribute to the pathologic aging observed in laminopathies, and in particular in HGPS [1]. In collaboration with researchers and clinicians of the Italian network for Laminopathies, we wondered whether it was possible to identify a pattern of cytokine expression that could discriminate laminopathy from other forms of muscular dystrophy and/or cardiomyopathy and a laminopathy with a cardiac involvement from one with only muscle involvement, with the final goal to identify biomarker(s) helpful for diagnosis, prognosis and evaluation of therapy efficacy. We analysed the cytokine profiles of sera collected from 37 patients affected by different forms of laminopathy (all LMNA mutations), 9 patients affected by genetically defined non-LMNA muscular dystrophy and 27 healthy individuals. Sera were screened for the expression levels of 16 cytokines, 6 chemokines, 5 growth factors and TGF-beta1, 2 and 3 by Luminex technology. Some pro-inflammatory cytokines were found to be differentially expressed in cardiopathic and non-cardiopathic patients compared to healthy controls, and among laminopathies with muscle and cardiac involvement, laminopathies without myopathy and muscular dystrophies. Interestingly, TGF-beta2 serum levels were higher in the LMNA patients than in healthy individuals and in patients with non-LMNA muscular dystrophy, suggesting a direct link between LMNA mutations and dysregulation of TGFbeta2 pathway, as indicated by previous and recent experimental studies [2,3].

References

O15
microRNA deregulation in Hutchinson-Gilford Progeria
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The Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare genetic disease characterized by an accelerated aging, due to the accumulation in nucleus of a toxic protein called progerin, leading to abnormal gene expression and potential microRNA (miRNA) deregulation. To evaluate the role of miRNAs in HGPS, we conducted an in vitro miRNAome analysis by RT-qPCR on dermal fibroblasts of 5 patients and 5 healthy individuals at early (P12+/2-) and for 5 individuals at late passages (P22+/2-). We found 29 deregulated miRNAs in more than 50% of patients (15 overexpressed, 14 underexpressed) presenting different deregulation profiles depending on their age and passage in vitro. We identified 4 interesting potential targeted pathways linked to aging/Progeria: cell cycle and proliferation, senescence, inflammation and autophagy for which 3 miRNAs target central actors of this pathway. No significant difference between patients and controls was detected for 3 autophagy makers on western blotting. However, using flow cytometry, allowing quantification of autophagy level cell by cell, we observed in a 14 yo patient exhibiting the most miRNA deregulated profile, a majority of cells presenting no autophagy. Our hypothesis is that the combined overexpression of the 3 autophagy inhibitor miRNAs acts as a "brake" on autophagy, leading to a decrease of progerin degradation, and finally to a pathophysiological vicious cycle. We are now confirming this hypothesis by transfecting antagoniRs on cellular model. We will also evaluate this mechanism in our HGPS LAKI mouse model and in the context of physiological aging, during which progerin is also produced at lower levels.
Emerinopathies include diseases caused by EMD gene mutations localized on chromosome X and encoding emerin, an integral protein of the nuclear envelope. The most frequent emerinopathy is the X-linked Emery-Dreifuss muscular dystrophy (X-EDMD) that was first reported in the 60ths by Emery and Dreifuss [1]. The disease is characterized by muscle weakness and wasting usually with a humeroperooneal distribution in the first stages, early joint contractures involving Achilles tendons, elbows, neck and the whole spine, and cardiac involvement featuring conduction defects, arrhythmias, subsequent cardiomyopathy (usually dilated) and frequently responsible of sudden death. Bone et al. [2] identified the first mutations of EMD gene encoding emerin to be responsible of X-EDMD. These mutations usually lead to absence or reduced level of emerin in different tissues of affected males including skeletal muscle, skin, oral mucosa cells and lymphocytes as it is demonstrated by immunocytochemical and histochemical methods [3,4]. Female carriers exhibit mosaic expression patterns with usually normal emerin amounts [3]. These methods may thus be used in the diagnostic strategy of X-EDMD prior to EMD gene analysis.

In a recent study (unpublished work from the French network and LBGM) aiming to assess the diagnostic utility of emerin study by western blot on lymphoblastoid cell lines, we looked at EMG mutation rate observed in a cohort of 269 male and female patients with variable emerin amounts. In male patients, absence or severe reduction of emerin (<5%) lead to EMD mutation identification in all cases, while moderate emerin amount reduction revealed EMD mutation in 75% of the patients. Interestingly, in all cases where emerin amounts were considered as normal, no EMD mutations were found. In female cases, all cases with emerin moderate or severe reduction harbored EMD mutation. When emerin is normal, EMD mutation was found in 58% of female cases. These results suggest that a diagnostic rate of 100% may be reached if emerin study by western blot is used in the diagnostic strategy of X-EDMD prior to EMD gene analysis.
Emery-Dreifuss muscular dystrophy (EDMD) is a hereditary muscular disorder characterized by early joint contractures, progressive muscular wasting and weakness of scapuloperoneal distribution, and at adult age, patients develop cardiac abnormalities with a high risk of sudden death [1]. EDMD encompasses both X-linked and autosomal inheritance due to mutations in the genes encoding the nuclear envelope proteins emerin, lamin A/C [2-4]. First mutations in the Four-and-half-LIM domain 1 gene (FHL1) being responsible for X-linked EDMD were described by Gueneau et al. [5]. The human FHL1 gene encodes three alternatively spliced isoforms, named FHL1A, FHL1B, and FHL1C, with FHL1A being the most abundantly expressed protein isoform in striated muscle. There is still little known about the precise localization and functions of the three different FHL1 isoforms in human skeletal muscle. Here, we describe for the first time the subcellular localization of FHL1A, FHL1B, and FHL1C in vitro in differentiating human primary myoblasts.

Localization of FHL1 protein isoforms was studied at the myoblast and myotube stages by confocal microscopy analysis. Endogenous FHL1B protein localization was detected by an anti-FHL1B specific antibody, while for FHL1A and FHL1C, as no efficient isoform-specific antibodies were available, an anti-Flag antibody was used to follow Flag-tagged FHL1A and Flag-tagged FHL1C protein expression, after lentiviral transduction of human primary myoblasts. Successful transduction was confirmed by western blotting of whole extracts from myoblasts and myotubes using an anti-Flag antibody. In human myoblasts, Flag-FHL1A and Flag-FHL1C showed both a cytoplasmic and a nuclear distribution, while the nuclear staining was more pronounced in Flag-FHL1C transduced myoblasts. Endogenous FHL1B protein gave a moderate cytoplasmic and a strong nuclear staining. During 6- and 12-days of human myoblast differentiation, localization of all three FHL1 protein isoforms shifted from the nucleus to the cytoplasm. In addition, all FHL1 protein isoforms were observed to co-localize with phalloidin-stained actin fibers. Collectively, these results indicate differentiation-related changes in expression and subcellular localization of the human FHL1 protein isoforms.

References

O19
LMNA-associated myopathies: the Italian experience in a large cohort of patients
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O19

We conducted a retrospective study in a large cohort of myopathic patients carrying LMNA gene mutations to evaluate clinical and molecular features associated with different phenotypes. To this purpose we included 90 LMNA-mutated myopathic patients and 36 LMNA-mutated familial cases without muscle involvement. Among the myopathic patients LGMD1B was by far the most frequent phenotype, observed in 43 (48%) patients, followed by L-CMD in 21 (23%), EMD2 in 20 (22%) and an atypical myopathy in 6 (7%). The different myopathic phenotypes shared a similar cardiac impairment. On the other hand comparing cardiac features between myopathic and familial cases without muscle involvement we observed that cardiodepressor defibrillator or pacemaker were implanted more frequently in myopathic patients (n=43) (p=0.006). In addition heart transplantation and death were observed only in myopathic subgroup, respectively in 8 (9%) and 10 (11%) patients. In conclusion LMNA-related myopathies represent a continuum clinical spectrum; their clinical course appears to be dominated by cardiac involvement, considering the relatively low frequency of other complications, including loss of ambulation, assisted ventilation, surgery for scoliosis or gastrostomy. Longitudinal studied are needed to better investigate their natural history and provide indications for early management of heart involvement, in particular in first decades of life, to prevent the risk of fatal events.

O20
Limb-girdle muscular dystrophy 1F is caused by a microdeletion in the transportin 3 gene
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O20

Whole genome sequencing strategy allowed identifying the gene responsible for autosomal dominant limb-girdle muscular dystrophy 1F, which was previously linked to locus 7q32.1-32.2. A large Spanish family spanning six generations with limb girdle muscular weakness and distal involvement was find to present a mutation in the stop codon of TNPO3 gene (c.2771delA). The mutation segregates with the clinical phenotype, and is absent in healthy relatives of the family as well as in genomic sequence databases. Histological abnormalities of the nuclei and altered TNPO3 expression assessed in muscle biopsy of the patients indicate impaired TNPO3 function. TNPO3 encodes transportin-3, a serine/arginine rich protein carrier through nuclear membrane. The function of transportin-3 in skeletal muscle has not been thoroughly characterized. The identification of this mutation as the cause of autosomal dominant limb-girdle muscular dystrophy highlights the importance of defects of nuclear envelope proteins as causes of inherited myopathies [1].

Reference

O21
Irisin levels in LMNA-mutated lipodystrophic syndromes
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O21

Sarcopenia is defined by decreased muscle mass and impaired muscle function, which may be associated with frailty and eventually higher mortality rates. It is physiologically induced by aging but also related to obesity by different mechanisms such as 1) diminished physical activity; 2) elevated oxidative stress; 3) inflammatory cytokines; 4) increased catabolic state, through hypothalamo-pituitary axis; 5) muscle insulin resistance; 6) abnormal muscle progenitor cells differentiation to an adipocyte-like phenotype as a result of paracrine signals from (adipo) cytokines.

Adipose tissue is classified as white adipose tissue (WAT), the major energy storing tissue, brown adipose tissue (BAT), which mediates non-shivering thermogenesis and brite adipocytes (brown in white). Increasing BAT and energy expenditure in adult humans could be a therapeutic strategy to combat obesity. Brown adipocytes are thought to originate from a precursor shared with skeletal muscle that expresses Myf5-Cre, while white adipocytes originate from a Myf5-negative precursors. This provides a rational explanation to why BAT is more metabolically favorable than WAT, even if the situation is more complex because subsets of white adipocytes also arise from Myf5-Cre expressing precursors. Differences in origin between adipocytes could explain metabolic heterogeneity between
depots and/or influence body fat patterning particularly in lipodystrophic disorders.

Irisin is a newly discovered myokine, associated with ‘browning’ of the WAT. It displays a day-night rhythm, is correlated with lean body mass, and increases after exercise in healthy young individuals, despite an association with major adverse cardiovascular events and polycystic ovary disease [1]. Deficiency of myostatin, and thus stimulation of muscle growth, has also been reported to induce irisin and its precursor FND5C expression in muscle and drive the browning of WAT in mice.

Familial partial lipodystrophy, Dunnigan variety (FPLD2), an autosomal dominant disorder caused by LMNA mutations, is characterized by fat loss from the extremities and apparent muscular hypotrophy. However, it is unclear whether these patients appear muscular because of a lack of subcutaneous fat or have an actual increase in muscle mass [2]. Moreover adipose tissue mitochondrial dysfunction triggers a lipodystrophic syndrome with insulin resistance, hepatosteatosis, and cardiovascular complications [3,4].

Therefore, our objective was to identify the status of lean mass in LMNA-mutated lipodystrophic syndromes and to determine simple biomarkers to differentiate LMNA-mutated and acquired lipodystrophies. To do so, we assessed the lean (as a surrogate of muscle mass) and fat mass with absorptiometry in FPLD2 patients, non-diabetic obese and control subjects using dual-energy x-ray absorptiometry and magnetic resonance imaging, and measured the myokine irisin and the adipokine leptin blood levels. Our hypothesis is that the rupture of balance between physiological lean and fat mass in lipodystrophic syndromes could explain the evolution towards insulin-resistance (Trial registration: Clínic.gov:2009-AO-1169-48/PHRC2009 09/04).

References


**O22 The nuclear lamina during human spermiogenesis**

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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O22

The nuclear lamina takes centre stage during spermiogenesis, the post-meiotic phase of spermatogenesis, when haploid round spermatids differentiate into spermatozoa: the acrosome and flagellum develop at opposite nuclear poles, the nucleus elongates and, as the nuclear histones are replaced with protamines, the chromatin condenses, to produce the highly compacted pyriform nucleus of the mature spermatozoon. In rodent spermatids, the nuclear lamina contains lamins B1 and lamins B3 a specific isoform of lamin B2 with a shortened rod domain, and A-type lamins are absent [1,2], but nothing is known about the structure of the nuclear lamina during human spermiogenesis. We are studying the nuclear lamina during human spermiogenesis. We have shown that the human nuclear lamina contains lamins B1 and, distinct from rodents, lamin B2. We also described a transcript potentially encoding a human lamin B3 that, like its mouse counterpart [3], induces severe nuclear deformation when expressed in HeLa cells [4]. In human, lamin B1 and B2 localize to the nuclear periphery in spermatids except in the region covered by the acrosome. They are seen to recede to the posterior pole of the nucleus as the spermatids progress through spermiogenesis. Lamina B1 was observed on 30-40% of ejaculated spermatozoa, while lamin B2 was not detected. The percentage of B1-labelled spermatozoa dropped at least 6-fold when spermatozoa with normal head density were selected, indicating that lamin B1 labels immature spermatozoa lacking a fully compacted nucleus, and may therefore be a marker of poor sperm quality. The comparison of the human nuclear lamina with that of the mouse suggests that lamin B1 and B3 have critical roles during mammalian spermiogenesis.

References


**O23 Laminopathies: clinical presentations and management**

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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O23

The number of laminopathies is large, and the variability is equally wide. OMIM mentions 10 different entities, but there are several additional reports of individuals with a lamin A/C mutation who have phenotypes that are still at variance of these. This variability can be explained in two ways. One is the widespread dissemination of the lamin A/C protein within our bodies and indeed within individual cells and the many functions that is has. The function of providing firmness to the nuclear envelop is a major function. A lack of that firmness due to the abnormal protein causes all structures and proteins in the envelope potentially to be disturbed. These have all kind of functions, sometimes also completely unrelated, and all can be disturbed by the abnormal lamin A/C only. The other explanation is the variability between individuals with changes in the same gene in general. Even brothers and sisters with exactly the same mutation in exactly the same gene can still show very different phenotypes. The background is that it will not be a single gene that explains the phenotype but also the background of genetic information of each person, and the exogenous influences on this, are important. Indeed, “monogenic disorder do not exist” [1]. So variability should in fact be expected and also explained to patients.

One can evaluate all laminopathies for their major manifestations, which are the heart, muscles, nerves, joints, fat tissue, skin, bone, morphology of the face, growth and endocrine functioning. Some laminopathies are explained by mainly heart and muscle abnormalities, other mainly by bone, fat, skin, growth and face abnormalities. However, it may be this distinction is artificial. It may be that in fact (almost) all laminopathies show signs or symptoms in all of the above tissues, but we fail to recognize this either because we haven’t looked carefully enough, or because patients die for one particularly affected tissue and therefore don’t have the time to show the other manifestations in other tissues. This can be important in evaluating patients with the various laminopathies, in providing optimal care to them, and in considerations if a management if applied for one of the consequences of an lamin A/C, as one cannot exclude others will then arise that have been unknown until then.

Some may argue that this means in fact all patients with a laminopathy might be better put under a single diagnosis. That seems not right. Detailed discussions about this are available in literature [2]. In addition, the WHO has decided in the development of the upcoming new International Classification of Diseases that what really counts is what a patient experiences from an entity. And surely it does make a difference if
one has an entity that leads to demise already around birth (restrictive dermopathy), leads to significant problems that will be fatal in puberty (Hutchinson-Gilford progeria), or allow you to live well into adulthood at least with only limited restrictions in well-being (mandibulo-acral dysostosis). So grouping all disorders under the umbrella laminopathy is very useful for our insights, but for patients subdivision into individual entities is still essential. The grouping into laminopathy has also advantages in considering various management strategies. In a very basic way one can divide management into influencing the abnormal DNA (gene therapy), influencing the abnormal RNA (mainly through morpholinos and other small molecules), decreasing the amount of abnormal protein and/or increasing the amount of normal protein (by farnesylation inhibition or increasing turnover of proteins), and by influencing the consequences on a cell or tissue level (for instance by statins). Gradually it becomes clear that the most effective way must be influencing the abnormal RNA as the other ways are either undesirable (gene therapy) or lack true, curative effectiveness (FTIs and statins). The advantage by working in this way is that studies for one laminopathy might have benefits for the other laminopathies as well, and in the end also for all patients.

References

O24
Management of congenital muscular dystrophies related to defects in the LMNA gene
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O25
Cardiac involvement in laminopathies
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Lamin A/C gene mutations can be associated with myocardial diseases, usually characterized by dilated cardiomyopathy and/or arrhythmic disorders. Phenotypic penetrance is age-related but expression is extremely heterogeneous, so that muscular and arrhythmic disease can be present in combination in the same patient, or one phenotypic manifestation can appear earlier than the other or even not become overt for a long time period [1]. From a cardiologic point of view, aetiologic diagnosis in dilated cardiomyopathy, and specifically the diagnosis of cardiomyopathies, is relevant, since clinical and prognostic implications as well as specific management strategies can be different, particularly with regard to prevention of sudden cardiac death. Patients can be diagnosed as being affected by a cardiomyopathy as a result of a cardiologic workup performed for symptoms of heart failure or for arrhythmic events or can be diagnosed incidentally or during family screening. Family history, physical examination, laboratory findings (specifically serum creatine kinase values) and ECG findings are important “red flags” to diagnose a cardiomyopathy. Patients with cardiomylopathies may present a wide range of arrhythmic disturbances, which include either bradycardias (conduction disturbances and atrio-ventricular blocks, sinus node dysfunction, atrial standstill) or tachyarrhythmias (atrial fibrillation, atrioventricular re-entrant tachycardia and atrioventricular re-entrant tachycardia), in variable combinations, and with frequent association with left ventricular dysfunction and heart failure (Figure 1). The presence and severity of arrhythmic disturbances is usually not related to the presence and degree of neuromuscular impairment [2-4]. The most common clinical manifestations are lightheadedness, syncope, palpitations, or even ischemic stroke due to cardioembolism (in case of atrial fibrillation or atrial standstill) or sudden death [2-5]. Implantation of a pacemaker protects from the consequences of bradycardias, while an implantable cardioverter defibrillator (ICD) is able to interrupt malignant ventricular tachyarrhythmias, thus preventing sudden cardiac death [6]. Biventricular pacing is a form of cardiac stimulation, referred as cardiac resynchronization therapy (CRT) that may improve cardiac function in case of heart failure, low ejection fraction and ventricular dyssynchrony [7]. Clinical decision making has to consider the risk and benefit of brady- and tachyarrhythmias, taking into account presence/absence of ventricular dysfunction, and the decision to implant a cardiac electrical device (pacemaker, ICD, with/without CRT) should consider potential risks and benefits (brignole EP). In a multicenter study a series of risk factors emerged as predictors of the occurrence of ventricular tachyarrhythmias (male gender, non-sustained ventricular tachycardia, left ventricular ejection fraction < 45% and non-missense mutation) and their presence or combination and should help for the decision to implant an ICD [8].

References
Lipodystrophic syndromes are rare diseases of acquired or genetic origin, associating a decreased amount of fat (with an altered distribution of body fat in partial forms) and the metabolic alterations usually observed in obesity, i.e. insulin resistance leading to diabetes, hypertriglyceridemia with the risk of acute pancreatitis, fatty liver with risk of cirrhosis, and precocious atherosclerosis. Mutations in more than 15 genes, including LMINA, have been shown to be responsible of monogenic forms of lipodystrophies. The decreased capacity of adipocytes to store excess energy as lipids and to perform physiological endocrine functions, is considered as the main pathophysiological determinant of lipodystrophies. The low circulating levels of leptin lead to an increased appetite and participate in the ectopic storage of lipids in the muscle and liver, which aggravates the metabolic alterations. Replacement leptin therapy was shown to strikingly improve insulin resistance, dyslipidemia and liver steatosis in patients with generalized form of lipatrophy, associated with very low endogenous secretion of leptin [1]. Recombinant leptin (metreleptin), administered in one daily subcutaneous injection, is well-tolerated, and, although it did not improve lipatrophy itself, demonstrated metabolic benefits in 55 lipodystrophic patients during a 3-year therapy [2]. Regarding laminopathies, two studies evaluated metreleptin therapy in 6 then 24 patients with the Dunnigan-type familial partial lipodystrophy [3,4]. Although metreleptin was efficient in decreasing circulating triglycerides and liver steatosis, the effects on glucose homeostasis did not reach statistical significance. Metreleptin, which is the first specific therapy for lipodystrophies, was approved in 2014 by the FDA for generalized forms, and is available in selected European centers through compassionate programs. Further studies are needed to clarify the therapeutic indications of metreleptin in partial lipodystrophies including laminopathies.

References

O28 Round Table: Discussion with families and lay associations
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O28

This session was dedicated to patients, their relatives and delegates of Family Associations (the Progeria Family Circle, the European Association of Progeria Families; AlProSAb, the Italian Association for Progeria, Sammy Basso: AIDMED, the Italian Association for Emery-Dreifuss Muscular Dystrophy; the Associazione Alessandro Proietti for Emery-Dreifuss Muscular Dystrophy), with the main objective to establish for the first time a direct interaction with the scientific international community working on laminopathies. Since the patient number is very low, and the clinical presentations of laminopathies vary greatly among the different subgroups, this was a good opportunity to confront the whole community to identify the ‘unmet needs’; besides the therapy to cure the disease, they include all the complementary aspects of the disease that worsen the patient quality of life. With the help of patients, the researchers may plan adequate strategies to address all the issues related to the disease and to give a ‘priority’ to each of them. Some examples of such positive interactions are the primary role of Patients Association in the production of the Standards of Care for a group
of neuromuscular disorders by TREAT-NMD, an European Organization, the involvement of Parent Project in DMD clinical trials planning by Pharma Industries and their inclusion in the process of drug approval by American and European Drug Agencies (FDA and EMA). In Italy, the Italian Association of Myology produced two consensus conferences, on vaccinations and on anesthesia procedures in neuromuscular patients, on the suggestion of Italian Neuromuscular Associations.

With the exception of patients with Progeria, who already benefit from a good network of expert that cover their needs, the other patients, in particular those with muscular dystrophy, are less characterized and their clinical protocols are less standardized.

Future possible areas of common interests, to be prioritized and supported by Patients Association, may include a consensus on precise criteria to define the different phenotypes; protocols to reduce diagnostic delay or misdiagnoses; physiotherapy indications; pain relevance and management; nutritional issues.

Patient participation during the round table allowed us to focus on their main issues: the need to spread knowledge on laminopathies and to foster research activity in the field (mentioned by an EMDMD parent and by an HGPS patient); the need of clear indications for follow-up reference centers (mentioned by an EMDMD patient); concerns about dietary advices for muscular and progeroid laminopathies (mentioned by an EMDMD parent with reference to the talk by Dr. Quijano-Roy and comments by Dr. D’Amico and by an HGPS parent).

### 2.3 Focus on registries and databases

#### O29

**Utility of patients’ registries to gather clinical, epidemiological and molecular information**

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*Orphanet Journal of Rare Diseases 2015, 10(Suppl 2)O29*

Rare disease patient registries are indispensable tools for translating research into improved care and therapeutic solutions. During the race to identify a safe and effective treatment, they come into play at many stages of the translational research cycle: collection of mutational data, description of the disease, support for patient recruitment for clinical trials and scientific studies (such as natural history studies), collection of epidemiological data, evaluation/monitoring of the efficacy/safety of a treatment, elaboration of guidelines for diagnosis and management of the disease, etc.

In the field of rare disease, the main challenges that patient registries face are sustainability, better interoperability with the establishment of common data standards (for data collection, data quality, data security, legal and ethical issues) and support for translational collaborations to constitute large cohorts of patients. To work out these questions, the IRDRRC (International Rare Disease Research Consortium) initiative is a major force to encourage cooperation at international level. In this process, patients and families are becoming more active participants and must continue to raise their voice to drive innovation in collaboration with all stakeholders.

#### O30

**Clinical aspects of cardiolaminopathies and prospects for a cardiolaminopathy registry**

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*Orphanet Journal of Rare Diseases 2015, 10(Suppl 2)O30*

Mutations in the LMNA gene, encoding nuclear proteins lamin A/C, have been associated with neurological and cardiac disease and a high risk of sudden death. The implant of a cardioverter defibrillator (ICD) is to date the only effective intervention, but no specific guidelines are available. We decided to create a common Italian database integrating clinical and genetic data of patients bearing LMNA gene mutations to improve knowledge of natural history of cardiopathy, define a risk stratification protocol for ICD implant and investigate genotype/phenotype correlations.

To date, 113 patients (age 47±18) from 11 Italian centers have been included in our database and followed for 7±11 years. We evaluated age at onset of different phenotypes. 70% developed cardiac symptoms, including both rhythm (atrial fibrillation, atrio-ventricular block, ventricular tachycardias) and structural defects (dilated cardiomyopathy, mitral insufficiency), which may or may not be preceded by neurological signs. Cardiac magnetic resonance was pathologic in 2/3 of studied patients. We also evaluated the occurrence of ICD implantation, appropriate shocks, cardiac transplantation and heart failure. Open questions include the identification of predictors of arrhythmias to allow early diagnosis and improve risk stratification and management of asymptomatic patients.
The European Consortium of Lipodystrophies (ECLip) is a network of relevant clinical and basic-science research groups in Europe involved in investigation of Lipodystrophic Syndromes (LS).

The goal of this Consortium is to enable intensive and effective collaboration among the various high-quality European research groups in order to promote the free exchange of ideas and information concerning research and clinical care among LS researchers. The principal benefit will be the advancement of patient care. It will also promote the public understanding of LS and its consequences in affected individuals. On the other hand, ECLip will lead to further growth and inclusion of novel aspects of LS research, making European investigators the leaders in this important but still poorly explored research area. Likewise, ECLip will try to give visibility and recognition of LS in society and among policy makers, and will help the promotion of advocacy groups in Europe and worldwide.

To date, ECLip is formed by 38 research groups coming from 15 European countries. The ECLip website (http://www.european-lipodystrophies.org) provides information on all groups involved in the consortium, with information about the researchers, the main research lines of each group, their clinical and basic research facilities, and contact details.

### 2.4 CLINICAL TRIAL FOR RARE DISEASES

#### 033 Which support from the French Foundation of rare disease towards clinical trial set up in rare diseases?
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O33

The French Foundation for rare diseases (Fondation maladies rares) is a new private non-profit organisation started in 2012 by Pr. Nicolas Lévy and Céline Hubert, which pooled together their complementary experiences in the field of rare diseases, from academia and the pharmaceutical industry respectively. Headquartered in Paris at the heart of the ‘Rare Diseases Platform’, the Foundation reaches out to the whole national territory with its network of regional delegates. The team is now composed of 14 dedicated professionals.

The Foundation was foreseen in the 2nd French National Rare Diseases Plan, as the flagship measure of the research axis. It was created and financially supported by 5 founders representing the patients, the research sector and the medical sector (AFM-Téléthon, Alliance Maladies Rares, National Institute of Health and Medical Research - Inserm, Conference of University Presidents – CPU and Conference of University Hospitals Directors-General).

The Foundation carries out a mission of general interest: it aims at accelerating rare diseases research programs by improving the coordination among rare diseases players, contributing to the understanding of rare diseases, the development of new treatments and the improvement of patient’s care and lives.

Since its creation, 168 research projects were funded, for an amount granted in excess of €4M, and over 100 “proofs of concepts” detected, half of which actively followed to help fill the gaps towards clinical development (e.g. strengthening of the proof of concept, orphan drug designation, agreement with a private partner, European funding, etc.).

#### 034 Applications of the PMO platform to genetic diseases
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Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O34

Genetic diseases are caused by a variety of mutations some of which lead to aberrant splicing of pre-mRNA and prevent proper mRNA translation of essential proteins or lead to translation of undesirable proteins. Such mRNA defects can be frequently repaired by appropriate targeting of oligonucleotides to modify splicing pathways and restore correct translation of desirable proteins.

Hutchinson-Gilford progeria syndrome (HGPS), the main topic of this conference, other laminopathies, as well as diseases such as Duchenne muscular dystrophy (DMD) and beta-thalassemia, are amenable to splicing manipulation or exon skipping. It has been shown in cell culture, in animal disease models or in case of DMD in clinical trials that oligonucleotides targeted to appropriate pre-mRNA splicing elements can restore correct splicing and allow production of desirable proteins, i.e. dystrophin in DMD, beta-globin in beta-thalassemia, or reduce the level of harmful proteins, such as progerin in HGPS.

Sarepta Therapeutics develops phosphorodiamidate morpholino oligomers (PMOs) and their derivatives as potential drugs for the treatment of rare diseases. After more than three years of treatment with PMO drug candidate, eteplirsen, stability of respiratory functions was observed and the results of the 6-minute walk test (6MWT) at 168 weeks showed continued ambulation across all patients evaluable on the test. Some decline in distance walked was observed since the week 144 time point. No significant treatment related adverse events were observed over the three-year course of this study.

Cite abstracts in this supplement using the relevant abstract number, e.g.: Kole: Applications of the PMO platform to genetic diseases. Orphanet Journal of Rare Diseases 2015, 10(Suppl 2):O34