4th International Meeting on Laminopathies

Madrid, 9-12 May, 2023

Abstract book

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Laminopathies are rare diseases caused by variants in genes encoding proteins of the nuclear envelope (mostly proteins of the nuclear lamina). The most frequent laminopathies are classified into 4 groups: muscular laminopathies (muscular dystrophies and cardiomyopathies), lipodystrophies, peripheral neuropathies, and progeroid disorders. Less frequent laminopathies affect the hematopoietic system, bones and joints, or skin.

Laminopathies have been linked to numerous gene variants, but the mechanisms underlying disease initiation and progression remain poorly understood. While the potential of several therapeutic approaches has been explored in experimental laminopathy models, only a few drugs have been approved for clinical use. Moreover, although some gene therapy strategies to correct the laminopathy-causing variants have shown benefits in animal models, none has advanced to the clinical arena.

The 4th International Meeting on Laminopathies, held in Madrid on May 9-12 2023, conveyed together basic researchers and physicians interested in these rare diseases, pharmaceutical industry representatives, and patients suffering laminopathies and patient associations from around the world. In all, the meeting hosted 166 participants from 24 countries. By providing a forum for the synergistic exchange of knowledge and ideas, the aim of the meeting was to improve understanding of the mechanisms underlying laminopathies and to identify avenues toward the development of new therapies.

The meeting included sessions on mechanistic and clinical aspects, the development of new experimental models, biomarker discovery, and drug-based and advanced therapies. Additionally, the participation of patients in two of the sessions helped promoting patient engagement and improved the experience of the research community, while providing a platform for patients to voice their concerns and describe the disease features that are most in need of interventions to improve quality of life. The meeting also included brainstorming sessions for the discussion of old and new ideas and unresolved hypotheses on mechanistic and clinical aspects of laminopathies. These dialogues were used to disseminate up-to-date information about the disease more widely, since a major limitation with nearly all rare diseases is that non-specialist clinicians are ill-equipped to recognize symptoms, so that patients often remain undiagnosed for decades. Thus, another output of the meeting was improved professional training, helping to ensure that patients are referred to specialist clinics and receive the best care possible.

The 4th International Meeting on Laminopathies has been made possible thanks to the generous support of our funding agencies, sponsors, and collaborators.

The Organizing Committee

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Rogier Veltrop, *LMNA Cardiac Foundation and Maastricht University, The Netherlands*

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"My history"

Elena Recio
AELIP, Spanish Association of Families and People Affected by Lipodystrophy

"A life with Progeria: a perspective of the patient role on research"

Sammy Basso, *Associazione Italiana Progeria Sammy Basso, Vicenza, Italy*

In this talk the focus will be on what to live with a rare genetic disease as Hutchinson-Gilford Progeria Syndrome means. Particularly, it will be highlighted the importance of the involvement of patients in patients' foundations and in the research. The purpose of this talk will be to show how patients and families are fundamental on spreading the knowledge on rare diseases, finding new patients and supporting researchers and doctors who have to manage the health of other patients. The call to action of this talk will be to encourage the involvement of patients and family on being the right connection between the scientific world and the rare disease daily life world. It will be then remarked the activities that progeria foundations are doing for disclosing and for sustaining the research.

- Opening key note lecture

"Oxygen and Heart Regeneration: An Evolutionary Tradeoff"

Yuji Nakada[1], Wataru Kimura[1, 2] **Hesham A. Sadek[1, 3, 4]**
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The adult mammalian heart is incapable of regeneration following cardiomyocyte loss, which underpins the lasting and severe effects of cardiomyopathy. Recently, it has become clear that the mammalian heart is not a post-mitotic organ. For example, the neonatal heart is capable of regenerating lost myocardium, and the adult heart is capable of modest self-renewal. In both of these scenarios, cardiomyocyte renewal occurs through proliferation of pre-existing cardiomyocytes, and is regulated by aerobic respiration-mediated oxidative DNA damage. Our group has demonstrated that the increase in oxygen tension postnatally mediates cardiomyocyte cell cycle arrest, and that exposure of adult mammals to hypoxia decreases oxidative DNA damage in the myocardium and can restore, in part, the endogenous regenerative capacity of the myocardium.

- Clinical aspects of laminopathies

"Type 2 Familial Partial Lipodystrophy: What about men?"

David Araújo-Vilar, Antía Fernández-Pombo, Sofía Sánchez-Iglesias.
Universidade de Santiago de Compostela, Spain.

Familial partial lipodystrophy type 2 is an autosomal dominant disorder generally due to heterozygous missense variants in the *LMNA* gene. In women, the phenotype begins to manifest before puberty, while in men the onset is later. It is striking that most of the cases reported in the literature are women. Specifically, in our cohort only 27% of these patients were men. Considering the pattern of inheritance of an autosomal dominant disease where a similar ratio between males and females would be expected, it is obvious that the majority of men with this disorder do not they are diagnosed. The causes of this discrepancy may seem obvious. On the one hand, the pattern

of lipodystrophy is not evident in men and hypermuscularity or phlebomegaly are not considered abnormal in men. On the other hand, the comorbidities associated with this disorder are less severe in men than in women. All of these evidences point out the need to establish two strategies in relation to FPLD2 in men, taking into account the large number of undiagnosed subjects, and the importance of an early diagnosis both for the subject himself and for his offspring. One would be to design indices that allow the clinician to be alerted about this disorder in men. The other is related to the identification of the pathogenetic mechanisms that explain the gender differences observed in the associated comorbidities. (This study was funded by the IISCIII (PI22/00514) and cofunded by the European Union).

"Metabolic laminopathies: a multi-faceted clinical approach"

Vatier Camille[1], Mosbah Héléna[1], Nobécourt Estelle[2], Donadille Bruno[1], Janmaat Sonja[1], Vantyghem Marie-Christine[3], **Vigouroux Corinne**[1]
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Laminopathies due to pathogenic variants in *LMNA* are characterized by a wide range of clinical manifestations. *LMNA*-linked familial partial lipodystrophy (Dunnigan syndrome) is associated with adipose tissue defects, leading to insulin resistance and metabolic complications. However, the tissue-specificity of the disease could be questioned, since phenotypic signs associated with muscular, cardiovascular and/ or ageing laminopathies can be observed in patients with *LMNA*-linked familial partial lipodystrophy. Therefore, patients should be routinely screened for cardiovascular involvement, which can include not only cardiomyopathy with rhythm and/or conduction disturbances, but also precocious atherosclerosis. This also implies multidisciplinary medical care. In addition, our recent studies, based on standardized quantitative autoquestionnaires and semi-structured interviews, point out that lipodystrophy has a strong impact on quality of life in affected patients. The burden of the disease also relates to its major psychological impact, due to altered body image, chronic pain, and medical constraints. With the French Lipodystrophy patient advocacy groups, we have set up a Patient Education Program dedicated to lipodystrophy. Preliminary results indicate that this therapeutic tool improves quality of self-image and self-esteem, and could increase the efficiency of non-specific metabolic treatments and/or adipose-centered specific therapies.

"Progeria: The Journey to the Cure"

Leslie Gordon
Boston Children's Hospital and Harvard Medical School, MA, USA; Hasbro Children's Hospital and Warren Alpert Medical School of Brown University, Providence, RI, USA; The Progeria Research Foundation, Peabody, MA, USA.

Hutchinson-Gilford progeria syndrome (HGPS) is an ultra- rare, fatal, autosomal dominant premature aging disease caused by progerin, an abnormal form of lamin A. Its prevalence is 1 in 20 million living individuals, with 142 currently identified through The Progeria Research Foundation (PRF) International Registry and an estimated 400 total population worldwide. The Progeroid Laminopathy population is collectively more prevalent, but still ultra-rare. The first clinical treatment trial for children with HGPS was initiated in 2007, just 4 years after the discovery of its causal variant. Since that time, PRF and Boston Children's Hospital (USA) have conducted serial trials, which facilitated uninterrupted treatment with the farnesyltransferase inhibitor lonafarnib. We utilized trial results together with data from the PRF International Patient Registry to demonstrate an average 4.2 years of lifespan benefit (29%) with lonafarnib therapy. This led to our first-ever drug approval by the FDA and EMA. Because the trials included children with progeroid laminopathies (PL) as well as HGPS, the indication also includes processing-deficient PL: pre-lamin A such as variants in ZMPSTE24. Children are living longer, but heart disease, often manifesting with severe aortic stenosis, is still the main cause of death. This presentation will provide an overview of the journey

from gene discovery to drug approval for these ultra-rare diseases, the importance of the newly defined plasma progerin biomarker in future treatment trial success, prospects for new treatment strategies to better treat and cure Progeria such as RNA therapy and genetic editing, and strategies for adapting treatments in HGPS to other progeroid laminopathies.

"A systematic review of metabolic drug efficacy in familial partial lipodystrophy type 2"

Rebecca Brown[1], Robert Semple[2], Kashyap Patel[3]
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Lipodystrophy syndromes are characterized by partial or generalized deficiency of adipose tissue, causing low leptin and severe metabolic disease including diabetes and dyslipidemia. The most common form of lipodystrophy is familial partial lipodystrophy type 2 (FPLD2) caused by pathogenic variants in *LMNA*, with prevalence as high as 1 per 9000. The efficacy of treatments for metabolic disease in FPLD2 has been poorly explored. We conducted a systemic review using Pubmed and Embase to assess safety and efficacy of metreleptin, thiazolidinediones, metformin, bariatric surgery, SGLT2 inhibitors, and GLP-1 receptor agonists in patients with lipodystrophy and known genotype. 7458 studies were identified; 43 met inclusion criteria. 22 were case reports, 8 case series, and 10 non-randomized experimental studies. Quality of evidence was rated as Fair for 15 studies and Poor for 28. 68 subjects with FPLD2 were included. 59 subjects with FPLD2 were treated with metreleptin, leading to 0.5±1.2% reduction in A1c (95% confidence interval -0.8 to -0.2), -1.1±1.5 kg/m2 reduction in BMI (95%CI -1.6 to -0.6), and median 71 mg/dL reduction in triglycerides (95%CI for log triglycerides -0.23 to -0.09). 9 subjects with FPLD2 were treated with thiazolidinediones, which did not significantly reduce A1c or BMI, but reduced triglycerides by median 141 mg/dL (95%CI for log triglycerides -0.42 to -0.13). Too few subjects were treated with other therapies to analyze. In conclusion, metreleptin led to modest but clinically significant improvements in metabolic parameters in FPLD2, with Fair to Poor evidence quality. More data is needed for other therapies.

"Clinical aspects of the pediatric laminopathies, an update"

Susana Quijano-Roy
AH-HP Hôpital Raymond Poincaré, Garches, France

The congenital form of laminopathies (L-CMD) was described in 2008 to complete the spectrum of skeletal muscle laminopathies (EDMD and LGMD1B) and since then a number of children have been identified all over the world, allowing a better understanding of the clinical features and course of this rare and severe disease. L-CMD patients show a spectrum of severities depending on age of onset and motor development, ranging from an early form in very hypotonic infants with absent head or trunk motor support to a later phenotype with typical development of neck weakness after acquisition of sitting or walking abilities. The course of the disease is progressive, with loss of autonomy, and frequent pulmonary and cardiac life-threatening complications before the adult age. Since early treatment and prevention of certain complications may have a major impact in survival, early diagnosis is a critical issue. The presentation will be directed to describing the most important clinical markers of the disease il order to alert clinicians and distinguish from other early onset myopathies. Typical additional complications during growth are orthopedic because it is a retractile myopathy which affects to joints and leads to scoliosis and stiffness of neck and spine. Finally, nutritional and metabolic issues are often reported and should be followed to better adapt global and specific management of the patients.

"Future clinical challenges in adult- onset cardiolaminopathies"

Karim Wahbi
AP-HP, Université de Paris, Hôpital Cochin, Paris, France.

The management of *LMNA* cardiomyopathy has greatly improved over the past 20 years, mainly with the implementation of sudden death preventive measures, based on the development of efficient malignant ventricular tachyarrhythmias prediction algorithms and the implantation of cardiac defibrillators. However, there remain major clinical challenges in the management of this population, including 1) improvement of the yield of our ventricular tachyarrhythmias and complete atrioventricular blocks prediction tools to reduce the burden of

sudden death and unnecessary defibrillators implantations, 2) strategies to treat supraventricular arrhythmias with prevention of thromboembolic events and arrhythmia recurrences, 3) prevention of progression of dilated cardiomyopathy and systolic dysfunction towards terminal heart failure. This talk will summarize the existing literature and prospects on these topics.

"Natural history studies in Skeletal Muscle Laminopathies- implications for clinical trials"

Luca Spiro Santovito[1, 2], Silvia Bonanno[2], Barbara Pasanisi[2], Annamaria Gallone[2], Federica Ricci[3], Irene Tramacere[4], Riccardo Zanin[5], Stefano C. Previtali[6], **Lorenzo Maggi**[2]

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Background: Skeletal muscle laminopathies (SMLs) belong to a group of rare disorders characterized by skeletal and cardiac muscle involvement and caused by a variant in LMNA. To date, the natural history of SMLs is mainly described by retrospective studies, which report mainly major events, and it is not well-defined yet. Conversely, promising therapeutic targets are currently under investigation in preclinical stages. Through a 2-year prospective study, using several clinical outcome measures, we aim to describe the natural history of SMLs. Methods: In the present study, we enrolled 26 SMLs, assessed with the following clinical outcomes measures: North Star Ambulatory Assessment scale (NSAA), timed tests, manual muscle testing, joint range of motion, six-minutes walking test (6MWT); respiratory evaluation including forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV1); individualized neuromuscular quality of life (INQoL) questionnaires. Results: Muscular performance with the aforementioned tools significantly correlated with phenotypes at the baseline, showing the worst outcome in EDMD2 patients. NSAA score significantly (p=0.0005) worsened during the 2-year follow-up, with higher decline in EDMD2 compared to LGMD1B patients. Surprisingly, the respiratory function through FVC and FEV1 significantly (p= 0.0086 and p=0.0290, respectively) deteriorated over the follow-up period. 6MWT and timed tests did not significantly change, as well as ankle, knee, and elbow contractures. Conclusions: This study showed a slow progression of motor and respiratory function in SMLs patients over a period of 2 years. Our data provide meaningful data for clinical trial readiness.

"Skeletal muscle laminopathies in children - questions, challenges and surprises"

Agnieszka Madej-Pilarczyk

The Children's Memorial Health Institute, Department of Medical Genetics Poland

The presentation of patients diagnosed with skeletal muscle laminopathy (SML) will be an illustration of a multi-faceted view of laminopathies in the youngest patients, including diagnostic, classification and genetic aspects, as well as challenges and still unmet needs in therapy. Clinical presentation of SML in children may include a characteristic phenotype, distinct from other laminopathies, typically consisting of early-onset head dropping and progressive hyperlordosis. Nevertheless, Emery-Dreifuss muscular dystrophy can also give its typical symptoms at an early age and then be associated with a severe course. SML in children may presents as limb-girdle involvement or myopathy, with inflammatory infiltrations mimicking myositis. All of this complicates the classification of SML in children both at the time of diagnosis and during the natural evolution of the disease over time. Large-scale studies have shown that laminopathy was the cause of skeletal muscle disease much more often than previously thought. Identification of new patients with SML allows for the expansion of known genotype-phenotype correlations: reporting of novel variants as well as surprising associations of known *LMNA* variants with phenotypes not previously associated with a given molecular defect. Cardiac symptoms and respiratory failure, observed since an early age in a significant percentage of children with SML, as well as deformities of the spine and chest, which additionally worsen of lung and motor functions, remain a therapeutic challenge. The growing awareness of rare dis-

eases gives hope for the improvement of coordinated multidisciplinary treatment approach and difficult transition from pediatric to adult care.

"Congenital *LMNA*: special patients, special cardiac features"

Georgia Sarquella- Brugada
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LMNA patients may present a wide variety of phenotypes. The congenital early phenotype, mostly related with drop-head syndrome, has special features concerning not only skeletal muscle, but especially cardiac disease. We present the early cardiac manifestations of a very young population of patients with congenital form of *LMNA*.

"Clinical and genetic characterisation of Lithuanian patients with muscle laminopathies"

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Background: Emery-Dreifuss muscular dystrophy type 2 (EDMD2; OMIM #181350) is a rare muscle disease characterized by the clinical phenotype of progressive proximal muscle weakness, early-onset joint contractures, and cardiac involvement. Here we delineate clinical and genetic features in three cases of EDMD2. Methods: The study subjects were recruited retrospectively from the database of our institution. We reviewed the clinical, laboratory and molecular findings. Results: Distinct heterozygous *LMNA* missense variants were found to segregate with the clinical phenotype in three subjects (S1, S2 and S3) from three unrelated families. All *LMNA* variants had occurred *de novo* and were reported previously. The disease manifested during infancy or early childhood and the age of onset ranged from 0 to 1.2 years of age. Two subjects (S1 and S2) presented with motor developmental delay. Meanwhile, S3 demonstrated proximal lower limb weakness characterized by gait abnormalities. All patients had skeletal system deformities. Contractures were observed in S3 at the ankle site. S2 had chest wall deformity (pectus excavatum). S1 and S3 had hyperlordosis. Joint hypermobility was present in 2/3 subjects. CK levels were elevated in all subjects. S1 and S3 had respiratory system involvement characterized by obstructive sleep apnea episodes. Cardiac assessments revealed a sinus tachycardia in each subject. Conclusions: These study results showed typical clinical characteristics in children with EDMD2. Early genetic diagnosis is important for management of possible associated complications like cardiac diseases, requiring regular cardiological follow-up.

"Different disease progression velocity in two female monozygotic twins diagnosed with *LMNA*-related congenital muscular dystrophy"

Sergi Cesar[1], Estefania Martínez- Barrios[1], José Cruzalegui[1], Fredy Chipa[1], Andrés Nascimento[2], Carlos Ortez[2], Daniel Natera[2], Oscar Campuzano[3], Georgia Sarquella- Brugada[1]
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LMNA-related congenital muscular dystrophy (L-CMD) is characterized by axial hypotonia, muscle weakness, joint contractures, spinal rigidity and progressive respiratory insufficiency. Life-threatening arrhythmias, initially without dilated cardiomyopathy (DCM), appear earlier than other phenotypes, leading into sudden cardiac death. We present the cardiac characterization in two female monochorionic diamniotic twins, enrolled in a comprehensive follow-up with implantable loop record with remote monitoring from 4 years of age. Twin 1 expressed earlier worsening neuromuscular impairment (weakness, gait problems) and earlier arrhythmias (multifocal atrial tachycardia -AT- at 7 years) than Twin 2. Intermittent AT started one year later in Twin 2. Both patients showed aggressive cardiac impairment, characterized by refractory multifocal AT despite pharmacological treatment. Rapidly progressive heart failure (HF) with DCM showed in both cases, leading to death at 8 and 9 years of age (respectively). AT and HF were related to right ventricular thrombus in twin 1 and a stroke in twin 2. Genetic testing showed the pathogenic variant *LMNA*: N39K of (exon 1). Additionally, other variants in other genes were identified: *CHRND*: P307S, *AGRN*: P325R and *DMD*: Q206L, all classified as having

uncertain significance. However, the *DMD*: Q206L variant has been reported (https://varsome.com/) in two men, one with a clear phenotype of Duchenne muscular dystrophy and the other with clinical findings compatible with Becker Muscular Dystrophy, suggesting a possible deleterious role. Differences in the functional role related with A-type lamins (i.e. modulation in skeletal muscle growth, DNA repair, cellular signaling pathway) and other modulatory factors (genetic, epigenetic or environmental), could be responsible of the differences in phenotype severity and progression speed, even in monozygotic twins.

"Quantification of skeletal muscle strength in laminopathies"

Valérie Decostre[1], Cathy Chikhaoui[1, 2], Corinne Vigouroux[3], Susana Quijano-Roy[4], Karim Wahbi[5], Bruno Eymard[1], Gisèle Bonne[2], Rabah Ben Yaou2, Jean-Yves Hogrel[1]

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BACKGROUND Skeletal muscle weakness is described in some laminopathies (myopathies of limb-girdle (LGMD1B) and Emery- Dreifuss muscular dystrophy (EDMD) types), but not in others (dilated cardiomyopathy with conduction disorder (DCM-CD) or partial lipodystrophy of the Dunnigan type (PLD)). We aimed to measure skeletal muscle weakness in various laminopathies as it is not quantified in the literature. METHODS The maximum isometric strength of handgrip and elbow/knee flexion/extension was measured using specific dynamometers. Strength and distance covered during a 6-minute walk test (6MWD) were expressed as a percentage of predicted value (%pred). The median (min, max) of the %pred values are presented here. RESULTS So far, 30 patients aged 53(24, 76) years, 20% male, have been included. All had a median elbow flexion strength below 100%pred regardless of phenotype: 17 (6, 44) for EDMD (n=3), 19 (2, 90) for myopathy + PLD (n=3), 51 (16, 65) for LGMD1B (n=9), 75 (59, 112) for PLD (n=9), 68 for Myopathy + DCM-CD (n=1) and 59 (41, 99) for DCM-CD (n=5). For all patients, elbow extension and flexion strengths were strongly correlated (rS=0.864, P).

"Long-term outcomes and arrhythmic presentations of *LMNA*-related heart disease: insights from a single-centre experience"

Davide Castagno[1], Veronica Dusi[1], Francesco Moscarini[2], Stefano Elia[2], Rosella Manai[1], Giulia Gobello U[1], Claudia Raineri[1], Stefano Pidello[1], Carla Giustetto[1], Matteo Anselmino[1], Filippo Angelini[1]

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Background Heart involvement induced by *LMNA* gene mutations is frequent and characterized by left ventricular (LV) dysfunction and a variety of arrhythmic presentations. Objectives To describe the clinical features and outcomes of a single-centre cohort of *LMNA* variant carriers. Methods Overall, 31 patients were enrolled and followed-up for a median of 9 years. Occurrence of advanced cardiac conduction system disease, supraventricular (SVA), ventricular arrhythmias (VA), need for cardiac device implantation (CDI) and advanced heart failure (AHF) were reported. All-cause mortality or heart transplantation was the main clinical endpoint. Results: The study comprised 31 patients with a mean age of 45 years at the time of genetic diagnosis and a family history of sudden cardiac death in 13 (42%) of cases. At first medical contact neuromuscular manifestations were observed in 13 (42%) patients and the main symptoms were dyspnea (32%), fatigue (29%) and palpitations (19%). At baseline, abnormal electrocardiogram findings were present in 19 (61%) patients, echocardiography showed a mean LV ejection fraction of 49%. During follow-up, SVAs and VAs occurred in 19 (61%) and 21 (68%) patients respectively and AHF developed in 39% (12 patients). CDI was performed in 22 (71%) patients (6 pacemaker, 8 ICD, 4 CRT and 4 ILR). An appropriate intervention (ATP/shock) was observed in 4 out of 11 ICD carriers (36%). During follow-up 6 (19%) patients died while 4 (13%) received heart transplantation. Conclusions: *LMNA* gene variants are associated with frequent arrhythmic events (both brady/ tachyarrhythmias) even in the context of mild impairment of LV systolic function.

"Cardiac features and genotype-phenotype correlations in patients with laminopathies: A single-center prospective study"

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Arrhythmic risk stratification in patients with *LMNA*-related cardiomyopathy influences clinical decisions concerning implantable cardioverter defibrillator (ICD) therapy. ICD should be considered in patients with estimated 5-year risk of malignant ventricular arrhythmia (MVA) ?10%. Risk prediction score for MVA includes non-missense mutations, whose role as an established risk factor for sudden cardiac death has often been questioned. Hence, we investigated the association among adverse outcomes and the *LMNA* variant type (missense versus non-missense) in a cohort of 54 patients. The study included 20 probands (37%). The median age at the first clinical manifestation was 37 ± 15 years. The type of *LMNA* gene variant was distributed as follows: missense in 26 patients (48%), insertions in 16 (30%), deletions in 5 (9%), nonsense in 6 (11%) and frameshift in 1 (2%). No alternative splicing variants were identified. Among the 26 (48%) missense mutation carriers, 2 (8%) died, 4 (15%) were admitted in the heart transplant list or underwent transplantation, 8 (31%) received appropriate ICD shocks (with a composite cardiovascular adverse event rate of 35%). It was also analyzed a possible relationship among the type of *LMNA* mutation and the dilated cardiomyopathy phenotype (DCM). DCM was identified in 16 (62%) of the 26 missense mutation carriers. No statistically significant differences in cardiovascular adverse events and in the DCM prevalence were identified according to missense and non-missense groups (p value=0.421 and 0.598 respectively). An interesting result that emerges from our study is that no association among non-missense variants and worse cardiac phenotype was identified.

- Mechanisms of laminopathies

"Endothelial YAP/TAZ activation in Hutchinson-Gilford progeria syndrome: From mechanisms to candidate therapies"

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Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare disease caused by progerin, an aberrant protein produced by a *de novo* point mutation in the *LMNA* gene. Patients show accelerated aging and die prematurely, mainly from atherosclerosis complications. Understanding vascular disease onset and progression in HGPS and uncovering new therapeutic targets critically depend on the identification of cell type-specific molecular and functional alterations in the highly heterogeneous cell subsets present in the arterial wall. We used single-cell RNA sequencing to characterize the cellular and molecular landscape of the aorta in progerin-expressing LmnaG609G/G609G mice and wild-type controls. Progeroid aortas showed transcriptional alterations in fibroblasts, vascular smooth muscle cells, immune cells, and endothelial cells (ECs) consistent with cell senescence, apoptosis, extracellular matrix (ECM) remodeling, defective contraction, and inflammation. HGPS ECs showed gene expression changes associated with ECM alterations, increased leukocyte extravasation, and activation of the mechanosensing pathway mediated by yes-associated protein 1 (YAP)/transcriptional activator with PDZ-binding domain (TAZ). Expression changes were validated by qPCR, western blot, and immunofluorescence. The aortas of progerin-expressing mice had a stiffened subendothelial ECM and disturbed blood flow, both key inducers of endothelial YAP/TAZ activation. YAP/TAZ inhibition with intraperitoneal verteporfin reduced leukocyte numbers in the aortic

intimal layer and decreased atherosclerosis burden in progeroid mice. Our findings provide a comprehensive cell-type- specific gene expression analysis of the mouse progeroid aorta, identify endothelial *YAP/TAZ* signaling as a key mechanism of HGPS-related vascular disease, and open a new avenue for the development of *YAP/TAZ* targeting drugs to ameliorate progerin-induced atherosclerosis.

"Endothelial-to-mesenchymal transition in progerin-driven accelerated atherosclerosis"

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Complications of atherosclerosis are the main medical problem in Hutchinson-Gilford progeria syndrome (HGPS), as they cause death in most patients. We previously showed that *Lmna* G609G/G609G mice with ubiquitous progerin expression develop aggravated atherosclerotic disease when crossed to an atherogenic background. We have also investigated the role of vascular smooth muscle cells (VSMCs) and myeloid cells in progerin-driven atherogenesis; however, the role of endothelial cells (ECs) in this process remains unexplored. In this study, we found altered EC phenotype, including augmented permeability for LDL and increased leukocyte recruitment, in two atheroprone mouse models of HGPS with either ubiquitous or VSMC-specific progerin expression (the latter without progerin expression in ECs). Furthermore, both models showed a substantial cell population expressing bona fide EC markers inside atherosclerotic plaques. A subset of these ECs expressed proliferation and mesenchymal markers, suggesting that luminal ECs in atheroma plaques of HGPS animals undergo endothelial-to-mesenchymal transition (EndMT). None of these alterations were observed in mice with EC-specific progerin expression, indicating that these processes stem from progerin-driven VSMC defects rather than from progerin expression in the ECs. We next analyzed TGFβ signaling, the most common trigger of EndMT. Atheroma plaques in both the ubiquitous and VSMC-specific progeria models showed upregulation of TGFβ1 and its downstream effector pSMAD3. Consistently, treatment with the pSMAD3 inhibitor SIS3 reduced leukocyte recruitment and alleviated the aortic phenotype in VSMC-specific progeria mice. In summary, progerin-induced VSMC alterations promote EC dysfunction and EndMT via TGFβ/pSMAD3, identifying this signaling pathway as a new candidate target for progeria treatment.

"Sterile inflammation in HGPS: mechanisms and targets for therapies"

Susana Gonzalo

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Hutchinson Gilford Progeria Syndrome (HGPS) patients' cells feature hallmarks of aging: DNA damage, telomere dysfunction, epigenetic changes, heterochromatin loss, mitochondrial dysfunction, loss of proteostasis, and early senescence. We also found that "progerin" expression causes replication stress, build-up of chromatin fragments and nuclear/mitochondrial DNA in the cytosol, and a robust sterile inflammation/interferon (IFN)-like response [1-3]. Sterile inflammation via the IFN response has emerged as a contributor to aging. However, the molecular mechanisms triggering the IFN response and its involvement in aging hallmarks remain poorly understood. Our new study demonstrates that STAT1 drives the IFN-like response and aging phenotypes in HGPS. In vitro, genetic, or pharmacological inhibition of STAT1 represses the IFN- like response in progeria fibroblasts and ameliorates cellular hallmarks of aging, including mitochondrial dysfunction, impaired autophagy, and proliferation defects, thus improving cellular fitness. Significantly, in vivo targeting of STAT1-IFN pathway with baricitinib has robust beneficial effects, maintaining tissue homeostasis and extending lifespan of LmnaG609G/G609G progeria mice. Huge improvements are observed in white adipose tissue and aortic smooth muscle cells, two tissues with high degeneration in HGPS. Importantly, the greatest benefit is obtained in progeria mice by combining baricitinib with a high-fat/high-caloric diet (HFD), or by Stat1 haploinsufficiency. These findings provide preclinical data that support testing an alternative treatment for HGPS. Specifically, HFD combined with baricitinib

(FDA approved anti-inflammatory) could be used to modulate phenotypes in HGPS patients such as lipodystrophy and vascular alterations, and perhaps the phenotypes of other aging-associated diseases that exhibit sterile inflammation.

"Structural characterization of Barrier-to-Autointegration Factor interaction with partners in health and diseases"

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Variants in A-type lamins are associated with a large number of diseases, including muscular dystrophies, lipodystrophies and premature aging diseases. A-type lamins are not only found at the inner nuclear envelope, but are also present in the nucleoplasm. They have a large number of partners. However, direct interaction was proven only for a few partners, and structural data describing the interaction is reported only in the case of lamin binding to Barrier-to-Autointegration Factor (BAF), a small protein encoded by the *BANF1* gene, that mediates the interaction between lamins and double-stranded DNA (dsDNA). BAF is an abundant, ubiquitously expressed and highly conserved metazoan protein. As a dimer, it is able to cross-bridge two dsDNA, thus favoring chromosome compaction. It participates in post-mitotic nuclear envelope reassembly and is essential for the repair of large nuclear ruptures. Based on an extensive structural analysis of BAF using X-ray crystallography, Nuclear Magnetic Resonance and Molecular Dynamics simulations, we will describe how BAF can simultaneously interact with lamins, LEM- domain proteins and dsDNA (Samson *et al.,* 2018; Essawy *et al.,* 2019), how phosphorylation by the mitotic kinase VRK1 regulates its binding properties (Marcelot *et al.,* 2021a; 2023a), and how missense disease- causing mutations in BAF impair specific functional mechanisms (Marcelot *et al.,* 2021b; 2023b; Janssen *et al.,* 2022). In particular, we will show that, while lamin recessive missense mutations causing progeroid syndromes impair BAF binding, the recessive progeria-associated mutation BAF Ala12Thr disrupts lamin binding, and we will discuss the impact of such defect on BAF functions.

"Long lifetime and tissue-specific accumulation of the A-type lamins in Hutchinson-Gilford progeria syndrome"

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Variants to the *LMNA* gene cause laminopathies including Hutchinson-Gilford progeria syndrome (HGPS). The origins of tissue specificity in these diseases are unclear, as the A-type Lamins are abundant and broadly expressed proteins. We show that A-type Lamin protein and transcript levels are uncorrelated across tissues. As protein-transcript discordance can be caused by variations in protein lifetime, we applied quantitative proteomics to profile protein turnover rates in healthy and progeroid tissues. We discover that tissue context and disease mutation each influence A-type Lamin protein lifetime. Lamin A/C has a weeks-long lifetime in the aorta, heart, and fat, but a days-long lifetime in tissues spared from disease. Progerin is even more long-lived than Lamin A/C in the cardiovascular system and accumulates there over time. These proteins are insoluble and densely bundled in cardiovascular tissues, which may present an energetic barrier to degradation. We find that Progerin expression causes a global decline in protein turnover flux, suggesting that accumulation of this long-lived toxic protein may interfere with protein homeostasis. These findings indicate that gene therapy interventions will have significant latency and limited potency in disrupting long-lived disease-linked proteins such as Progerin.

"Recent insights in the pathophysiological mechanisms of striated muscle laminopathies"

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Variants of the *LMNA* gene, encoding A-type lamins, give rise to a diverse and complex group of rare genetic conditions, the laminopathies, that affect single tissues (mainly striated skeletal and cardiac muscles, adipose tissue, and peripheral nerve) or multiple organs. Hence, laminopathies can be included in 4 different disease groups: striated muscle diseases, lipodystrophies, peripheral neuropathies and premature aging syndromes. Striated muscle laminopathies (SML) that affect skeletal and/or cardiac muscle, are the most frequent type of laminopathies, while premature aging syndromes (PAS) are much rarer (L≈60% for SML vs 5% for PAS of all laminopathies published cases). SML comprise *LMNA*-related congenital muscular dystrophy (L-CMD), Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy, type 1B (LGMD1B) and dilated cardiomyopathy with conduction system disease (DCM-CD) but also atypical forms with variable muscle and cardiac impairments. Associated with this wide clinical heterogeneity, there is also a large genetic variability as more than 500 different *LMNA* variants have been reported so far (www.umd.be/LMNA/, OPALE registry (NCT#03058185) and unpublished data). To study the role of lamin A/C in skeletal and cardiac muscles, and to understand the pathophysiological processes induced by *LMNA* variants, we explore both patients' biological material and knock-in mouse models that we have created, reproducing *LMNA* variants identified in SML patients. Exploring these materials, we and others demonstrated that higher susceptibility to mechanical stress and defective regulation of gene expression are the two main hypotheses to explain the mechanisms underlying the development and progression of these diseases. Insights of the clinic-genetic spectrum and of the pathophysiological mechanisms of *LMNA* variants in SML will be presented.

"Role of DNA conformation in laminopathies"

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The correct 3D organization of the genome is known to influence the spatiotemporal expression of lineage-specific genes during stem cell differentiation and aging processes. The genome conformation is established and maintained by a plethora of epigenetic factors, including Lamin A, a component of the inner nuclear membrane. Due to its key role in the control of genome architecture, it is not surprising that the structural organization and epigenetic regulation of chromatin are altered in lamin mutated background. We set up an advanced chromatin fractionation technique, named 4 fractions Sequential Analysis of MacroMolecules accessibilitY (4fSAMMY-seq), capable of precisely map genomic regions separated by their biochemical properties. This single- handedly technique enables the identification of heterochromatic and euchromatic domains and their compartmentalization in the nuclear space. We used this technique to systematically dissect chromatin conformation alterations of lamin dependent diseases. Our extensive characterization of the chromatin organization in distinct models, will expand our understanding of stemness and aging processes, laying the groundwork for defining new pathways of investigation for understanding early events of premature senescence.

"Chromatin (de)regulation in lipodystrophic laminopathies"

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Variants in lamin A/C (*LMNA/C*) cause FLPD2. Differentiation of adipose stem cells (ASCs) into adipocytes is a robust system to study the impact of *LMNA/C* variants on the epigenetic regulation of adipogenesis. We have reported defects in adipogenic differentiation linked to defective *LMNA/C* binding, abrogation of Polycomb repression, epigenetic activation and chromatin conformation changes at enhancers of the anti- adipogenic gene MIR335, promoting its overexpression. In FPLD2 patient cells, genes important for adipocyte function are bound by LMNA/C as a result of repositioning of lamina-associated domains (LADs). Providing a deeper understanding of gene regulation in LADs, we have identified 244 expressed genes in conserved LADs during adipogenic differentiation, within nar-

row open euchromatic regions of low contact frequency with LMNA/C or LMNB1. Analysis of published enhancer-capture Hi-C data during adipogenesis reveals that enhancers of active genes in LADs can form connections with other enhancers, within and outside LADs. LADs also display lower frequency of connections between heterochromatic sites. Down-regulation of LMNA/C (but not LMNB1) elicits expression of silent genes flanking these in-LAD active sites, implicating A-type lamins in regulating gene expression in or near these sites. Our data altogether suggest a view of dynamic and intricate 3D chromatin looping patterns functionally shaping the genome at the nuclear lamina. Determination of how FPLD2-causing LMNA/C mutations affect these loops is our next challenge.

"Structural insight into lamin- chromatin interactions"

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Nuclear lamins are the major building blocks of the nuclear lamina, a proteinous layer at the nucleoplasmic aspect of the inner nuclear membrane of metazoan cells. They are classified as type V intermediate filament proteins, based on their sequences, and assembled into 3.5 nm thick filaments, at the nuclear lamina. In mammals, the principal constituent of the fibrillar architecture of the nuclear lamina are the lamins, comprising of A- and B-type lamin proteins. Lamins are at the interface of chromatin and the nuclear membrane, that primarily function as a mechanical scaffold of the nucleus. However, the molecular basis for lamin interactions with chromatin is still elusive. In this work, we aim at identify the interplay between lamins and chromatin, at high-resolution. Using variety of electron microscopy modalities and chromatin solubility assays, we investigated the organization of lamins and their interactions with chromatin, *in cellulo* and *in vitro*. We employed cryo-electron tomography in combination with image processing to reveal the organization of lamin filaments and their interactions with chromatin, in cells expressing specific lamin isoforms. Moreover, we studied the alterations of lamin organization caused by laminopathies variants and how variants may alter the lamin- chromatin interactions. Finally, we use cryo-EM approaches to define lamin-nucleosomes interactions. These results provide a first glance into the interactions between lamins and chromatin and indicate the variability of lamina alterations in laminopathies

"NET39 knockout yields strong muscular dystrophy phenotype in mice"

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Emery Dreyfuss muscular dystrophy is a rare disease with symptoms including slowly progressive muscle wasting and life-threatening heart arrhythmias. Although the full mechanism of these heterogeneous groups of diseases is unknown, a significant amount of data suggests genome mis-organization as a cause. NET39 is a nuclear envelope transmembrane protein known for its role in muscle differentiation and genome organization. Our recent study identified patients carrying NET39 variants with muscular dystrophy phenotype. To investigate these variants, we generated muscle-specific NET39 mouse knockout as well as CRISPR-generated NET39 point mutant. Both models show strong muscle wasting phenotype making this mouse an attractive model for studying the disease. A complex metabolic trait of these animals might further explain the heterologous metabolic status of patients with muscular dystrophy.

"Knockdown of microtubule and lysosomal regulators alleviates embryonic lethality in a Nestor Guillermo Progeria *C. elegans* model"

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Nestor-Guillermo Progeria Syndrome (NGPS) is a premature ageing illness that affects a variety of tissues, leading to growth retardation, and severe skeletal defects. The syndrome is caused by a single amino acid substitution (A12T) in BAF1 (Barrier to

Autointegration Factor 1), a highly conserved chromatin binding protein implicated in nuclear envelope (NE) breakdown, assembly and repair as well as chromatin compaction. We have modified the baf-1 locus in Caenorhabditis elegans to mimic the human NGPS mutation (baf-1(G12T)). We report that NE levels of lamin/LMN-1 and emerin/EMR-1 are reduced in baf-1(G12T) mutants, whereas errors in chromosome segregation are increased. The baf-1(G12T) variant reduces fertility, lifespan and accelerates age- dependent nuclear morphology deterioration. Moreover, we found that baf-1(G12T) mutants are hypersensitive to NE perturbations, particularly to modifications affecting lamin/LMN-1. CRISPR-mediated gene knockout in NGPS fibroblasts unveiled a set of genes whose depletion alleviates the nuclear associated defects. When orthologs were silenced by RNAi in C. elegans, lis-1(PAFAH1B1/ LIS1), vps-16(VPS16), smu-1(SMU1) and rps-1(RPS3A) reduced the embryonic lethality of sensitized baf-1(G12T) mutants. LIS1 is necessary for the correct differentiation and function of osteoclasts, regulating microtubule network and lysosomal dynamics. This offers a working model to explain the severe skeletal defects of NGPS patients. In support of these observations, we uncover that depletion of dlc-1(DYNLL2), vps-11(VPS11) and LINC (Linker of nucleoskeleton to cytoskeleton) complex subunits sun-1(SUN1) and zyg-12(HOOK1/2) also decreased the proportion of dead eggs of sensitized baf-1(G12T) worms. These results represent an encouraging list of genes to be further explored for the development of NGPS therapies.

"Identification of potential genetic modifiers underlying phenotypic variability in a French family with striated muscle laminopathies"

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LMNA gene variants are responsible for a wide spectrum of disorders called laminopathies, the majority of which affecting striated muscles. Among them, Emery-Dreifuss muscular dystrophy (EDMD) and Limb- Girdle muscular type 1B (LGMD1B) show skeletal muscle involvement of different severity but share the same cardiac involvement, i.e., dilated cardiomyopathy with conduction system defect (DCM-CD) that can also be present in an isolated manner. Clinical heterogeneity is well known among the *LMNA* mutation carriers. Modifier genes have been suggested to explain such variability. The *LMNA* variant (p.Gln6*), identified in a large French family (named here EMD1), is associated with a wide range of age at onset of myopathic symptoms (AOMS). According to this latter, three phenotypic subgroups have been described within the family: AOMS before 20 years (early AOMS), AOMS after 30 years (late AOMS) and isolated cardiac disease without skeletal muscle symptoms. Our objective was to identify genetic modifiers underlying the intrafamilial phenotypic variability within EMD1 family. Whole genome sequencing (WGS) performed in EMD1 family enabled us to identified 2 splice variants with a potential aggravating effect for which functional validation has been performed. Moreover, 4 structural variants have been detected only in early AOMS patients. An identity by descent analysis specific to phenotypic subgroups was performed and identified one region shared on chromosome 1, containing the *LMNA* gene. Our results suggest that a single genetic modifier may not be solely responsible for phenotypic variability in this family, but that a combination of several factors is more likely.

"Effects of DCM mutants of lamin A on nuclear architecture and function"

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Dilated Cardiomyopathy (DCM) is one of the different types of laminopathies caused by variants in A-type lamins in somatic cells. The normal physiological cyclic stretching of cardiac muscle cells is significantly perturbed in DCM. It is already established that A-type lamins are principal components in nuclear mechanics. Therefore we have investigated the effect of the DCM-causing mutants- K97E, E161K, and R190W on nuclear stretching and deformation by static and dynamic strain-inducing experiments. The mutants exhibited

differential nuclear structural aberrations along with a tilt in the nuclear axis compared to the direction of the cell axis and a progressive thinning of the nuclear lamina which could possibly account for reduced mechanical rigidity. These phenotypes could potentially lead to defects in nuclear anchorage to the actin filaments thereby resulting in a misshapen and misaligned nucleus. We are also investigating the effect of compressive force on the myocytes in presence of these mutations where we observe significant changes in the circularity of the nucleus. More elaborate experiments are being performed on PAA and PDMS substrates of varying stiffness to monitor any changes in the nuclear architecture. Forces of varying magnitude exerted on the matrices by the wild type and mutant lamin A transfected cells are being followed by traction force microscopy. Taken together my laboratory aims to elucidate any perturbation of mechanotransduction from ECM to the nucleus as a sequel to variants in lamin A that cause DCM.

"Lamin A/C expression in hematopoietic cells: Regulation during aging and role in mouse atherosclerosis"

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Aim: Nuclear lamin A/C play key structural and functional roles in many cell types. We have shown that immune cell function is regulated by lamin A/C, which undergoes age-dependent downregulation in these cells. Since aging is the main cardiovascular risk factor, we aimed to investigate whether atherosclerosis is affected by changes in Lamin A/C expression in immune cells, major regulators of atherosclerotic plaque development. Methods: We generated by CRISPR-Cas9 a new transgenic mouse model with Cre-LoxP driven inducible lamin A overexpression (LAO). For atherosclerosis studies, we reconstituted lethally-irradiated Ldlr-KO mice with control, lamin A/C-KO (LKO) or LAO bone marrow (BM). After recovery, transplanted mice were challenged with a high-fat diet for 6 weeks and aortas were processed for characterization of plaque size and composition, and for sc-RNAseq. We also performed intravital microscopy to assess leukocyte/endothelium interactions *in vivo*. Results: Circulating blood cell populations, body weight, and lipid profile were undistinguishable between experimental groups; however, atherosclerosis burden was increased and reduced in Ldlr-KO mice transplanted with LKO and LAO BM, respectively. These results were associated with changes in the expression of genes involved in leukocyte migration (sc-RNAseq studies), and with increased and reduced number of extravasated leukocytes in mice receiving LKO and LAO BM, respectively (intravital microscopy). Conclusion: These findings highlight an important role of lamin A/C in atherosclerosis development, mediated at least in part through regulation of leukocyte extravasation. We propose age-dependent downregulation of lamin A/C in immune cells as a new mechanism contributing to atherosclerosis development during aging.

"Lamins dysfunction-induced replication fork instability and its consequences"

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Lamins provide a scaffold for compartmentalization of chromatin and protein complexes regulating genome integrity and function. *LMNA* gene variants elicit degenerative disorders termed laminopathies, including Hutchinson-Gilford Progeria Syndrome (HGPS), a premature aging disease caused by a truncated lamin-A called "progerin". In addition, alterations in lamins-A/C levels are associated with cancer. Mechanisms whereby lamins-A/C and truncated forms impact genome stability and function remain poorly understood. We find that lamins-A/C are critical for DNA replication, maintaining the stability of the replication fork during replication stress (RS). Lamins-A/C bind to nascent DNA and help recruit RPA and RAD51, essential factors for stalled replication fork stability, remodeling, and restart. Loss of lamins-A/C elicits replication fork instability (RFI) characterized by nuclease-mediated degradation of nascent DNA, DNA damage, and sensitivity to replication inhibitors. Consistently, RFI is rescued by overexpression of RPA or RAD51. We also find that progerin causes profound RFI, beyond that of lamins- A/C loss, with fork stalling and degradation of nascent DNA. However, the mechanisms underlying RS in progeria are different from those of lamins-A/C depletion, featuring a marked

downregulation of RAD51. Interestingly, calcitriol treatment rescues RFI in lamins-A/C depleted and progerin-expressing cells, despite the mechanistic differences. Our studies are now focused on understanding the causes and consequences of RS in progeria and other laminopathies, to identify new therapeutic targets. We find that a consequence of RFI is the accumulation of cytosolic DNAs and activation of an interferon (IFN)-like response, which can represent a feedback mechanism that further exacerbates genomic instability and cellular decline.

- Biomarkers

"The Search for Biomarkers for the Skeletal Muscle Laminopathies"

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There is an unmet need of biomarkers for the skeletal muscle laminopathies both for primary diagnoses and for prognostics. For example, Emery- Dreifuss muscular dystrophy (EDMD) pathology is often not initially clear, leading to sometimes decades of waiting for diagnosis; so combining clinical evaluation with biomarkers would enable conclusive early diagnosis. Prognostically, LMNA-related congenital Muscular Dystrophy (L-CMD) can differ in degree of scoliosis, so markers could enable earlier interventions. For EDMD the pathology ranges from patients ambulant their whole lives to those needing a wheelchair in their teens. Separate recent studies searched for biomarkers of EDMD in patient serum and patient myoblasts differentiated in vitro. Both studies found changes in cytokines and inflammatory pathways and changes in miRNAs produced. The in vitro differentiation study additionally found loss of several muscle-specific splice variants, some of which were not previously identified and loss of cell cycle regulation seemingly with respect to the timing of myotube fusion. In some cases, the same functional pathway was altered in all patients, but the specific candidate biomarkers segregated amongst different patient subgroups that may correlate with disease severity. More work needs to be done to determine how specific these candidate biomarkers are for EDMD and they need to be tested amongst a greater number of patients with comprehensive clinical histories to determine if they can relate to severity, but we predict that amongst the candidates in the intersect of these different approaches are novel biomarkers that can be used for diagnosis, prognosis and clinical trials in skeletal muscle laminopathies.

"Biomarkers in *LMNA*-related Cardiomyopathy"

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LMNA-related cardiomyopathy is an important concern for clinical cardiologists due to its aggressiveness and is highly arrhythmogenic. Biomarkers research in this entity is scarce. In daily practice electrocardiograms and transthoracic echocardiograms provide us with important information regarding *LMNA* carriers' outcomes. We perform a quick review of circulating biomarkers and the information they have shown to provide to the clinician. Afterwards, we will expose the usefulness of non-coding RNAs in *LMNA*-related cardiomyopathy. This novel family may provide potential as biomarkers to diagnose and stratify risk in these patients as well as help to understand molecular and cellular pathways underlying.

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"Enhanced cell viscosity: a new phenotype associated with lamin A/C alterations"

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Lamin A/C is a well-established contributor to nuclear stiffness, chromatin organization, and gene expression. Due to its fundamental and ubiquitous roles, alterations to lamin A/C can result in diverse cellular features, such as abnormal nuclei, proliferation defects, and premature senescence. Genetic diseases associated with lamin A/C mutations, known as laminopathies, are severe and clinically heterogeneous. The involvement of lamins in nuclear mechanical properties has been extensively studied through a variety of techniques applied to different cell types and various lamin modifications, such as depletion or genetic variants. However, the diversity of approaches has resulted in a large panel of results that are rather difficult to compare. Furthermore, the impact on the whole cell mechanical properties has been poorly described in terms of measurable physical parameters, as most studies have focused solely on nucleus investigations. In this study, we combined measurements of cell entry time in constrictions with the application of a rheological model to extract the viscoelastic properties of cells affected by lamin A/C alterations resulting from Atazanavir treatment or the FPLD2-associated variants R482W. Overall, our microfluidic test provides a quantitative estimation of the whole cell effective mechanical properties and reveals an increase in the long-time effective viscosity as a signature of cells affected by lamin A/C alterations. Additionally, we demonstrate that the whole cell response to mechanical stress is driven not only by the nucleus but also by the nucleo- cytoskeleton links and that the microtubule network plays a critical role in this link in cases of lamin A/C alterations.

"Muscle MRI as biomarkers for Straited muscle laminopathies"

Robert Carlier
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No Abstract provided.

"Sex differences in lamin A levels in immune cells"

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Biological sex is a major factor effecting inflammation and immunity, yet the mechanisms involved remain largely unclear. Lamin A plays a central role in determining cell behavior, including the inflammatory potential of immune cells called macrophages, by regulating gene expression and integrating mechanical signals. Lamin-linked Emery- Dreifuss muscular dystrophy (EDMD) generally presents earlier in males while the lipodystrophies present earlier and stronger in females, yet sex differences in Lamin A expression have not been investigated. We have observed higher expression of lamin A in male vs female murine serous cavity macrophages at both gene and protein level. This exciting observation could explain stronger inflammatory responses but weaker infection resistance in males vs females in these sites and opens the possibility that lamin sex differences might explain dimorphisms in clinical presentation in nuclear envelope-linked disorders. We have begun to investigate the relative importance of sex-differences in the tissue environment versus cell-intrinsic effects of sex in regulating lamin A expression in tissue macrophages, whether these differences extend to other types of immune cells and to cells from humans, and the potential contribution this makes to sex-differences in macrophage function.

- Laminopathies Models

"*LMNA* and beyond: iPSC-based cardiac models to study Cardiolaminopathy"

Ann-Kathrin Vlacil[1*], Silvia Crastoa[1*], Cecilia Thairi[1], Marta Mazzola[1], Martina Rabino[1], Camilla Galli[1], Alex H Christensen[2], Ju Chen[3], Nicolò Salvarani[1, 4], **Elisa Di Pasquale**[1, 4].
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Variants in *LMNA* gene, encoding the nuclear envelope proteins Lamin A/C, are the main cause of laminopathies. In the last few years many research groups, including ours, have significantly contributed to the advancement of the state-of-the-art knowledge on the role of Lamin A/C in the pathogenesis of cardiomyopathy and conduction disorders. Thanks to the availability of human cardiac models obtained through the differentiation of induced pluripotent

stem cells (iPSCs) into cardiomyocytes (CMs), several molecular determinants regulating different pathways (i.e. contractility, conduction and metabolisms) have been found contributing to the final disease outcome, and studies are now ongoing to identify molecular targets suitable for development of specific therapeutics. However, mutations in other genes other than *LMNA* have been associated to laminopathies. Among these, variants in *TMEM43* gene encoding LUMA have been found in patients with Emery-Dreifuss muscular dystrophy and Arrhythmogenic Right Ventricular Dysplasia; however, functional and molecular studies aimed to determine their effect on cardiac function in humans are still missing. By the use of iPSC- CMs carrying the p. S358L LUMA variant, we explored morphological, functional and molecular consequences of LUMA defect in cardiac cells and found an alteration of the nuclear morphology and loss of heterochromatin associated to the mutations. No major functional defects were identified in spontaneously active LUMA-CMs, with the exception of a more depolarized phenotype when forced to a -80 mV potential. We are now conducting epi-transcriptomic studies on isogenic pairs generated by CRISPR/Cas9 base editing to elucidate the molecular mechanisms underlying the observed phenotypes and the disease pathogenesis.

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"Endothelial and paracrine senescence pathways contribute to cardiovascular disease in progeria"

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Hutchinson Gilford progeria syndrome (HGPS) is a premature aging disease linked to a variant in the *LMNA* gene that encodes nuclear proteins lamins A and C. Cardiovascular disease (CVD) is a severe pathology in HGPS. In order to understand, if and how the endothelium contributes to CVD in progeria, we generated a transgenic mouse model, specifically expressing a disease-linked human lamin A mutant, called progerin, in the endothelium (Prog-Tg mice) and analyzed deregulated pathways and genes in tissues and primary cells. Prog-Tg mice develop interstitial myocardial and perivascular fibrosis and left ventricular hypertrophy associated with diastolic dysfunction and premature death. Prog-Tg endothelial cells show impaired shear stress response due to accumulation of progerin at the nuclear envelope, and initiate a p53- linked senescence pathway with a profibrotic and pro-inflammatory senescence-associated secretory phenotype that involves senescence-associated microRNAs (miR), such as miR34a-5p. Prog-Tg endothelial cells exert profound cell-non-autonomous effects initiating senescence in non-endothelial cells linked to miR34a-5p upregulation in endothelial and non-endothelial cell populations and in plasma of mice. miR34a- 5p knockdown in endothelial cells reduced p53 levels and late-stage senescence regulator p16, while p53 knockdown reduced miR34a-5p and partially rescued p21-mediated cell cycle inhibition. Overall, our data show that progerin-mediated impairment of mechanoresponsive pathways in endothelial cells activates cellular and paracrine senescence and pro-fibrotic and pro-inflammatory signaling involving miR34a-5p that reinforces and maintains senescence pathways. These pathways contribute to CVD pathologies including cardio-vascular fibrosis and cardiac pathologies. Thus, a dysfunctional endothelium contributes to CVD in HGPS.

"Altered adipose tissue dynamics associated to *LMNA* variants"

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FPLD2, a rare lipodystrophy caused by *LMNA* variants, is characterized by loss of subcutaneous fat and excess accumulation of adipose tissue in the neck and face. Several studies have reported that activation of the mineralocorticoid receptor (MR) plays an essential role in white adipose tissue, while inhibition of MR promotes brown adipogenesis associated with retention of MR in the cytoplasm. We previously showed that preadipocytes isolated from FPLD2 patient neck aberrantly differentiate towards the white lineage while preadipocytes from subcutaneous fat differentiate towards the brown lineage. As

this condition may be related to MR activation, we suspected altered MR dynamics in FPLD2 preadipocytes. We observed a strong nuclear accumulation of MR in FPLD2 brown adipocyte nuclei and we found increased production of perilipin, interleukin 6 and interleukin 8 and reduced UCP1 uncoupling activity in these cells, consistent with partial MR activation and induction of an aberrant white differentiation program. Conversely, in white FPLD2 adipocytes, MR localized principally in the cytoplasm leading to differentiation towards the brown lineage. Interestingly we observed the same trend in HGPS cells. HGPS is caused by variants in *LMNA* gene and is characterized by generalized lipodystrophy in addition to accelerating aging, bone and cardiovascular disorders. We found that HGPS adipocyte precursors induced to differentiate towards the white lineage show low nuclear MR signal and small lipid vesicles that resembles the brown adipocyte phenotype. These findings identify MR as a new player in lamin A-linked lipodystrophies and suggest exploring MR modulators for the treatment of these diseases.

"A novel mouse model of nesprin-1 associated dilated cardiomyopathy"

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Cardiomyopathies are an important cause of heart failure and sudden cardiac death. Emerging evidence demonstrated the importance of the mechanical properties of cardiomyocytes as new causes for dilated cardiomyopathy (DCM). Nesprin-1/2 are highly expressed in skeletal and cardiac muscle and together with SUN1/2, lamin A/C and emerin form the LInker of Nucleoskeleton and Cytoskeleton (LINC) complex at the nuclear envelope (NE), mechanically couples the nucleus to the cytoskeleton networks. Our recent data showed nesprin-1 mutations in DCM patients cause increased NE fragility and compromise LINC complex function in vitro. We aim to investigate mechanisms through which these mutations lead to DCM. Therefore, we have generated a nesprin-1 mutant knock-in (KI) mouse line as the first clinically relevant animal model. Preliminary mouse echocardiography data showed significantly reduced thickness of left ventricle (LV) posterior wall in diastole and reduced % ejection fraction in the KIs, suggesting LV dysfunction and a tendency of DCM, which is consistent with echo observations in DCM patients harbouring the same mutation. Immunofluorescence showed misshaped nuclei, reduced perinuclear microtubule (MT) intensity and abnormal nuclear positioning in KI cardiomyocytes. The data suggests a novel role for nesprin-1, in particular nesprin 1?2 isoform, in perinuclear MT organisation, nuclear positioning and cardiomyocyte homeostasis, thus serving as a platform to investigate novel pathological mechanism of NE-related cardiomyopathies.

"Learning Mechanisms of fat loss in Lamin a related Lipodystrophy"

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Lipodystrophy syndromes are disorders characterized by adipose tissue loss and redistribution, with associated metabolic complications including diabetes. The most common form of monogenic lipodystrophy is familial partial lipodystrophy type 2 (FPLD2), which is caused by a mutation in the *LMNA* gene. The mechanisms for how adipose tissues are lost through adolescence are unknown. To address this, we selectively deleted *Lmna* in adipocytes (*Lmna*ADKO) of mice. We observed a striking loss of white adipose tissue (WAT) in adult *Lmna*ADKO mice, along with increased fat deposition in the liver, hyperglycemia, and insulin resistance. Analyses of young mice revealed development of WAT loss progressively in *Lmna*ADKO mice, coincident with puberty. These phenotypes closely mirror those observed in FPLD2. To probe the mechanisms by which adipocytes are lost, we have now developed inducible *Lmna*iADKO mice as well as seven knock-in mouse lines, each containing a mutation that causes lipodystrophy. We hypothesize that lamin A/C is required to maintain mature adipocyte characteristics and are investigating molecular and cellular mechanisms that underly loss of mature adipocytes in mouse models and in patients. To test our hypotheses, ongoing work focuses on three goals: 1) to determine in *Lmna*iADKO mice whether loss of adipose tissues is due to dysregulation of

genes associated with lipogenesis, lipolysis, or inflammation 2) to characterize mice line that contain human pathogenic variants 3) to study effects of *LMNA* variants on adipocyte and nuclear morphology, gene expression, cellular composition of adipose tissue depots, and chromatin architecture longitudinally in patients and controls.

Enabled via NIH grant R01DK125513. Mechanisms of adipocyte loss in laminopathy- induced lipodystrophy in mice and humans.

"A 3D myotube chip to study muscular diseases"

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Quantification of skeletal muscle functional contraction is essential to assess the outcomes of therapeutic procedures for neuromuscular disorders. Muscle three-dimensional "Organ-on-chip" models usually require a substantial amount of biological material, which rarely can be obtained from patient biopsies. Here, we developed a miniaturized 3D myotube culture chip with contraction monitoring capacity at the single cell level. Optimized micropatterned substrate design enabled to obtain high culture yields in tightly controlled microenvironments, with myotubes derived from primary human myoblasts displaying spontaneous contractions. Analysis of nuclear morphology confirmed similar myonuclei structure between obtained myotubes and in vivo myofibers, as compared to 2D monolayers. *LMNA*-related Congenital Muscular Dystrophy (L-CMD) was modeled with successful development of diseased 3D myotubes displaying reduced contraction. The miniaturized myotube technology can thus be used to study contraction characteristics and evaluate how diseases affect muscle organization and force generation. Importantly, it requires significantly fewer starting materials than current systems, which should substantially improve drug screening capability.

"Using patient iPSC-derived skeletal muscle models for development of a CRISPR-based exon removal therapeutic strategy"

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Skeletal muscle laminopathies are a clinically diverse group of severe diseases caused by variants in the *LMNA* gene which encodes nuclear lamins A and C. Together with lamins B1 and B2, these assemble to form a meshwork-like structure called the nuclear lamina that resides beneath the inner nuclear membrane. This maintains nuclear and cellular homeostasis by providing structural support to the nucleus, anchoring nuclear transmembrane complexes, organizing chromatin conformation, and regulating gene transcription. Mechanistic and therapeutic studies of laminopathies are hindered by limited patient biopsy samples, unclear genotype-phenotype correlations and lack of effective humanized models. Our lab has demonstrated the potential of patient-derived induced pluripotent stem cells (iPSCs) to model disease-associated phenotypes such as abnormal nuclear shape in muscle cells, using a transgene-based protocol for differentiation. To more faithfully mimic in vivo muscle development, here we use a small-molecule based, transgene free approach to differentiate three *LMNA*-mutant patient lines into skeletal muscle cells, to then model disease-associated phenotypes in 2D and 3D artificial muscle platforms. We found no significant defect in myogenic capacity of *LMNA*-mutant lines but presence of hallmark nuclear shape abnormalities and lamin A/C mis-localization. Furthermore, a CRISPR-based exon removal strategy was developed to excise pathogenic mutations and create internally-truncated mRNA and protein lamin A/C isoforms. Such CRISPR-edited cells produce internally-truncated lamin A/C proteins that localize correctly. Current work focuses on assessing amelioration of disease-associate phenotypes in CRISPR-edited cells.

- Drug-based Therapies

"Alteration of cytoskeleton in Cardiolaminopathy"

Antoine Muchir
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Dilated cardiomyopathy caused by variants in *LMNA*, encoding A-type lamins (i.e., *LMNA* cardiomyopathy), is characterized by a left ventricle enlargement and ultimately results in poor cardiac contractility associated with conduction defects. Despite current strategies to aggressively manage the symptoms, the disorder remains a common cause of sudden death and heart failure with decreased ejection fraction. A-type lamins are intermediate filaments and are the main components of the nuclear lamina, a meshwork underlying the inner nuclear membrane, which plays an essential role in both maintaining the nuclear structure and organizing the cytoskeletal structures within the cell. Cytoskeletal proteins function as scaffold to resist external mechanical stress. An increasing amount of evidence demonstrates that *LMNA* variants can lead to disturbances in several structural and cytoskeletal components of the cell such as microtubules, actin cytoskeleton and intermediate filaments. Molecular tuning of cytoskeletal dynamics has been successfully used in preclinical models and provides adequate grounds for a therapeutic approach for patients with *LMNA* cardiomyopathy.

"Nuclear receptor dynamics in response to drug treatments in progeroid laminopathies"

Elisa Schena, Stefano Squarzoni, Cristina Capanni, Elisabetta Mattioli, **Giovanna Lattanzi**
CNR Institute of Molecular Genetics "Luigi-Luca Cavalli Sforza, Unit of Bologna, Italy.

Nuclear receptors have been implicated in the pathogenetic pathway of laminopathies featuring lipodystrophy and metabolic abnormalities as Familial Partial Lipodystrophy (FPLD2), Mandibuloacral Dysplasia and Hutchinson-Gilford Progeria (HGPS). As all nuclear receptors are characterized by their ability to directly bind DNA and modulate different gene sets in different cell types, nuclear receptor-lamin A interplay warrants deep investigation. Mechanisms affected by progerin or other prelamin A forms involve PPARgamma, the retinoic acid receptor and the vitamin D receptor. We recently found that the mineralocorticoid receptor subcellular distribution is affected by lamin A or prelamin A levels and mineralocorticoid receptor localization is altered in FPLD2. These results and those previously published by us and other research groups suggest that a specific domain in nuclear receptors could be a prelamin A binding site and *LMNA* variants could either exacerbate prelamin A-receptor interaction or reduce protein binding affinity. In this context, it is not surprising that drugs acting as nuclear receptor ligands (as glitazones, calcitriol or retinoic acid) or antagonists (as spironolactone) appear to improve the disease phenotype in preclinical and clinical studies of lipodystrophic and progeroid laminopathies. Less expected are the indirect effects of prelamin A inhibitors and cytokine neutralizing antibodies in the rescue of nuclear receptor dynamics, as we observed with statins and the interleukin 6 antibody tocilizumab. These results and further in vivo testing of nuclear receptor agonists/ antagonists may open new therapeutic perspectives.

"Testing genetic drugs for gene therapy strategies for Hutchison-Gilford Progeria Syndrome"

Volha Dzianisava, Katarzyna Piekarowicz, Magdalena Machowska, **Ryszard Rzepecki**
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The "Golden Standard" therapy for HGPS progeria should be gene therapy based on virus-delivered, single-dose genetic drug. We have been testing gene therapy strategies based on working hypothesis that efficient knocking down of progerin protein, eventually combined with delivery of exogenous copy of wt *LMNA* ORF, should have been sufficient to reverse all the symptoms of the disease. In order to reach the final target the designed, tested and selected five, the most promising and efficient siRNA sequences for progerin knockdown were selected following the rule of high efficiency against progerin and no decrease (or increased level) of endogenous lamin A protein level using our new cellular model for quantitative drug selection. We confirmed the efficiency of the drugs to be effective (50-80% of knockdown) against progerin in patient's cellular model. We demonstrated that siRNA can be combined with FTI inhibitor to get additive effect of progerin knockdown. Modification of siRNAs for increased stability affected specificity and efficiency for some of them but we were able to positively se-

lect three modified siRNAs with prolonged stability and up to 80% efficiency against progerin. Long term studies in patient model confirmed their activity and efficiency. Most efficient siRNAs has been used as a "seed sequences" in designed shRNAs and micro RNAs for testing for their suitability for pol II dependent, virus mediated, tissue specific targeted gene therapy. Preliminary tests with constructed expression plasmids and lentivirus delivery of the constructs into tissue culture cells confirm their expression and selectivity. E-Rare-3 Treat HGPS (ERA-NET-E-RARE-3/III/TREATHGPS.

"NAT10 inhibition in Cardiolaminopathy: investigation of the effect of Remodelin on iPSC-derived laminopathic cardiomyocytes"

Cecilia Thairi[1], Nicolò Salvarani[1], Silvia Crasto[1], Paolo Kunderfranco[1], Ann-Kathrin Vlacil[1], Camilla Galli[1], Michele Miragoli[2], Carla Lucarelli[3], Matteo Dal Ferro[4], Elisa Di Pasquale[1].

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Mutations of Lamin A/C gene (*LMNA*) are common causes of *LMNA*-dependent cardiomyopathy (LMNA-CMP), a form of dilated cardiomyopathy typically manifesting with conduction disorders and arrhythmias. Inhibition of N-acetyltransferase 10 (NAT10) by Remodelin has been recently shown to ameliorate the phenotype of laminopathic mice and to improve nuclear abnormalities of either progeric or Lamin A/C-depleted cells. However, studies on cardiac cells are still lacking. Our aim is to fill this gap by investigating the effect of Remodelin on models of LMNA-CMP, obtained through differentiation into cardiomyocytes (CMs) of induced pluripotent stem cells (iPSCs) carrying *LMNA* variants (*LMNA*-CMs). Starting from previous electrophysiological data by our group indicating a significant impairment of electrical excitability of *LMNA*-CMs, we next tested the effect of Remodelin on these cells. Our results showed that Remodelin treatment restores peak sodium currents density and its related action potential parameters, concurrently boosting junctional conductance. A positive drug response, in terms of cardiac conduction and action potential, was also observed in control CMs (CNTR-CMs). This might be due to modulation by Remodelin of cardiac biological processes, including microtubule stability and metabolism, as emerged from RNA sequencing experiments. Notably, genes involved in CMs metabolic switch and contractility have been found significantly modulated in LMNA-CMs compared to CNTR-CMs. Coherently, *LMNA*-CMs models showed defects in calcium dynamics and contractility. Testing of Remodelin on these pathways is ongoing. In conclusion, although underlying mechanisms are yet to be demonstrated, our study reinforces the evidence indicating NAT10 inhibition as a promising therapeutic target for *LMNA*-CMP.

- Advanced therapies for laminopathies

"AAV (finally) flexes its muscles - novel myotropic vectors for treatment of laminopathies and other muscle disorders"

Dirk Grimm

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Adeno-associated virus (AAV) has taken center stage as a template for the development of viral gene transfer vectors for the treatment of infectious, inherited or acquired disorders. A highly relevant indication for AAV- based gene therapies are diseases affecting the human musculature, yet they are also particularly challenging owing to the sheer mass and wide distribution of the target tissue, including skeletal muscle, heart and diaphragm. Fortunately, over the past two decades, the AAV community has successfully implemented an arsenal of technologies for the diversification of naturally occurring AAV capsid variants and the subsequent high-throughput interrogation of the resulting virus libraries for capsids that fulfill a set of disease-specific requirements. Most recently, these techniques have been complemented by an also rapidly increasing battery of methodologies for the bottom-up, rational design of optimized AAV capsid variants including machine learning. This presentation will provide an overview over selected examples of these top-down or bottom-up technologies together with representative cases

where they have been applied to identify novel AAV capsid variants for use in muscle gene therapy. Most notable candidates comprise the recently reported families of AAVMYO (1, 2) or MyoAAV (3) capsids that exhibit unprecedented degrees of efficiency combined with specificity of gene transfer in the whole musculature following systemic delivery in animals. The presentation will conclude with an outlook into possible improvements in the strategies for AAV capsid bioengineering and selection that promise to further facilitate and accelerate clincial translation of AAV-mediated human gene therapy of laminopathies and other devastating muscle disorders.

1. Weinmann et al., Nat. Commun., 2020, 11:5432 ; 2. El Andari et al., Sci. Adv., 2022, 8:eabn4704 ; 3. Tabebordbar et al., Cell, 2021, 184:4919-4938.

"Challenges in gene therapy for striated muscle laminopathy"

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Lamins A/C, encoded by *LMNA*, are components of the nuclear lamina underlying the inner nuclear envelope. *LMNA* variants lead to a great phenotype variability, mainly affecting striated muscles, called laminopathies. At the most severe spectrum of striated muscle laminopathies, *LMNA*-related congenital muscular dystrophy (L-CMD) is characterized by severe muscle atrophy and weakness, joint contracture and dilated cardiomyopathy. Dilated cardiomyopathy can also be the sole symptom. There is currently no curative treatment available. Taking advantage of our KI-LmnaK32del mouse model that develop a L-CMD-like phenotype at the homozygous state and isolated dilated cardiomyopathy at the heterozygous state, we assessed a therapeutic approach aiming both at reducing mutant lamin A/C expression and restoring normal lamin A/C levels. We first evaluated the potential of allele-specific lamin A/C knock down by siRNA, as well as human lamin A or C overexpression, to correct defects of KI-LmnaK32del primary myotubes in vitro. We then evaluated the therapeutic efficacy of systemic delivery of different AAV2/9 vectors, either containing human mature lamin A cDNA alone, or in combination with one of 2 different shRNAs against *Lmna* mRNA in homozygous and heterozygous mice. Our results show a moderate benefit in term of survival and a sustained human lamin A overexpression. However, we also observed a lack of mouse *Lmna* mRNA knock-down due to decreased expression of several proteins involved in miRNA/ shRNA maturation pathway, and side effects in the liver preventing a better efficacy of the gene therapy. We investigate further these defects and pursue optimization of this therapeutic strategy.

"Heterogeneous responses to the application of different gene therapy strategies on an *Lmna*-R249W mouse of *LMNA*-related congenital muscular dystrophy"

Carolina Epifano[1, 2], Déborah Gómez-Domínguez[1], Iván Hernández[1], Miguel Sena-Esteves[3], Sergi Cesar [4], Georgia Sarquella-Brugada[4], Antonio de Molina-Iracheta[5], **Ignacio Perez de Castro**[1]

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LMNA-related congenital muscular dystrophy (L-CMD) is a genetic disease caused by point variants in the *LMNA* gene, for which there is currently no cure. This rare disease is characterized by hypotonia, muscle weakness, joint contractures, spinal rigidity, respiratory insufficiency, and cardiac anomalies that can lead to sudden death. The goal of this study is to develop effective therapies for L-CMD. Three different genetic therapies were explored, including replacement therapy, HITI, and specific elimination of mutant alleles suing CRISPR/Cas9 complexes. Using mice carrying *LMNA* c.745C>T, p.R249W variants, the study evaluated the efficacy of these therapies in a metabolic scenario or a cardiomyopathy background. The results showed that one dose of AAV9 vectors for any of the three gene therapy approaches significantly increased the median survival of homozygous *Lmna*R249W/R249W mice (metabolic scenario). However, heterozygous mice (cardiomyopathy phenotype) responded differently to each therapy. Replacement therapy led to worse survival, while HITI had no major effect. AAV9 deliv-

ery of Cas9 plus sgRNA specific for the c.745C>T mutation showed clear survival benefits. These findings confirm the potential of gene therapy for L-CMD treatment and highlight the importance of careful evaluation and selection of the appropriate approach.

"Base editing and antisense therapy in progeria"

Gwladys Revêchon, Maria Eriksson
Karolinska Institutet Sweden

Hutchinson-Gilford progeria syndrome (HGPS) is a rare disease caused by a point variant in the *LMNA* gene (*LMNA* c.1824 C>T), leading to cardiovascular complications and patients' premature death in their teens. Many treatment strategies have shown great promise in preclinical models of the disease, including the use of base editing and antisense therapies, which bring exciting future perspectives for patients. In line with this, our lab recently demonstrated that transient expression of an adenine base editor delivered by a non-integrative viral vector is a plausible approach for future gene-editing therapies. Using a skin- specific HGPS mouse model we achieved mutation correction in 20.8%- 24.1% of the keratinocytes, which resulted in long-term improvement of the skin phenotype. But the effectiveness and safety of such therapeutic tool in humans remain unknown, therefore there is still a need to develop novel treatment approaches. The vascular phenotype being detrimental in HGPS, understanding the molecular mechanisms at play in the vascular wall could help identifying new treatment targets. We previously demonstrated that antisense oligonucleotides targeting long non-coding RNAs (lncRNAs) at telomeres could improve the disease phenotype and extend the lifespan of HGPS mice. Here, we performed transcriptomic analysis at the single-cell level on the aorta of HGPS mice. This revealed that a subpopulation of vascular smooth muscle cells enriches during disease progression, and that several lncRNAs were misregulated in the vascular wall of HGPS mice. Additional analysis will show whether using antisense therapy against these lncRNAs could be an effective treatment approach for HGPS.

"CRISPR/Cas9-based genome editing for correction of X-linked Emery-Dreifuss Muscular Dystrophy"

Eleonora Cattin[1], Elisa Schena[2], Elisabetta Mattioli[2] Federico Corradi[3], Giovanna Lattanzi[2], Alessandra Recchia[3]

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Type I EDMD is a rare genetic X-linked disease caused by variants in the EMD gene, encoding emerin. A cure for this disease is not available to date and the molecular pathogenesis of EDMD1 is not entirely elucidated. The identification of biomarkers for the evaluation of disease progression is mandatory. Herein, we corrected two genetic variants in the EMD gene by CRISPR/ Cas9 technology. Cytidine Base Editing (CBE) was used to correct a non- sense variant in the N-terminal domain. To evaluate the genomic correction, we took advantage from a RFLP. This assay revealed 60% correction of the variant in patient fibroblasts and myoblasts. The corrected EDMD1 cells showed the restoration of emerin expression and the protein was properly localized in the nuclear membrane. As the LINC complex is considered a main driver of nuclear envelope-related mechanisms in developing skeletal muscle, we investigated SUN1 localization in EDMD1 myotubes. SUN1, farnesylated prelamin A and pericentrin were mislocalized and, after CBE, a complete rescue was observed. We also corrected by CRISPR strategy a mutation affecting the C-terminal domain of emerin. The mutation led to a frame shift of *EMD* gene generating a truncated protein missing the transmembrane domain. We aimed to re-establish the correct frame of the gene in patient's tenocytes. After CRISPR treatment, emerin localization in the nuclear envelope was obtained in approximately 15% of treated cells. In conclusion, we corrected genetic defects in EDMD1 cells that could represent isogenic controls used to identify biomarkers for the evaluation of disease progression.

- Closing key note lecture

"The Lamins in Development and disease – a 40-year journey from basic science to gene therapy"

Colin Stewart
ASTAR Skin Research Laboratories, Singapore

The A-type lamins are absent during the early stages of mouse embryo development and in embryonic stem (ES) cells. LaminA expression begins with ES differentiation and gradually appears during em-

bryogenesis. *Lmna* null mouse embryos develop to birth, with postnatal growth seemingly normal. After 3-weeks, growth ceases, and the mice die from muscular dystrophy and dilated cardiomyopathy (DCM), suggesting that *Lmna* is unnecessary for cell differentiation during embryogenesis. Why A-type lamins are absent in pluripotent ES cells and embryos is still unclear, as we derived developmentally competent ES cells expressing either LaminA or progerin. Intriguingly ES cells lacking Tert, required for telomere maintenance in stem cells, do not tolerate *Lmna* expression, revealing a link between LaminA, telomere maintenance. The derivation of the mice lacking *Lmna* coincided with the discovery that *LMNA* variants in humans cause EDMD and were the 2nd most frequent cause of congenital DCM. Other diseases; - the laminopathies are caused by specific mutations in the *LMNA* gene, making the *LMNA* gene unique, as different variants in the same gene cause various tissue-specific diseases. We derived mouse lines, each carrying a specific *Lmna* mutation, to model the specific laminopathy to understand their molecular basis. An unexpected finding was that loss of the LINC-complex protein SUN1, suppresses the pathology with significant lifespan extension. Disrupting SUN1 led to developing a novel approach to treating DCM using AAV gene therapy. Our company, Nuevocor, is refining this approach to treat DCM, with which we hope to start clinical trials soon.

- Regeneron Satellite Talk

“From bench to bedside: development of REGN4461, a novel leptin receptor antibody for leptin deficiency”

Judith Altarejos
Director of Research- Obesity Metabolism & Muscle Diseases, Regeneron

- AELIP Satellite Talk

“Social and health resources for individuals and families affected by lipodystrophies”

POSTERS

- Clinical aspects of laminopathies

P1. "YAP/TAZ activation in endothelial cells promotes atherosclerosis in Hutchinson-Gilford progeria syndrome"

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Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder caused by the expression of progerin, an aberrant protein produced as the result of a de novo point variant in the *LMNA* gene. HGPS is currently an incurable disease with vascular pathology as the main driver of patients' premature death. The HGPS vascular phenotype is likely consequence of vessel wall malfunction derived from deregulation of molecular signalling pathways in its very heterogeneous cellular components. Using single cell RNA sequencing, we have exhaustively characterized the cellular heterogeneity in aortas from a mouse model of progeria, and the associated pathological changes. Furthermore, we have delved into the mechanisms that direct the changes in the expression profile of progeric endothelial cells, identifying the activation of the YAP/TAZ mechanosensing pathway, which was validated by qPCR, western blot, and immunofluorescence assays. En face atomic force microscopy experiments on decellularized aortas demonstrated stiffer subendothelial extracellular matrix in progerin-expressing mice, and ultrasound assessment of the aortas of live HGPS mice revealed disturbed blood flow, both potential inducers of the YAP/TAZ pathway in endothelial cells. Drug-based in vivo YAP/TAZ pathway inhibition attenuated leukocyte infiltration into the tunica intima in HGPS aortas and decreased atherosclerosis burden in atheroprone Apoe-/- HGPS mice. Our findings identify YAP/TAZ signaling as a potential therapeutic target for HGPS-associated atherosclerosis and open a new avenue for drug development.

P3. Progerin expression in endothelial cells is not a causal driver of cardiovascular alterations and premature death in progeria"

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disorder caused by a variant in the *LMNA* gene that results in the synthesis of an aberrant protein called progerin, which provokes accelerated aging and dramatically reduces lifespan. The most clinically relevant feature of HGPS is the development of severe cardiovascular alterations, including massive loss of vascular smooth muscle cells (VSMCs), vessel stiffening, vascular calcification and fibrosis, and generalized atherosclerosis, as well as electrical, structural, and functional anomalies in the heart. To study whether progerin expression in endothelial cells (ECs) has a direct causal role in HGPS, we generated mouse models with EC-specific progerin expression (LCS iEC) and suppression (HGPSrev sEC), the latter together with lamin A restoration. LCS iEC mice did not develop heart fibrosis compared with progerin-free controls and showed normal cardiac electrical and functional properties, body weight, and lifespan. Moreover, atheroprone Apoe-/-LCS iEC mice did not show aggravated atherosclerosis compared to Apoe-/- controls. HGPSrev sEC mice exhibited HGPS-associated cardiac electrical and functional alterations characteristic of ubiquitous progerin expression, and did not show improved body weight and survival. Excessive atherosclerosis burden was undistinguishable between HGPSrev mice with ubiquitous progerin expression and HGPSrev sEC mice. In contrast, suppressing progerin and restoring lamin A in VSMCs was sufficient to reduce atherosclerosis burden. Our studies strongly suggest that progerin expression in ECs is not a direct cause of the HGPS-associated cardiovascular phenotype and premature death, and that progerin suppression only in

ECs does not ameliorate cardiovascular pathology and fails to prolong survival.

P8. "Lamin A/C ablation causes vascular defects that may contribute significantly to the pathophysiology of cardiolaminopathies"

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Variants in the *LMNA* gene (encoding lamin A/C proteins) cause several human cardiac diseases, including *LMNA*-related dilated cardiomyopathies (*LMNA*-DCM). The main clinical risk in *LMNA*- DCM patients is sudden cardiac death, and therefore most human and animal studies have sought to define the mechanisms through which *LMNA* mutations provoke cardiac alterations, with particular focus on cardiomyocytes. To investigate if *LMNA* mutations also cause vascular alterations that might contribute to the etiopathogenesis of *LMNA*-DCM, we have generated and characterized Lmnaflox/floxSM22?-Cre mice, which have constitutive lamin A/C deficiency in vascular smooth muscle cells (VSMCs), cardiac fibroblasts, and cardiomyocytes. Like mice with ubiquitous or cardiomyocyte-specific lamin A/C ablation, Lmnaflox/ floxSM22?-Cre mice recapitulated the main hallmarks of human *LMNA*- DCM, including ventricular systolic dysfunction, cardiac conduction defects, cardiac fibrosis, and premature death. These alterations were associated with hyperactivation of SMAD3 and higher expression of the pro-apoptotic caspase 3 protein in the heart. The mice also exhibited perivascular fibrosis in coronary arteries, a phenotypic switch of aortic VSMCs from the 'contractile' to the 'synthetic' phenotype, and elevated systolic blood pressure. Ex vivo wire myography in isolated aortic rings revealed impaired maximum contraction capacity and an altered response to vasoconstrictor and vasodilator agents in Lmnaflox/ floxSM22?- Cre mice. To our knowledge, our results provide the first evidence of phenotypic alterations in VSMCs that may contribute significantly to the pathophysiology of some forms of *LMNA*-DCM. Future work addressing the mechanisms underlying vascular defects in *LMNA*-DCM may open new therapeutic avenues for the treatment of these diseases.

P11. "A heterozygous pathogenic variant in ZMPSTE24 is responsible for severe diabetes, central obesity and hepatic steatosis in patients from Wallis Island with a possible funder effect"

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ZMPSTE24 encodes the metalloprotease transforming prelamin A into mature lamin A. A single case of a severe cardio-metabolic syndrome associated with the variant p.(Leu438Phe) in *ZMPSTE24* has been previously reported in a patient from Wallis. The objectives of this study were 1) to describe a second family from Wallis with severe diabetes and carrying the same variant; 2) to investigate the pathogenicity of this variant in patients'' fibroblasts. The propositus presented with type 2 diabetes at 35 years, central obesity, and hepatic steatosis. The heterozygous pathogenic variant p.(Leu438Phe) in *ZMPSTE24* was found by whole exome sequencing. One of her sisters also carried this variant and presented type 2 diabetes at 27 years with central obesity. Functional studies (quantification of nuclear anomalies by immunofluorescence, of senescence by Beta-Galactosidase assay, and of cellular replication by BrdU labeling) were conducted in the patient fibroblasts and compared with controls and results obtained from the previous case. An increase in cellular senescence, nuclear anomalies, and prelamin A labeling and a decrease in cellular replication were evidenced in the propositus cells compared with controls as for the first patient reported.

P12. "Progerin suppression and lamin A restoration in adipose tissue reduces lipodystrophy, ameliorates vascular alterations, and extends lifespan in progeroid mice"

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disease caused by the expression of proger-

in, a mutant form of prelamin A (*LMNA* gene). Patients exhibit cardiometabolic alterations and premature aging. Although lipodystrophy is severe in HGPS, its role in triggering progerin-dependent cardiometabolic alterations and premature death remains largely unexplored. Here, we crossed HGPSrev mice with ubiquitous progerin expression with mice that express Cre recombinase in adipocytes to generate HGPSrev-FABP4Cre mice with progerin suppression and lamin A restoration mainly in fat depots. qPCR and immunofluorescence studies confirmed prominent progerin suppression in adipose tissue from HGPSrev-FABP-4Cre mice, which showed improvement in fat content, adipocyte morphology, body weight, and a 37% increase in longevity compared with HGPSrev mice. Lifespan extension in HGPSrev-FABP4Cre mice occurred without amelioration in electrocardiographic disturbances, but correlated with an improvement in aortic structural alterations and perivascular fat-mediated effects on maximal aortic contraction. Transplantation of wild-type subcutaneous adipose tissue into progeroid HGPSrev mice ameliorated body weight loss and extended healthspan. Our results suggest that lipodystrophy contributes to HGPS through an imbalance in the secretion of adipokines by damaged adipocytes, and that progerin suppression in adipocytes is sufficient to ameliorate lipodystrophy, reduce vascular damage, and extend lifespan. Our current transcriptomic and metabolomic analysis will shed light on the mechanisms underlying the cross-talk between adipose tissue and the cardiovascular system in HGPS and to identify potential novel therapeutic targets.

P22. "Prevalence and clinical outcomes of lipodystrophy: Cross-sectional analyses of a US national cohort"

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Background: Limited information is available on the population-based prevalence and clinical characteristics of lipodystrophy (LD). Methods: A cross-sectional study was conducted using the 2007–2019 Clinformatics® Data Mart, an integrated commercial healthcare claims database in the US. Continuously enrolled adult LD cohorts with ?1 inpatient or ?2 outpatient LD diagnoses were included; non-HIV-associated LD (non-HIV-LD) and HIV-associated LD (HIV-LD) subgroups were assessed. Standardized annual LD prevalence based on the age and sex distribution of the 2019 US population was calculated. The prevalence of clinical outcomes in 2018–2019 was estimated among subgroups, versus age- and sex-matched control groups. Results: The prevalence of non-HIV- LD in the general adult population slightly increased from 2.3/100.000 in 2007 to 2.9/100.000 in 2019, while the prevalence of HIV-LD stayed stable (1.9/100.000 in 2019). We identified 546 individuals with non- HIV-LD (mean age 60.3 years, 67.6% were women) and 334 individuals with HIV-LD (mean age 59.2 years, 15.0% were women) in 2018– 2019. Compared to the general population, individuals with LD in both subgroups had higher risks of hyperlipidemia, hypertension, diabetes, kidney disease, liver fibrosis and cirrhosis, cancer, and serious infections resulting in hospitalization. Increased risk for autoimmune diseases, acute pancreatitis, and polycystic ovary syndrome was identified only in individuals with non-HIV-LD. Conclusions: LD bears a substantial burden on affected individuals due to a high prevalence of metabolic comorbidities and complications, autoimmune diseases, cancers, and serious infections resulting in hospital admissions. Further studies are warranted to investigate the causality between LD and observed clinical outcomes.

P39. "Regulation of A-type lamins in endothelial cells during ageing and role in age-related endothelial dysfunction"

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Ageing is the main risk factor for cardiovascular disease, including arterial stiffening and atherosclerosis. A-type lamins (lamin A/C; *LMNA* gene) are nuclear envelope proteins implicated in structural and functional roles, including chromatin organization, transcription, signal transduction, cell proliferation, migration and differentiation. Previous in vitro studies have associated low lamin A/C expression level with endothelial cell (EC) dysfunction and increased subendothelial migration of immune cells. However, it remains unknown whether EC-specific lamin A/C expression could play a role in age-related endothelial dysfunction and atherosclerosis.

Western blot analysis of human coronary arteries of subjects -30 years and -58 years of age, and aorta of young and old mice (3 weeks, 65 weeks and 109 weeks

of age), demonstrated a significant age-dependent downregulation of lamin A/C expression. FACS analysis in mouse aorta demonstrated age-associated lamin A/C downregulation in ECs and vascular smooth muscle cells, but not in adventitial cells. We generated an atheroprone mouse model with EC-specific *Lmna* disruption (Cdh5- Cre/ERT2 Lmnaflox/flox LDLr-/-). Compared with wild-type controls, these mice showed normal body weight, peripheral blood cell counts and survival. In contrast, aortic rings isolated from fat-fed mice with EC-specific *Lmna* ablation showed impaired endothelial-dependent vasorelaxation in ex vivo wire myography studies. Our findings suggest that downregulation of lamin A/C expression in ECs could play a prominent role in age-associated endothelial dysfunction. Ongoing studies will assess the effects of EC-specific lamin A/C ablation on atherosclerosis development, EC permeability, leukocyte recruitment, angiogenesis, and gene expression.

P40. "Deflazacort treatment in *LMNA*-related congenital muscular dystrophy: the ongoing study of clinical effectiveness searching for reliable biomarkers"

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LMNA-related congenital muscular dystrophy (L-CMD) and *LMNA*-related Emery-Dreifuss muscular dystrophy (EDMD2) with early onset (<5 years) may be considered a continuum phenotype. There is no cure for patients with these neuromuscular diseases. Literature data and ongoing clinical approaches suggest the use of steroids. Starting from the collaborative approach and the multidisciplinary effort of the Italian Network for Laminopathies (NIL), we are performing an open-label prospective cohort pilot study aimed to: 1) evaluate the effect of the treatment with deflazacort in a cohort of maximum 20 L-CMD or EDMD2 patients with infantile onset, aged 3-40 years; 2) analyse the secretome profile at basal condition and during steroid treatment to evaluate variations and establish a correlation between steroid treatment and clinical outcome; 3) validate selected cytokines as biomarkers for L-CMD and EDMD2 and to study the effect of serum biomarkers in cellular models of control human myoblasts and fibroblasts in order to assess the fibrogenic and anti-differentiation activity of patients' serum. So far, we've enrolled four patients, 3 male and 1 female, with an average age of 15,5 years (s.d. 11,6 y). Our project is the first therapeutic approach attempted in Italy so far for this debilitating disease, and it appears crucial to verify the hypotheses that emerge from literature and empirical data concerning the use of steroids. Importantly, our study is also aimed at defining a panel of altered cytokines as disease biomarkers so that future therapies can benefit from such parameters.

P49. "Quantification of skeletal muscle strength in laminopathies"

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BACKGROUND Skeletal muscle weakness is described in some laminopathies (myopathies of limb-girdle (LGMD1B) and Emery- Dreifuss muscular dystrophy (EDMD) types), but not in others (dilated cardiomyopathy with conduction disorder (DCM-CD) or partial lipodystrophy of the Dunnigan type (PLD)). We aimed to measure skeletal muscle weakness in various laminopathies as it is not quantified in the literature. METHODS The maximum isometric strength of handgrip and elbow/knee flexion/extension was measured using specific dynamometers. Strength and distance covered during a 6-minute walk test (6MWD) were expressed as a percentage of predicted value (%pred). The median (min, max) of the %pred values are presented here. RESULTS So far, 30 patients aged 53(24, 76) years, 20% male, have been included. All had a median elbow flexion strength below 100%pred regardless of phenotype: 17(6, 44) for EDMD (n=3), 19(2, 90) for myopathy+PLD (n=3), 51(16, 65) for LGMD1B (n=9), 75(59, 112) for PLD (n=9), 68 for Myopathy+DCM-CD (n=1) and 59(41, 99) for DCM-CD (n=5). For all patients, elbow extension and flexion strengths were strongly correlated (rS=0.864, P).

P51. "Long-term outcomes and arrhythmic presentations of *LMNA*-related heart disease: insights from a single-centre experience"

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Background Heart involvement induced by *LMNA* gene variants is frequent and characterized by left ventricular (LV) dysfunction and a variety of arrhythmic presentations. Objectives To describe the clinical features and outcomes of a single-centre cohort of *LMNA* variant carriers. Methods Overall, 31 patients were enrolled and followed-up for a median of 9 years. Occurrence of advanced cardiac conduction system disease, supraventricular (SVA), ventricular arrhythmias (VA), need for cardiac device implantation (CDI) and advanced heart failure (AHF) were reported. All-cause mortality or heart transplantation was the main clinical endpoint. Results The study comprised 31 patients with a mean age of 45 years at the time of genetic diagnosis and a family history of sudden cardiac death in 13 (42%) of cases. At first medical contact neuromuscular manifestations were observed in 13 (42%) patients and the main symptoms were dyspnoea (32%), fatigue (29%) and palpitations (19%). At baseline, abnormal electrocardiogram findings were present in 19 (61%) patients, echocardiography showed a mean LV ejection fraction of 49%. During follow-up, SVAs and VAs occurred in 19 (61%) and 21 (68%) patients respectively and AHF developed in 39% (12 patients). CDI was performed in 22 (71%) patients (6 pacemaker, 8 ICD, 4 CRT and 4 ILR). An appropriate intervention (ATP/shock) was observed in 4 out of 11 ICD carriers (36%). During follow-up 6 (19%) patients died while 4 (13%) received heart transplantation. Conclusions *LMNA* gene variants are associated with frequent arrhythmic events (both brady/ tachyarrhythmias) even in the context of mild impairment of LV systolic function.

- Mechanisms of laminopathies

P2. "Validation of Myo-converted fibroblasts as a relevant model to study chromatin organization defects in striated muscle laminopathies"

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Lamins are the main constituents of the nuclear lamina, a protein meshwork underlining the inner face of the nuclear envelope (NE) and facing chromatin and nucleoplasm. A-type lamins (lamin A and C), encoded by the *LMNA* gene, have roles in the chromatin organization, through domains called Lamin-Associated Domains (LADs). Proper maintenance of chromatin organization is essential for normal cell function, and depends on nuclear envelope stability and tissue-specific nuclear envelope proteins. *LMNA* gene variants are responsible for a wide spectrum of disorders called laminopathies, most of them affecting striated muscles. *LMNA* variants have been associated with defects in LAD organization and gene expression at the nuclear periphery, contributing in the pathophysiological mechanisms of laminopathies. Laminopathies are characterized by a strong clinical variability and genetic or epigenetic factors, such as modifier genes or specific chromatin organization defects could explain such variability. We collected biological materials, through national and international collaborations and we received a majority of fibroblasts from *LMNA*-mutated patients. Genome organization being highly different between cell types, we myo-converted immortalized skin fibroblasts into myogenic cells, via overexpression of murine MyoD. We performed a preliminary study to validate this model as relevant to investigate LAD organization, by combining RNA-seq and ChIP-seq targeting Lamin A/C and histone marks. We showed that myotubes derived from these myo-converted fibroblasts underwent a clear phenotypic switch to myogenic cell type, at a transcriptomic and at a chromatin level, hence validating this cell system to study LAD organization in muscle cells.

P4. "The ESCRT machinery at the nuclear envelope controls telomere stability"

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The ESCRT machinery is a complex macrostructure devoted to membrane reparation. A prominent role is played by the ESCRT machinery at the nuclear envelope. The components of the ESCRT machinery have been largely studied, however, not exhaustively. Many

open questions concern the composition of this machinery at the nuclear envelope and its role in intranuclear damage. In our work we pursued two aims: i) extending the dissection of the ESCRT machinery at the nuclear envelope; ii) studying the impact of this machinery on the genome, focusing on telomeric structures. We focused on telomeres because these are chromosomal regions with a pivotal role in genome organization, they are connected to the nuclear envelope, and, finally, they are dysfunctional in laminopathic cells. Using super-resolution microscopy and FRET we identified two new ESCRT members at the nuclear envelope: AKTIP and TSG101. These ESCRT subunits are both organized in foci at the nuclear rim, in close association with one another, and with the lamina. We also revealed that the depletion of ESCRT subunits generates telomere aberrations, including telomere-free ends, sister telomere associations, and multiple telomeric signals. Our results prove that the ESCRT machinery at the nuclear envelope plays a role in telomere stability and add new information to the characterization of the ESCRT machinery players at the nuclear envelope. Finally, based on our work we suggest the usage of the ESCRT machinery for the control of the nuclear envelope in laminopathies, and hypothesize that ESCRT variants could be associated with nucleopathies.

P5. "Nuclear fragility in Familial Partial Lipodystrophy of Dunnigan-type (FPLD2)"

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Familial partial lipodystrophy of Dunnigan-type (FPLD2) is a nuclear envelopathy characterized by atrophy of lower adipose tissue and concomitant upper-body fat accumulation. FPLD2 is caused by dominant variants in the *LMNA* gene (p.R482W/Q), a critical nuclear lamina component with pleiotropic functions in gene regulation, chromatin architecture, and nuclear plasticity. Molecular insight into the etiology of FPLD2 is limited and hampered by a lack of tractable cell models reflecting lower-body adipose depots, and the wide range of lamin A functions. To address this, we have established human gluteal primary adipose-derived stem cell (ASC) FPLD2 models. Using these models, we have mapped stage-specific defects in proliferating ASCs, and throughout differentiation into mature adipocytes. We find that nuclear herniations, honeycomb structures, and nuclear ruptures of p.R482W cells prominently manifest as mature adipocyte-specific phenotypes. Importantly, we show that intracellular lipid droplets provide a prominent source of nuclear mechanical stress. Through manipulation of lipid droplet size, abundance and intracellular pressures, we demonstrate that lipid droplets directly drive nuclear ruptures and re-ruptures with extended repair half-life and cumulative nuclear stress load. Nutrient deprivation suppresses these phenotypes in mature p.R482W adipocytes, and can be re-introduced by refeeding the cells. Lastly, we find that the massive cumulative ruptures stress the mature p.R482W cells are experiencing, subsequently results in DNA damage, cell death, and prevents re-engagement of adipocyte differentiation. Our data argue that lipid droplet-mediated mechanical force on nuclei of mature Lamin A p.R482W-expressing adipocytes could present a major factor in the etiology of FPLD2. *Corresponding author: coen.campsteijn@medisin.uio.no

P6 "STAT1 is a major driver of cellular aging and organismal decline in Progeria"

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Accumulation of cytosolic DNAs is a new hallmark of aging that triggers sterile inflammation and tissue deterioration. In Hutchinson-Gilford Progeria Syndrome (HGPS), a truncated lamin A protein named "progerin" causes genomic instability and accumulation of cytosolic DNAs, triggering an inflammatory pathway characterized by a robust interferon (IFN)-like response. This persistent inflammatory signature is a common hallmark of different metabolic diseases, senescence, and aging-related disorders. However, the specific mechanisms driving this cytosolic DNAs-induced sterile inflammation and how it leads to metabolic dysfunction and tissue degeneration in HGPS are unknown. Here we show that STAT1, a key factor in the IFN response, drives aging phenotypes in HGPS cellular and mouse models. Targeting STAT1 pharmacologically with baricitinib and calcitriol, we repress the sterile inflammation/IFN-like response in progerin-expressing cells, which significantly ameliorates progeria phenotypes such as mitochondrial dysfunction, autophagy

deficiency, and proliferation. In vivo, either calcitriol or baricitinib extends the lifespan of LmnaG609G/G609G progeria mice. Importantly, progeria mice treated with baricitinib alone or in combination with a high-caloric/high-fat diet exhibit an improvement of healthspan, with a remarkable amelioration of skin, aortic and adipose tissue degeneration. Critically, Stat1 haploinsufficiency is sufficient to reduce tissue degeneration and extend lifespan of progeria mice. Our study unveils STAT1 as a major driver of HGPS pathology and suggests that aberrant STAT1 signaling contributes to organismal aging. In addition, our data provide new therapeutic avenues for HGPS and possibly other laminopathies as well as diseases associated with sterile inflammation/IFN response.

P10. "Tissue-specific chromatin remodeling in Hutchinson–Gilford progeria syndrome"

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Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disease with symptoms that recapitulate accelerated ageing. The disease is caused by progerin, a truncated form of Lamin A that accumulates on the nuclear lamin mesh causing substantial rearrangements in the nuclear architecture, ultimately leading to chromatin changes that affect cell homeostasis. Studies of eu- and heterochromatin pathological remodelling in progeria, especially in early phases, could elucidate mechanisms underlying the disease progression. In our lab, we develop Sequential Analysis of MacroMolecules accessibilitY (SAMMY-seq), a chromatin fractionation technique to separate multiple types of -based on its accessibility. In progeria, SAMMY-seq can detect chromatin early heterochromatin changes with as little as 50K cells. Building on this earlier work, we further developed the SAMMY-seq implementing a novel experimental protocol and data analysis algorithms that allow mapping the position of both open and closed chromatin regions along the genome, in addition to their 3D spatial segregation in distinct chromatin compartments. To unravel tissue-specific chromatin changes, we used the mouse HGPS model G609G, and we extracted and sequenced multiple cell populations from single animals, selecting tissues where symptoms are more prominent as skin, aorta and muscle. Moreover, we used the age of 1 and 3 months to depict the onset and the evolution of chromatin remodelling. We found that compartmentalization diverges increasingly with age in a cell-specific manner. Our work will build an extensive representation of chromatin reorganization over time, disease progression, and tissues.

P15. "Proteomic characterisation of human *LMNA*-related congenital muscular dystrophy muscle cells"

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Variants in the *LMNA* gene, encoding lamin A/C, cause a rare form of congenital muscular dystrophy (L-CMD), characterised by severe muscle weakness and wasting, delayed motor milestone achievement, often with cardiac abnormalities and respiratory insufficiency. The molecular mechanisms downstream of the *LMNA* variants remain unclear, hindering the development of non-mutation specific therapies for L-CMD. Here, proteomic and bioinformatics analyses were conducted on immortalized myoblasts and myotubes from three individuals with L-CMD, each harbouring a different *LMNA* variant (i.e., R249W, del.32K and L380S), and compared to unaffected control cells. Abnormal nuclear morphology was evident in all three L-CMD cell lines, while nucleoplasmic aggregation of lamin A/C was restricted to the del.32K cell line, and mislocalisation of the inner nuclear membrane protein, emerin, was seen only in the R249W cell line. Across all three L-CMD cell lines, mass spectrometry analysis identified 124 and 228 differentially expressed proteins in the myoblasts and myotubes, respectively. Enriched signalling pathways associated with these proteins include synaptogenesis and necroptosis in L-CMD myoblasts, and Huntington's disease and insulin secretion in L-CMD myotubes. Abnormal nuclear morphology indicates loss of nuclear lamina integrity, likely render-

ing muscle cells vulnerable to mechanically induced stress. Emerin mislocalisation and nucleoplasmic aggregation of lamin A, seen only in one of three cell lines, suggests that some molecular pathways associated with L-CMD may differ, depending on the specific *LMNA* variant. Nonetheless, the identification of common proteomic alterations and associated molecular pathways across all three L-CMD lines, highlighted potential targets for the development of non-mutation specific therapies.

P17. "Increased DNA binding of a de novo variant of Barrier-to-autointegration factor is associated with dominant motor neuronopathy."

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Barrier-to-autointegration factor (BAF) is an essential component of the nuclear lamina. Encoded by *BANF1*, this DNA binding protein regulates gene expression, cell cycle progression, and nuclear integrity. A complete loss of BAF is lethal in multiple organisms. By contrast, an Ala12Thr missense variant of *BANF1* causes a recessive premature aging syndrome, called Néstor-Guillermo Progeria Syndrome (NGPS). Here, we report the identification of a dominant pathogenic variant of BAF, Gly16Arg. This variant was identified in an individual presenting with progressive neuromuscular weakness. Cellular and biochemical properties of this novel variant are distinct. Whereas NGPS patient fibroblasts show altered lamin and emerin localization, and a distorted nuclear shape, BAF Gly16Arg patient fibroblasts retain lamins and emerin at the nuclear periphery and show modest changes in nuclear shape. Solution structural analyses of BAF Gly16Arg revealed significantly reduced dynamics of its N-terminal region, a region that regulates DNA binding. The stabilized BAF Gly16Arg structure results from the formation of an inter-monomer salt brige between Arg16 of one monomer and the carboxyl group of the terminal Leu89 of the second monomer. This structural change increases DNA binding affinity and elevated levels of repressive chromatin modifications in patient fibroblasts. Taken together, our studies suggest that the BAF Gly16Arg variant has increased genome occupancy, which imparts epigenetic changes that impact nuclear functions. These studies provide a new example of how a missense mutation can change a protein conformational equilibrium to cause a dominant disease and extend our understanding of mechanisms by which BAF function impacts human health.

P20. "miR-376a-3p and miR-376b-3p: 2 miRNAs involved in pathophysiology of Hutchinson-Gilford progeria."

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Hutchinson-Gilford progeria syndrome (HGPS) is a very rare genetic disease, characterized by accelerated and premature aging. The *de novo* point variant in the *LMNA* gene (c.1824C > T in classical form) leads to production of abnormal and toxic protein called progerin, which accumulates in cell nuclei, leading to major cellular defects. Among them, chromatin remodeling drives gene expression changes, including miRNA dysregulation. We have investigated miRNA expression profiles in HGPS and control fibroblasts, highlighting an enrichment of overexpressed miRNAs belonging to the 14q32.2-14q32.3 miRNA cluster, linked to chromatin remodeling at this specific locus in HGPS fibroblasts. The role of miR-376b-3p and miR-376a-3p, both overexpressed in HGPS fibroblasts and belonging to this cluster, was then investigated. We generated models of induced overexpression of these miRNAs in control fibroblasts and their inhibition in HGPS fibroblasts. We demonstrated that miR-376b- 3p and miR-376a-3p inhibited cell proliferation, enhanced senescence, and prevented progerin degradation. By targeting these major processes linked to premature aging, these two miRNAs may play a pivotal role in the pathophysiology of HGPS. This presentation describes our results published in the journal iScience in 2022.

P21. "Single-cell RNA sequencing revealed alterations in early steps of differentiation of C2C12 myoblasts bearing *LMNA* variants"

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Background. Skeletal and cardiac muscle appeared to be more susceptible to different *LMNA* variants. Previously we showed that skeletal muscle stem precursors, myoblasts, bearing *LMNA* mutations R482L/G232E, exhibit altered expression profiles, bioenergetics and dysregulation of the differentiation progress. The purpose of this study is to describe transcriptional and populational changes under different *LMNA* variants on the single- cell level at the early stages of myoblasts differentiation. Methods. C2C12 mouse myoblast cell line was transduced with lentivirus bearing human *LMNA* gene with next variants: *LMNA*-WT as a wild-type; *LMNA*-G232E causing severe muscular dystrophy (MD) with signs of LGMD and EDMD; *LMNA*-R249Q causing EDMD accompanied by cardiovascular complications; *LMNA*-R482L causing FPLD. Cells were collected on hours 0, 6, 12, 24 after the start of differentiation. Libraries for scRNA-seq were prepared using 10X Genomics. Data was processed with CellRanger, R packages seurat, monocle3; FDR=0.01. Results. In total 36574 cells were obtained for 16 samples; 12 cell clusters were revealed. Cells are visually divided in 2 "global" populations connected via small cluster expressing Myog/Mymk. At the time 0h in WT there are 2 proliferated cell populations in both "global" branches; during the process of differentiation one proliferated population replaced by another reaching the maximum density at 12h. Cell lines *LMNA*-G232E and *LMNA*-R482L show similar to WT dynamics with slight compositional changes; *LMNA*-R249Q at 0h looks like WT at 12h. These findings could help in understanding the pathogenesis of laminopathies in muscle differentiation.

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P24. "Dynamics and gene regulatory interactions at the nuclear periphery during adipose differentiation"

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Accurate control of gene expression at the nuclear periphery is critical for proper regulation of differentiation. Chromatin interacts with the nuclear lamina via lamina-associated domains (LADs) at the nuclear periphery. LADs are in general heterochromatic, with low gene density and transcriptionally inactive. We examined changes in the association of lamins with the genome in the first 72 hours of differentiation of adipose stem cells into adipocytes. We demonstrate a repositioning of entire stand- alone LADs and of LAD edges as a prominent nuclear structural feature of early adipogenesis. Adipogenic genes are released from LADs, while LADs sequester genes involved in non-adipogenic lineages. However, LAD repositioning only partly concurs with gene expression changes. Further, we identify expressed genes in constitutive LADs (cLADs), which reside in local euchromatic and lamin-depleted regions, marked by active histone marks and accessible chromatin. By using publicly available enhancer-capture Hi-C data, we show that expressed genes in LADs are connected to active enhancers in LADs and outside LADs. Fluorescence in situ hybridization confirms active enhancer-gene proximity in LADs at the cellular level. We also provide evidence that lamin A/C, but not lamin B1, plays a role in repressing genes flanking active regions within cLADs. This favors a model where spatial topology of chromatin at the nuclear lamina is compatible with gene expression. Next, we aim to test to what extent gene expression and spatial chromatin organization at the lamina are regulated by lamin A/C in models of laminopathies, and more precisely of partial lipodystrophy and muscular dystrophy.

P25. "The first multiparametric anti-ageing CRISPR screen uncovers new targets in Progeria"

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Progeria syndromes are very rare, incurable premature aging conditions recapitulating most ageing features. We have recently carried out the first whole genome, multiparametric CRISPR anti-ageing screen, identifying 43 new genes that can reverse multiple cellular ageing phenotypes in progeria. The screen was implemented in fibroblasts from Néstor- Guillermo Progeria Syndrome (NGPS) patients, carrying a homozygous p.Ala12Thr variant in barrier-to-autointegration factor (BAF A12T). The hits were enriched for genes involved in protein translation, protein and RNA transport and osteoclast formation. We further confirmed that BAF A12T drives increased protein translation and translational errors that could directly contribute to premature ageing in patients. This work has highlighted the power of multiparametric whole genome synthetic rescue screens to identify new anti-ageing genes and therapeutic avenues in Progeria and to uncover novel biology behind progeria-associated cellular dysfunction.

P26. "Nuclear platform reorganization and nuclei orientation are lost in EDMD2 human myoblasts subjected to mechanical stimulation"

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In muscle cells subjected to mechanical stimulation, LINC complex and cytoskeletal proteins are basic to reorganize nucleus-cytoskeleton architecture and to maintain nuclei orientation. In this context the lamin A/C, plays a role in the remodeling of nuclear protein scaffold, with a mechanism that remains mostly elusive. This study demonstrates that in human myoblasts subjected to mechanical stretching, lamin A/C is able to recruit desmin and plectin to the nuclear envelope, allowing a proper spatial orientation of the nuclei. Interestingly, in human myoblasts carrying EDMD2-causative *LMNA* variants exposed to mechanical stimulation, the recruitment of desmin and plectin to the nucleus and nuclear orientation were impaired, suggesting that a functional lamin A/C is crucial for the response to muscular strain. By describing a new mechanism of action headed by lamin A/C in the nuclear platform remodeling during mechanical stress, these findings show a structural alteration that could be involved in the onset of the muscular defects observed in muscular laminopathies.

P27. "DNA damage repair in *LMNA*-related congenital muscular dystrophy"

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Non-Homologous End Joining (NHEJ) repair pathway is a multi-step process, comprising phosphorylation of histone H2AX (γH2AX) at DNA breaks, followed by additional post-translational modifications (methylation, ubiquitination and deacetylation) of histones required for the recruitment of 53BP1 at double-strand breaks. Growing evidence are pointing to an accumulation of DNA damage in *LMNA*-related muscular dystrophies, and more particularly in the most severe one, the *LMNA*-related congenital muscular dystrophy (L-CMD). *LMNA* gene encodes for the nuclear envelop proteins lamin A/C. These proteins have multiple functions, including nuclear envelop resistance, genome organization and sequestration of nuclear envelop proteins at the inner nuclear membrane. Interestingly, lamin A/C has been shown to interact with several histone acetylases and deacetylases and with 53BP1. We therefore speculate that DNA breaks accumulation in *LMNA*-related muscular dystrophies might be due to defective DNA repair through NHEJ. We used L-CMD patient myotubes in culture to analyze their ability to repair DNA following etoposide-induced double-strand breaks. Our preliminary data points to an increased sensitivity of L-CMD cells to etoposide, evidenced by increased γH2AX foci after 2h etoposide treatment and cell death following 2h recovery post-treatment. This might be due to the inability of L-CMD myotubes to recruit 53BP1 to DNA breaks, hence abrogating DNA repair through NHEJ.

P29. "Characterization of White Adipose Tissue in Laminopathic Patients: The Dual Nature of WAT Overaccumulation"

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Pathogenic variants in the *LMNA* gene can result in abnormal fat distribution, including overaccumulation of white adipose tissue (WAT) in the neck/face region, often associated with lipoatrophy in the limbs and buttocks. To investigate into this as yet unexplained regional discrepancy in WAT distribution, we characterized some biological properties of WAT overaccumulated around neck/face of two female patients, respectively carrying the R482Q and R545H *LMNA* variants, in comparison with that of age/sex-matched healthy subjects. The former patient exhibits a typical Familial Partial Lipodystrophy 2 phenotype, while in the latter lipoatrophy is not reported. We analyzed: 1. gene expression of WAT biopsies, 2. proliferative, differentiation potential and response to insult of preadipocytes isolated from their stromal vascular fraction (SVF). When laminopathic patients are considered together we observe increased expression of fibrosis related genes (collagens and TGFbeta), and a moderate upregulation in inflammation-related transcripts (Haptoglobin, MCP-1, IL-6), with respect to controls. Differences are more pronounced for the R482Q compared to the R545Q biopsy. The expression of miRNA 196a-5p, an inhibitor of various collagens expression, is upregulated in the laminopathic WAT. Cells from the SVF

of laminopathic patients showed a higher proliferation rate, and a greater adipogenic capacity as assessed by Oil Red O staining, and higher expression of mature adipocyte markers such as ap2/FABP4 and leptin. Conclusions: WAT over-accumulating around the neck/facial region of laminopathic patients exhibits signatures of increased fibrosis and inflammation, suggesting pathological changes in the tissue, while cultured preadipocytes isolated from this tissue exhibit a greater potential to become functional adipocytes.

P30. "Endothelial-to-mesenchymal transition triggered by dysfunctional vascular smooth muscle cells contributes to accelerated atherosclerosis in progeria"

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Hutchinson-Gilford progeria syndrome (HGPS) is a rare disease caused by a mutant form of lamin A called progerin. HGPS patients manifest premature aging and die during adolescence, predominantly from complications of atherosclerosis. Previously, we found that progerin expression in vascular smooth muscle cells (VSMCs) triggers accelerated atherosclerosis in progeria; however, the impact of progerin-triggered VSMC alterations on endothelial cells (ECs) remains to be elucidated. Here, we investigated EC phenotypes in two atheroprone mouse models of HGPS, with ubiquitous or VSMC-specific progerin expression. Immunofluorescence studies showed altered EC morphology, augmented LDL permeability and leukocyte recruitment, and abnormal accumulation of cells expressing bona fide EC markers inside atherosclerotic lesions in both progeroid mouse models. These EC-like cells showed higher proliferation and expressed mesenchymal markers, including N-cadherin and collagen III, suggestive of endothelial-to-mesenchymal transition (endMT). Furthermore, RT-qPCR analysis showed upregulation of the transcription factors Snai1 and Zeb2 involved in endMT. We next explored TGFβ signaling, a well-known endMT trigger, in HGPS- associated atherosclerosis. Atheroma plaques in both progeria models presented increased expression of TGFβ1 together with activation of its downstream mediator phospho-SMAD3, and in vivo treatment with SIS3 (SMAD3 phosphorylation inhibitor) reduced leukocyte recruitment, adventitial thickening, VSMC loss, and atherosclerotic lesions in aorta in VSMC- specific progeria mice. Additionally, SIS3 treatment decreased collagen III and IV, and the number of CD31-positive cells within HGPS atheroma plaques. Our results indicate that TGF β1/SMAD3-induced endMT contributes to accelerated atherosclerosis in progeria.

P31. "Extending evidence for a relevant contribution of nuclear envelope proteins in myotonic dystrophy"

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Myotonic dystrophy type 1 (DM1) is a multisystemic disorder with predominant muscle and neurological involvement. Despite a well described pathomechanism, which is primarily a global missplicing due to sequestration of RNA-binding proteins, there are still many unsolved questions regarding additional factors contributing to the disease development. One such question is the disease etiology in the different affected tissues. We observed alterations at the nuclear envelope (NE) in primary muscle cell cultures of DM1 patients before. This led us to reanalyze a published RNA-sequencing dataset of DM1 and control muscle biopsies regarding the misregulation of NE proteins. We could identify several muscle NE proteins encoding genes to be misregulated depending on the severity of the muscle phenotype. Among these misregulated genes were NE transmembrane proteins (NETs) involved in nuclear-cytoskeletal coupling as well as genome organization. For selected genes, we could confirm that observed gene-misregulation led to protein expression changes. Furthermore, we investigated if genes known to be under expression-regulation by genome organization NETs were also misregulated in DM1 biopsies, which revealed that misregulation of two NETs alone is likely responsible for differential expression of about 10 % of all genes being differentially expressed in DM1. Notably, the majority of NETs identified here to be misregulated in DM1 muscle are mutated in Emery-Dreifuss muscular dystrophy (EDMD) or clinical similar muscular dystrophies, suggesting a broader similarity on the molecular level for muscular dystrophies.

P33. "Nuclear envelope stress in glioblastoma multiforme"

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Glioblastoma multiforme (GBM) is the most aggressive, primary brain tumor in adults, due to its high heterogeneity and extensive infiltration in surrounding tissues. Recurrence is almost universal, and there is no cure, urging for novel research angles. Owing to the stiff tumor microenvironment and limited migration space, the nuclei of GBM cells are exposed to significant mechanical forces. We hypothesize this renders them vulnerable to nuclear envelope (NE) stress, a process that promotes DNA damage and contributes to tumor aggressiveness. To investigate the role of NE stress in GBM progression, we first quantified nuclear morphology, a hallmark of patient survival, in a panel of widely used GBM cell lines. We found that they display nuclear dysmorphy with phenotypes ranging from blebbed to polylobed, depending on the cell type. The same phenotypes were observed in patient-derived glioblastoma cells and in GBM patient biopsies. We observed altered lamin levels in the GBM cell lines. Using live cell imaging, we found that the GBM cells with higher levels of nuclear dysmorphy were more prone to repetitive NE rupture both spontaneously and after exposure to mechanical confinement. To verify whether NE stress was recapitulated in a physiologically more relevant context, we visualized GBM cells integrated in cerebral organoids and identified several cells with loss of nuclear compartmentalization. Finally, we confirmed this loss of compartmentalization in the form of focal nuclear BAF staining in GBM patient biopsies. Thus, we conclude that GBM cells experience NE stress in vitro and in vivo.

P34. "Molecular imaging of Lamin A/C and Src proteins in the nucleus of SaOS- 2 cells: a cellular model for studying nuclear dysmorphisms associated to laminopathies"

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SaOS-2 osteosarcoma cells, commonly recognized as low aggressive osteoblast-like cells, have shown high nuclear Lamin A/C expression and all the hallmarks of laminopathic nuclear phenotype, as folds, honeycombs and donut nuclei. Moreover, we previously described SaOS-2 cells showing a high nuclear localization of Src, a tyrosine- kinase involved in several cellular processes, such as cell proliferation, migration and cell response to mechanical stimulation. In this study, we demonstrated a tight relationship between lamin A/C and Src in SaOS-2 cell nuclei, assessed by advanced imaging-based microscopy techniques. With confocal laser scanning and STED microscopy, a statistically significant co-distribution between the two proteins was observed, especially in the nuclear rim rather than in the nuclear matrix. To deepen the Src-Lamin A/C colocalization at the nanoscale level, we performed Forster''s resonance energy transfer after bleaching (FRET- AB) experiments, revealing a FRET efficiency of 14% in the nuclear rim and folds, of the Src/Lamin A/C antibody pair. Then, we used the time- domain fluorescence lifetime imaging microscopy (FLIM), a sensitive molecular imaging method to detect protein-protein interactions, combined with FRET detection (FLIM-FRET technique), demonstrating a decreased lifetime value of Src (as donor antibody) in the presence of Lamin A/C (as acceptor antibody) in double-stained SaOS-2 nuclei, with a 19% FRET efficiency. These results suggest a close relationship between Src and Lamin A/C in SaOS-2 cells that needs to be confirm in cells from laminopathic patients, thereby confirming SaOS-2 cells as a cellular model for studying laminopathic nuclear dysmorphisms and opening to new therapeutic approaches for patients.

P35. "Nuclear fragility in Familial Partial Lipodystrophy of Dunnigan-type (FPLD2)"

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Familial partial lipodystrophy of Dunnigan-type (FPLD2) is a nuclear envelopathy characterized by atrophy of lower adipose tissue and concomitant upper-body fat accumulation. FPLD2 is caused by dominant variants in the *LMNA* gene (p.R482W/Q), a critical nuclear lamina component with pleiotropic functions in gene regulation, chromatin architecture, and nuclear

plasticity. Molecular insight into the etiology of FPLD2 is limited and hampered by a lack of tractable cell models reflecting lower-body adipose depots, and the wide range of lamin A functions. To address this, we have established human gluteal primary adipose-derived stem cell (ASC) FPLD2 models. Using these models, we have mapped stage-specific defects in proliferating ASCs, and throughout differentiation into mature adipocytes. We find that nuclear herniations, honeycomb structures, and nuclear ruptures of p.R482W cells prominently manifest as mature adipocyte-specific phenotypes. Importantly, we show that intracellular lipid droplets provide a prominent source of nuclear mechanical stress. Through manipulation of lipid droplet size, abundance and intracellular pressures, we demonstrate that lipid droplets directly drive nuclear ruptures and re-ruptures with extended repair half-life and cumulative nuclear stress load. Nutrient deprivation suppresses these phenotypes in mature p.R482W adipocytes, and can be re-introduced by refeeding the cells. Lastly, we find that the massive cumulative ruptures stress the mature p.R482W cells are experiencing, subsequently results in DNA damage, cell death, and prevents re-engagement of adipocyte differentiation. Our data argue that lipid droplet-mediated mechanical force on nuclei of mature Lamin A p.R482W-expressing adipocytes could present a major factor in the etiology of FPLD2.

P37. "The microtubules plus-end tracking proteins CLIP-170 mediates nuclear shape in Emery-Dreifuss muscular dystrophy"

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Variants in lamin A/C gene (*LMNA*) cause Emery-Dreifuss muscular dystrophy (EDMD). We set out to unravel the molecular and cellular causes of EDMD in Lmnap.H222P/H222P mice, a model for the disease. We recently showed that a decreased acetylation of microtubules, a post- translational modification, was altering the microtubule organization in EDMD. Given that it has been described that microtubules control nuclear shape, we asked how abnormal microtubules participate in the nuclear elongation, a cellular phenotype of EDMD. Microtubules are highly dynamic components of the cytoskeleton. CLIP-170 is one of the microtubules plus-end tracking proteins, which binds to the plus-end of microtubules to protect them from depolymerizing. We found that the expression of CLIP-170 was increased in striated muscles from Lmnap. H222P/H222P mice, and this was dependent of tubulin acetylation. We next found that CLIP-170 displays a punctiform localization at the poles of elongated nuclei in striated muscles from Lmnap.H222P/H222P mice, while it is localized around the nuclei in the wild type animals. CLIP- 170's activities are determined by conformational changes. A folded conformation of CLIP170 (phosphorylated form), dissociates from microtubule plus ends. CLIP-170 in the open extended conformation (unphosphorylated form) binds microtubule more readily. We studied the action of Pregnenolone, a molecule that activated CLIP-170 by changing CLIP-170's conformation, in EDMD. We found that Pregnenolone removes CLIP-170 from the poles of the elongated nuclei and regulates nuclear shape. These results suggest that CLIP-170 plays a crucial role in nuclear shape, a cellular phenotype of EDMD.

P38. "Disruption of nuclear envelope integrity as a possible initiating event in tauopathies"

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The microtubule-associated protein tau is an abundant component of neurons of the central nervous system. In Alzheimer's disease and other neurodegenerative tauopathies, tau is found hyperphosphorylated and aggregated in neurofibrillary tangles. To obtain a better understanding of the cellular perturbations that initiate tau pathogenesis, we first performed a CRISPR-Cas9 screen for genetic modifiers that enhance tau aggregation. This initial screen yielded three genes, *BANF1, PPP2CA* and *ANKLE2*, whose inactivation promoted the accumulation of tau in a phosphorylated and insoluble form. In a complementary screen, we identified three additional genes, *LEMD2, LEMD3* and *CHMP7*, that when overexpressed provided protection against tau aggregation. The proteins encoded by the identified genes are mechanistically linked and recognized for their roles in the maintenance and repair of the nuclear envelope. These studies implicate disruption of nuclear envelope integrity as a possible initiating event in tauopathies and reveal new targets for therapeutic intervention.

P42. "New lamin interactions networks- analyses of interactomes, transcriptomes and chromatin interactions"

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We have been using fly model system to study the function of lamins and topo II. One project has been focused on lamins function in development using initially GAL4–Mef2 driver for lamin selective knockdown and analyses of bodywall muscles at the stage of 3rd instar larvae. Lamin C downregulation results in strong larval phenotype and 100% lethality up to imago stage. At the ultrastructural level we detected abnormal distribution of actin in cytoplasm next to cell nuclei, abnormally shaped nuclei, NL and NE, depositions of polymerized actin inside cell nuclei. Surprisingly, larval expression of lamin C in muscles did not result in incorporation of protein into the cell nuclei in larval muscles. For lamin Dm knockdown phenotype was milder with disturbed M and Z lines and lower mobility of adult flies. The second project has been focused on lamins and topo II interactomes, transcriptomes and chromatin binding (ChIP-seq) and their modulations during heat shock and recovery. We detected strong changes in interactions of each protein during HS as well proteins properties and chromatin binding abilities. We have identified new protein zcomplexes and new functions of lamins and topo II both in normal conditions and during HS.
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P43. "Lem2 is essential for cardiac development by maintaining nuclear integrity"

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Nuclear envelope (NE) integrity is essential for compartmentalisation of nucleus and cytoplasm. Importantly, mutations in genes encoding NE and associated proteins are the second-highest cause of familial dilated cardiomyopathy. One such protein that causes cardiomyopathy in humans and affects mouse heart development is Lem2. However, its role in heart remains poorly understood. We generated mice in which *Lem2* was specifically ablated either in embryonic cardiomyocytes (*Lem2* cKO) or adult cardiomyocytes (*Lem2* iCKO) and carried out physiological, tissue and cellular analyses. High resolution episcopic microscopy was used for 3D reconstructions and detailed morphological analyses. RNA- sequencing and immunofluorescence identified altered pathways and cellular phenotypes, and cardiomyocytes were isolated to interrogate nuclear integrity in more detail. In addition, echocardiography provided physiological assessment of *Lem2* iCKO adult mice. We found that *Lem2* was essential for cardiac development, and hearts from *Lem2* cKO mice were morphologically and transcriptionally underdeveloped. *Lem2* cKO hearts displayed high levels of DNA damage, nuclear rupture, and apoptosis. Crucially, we found that these defects were driven by muscle contraction as they were ameliorated by inhibiting myosin contraction and L-type calcium channels. Our data suggest that *Lem2* is critical for integrity at the nascent nuclear envelope in fetal hearts, and protects the nucleus from the mechanical forces of muscle contraction. In contrast, the adult heart is not detectably affected by partial *Lem2* depletion. Taken together, these data provide insights into mechanisms underlying cardiomyopathy in patients with variants in *Lem2* and cardio- laminopathies in general.

P45. "Functional studies of two microRNAs overexpressed in Hutchinson-Gilford Progeria and related syndromes"

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Hutchinson-Gilford progeria (HGPS) is a very rare genetic disease in which an abnormal protein, called progerin, accumulates in the nucleus with a dose-dependent toxicity. HGPS is characterized by accelerated and prematureaging resulting in death at around 14 years of age. The patients develop numerous bone anomalies. We have identified by NGS the overexpression of two miRNAs from the same precursor in dermal fibroblasts of patients. The first one is known to target transcripts of key molecules involved in chondrocyte differentiation and in the regulation of oxidative stress. Both of these miRNAs regulate osteoblastic differentiation. To elucidate the role of these two miRNAs in HGPS, we used human and mouse models: In vitro modulation of the two miRNAs by transfection in fibroblasts from HGPS patients and controls; differentiation of HGPS and control human mesenchymal stem cells (MSCs) derived from iPSCs into osteoblasts and chondrocytes; osteoblasts and chondrocytes harvested from WT (wild type) and HGPS (KI LmnaG609G/G609G) newborn mice; tissues collected from the HGPS and WT mice at different ages. This work re-

vealed a decrease in the expression of these two miRNAs in the aorta, aortic arch, adipose tissue and bone of old HGPS mice (4 months), whereas their expression was found to be increased in VSMCs isolated from aortas of young HGPS mice (1 month). The identification of the mechanisms in which these 2 miRNAs are involved could improve the understanding of the pathophysiology of progeria, paving the way to new therapeutics.

P46. "Muscle markers expression in Emery- Dreifuss muscular dystrophy type 1 iPSC-derived satellite-like cells and myoblasts"

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Emery-Dreifuss muscular dystrophy (EDMD) remains an untreatable disease caused by variants in genes coding for nuclear lamina proteins, e.g. EMD encoding emerin (EDMD1 phenotype), or proteins directly interacting with them. The results obtained so far seem to be highly dependent on used research model, therefore we propose the utilization of human-derived induced pluripotent cells (iPSCs) differentiated into subsequent stages of myogenesis using adequate growth media. In the presented study, we concentrated on the first two stages of the differentiation process, represented by satellite-like cells and myoblasts. Our goal was to answer if a mutation in EMD gene distorts expression levels of muscle markers and if observed alterations might result from diversification between analyzed clones. iPSCs derived from EDMD1 patients and healthy donors were cultivated according to the protocol and collected at each stage. The main focus was on muscle markers, like *pax3* and *pax7*, characteristic for satellite cells, *myf5* and *myod*, typical for myoblasts. Genes were analyzed for relative gene expression against 3 housekeeping genes. Results gained in our research may help understand the emerin role in muscle differentiation and optimize iPSC differentiation into muscle cells protocol by minimization of initial differences in gene expression levels.

P47. "Lamin B1 governs a cell fate decision switch in human neuroblastoma cells"

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The nuclear lamina is a versatile coordinator of cell function with high tissue specificity. While having initially received less attention than A-type lamins, it has now become clear that B-type lamins, and in particular lamin B1, play a crucial role in neuronal and brain development [1-3]. To shed light on their natural evolution during brain aging, we quantified the protein levels of all major lamins in aging mouse brain. We found attenuated levels of lamin B1 and B2 in postnatal cortices compared to embryonic brains. To further dissect the role of lamin B1 attrition in the human context, we evaluated the cellular proteomic responses that take place upon selective *LMNB1* knockdown in human SH-SY5Y neuroblastoma cells. Downregulation of *LMNB1* significantly induced the upregulation of proteins involved in the biosynthesis of a specific subset of amino acids as well as specific tRNA synthetases reminiscent of a signature of an endoplasmic reticulum stress response [4]. When further scrutinizing the reactive proteome of *LMNB1* knockdown cells that were treated with retinoic acid to induce neuronal differentiation, we observed a strong dysregulation of pathways involved in cell cycle regulation, DNA synthesis and DNA replication. This suggests that lamin B1 depletion induces a stress response that prematurely diverts cells towards a non- proliferative state and thereby prevents their proper differentiation into the neuronal lineage. [1] Coffinier et al. (2011) Mol Biol Cell [2] Bedrosian et al. (2021) EMBO J [3] Bin Imtiaz et al. (2021) Cell Stem Cell [4] Gonen et al (2019) iScience.

P48. "Lamin-mediated chromatin condensation in dilated cardiomyopathy"

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Variants in the *LMNA* gene, which encodes A-type lamins (Lamin A/C), are the most prevalent genetic cause of familial dilated cardiomyopathy (DCM), a disease that progressively affects heart muscle function. A-type lamins structurally support the nucleus and their loss or faulty maturation compromise the stability of the nu-

clear envelope (NE) leading to nuclear dysmorphy and NE ruptures. These features are accompanied by local changes in the chromatin landscape but the causal mechanisms and relation to disease progression are not well defined. To interrogate the interplay between chromatins and lamins, we first quantified nuclear parameters of stable HeLa knockouts (ko) clones for each of the three lamin-encoding genes (*LMNA, LMNB1* and *LMNB2*), as well as the ZMPSTE24 gene. While the individual knockouts showed very distinct changes in nuclear morphology (e.g., irregular shaped nuclei in *LMNA* ko or nuclear bleb formation in *LMNB1* ko and ZMPSTE24 ko), all cell lines displayed a global change towards a more heterochromatic state. To understand whether similar rules apply to cardiomyocytes, we established a protocol to generate mature iPSC-derived cardiomyocytes. While we are optimizing targeted knockout of *LMNA* and ZMPSTE24 in this model, and generating DCM patient-derived cardiomyocytes, we have already tested pharmacological treatments that interfere with lamin A/C levels or maturation. In sum, our data suggest that a finely regulated lamina composition ensures a transcriptionally active chromatin state. Thus, we anticipate that DCM causing *LMNA* variants will affect gene expression patterns, which may add to the pathogenic process.

- Laminopathies Models

P7. "Models for the investigation of the pathophysiology of the progeroid MADaM syndrome"

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We have recently described a novel progeroid syndrome we called MADaM - Mandibuloacral Dysplasia associated to MTX2 - characterized by growth retardation, bone resorption, arterial calcification, renal glomerulosclerosis and severe hypertension leading to early and accelerated aging and a premature death, showing a remarkable similarity with HGPS symptoms. However, while HGPS patients carry variants in the Lamin A/C gene resulting in nuclear morphology abnormalities and dysfunctions, MADaM syndrome is due to an autosomal recessive null mutation in Metaxin-2 (MTX2) gene, an outer mitochondrial membrane protein. Interestingly, MTX2-mutant fibroblasts show secondary nuclear morphological defects, revealing an unsuspected pathophysiological link between mitochondrial function and nuclear morphology. The aim of our work is to explore potential convergent molecular mechanisms underlying the pathophysiology of MADaM and HGPS in cellular models relevant to the disease. For that purpose, using Sendai virus reprogramming with an integration-free vector, we generated induced pluripotent stem cells (iPSCs) from two different MADaM-derived fibroblast cell lines, for which we verified the expression of pluripotency markers and genome integrity. In parallel, using CRISPR/Cas9-mediated gene editing, we are generating MTX2 KO iPSCs and their isogenic controls to ascertain the molecular origin of the observed defects. As a loss of vascular smooth muscle cells (VSMCs) was proposed to be the cause of arterial fibrosis/ atherosclerosis in progeria models, we are currently optimizing protocols for differentiating MADaM patients-derived and MTX2 KO iPSCs into VSMCs. These models will be key to determine at the cellular, genomic and epigenomic levels what pathways determine the pathophysiology of MADaM syndrome.

P9. "Bone phenotype of the *Lmna*G609G/ G609G-mouse model"

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Hutchison-Gilford progeria syndrome (HGPS) is a rare genetic premature aging disease caused by a variant in *LMNA*, the gene encoding A-type lamins. HGPS is a multi-systemic disorder where patients also develop a bone phenotype. In addition, also progeria mouse models develop bone abnormalities including growth retardation, osteolysis, cervicothoracic lordokyphosis, reduced bone volume and rip fractures. Our aim was to characterize for the first time in detail the mineralisation in such a mouse model. We analysed humeri from 15 weeks old *Lmna*G609G/ G609G mice using quantitative backscattered electron imaging (qBEI) for mineral content and histomorphometry information, Giemsa and Goldner trichrome staining for histology. Interestingly, qBEI analysis did not reveal any differences in the mineralization of either epiphyseal, trabecular or cortical bone between mutant and age matched wild- type mice. However, it seems that the *Lmna*G609G/G609G mice exhibit a growth plate dysplasia, as they have a thinner unmineralized resting and proliferative zone compared to control mice. Furthermore, histomorphometric analysis of qBEI images show a highly significant decrease of mineralized

matrix volume per tissue volume from the region combining mineralized hypertrophic zone and primary spongiosa in the *Lmna*G609G/G609G mice. A trend to a reduced bone volume/tissue volume was also observed in the secondary spongiosa. This is confirmed by the histological staining which also suggests a thinner non-mineralized and mineralized cartilage in the mutant mice. Summing up it appears that mainly the cartilage is affected in this progeria mouse model. It results in a reduced bone volume even though its mineralization is not affected.

P14. "A progerin-inducible human Induced Pluripotent Stem Cell line to study cardiac cell aging"

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Aging is a major risk factor for heart disease, but how exactly aging contributes to cardiac dysfunction is not well understood at the molecular level. This is mainly due to a lack of human-relevant models. Patients with Hutchinson-Gilford Progeria Syndrome, carriers of a *LMNA* variant that express a pathogenic lamin A variant called progerin, show accelerated aging with cardiovascular defects similar to those observed in natural aging. Hence, we aimed to leverage progerin-induced accelerated aging to establish a human cardiac cell aging model. We generated a human induced pluripotent stem cell (hiPSC) line with a doxycycline- inducible expression system of progerin inserted in the AAVS1 locus via recombinase mediated cassette exchange and developed a multifactorial maturation strategy of hiPSC-derived cardiomyocytes (iCM) to obtain the most consistent and faithful model. Deep phenotypic characterization using high content microscopy was used to evaluate maturation and progerin-induced changes. We found that the combination of epigenetic priming, hormonal stimulation, and metabolic switch induction had a synergistic effect on CM maturation. In parallel, induction of progerin expression in immature iCM recapitulated senescence-related features (genomic damage accumulation, SERCA2A changes among others). Thus, we have generated a maturation strategy that closer recapitulates the human adult ventricular phenotype, and defined the conditions to induce progerin so as to mimic the aging process. The later will guide our future work on mature iCM to better replicate cardiac cell aging from adulthood. We expect this model will help us better understand the molecular pathways that contribute to age-related human cardiac dysfunction.

P18. "Using a conditional Lmna R249W mouse model to determine tissue involvement in *LMNA*-Associated Muscular Dystrophy development"

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Laminopathies refer to a group of diseases caused by variants in the *LMNA* gene. To date, over 400 variants have been identified in this gene, resulting in various pathologies that can be classified based on the affected tissue. One specific variant, the substitution of cytosine to adenine at position 745 in exon 4, which leads to the *LMNA*-R249W variant is associated with dilated cardiomyopathy and muscular dystrophy in patients, but the molecular mechanisms underlying these symptoms remains unclear, and the specific tissue that triggers the disease is still unknown. To address this question, we generated a conditional *Lmna* mouse model by introducing the c.745 C>T mutation at exon 4 plus an upstream, floxed cassette containing wild type exons 3 to 12. Using this model, we have demonstrated that the constitutive and ubiquitous expression of two copies of the *LMNA*-R249W variant is associated with premature dead due to a metabolic phenotype. The *Lmna*R249W mouse model allowed us to investigate the effects of the R249W variant in different tissues by expressing the Cre recombinase gene under tissue-specific promoters. We created and studied new mouse lines for the expression of the *LMNA*-R249W variant at hepatocytes, white adipose tissue and striated muscle plus brown adipose tissue. Only the *Lmna*R249W/R249W-Pax7-Cre+ mice recapitulated the defective survival of constitutive *Lmna*R249W/R249W mice, which pointed out to striated muscle/brown adipocyte precursors to the cell of origin of the disease. These results are critical to define the target tissue for future therapies against *LMNA*-R249W associated diseases.

P19. "A mechanobiological model for progerin-induced nuclear blebbing based on characterization with optical tweezers poroelastic indentation"

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Hutchinson-Gilford progeria syndrome (HGPS) is caused by erroneous processing of prelamin A, which leads to the deletion of the cleavage site for the endoprotease *ZMPSTE24*. As a result, a farnesyl group remains attached to the truncated version of the mature lamin A. The accumulation of this defective lamin A, named progerin, promotes nuclear bleb formation. It has been demonstrated that the ability of progerin to attach to the nuclear envelope through its farnesyl moiety is directly related to the process of bleb formation. In this context, we conducted optical tweezers indentation assays to characterize the mechanical alterations over the nuclear envelope expressing progerin. Combining these data with confocal imaging, osmotic shocks, and mathematical modeling, we built a mechanistic model for progerin-induced blebbing based on alterations of the lamina-chromatin crosstalk.

P23. "Investigating lineage-specific phenotypes of laminopathies using induced pluripotent stem cells"

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Lamins A and C are type V intermediate filament proteins encoded by the *LMNA* gene which, along with proteins lamin B1 and B2, form the nuclear lamina. Variants in *LMNA* result in a group of heterogeneous disorders called laminopathies, which are tissue-specific conditions affecting various tissues. The same *LMNA* variant can result in different disorders, resulting in unclear genotype-phenotype correlations. Although progress has been made in determining mechanisms by which tissue-specific phenotypes arise in striated muscle, little is known of the effects of mutant-*LMNA* in other tissues. To analyse lineage- specific phenotypes of laminopathies, we used pluripotent stem cells from laminopathy patients which were differentiated into various cell types affected in these disorders. As perturbed nuclear morphology is a hallmark of laminopathies, it was investigated as a phenotypic readout to determine if nuclear morphology is a suitable output for studying laminopathies, and to determine lineage-specificity of various mutant Lamin A/C isoforms. Results indicate that perturbed nuclear morphology may be a lineage-specific phenotype of mechanically challenged tissues. No difference in the nuclear contour ratio was detected in cell types other than in *LMNA*-mutant congenital muscular dystrophy skeletal myotubes. Preliminary data showed reduced axon length in subsets of *LMNA*-mutant motor neurons, suggesting expansion of the spectrum of *LMNA* variants causing peripheral neuropathies. Finally, we present evidence confirming motor neurons are one of the few somatic cells which do not express Lamin A/C, suggesting defective *LMNA* downregulation upon differentiation of neural progenitors into motor neurons could contribute to the pathomechanism of peripheral nerve laminopathies.

P28. "Application of Human Pluripotent Stem Cells to Study *Lmna*-Related Cardiomyopathy"

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Human induced pluripotent stem derived from patients serve as a unique model of the disease for pathogenetic studies. In our study, we have focused on a group of cardiomyopathies with defect genes associated with nuclear lamina. We have generated hiPSC-derived cardiomyocytes from cardiac patients who harbored different variants in genes associated with nuclear lamina. Patients were diagnosed with severe laminopathy and show different clinical outcomes. Using iPSC-derived cardiomyocytes, we have characterized production and localization of mutant nuclear lamina associated proteins including lamin, emerin and thymopoetin. We have observed *LMNA* gene variants associated with abnormalities of nuclear membrane architecture and

nuclear lamina proteins localization. Upon treatment with lamin pharnesylation inhibitor we have found limited rescue effect on aberrant nuclear morphology of iPSC-derived cardiomyocytes. Our results illustrate that iPScells are a valuable tool for generation of patient-specific models allowing to improve insight into the effects of genetic variants on disease pathogenesis.

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P36. "A preclinical model for Emery- Dreifuss muscular dystrophy type 1 based on reprogrammed primary cells for the analysis of myogenesis in patients cells."

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Ryszard Rzepecki University of Wroclaw Poland Emery-Dreifuss muscular dystrophy is a genetic disease caused by variants in genes encoding nuclear proteins emerin (EDMD1), lamin or associated proteins, characterized by skeletal muscle wasting, contractures of major tendons and cardiac conduction defects. The variants found in EDMD1 cells may result in loss of emerin, protein loss of function or gain of toxic properties by changing interaction networks. Most of the analysed EDMD1 patients' cells were reported to be emerin null, however this conclusion might have come from a single immunostaining approach. We created and analysed a unique collection of patient-derived fibroblasts with variants in EMD gene encoding emerin. We performed a sequencing of all emerin exons, analysis of transcripts lengths and expression levels and examination of the particular peptide presence. The obtained results let us conclude how particular mutations lead to changes in EMD expression, splicing patterns, protein level and modification. Additionally, we reprogrammed EDMD1 fibroblasts together with healthy donor cells to obtain induced pluripotent stem cells. Clones were broadly validated and then differentiated using transgene-free protocol to muscle cells at various developmental stages: satellite-like cells, myoblasts and multinucleated myotubes. Expression of muscle markers was confirmed with immunostaining and qPCR. We created and validated the new model to investigate myogenesis and molecular background of the disease in emerin-null patients-derived cells which allows the genetic background to be taken into account. This may bring new insight on the emerin role in muscle cells differentiation and maintenance. Additionally, our model allows us to nest new therapeutic approaches.

P41. "Modeling of *LMNA* p.H222P mutation- related cardiomyopathy using human induced pluripotent stem cells"

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Variants in the *LMNA* gene, which encodes the nuclear lamins A/C, can cause a diverse range of diseases, called laminopathies, which can affect different tissues. The genotype-phenotype link for these variants is still unclear and no mutation-specific therapy currently exists. In this project, we focus on the *LMNA* H222P mutation, which leads to an Emery-Dreifuss Muscular Dystrophy inducing a dilated cardiomyopathy and a muscular dystrophy. In order to develop a disease model, we used induced pluripotent stem cells (iPSCs) from a heterozygous patient for the *LMNA* H222P variant. We have developed a mutation-specific CRISPR/Cas9-based gene editing therapy and obtained 2 corrected iPSCs clones. The cardiac phenotype associated with this variant was characterized by comparing cardiomyocytes derived from the mutated and corrected iPSC lines (iPSC-CMs). Calcium transient measurements showed that the calcium release and recapture was slower in the mutated cardiomyocytes, and was restored in the corrected cell lines. Moreover, patch-clamp experiments in the mutated cells showed an impaired sodium current (INa), which was restored in the corrected cell lines. qPCR data suggests that the expression of SCN5A, coding for the sodium channel Nav1.5 is unchanged in the *LMNA* H222P cardiomyocytes compared to wild type and mutated cardiomyocytes. Therefore, we suppose that the reduction of INa density is not due to a downregulation of SCN5A but may rather be linked to a disruption of Nav1.5 trafficking at the membrane. Further experiments are carried out to explore this mechanism.

P50. "Contributions of genetic variation to *LMNA*-associated muscle disease"

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Variants in the *LMNA* gene cause a collection of diseases called laminopathies that includes three types of muscular dystrophies. Individuals with the same *LMNA* mutation can exhibit a range of muscle defects, even among closely related family members. This suggests that genetic background influences disease severity. To identify DNA sequence variants that modify *LMNA*-associated disease, we mined whole genome sequence data from closely related family members who display dramatically different muscle disease phenotypes. A predicted pathogenic variant in the *SMAD7* gene was identified and found to segregate with severe muscle defects. *SMAD7* encodes a repressor of Smad signaling; activation of this pathway is deleterious to differentiated muscle cells. We extended our analysis by sequencing the *SMAD7* gene in a cohort of 45 individuals with *LMNA*-associated muscular dystrophy. We identified six additional variants in SMAD7. Interestingly, two of these variants reside within a domain of Smad7 known to bind the ubiquitin ligase Smurf2, an interaction required for translocation of the Smad7-Smurf2 complex out of the nucleus to ubiquinate and degrade the TGF? receptor. To access whether these variants affect *LMNA*-associated muscular dystrophy, we are using Drosophila which permits robust quantitative analyses of indirect flight muscle (IFM) function. Expression of mutant lamins in IFM causes wing posturing defects. Variation in *SMAD7* increases wing posturing defects when co-expressed with mutant lamins, while having minimal effects on its own. These findings demonstrate that variation in *SMAD7* can enhance muscle defects caused by mutant lamins and loss of interaction with Smurf2 is a potential mechanism.

- Drug-based Therapies

P13. "Study of the therapeutic potential of the compound ARRY-371797 in congenital dystrophy associated to *LMNA* (L-CMD)"

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Congenital muscular dystrophy associated to *LMNA* (L-CMD) is a rare disease that present at birth or early infancy. It is characterized by progressive muscle wasting, abnormalities in the atrioventricular conduction system, fibrosis of cardiac tissue and respiratory failure. Variants in *LMNA* are causally associated with L-CMD. Unfortunately, no cure exists for L-CMD patients. Our team aims to advance knowledge of this disease and obtain effective therapies. We focused our studies on the *LMNA*-R249W mutation, the most prevalent in L-CMD. Here, we explored the therapeutic potential of ARRY-371797, a specific inhibitor of p38α that has shown beneficial effects in the cardiac phenotype of an *Lmna*H222P mouse model (PMID: 22773734) and in phase II clinical trial for Lamin A/C–Related Dilated Cardiomyopathy (PMID: 36515663). We assessed its effect in different models carrying an R249W or equivalent *LMNA* variant. We demonstrate that ARRY- 371797 improves nuclear morphology abnormalities of human myoblasts carrying a *LMNA*-R249W variant. Using an LmnaR264W fly model, we showed that ARRY-797 treatment rescues the muscle phenotype induced by *LMNA*-R249W. Finally, ARRY-371797 treatment significantly increases the survival of homozygous LmnaR249/R249W mice. However, preventive and curative ARRY-371797 treatment worsen survival of heterozygous *Lmna*+/R249W mice. These results confirm the potential of ARRY-797 for some of the phenotypes induced by *LMNA* variants and highlight the importance of exploring all possible scenarios in which a putative drug could be used to treat L-CMD. Our study provides valuable insights into potential therapeutic strategies for L-CMD and contributes to the understanding of the molecular mechanisms underlying the disease.

P16. "Steroid treatment may change natural history in congenital laminopathies"

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LMNA gene variants cause a broad clinical from congenital (L-CMD) to later forms (Emery Dreifuss type, EDMD) and non-retractile proximal forms. L-CMD forms have a rapidly progressive evolution, starting with cervico-axial weakness (Dropped Head Syndrome, DHS), followed by extremities and respiratory muscles, which may associate cardiac involvement. Treatment with corticosteroids was suggested because of inflammatory signs observed on biopsy. We describe the experience treating with corticosteroids children with L-CMD in three French Neuromuscular Pediatric Centers Methods: Retrospective study in 7 children (6 males) with genetically confirmed L-CMD treated by oral corticosteroids for at least one year. Collected data included genetics, phenotype, clinical evolution (respiratory, motor, cardiology) and ancillary tests (biopsy, muscle imaging, biochemistry) Results: 7 children (6 DHS, 1 EDMD) were treated with prednisone (0.75 mg/Kg/day) at a mean age of 4 years (2-8) for 3 years (1-7). All of them carried the novo mutations in *LMNA* gene. Inflammatory signs were observed on biopsy (2) or whole-body muscle MRI (5). Three patients who had never walked acquired walking after treatment (2 weeks-1 year). One patient recovered walking ability 2 years after starting corticosteroids. The 2 patients with ambulatory DHS phenotype remained stable. Corticosteroids were stopped in the EDMD patient after 3 years of treatment after worsening motor and cardiac status. Conclusion: Corticosteroids seem to be beneficial in young children with *LMNA* variants, particularly in those with DHS phenotype treated before the age of four. Muscle MRI may be useful to assess the presence of inflammation before treatment and during progression.

P32. "Gene therapy for striated muscle laminopathy (*in vivo* study)"

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LMNA encodes for the nuclear envelope proteins: lamin A/C. *LMNA* mutation induces numerous disorders called laminopathies, mainly affecting striated muscles. All striated muscle laminopathies are characterized by the development of a life-threatening dilated cardiomyopathy. We developed a mouse model mimicking a human *LMNA* variant. Heterozygous LmnaK32del mice develop dilated cardiomyopathy due to a combination of lamin haploinsufficiency and expression of toxic mutant. Based on these facts, we develop therapeutic approach aiming both at reducing the expression of the mutant proteins and restoring the normal lamin level. We produced different AAV2/9 vectors containing human mature lamin A under control of a CMV promoter, either alone, or in combination with shRNA specifically targeting K32del *Lmna* mRNA or WT and mutated allele under a H1 promoter. These AAVs were injected intravenously in WT and heterozygous *Lmna*K32del new-born mice. All treatments showed similar results: transient benefit in term of survival but no effect on cardiac function. Absence of benefit at long term is neither due to a loss of AAV genome particle nor to a loss of its expression with time as we observed maintenance of AAV particle number and sustained human lamin A mRNA and protein expression in heart of injected mice. Rather, it is due to the absence of mouse *Lmna* mRNA knock-down and side effect in the liver, strongly targeted by AAV2/9. Future development of our gene therapy will include new shRNA design, new promoters and AAV capsids to increase mouse *Lmna* mRNA knock-down and tissue specificity.

P44. "Improving the quality of life in Progeroid *Lmna* G609G/G609G mice"

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Hutchinson–Gilford progeria syndrome (HGPS) causes premature aging in children, with adipose tissue, skin and bone deterioration, and cardiovascular impairment. The quality of HGPS patients' life is compromised under many aspects requiring continuous and skillful effort to be managed and overcome. The final goal of this project is identifying a therapeutic strategy able to improve the quality of life in progeria. Recently, we demonstrated that inhibition of interleukin-6 activity by Tocilizumab, a neutralizing antibody raised against interleukin-6 receptors, counteracts progeroid features in both HGPS fibroblasts and *Lmna* G609G/G609G progeroid mice and extends the life span of *Lmna* G609G/G609G progeroid mice. We had also noticed that locomotor activity was preserved, while skin and

hair deterioration and kyphosis were delayed in Tocilizumab-treated *Lmna* G609G/G609G mice. Based on these results, we decided to test the possibility that adding Tocilizumab to currently used clinical protocols for progeria, based on Lonafarnib or Everolimus, could at least improve the quality of life of HGPS patients. Thus, we treated *Lmna* G609G/+ and *Lmna* G609G/G609G progeroid mice with Tocilizumab in combination with Lonafarnib or Everolimus. Surprisingly, we observed that both combined treatments improve the quality of life in progeroid mice as assessed by frailty index. This work suggests to explore the combination of Tocilizumab and Lonafarnib or Tocilizumab and Everolimus in clinical trials for HGPS.

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