Institut
Marseille
Maladies rares
Aix*Marseille Université

ANNUAL SYMPOSIUM

Rare diseases' patient journey: from diagnosis to therapeutic developments

8-9 JUNE 2023

Hexagone auditorium, Luminy Campus

CONTENT

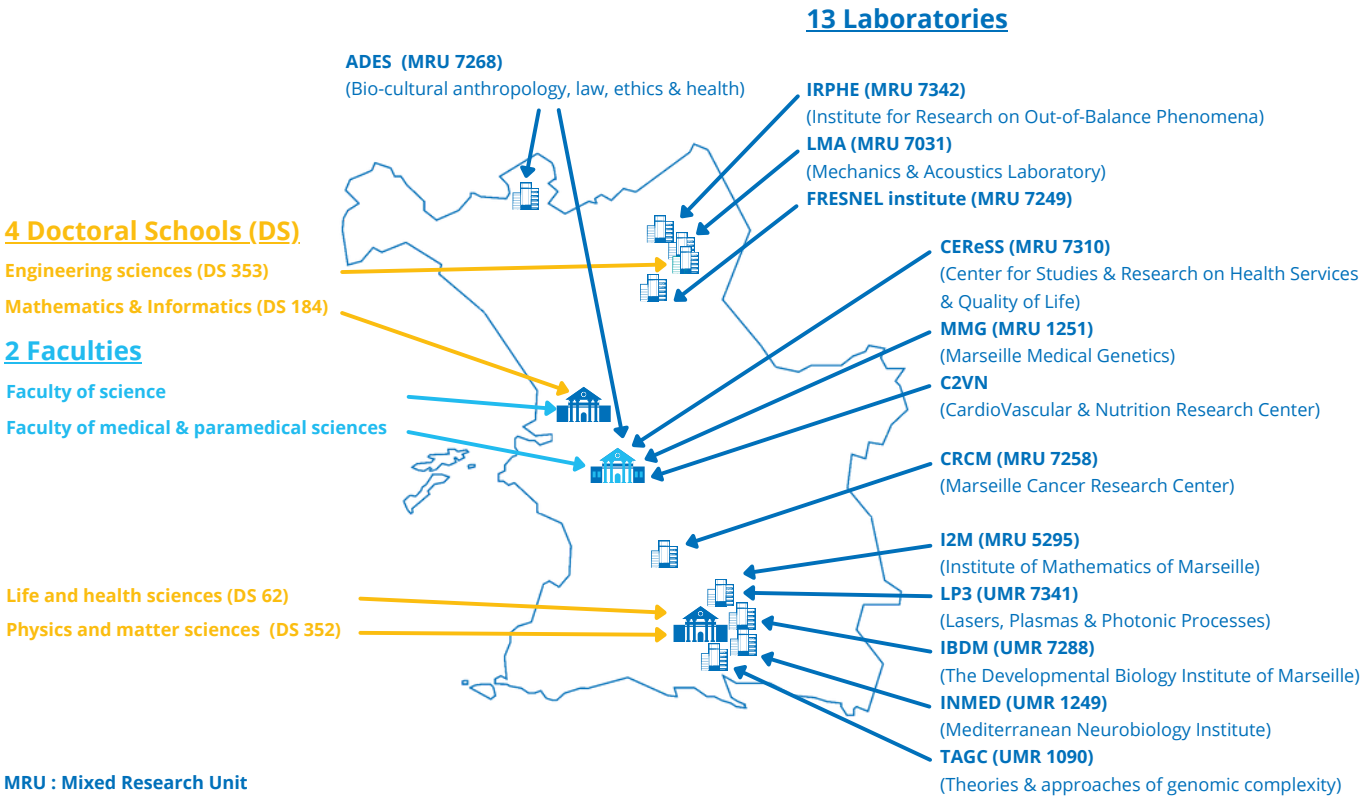
Institut Marseille Maladies rares
Aix*Marseille Université

GENERAL INFORMATION

The Marseille Rare Diseases Institute- MarMaRa is one of the Aix-Marseille Université institutes for research and education. AMU institutes aim at developing interdisciplinary training and research by gathering cutting edge research and faculties and the involvement of socio-economic partners, to strengthen its international outreach.

The objective of the MarMaRa institute is to gather research laboratories, medical departments, educational teams and industrial partners around cross-cutting actions to develop cutting-edge research for the benefit of patients. By federating new stakeholders, the MarMaRa institute aims at promoting, at the local, national and international levels, multidisciplinary research and training in connection with the socio-economic and cultural environment in the field of rare diseases.

Involved structures



MarMaRa 2023 Symposium

Rare diseases' patient journey: from diagnosis to therapeutic developments

The 2023 Marseille Rare Diseases Institute - MarMaRa annual symposium will focus on rare disease patients' journey.

The event takes place on **Thursday 8 and Friday 9 June 2023** at the auditorium of the **Hexagone on the Luminy Campus**.

Divided into eight sessions, the symposium will address issues related to diagnostic, patient participation, proof of concept as well as ethical, legal and societal issues.

We are pleased to welcome this year **Maria-Popa Roch**, associate professor in social psychology at the University of Strasbourg for the lecture of a STAB member.

This year, the institute organises **a prize ceremony** and two prizes for the best oral presentation and the best poster will be distributed.

This event will be an opportunity to **discuss the current and future actions** of the institute as well.

Hexagone, Campus Luminy

STEERING COMMITTEE

Thierry Brue, Director
Frédérique Magdinier, Deputy Director for Research
Denis Puthier, Deputy Director for Education
Laurence Colleaux, Grants and calls for tender coordinator
Cécile Bernard, Project manager
Rodolphe Moreau, Administrative and financial manager MMG

SCIENTIFIC COMMITTEE

Thierry Brue, MMG
Frédérique Magdinier, MMG
Denis Puthier, TAGC
Laurence Colleaux, MMG
Cécile Bernard, MMG
Rodolphe Moreau, MMG
Pascal Auquier, CEReSS
Adrien Casanova, LP3
Christophe Chevillard, TAGC
Laurent Fasano, IBDM
Nathalie Lalevée, C2VN
Fabienne Lescroart, MMG
Serge Mensah, LMA
Françoise Muscatelli, INMED
Jean-Christophe Roux, MMG

JURY FOR POSTERS AND SHORT TALKS

Christophe Chevillard, TAGC
Fabienne Lescroart, MMG
Françoise Muscatelli, INMED
Jean-Christophe Roux, MMG

PROGRAM

1st DAY | Thursday 8th June 2023

09:00-09:30 Welcome coffee

09:30-10:00 MarMaRa news
Thierry Brue, Director (RST)
Frédérique Magdinier, Deputy Director for Research
Denis Puthier, Deputy Director for Education

SESSION I : Journey to diagnosis

Moderator : Thierry Brue

10:00-10:20 **Registries and cohorts,** Pascal Auquier, CEReSS AP-HM

10:25-10:45 **The organisation of care for rare diseases in France: the example of neuromuscular diseases,** Shahram Attarian, MMG AP-HM

10:50-11:10 **Genomic Medicine 2025 Plan - what contribution for MarMaRa?**
Anne Barlier, MMG AP-HM

11:15-12:00 **Break & posters**

SESSION II : Patients engagement

Moderator : Julien Fromonot

12:00-12:15 **Involving patient organisations in the design of clinical research projects. Why and how?,**
Ben Braithwaite, Sanoïa Digital CRO

12:20-12:35 **A more accessible consent to research and genetic testing for the persons concerned: a co-constructed approach between associations, institutions and researchers,**
Sandrine de Montgolfier, INCa - AMU -IPC

12:40-12:55 **Modelling Laminin α2-deficiency congenital muscular dystrophy using patients'pluripotent stem cells,** Leslie Caron, MMG

13:00-13:15 **Spatial transcriptomic profiling of a rare somatic RASopathy,** Heather Etchevers, MMG

13:20-14:30 **Lunch break**

Institut Marseille Maladies rares
Aix*Marseille Université

SESSION III : Therapeutic Proof of concept

Moderator : Fabienne Lescroart

14:30-14:45	**Implementation of therapeutic treatments in Rett syndrome**, Jean-Christophe Roux, MMG
14:50-15:05	**Oxytocin and suckling in neonates**, Françoise Muscatelli, INMED
15:10-15:25	**Identification of a novel therapeutical target for the treatment of dilated cardiomyopathies**, Francesca Rochais, MMG
15:30-15:50	**Break & posters**

SESSION IV : Lecture from a STAB member

15:50-16:40	**Social attitudes and judgments: what impact on the academic success of children with (rare) diseases,** Maria Popa-Roch, Université de Strasbourg
16:40-16:55	**Conclusion of the 1st day**
17:00-18:00	**Closed meeting with STAB members**

2nd DAY | Friday 9th June 2023

09:00-09:30 **Welcome coffee**

SESSION V : MarMaRa Training actions

09:30-10:00 Denis Puthier, Deputy Director Education

SESSION VI : Interdisciplinarity call awarded projects

Moderator : Adrien Casanova

10:00-10:15	**Molecular, Imaging and Clinical Data integration in FacioScapuloHumeral Muscular Dystrophy: towards understanding genotype-phenotype correlations,** Jean-Philippe Trani, MMG
10:20-10:35	**Human and Social consequences of the announcement of the diagnosis of genetically and non-genetically determined pituitary neoplasms in the young,** Marie Vermalle, AP HM
10:40-10:55	**Modeling complex behavioral phenotypes in mouse models of rare neurodevelopmental disorders,** Laurent Fasano, IBDM

SESSION VII : Ethical, legal and social issues in rare diseases

Moderator : Françoise Muscatelli

11:00-11:20 **Ethical aspect of genetic testing : update on the August 2021 bioethics french law,** Péririne Malzac, ADES Espace Ethique, AP-HM

11:30-11:50 **Studying the socio-scientific trajectory of human embryo editing: the INTEMBRYO project,** Thémis Apostolidis, Institut ISSPAM, LPS

12:00-12:20 **"APPART" a transition space at Timone hospital: transition, youth and chronic illness,** Floriane Poubanne, APPART AP-HM

12:30-14:00 **Lunch break**

SESSION VIII : Short talks selected from submitted abstracts

Moderators : Nathalie Lalevée & Laurence Colleaux

14:00-14:15 **Biomarkers and putative drugs identification for Chagas disease Cardiomyopathy,** Pauline Brochet, TAGC

14:20-14:35 **Cardiotoxicity is mediated by the NLRP3 inflammasome in hiPSC-derived endothelial cells from patients with immune checkpoint inhibitor-related myocarditis,** Samantha Conte, C2VN

14:40-14:55 **Investigating the regulatory program controlling trapezius muscle development at the head trunk interface** Camille Dumas, IBDM

15:00-15:15 **Combination of laser assisted bio printing and laser structuration for the creation of bio models,** Lucas Duvert, MMG-LP3

15:20-15:35 **2017-2023 state of the art of clinical R&D of gene therapies in rare diseases: european dynamics, new approved treatments and expected therapies in the pipelines,** Tristan Gicquel, OrphanDev

15:40 -15:55 **A rare case of monogenic KCC2-related autistic spectrum disorder,** Mira Hamze, INMED

CLOSING SESSION

16:00 -16:30 **Discussion with the participants on 2023 main actions**

16:30-17:00 **Conclusions by Thierry Brue, Director & awards ceremony**

Institut Marseille Maladies rares
Aix*Marseille Université

JOURNEY TO DIAGNOSIS

Genomic Medicine 2025 Plan -what contribution for MarMaRa ?

Anne Barlier, MMG AP HM

The plan Genomique 2025 responds to a request made by the Prime Minister to the Aviesan Alliance in April 2015, in order to examine the implementation of access to genetic diagnosis in our country.

The plan addresses 3 objectives:

1. Prepare the integration of genomic medicine in the current care pathway and the management of diseases. The aim is to ensure access to genomic medicine to patients who need it, suffering from cancer, a rare disease, or in the future, a common disease.
2. Set up a national sector of genomic medicine to serve patients, capable of being a lever for scientific and technological innovation, industrial valorisation and economic growth.
3. Place France among the world's leading countries committed to personalised medicine, with a capability to export the know-how of our medical and industrial sector in genomic medicine.
4. Today, two LBM-FMG (PFMG medical biology laboratory) for very high throughput genomic sequencing are operational.

They cover all the needs of the national territory. The distribution of analyses on SeqOIA or AURAGEN is done according to the location of the prescriber.
– SEQOiA led by Assistance Publique - Hôpitaux de Paris (APHP), l'Institut Curie and l'Institut Gustave Roussy
– AURAGEN led by les Hospices Civils de Lyon, le CHU de Grenoble, le CHU de Saint-Etienne, le CHU de Clermont-Ferrand, le Centre Léon Bérard, le Centre Jean Perrin et l'Institut de cancérologie de la Loire.

PATIENTS ENGAGEMENT

Involving patient organisations in the design of clinical research projects. Why and how?

Ben Braithwaite, Sanoïa Digital CRO

Though patients play an increasingly active role in the health care system, they continue to be seen primarily as the source of data, not as possible protagonists of research projects. However, patients can contribute much more to a project than just data, with potentially invaluable contributions before, during and after a study.

Modelling Laminin α2-deficiency congenital muscular dystrophy using patients' pluripotent stem cells.

Leslie Caron, AMU-MMG

Laminin α2 deficient muscular dystrophy (Lama2-CMD) is rare genetic disorders that affect skeletal muscle function and can be life threatening due to respiratory complications. It is caused by autosomal recessive mutations in the LAMA2 gene, encoding laminin α2 chain (Lm-α2). Complete deficiency of this protein leads to the most severe, non-ambulatory phenotypes, while partial deficiency causes milder clinical forms. Although much is known about the genetic defects causing these invalidating disorders whose manifestations (hypotonia, muscle weakness, contractures) occur in the first few months of life, to date there are no curative treatments. Laminin α2 (Lm-α2) is a major component of skeletal muscle basement membrane (BM). Lm-α2 deficiency perturbs alters cell-ECM communication, thereby disrupting cellular homeostasis and ultimately leading to myofiber death. Mouse models of LAMA2-CMD have helped unveil some disease mechanisms and have been used as pre- clinical platforms to test therapeutic options with some promising results. However, despite the tremendous efforts and progress in understanding defective molecular pathways in this disease, none of these therapeutic strategies have been translated into the clinic so far. LAMA2-CMD represents an urgent unmet clinical need. The development of more physiologically relevant disease models, in which to study human muscle wasting and weakness, are necessary to develop and test innovative therapeutic options. Therefore, our project aims to1) develop cutting-edge in vitro patient-derived induced pluripotent stem cell (hiPSC) models and 2) unveil the molecular mechanisms at play in LAMA2-CMD patients. Overall, these stem cell models will lead to a better understanding of LAMA2-CMD mechanisms and provide a versatile platform that will ultimately assist in the development of novel and more effective therapeutic approaches.

Spatial transcriptomic profiling of a rare somatic RASopathy

Heather C. Etchevers , MMG team DIP-NET (Differentiation and Proliferation of NeuroEndocrine Tissues)

Melanoma in childhood and adolescence lacks adequate preventive, diagnostic, and therapeutic strategies. A rare condition, its incidence is reported to be about 1.3-1.6 per million in children under 15 years of age and 15 per million in 15-19 y.o., with increasing incidence in adolescents by 4.1% annually since 1997. Pediatric melanoma is a distinct entity from melanoma in adulthood, which is clearly linked to ultraviolet radiation damage to the skin. Little is known about the observed progression from some benign melanocytic lesions to melanoma.

Large and giant congenital melanocytic nevi (GCMN) are also very rare, observed in an estimated 1 in 20-50,000 births. Unlike small CMN, they are heterogeneous, conspicuous skin tumors in terms of appearance, genetic background, and complications. As many as one of 20 children with a GCMN will develop cutaneous or extracutaneous melanoma before adulthood, with poor prognosis. This means that closer study of GCMN should provide clues not only into the early transformation events of melanoma in children, adolescents and young adults (CAYA), but also into the mechanisms of more common melanoma formation.

Like many other individually rare mosaic cutaneous disorders, young patients with GCMN have also unmet needs in terms of therapies, particularly concerning the syndromic forms that can have devastating effects in the central nervous system (kysts, tumors, epilepsies, hydrocephalus). I will present preliminary results that were obtained thanks to a long collaboration with European and American GCMN patient organizations. My most continuous partner of over two decades among these has been Naevus 2000 France-Europe. Our results implicate a much less visible cellular component of GCMN in vulnerability to oncogenic changes. The entire initiative has been a model to organize a pan-European effort called MELCAYA, to boost research into these rare diseases hand in hand with patient groups and better understand the links between developmental biology and predisposition to cancers.

LECTURE FROM A STAB MEMBER

MARIA POPA-ROCH

Maria Popa-Roch is currently an assistant professor in social psychology at the University of Strasbourg.
Her research activities are generally organized around the theme of social perceptions of individuals because of their group membership. More specifically, she is interested in the psychosocial mechanisms involved in the stigmatization of minority social groups, as well as its consequences.

Models of social cognition and those derived from social identity theory provide the theoretical basis for her research. In her recent work, she has been interested in the negative attitudes that children with disabilities face in educational settings, as well as the implications of these attitudes in the current context of inclusive education. The role of implicit and explicit stereotypes and prejudices is addressed in this work.

Talk: Social attitudes and judgements: what impact on the academic success of children with (rare) diseases ?

The overall aim of our research program is to enhance the school inclusion of children with juvenile arthritis (JA). The parents of children with JA and their physicians, witnessed an important range of difficulties that children regularly face at school, which refrain them from experiencing a sense of fulfilment. They frequently report that teachers are reluctant to include children in their regular classes. As children disabilities are often invisible and their difficulties unidentified, teachers might feel lacking of control and notable discomfort.

However, social psychology literature shows that teachers' negative attitudes are associated with pupils' reduced academic achievement. In our research, we assessed teachers' attitudes and cognitive abilities of children with health condition compared with children without health condition. We hypothesized that potential poorer academic performance for children with rare diseases is not caused by cognitive abities but rather by how teachers perceive children. Our results provide basis of teachers' training in order to reduce their resistance and their prejudice when teaching to children with invisible, rare and poorly known diseases.

INTERDISCIPLINARITY CALL AWARDED PROJECTS

Molecular, Imaging and Clinical Data integration in FacioScapuloHumeral Muscular Dystrophy: towards understanding genotype-phenotype correlations

Jean-Philippe Trani, MMG

FacioScapuloHumeral muscular Dystrophy (FSHD,) one of the most common muscle dystrophies, is characterized by asymmetric weakness of specific muscles with variable onset, penetrance, and severity .
By exploring a large cohort of FSHD patients followed for years in Marseille, we aim to identify biomarkers of FSHD severity, progression, in a disease where a cure is still missing. We will develop innovative methods for the integration of molecular diagnosis, medical imaging data, and clinical information together with epigenetic parameters susceptible to modify the disease severity.
This strongly collaborative and interdisciplinary project involves clinicians in charge of the management and evaluation of FHSD patients (Pr. S. Attarian, Dr. E. Salort Campana), an expert in MRI and development of algorithm for image analysis and segmentation (Dr. D. Bendahan), an expert in computational biology and multimodal data integration (Dr. A. Baudot) and an expert in the molecular exploration of FSHD patients, in particular at the epigenetic level (Dr. F. Magdinier). To the best of our knowledge, integrative exploration of diverse data modality has never been conducted in FHSD. It is however the key to understand and manage this highly heterogeneous disease, identify biomarkers for FSHD disease severity, progression, and response to therapeutic intervention.

Human and Social consequences of the announcement of the diagnosis of genetically and non-genetically determined pituitary neoplasms in the young

Marie Vermalle, AP HM

The majority of pituitary neoplasms are benign. While they have long been termed “pituitary adenomas”, emphasizing their benign nature, a recent controversy has suggested replacing the term “adenoma” by the term “neuroendocrine tumor”. As the term “tumor” is usually associated with an overall idea of malignancy and cancer, the way this diagnosis is announced to the patient could have a deep impact on their perception of the disease. This project will focus on young patients (younger than age 35 years at diagnosis), presenting with a pituitary neoplasm. This is also a specific population because of the risk of genetic inheritance of the disease. We will perform a qualitative (interview based) and quantitative (self-administered questionnaire based) study to better understand the psychosocial consequences of the announcement of the diagnosis for both the patient and their family member/partner, focusing on the term used (and the difference of perception it could lead to) and the perception of the risk of genetic inheritance. This transdisciplinary project (endocrinology, molecular biology, social psychology) should improve our clinical approach to this rare disease and could also pave the way for further improvements in the management of other rare diseases, including benign tumors, and/or those that are genetically inherited.

MEASURED: Modeling complex behavioral phenotypes in mouse models of rare neurodevelopmental disorders

Yasmine Belaidouni *1,4*, Raul De Sousa Silva *2*, Idrisse Kabore *4*, Jean-Christophe Roux *3*, Françoise Muscatelli *1*, Séverine Dubuisson *2*, Laurent Villard *3*, **Laurent Fasano** *4*.

*1 Institute of Mediterranean Neurobiology, **INMED** UMR 1249 AMU – INSERM*
*2 Computer Science and Systems Laboratory, **LIS** UMR 7020 AMU - CNRS*
*3 Marseille Medical Genetics Center, **MMG** AMU - INSERM U1251*
*4 Marseille Developmental Biology Institute, **IBDM** UMR7288 AMU-CNRS*

Neurodevelopmental disorders (NDDs) include impairments in one or more domains of functioning (e.g. social interaction, cognition, language, motor skills), and their diagnoses are based on descriptive behavioral analysis with developmental acquisition lags. The analysis of mouse models of NDD, which is essential for identifying pathological mechanisms and improving the treatment of NDD, requires the identification of a wide range of complex behaviors. We previously characterized different mouse models of NDDs using state-of-the-art conventional mouse behavioral tests. However, these tests, although powerful, are restricted to single actions involving one or two mice and do not allow for the analysis of the complex behavior of several mice moving in a “natural” environment. Advancements in computer vision and deep learning have opened the door to overcome these restrictions.

In this project, we propose to use and improve an automated video processing and behavior analysis systems (Live Mouse Tracker; LMT) to further characterize mouse models of NDDs.

ETHICAL, LEGAL AND SOCIAL ISSUES IN RARE DISEASES

Ethical aspect of genetic testing : update on the August 2021 bioethics french law

Pérrine Malzac, ADES Espace Ethique AP HM

The practice of genetic testing in the medical field has been particularly regulated, since 1994, by the bioethics law. Today, with the development of sequencing, the border between research and clinical practice is not always very clear when implementing genetic tests.

The purpose of this presentation is to describe the legal framework for genetic testing, according to the consequences for the persons et their families.

Studying the socio-scientific trajectory of human embryo editing: the INTEMBRYO project

Thémis Apostolidis and the INTEMBRYO Consortium
Institut ISSPAM LPS (UR 849)

In recent years, emerging biotechnologies, especially gene editing concerning the human embryo has become one of the most dynamic scientific-technological areas but also one of the most publicly and scientists contested issues. Controversies around emerging scientific issues constitute a privileged opportunity to study scientific practices in the making as well as the circulation and the transformation of science and new scientific insights in the society. INTEMBRYO research program, using mixed methodologies, aims to analyze the socio-scientific trajectory of human embryo editing and to study the social representations of this object and the impact of this knowledge on social practices. Human embryo editing appears as a tensional object in the scientific arena as in the social one, an object that generates conflicts between different systems of social thinking and scientific practices. We will discuss social and bioethical implications of human genetic editing and moreover social acceptability of scientific innovation.

“APPART” a transition space at Timone hospital: transition, youth and chronic illness

Prof. Mathieu Milh , Ms. Floriane Poubanne

L’APPART’ is a space dedicated to transition and adolescents. Located between the pediatric hospital and the Timone adult hospital, it was funded by the Hospital Foundation, the PACA region and the APHM. A real place of care, it is dedicated to adolescents over the age of 14 who are carriers of a chronic disease, a rare disease or a disability. They are received as part of their medical follow-up for a consultation, a day hospital, a duo transfer consultation (paediatrician / adult doctor) or an annual check-up, for example. L'APPART', thought of as a real resource place for adolescents in the hospital, is available to teams to also organize therapeutic education sessions, educational assessments, workshops, special days and invite associations, patients experts or non-hospital caregivers.

At L’APPART’, patients are seen by a psychologist for a transition assessment, by the referring doctor and if necessary by other caregivers for multidisciplinary care. The transition space project was designed around the question of autonomy and charity, medical information and transition in the broad sense.

SHORT TALKS SELECTED FROM SUBMITTED ABSTRACTS

Biomarkers and putative drugs identification for Chagas disease Cardiomyopathy

Pauline Brochet *1*, Pauline Andrieux *1*, João Paulo Nunes *2*, Lionel Spinelli* *1*, Edecio Cunha-Neto* *2*, Christophe Chevillard* *1*
*Authors had an equal contribution

1 Institut National de la Santé Et de la Recherche Médicale (INSERM), Unité Mixte de Recherche (UMR)_1090, Aix Marseille Université, TAGC Theories and Approaches of Genomic Complexity, Institut MarMaRa, Marseille 13288, France
2 Laboratory of Immunology, Heart Institute Instituto do Coração (InCor), University of São Paulo, School of Medicine, São Paulo 05403-900, Brazil

Chagas disease is a parasitic disease caused by Trypanosoma cruzi, which is endemic from South America but affects around 7 million people worldwide. Decades after the infection, 30% of people develop chronic forms, including Chronic Chagas Cardiomyopathy (CCC), a severe inflammatory dilated cardiomyopathy. It presents a worse prognosis compared to other cardiomyopathies, and no treatments exist at this time.

The immune infiltrate in the heart tissue of CCC is mainly composed of Th1 cells, which produce a significant amount of IFN-γ and TNF-α. These molecules stimulate the production of reactive oxygen species (ROS) through the NF-κB pathway. While ROS production is necessary for parasite control, the continuous production of IFN-γ and TNF-α results in the accumulation of ROS, leading to dysfunction of mitochondria and disruption of cardiac function. Our team confirmed the impact of IFN-γ and TNF-α on mitochondria using AC16 human cardiomyocyte cell lines, demonstrating that these molecules decrease the membrane potential, ATP production, and mitochondria copies. Furthermore, promising results were obtained through the use of different drugs that target the IFN-γ and TNF-α pathways, rescuing mitochondrial function.

Furthermore, blood methylation data has been utilized to identify methylation sites that can be used as biomarkers of CCC, and of its severity. Machine learning algorithms are employed in this analysis, which predicts the phenotype with over 95% accuracy in a test group. Additionally, these methylation sites are located close to genes that play a role in immune response, nervous system, ion transport, or ATP synthesis, highlighting the importance of those biological mechanisms in CCC.

Taken together, these diverse approaches suggest potential candidates for the diagnosis or treatment of CCC, offering promise for the management of this disease.

Keywords : Biomarkers, Drug, Chagas, Cardiomyopathy, Mitochondria

Cardiotoxicity is mediated by the NLRP3 inflammasome in hiPSC-derived endothelial cells from patients with immune checkpoint inhibitor-related myocarditis

Samantha Conte *1,2* ; Thi Thom Tran *1,2* ; Isaure Firoaguer *1* ; Zohra Rebaoui *1,2* ; Stéphane Robert *3* ; Emilie Brotin *5* ; Magdinier Frédérique *2,4* ; Denis Puthier *2,6* ; Franck Thuny *1,7,8* ; Jennifer Cautela *1,7,8* ; and Nathalie Lalevée *1,2,7*

1 Equipe 5, C2VN INSERM 1263, Marseille, France
2 Institut MarMaRa, Aix-Marseille Université, Marseille, France
3 AMUTICYT facility, C2VN, Marseille, France
4 Cell reprogramming and differenciation facitily, Marseille Medical Genetics, Marseille, France
5 ImpedanCELL facility, ANTICIPE U1086, BioTICLA, Normandie Université, Caen, France
6 UMR 1090, TAGC, Marseille, France
7 University Mediterranean Center of Cardio-Oncology, Aix-Marseille Université, Marseille, France
8 Unit of Heart Failure and Valvular Heart Diseases, Department of Cardiology, North Hospital, Hôpitaux Universitaires de Marseille – AP-HM, Marseille, France

Introduction: In association with immune checkpoint inhibitors (ICI) anti-cancer therapy, rare cardiovascular complications (<2%), particularly myocarditis, have emerged with a mortality rate reaching 50%. The IFN-γ pathway and inflammasome regulatory proteins seem to be specifically involved in ICI-related myocarditis, but the mechanism of action remains unclear. In the cardiovascular system, the endocardial and vascular endothelium is involved in the regulation of the contractile function of cardiomyocytes. Our hypothesis is that the cardiotoxicity induced by the infiltration of immune cells into the myocardium is partly due to an exacerbation of inflammasome activation in endothelial cells, which subsequently affects the cardiomyocytes function.

Objective: Our aim is to characterise the role of endothelial cells in ICI-related myocarditis by comparing results obtained in cells from patients who developed myocarditis after ICI treatment with cells from patients who did not develop cardiotoxicity.

Methods: Induced pluripotent stem cells (hiPSC) were generated from samples taken from melanoma patients treated with anti-PD-1/anti-CTLA-4 immunotherapy and followed at the Cardio-Oncology Centre (North Hospital, Marseille). Twelve hiPSC clones were generated from patients who developed immune-induced myocarditis following immunotherapy (ICI-M, 6 clones from 3 patients), and patients who did not develop cardiotoxicity (non-ICI-M, 6 clones from 3 patients). hiPSC-derived endothelial cells (hiPSC-EC) were differentiated from hiPSC clones and stimulated by 10 ng/ml of IFN-γ during 48h.

Results: IFN-γ induced a decrease in cell viability, as well as an increase in the expression of transcripts of genes associated with the JAK/STAT and NLRP3 inflammasome pathways in hiPSC-EC. However, the decrease in cell viability was significantly greater in hiPSC-EC from the ICI-M group compared to the non-ICI-M group. IFN-γ activation of inflammatory intracellular signalling is greater in the ICI-M group, in particular overexpression of the jak1, stat3, tlr3/4, casp1, il-18, il-1B and gbp6 transcripts. At the protein level, in this ICI-M group, the IFN-γ-induced increase of MHC-I and PD-L1 is greater than in the non-ICI-M group.

Conclusion: IFN-γ stimulation revealed specific regulations in endothelial cells derived from hiPSC clones from patients with ICI-related myocarditis. We are now analysing the apoptotic and inflammatory activity of hiPSC-EC by real-time microscopy monitoring of caspases (Incucyte S3) and by assaying pro-inflammatory molecules in the supernatants (ELISA). This study will allow to establish the role played by endothelial cells in cardiac toxicity induced by ICI immunotherapy.

Investigating the regulatory program controlling trapezius muscle development at the head trunk interface

Camille E. Dumas *1*, Celia Rousset *1*, Christopher De Bono *1*, Claudio Cortès *1*, Fabienne Lescroart *2*, Stéphane Zaffran *2*, Noritaka Adachi *1*, Robert G. Kelly *1*

1 IBDM, CNRS UMR7288, Aix-Marseille University, Marseille (France)
2 MMG, Inserm U1251, Aix-Marseille University, Marseille (France)

Characterizing muscle diversity is an essential step towards understanding the origins of myopathies that affect specific muscle groups. Skeletal muscles of the head and trunk originate in distinct lineages with divergent regulatory programs converging on activation of myogenic determination factors of the MyoD family. Branchiomeric head and neck muscles share a common origin with second heart field cardiac progenitor cells in cardiopharyngeal mesoderm (CPM) associated with the branchial arches of the early embryo. Clonal analysis has identified a series of common skeletal and cardiac muscle lineages along the antero-posterior axis of the developing pharynx. Progenitor cells giving rise to jaw opening muscles and the right ventricle, or facial expression muscles and the cardiac outflow tract, are found in anterior CPM. Posterior CPM gives rise to neck muscles, including the trapezius, and both arterial and venous pole myocardium. The retinoic acid (RA) signalling pathway is required for normal deployment of cardiac progenitor cells in posterior CPM and blocking RA signaling during a defined early time window leads to conotruncal and atrial septation defects.

Here, we show using pharmacological approaches that blocking RA signalling also results in strikingly selective loss of the trapezius muscle, without affecting other branchiomeric or somitic muscles. This reveals that, although CPM regulators such as transcription factor and 22q11.2 deletion syndrome gene TBX1 are broadly required for branchiomeric myogenesis, the regulatory subprogram differs in posterior and anterior arches. RA is required to specify the derived trapezius muscle anlagen upstream of myogenic determination factor gene expression. Moreover, a genetic approach using lineage specific activation of a dominant negative RA receptor suggests that, unexpectedly, this effect is not mediated by direct RA signalling to trapezius progenitor cells in CPM but indirectly through activation of secondary signalling events in cells in the trunk lineage.

These findings suggest a model in which trapezius development is dependant on a signalling cascade between cell types across the head/trunk interface. Our results provide insights into basic mechanisms driving muscle development and contribute to a better understanding of muscle pathology and evolution.

Keywords: branchiomeric muscles, cardipharyngeal mesoderm, retinoic acid, trapezius muscle.

Combination of laser assisted bio printing and laser structuration for the creation of bio models

Lucas Duvert *1* ; Clarissa Muru *1* ; Stefano Testa *2*; Adrien Casanova *1* ; Frédérique Magdinier *2* ; Anne Patricia Alloncle *1*

1 Aix Marseille University, CNRS, UMR 7341, LP3, Campus de Luminy,
Case 917, 13288, Marseille cedex 9, France
2 Aix Marseille University, INSERM, Marseille Medical Genetics, MMG, 27 Bd Jean moulin
13385 Marseille, France

Printing techniques applied to biology have begun to develop in the 2000s and have since been greatly improved and perfected [1]. Based on interdisciplinary approaches they make use of cell biology, chemistry, engineering, and a combination of sophisticated protocols to create organized 2D and 3D patterns of a chosen bio i nk. Their application fields range from tissue engineering or organ creation to regenerative medicine and new drug discovery.

In that scope, it has been more than a decade since Laser Induced Forward Transfer (LIFT) is studied in lab scale for its ability to print biomaterials and more specifically living cells [2]. This technique uses a short laser pulse to transfer tiny amounts of material from a thin film donor to a receptor substrate. Under appropriate conditions, the laser pulse induces the formation of a jet propagating perpendicularly to the donor substrate. The bio ink previously spread as a thin film (few tens of microns) on this donor substrate is thus deposited as a droplet on the collector slide. In this context, at LP3 and in close collaborati on with MMG , we took advantage of our expertise on LIFT process [3] to print bio inks containing living cells for the creation of in vitro bio models. This work will be focused on the printing of muscular stem cells and the process optimisation for stem cell d ifferentiation in the prospect of creating a versatile tool aim ing at improving differentiation toward the skeletal muscle lineage and formation of neuromuscular junctions (NMJ).

Here, we will present the LIFT process and its optimisation allowing us to achieve a controlled, reliable, precise printing of muscle progenitor cells, ensuring a high post printing cell survival rate and proliferation. Making use of the resolution and reproducibility of the LIFT process, we will present the potential of co cultu re printing that further improve the fidelity of the bio models created with precise printing of different cell type on a single substrate.

In parallel to the printing process, laser structuration by direct laser ablation of hydrogels was developed to modi fy the receiver substrate topography [4]. Conditions t o crea te microchannel arrays have been determined and we highlighted that this ki nd of surface structuration promote s proliferation, adhesion , and differentiation of the cells. The nature and versatility of our experimental set up allows us to combine both processes, laser printing and laser structuration , in an extremely fast and efficient manner . A hydrogel coating deposited on the receiver is structured in accor dance with the desired printing pattern. The c ells printed precisely in pre made micro structured channels shows a n alignment according to the channels orientations and a higher differentiation rate Based on this combin ation of laser processes we proved our ability to produce high ordered muscle fibres of about 200 m width and several millimetres long from h uman myoblast cells.

[1] Tarassoli et al. Skin tissue engineering using 3D bioprinting: An evolving research field. J Plast Reconstr Aesthet Surg. 2018 May;71(5):615 623. doi: 10.1016/j.bjps.2017.12.006. Epub 2017 Dec 13. PMID: 29306639.
[2] Koch, Lothar & Deiwick, Andrea & Chichkov, Boris. (2021). Capillary Like Formations of Endothelial Cells in Defined Patterns Generated by Laser Bioprinting. Micromachines. 12. 1538, doi: 10.3390/mi12121538.
[3] P. Delaporte et A.P. Alloncle, «Laser induced forward transfer: A high resolution additive manufac turing technology »,Opt. Laser Technol.,2016.
[4] Al Kattan, et al. «Short Pulse Lasers: A Versatile Tool in Creating Novel Nano --/Micro Structures and Compositional Analysis for Healthcare and Wellbeing Challenges », Nanomaterials, 2021.

2017-2023 state of the art of clinical R&D of gene therapies in rare diseases: european dynamics, new approved treatments and expected therapies in the pipelines.

Tristan Gicquel ; Florence Cocqueel, Lucas Cortial, Karyna Lutsyk, Olivier Blin

This study provides a detailed analysis of current trends in the clinical development of gene therapies for rare diseases in Europe. The data are based on the GENOTRIAL database developed by OrphanDev, which gathers information on Phase I to III gene therapy clinical trials in rare diseases conducted, started, or completed between 2017 and 2023. The database contains 300 investigational gene therapy clinical trials for rare diseases corresponding to 93 sponsored clinical protocols around U.E.

At the temporal level, the analysis reveals that rare diseases represent a promising area for clinical development of gene therapies, with a number of protocols launched per year remaining stable and significant with an average of 8 related rare disease gene therapy protocol launch a year.

The survey of sponsors reveals that the United States leads the way in funding the study of clinical protocols using gene therapies for rare diseases, followed by the United Kingdom and France. The analysis further reveals that 91% of clinical protocols were conducted by industry-sponsored entities, and only 9% were conducted by entities with non-commercial academic promotion. In addition, analysis of the investigator sites associated with the 93 protocols identified in the database show that the United Kingdom is the leader in the clinical investigation of gene therapy for rare diseases, followed by France, Italy, Spain and Germany within Europe.

The analysis also reveals that nutritional and metabolic diseases are the most represented therapeutic area in the development of gene therapy in rare diseases, followed by rare oncology, blood and lymphatic diseases, and ocular diseases. Furthermore, the detailed analysis of the 35 rare diseases studied in clinical protocols using gene therapies reveals a heterogeneous distribution with a more or less advanced stage of development depending on the pathology.

Overall, the analysis highlights the promising potential of gene therapies for rare diseases, with several therapies having already shown positive results in clinical trials and many others being in various stages of advanced development.

In this sense, this study identifies 73 gene therapy medical products covering all 35 diseases and at various stages of development from phase I to market authorization. Among them, 13 new therapies have obtained marketing approval in recent years, between 2017 and early 2023, in Europe and/or in the United States for 9 rare diseases while 14 other therapies are at an advanced stage of phase III in their development plan for 10 other rare diseases suggesting their future approval in the United States, in Europe or in both regions by the end of 2023 and in the years to come highlighting that the development of gene therapies for rare diseases represents a promising and rapidly expanding field of research.

A rare case of monogenic KCC2-related autistic spectrum disorder

Mira Hamze *1*, Christophe Porcher *1*, Igor Medina *1*, Gaetan Lesca *2*

1 Institut de Neurobiologie de la Méditerranée (INMED), INSERM, UMR1249, Marseille.
2 INMG, CNRS UMR 5310 - INSERM U1217, Lyon.

Rare human neurodevelopmental diseases - related mutation is a powerful tool to characterize novel signaling pathways controlling disease formation and developing novel therapeutics. Recently, Dr.Lesca and collaborators have identified among patients suffering from different NDD one family associated with 2 missense variants of SLC12A5 gene that codes a neuron-restricted K+/Cl- co-transporter KCC2 that is critically involved in etiology of different NDD. The discovered variants of SLC12A5 produce previously unknown mutations in the coding region of KCC2 (V180M and R590H, respectively). The long-term objective of the project is to evaluate mutation's impact on KCC2 function and consequences of this impact on the level of single neuron, neural network and entire organism.

The short-term task of my work supported by MarMaRa was analysis of the properties of KCC2 constructs harboring V180M and R590H mutations as well as performs preliminary phenotyping of the transgenic mouse harboring both mutations to investigate the impact of mutations on the level of the organism.
We have found that both V180M and R590H mutations of human KCC2 construct transiently expressed in murine neuroblastoma cells N2a produced highly significant change in resting [Cl-]i and ion-transport ability of KCC2. Both mutated constructs also showed deficiency in surface expression.

The transgenic mouse harboring V180M and R590H mutations in different alleles and named thereafter KCC2 V180M/ R590H, showed no apparent difference in the developing during first fifty postnatal days (eye opening, weight gain, other general milestones). During the postnatal period from P50 to P55 all studied KCC2 V180M/ R590H mice (n=8) exhibited spontaneous seizures and died shortly after. Thus, the KCC2 V180M/ R590H mouse represents a valuable model to study mutations impact on organism level. The comprehensive study of KCC2 V180M/ R590H mouse behavior and neuronal network activity is in process thanks to support provided by MarMaRa.

POSTERS SELECTED FROM SUBMITTED ABSTRACTS

Consider rare diseases as a social issue and not just a medical issue

Florence Cocqueel *1* ; Olivier Blin *1*

Introduction
Tackling rare diseases cannot only be based on an approach centered on the disease and on the means of dealing with it or on strengthening scientific research on the subject. There are many other useful and representative points of view and expertise to take into account and many other ways of approaching the world of rare diseases in a global and cross-functional perspective.

Approach and methods
In this work, authors chose to follow a sociological approach, to reach a holistic overview of rare diseases issue. Around thirty interviews have been performed by a sociologist, covering all the parties concerned: two thirds of which were with patients or more often parents of patients, researchers and clinicians, entrepreneurs and decision/policy makers. The sociological analysis consisted in crossing expectations and constraints expressed by the panel to identify key priority recommendations. This resulted in a report combining testimonials, pedagogy and proposals.

Results
Listening to and understanding patients and caregivers is an opportunity to progress in science, to improve treatments, to adapt care, to provide better support. Panel members expressed priorities in two issues:
- the quality of life of RD patients, with attention to:
  - need od improved comfort in daily life
  - need of better consideration of issues surrounding side effects of treatments
  - seeking best possible dialogue with parents
  - considering all carers as a whole around the patient
  - improved participation of patients' representatives in governance bodies of care specialized centers
  - anticipation of the increase in life expectancy of patients
- the research and RD care, with identified challenges:
  - access to treatments
  - diagnostic odyssey and information of primary care providers
  - actual allocation of dedicated funding for RD hospital services
  - improved access to real world data

Recommendations
Major recommendations concern:
- the proposals for recognition of the expertise of patients and their carers to enable them to put it at the service of society: set up validation of acquired experience specific to rare diseases for parents and patients; create a rare sick employee status; strengthen the representativeness of rare diseases patients and carers in expertise centers.
- the establishment of a cross-ministerial delegation for rare diseases, involving, beyond Health, the Ministries of Education, Labour, Transport and Housing.

Improvements of gene therapy in a mouse model of Rett syndrome.

Marie-Solenne Felix *1,2*, Emilie Borloz *1*, Yann Ehinger *1*, Valérie Matagne *1*, Jean-Christophe Roux *1*, Laurent Villard *1,3*

1 – Aix Marseille Univ, Inserm, MMG, U1251 – Marseille, FR.
2– Inovarion, Paris, FR
3– Service de Génétique Médicale, AP-HM, Marseille, FR

Rett syndrome (RTT) is an X-linked neurogenetic disorder caused by mutations of the Mecp2 (methyl-CpG-binding protein 2) gene. Mecp2 has been described as a protein involved in the regulation of neuronal physiology, synaptic communication, and behavior. Given the genetic origin of RTT and the proof of concept of its reversal in a mouse model (Guy et al., 2007), considerable efforts have been made to design therapeutic approaches to re-express Mecp2. To perform a translational study, we initially decided to inject AAV9 containing an optimized codon version of Mecp2 into the blood of our mouse models. This protocol showed improvements in survival and breathing despite a limitation regarding the percentage of infected CNS cells (5-8%) (Matagne et al. 2017). In the current study we used again an AAV9 expressing a Mecp2-codon-optimized injected in retro-orbital in Mecp2 knock-out mice Here, the AAV9 injection is coupled with a focus ultrasound (FUS) technology allowing temporarily the blood brain barrier (BBB) opening and resulting in a better infection rate (Felix et al. 2021). The survival is improved in comparison with only AAV9-treated KO mice. We observed an improvment of breathing parameters and a much higher number of cells infected (35-50%), expressing Mecp2 in all the brain. All together these results show that FUS strongly improves CNS infection and represents a major alternative to intrathecal or intracerebral injection for neurological diseases.

KCTD11: a new gene in Charcot-Marie-Tooth disease?

Jihane Gadacha *1*, Zahraa Haidar *1*, Amandine Pauset *1*, Nathalie Roeckel-Trévisiol *1*, Christel Castro *1*, Nicolas Lenfant *1*, André Mégarbané *2*, Rosette Jabbour *3*, Nathalie Bernard-Marissal *1*, Valérie Delague *1*

1 Aix Marseille Univ, INSERM, MMG, U 1251, Marseille, France
2 Department of Human Genetics, Gilbert and Rose-Marie Chagoury School of Medicine, Lebanese American University, Lebanon
3 Neurology Division, Department of Internal Medicine, St George Hospital University Medical Center, University of Balamand, Beirut, Lebanon

Charcot-Marie-Tooth (CMT) diseases is one of the most common neuromuscular disorders, characterized by extensive phenotypic and genetic heterogeneity with more than 100 defective genes reported to date.

We have performed genetic studies, using Whole Genome Sequencing in 3 sibs affected with demyelinating CMT and two unaffected relatives, from an inbred family originating from the Middle east. This allowed us to identify a mutation in KCTD11, a gene encoding KCTD11/REN, a protein, which function in the Peripheral Nervous System (PNS) has never been investigated, although it interacts HDAC1 and mTOR, two main regulators of myelination in the PNS.

Here, we want to i) demonstrate that KCTD11 is a new defective gene in CMT and study the pathophysiological mechanisms underlying the disease, and ii) study its role in the PNS.

To this aim, we used an in vitro model of myelination, based on the coculture of mouse Dorsal Root Ganglia neurons and Schwann cells, after knock-down of kctd11 by infection with lentivirus expressing a shRNA targeting kctd11 transcripts. Our result showed that the loss of kctd11 leads to decreased myelination. This observation was also verified by using the same in vitro model derived from kctd11-/- knock-out mice.

In-vivo level, in sciatic and tibial nerves from these mice, we observe an increase of myelin abnormalities and signs of myelin degradation, at 18 months of age.

In conclusion, we have identified KCTD11 as a new causative gene in Charcot-Marie-disease and we are bringing new insights into the underlying pathophysiological mechanisms and the role of KCTD11 in PNS myelination.

Pathophysiological mechanisms in a new form of Charcot Marie Tooth due to a mutation in PDXK.

Zeinab Hamze *1*, Patrice Quintana *1*, André Mégarbané *2*, Valérie Delague *1*

1 Aix Marseille Univ, INSERM, MMG, U 1251, Marseille, France
2 Department of Human Genetics, Gilbert and Rose-Marie Chagoury School of Medicine, Lebanese American University, Lebanon

Introduction
Charcot Marie Tooth (CMT) diseases is the commonest inherited group of neuromuscular diseases. This group of diseases, affecting the peripheral nervous system, is characterized by wide clinical and genetic heterogeneity, with around 100 genes identified to date. Here, we describe 4 new patients, from two consanguineous Middle Eastern families, presenting with a rare subtype of CMT, due to a homozygous missense p. Ala228Thr mutation in PDXK, previously described by Chelban et al 2019. While the patients described by Chelban et al are affected with AR axonal CMT associated with visual loss, our patients have demyelinating CMT and no visual impairment. Here, we used patients' cells, including hiPSC-derived Motor Neurons (hiPSC-MN) to study the pathomechanisms in this new form of CMT.

Methods
We evaluated the levels of PDXK by QRT-PCR and Western-Blot in the different cell types used the Multi Electrode Array (MEA) technology on hiPSC-MNs to assess potential electrical defects in patients.

Results
In patients' lymphocytes and hiPSCs, we have found 40% decrease in PDXK protein levels. Interestingly, in patient's hiPSC-MNs, we observed no such decrease. At the mRNA levels no significant differences were identified suggesting a post-translational degradation of PDXK. Using MEA technology, we studied the general electrical activity of hiPSC-MNs by measuring Action Potential frequencies and amplitudes. Our preliminary results suggest increased global electrical activity in patients' hiPSC-MNs, as compared to controls. These results will be completed by additional measurements in hiPSC-DRG neurons, but they are very encouraging toward using hiPSC-derived neurons from the PNS to study the pathogenicity of mutations in this specific subtype of CMT and in CMT in general.

Conclusion
Here, we describe new patients with the homozygous p. Ala228Thr mutation in PDXK. Interestingly, our patients present a very different phenotype and our preliminary results from MEA technology suggest that our model enables to study this phenotypical variability.

Novel functional test for NEK10 involved in pediatric bronchiectasis

Ambrine Peyran, Julie Mazenq, Nathalie Da Silva, Bénédicte Allary, Martin Krahn, Marc Bartoli, Jean-Christophe Dubus, Svetlana Gorokhova

Pediatric bronchiectasis are rare pulmonary diseases with multiple causes including cystic fibrosis and primary ciliary dyskinesia. We have recently identified a previously unreported homozygous missense variant in the gene NEK10 in two siblings with early onset bronchiectasis. It has been shown that NEK10 is involved in a pericentriolar multimeric complex and that bi-allelic pathogenic variants can result in abnormally short motile cilia. Only a handful of bronchiectasis patients with variants in this gene have been reported to date.

The variant identified in our patients is absent from the general population. Examining the predicted 3D structure of the protein revealed that this variant is located in close proximity of a known pathogenic variant. However, further functional studies are still necessary to confirm the pathogenic nature of our variant.

In order to establish a functional test for variants in NEK10 gene, we first screened several cell culture lines for presence of primary cilia. We show that RPE-1 cells are suitable for this analysis since numerous primary cilia can be observed after staining with antibodies specific to nek10 or to acetylated tubulin. We are now in the process of testing different methods to knock down the expression of the endogenous NEK10 and then rescue the observed phenotypes with constructs carrying variants from patients.

Our study describing two patients with a novel variant in NEK10 provides valuable phenotypic information and expands the mutational spectrum for this recently described gene. Moreover, the functional analysis developed during our project will be a valuable resource to study the pathogenicity of variants in NEK10 gene. It will also help development of similar tests for other genes involved in primary ciliary dyskinesia.

Characterization of the axon initial segment and electrophysiological properties of human iPSC-neurons carrying KCNQ2 pathogenic variants

Cécile Mignon-Ravix (1), Florence Riccardi (1,2), Mathieu Milh (3), Alexandra Benchoua (4) Laurent Villard (1,5), Florence Molinari (1)

1 Aix Marseille Univ, INSERM, MMG, Marseille, France
2 CHITS, Hôpital Ste Musse, Département de Génétique Médicale, Toulon, France
3 AP-HM, Hôpital Timone Enfants, Service de Neurologie Pédiatrique, Marseille, France
4 CECS, I-STEM, AFM- Téléthon, Neuroplasticité et Thérapeutique, Corbeil-Essonnes,France
5 AP-HM, Hôpital Timone Enfants, Département de Génétique Médicale, Marseille, France

Kv7.2, encoded by the KCNQ2 gene, is one of the subunits composing the voltage-gated potassium channel Kv7. This channel is widely expressed in the brain and responsible for the M current, which controls neuronal excitability. Kv7 channels are located at the axon-initial segment (AIS), a key site for the generation and propagation of action potentials along the axon.

Pathogenic variants of KCNQ2 are associated with several epileptic conditions, the most severe of which is an early epileptic and developmental encephalopathy (KCNQ2-NEO-DEE) characterized by resistant seizures and a severe neurodevelopmental disorder.

To better understand the consequences of KCNQ2 pathogenic variants on neurons and neuronal networks, we used induced pluripotent stem cells (iPSC)-derived neurons from one control and 3 patients carrying distinct variants: p.(Leu203Pro), p.(Thr287Asn) and p.(Thr274Met), known to be loss of function with a dominant negative effect on the M current (Orhan et al 2014, Zhang et al 2020 and personal data). We monitored neuronal differentiation during 50 days using immunostaining and we observed a decrease of neural progenitors (Sox 2 positive staining) in favor of mature neurons (HuC/D positive staining) with progressive neural network development (MAP2 and b-tubulin III staining).

Because Kv7 is expressed at the AIS which shows plasticity correlated to the electrical activity, we analyzed AIS position and length during neuronal differentiation. We used Ankyrin G labelling to visualize AIS in inhibitory (GABA+) and excitatory (GABA-) neurons at days 15, 30 and 50. According to our findings, the AIS of neurons containing the p.(Thr287Asn) was shorter compared to control in excitatory neurons at day 50. In neurons carrying the two other pathogenic variants, p.(Leu203Pro) and p.(Thr274Met), we did not observe any difference in length or position of the AIS.

In parallel, we performed electrophysiological recordings using multielectrode arrays from 10 to 90 days of differentiation. We observed that spontaneous neuronal network activity of the p.(Thr287Asn) cell line was lower whereas the p.(Leu203Pro) and p.(Thr274Met) cell lines showed delayed activities compared to a control cell line.

These results suggest that iPSC-derived neurons carrying KCNQ2 pathogenic variants are all distinct from a control cell line but do not display the same morphological and electrophysiological characteristics. New experiments will be done to confirm these preliminary results.

Characterizing the molecular and pathophysiological mechanisms of T cells associated with immune-related myocarditis

Rebaoui Z., Tran TT., Conte S., Robert S., Cautela J., Thuny F. and Lalevée N.

Background

Immune checkpoint inhibitors (ICIs) represent a major advance in cancer treatment, allowing better recognition of cancer cells by the immune system and improving outcomes of various types of cancers. However, ICI-related cardiovascular adverse events (irAEs) are associated with a very high case-fatality rate. Among these irAEs, severe ICIinduced myocarditis (ICI-M), the most common and serious cardiovascular complication with a mortality rate of up to 40%, occur in 1-2% of patients. In addition, necrosis and infiltration of CD4+, CD8+ T cells, and macrophages within the myocardium and conduction system of ICI-M patients, treated for melanoma with ICI bi-therapy were reported in an indepth post-mortem study.

Goal

The aim of my thesis work is to identify pathological immune subsets and molecular changes in the immune infiltrate causing myocarditis using PBMC from melanoma patients who reported ICI-M. Identified pathways will be characterized in a unique cellular model of toxicity in which immune and cardiac cells interact.

Methods

Blood samples from melanoma patients of the GMEDICO cohort (Cardio-Oncology Centre of the North Hospital) are collected prior immunotherapy, during treatment in case of ICI-M, and at 3 months for patients who have no sign of toxicity. PBMC are isolated using Ficoll gradient and stored in liquid nitrogen to perform single-cell RNA sequencing coupled with single-cell TCR sequencing. The “in vitro model of cardiotoxicity” is under development with PBMC from healthy donors. CD4+ /CD8+ T cells amplification was performed using CD3/CD28 antibodies (ratio 1:1) and rh-IL2 (30 UI/ml). Before coculture, T cells were previously stimulated with anti-PD-1 (1 or 10 µg/ml) and anti-CTLA-4 (3 or 30 µg/ml). Endothelial cells (hiPSC-EC) are differentiated from induced pluripotent stem cells (hiPSC) generated from the same patients as those studied in the transcriptomic study.

Results

PBMCs from 83 melanoma patients have been collected: 33 prior ICI, 27 when ICI-M occurred, and 23 at 3 months of ICI treatment for patients with no sign of ICI-M. I’m waiting for the outcome of the 33 patients collected prior ICI to start the sequencing. Regarding preliminary data in the “in vitro model of cardiotoxicity”, CD4+/CD8+ T cells have been co-cultured during 24 hours with human melanoma cells (DM6), ratio 1:1. Co-cultured conditions led to a decrease in T cells activation markers (CD25 and MHC-II) and an increase in DM6 inflammation mediators (MHC-I, MHC-II, PD-L1). Regarding T Cells/hiPSC co-cultures, an increased expression of inflammation mediators (MHC-I, MHC-II, PD-L1) after 6 hours of co-culture with CD4+/CD8+ T cells (ratio 3:1 for T cells and EC respectively) has been observed in endothelial cells. ELISA essays will be used to quantify secreted pro-inflammatory cytokines (IFN-ɣ, IL-1β, IL-18).

Perspectives and conclusion

To complete the in vitro model of cardiotoxicity, T cells, previously amplified in melanoma cells cocultures, will be then transferred to hiPSC-EC to decipher mechanisms of cardiotoxicity. T cells from the same donors as those used for hiPSC generation and NGS sequencing will be used. Identified genes and pathways from single-cell/TCR sequencings will be validated in this in vitro model. This work should ultimately lead to the identification of markers of immunerelated myocarditis.

Loss of the Tbx5 gene in the ventricular conduction system induces spontaneous ventricular arrhythmias

J. Vahdat (1) ; J. Fromonot (2) ; N. Lalevée (2) ; L. Miquerol (1)

(1) CNRS UMR 7288, IBDM, Marseille, France ;
(2) Inserm 1263, C2VN, Marseille, France

The ventricular conduction system (VCS) and the working myocardium (WM) derive from the same myogenic progenitors during the embryonic development. The development of the VCS is regulated by a complex gene regulatory network including the T-box transcription factor 5 (TBX5). In human, TBX5 mutations cause Holt-Oram syndrome, an autosomal dominant disease associated with heart abnormalities such as arrhythmias. In adult mice, the deletion of the Tbx5 gene provoked a slowing of ventricular conduction due to a loss of the gap junction Connexin 40 and dysfunction of ionic channels. However, it is still unknown how TBX5 deletion affects the development and the function of the VCS.

The aim is to study the consequences of the Tbx5 deletion in embryonic progenitors of the ventricular conduction system on cardiac function in adult mice. Conditional deletion of Tbx5 alleles in mice was induced by expressing the Cre recombinase under the control of the Connexin 40 (Cx40) gene. We studied two groups of mutants: Tbx5 deletion at embryonic days E13-14 targeting both the ventricular conduction system and the working myocardium ("VCS+WM" group ; n=40) and mice deleted at E18-P2 targeting only the ventricular conduction system ("VCS" group ; n=17). Surface ECGs were achieved monthly until 3 months of age, when immunostaining was performed on the left VCS.

Immunostaining showed a reduced expression of the two major connexines in the ventricle (Cx40 and Cx43) and a reduced number of conductive cells in both mutants, suggesting a defective maturation of the VCS (Figure 1). Furthermore, the loss of Cx43 was correlated with that of Tbx5, reflecting a direct transcriptional effect. Surface ECG revealed a significant increase of the ventricular activation duration in both mutants ($p<0.01$). Moreover, premature ventricular contractions (PVCs) and a left QRS axis deviation were observed in both mutants. Finally, these PVCs disappeared with age in both mutants under resting conditions, and are unmasked when the heart is challenged with a β-1 adrenergic agonist.

Our results provide new insights into Tbx5 targets in the VCS. Tbx5 deletion impacts the maturation and the anatomy of the VCS as proved by the deviation of the ventricular electrical axis, which even occurs when the VCS is already formed. Tbx5 appears to have a direct impact on the Gja1 gene, encoding the gap junction Cx43, which is reduced at the junction between the VCS and the WM in both mutants and may be responsible for PVCs.

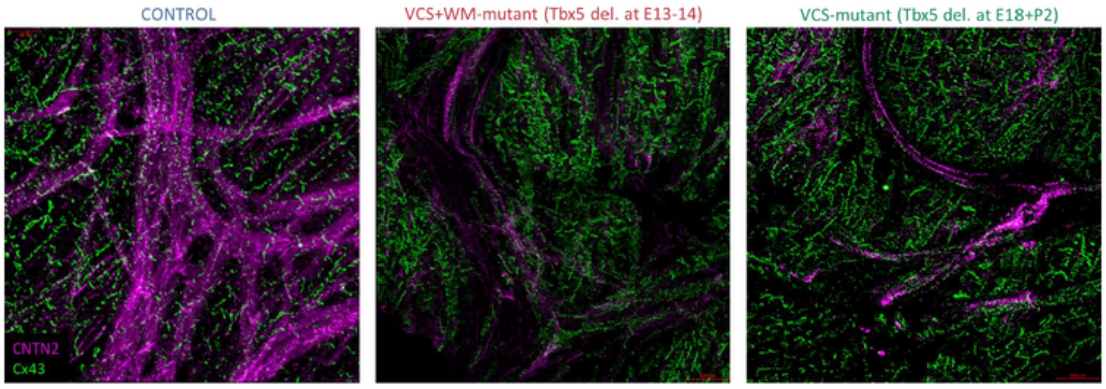


Figure 1 : In mutants, Tbx5 deletion induces a loss of Contactin-2 and gap junction Cx43 in the VCS.

Key words: Ventricular arrhythmias, Purkinje fibers.

SPEAKERS - POSTERS

APOSTOLIDIS Thémis
LPS, Institut ISSPAM, Campus Schuman
themistoklis.apostolidis@univ-amu.fr

ATTARIAN Shahram
MMG AP-HM, Campus Timone
shahram.attarian@ap-hm.fr

AUQUIER Pascal
CEReSS, AP-HM, Campus Timone
pascal.auquier@univ-amu.fr

BARLIER Anne
MMG AP-HM, Campus TImone
anne.barlier@univ-amu.fr

BRAITHWAITE Ben
Sanoïa Digital CRO
bbraithwaite@sanoia.com

BROCHET Pauline
TAGC, Campus Luminy
pauline.brochet@univ-amu.fr

BRUE Thierry
MMG – AP HM, Campus Timone
thierry.brue@ap-hm.fr

CARON Leslie
MMG, Campus Timone
leslie.caron@univ-amu.fr

COCQUEEL Florence
OrphanDev, Campus Timone
florence.cocqueel@orphan-dev.org

CONTE Samantha
C2VN, Campus Timone
samantha.conte@etu.univ-amu.fr

DE MONTGOLFIER Sandrine
INCa - AMU - IPC
sandrine.DE-MONTGOLFIER@univ-amu.fr

DUMAS Camille
IBDM, Campus Luminy
camille.dumas@univ-amu.fr

DUVERT Lucas
MMG, Campus Timone, LP3, Campus Luminy
lucas.duvert@univ-amu.fr

ETCHEVERS Heather
MMG, Campus Timone
heather.etchevers@univ-amu.fr

FASANO Laurent
IBDM, Campus Luminy
laurent.fasano@univ-amu.fr

FELIX Marie-Solenne
MMG, Campus Timone
marie-solenne.felix@univ-amu.fr

GADACHA Jihane
MMG, Campus Timone
jihane.gadacha@univ-amu.fr

GICQUEL Tristan
OrphanDev, Campus Timone
tristan.gicquel@orphan-dev.org

HAMZE Mira
INMED, Campus Luminy
mira.hamze@inserm.fr

HAMZE Zeinab
MMG, Campus Timone
zeinab.hamze@univ-amu.fr

LOUIS Jordane
MMG, Campus Timone
jordane.louis@univ-amu.fr

MALZAC Pérrine
Espace Ethique, AP-HM, Campus Timone
perrine.malzac@ap-hm.fr

MIGNON-RAVIX Cécile
MMG, Campus Timone
cecile.ravix@univ-amu.fr

MUSCATELLI Françoise
INMED, Campus Luminy
francoise.muscatelli@inserm.fr

PEYRAN Ambrine
MMG, Campus Timone
ambrine.peyran@gmail.com

POPA-ROCH Maria
INSPE, Université de Strasbourg
poparoch@unistra.fr

POUBANNE Floriane
APPART, AP-HM, Campus Timone
floriane.poubanne@ap-hm.fr

PUTHIER Denis
TAGC, Campus Luminy
denis.puthier@univ-amu.fr

REBAOUI Zohra
C2VN, Campus Timone
zohra.rebaoui@etu.univ-amu.fr

ROCHAIS Francesca
MMG, Campus Timone
francesca.rochais@univ-amu.fr

ROUX Jean-Christophe
MMG, Campus Timone
jean-christopheroux@univ-amu.fr

TRANI Jean-Philippe
MMG, Campus Timone
jean-philippe.trani@univ-amu.fr

VAHDAT Juliette
MMG, Campus Timone
juliette.vahdat@univ-amu.fr

VERMALLE Marie
AP-HM, Campus Timone
marie.vermalle@ap-hm.fr

Institut
Marseille
Maladies rares
Aix*Marseille Université

Thank you all for your participation !

Let's stay in touch !

institut-marmara-contact@univ-amu.fr

www.univ-amu.fr/marmara

@marmara_amu

https://www.linkedin.com/company/marmara_amu/

Crédit image: Escalenta