Deliverable

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Explanation according to GA Annex I:

Functional validation and identification of therapeutic leads via a novel and unique brokerage system. This is using the successful Canadian blueprint as the role model Solve-RD will establish a European Rare Diseases: Models & Mechanisms Network (Solve-RDMM) to catalyse connections between people discovering new genes in patients with rare diseases, and basic scientists, also from outside Solve-RD, who can analyse equivalent genes and pathways in model organisms.

Parts of report are based on the publication:
Ellwanger, K., Brill, J.A., de Boer, E. *et al.* Model matchmaking via the Solve-RD Rare Disease Models & Mechanisms Network (RDMM-Europe). *Lab Anim* (2024).
https://doi.org/10.1038/s41684-024-01395-2

Abstract:

In biomedical research and in particular for rare diseases, there is a critical need for model organisms aiming to unravel the mechanistic basis of diseases, to perform biomarker studies, and to develop potential therapeutic interventions. In Solve-RD we have established the European Rare Disease Models & Mechanisms Network (RDMM-Europe) to promote fruitful collaborations between clinicians and model organism experts. The main principle of this brokerage service is to fill the gap between RD gene discovery and functional validation of potentially new disease genes and/or novel disease mechanisms. For this purpose, RDMM-Europe catalyzes the connection of Solve-RD clinicians and scientists, who have discovered new disease-causing genes, with Model Organism Investigators (MOI), who are experts for the given genes, the proposed model organism and/or cell culture systems. Solve-RD provided Seeding Grants for selected validation projects to be conducted by researchers outside the consortium across the world.

Introduction:

RDMM-Europe is based on the initial idea and makes use of the infrastructure provided by the innovative and successful Canadian RDMM Network[1]. Both networks are built on a registry used as the central model matchmaking platform. The RDMM-Europe Registry (https://rdmm.imgag.de) is a database that allows all interested model organism investigators to register the genes and the respective model organisms or systems they work with. Registrants thereby express interest in getting connected with scientists and/or clinicians presenting patients with RD, and in collaborating in seed-funded validation projects provided by Solve-RD. Registered users have the option to share their data publicly and these data are accessible to any interested user through the public search interface. The RDMM-Europe Registry is also linked to other international partner network registries such as the Canadian RDMM network (http://www.rare-diseases-catalyst-network.ca/), the Australian Functional Genomics Network (https://www.functionalgenomics.org.au/), the Japanese RDMM network (https://j-rdmm.org/), and the global matchmaking platform ModelMatcher[2] forming a global network of RDMM networks (https://rdmminternational.org/) and allowing highest interconnectivity across the world.

RDMM-Europe is a brokerage service that (i) actively helps to recruit scientific expertise that is not present within the Solve-RD consortium (according to its primary mission), particularly with regards to molecular and functional validation of newly identified genes or novel disease mechanisms, and (ii) supports these collaborative projects by providing Seeding Grant funding (20,000 Euro per project/gene).

RDMM-Europe has been established by Solve-RD (https://solve-rd.eu/), an EU-funded research project with the primary goal to decipher large numbers of unsolved rare diseases (RD) for which a molecular cause has not been identified yet[3]. More than 70% of all patients with RD are predicted to have a genetic cause[4]. However, less than 50% of all RD patients receive

a genetically confirmed diagnosis with the currently applied diagnostic methodologies[5], leaving more than 15 million European RD patients without a diagnosis. Consequently, the primary focus of Solve-RD is to explore the full diagnostic potential of re-analysing existing exome and genome data by standardized and novel bioinformatic algorithms, and to investigate the use of novel omics technologies and methods such as RNA sequencing, Whole Genome Sequencing (WGS), long read sequencing, metabolomics for RD research. In Solve-RD and beyond, this combined approach has successfully led to a strong increase of diagnostic sensitivity of WES/WGS analysis[6] but also to an ongoing discovery of potential novel disease genes[7,8]. Newly identified disease-causing genetic variants are extremely rare, stimulating worldwide efforts to identify further affected families and to jointly decipher the role of a novel gene as a disease-causing gene via the matchmaker exchange platform, a federated network of RD databases allowing novel disease-gene relationship discoveries[9,10]. Even with these collaborative efforts, additional families are frequently not identified, partially because ultra-rare diseases and their phenotypes are not generally known by most clinicians, and also because the associated phenotypes are often highly variable, leaving doubts as to the causality of the identified variants and/or genes. In addition, exome/genome-wide NGS analysis is not globally available and we are not yet able to offer genome-wide analysis to a significant number of RD patients. Thus, further functional analyses are urgently needed to provide additional evidence that a candidate gene is causative for the respective disease. Here RDMM-Europe plays a pivotal role by catalysing new collaborative projects with world-experts on RD and supporting research with model organisms and systems for functional validation of genes of interest.

The clinical core of Solve-RD is formed by expert centres from four European Reference Networks (ERN) for Rare Diseases, including Rare Neurological Diseases (RND, https://www.ern-rnd.eu), Rare Malformation Syndromes, Intellectual and Other Neurodevelopmental Disorders (ITHACA, https://ern-ithaca.eu), Neuromuscular Diseases (EURO-NMD, https://ern-euro-nmd.eu), and Genetic Tumour Risk Syndromes (GENTURIS, https://www.genturis.eu). In total 24 ERNs, each focusing on a specific group of rare or low-prevalence complex diseases, were formed in Europe to improve the care for patients with RD[11]. In Solve-RD, the ERNs provided clinical expertise, infrastructure and patient cohorts, and as such contributed use cases for functional gene validation in the RDMM-Europe program[3].

Report:

Europe contributes to the international RDMM network

To promote the connection of rare disease gene discovery groups with functional validation researchers, we have implemented a two-committee process (Figure 1A). Connection applications on novel RD candidate genes are submitted by Solve-RD clinicians and scientists. Connection applications are evaluated and approved by the Clinical Advisory Committee (CAC) in a process that starts with peer-review, which is followed by general discussion by the whole CAC. The CAC is composed of clinical genomics experts from the European Reference Networks for Rare Diseases (ERNs) and the undiagnosed rare disease programs involved in Solve-RD (https://solve-rd.eu/the-group/consortium/). The committee evaluates the proposals based on defined criteria (see Supplement) and decides which proposed candidate genes should be considered for connection via the RDMM program. Upon approval of candidate genes, the RDMM-Europe management office opens a call for tender to identify the best matching model organism scientists for the requested functional validation, who are invited to submit a short Seeding Grant Application. For model matchmaking and to identify the best MOI, we use the existing RDMM registries including the search option via international partner networks and other model matchmaking initiatives that we are connected with[12], but we also apply classical search options, e.g., via publication databases. The Scientific Advisory Committee (SAC) evaluates the Seeding Grant Applications that were received and approves projects for funding. In general, only one Seeding Grant is promoted per disease gene.

Overall, starting from December 2019, we have opened ten calls for Connection Applications in a three-month interval. Proposals were selected according to the above-mentioned process

(Figure 1). In total, 65 Connection Applications have been submitted and 40 of them have been positively evaluated and approved by the CAC (Figure 1B). The RDMM-Europe Office has opened a call for tender for each of the approved genes. To be in line with German procurement law (the coordinating office of RDMM-Europe is located in Germany) and to increase the chance of finding the best possible match, we invited at least three independent model organism investigators to propose functional validation projects. In total, 178 researchers were invited to submit a Seeding Grant Application and 73 of them submitted proposals. These applications covered 39 out of the 40 approved genes. The SAC evaluated all received Seeding Grant proposals and approved 36 projects that best addressed the aim of validation requested by the respective clinical research group and offered the highest likelihood in providing new functional evidence for the respective gene variants in the context of the given disease. Upon approval by the SAC, the RDMM-Europe Office initiated the conclusion of a subcontract between the Solve-RD lead beneficiary and the institution of the scientific modelling group to get the Seeding Grant of up to 20,000 Euro formally awarded. Thus, Seeding Grant funding can in principle be disbursed to any research institution in the world. The grants can be used for consumables, direct model generation costs, but also for personal. Of note, 3 projects failed subcontracting, ending in a total of 33 collaborative modelling projects funded by Solve-RD budget.

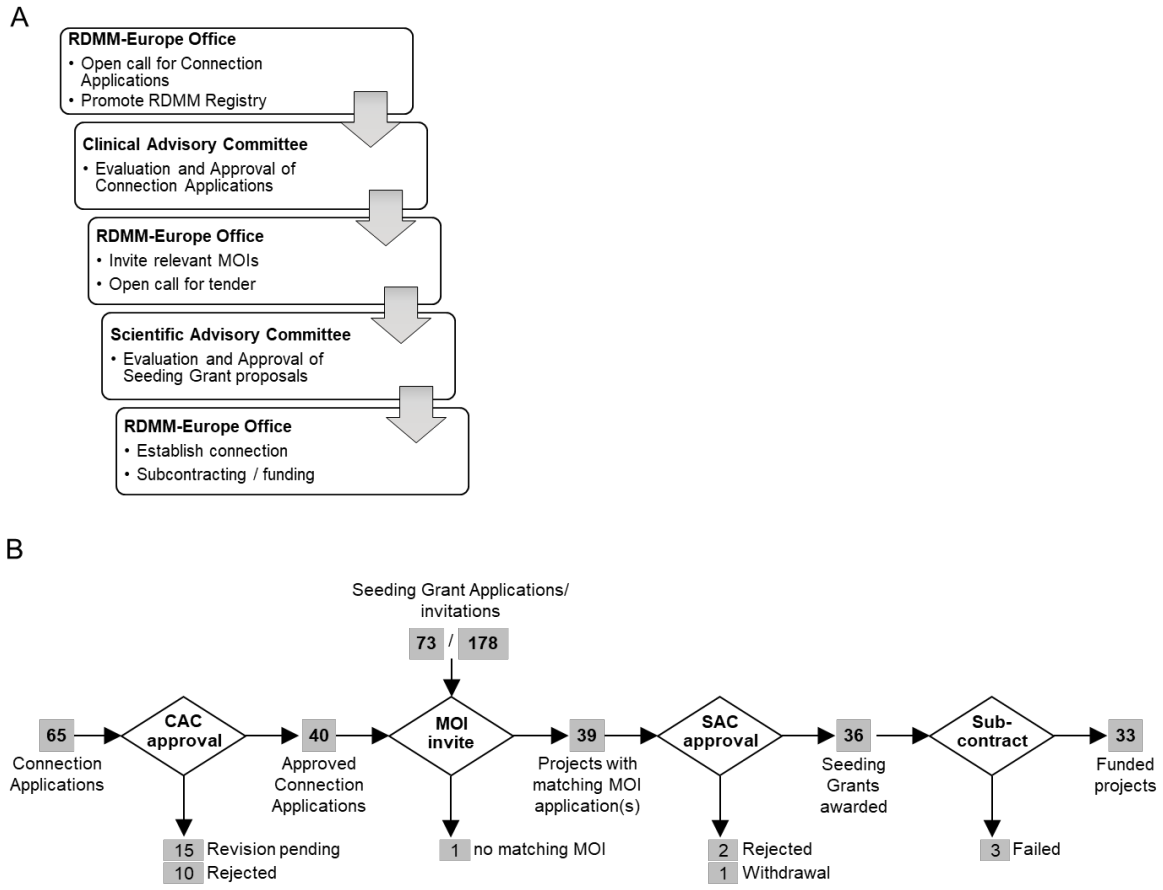


Figure 1: RDMM-Europe Model Matchmaking Pipeline. (A) Steps of the application and selection process managed by the RDMM-Europe Office and two selection committees. (B) From year 2019 to 2022 the RDMM-Europe Office opened 10 calls for Connection Applications and received a total of 65 proposals. All Connection Applications were reviewed by the Clinical Advisory Committee (CAC) which either approved (n=40), rejected (n=10) or requested to revise (n=15) the proposals. The RDMM-Europe Office opened a call for tender for 39 of the approved Connection Applications and invited in total 178 scientists of which 73 submitted a Seeding Grant Application. Those Seeding Grant Applications covered 39 of the genes that were approved for functional validation within the RDMM program. The Scientific Advisory Committee (SAC) evaluated all 73 Seeding Grant Applications and approved 36 proposals for funding. For one approved candidate gene the clinical group withdrew their original validation request. Other two validation requests were rejected by the Scientific Advisory Committee. For 3 projects subcontracting failed, ending in a total of 33 fund-ed projects.

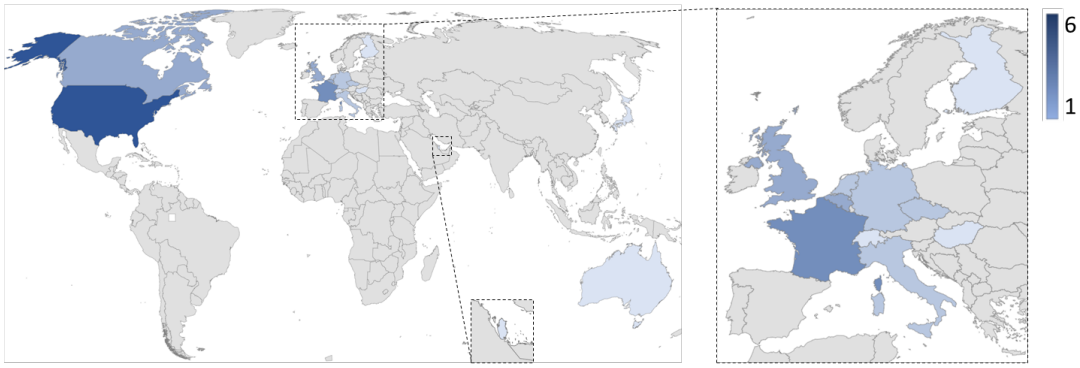
New international connections to advance RD research

Here, we showcase the main objectives of the different projects, highlight examples of dedicated validation projects in different models and link to primary research articles of the first projects that have recently been accepted for publication[13-17]. (Table 1 and Table 2). The projects can be classified based on the scope or key objectives. All projects aim to achieve functional gene validation in respect to the given pathology (Table 1). Some projects are expected to provide additional insights in regard to the mechanism of pathogenicity (mechanism) and/or therapeutic avenues (treatment). Two projects have successfully applied for competitive follow-up funding by the Telethon Foundation (Table 2).

Project partners from all four core ERNs represented in Solve-RD have used the RDMM-Europe program by submitting new genes for validation via Connection Applications and have benefited from Seeding Grant funding awarded to MOIs. Though, some of the pathologies under study can be associated with different ERNs, projects submitted by partners of ERN-ITHACA have been most successful with a total of 16 Seeding Grants awarded.

Seeding Grant funding by Solve-RD has been provided for 33 projects and the initiative has connected Solve-RD clinicians and scientists to model researchers in ten European countries (Belgium (n=3), Czech Republic (n=2), Germany (n=2), Finland (n=1), France (n=4), Italy (n=2), the Netherlands (n=2), Hungary (n=1), Switzerland (n=1) and UK (n=3)), but also to the US (n=6), Canada (n=3), Qatar (n=1), Japan (n=1), and Australia (n=1) (Figure 2A). In terms of model organisms and systems, zebrafish proved to be the most popular model, followed by mouse and Drosophila (Figure 2B). Four projects applied combined approaches using more than one model system.

A



B

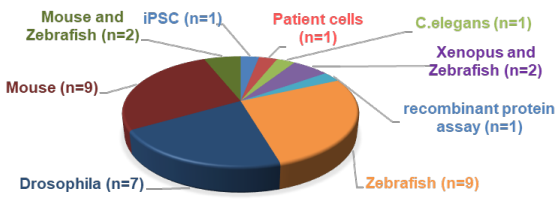


Figure 2: New international connections to advance RD research. (A) Geographical distribution of RDMM-Europe Seeding Grant recipients per country in the world. Blow-ups show Europe and Qatar. (B) Distribution of RDMM-Europe funded projects by disease type according to the European Reference Networks (ERN) for rare diseases.

Once all projects have been concluded we will assess the success of differential modelling approaches to accelerate new disease-gene associations, to better understand the pathophysiological mechanisms of ultra-rare and complex diseases and to identify and develop potential therapeutic approaches for the benefit of patients and families living with RD.

Table 1: New international connections to advance RD research. Details of projects established and supported by RDMM-Europe. Disease description, European Reference Network for rare diseases (ERN) of clinical partner that proposed the respective gene for validation, geographic location of Seeding Grant recipients (subcontractor), projects main scopes, model organism(s) approach and references are given. Projects are in the order of the formal start dates defined by the conclusion of the subcontracts.

Disease	ERN	Location of sub-contractor	Scope	Model organ-ism(s)	Reference
Cortical dysplasia, complex, with other brain malfor-mations	ITHACA	Dijon, France,	validation, mechanism	mouse	
Demyelinating neuropathy with central involvement	RND	Oxon, UK	validation, mechanism	mouse	
Acute middle age onset respiratory insufficiency with selective muscle involvement	EuroNMD	Washington, D.C., US	validation	mouse	Weihl et al., 2022[13]
Malformative syndrome without intellectual disability reprenting distinct entity from Pitt-Hopkins syndrome	ITHACA	Pisa, Italy	validation, mechanism, treatment	*Xenopus* & zebrafish	
Developmental disorder	ITHACA	Rotterdam, the Netherlands	validation, mechanism	mouse embryo electroporation	Lecoquierre et al., 2024[17]
Polymalformative syndrome	ITHACA	Toronto, Canada	validation, mechanism	Drosophila	
Neurodevelopmental disorder	ITHACA	Oklahoma City. US	validation	zebrafish	Kaiyrzhanov et al., 2023[16]
Distal myopathy with some proximal involvement	EuroNMD	Helsinki, Finland	validation	zebrafish	
Neurodevelopmental disorders	ITHACA	Ghent, Belgium	validation, mechanism	Drosophila	Bogaert et al., 2023[15]
Hereditary spastic paraplegia, subtype SPG32	RND	Paris, France	validation	zebrafish	
Intellectual Disability and Autism spectrum disorder	ITHACA	Lyon, France	validation	*C.elegans*	
Autosomal recessive hereditary spastic paraplegia (HSP)	RND	Miami, US	validation, mechanism, treatment	Drosophila	
Neurodevelopmental disorder	ITHACA	Milano, Italy	validation	iPSC	
Microcephaly, developmental delay and facial dys-morphisms	ITHACA	Toronto, Canada	validation, mechanism	Drosophila	
Serrated Polyposis Syndrome (SPS)	GENTURIS	Cleveland, US	validation	cell-cell adhe-sion assay, mouse	

Myopathy with severe (lethal) cardiac involvement	EuroNMD	Sydney, Australia	validation	patient derived cells	
Intellectual disability	ITHACA	Oxon, UK	validation	mouse	
Cornelia de Lange-related disorder	ITHACA	Doha, Katar	validation	zebrafish	
Epileptic encephalopathy	EuroNMD	Osaka, Japan	validation, mechanism	mouse	
Global developmental delay, cardiac involvement and facial dysmorphism	ITHACA	Prague, Czech Republic	validation	mouse & zebrafish	
Severe developmental delay, seizures and facial dysmorphisms	ITHACA	Prague, Czech Republic	validation	mouse & zebrafish	
Developmental disorder	ITHACA	Munich, Germany	validation	mouse	
Neurodevelopmental disorder (NDD)	GENTURIS	London, UK	validation, mechanism, treatment	zebrafish	
Microcephaly, global developmental delay and craniofacial abnormalities	ITHACA	Paris, France	validation, mechanism	zebrafish	
Complex dystonia-parkinsonism-ataxia phenotype	EuroNMD	Oklahoma City, US	validation	zebrafish	
A new genetic form of Hereditary Spastic Paraplegia (HSP)	EuroNMD	Rotterdam, the Netherlands	validation, treatment	zebrafish	Deng et al, 2023[14]
Polymalformative syndrome	ITHACA	Ghent, Belgium	validation, mechanism	Drosophila	
Medullary Thyroid cancer and Breast cancer	GENTURIS	Budapest, Hungary	validation	zebrafish	
Autosomal-dominant cerebellar ataxia	RND	Massachusetts, US	validation	recombinant protein assay	
Epileptic encephalopathy with severe brain degeneration	EuroNMD	Geneva, Switzerland	validation, treatment	Drosophila	
Non-syndromic sensorineural hearing impairment	EuroNMD	Göttingen, Germany	validation	mouse	
Developmental and epileptic encephalopathy	EuroNMD	Manitoba, Canada	validation, mechanism, treatment	Drosophila	
A distinct syndromic neurodevelopmental disorder	EuroNMD	Ghent, Belgium	validation	*Xenopus* & zebrafish	

Table 2: Descriptions of projects that have received Seeding Grant funding by the Solve-RD RDMM-Europe program as published on the website (https://solve-rd.eu/rdmm-europe/).

Binnaz Yalcin | Lisenka Vissers (ERN-ITHACA)
The group of Lisenka Vissers and her colleagues at Radboud University Medical Centre identified novel variants of a gene associated with complex cortical dysplasia and other brain malformations. Affected patients are clinically characterized by neurodevelopmental delay, intellectual disability and refractory epilepsy. The RDMM Europe Seeding Grant will facilitate the characterisation of a knock-in mouse model by Binnaz Yalcin and her group at University of Burgundy, Dijon, France as part of Inserm U1231. For the validation of the novel gene variants the mouse model will be characterized by histological and neuroanatomical methods as well as in behavioural tests. By modelling this novel rare human disease we expect to improve diagnosis and future treatment possibilities for affected patients. Start date: March 2020
Sara Wells | Stephanie Efthymiou (ERN-RND)
The group of Stephanie Efthymiou and her colleagues at the UCL Institute of Neurology identified novel variants of a gene associated with severe neurodevelopmental disorders. Homozygous carriers of the alleles come up with severely delayed psychomotor development. Neurophysiological investigations indicated severe demyelination, axonal neuropathy and loss of cerebral white matter. The RDMM-Europe seeding grant will facilitate the generation of a mouse model by Sara Wells at the Mary Lyon Centre, MRC Harwell Institute by using CRISPR/Cas9 technology to alter this novel gene. The model will play an important role in understanding the pathological consequences of the novel gene variants. In particular, the mutants will be studied for phenotypic features observed in the patient, such as neurological development, locomotor activity and behaviour. Modelling this human disease will not only help to provide further evidence on this new rare genetic disease and hence to improve diagnostic and management strategies, but will also facilitate assessment of potential treatment possibilities for patients. Start date: July 2020
Conrad Weihl | Ana Töpf (ERN-EURO NMD)
The group of Ana Töpf and Volker Straub at the John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, Newcastle University identified new vary rare variants of a gene in patients with severe respiratory failure, dyspnoea and spine rigidity. The RDMM-Europe Seeding Grant of 20,000 EUR awarded to Conrad Weihl and his group at the Washington University School of Medicine will enable the functional validation of the disease-causing gene in a knockout mouse model. The group has vast experience in working with mouse models of neuromuscular disorders. In addition, biochemical studies will be carried out in knockout myoblasts to unravel putative mechanism of pathogenicity.

Start date: July 2020

Publication: Weihl, C.C., Töpf, A., Bengoechea, R. et al. Loss of function variants in DNAJB4 cause a myopathy with early respiratory failure. Acta Neuropathol (2022). https://doi.org/10.1007/s00401-022-02510-8

Ype Elgersma | Laurence Faivre (ERN-ITHACA)

Solve-RD partner Laurence Faivre from CHU Dijon and colleagues discovered a novel de novo missense variant in a gene in four individuals affected with a severe neurodevelopmental disorder of unknown cause.

The RDMM-Europe Seeding Grant awarded to Ype Elgersma at Erasmus University Medical Center in Rotterdam will allow to assess the pathogenicity of this rare genetic variant. The project involves expression of the mutant form in developing mouse embryos and in primary neuronal cultures and analysis of the resulting phenotypes. Ype has great expertise in functional genomic screenings to assess the pathogenicity of genetic variants identified in individuals with neurological development disorder.

Start date: August 2020

Publication: François Lecoquierre, […] Laurence Faivre, Ype Elgersma, Antonio Vitobello. A recurrent missense variant in the E3 ubiquitin ligase substrate recognition subunit FEM1B causes a rare syndromic neurodevelopmental disorder. Genetics in Medicine, Volume 26, Issue 6, 2024, 101119, ISSN 1098-3600, https://doi.org/10.1016/j.gim.2024.101119

Michela Ori | Antonio Vitobello (ERN-ITHACA)

The group of Antonio Vitobello and Estelle Colin from CHU Dijon identified novel gene variants in patients with facial dysmorphism. Loss-of-function mutations of the respective candidate gene have previously been described as causative in a rare genetic disorder characterized by developmental delay and intellectual disability.

The Seeding Grant will allow the generation of Zebrafish and Xenopus mutants by Michela Ori and her group at the Department of Biology, University of Pisa, Italy to model the dysmorphic phenotype. Michela has extensive experience in molecular embrology and has previously used Xenopus and Zebrafish animal models to study craniofacial development.

Start date: August 2020

Follow-up funding granted by the Telethon Foundation (Italy) to continue the analysis (link).

Howard Lipshitz | Antonio Vitobello (ERN-ITHACA)

The group of Antonio Vitobello from CHU Dijon identified a novel gene variant in two unrelated patients with polymalformative syndrome. The patients both present with developmental delay and multiple malformations and exome sequencing revealed that both patients carry the same rare missense variant in a gene that has not been associated with human disease so far. To answer it´s causative implication in the disease the Solve-RD RDMM-Europe Seeding Grant will allow the generation of a Drosophila melanogaster model by Howard Lipshitz and his group at the Department of Molecular Genetics, University of Toronto, Canada. The applicant has extensive experience and a proven track record in the functional validation of human genes in Drosophila. This is the first Solve-RD Seeding Grant awarded to a scientist identified via our partner network RDMM in Canada. Start date: September 2020
Gaurav K. Varshney | Siddharth Banka (ERN-ITHACA)
The group of Siddharth Banka at the Manchester Centre for Genomic Medicine identified novel missense gene variants in several unrelated patients with neurodevelopmental disorders. The patients presented moderate to severe intellectual disability, developmental delay, behavioural problems and in addition occasional congenital malformations. Exome sequencing revealed that all patients carry rare missense variants in a novel candidate gene. The Solve-RD RDMM-Europe Seeding grant will allow the generation of a Zebrafish model by Gaurav K. Varshney and his group at the Oklahoma Medical Research Foundation. The applicant has extensive experience with clinical collaborations, and his primary focus is the use of zebrafish and gene editing technologies to study human disease models. Start date: October 2020
Pertti Panula | Bjarne Udd (ERN-EURO NMD)
The group of Bjarne Udd at the Folkhälsan Research Center (University of Helsinki) / Tampere Neuromuscular Center, Finland identified a novel disease gene in patients with adult onset distal myopathy. Solve-RD RDMM-Europe Seeding Grant funding will now allow the generation of a Zebrafish model by the group of Pertti Panula at the University of Helsinki. Zebrafish (Danio rerio) is preferred as model organism as it has an ortholog of the gene of interest. The lab of Pertti Panula has extensive experience with genetically modified zebrafish models and quantitative analysis to identify motor, sensory and social anomalies. Start date: November 2020
Bart Dermaut & Elke Bogaert | Antonio Vitobello (ERN-ITHACA)
The group of Antonio Vitobello at the University Hospital Dijon-Bourgogne identified novel gene variants in several patients with symptoms like developmental delay, skeletal abnormalities, heart defects and motoric and intellectual impairments. The symptoms are different in severity, but

featured in similar patient phenotypes. Most variants emerged by *de novo* mutations of a gene which is involved in the regulation of posttranscriptional mechanisms.

The Solve-RD RDMM-Europe Seeding Grant will allow to study the impact of the variants in a Drosophila model by the team of Bart Dermaut and Elke Bogaert at Ghent University in Belgium. The group has vast experience in studying flies for Alzheimer's and several other neurological disorders. In addition, Drosophila is the best suited model organism for the approach as the respective gene is highly conserved between fly and human.

Start date: January 2021

Publication: Elke Bogaert, Aurore Garde, […] Bart Dermaut, Antonio Vitobello: SRSF1 haploinsufficiency is responsible for a syndromic developmental disorder associated with intellectual disability. The American Journal of Human Genetics (2023). https://doi.org/10.1016/j.ajhg.2023.03.016

Jamile Hazan | Giovanni Stevanin (ERN-RND)

By exome analysis Giovanni Stevanin and his colleagues at INSERM-ICM in Paris identified rare homozygous missense variants in a new candidate gene in three patients of a consanguineous family with symptoms of a specific type of hereditary spastic paraplegia (HSP) and mild mental retardation and learning problems.

The Solve-RD RDMM-Europe Seeding Grant will allow the generation of a Zebrafish model by Jamile Hazan and her group at Sorbonne University (Paris). The successful applicant has extensive experience with the examination of the physiopathology of HSP in vertebrate models and has already done preliminary work to clarify the function of the new candidate gene.

Start date: February 2021

Alessandro Sessa | Siddharth Banka (ERN-ITHACA)

The group of Siddharth Banka at the Manchester Centre for Genomic Medicine identified missense variants in a gene involved in the epigenetic machinery in several unrelated patients with neurodevelopmental disorders. The patients presented intellectual disability, learning and behavioural difficulties, facial dysmorphism and occasional heart defects.

Exome sequencing revealed that all patients carry rare missense variants in the novel candidate gene. The Solve-RD RDMM-Europe Seeding Grant will support a validation study in iPSCs performed by Alessandro Sessa and his group at the IRCCS Ospedale San Raffaele, Milano, Italy. The applicant has extensive research experience with stem cells and the analysis of the molecular basis of neurological disorders. One focus of his work is to study how epigenetic factors impact pathological conditions in humans.

Start date: April 2021

Follow-up funding granted by the Telethon/CARIPLO alliance (Italy) to continue the analysis (link).

Julie Brill | Manuela Morleo (ERN-ITHACA)
Solve RD partner Manuela Morleo from Telethon Institute of Genetics and Medicine, Naples, Italy has discovered previously unknown de novo variants of a gene in six patients with severe neurodevelopmental disorders. Interestingly, loss of function mutations in two known interactors of the respective gene product have been described to result in clinical manifestations with largely overlapping phenotypes. Via the Canadian RDMM registry we have found Julie Brill from The Hospital for Sick Children (SickKids) in Toronto, Canada as model researcher with expertise for the given gene to answer the causative implication of the new variants in the disease. Julie will introduce variants in the Drosophila orthologue gene in S2 cells, tissues and flies. She proposed a very straightforward approach with high potential to yield clear results on the pathomechanism of the new variants. Start date: April 2021
R. Grace Zhai | Rebecca Schüle (ERN-RND)
Rebecca Schüle (Center of Neurology, Tübingen, Germany) recently identified a rare missense variant in the Solve-RD cohort of patients with hereditary spastic paraplegia (HSP), a group of inherited disorders that are characterized by progressive weakness and spasticity of the legs. By collaborative analysis of phenotypic and genetic data in the Solve-RD project and via the GENESIS platform (collaborator Stephan Züchner) they have been able to confirm the impact of the variant in additional patients with similar phenotypes. Grace Zhai (Miller School of Medicine, University of Miami, USA) will receive Seeding Grant funding by Solve-RD to support the investigation of the respective gene variant in respect to its pathological phenotype. Morphological, pathological and developmental studies will be conducted by using loss-of-function and transgenic approaches in Drosophila melanogaster with the aim to establish the functional causality of this new candidate gene in HSP. Start date: April 2021
Thomas Boulin | Alessandra Renieri (ERN-ITHACA)
Solve-RD partner Alessandra Renieri and her team (University of Siena, Italy) recently identified a *de novo* mutation of a highly conserved gene in a patient with intellectual disability and autism spectrum disorders. The respective gene product is crucial for cell signalling, especially in the neural and motor system. Interestingly, additional variants of the same gene were found in unrelated patients with similar phenotypes. The molecular relationship between these gene variants and the disease will be investigated by Thomas Boulin (Institut NeuroMyoGène, Lyon, France), supported by Solve-RD Seeding Grant funding. Thomas will make use of CRISPR/Cas9-based genome editing technologies to engineer and validate the patient variants in the invertebrate model *Caenorhabditis elegans*. Start date: April 2021

Zhenghe Wang | Stefan Aretz (ERN-GENTURIS)
Analysing leukocyte WES data, the group of Stefan Aretz at the University Hospital Bonn identified three different germline missense variants of a tumour suppressor gene in five patients from three families with Serrated Polyposis Syndrome, a colorectal cancer predisposition. The implicated protein is part of a family of signalling molecules that regulate different cellular processes such as cell growth and differentiation. The patients were diagnosed with several serrated polyps through the whole colon with a size of ~10mm. The Solve-RD RDMM-Europe Seeding Grant will support a validation study in knockout mice performed by Zhenghe Wang and his group at the Case Western Reserve University, Cleveland, USA. The applicant is a cancer biologist with perfect expertise to model colorectal cancers in knockout mice. Start date: July 2021
Sahar Isa Da'as | Antonio Vitobello (ERN-ITHACA)
By exome and Sanger sequencing, the group of Antonio Vitobello at the CHU Dijon identified a de novo variant in a gene implicated in the regulation of natural killer cell effector functions. The variant was identified in a cohort of three unrelated patients with severe malformation phenotypes described as Cornelia de Lange-like syndrome. The Solve-RD RDMM-Europe Seeding Grant will support a validation study in a knock-in zebrafish line proposed by Sahar Isa Da'as at the Sidra Medical and Research Centre, Doha, Qatar. Sahar Isa Da'as and her group will use the zebrafish model to analyse effects of the variant on skeletal and nervous system development and locomotor activity. Sahar Isa Da'as leads the Functional Genomics Core Facility at Sidra Medicine and has extensive expertise with modelling of human disease in zebrafish. Start date: September 2021
Sandra Cooper | Ana Töpf & Volker Straub (ERN-EURO NMD)
The group of Ana Töpf and Volker Straub at the John Walton Muscular Dystrophy Research Centre in Newcastle upon Tyne, UK have identified novel recessive gene variants in three individuals from two families that showed myopathy with severe cardiac involvement. With a disease onset in the second decade of life, the patients showed remarkably similar, rapidly progressing proximal upper and lower muscle weakness and developed a severe cardiomyopathy resulting in early death. A Solve-RD RDMM-Europe Seeding Grant was awarded to Sandra Cooper and her group at the Sydney Children's Hospitals Network, University of Sydney, Australia. Due to her expertise and previous research, Sandra is an excellent candidate to investigate the causative implication of the gene. The group will analyse muscles samples of their huge biobank and biopsies from the patients and will apply pre-mRNA splicing studies and Western blot. Furthermore, they will conduct rescue experiments in CRISPR/Cas9 KO cells of the given gene. Start date: September 2021
Sara Wells | Lisenka Vissers & Tjitske Kleefstra (ERN-ITHACA)

By combining de novo calls identified by different algorithms and prioritizing candidate genes for intellectual disability, the groups of Lisenka Vissers and Tjitske Kleefstra at the Radboudumc, Nijmegen, the Netherlands identified five different de novo missense variants and one de novo truncating variant in a protein coding gene in patients with mild to moderate intellectual disability and additional medical problems such as obesity, cerebral palsy or seizures. By searching the Solve-RD cohort and using GeneMatcher exchange tools, they identified additional patients from unrelated families with overlapping phenotypes and mutations in the same gene. The Solve-RD RDMM-Europe Seeding Grant will support a validation study by introducing a human variant into the mouse genome performed by Sara Wells and her group at the Mary Lyon Centre, MRC Harwell Institute, UK.

Sara Wells is the director of the Mary Lyon Centre that works in synergy with academic institutions as well as large global programmes such as the International Mouse Phenotyping Consortium (IMPC) in order to provide expertise in mouse genetics and resources such as innovative and transformative phenotyping platforms that deliver relevant, translatable and reproducible data.

Start date: September 2021

Tamotsu Yoshimori | Henry Houlden (ERN-Euro NMD)

By using WES bioinformatics filtering, the group of Henry Houlden at the UCL Queen Square Institute of Neurology identified five homozygous or compound heterozygous frameshift or missense variants in an autophagy regulator gene in a cohort of patients from unrelated families with severely delayed psychomotor development.

Tamotsu Yoshimori and his group at the Department of Genetics, Graduate School of Medicine, Osaka University, Japan received Solve-RD RDMM-Europe Seeding Grant funding to support a validation study in a conditional knockout mouse model. Tamotsu Yoshimori is an expert in studying autophagy in vivo and has already generated and analysed tissue-specific knockout mice.

Start date: November 2021

Radislav Sedlacek | Manuela Morleo (ERN-ITHACA)

Solve RD partner Manuela Morleo from Telethon Institute of Genetics and Medicine, Naples, Italy identified two new gene variants in cohorts of patients suffering from severe developmental delay, facial dysmorphisms and seizures or cardiac anomalies, respectively.

Two Solve-RD RDMM-Europe Seeding grants will facilitate validation of pathogenicity for both gene variants by supporting combined functional studies in zebrafish and mouse models of the respective genes. Radislav Sedlacek and his group at the Czech Centre for Phenogenomics (CCP), Institute of Molecular Genetics of the Czech Academy of Sciences in Prague will do comprehensive phenotyping of the models using high resolution microCT scanning. The CCP is an outstanding facility in combining genetic engineering capabilities and advanced phenotyping. Therefore, Radislav Sedlacek and team will be ideal to uncover the molecular mechanisms and the underlying pathological conditions of the given gene variants.

Start date: January 2022

Martin Hrabě de Angelis | Laurence Faivre (ERN-ITHACA)
Laurence Faivre and colleagues at the CHU Dijon identified three different *de novo* start-loss codon variants of a gene encoding a RNA-binding-protein. Similar variants of the gene were found through international collaborations in nine patients from unrelated families with a clinical spectrum including variable associations of altered growth parameters, skeletal anomalies, impaired neurodevelopmental and more. The Solve-RD RDMM-Europe Seeding grant will support a validation studies in mutant mouse lines proposed by Martin Hrabě de Angelis at the Institute of Experimental Genetics, German Mouse Clinic, Helmholtz Zentrum München, Germany. The German Mouse Clinic provides an advanced phenotyping platform for mouse models that covers all disease relevant organs. Start date: March 2022
Caroline Hill | Arne Jahn (ERN-GENTURIS)
Solve RD partner Arne Jahn from the Institute of Clinical Genetics at the University Hospital Dresden in Germany has identified *de novo* loss-of-function (LOF) mutations of a gene in a cohort of patients with neurodevelopmental disorder. Phenotypic data and NGS samples of affected families were collected through different networks (Decipher, ClinVar, GeneMatcher and Solve-RD). The Solve-RD RDMM-Europe Seeding Grant will support a validation study in CRISPR/Cas9 knock-out zebrafish performed by Caroline Hill, at the Francis Crick Institute London, UK. Caroline has an excellent track record and extensive experience with genetically modified zebrafish models and the analysis of neuronal morphology, migration and behavioural anomalies. Start date: April 2022
Filippo Del Bene | Manuela Morleo (ERN-ITHACA)
The group of Manuela Morleo at the Telethon Institute of Genetics and Medicine, Naples, Italy have performed Trio-based WES analysis in a patient with perinatal complications, developmental delay and dysmorphisms and identified a heterozygous *de novo* gene variant that is not present in the non-consanguineous healthy parents. By querying the Match Maker Exchange databases, they identified 3 additional families with the recurrent variant and the patients sharing a phenotype of global developmental delay, intellectual disability, microcephaly and craniofacial abnormalities. A Solve-RD RDMM-Europe Seeding Grant was awarded to Filippo Del Bene at Institut de la Vision, Sorbonne Université, Paris, France to support a project that is extremely well aligned with the validation need of the clinical group. Filippo will generate two complementary zebrafish models and study the effect of the human variant and of the homologous mutation in the respective zebrafish gene on brain size and structure, locomotor activity and behaviour. Gene expression analysis and immunohistochemistry will be done as well. Start date: May 2022

Gaurav K. Varshney | Henry Houlden (ERN-RND)
The group of Henry Houlden at the UCL Queen Square Institute of Neurology identified novel missense gene variants in several unrelated patients with complex dystonia parkinsonism ataxia phenotype. The patients presented moderate-to-severe delay in psychomotor development predominantly affecting the acquisition of walking skills, speech, intellectual functioning (global developmental delay, moderate to severe intellectual disability, severe speech disorder, diverse behavioural abnormalities), variable dysmorphisms and seizures. The Solve-RD RDMM-Europe Seeding Grant supports a study to elucidate the functional role of the gene in disease pathogenesis using a zebrafish model by Gaurav K. Varshney and his group at the Oklahoma Medical Research Foundation. The group will study morphological and behavioural effects of the gene knockout in zebrafish and will try to rescue the phenotype(s) by using wildtype and variant mRNA overexpression. Start date: May 2022 Publication: Rauan Kaiyrzhanov, Aboulfazl Rad, Sheng-Jia Lin, […], Gaurav K Varshney, Henry Houlden, Reza Maroofian: Bi-allelic *ACBD6* variants lead to a neurodevelopmental syndrome with progressive and complex movement disorders. *Brain* (2023) https://doi.org/10.1093/brain/awad380
Bart Dermaut & Elke Bogaert | Antonio Vitobello (ERN-ITHACA)
The group of Antonio Vitobello from CHU Dijon identified the same *de novo* missense variant in several unrelated patients with polymalformative syndromes. The patients are seen in different medical centres and present concordant phenotypes with developmental delay, major birth defects and considerable medical need. The gene has not been associated with developmental disorders so far. Bart Dermaut and Elke Bogaert (Ghent University, Belgium) will receive Seeding Grant funding by Solve-RD to support the investigation of the gene variant in respect to its pathological phenotype in fly models. Wing and eye phenotype studies will be conducted to investigate whether the overexpression of the human ortholog will phenocopy the fly protein. Additional loss-of-function and transgenic approaches in Drosophila melanogaster will help to evaluate the impact of the gene variant on morphological, pathological and developmental processes. Start date: May 2022
Stefan Barakat & Tjakko van Ham | Henry Houlden (ERN-RND)
By WGS analysis of two affected siblings suffering from Hereditary Spastic Paraplegia (HSP) the group of Henry Houlden at the UCL Queen Square Institute of Neurology identified a homozygous frameshift mutation within a shared RoH region. Biallelic loss of function mutations of the same gene were found in 14 additional affected individuals from 6 consanguineous families. The RDMM-Europe Seeding Grant awarded to Stefan Barakat and Tjakko van Ham at Erasmus University Medical Center in Rotterdam will allow to assess the pathogenicity of the new HSP candidate gene using zebrafish models. The group has been able to show in pilot experiments that the respective zebrafish Crispants show reduced locomotor activity compared to wild-type, indicating that the larvae phenocopy symptoms seen

in patients. The group will perform detailed investigations of cellular abnormalities and neuronal functions in these zebrafish and will explore options to modulate the phenotype by exposing zebrafish larvae to a selected library of chemical compounds, incl. FDA approved drugs, to study whether specific compounds suppress locomotor and cellular abnormalities identified in mutants.

Start date: May 2022

Publication: Ruizhi Deng, Eva Medico-Salsench, […], Tahsin Stefan Barakat: AMFR dysfunction causes autosomal recessive spastic paraplegia in human that is amenable to statin treatment in a preclinical model. *Acta Neuropathol* (2023). https://doi.org/10.1007/s00401-023-02579-9

Kris Vleminckx & Sarah Vergult | Rauan Kaiyrzhanov & Henry Houlden (ERN-Euro NMD)

The group of Henry Houlden at the UCL Queen Square Institute of Neurology identified three unrelated families with four affected individuals presenting with novel ultrarare variants in a gene that encodes an RNA binding protein. All affected individuals have overlapping phenotypes such as facial and skeletal abnormalities, some individuals also suffer from hypogammaglobulinemia with recurrent chest infections.

Kris Vleminckx and Sarah Vergult (Ghent University, Belgium) will receive seeding grant funding by Solve-RD to support the investigation of the gene in *Xenopus tropicalis* and zebrafish models. Both scientists are part of the Rare-Med consortium that has recently been initiated at Ghent University and both have excellent expertise in their respective animal models. Phenotypic assessment will include histological determination of the craniofacial and skeletal development as well as RNAseq of the brains of frogs and fish.

Start date: June 2022

Máté Varga | Iris te Paske & Richarda der Voer (ERN-GENTURIS)

Solve RD partners Iris te Paske & Richarda de Voer at the Radboudumc, Nijmegen, the Netherlands have identified a homozygous missense variant in a new gene in a female patient with early-onset thyroid and breast cancer. Regions-of-Homozygosity (ROH) analysis performed by the ROH and Relatedness working group within Solve-RD predicted that the proband has consanguineous parents. The affected gene product is a protein known to be involved in pathways to maintain genetic stability.

The Solve-RD RDMM-Europe Seeding Grant will support a validation project performed by Máté Varga, at the Department of Genetics, Eötvös Loránd University in Budapest, Hungary. The applicant will generate knockout zebrafish and strains that express the patient´s variant. Phenotyping will be done based on morphology, changes in double-strand break frequency, and sensitivity to genotoxic agents. Máté has an excellent track record and extensive experience with the generation and phenotyping of genetically modified zebrafish models to study morphological or behavioural abnormalities and DNA damage.

Start date: June 2022

Paul C Marcogliese | Stephanie Efthymiou (ERN-Euro NMD)

Stephanie Efthymiou and colleagues at the UCL Queen Square Institute of Neurology have identified a cohort of paediatric patients that share variable developmental delay, epileptic abnormalities, and occasionally craniofacial features. WES filtering identified homozygous or compound heterozygous missense variants in a gene with implicated relevance for neuropediatric pathology.

Paul C Marcogliese**,** trained in the Bellen lab at the Baylor College of Medicine, who has now started his own group at the University of Manitoba in Canada will receive RDMM-Europe Seeding Grant funding by Solve-RD to support a validation study in Drosophila. Paul provides an excellent match to model the respective gene, that has a highly conserved one-to-one orthologue in the fly. The group has already produced a knockout fly line, which has reduced viability, reduced lifespan, and behavioral phenotypes. They will now test whether wild-type or patient variants can rescue the null phenotype. In addition, they plan to apply drugs to test if the null phenotype can be ameliorated.

Start date: June 2022

Nicola Strenzke | Henry Houlden (ERN-Euro NMD)

The group of Henry Houlden at the UCL Queen Square Institute of Neurology has identified a novel candidate gene with suspected implication in non-syndromic sensorineural hearing loss with prelingual onset.

Nicola Strenzke (Department of Otolaryngology and Institute for Auditory Neuroscience, University Medical Center Göttingen, Göttingen, Germany) receive Seeding Grant funding by Solve-RD to support the investigation of the gene in a mouse model with a targeted deletion of the respective gene. Most valuable to this study is the analysis of homozygous mice, which should mirror the human null mutation. Phenotypic analysis of the mice will focus on the assessment of hearing functions complemented by comprehensive morphological studies of inner and outer hair cells, their stereocilia, and ribbon synapses. The highly complex inner ear of the mouse is strikingly similar to human in terms of anatomy, physiology and genetics. Despite the availability and successful use of other animal models in hearing research, the mouse still continues to be the predominant and preferred model organism in the auditory field, especially with regards to genetic deafness.

Start date: June 2022

Vladimir Katanaev | Elisa Cali (ERN-RND)

Elisa Cali and colleagues at the UCL Queen Square Institute of Neurology described eight affected individuals from five unrelated families with a recurrent segregating homozygous truncating gene variant. All affected presented at birth with microcephaly and respiratory failure requiring intubation and constant ventilation assistance for the rest of their life. Concerning psychomotor development, the impairment was profound with lack of achievement of any developmental milestone. Axial hypotonia and appendicular hypertonia were observed in all individuals. Seizures were present in all patients and have been described as myoclonic seizures or stimulus-related seizures.

The Solve-RD RDMM-Europe Seeding grant supports a study to characterize the gene variant in the Drosophila orthologue. Vladimir Katanaev and his team at the University of Geneva, Switzerland will perform gene editing using the CRISPR/Cas9 technology to introduce the mutation. Established lines will first be characterized with basic parameters such as viability/ lethality in the homo- and heterozygous state, longevity and any clear visible phenotype (e.g. morphological deviations). Further experiments will focus on the analysis of the movement behaviour and

eventual seizures. The successful establishment of a Drosophila model with the truncating variant can then be followed by two types of translational investigations: a suppressor-enhancer screening and a drug candidate screening.

Start date: June 2022

David Lambright | Matthis Synofzik (ERN-RND)

The team of Matthis Synofizik (Center of Neurology, Tübingen, Germany) identified a strong heterozygous gene variant segregating with disease in a large family with autosomal-dominant cerebellar ataxia (ATX). The WES datasets of two affected family members available in RD-Connect GPAP were taken as a starting point, complemented by subsequent Sanger segregation in 11 family members in total. Screening of several genomic databases (e.g. RD-Connect, GENESIS) with a total of more than 2000 WES/WGS ATX datasets led to identification of two additional unrelated ATX index cases with the same gene variant as identified in the first family. All three index cases share a uniform phenotype of early onset slowly progressive cerebellar ataxia, remarkably without relevant signs of extra-cerebellar systems affection.

The Solve-RD RDMM-Europe Seeding Grant will support quantitative in vitro assays with purified proteins performed by David Lambright, University of Massachusetts Chan Medical School in Worcester, USA. The biochemical effects of the variants will be interpreted in the context of available structural models, allowing structural and molecular understanding of the functional impact of the variants. This information will further support design and interpretation of subsequent cell-based and/or in vivo experiments to examine specific mechanism-based hypotheses.

Start date: June 2022

Conclusion:

RDMM-Europe with its international integration into the worldwide RDMM network has proven to be an important factor in achieving the aims of Solve-RD, to find novel disease genes and define disease mechanisms for ultra-rare diseases. Major advantages of RDMM are: a) low (no) hurdles to receive Seeding Grants for modelling, b) concise applications, c) short decision times in terms of what disease gene/disease mechanism is being supported, what model will be favoured and what model group will be partnered, d) standardized evaluation protocols and boards in a transparent decision process, and e) bringing world experts and expertise together.

In the end, by experimentally confirming new diseases genes, RDMM serves the European and international goals to improve knowledge on RD and decrease time until diagnosis for RD patients. Although the number of newly generated models was restricted by the end of the Solve-RD funding period (33 model projects were approved), the general demand may be enormous, as basically all of the todays identified new causes of RD are ultra-rare[18]. Due to the significance of a genetic finding in diagnostics and potentially even in decisions of life such as in prenatal conflict situations, a potential disease gene needs to be confirmed beyond a description in a single family. In Solve-RD, we could only serve major clinical indications as defined by the core ERNs (ERN-ITHACA, ERN-RND, ERN-Euro NMD, ERN-GENTURIS), but over time Solve-RD integrated many more ERNs and Undiagnosed Diseases Networks (UDN) as partners. Ultimately, 24 ERNs have been established in Europe and numerous clinicians and scientists have successfully identified specific genes involved in ultra-rare diseases. Thus, the need for modelling to functionally validate their findings is steadily increasing. Based on the experiences in Solve-RD and other RDMM initiatives, we need to retrospectively evaluate what types of models were most successful for instance in terms of publications, and, probably even more important, what seed funding was not successful and what we can learn from this.

Based on the genome data and (long read) WGS, we will see a trend towards novel disease mechanisms of known genes (such as mutations within non-coding regions, enhancers, topologically associating domains (TADs)), which may be more difficult to study compared to open reading frame alterations, but also a strong movement into epigenomics. It may therefore be anticipated that hundreds, or even thousands, of novel disease genes and/or disease mechanisms will be discovered over the next decade. It is also expected that the majority of these diseases are ultra-rare, making it difficult to establish cohorts of moderate sizes with the same genetic cause requiring modelling in cell systems or animals even more. Also, disease modification is an increasing important issue in discovery. Numerous diseases such as dystonia (reduced penetrance) or neurofibromatosis (reduced expressivity) may barely manifest in some mutation carriers but may have severe phenotypes even in the same family. As no obvious environmental factors have been found, one may conclude genetic modifiers as the cause of this phenotypic diversity. Identification of these variants and modelling them might become crucial even for treatment concepts.

We also need to discuss whether the existing call for models and model groups will meet the requirements of the next years. For instance, it may be more efficient in terms of time and money to select constant partners for model organisms or specific disease mechanisms. And finally, in the interest of the patients waiting for a diagnosis, we also have the responsibility to functionally investigate variants of clinically unknown significance (VUS) of known disease genes to improve diagnostic sensitivity and specificity. Efficient concepts in this direction have to be developed and enrolled across the world.

Ultimately, the need to validate newly identified variants and genes is linked to RD patients without diagnosis. The vast majority of these patients are being seen by the clinical expertise centres linked to ERNs or Undiagnosed Disease Programs that are involved in Solve-RD. Generally speaking, Solve-RD showcases that European collaboration can be a game changer when it comes to addressing rare disease needs. In this sense and more concretely, RDMM-Europe can also be considered as a collaboration broker, one, however, that goes beyond European borders.

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