

SEVENTH FRAMEWORK PROGRAMME Health

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Clinical utility of -omics for better diagnosis of rare diseases



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NeurOmics

Integrated European –omics research project for diagnosis and therapy in rare neuromuscular and neurodegenerative diseases

Instrument: **Collaborative Project**

Periodic report

Period covered: from 01.10.2014 to 30.09.2015

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Duration: 60 months

Project coordinator name: Prof. Dr. Olaf Riess

Project coordinator organisation name: Eberhard Karls Universität Tübingen (EKUT)

NeurOmics: Integrated European –omics research project for diagnosis and therapy in rare neuromuscular and neurodegenerative diseases**PUBLISHABLE EXECUTIVE SUMMARY FOR PERIODIC REPORT****1. Summary description of project context and main objectives**

Neurodegenerative (ND) and neuromuscular (NM) diseases are amongst the most frequent classes of rare diseases, affecting life and mobility of 500,000 patients in Europe and millions of their caregivers, family members and employers. This NeurOmics project brings together the leading research groups in Europe, five highly innovative SMEs and relevant overseas experts using the most sophisticated Omics technologies to revolutionize diagnostics and to develop pathomechanism-based treatment for ten major ND and NM diseases. Specifically we aim to:

- (i) use next generation WES to increase the number of known gene loci for the most heterogeneous disease groups from about 50% to 80%,
- (ii) increase patient cohorts by large scale genotyping by enriched gene variant panels and NGS of so far unclassified patients and subsequent phenotyping,
- (iii) develop biomarkers for clinical application with a strong emphasis on presymptomatic utility and cohort stratification,
- (iv) combine -omics approaches to better understand pathophysiology and identify therapeutic targets,
- (v) identify disease modifiers in disease subgroups cohorts with extreme age of onset
- (vi) develop targeted therapies (to groups or personalized) using antisense oligos and histone deacetylase inhibitors, translating the consortiums expertise in clinical development from ongoing trials toward other disease groups, notably the PolyQ diseases and other NMD.

To warrant that advances affect a large fraction of patients we limited the selection to a number of major categories, some of which are in a promising stage of etiological and therapeutic research while some others are in great need of further classification. The efforts will be connected through a NeurOmics platform for impact, communication and innovation that will provide tools and procedures for ensuring trial-readiness, WP performance, sustainability, interaction with the chosen Support IRDiRC and RD-Connect project and involvement of stakeholders in the NDD/NMD field.

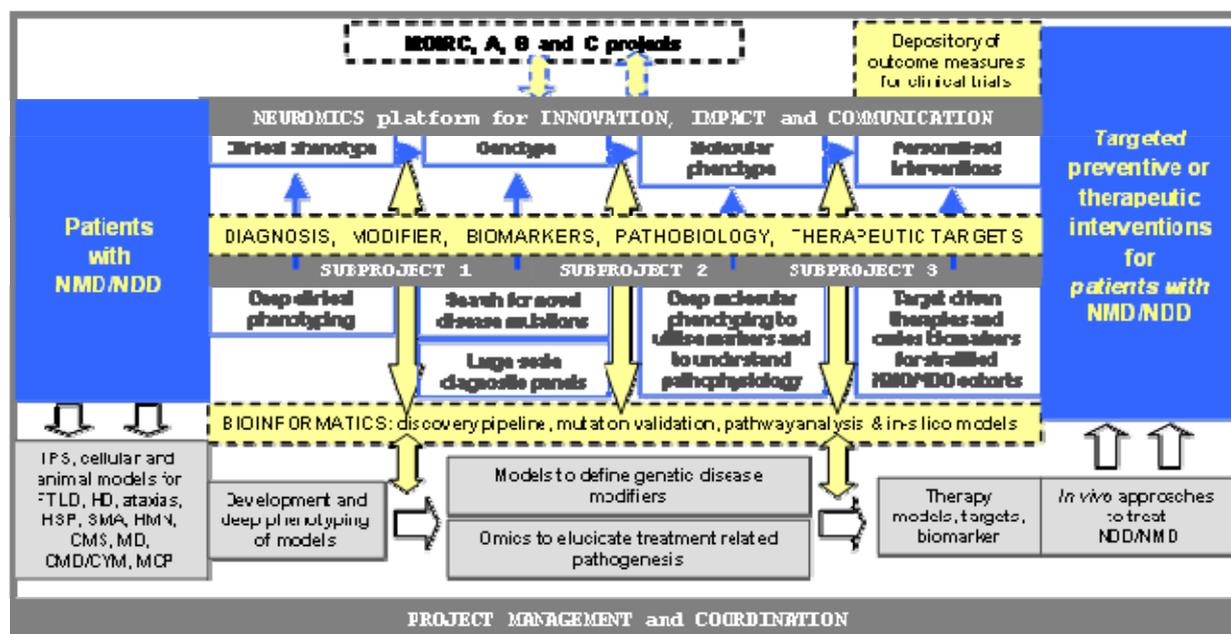


Figure 1: Concept of clinical-genetic and basic-applied research of NeurOmics.

2. Description of the work performed since the beginning of the project and the main results achieved so far

Broken down to aims, the work performed and results achieved can be summarised as follows:

Ad aim i: 589 samples have been exome sequenced. In June 2015, it has been decided to switch over to WGS. So far, 54 samples have been WGS sequenced. In total, >75 new genes have been identified of which 52 have been published. Further novel candidate genes are being functionally validated in cell and animal models.

The NeurOmics data sharing policy has been implemented and allows sharing of genomic data after the expiry of embargo and hold periods within the wider RD research community via controlled access to the RD-Connect platform to variant lists and via application to the NeurOmics DAC to the raw data stored at EGA.

Ad aim ii: Clinical information is being collected in PhenoTips in a standardised way using clinical data sheets for each disease group studied. CDS have been mapped to the HPO. Mapping of HPO terms has been improved in a workshop led by P. Robinson (HPO, Berlin) and M. Brudno (PhenoTips, Toronto), with all NeurOmics disease coordinators attending. >1205 patients have been entered in PhenoTips by date. 98% of all sequenced samples have a complete PhenoTips entry. The CTSR was expanded and holds now 328 sites in 48 countries of which 72 sites are registered for NDD.

Targeted NGS panels have been designed for ataxia, HSP, NMD and SMA/LMND. A “supercapture” panel for neurogenetic and cardiomyopathy diseases has been developed to meet the needs of the smaller Australian population. All panels have been improved to also cover recently identified new genes. >432 patients have been sequenced with the different disease panels. The Australian panel has been run >900 times. The alignment pipeline of the 3DM mutation prediction system developed by Bio-Product has improved significantly over the previous version. 3DM systems have been generated for 30 genes in which variants have been found that were (possibly) linked to diseases covered in NeurOmics.

Ad aim iii: SOPs have been developed for HD, SCA, FTLN and HSP patient sampling for biomarker studies. Recruitment and sample collection of SCA cohort (2nd timepoint), FTLN

and HSP patients and presymptomatic individuals for transcriptomics and/or lipidomics analysis is ongoing. Samples of FTLD patients, presymptomatic carriers and controls have been analysed using lipidomics and lipid profiles are currently being compared.

RNAseq of 52 premanifest, 63 early stage HD patients and 23 controls has been completed. No differentially expressed genes were found between HD and control. Pathway analysis has suggested possible dysregulation in immune pathways. RNAseq in myeloid cells from 30 manifest HD patients and 33 controls has been completed. 73 genes were found to be differentially expressed between the unstimulated HD and control samples, while 25 genes were found to be differentially expressed between the stimulated HD and control samples.

Sample collection of HD, HSP and NMD patients for biomarker validation studies is ongoing. Sample collection of 100 SCA samples has been completed and has been sent for BCAA validation analysis.

Study protocols for two therapeutic trials with SPG5 patients have been approved and are currently recruiting patients.

Ad aim iv: 12 human cell lines from SMA patients and unaffected PLS3-discordant family members have successfully been reprogrammed and validated. iPSC lines have been fully characterised. 6 hiPSC lines from HD patients carrying different numbers of CAG repeats and 4 control lines have been differentiated into striatal neurons. Characterisation is ongoing. 2 new SPG4 and 3 new SPG5 iPSC lines have been generated. SPG5 iPSC lines have been re-programmed into hepatocytes and are now being evaluated.

A lipidomics pilot study has been performed to validate the time points of sample collection from a SPG11 mouse model. Characterisation of the KI/KO HSPB8 and the Rosa26 HSPB1 transgenic mice is ongoing; transcriptomics analyses have been performed. A double-mutant (Parkin-Ataxin-3 transgenic) mouse model has been generated and characterised with different behavioural and neuropathological analyses. QPCT has been identified in a siRNA screen in cell-based systems as a target for modifying polyQ toxicity/aggregation. Druggability has been confirmed and the mechanism of action has been identified. Ariadne analysed several pathways involved in HD to identify biomarkers to monitor disease progression.

Ad aim v: 48 HD samples from the deeply phenotyped Track-HD study were selected for WES on the basis of having atypically fast or slow disease progression. Initial case control analysis has yielded interesting results but none reach statistical significance. Therefore, the findings are being integrated with other datasets to increase power to detect rare variants influencing HD progression.

20 SPG4 parent-offspring pairs with discrepant age of onset of >25 years have been exome sequenced. WES data analysis revealed two hits which are currently being functionally analysed. Additional hits have been identified in an interactome analysis by Ariadne and are currently being further investigated.

20 Bulgarian patients with the same mutation (CHRNE1267delG) but differences in phenotype (10 mild, 10 severe) have been whole genome and RNA sequenced.

Sequencing data from large patient cohorts are required to find putative modifying variants for NMD/NDD. Thus, WGS, WES and targeted NGS genomic data is being collected via the RD database at EGA and will be analysed once more data is available.

Ad aim vi: AON-mediated exon skipping to block TGF-beta and myostatin signalling by targeting their type I receptors ALK5 and ALK4 has been confirmed *in vitro* and in *mdx* mice. Characterisation of muscular dystrophy progression in four additional mouse models is ongoing. Metabolomics and lipidomics analysis of 4 dystrophic mouse models have been completed.

The LGMD2B mouse model has been generated and characterised. AON-mediated exon 32 skipping has been shown *in vitro*. The presence of dysferlin at the protein level has now also

been demonstrated. Skipping of other exons in LGMD2B patient-derived cells is currently tested *in vitro*.

A pilot study has been performed in the YAC128 HD mouse model. SCA3 exon skipping in *in vitro* experiments is ongoing to show reduced calpain cleavage of the ataxin-3 protein.

Screening of ~30,000 chemical compounds has been completed and 12 compounds have been identified to be able to increase the glycosylation of α -DG.

3. Description of the expected final results and their potential impacts and use

NeurOmics will have significant impact for a large group of patients with rare diseases - 120-150 patients/100,000, (500,000 patients in Europe). The project will integrate and extend existing networks and tools within the NDD/NMD field to ensure maximum impact and benefit from these and avoid duplication of efforts.

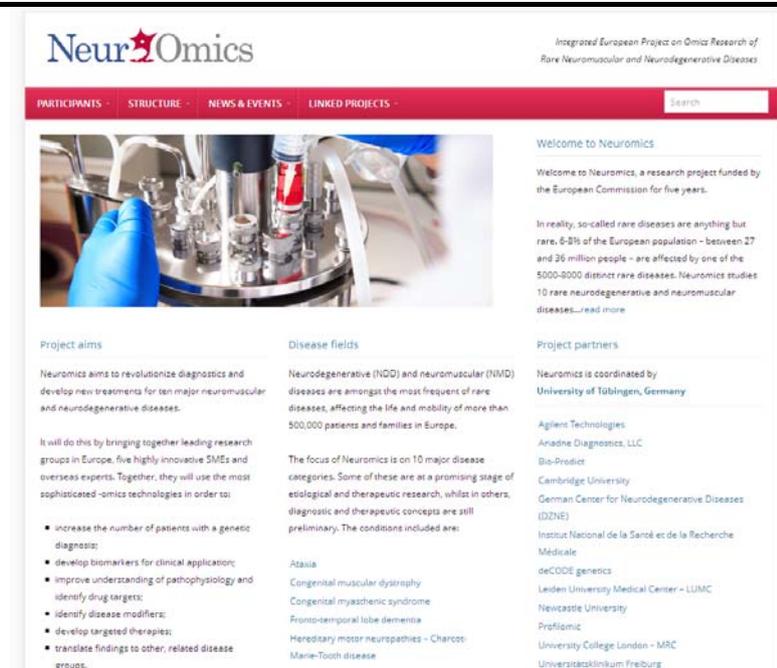
This will be done in close **interaction with the RD-Connect** project to address the major challenges identified through the IRDiRC initiative in the field of rare neurological diseases. Specifically, WP13 will extend the care and trial site registry (CTSR) developed for the neuromuscular field in order to incorporate neurodegenerative centres and ensure that the information gathered includes that needed for –omics research, supporting future clinical trials in these areas. The extended CTSR will also enable members to search for undiagnosed patients in other centres across the world fitting a particular phenotype profile. With RD-Connect, we will ensure that high quality, well annotated biospecimens are collected through harmonized biobanks and registries. Through its bioinformatics work, NeurOmics will provide a comprehensive knowledgebase for pathway informed target and biomarker search, to increase our understanding of unique and shared pathogenic mechanisms for rare and common NDDs/NMDs. Stakeholders including patient organizations, regulatory authorities and industry will be integrated into the study to support joint studies and widen the project's reach. All **major outcomes** of NeurOmics will have an impact directly linked to clinical utility:

- **Diagnostic kits** for up to 80 % of NMD/NDD will result in the availability of targeted genetic testing for familial NMD/NDD mutations This will help physicians provide the most appropriate treatment, allow affected families to make informed family planning decisions, form better-stratified patient cohorts for interventional trials, shorten the time to diagnosis and avoid unnecessary or invasive test procedures.
- Discovery and validation of **biomarkers** will increase implementation and appropriateness of new treatments by better stratifying patient cohorts. Monitoring of treatments will be improved. Better insight into pathogenic commonalities for NMD/NDD/RD will be achieved.
- Proof of concept of **new therapeutic approaches** for NDDs/NMDs will lead the way towards clinical trials of therapeutic interventions for up to 10 diseases eventually improving the quality of life of thousands of patients with NDDs/NMDs. Not only may patients already benefit from participating in clinical trials, but the resulting new therapies will enable physicians worldwide to treat currently incurable diseases.
- NeurOmics will **improve health and quality of life** of NMD/NDD patients and decrease disease related costs through improved and early diagnoses, characterization of stratified patient cohorts, improved understanding of disease complications through deep phenotyping and development of new treatments.
- NeurOmics' **communication tools** and procedures across all stakeholder groups will help to ensure trial-readiness, efficient and effective testing of new hypotheses and the rapid implementation of new recommendations by disseminating the latest findings, promoting data-sharing, extending existing tools and networks and developing appropriate SOPs (see Neuromics website at www.rd-neuromics.eu). This will reduce time-to-trials significantly, reach most appropriate patients to form specific

cohorts and ensure standards of care throughout the entire care and trial sites network through tailored training programs.

NeurOmics web site

NeurOmics logo



NeurOmics
Integrated European Project on Omics Research of Rare Neuromuscular and Neurodegenerative Diseases

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Welcome to Neuromics

Welcome to Neuromics, a research project funded by the European Commission for five years.

In reality, so-called rare diseases are anything but rare. 6-8% of the European population - between 27 and 26 million people - are affected by one of the 5000-8000 distinct rare diseases. Neuromics studies 10 rare neurodegenerative and neuromuscular diseases...[read more](#)

Project aims

Neuromics aims to revolutionize diagnostics and develop new treatments for ten major neuromuscular and neurodegenerative diseases.

It will do this by bringing together leading research groups in Europe, five highly innovative SMEs and overseas experts. Together, they will use the most sophisticated -omics technologies in order to:

- increase the number of patients with a genetic diagnosis;
- develop biomarkers for clinical application;
- improve understanding of pathophysiology and identify drug targets;
- identify disease modifiers;
- develop targeted therapies;
- translate findings to other, related disease groups.

Disease fields

Neurodegenerative (NDD) and neuromuscular (NMD) diseases are amongst the most frequent of rare diseases, affecting the life and mobility of more than 500,000 patients and families in Europe.

The focus of Neuromics is on 10 major disease categories. Some of these are at a promising stage of etiological and therapeutic research, whilst in others, diagnostic and therapeutic concepts are still preliminary. The conditions included are:

Ataxia
Congenital muscular dystrophy
Congenital myasthenic syndrome
Fronto-temporal lobe dementia
Hereditary motor neuropathies - Charcot-Marie-Tooth disease

Project partners

Neuromics is coordinated by University of Tübingen, Germany.

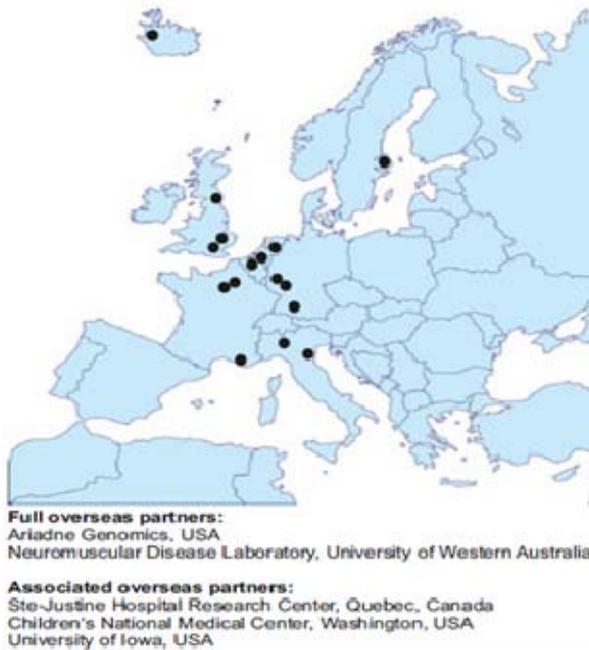
Agilent Technologies
Anadine Diagnostics, LLC
Bio-Product
Cambridge University
German Center for Neurodegenerative Diseases (DZNE)
Institut National de la Santé et de la Recherche Médicale
deCODE genetics
Leiden University Medical Center - LUMC
Newcastle University
Proflomic
University College London - MRC
Universitätsklinikum Freiburg



www.rd-neuromics.eu

4. Participants involved in NeurOmics

| No | Participant | Country | PIs |
|--------------------|--|-----------------|---|
| 1 (coordinator) | Eberhard Karls Universitaet Tuebingen (EKUT) | Germany | Olaf Riess, Ludger Schöls, Holm Graessner |
| 2 | Academisch Ziekenhuis Leiden – Leids Universitair Medisch Centrum (LUMC) | The Netherlands | Gert-Jan van Ommen, Annemieke Aartsma-Rus, Willeke van Roon-Mom |
| 3 | Klinikum der Universitaet zu Koeln (UK Cologne) | Germany | Brunhilde Wirth |
| 4 | University of Newcastle upon Tyne (UNEW) | UK | Volker Straub, Hanns Lochmüller, Kate Bushby |
| 5 | Deutsches Zentrum fuer Neurodegenerative Erkrankungen EV (DZNE) | Germany | Thomas Klockgether |
| 6A | University College London, Institute of Child Health (UCL-ICH) | UK | Mike Hanna, Henry Houlden |
| 6B | University College London, MRC Centre for Neuromuscular Diseases (UCL-IoN) | UK | Francesco Muntoni |
| 6C | University College London, Institute of Neurology (UCL-IoN) | UK | Sarah Tabrizi |
| 7 | Universite d'Aix Marseille (AMUMS) | France | Nicolas Levy |
| 8 | Institut National de la Sante et de la Recherche Medicale (INSERM) | France | Alexis Brice, Alexandra Durr |
| 9 | VIB (VIB) | Belgium | Vincent Timmerman |
| 10 | Universita Degli Studi di Milano (UMIL) | Italy | Elena Cattaneo |
| 11 | Universita Degli Studi di Ferrara (UNIFE) | Italy | Alessandra Ferlini |
| 12 | The Chancellor, Masters and Scholars of the University of Cambridge (UNICAM) | UK | David Rubinsztein |
| 13 | Islensk Erfdagreining EHF (deCODE) | Iceland | Hreinn Stefansson |
| 14 | Ariadne Diagnostics LLC (Ariadne) | USA | Elena Schwartz |
| 15 | Profilomic SA (Profilomic) | France | Bruno Corman |
| 16 | Agilent Technologies Sweden AB (Agilent) | Sweden | Henrik Johansson |
| 17 | Bio-Product BV (Bio-product) | The Netherlands | Henk-Jan Joosten |
| 18 | The University of Western Australia (UWA) | Australia | Nigel Laing |
| 19 | Universitaetsklinikum Freiburg (UKL-FR) | Germany | Janbernd Kirschner |



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Figure 2: Involved European countries in NeurOmics