SEVENTH FRAMEWORK PROGRAMME Health

Theme: HEALTH.2012.2.1.1-1-B

Clinical utility of -omics for better diagnosis of rare diseases

Grant agreement no.: 305121

NeurOmics

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

Instrument: **Collaborative Project**

Periodic report

Period covered: from 01.10.2013 to 30.09.2014 Date of preparation: 31.10.2014

Start date of project: 01.10.2012 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 oversea 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.

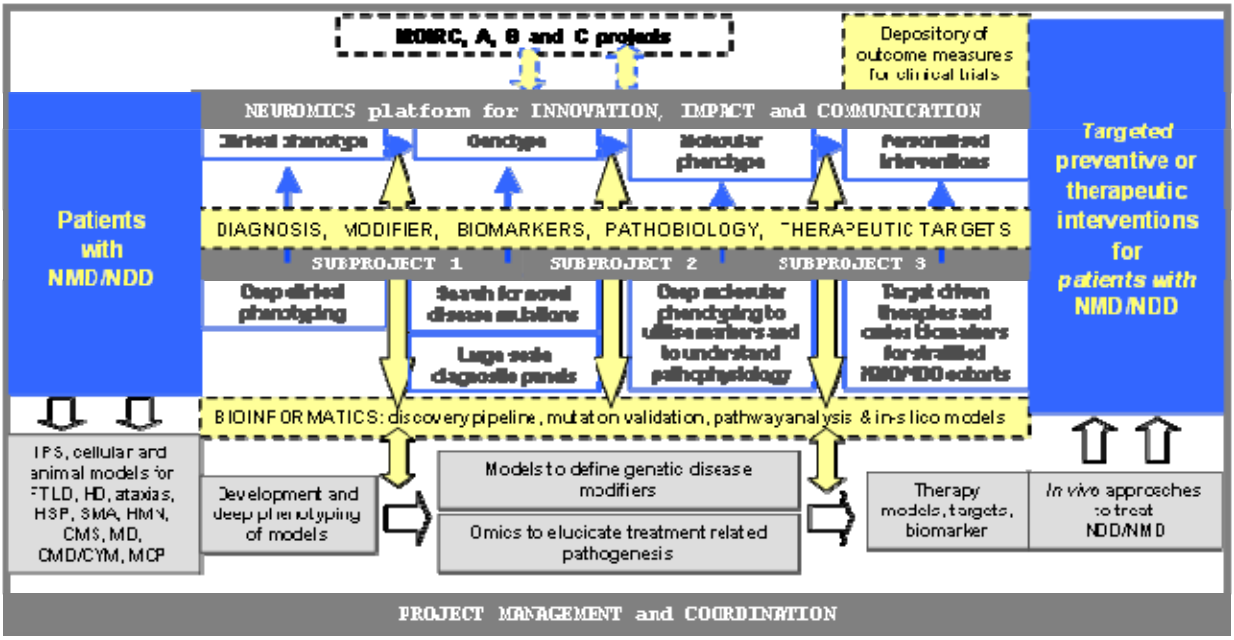


Figure 1: Concept of clinical-genetic and basic-applied research of NeurOmics.

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: 526 samples from patients and healthy family members have been whole exome sequenced at deCODE using the Illumina Nextera technology. 52 novel disease-causing genes have been identified of which 39 have already been published. Novel candidate genes are currently validated in ongoing functional testing including cellular and animal models.

A data sharing policy has been adopted and first batches of data (WES and clinical) are now being shared within the consortium. After expiry of a 12 months “hold period”, NeurOmics data will be shared within the wider rare disease (RD) research community via controlled access to the RD-Connect RD database. The database has been established at the European Genome-phenome Archive (EGA) and raw data from deCODE is now routinely copied to this database in order that it can be safely stored.

Ad aim ii: Clinical data sheets (CDS) have been developed for all 10 diseases studied in NeurOmics and the Human Phenotype Ontology (HPO) has been used to map the clinical features requested with these CDSs in order to get standardised terms. The standardised terms have then been used to create a phenotypic database at PhenoTips which went live in December 2013. Clinical data are now being entered and stored at PhenoTips. More than 1043 NeurOmics patients have been entered so far. The Care and Trial Site Registry was expanded and holds now 316 sites in 45 countries of which 50 sites are registered for NDD. A ‘phenotype search facility’ has been implemented in the CTSR as well.

Targeted NGS panels have been designed for SCA and 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. The panels have been improved to also cover recently identified new genes. More than 220 patients have been sequenced with the different disease panels. The Australian “supercapture” 336-gene panel has been run >450 times. The automated 3DM mutation prediction system developed by Bio-Prodict has been further improved and is now able to deal with transcript-based information systems and the resulting data.

Ad aim iii: SOPs have been developed for HD, SCA, FTLD and HSP patient sampling for biomarker studies. SCA, HSP and FTLD samples from patients and presymptomatic individuals are currently being collected. For the HD project, all samples have been obtained and are currently being RNA sequenced. For the validation of existing biomarkers in NMD patients, different approaches have been tested and compared. Sample collection is ongoing.

Profiling methods for metabolites and lipids in plasma and CSF based on UPLC-HRMS have been developed. This database now includes >2000 unique lipid signals from 6 families and 22 different classes.

Ad aim iv: hiPS cell lines have been generated from patients with SPG4 and SPG5 subtype of HSP, from selected distal HMN patients, from SMA patients and unaffected PLS3-discordant family members, and from HD patients carrying different numbers of CAG repeats. hiPS cells have been and are being differentiated into specialised cells of interest (such as glutaminergic cortical neurons, hepatocytes, motoneurons, striatal neurons or astrocytes) using various protocols. Disease-phenotypes are further analysed in these cells. The underlying mechanism causing responsiveness to VPA in SMA patients has been solved and the differential response in GABA-ergic neurons derived from hiPSC of these patients has been confirmed. In a novel conditional transgenic animal overexpressing the protective modifier for SMA, the underlying protective mechanism has been identified.

Different mouse models are generated, characterised and used for transcriptomics and/or lipidomics analysis to study e.g. SPG11, HMN or SCA3. A target, identified in a large siRNA screen with possible effects on polyQ toxicity/aggregation, has been analysed further. It has been genetically validated as a suppressor of polyQ toxicity/aggregation in different cellular and animal models, the druggability has been confirmed and the mechanism of action has been unravelled.

Data sets available in public databases and provided by NeurOmics partners have been used to improve HD and SCA and to generate HSP pathway maps. These pathway maps have identified a list of potential genes involved in modulation of mutant polyQ protein aggregation and toxicity. Proteins that are shared binding partners of Htt and ataxins have been identified which suggests common mechanisms and points of intervention for different polyQ disorders. SAGE data provide further evidence for microglia activation in HD and suggest a mechanism for down-regulation of T-cell response and microglia activation.

Ad aim v: 48 Huntington's disease samples from the Track-HD study with atypical disease progression have been sequenced by WES. Findings are 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 sequenced by WES and are now being analysed.

Sequencing data from large patient cohorts are required to find 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 inhibit the function of type I TGFβ receptors has been confirmed *in vitro* and in *mdx* mice. The effects of AON-mediated down-regulation of ALK4 and/or ALK5 receptors on TGF-β and MSTN signalling and its target genes, muscle histology and muscle mass and function will be validated in other NMD mouse models. The LGMD2B mouse model has been generated and characterised. AON-mediated exon skipping of dysferlin exon 32 has been demonstrated *in vitro* and is now tested in the mouse model. Skipping of other exons in LGMD2B patient-derived cells is currently tested *in vitro*. Exon skipping proof of concept for HD has been published: skipping of exon 12 in huntingtin pre-mRNA removes important caspase cleavage sites. 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.

High-throughput screening with Chemibank library consisting of 30,000 chemical compounds has been established and a pre-screen has been run to identify compounds increasing glycosylation of α-DG.

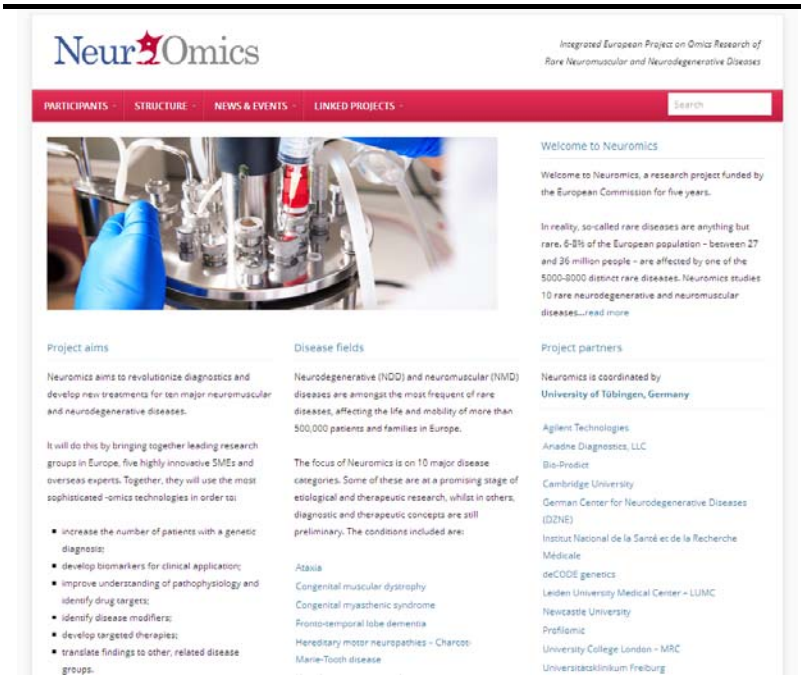
3. Description of the expected final results and their potential impacts and use

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



www.rd-neuromics.eu

NeurOmics logo

NeurOmics

4. Participants involved in NeurOmics

No	Participant	Country	PIs
1 (coordi-nator)	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 Neurodegenrative 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-Prodict BV (Bio-prodict)	The Netherlands	Henk-Jan Joosten
18	The University of Western Australia (UWA)	Australia	Nigel Laing
19	Universitaetsklinikum Freiburg (UKL-FR)	Germany	Janbernd Kirschner

Figure 2: Involved European countries in NeurOmics

Address of the Coordinator

Prof. Dr. Olaf Riess

Institute of Medical Genetics and Applied Genomics

University of Tübingen

Calwerstrasse 7

D 72076 Tübingen, Germany

Phone: +49-7071-2976458

Email: olaf.riess@med.uni-tuebingen.de