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

Reporting

Project Information

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Final Report Summary - NEUROMICS (Integrated European –omics research project for diagnosis and therapy in rare neuromuscular and neurodegenerative diseases)

Executive Summary:
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 brought together the leading research groups in Europe,
five highly innovative SMEs and relevant oversea experts using the most sophisticated Omics technologies. NeurOmics partners elaborated diagnostic approaches which are now standards and widely used for diagnosing rare ND and NM diseases. Grand progress has also been made in the development of disease specific biomarkers and pathomechanism-based treatment approaches of those diseases.

Key achievements of the NeurOmics project:
(i) 1105 patient samples were analysed by next generation exome (590 samples) and genome sequencing (515 samples). Thereby, disease causing mutations have been identified in more than 100 new genes. 81 modifications have been functionally validated and published by end of the project.

(ii) Disease specific enriched gene panels were designed and improved within the NeurOmics project. 3840 so far undiagnosed NDD and NMD patients were analysed by those panels and genetic causes were identified in almost 30 % of tested patients. Additionally, this large scale genotyping approach increased the patient cohorts available for further studies. Guidelines for genetic testing of NDD/NMD have been established and the developed gene panels have been implemented into diagnostic routine.

(iii) Lipidomics, metabolomics and transcriptomics analyses were carried out in more than 850 NDD/NMD patients and controls. A variety of variations between controls, presymptomatic and symptomatic patients have been identified and can be used as biomarkers for clinical application after further validation. In addition, these analyses also led to the identification of new pathways and mechanisms involved in disease progression. These findings revealed new common mechanisms shared between several NDDs and can be used as future targets for therapeutic approaches.

(iv) Genetic disease modifiers have been identified for HD, HSP, CMS and SMA by combined approaches of genome sequencing, transcriptome analyses and deep phenotyping. After completion of functional validations these modifiers will allow more precise sub-classification and prediction of severity and age of onset of those diseases.

(v) Exon skipping approaches have been tested as potential therapeutic approaches in HD, SCA3 and MD. Proof of concept studies have been completed and patents have been filed. These approaches will be further developed and brought to clinical trials in close cooperation with pharma companies.

(vi) At least 353 articles have been published in peer reviewed journals, 4 article sections have been contributed to edited books and a minimum of 9 PhD theses have been submitted. Several additional publications will follow within the next months. More than 400 times NeurOmics researchers presented their work at national or international conferences (poster or oral presentation) and more than 35 interviews were given and articles were published in popular press. In addition, 3 patents have been filed. A list of all dissemination activities can be found attached to this report.

(vii) Close and fruitful collaborations between NeurOmics, RD-Connect, EURenOmics and IRDiRC were established and maintained. NeurOmics contributed huge amounts of data and tools to the RD-Connect platform, both projects harmonised their consents and data sharing policy and joint meetings were held. Taskforces combining researchers from all projects were established and joint articles were submitted.
(viii) Awareness of different stakeholder groups for needs of NDD/NMD patients was significantly raised by regular Newsletters which reached more than 1000 subscribers (together with RD-Connect), a multitude of presentations at conferences and an Outreach Day (together with RD Connect and EURenOmics) which was attended by more than 300 representatives from politics, industry, research and patient organisations.

Project Context and 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.

We will significantly increase the knowledge on NDD/NMD in respect of novel disease genes and understanding underlying pathomechanisms, use this knowledge to develop biomarkers towards potential use in treatment monitoring, and use gene panels to significantly improve current diagnostic strategies. With our objectives, which are structured into three scientific main projects (sub-projects 1-3) we will significantly contribute to the IRDiRC goals for 2020: identifying the cause of most rare diseases, and developing novel treatments. Specifically, we will increase the number of diagnosable NDD/NMD from about 50% to 80% (SP1), transfer this information into novel tools of the routine diagnostics (SP2) and developing at the same time Omics-based therapeutics (SP3).

As most of the NDD/NMD have varying age at onsets determined by other genetic factors (e.g. Plastin 3 in SMA), and as disease modifying pathways may be potential targets for treatment strategies, we will also have a strong commitment to develop and verify Omics-based modifiers. Subsequently, preclinical experiments in cellular and animal models, development of tools such as iPS cells and first treatment studies of patients will guide us to explore the potential of Omics technologies to change the devastating situation of patients suffering from NDD/NMD over the coming years. This translational research approach is associated with further development of biobanks, patient registries, and a care and trial site network.

Objectives of subproject 1: Deep phenotype analysis and Omics-based identification of novel genes, modifiers, and biomarkers
SP1 is aimed towards discovery. Sophisticated Omics technologies will be applied in clinically very well characterized patients with NDD/NMD to discover novel genes, genetic modifying factors and biomarkers.

WP1 – Deep phenotype analysis in presymptomatic and symptomatic NDD/NMD patients
• To recruit NDD/NMD patients and presymptomatic gene carriers with a specific emphasis on disorders with an unknown genetic basis
• To obtain biomaterials of the study participants, store them, and make them available for genomic and biomarker studies
• To longitudinally follow the study participants and obtain biomaterials at repeated time points to be used for biomarker development and validation, and integration into existing registries and biobanks

WP2 – Identification of novel disease genes in NDD/NMD patients
• To identify up to 100 novel disease genes in NDD/NMD patients by WES
• To decipher the genetic basis of ~ 80% of all NDD/NMD cases
• To identify the cellular and functional relevance of novel disease genes
• To define effectiveness, turn-around time and costs of WES in an early diagnostic setting
• To identify potential modifiers across NDD/NMD groups analysing all WES datasets
• To integrate genetic data into clinic, family registries, biobanks and public database

WP3 – Identification of modifying factors in cohorts enriched by deep phenotyping
• To identify genetic variations that influence onset/progression in HD & SPG4 (5 per disease) and CMS (1 major variant, given expectation of founder effect)
• To identify and study pathways associated with phenotypic alteration

WP4 – Identification of hypothesis-driven biomarkers for disease progression (prospective studies in genetically diagnosed patients and presymptomatic carriers)
• To identify transcriptomic and metabolomic disease biomarkers in FTLD, HD, SCA, HSP and MD patients deciphering the functional pathway affected and facilitating new disease gene identification

Objectives of subproject 2: Clinico-genetic diagnostics and clinically relevant Omics-based biomarkers
Knowledge gathered by SP1 and in the general scientific community will be transferred into clinical routine, implementing extensive genetic characterization of NDD/NMD patients, and validating diagnostic and therapeutic biomarkers for their clinical use.

WP5 – Development and implementation of disease group spanning NGS-based diagnostic tools
• To set up recommendations of a diagnostic sequence for each of the diseases
• To design NDD (103 genes: 17 SCA genes, 16 ARCA, 5 episodic ataxias, 40 CA, 25 SPG), NMD (159 genes) and SMA-LMND (38 known genes) genetic diagnostic panels and algorithms
• To sequence 100 pre-screened patients of each disease group with unknown genetic cause using gene panels (targeted NGS)
• To define limitations of the technology for the respective approach and for each of the tested genes
• To set up a bioinformatic strategy for routine diagnostics
• To expand the genotype/phenotype correlations for all identified mutation carriers
• To compare cost, validity and efficacy between whole exome sequencing (or WGS) and a selected disease panel approach
• To integrate genetic data into the clinic, registries, biobanks and a public database (RD-Connect project)

WP6 – Diagnostic readouts for predicting disease modification
• To collect WGS-based, WES-based and targeted NGS-based mutation data from WP5 and identify potential disease modifying effects based on bioinformatics

WP7 – Omics-based biomarkers for progression and therapy monitoring based on disease pathways
• To validate and optimize known biomarkers in SPG, SCA1,2,3,7, HD, DMD, FSHD, and other NMD
• To validate newly identified biomarkers from WP4

WP8 – Bioinformatic tools for diagnostic prediction
• To develop 3DM automated mutation prediction systems, apply them to multiple target proteins and prioritize pathogenicity of variants identified by NGS

Objectives of subproject 3: Omics to elucidate pathogenesis and guide therapy
Profound knowledge of NeurOmics partners will be exploited for deep characterization of additional disease pathways and will guide future treatment strategies. Thus we focus on pathogenesis research which has the potential to identify druggable targets.

WP9 – Omics-assisted therapy development
• To validate AO-mediated improvement of muscle quality in vivo
• To validate the therapeutic applicability of AO-mediated exon skipping for LGMD2B in vivo
• To validate exon skipping of caspase cleavage domains as a therapy for PolyQ diseases
• To validate compounds that induce hyperglycosylation for CMD

WP10 – Elucidation of pathogenesis and monitoring of treatment
• To establish standardized disease-based cell models (hiPSCs and others)
• To correlate Omics profiling of hiPS derived cells and in vivo animal models/patient samples
• To identify, prioritize and test modifiers in cell assays and animal models
• To develop molecular therapies for plausible targets cell and animal models
• To facilitate translation of preclinical approaches to clinical trial stage

WP11 – Modifier gene identification and study in proteinopathies
• To develop disease pathway maps for HD and various SCAs
• To identify and characterize genes that modulate aggregation of polyglutamine and other neurotoxic proteins

Objectives of the innovation, impact, communication and management work packages
WP12 – Impact and communication (IC)
• Develop an internal and external communication platform for the project to ensure effective information exchange within the consortium and with the wider neuro-field and establish the project’s profile and visibility
• Maintain close links with stakeholders, in particular patient organisations, industry and regulators, building on the already highly advanced existing stakeholder integration mechanisms developed by the consortium partners
• Deal with ethical issues raised by the project through a Project Ethics Council in close collaboration with a Patient Association Council, and ensuring the implementation of ethical and consent standards generated within RD-Connect
• Develop a training plan to educate additional sites in cutting-edge techniques to broaden the impact base of the project results to reach as many parts of Europe as possible (including E. Europe).

WP13 – Research Infrastructure
• Integrate and utilise facilities and tools that have been developed over recent years in the field of neuromuscular and neurodegenerative diseases with the aim of maximising the output and impact of the
Develop the interface with the RD-Connect (including exchange of reference data and standards and harmonisation of resources) and the Support IRDiRC (for IRDiRC links and information exchange).

Develop a plan for the integrated scientists and clinicians to improve clinical interpretation of Omics results.

WP14 – Project Management

- Ensure that all budgetary actions are performed correctly and according to the rules and regulations established by the European Commission and the consortium agreement; ensure that the received funds are correctly distributed, and accounted for, including independent auditing.
- Provide the administrative and coordinative measures that help to ensure that the work and tasks are completed on time and within the budget and satisfy the high quality requirements as laid out in the work plan.
- Ensure that reporting is performed on a regular basis, in the most efficient and pragmatic way, according to EU Commission guidelines. The objective is to provide all consortium members with all important, relevant and impacting information that can influence the project’s outcome.
- Ensure appropriate links and coordination with Support IRDiRC, EURenOmics and RD-Connect projects and future related IRDiRC projects.
- Ensure that the knowledge required for the work is accessible and,
- Ensure that the newly generated knowledge will be adequately used and/or exploited.
- Ensure that all ethical requirements needed for the research are in place.

Project Results:

Summary WP1 – Deep phenotype analysis in presymptomatic and symptomatic NDD/NMD patients

This work package aimed to:
- Continuously recruit and phenotypically characterise NDD/NMD patients with a specific emphasis on patients with an unknown genetic basis or modifiers.
- Obtain biomaterials, to store them, and to make them available for gene identification (WP2), biomarker studies (WP4) and integration into family registries (WP13, RD-Connect).
- Continuously recruit and phenotypically characterise patients and presymptomatic gene carriers with FTLD and SCA for identification of early diagnostic biomarkers.
- Longitudinally follow the study participants (FTLD; SCA) and repeatedly obtain biomaterials that can be used for biomarker development and validation (WP4, WP7, WP13, RD-Connect).

Recruitment, clinical characterisation of patients and collection of biomaterial from patients and their family members for all 10 NDD/NMD disease groups studied in NeurOmics was emphasised throughout the whole project period. PhenoTips, an online platform for standardised collection of clinical data was developed and improved in cooperation with RD-Connect. Clinical data sheets (CDS) have been established for each of the studied disease groups and have been mapped to the Human Phenotype Ontology (HPO).

To date, more than 1500 NeurOmics patients have been entered in PhenoTips (Table 1). Of all samples sequenced by deCODE using WES, WGS or RNAseq, complete PhenoTips entries are available for more than 99 % of samples. 1 % of samples have no patient consent for data sharing although most partners put much effort in re-contacting and re-consenting their patients.
The continuous improvement of PhenoTips in close cooperation with NeurOmics researchers and the possibility to connect PhenoTips data with entries at the European Genome-phenome Archive (EGA) made PhenoTips a well-established and elaborate platform for collecting clinical data. Enhancing PhenoTips functions and user friendliness is still ongoing and will be continued after the project finished. Two recent enhancements are the implementation of a pedigree editor and family identifier. This means that you can now see family relationships more easily and link families together via a pedigree. Additionally, PhenoTips developers are currently developing methods to easily implement data from other databases. PhenoTips and its advantages are well known within the rare disease community and hence, this platform will be used for data collection in future projects (e.g. Solve-RD).

Deep phenotyping efforts, as well as collection and sharing of all the above mentioned clinical data within the NeurOmics community lead to several publications. Three deep phenotyping studies have been published for HMN (Echaniz-Laguna et al. 2017; Tsai et al. 2017; Salter et al. (under review). The SPORTAX data lead to a manuscript on clinical and genetic characteristics of sporadic adult onset degenerative ataxia which has been published by Giordano et al., Neurology 2017. Deep clinical phenotyping and sample collection of a Bulgarian CMS cohort (CHRNE1267delG) has been completed.

Summary WP2 – Identification of novel disease genes in NDD/NMD patients

This work package aimed to:

• Identify up to ~100 novel disease genes in NDD/NMD patients by WES
• Enable to decipher up to ~80 % of genetic caused NDD/NMD patients
• Define cellular and functional relevance
• Define effectiveness, turn-around time and costs of WES in an early diagnostic setting
• Identify potential modifiers across NDD/NMD groups analysing all WES datasets
• Integrate genetic data into clinic, family registry, biobanks and public database

In an effort to identify the underlying genetic cause for NDD/NMD patients, 1105 WES/WGS analyses were performed at deCODE genetics (Iceland) during the NeurOmics research period. Since the switch from WES to WGS in mid-2015, 515 samples have been whole genome sequenced. WES has been performed using the Illumina Nextera technology. Illumina’s HiSeqX platform has been used for WGS by generating sequencing libraries using the so-called PCR-free sample preparation method. Samples are in general sequenced to a mean depth of at least 30X.

Table 2 provides a summary of the distribution of all available WES/WGS analyses per disease group. For each disease group, patient recruitment progressed effectively and during the 60 months of research period, all available slots for analysis have been used.

Table 2: Distribution of available and used WES/WGS analyses per disease group (see attachment)

The number of performed analyses is not equal to the number of patients/families analysed. Depending on the expected mode of inheritance, further affected family members, but also unaffected family members were frequently included to analysis.
Results obtained from WES/WGS and the identification of novel disease-causing genes is often not clear. Researchers cannot be perfectly certain as to the pathogenic nature of a variant, or analyses yield multiple possibly disease-causing variants. Hence a classification system dividing samples into “Solved with novel disease gene”, “Solved with known disease gene”, “Cases with VUS3 and candidates” and “Unsolved cases”. This classification enabled us to calculate a success rate for each disease group (Table 3). Success rates range from 1.5% to 45% with average of 30%. The huge differences can be explained by the different approaches applied by NeurOmics researchers. Some groups mainly sent samples of index patient whereas other partners preferred trio analysis. While increasing the per patient cost, trio analyses enable efficient calling of real homozygous and compound heterozygous variants without the need for multiple and laborious segregation analyses based on Sanger sequencing. Trio analysis it enables detection of de novo variants and increases the overall success rate. In addition, researchers also used different bioinformatics tools for variant detection.

Table 3: Success rate of WES/WGS per disease group (see attachment)

More than 100 novel disease causing genes of which 81 are already published mostly in high ranking journals were identified using this WES/WGS approach (Table 4). For 43 genes we have established novel phenotypical associations in 50 independent publications (Table 5). Strikingly, up to 81 newly identified disease-causing genes were analysed functionally using cellular and animal model systems. For newly identified disease-causing genes we can provide first insights into the molecular pathomechanism of disease including Limb-Girdle Muscular Dystrophy 2X/POPDC1 (Schindler et al., 2016), Congenital Myasthenic Syndrome/SLC5A7, VAMP1 and MYO9A (Bauche et al., 2016; O’Connor et al., 2016; Salpietro et al., 2017), Myofibrillar Myopathy 8/PYROXD1 (O’Grady et al., 2016); Autosomal recessive cerebellar ataxia/CHP1 and CAPN1 (Ferreira-Mendoza et al., 2017,) (Wang et al., 2016), autosomal dominant spinal muscular atrophy/BICD2 (Neveling et al 2013, Peeters et al 2013, Storbeck et al., 2017); hereditary motor neuropathy/WARS (Tsai et al., 2017); and congenital myopathy/MSTO1 (Nasca et al., 2017). Numerous novel genes have been identified in collaborations between NeurOmics partners highlighting the importance of data sharing.

Table 4: Published novel genes (see attachment)

Table 5: Genes with novel phenotypical associations (see attachment)

Sequencing data was transferred to the EGA archive where it will be made accessible by a controlled access mechanism to other scientists in the field. Work is currently ongoing to create disease- and approach-specific data sets at the EGA. Clinical data of all patients analysed in WP2 has been entered in PhenoTips as described in the summary of WP1. Additionally, the majority of samples has been integrated into local and international biobanks and a paroxysmal neurological disorder clinical and genetic disorders database has been developed. This has captured patients with episodic neuromuscular and neurogenetic disorders, in an often difficult to diagnose and neglected group for future genetic analysis and collaboration.

Summary WP3 – Identification of modifying factors in cohorts enriched by deep phenotyping

This work package aimed to:
• Identify genetic variations that influence onset/progression in HD & HSP (5 per disease) and CMS (1 major variant, given expectation of founder effect)
• Identify and study pathways associated with phenotypic alteration (same numbers)

HD: While the classical case-control design in complex disease has yielded multiple genetic associations highlighting relevant biology for novel treatment design, studies of potential genetic modifiers in genetically simple Mendelian diseases have been difficult to conduct. The diseases are rare and show gene and locus heterogeneity, thus finding genuine modifying associations in such a noisy background is inherently difficult.

However, 48 Huntington’s disease samples from the deeply phenotyped Track-HD study were selected for WES on the basis of having atypically fast or slow disease progression. All samples have been sequenced at deCODE. Initial case control analysis has yielded interesting results but none reach statistical significance. Therefore, the findings have been integrated with other datasets (e.g. GeM-HD GWAS study, HD REGISTRY) to increase power to detect rare variants influencing Huntington’s disease progression. Variation in the DNA repair gene MSH3 has been identified to be associated with the rate of Huntington’s disease progression (Hensman Moss et al. Lancet Neurology. 2017).

The role of variants in DNA repair genes to modify age at onset in Huntington’s disease has been confirmed and extended to other polyQ repeat disease (published in Bettencourt et al. Ann Neurol 2016). To investigate the relationship between age at onset and disease progression 216 TRACK-HD subjects have been genotyped. Variants in FAN1 have been found to be associated with not only HD onset but also HD progression. Several coding variants in FAN1 identified in the TRACK-HD cohort are being investigated.

SPG: 20 SPG4 parent-offspring pairs with discrepant age of onset of more than 25 years have been whole exome sequenced (40 samples in total). However, with only 20 parent-offspring pairs the sample is insufficiently powered to suggest any definite modifiers. Sample size will be increased and WES data will be re-analysed.

CMS: 20 Bulgarian patients with the same mutation (CHRNE1267delG) but differences in phenotype (10 mild, 10 severe) have been whole genome and RNA sequenced. Analysis of the transcriptome data identified a total of 53 genes that are differentially up- or down- regulated in the severe vs mild cohort. Ariadne analysed exome and transcriptome data to suggest variants affecting disease severity. Functional validation is still ongoing and other strategies are needed to analyse the data.

Summary WP4 – Identification of hypothesis-driven biomarkers for disease progression
This work package aimed to:
• Identify disease progression biomarkers in FTLD, HD, SCA and HSP patients
• Highlight the functional pathways affected in these diseases
• Facilitate new disease gene identification derived from a disease interactome

HD: SAGEseq analysis of an HD patient cohort of 124 samples revealed a biomarker panel consisting of 5 genes. Blood samples were collected 4 years later from this primary cohort and RNA has been sequenced by deCODE. Unfortunately, earlier results obtained with SAGEseq could not be reproduced. 24 samples from the first time point have now also been RNA sequenced. These 24 samples to identify the difference between SAGEseq and RNAseq and these results will be used to perform a longitudinal study in this cohort.
Whole blood RNAseq of 52 premanifest HD patients, 63 early stage HD patients and 23 controls has been completed. No differentially expressed genes were found between HD and control. Data from LUMC and UCL have been combined to increase the power of the analysis. It has been found that peripheral blood transcriptome in HD parallels that in the most affected regions of the HD brain. Immune upregulation shows common pathogenic mechanism of HD and Alzheimer’s disease involving macrophage phagocytosis and microglial synaptic pruning, raising potential for shared therapeutic approaches. The joint analysis has been published in Hensman Moss et al., Nature Scientific Reports 2017.

RNAseq in myeloid cells from 30 manifest HD patients and 33 controls has been completed. Transcriptional dysregulation in HD myeloid cells is characterised by increased expression of proinflammatory mediators, even in the absence of stimulation, and is driven by activation of the NFkB signalling pathway. Results have been published by Miller et al. Hum Mol Genet 2016.

SCA: Plasma samples from two time points (2 year interval) of SCA patients, presymptomatic individuals and controls have been analysed using lipidomics. Over-expression of components has been detected in SCA7 patients at baseline compared to controls and other SCAs but has not been observed in the same cohort 2 years later. Ariadne compared lipid profiles of different SCA groups of the first cohort using Subnetwork Enrichment analysis (SNEA) and identified up- and down-regulated candidates. Pathways have been built based on the connections between these candidates. A comprehensive Lipidome database and lipidome analysis tools are now in place.

TR-FRET-based assays have been established to measure alpha-synuclein, parkin and ataxin-3 levels in lymphocytes. Measurement of parkin and alpha-synuclein in lymphocytes from 25 SCA3 patients, 7 presymptomatic carrier and 25 controls have been carried out and a publication is under preparation.

FTLD: Plasma (for lipidomics), lymphoblasts and lymphocytes (for RNAseq) of 78 FTLD patients and presymptomatic individuals carrying C9ORF72, PGRN and MAPT mutations, as well as non-genetic tauopathies and presymptomatic carriers have been collected. Lipidomic and metabolomic analyses of plasma samples of FTLD patients do not seem to be efficient FTLD biomarkers to discriminate between presymptomatic and symptomatic FTLD mutation carriers.

HSP: HSP sample collection has been completed and lipidomics analysis has been done. Four disease specific biomarkers in HSP have been identified (unpublished data) In SPG26 patients, lipidomic analyses showed a significant increase of three components compared with controls (unpublished data). Additional patients have been obtained in order to confirm this finding in SPG26 patients – analyses are pending.

Summary WP5 – Development and implementation of disease group overlapping NGS-based diagnostic panels

This work package aimed to:

- Development of recommendations of a diagnostic sequence for each of the diseases
- Design NDD (103 genes: 17 SCA genes, 16 ARCA, 5 episodic ataxias, 40 CA, 25 SPG), NMD (159 genes) and SMA-LMND (38 genes) panels and algorithms. New genes identified in WP2 or meanwhile published will be included in updated version
- Sequencing of 100 pre-screened patients of each disease group with unknown genetic cause using gene panels (Targeted NGS)
- Recommendation of families for second WES mutation screening (WP5), for which no disease causing mutation has been identified using the targeted approach
- Define limitations of technology for the respective approach and for each gene
- Bioinformatics strategy for routine diagnostics (WP8)
• Recommendations and guidelines for genetic testing  
• Expanding the genotype/phenotype correlations for all identified mutation carriers (WP1)  
• Report variants as potential modifier variants (WP6)  
• Cost, validity and efficacy comparison between WES, WGS and disease panel approach

Targeted NGS panels have been designed for ataxia and HSP, NMD and SMA/LMND. Inclusion criteria have been defined for all three disease groups separately to match the specificities of each disease. A “supercapture” panel developed to meet the needs of the smaller Australian population has been split into a muscle (myogenic disease) panel and a nerve (neurogenic disease) panel. Panels are regularly updated to include novel identified genes and to adjust target enrichment. The LMND panel with 65 genes has been discontinued as the diagnostic output was too low (>80 % unsolved). WES/WGS of unsolved patients identified known muscular disease genes in >1/3 of cases underlining a strong clinical overlap. Thus, a novel gene-panel approach for all neuromuscular diseases (443 genes) using Agilent SureSelect enrichment and Illumina HiSeq4000 instruments has been established and validated in Cologne. Since the start of the project, 68 HSP and 107 ataxia panels have been run on Illumina MiSeq using the Haloplex enrichment method. In 37 % and 19 % of patients the disease-causing mutation has been identified, respectively. 112 NMD panels have been run with the improved panel on Illumina NextSeq500 using the Haloplex enrichment method (33 % have been solved). 35 SMA/LMND patients have been sequenced using the AmpliSeq enrichment protocol on the IonTorrent PGM and 62 with the recently implemented NMD panel using Agilent SureSelect on Illumina HiSeq4000. 17 % and 45 % of patients have been solved, respectively.  
2270 patients have been sequenced with the three versions of the PathWest targeted panels for muscle and nerve. 31.8 % of cases have been solved.  
774 patients have been analysed with the second HSP panel in Paris using a combination of capture with Nimblegen/ROCHE probes and sequencing with Illumina technology (Morais et al. EJHG 2017). 30 % of cases have been solved. Screening of a cohort of 25 consanguineous families from Sudan with this panel led to a genetic diagnosis in six families with autosomal recessive HSP (SPG11 in three families; TFG/SPG57, SACS, and ALS2 in one family each) and in one family with autosomal dominant HSP (heterozygous mutation in AT1/SPG3A). The TFG/SPG57 variant was the second worldwide and has been published in Elsayed et al., Eur J Hum Genet 2016. Diagnosis kit of dominant ataxias: An amplicon strategy to amplify and sequence 34 genes involved in dominant ataxias has been designed. 412 dominant ataxia cases excluded for polyQ repeat expansions have been analysed, 14 % have been solved (Coutelier et al. Brain 2017).

Table 6: Disease specific gene panels developed and applied by NeurOmics (see attachment)

All panel sequencing data from EKUT, UK Cologne and AMUMS and corresponding meta-data have been transferred to EGA. Cologne and EKUT are currently preparing two independent guidelines for using gene panels for genetic testing of SCA/HSP and SMA/LMND patients, respectively. In addition a comparison paper highlighting advantages and limitations of gene panels combining the results from all partners involved in WP5 is currently being prepared with EKUT as leading partner.

Summary WP6 – Diagnostic read outs for predicting disease modification
This work package aimed to:
• Collect WGS-based, WES-based or targeted NGS-based mutational data from WP5 and enter into data base (interaction with RD-Connect)
• Identify potential disease-modifying effects based on bioinformatics analyses

Guidelines for the sharing and protection of data have been developed and adopted by the NeurOmics Steering Committee. Collection of WGS-based and WES-based mutational data from WP2 at the EGA has been completed: deCODE transferred the data directly to the EGA. Meta-data of the patient samples sequenced by WES, WGS and RNAseq has been collected and also uploaded to the EGA. Transfer of sequencing data from WP5 (panel sequencing) as well as corresponding meta-data have been transferred to EGA.

At the annual meeting 2014 in Heidelberg, an overall data-workflow has been suggested, but unfortunately could not be made functional until the end of the project period. Insufficient sample data for the constitution of cohorts with a clear identification of differential phenotypes within same disease groups constituted the main drawback hindering to reach WP6 objectives. This remains an overall problematic faced across different disease thematics in the field of rare genetic diseases.

Summary WP7 – Omics-based biomarkers for progression and therapy monitoring related to disease pathways
This work package aimed to:
• Validate and optimize known biomarkers in 300 SPG patients
• Apply lipid biomarkers in 15 SPG5 patients as readouts in therapeutic trials
• Screen newly identified lipid biomarkers in SPG
• Validate and optimize known biomarkers in 100 SCA patients and persons at risk, as well as 220 HD patients
• Validate existing and screen of newly identified biomarkers in more than 800 NMD patients

SPG: Two therapeutic trials with SPG5 patients have been completed by EKUT (Schöls et al 2017) and INSERM (Marelli et al. 2017) and submitted back to back to Brain (both in press):
Hereditary spastic paraplegia type 5 (SPG5) is caused by bi-allelic mutations in the oxysterol-7α-hydroxylase gene CYP7B1. This enzyme is involved in the degradation of cholesterol into primary bile acids. CYP7B1 deficiency in SPG5 leads to marked accumulation of CYP7B1 substrates like 27-hydroxy-cholesterol (27-OHC) and 25-hydroxy-cholesterol (25-OHC) in serum and cerebrospinal fluid (CSF). In cell cultures of motoneuron-like cells (NSC-34) and human induced pluripotent stem cell (hiPSC)-derived cortical neurons we proved side-chain oxysterols like 27-hydroxy-cholesterol (27-OHC), 25-hydroxy-cholesterol (25-OHC) and 24-hydroxy-cholesterol (24-OHC) to be toxic. Especially 27-OHC was found to be neurotoxic in concentrations close to those found in SPG5 patients. As cholesterol and 27-OHC levels are closely related, we hypothesized that treatment of SPG5 patients with HMG-CoA reductase inhibitors can lower the pathologically elevated levels of 25-OHC and 27-OHC.

Given the substantially increased levels of 27-OHC in serum as well as CSF of SPG5 patients, EKUT and INSERM performed two independent randomized placebo-controlled trials with Atorvastatin. Atorvastatin but not placebo treatment resulted in approximately 31 % (EKUT 31.5 %, INSERM 30 %) reduced serum 27-OHC. Similarly, 25-hydroxycholesterol levels in serum were reduced. In CSF 27-OHC was reduced by 8.4 % but this did not significantly differ from placebo.
The confirmation of an abnormal bile acids profile in SPG5 patients, which can be improved by chenodeoxycholic acid, also suggests that it may be worth combining atorvastatin (40mg) and chenodeoxycholic acid (500mg) for the treatment of SPG5 patients. The neurological benefit of these metabolic interventions remains to be evaluated.

HSP: In 268 samples from patients with undiagnosed HSP VLCFA, cholestanol and oxysterols have been measured. Thanks to those known biomarkers, 8 % of this cohort have now a diagnostic.

HD: 133 samples from HD patients and 255 samples from SCA patients have been sent for BCAA analysis. Longitudinal sampling was obtained in 92 patients (52 with 2 time-points, 36 with 3 time points and 4 with 4 time-points). BCAAs mean levels were lower in the disease groups compared to controls. However, BCAA levels remain stable over time in all disease groups, indicating that BCAA level cannot be used as disease biomarker. These findings will be confirmed in a larger cohort.

NMD: Given the promising data obtained in WP9 by analysing metabolomics and lipidomics profiles in dystrophic mice, the same analysis has been done in fasted NMD patients. Differences between patients and controls as well as correlation with outcome measures have been identified. A more refined data analysis to identify the affected pathways and to bridge the findings obtained in patients and animal models has been performed.

Summary WP8 – Bioinformatic tools for diagnostic prediction
This work package aimed to:
- Develop 3DM automated mutation prediction systems
- Generate 3DM mutation prediction systems for multiple target proteins
- Use 3DM to link variants to diseases
- Prioritise pathogenicity of variants that result from NGS

An alignment pipeline has been generated and continuously improved over the course of the project. It is now possible to build 3DM systems for human transcripts completely automatically.

For 301 genes 3DM systems are available to the NeurOmics community. A Prioritisation algorithm has been developed that outperforms current state of the art tools. High-quality predictions have been generated for all possible missense variants.

Integration with RD-Connect has been initialized by Bio-Prodict. The goal of this collaboration is to provide users of the RD-Connect platform with 3DM pathogenicity predictions and the ability to navigate to 3DM information systems if they want to dive deeper into specific variants and enable them to generate reports about different aspects (conservation, structure, literature, etc.) of their variants.

Summary WP9 – Omics-assisted therapy development
This work package aimed to:
- Validate AO-mediated improvement of muscle quality in vivo
- Validate therapeutic applicability of AO-mediated exon skipping for LGMD2B in vivo
- Proof of concept for exon skipping of caspase cleavage domains as a therapy for PolyQ diseases
- Assess the Omics effects of compounds that induce hyperglycosylation for CMD

LGMD: To generally support muscle quality, LUMC developed an efficient and selective method to block TGF-beta and myostatin signalling by targeting their type I receptors ALK5 and ALK4 with antisense oligonucleotides (AONs). ALK4 inhibition increases myogenesis, but also regulates the tight balance of
protein synthesis and degradation. Alk4 AON-mediated inhibition led to muscle atrophy rather than the expected hypertrophy (published by Pasteuning-Vuhman et al. FASEB J 2016). Thus Alk 4 and Alk 5 skipping is not therapeutically beneficial as muscle disease therapy.

Natural disease history of LGMD2D and 2F (α- and δ- sarcoglycan deficient) mice has been characterised based on an SOP provided by the TREAD-NMD network and published by Pasteuning-Vuhman et al. PLoS One 2017.

The LGMD2B mouse model (dysferlin KI mouse carrying a stop codon in exon 32 of DYSF) has been generated and characterised. AON-mediated exon skipping has been shown in vitro and presence of dysferlin at the protein level has been demonstrated. Testing is ongoing if exon 32 skipping can rescue the mice phenotype at a functional level. Encouraging results in lipidomics in dysferlin deficient mice have been obtained. In parallel with the in vivo work for dysferlin exon 32 skipping, a second project focuses on in vitro exon 30 and 34 skipping. LUMC has been able to show a shorter dysferlin protein on Western blot after exon 30 skipping in cultured control cells but not restoration of dysferlin in patient-derived cells. Work on exon 34 skipping is ongoing.

HD: Exon skipping Proof of Concept for HD has been published by Evers et al. 2014. A pilot study has been performed in the YAC128 HD mouse model showing detectable exon 12 skipping on RNA level after one intracerebral ventricular injection. The following larger study gave conflicting results. AON will have to be modified to improve AON efficiency and effect on protein level. A patent has been filed and is currently explored with ProQR startup.

SCA3: For SCA3, calpain and caspase cleavage sites implicated in disease pathology are coded for in exons 8 and 9 of ataxin-3 pre-mRNA. Results of the double skip of exon 8 and 9 as a strategy to reduce proteolytic cleavage of ataxin-3 have been published in Toonen et al. 2016. Skipping of ATXN3 exon 10 led to formation of a truncated ataxin-3 protein lacking the toxic polyQ expansion. Proof of concept has been confirmed in mouse (Toonen et al. Mol Ther Nucleic Acids. 2017). A Patent has been filed and the approach will be developed further with Ionis Pharmaceuticals.

CMD: 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. Further analysis resulted in one promising hit which showed increased α-DG glycosylation in a second cell line, in patient fibroblasts and iPSC-derived cortical neurons.

Summary WP10 – Elucidation of pathogenesis and monitoring of treatment

The aims of this work package are to:

• Establish standardized disease-based cell models (hiPSCs and others)
• Correlate Omics profiling of hiPS derived cells and in vivo animal models/patient samples
• Identify, prioritize and test disease modifiers in cell assays and animal models
• Develop molecular therapies for plausible targets cell and animal models
• Facilitate translation of preclinical approaches to clinical trial stage

SPG: SPG5 iPSC were successfully differentiated into hepatocyte-like cells (HLCs). These SPG5-hepatocytes secrete higher 27-OHC concentrations compared to CTR in the supernatant recapitulating the in vivo disease phenotype. Oxysterols (like 27-hydroxy-cholesterol) have been established as reliable serum and CSF biomarkers related to pathogenesis in SPG5 (Schöls et al. Brain 2017, in press).

Lipidomics analysis of different brain regions of SPG11 KO mice (SPG11 +/+, +/-, -/-) has been completed. Accumulation of specific lipidic species in the brains of SPG11 KO mice has been validated.
and published in Branchu et al. 2017. In addition, a SPG56 KO mouse model has been characterised. Mice showed an abnormal cognitive behaviour at 2 months of age as well as a decrease trend for locomotor activity at 4 months. Lipidomics has been performed on spinal cord and hippocampus. Validation of two lipidic products accumulating in the KO animals is ongoing.

SMA: Generation and full characterization of iPSC-derived motor neurons of SMA patients and fully asymptomatic discordant family members has been published in Heesen et al. 2016. Lipidomics, transcriptomics, and proteomics analysis on patient-derived motor neurons generated from iPSCs has been completed. This identified genes that are upregulated in unaffected discordant family members (including PLS3 and others). The mechanism for the modifying effect of PLS3 has been unravelled and CORO1C identified as a second modifier and PLS3-interactor that rescues actin cytoskeleton dysregulation and endocytosis in SMA models. Results have been published in Hosseinibarkooie et al. 2016.

HSP: Phenotypic characterisation of the KI/KO HSPB8_K141N and the results of the phenotypic analysis of Rosa26 HSPB1 transgenic mice have been published in Bouhy et al. 2017 and Bouhy et al. 2016, respectively. iPSCs from patient fibroblasts have been generated by a partner lab for the most frequent mutations observed in distal HMN and axonal CMT (MFN2, HSPB1, HSPB8, and NEFL). Differentiation of iPSC into motor neurons (iNeurons) and characterisation for correct neuronal profiling has been completed. HSPB1 KO cell lines have been generated in the neuroblastoma cell line SH-SY5Y using Crispr/Cas9. HSPB1 interacting poly-C binding protein, PCBP1, an RNA binding protein with a possible role in neurodegenerative disease has been published by Geuens et al. 2017.

HD: In the HD-project, three hiPS control cell lines with 21, 28 or 33 CAG repeats and three HD cell line carrying 60, 109 or 180 CAG repeats have been differentiated into striatal neurons. HD lines show defects in striatal and cortical differentiation. In addition, self-renewing HD-iPS cells show an increased number of huntingtin (Htt) aggregates and an increase in the number of autophagosomes. But despite the impairment of the autophagic process, control and HD-iPS cells seem to exhibit no differences at the level of the autophagic flux.

Summary WP11 – Modifier gene identification, prioritization and study

This work package aimed to:
- Develop disease pathway maps for HD and various SCAs (in liaison with WP7 and WP10)
- Identify and characterise genes that modulate aggregation of polyglutamine and other neurotoxic proteins
- Test the genetic interaction in vivo between Parkin and mutant ataxin 3

Ariadne analysed several pathways involved in Huntington disease to identify biomarkers to monitor HD disease progression. One HD-related pathway that was identified in the knowledgebase is axonal transport. A key player here is HAP1. Several intracellular transport functions may be impaired through altered interaction of mutant HTT with HAP1, either directly or through reduced palmitoylation of pathway components.

12 hits with possible effects on polyQ toxicity/aggregation have been identified in a siRNA screen in cell-based systems. QPCT has been identified as a target for modifying polyQ toxicity/aggregation in this screen, and validated in mammalian cells, primary neurons, Drosophila and zebrafish HD models. Druggability of this hit has been confirmed by using specific inhibitors in mammalian cells, primary neurons, Drosophila and zebrafish HD models. The mechanism of action has been identified: modification
of general protein aggregation by inducing the levels of a molecular chaperone, alpha B-crystallin. Results have been published (Jimenez-Sanchez et al. 2015). A novel target that modulates polyQ aggregation and toxicity by regulating autophagy has been identified and is currently being validated. RNAseq, metabolomics and lipidomics analyses have been done upon autophagy perturbation. Transcriptional profiling has been performed in brain tissue of an autophagy hypomorphic mouse model upon starvation-induced autophagy. Data is currently being analysed.

A double-mutant (Parkin-Ataxin-3 transgenic) mouse model has been generated and fully characterised with different behavioural and neuropathological analyses. From 6 to 15 months in age these mice show significantly reduced coordination compared to controls. From the beginning of 3 months in age a different fragmentation pattern of ataxin-3 has been found if parkin is knocked out but no differences in aggregation up to 15 months in age have been observed. RNAseq, metabolomics and transcriptomics have been done. Data is currently being analysed.

Three SCA3 knock-in founder lines with 92Q, 150Q and 223Q have been generated. No neurological phenotype in all lines at the age of 12 months has been observed but more aggregates were found in the first line (92Q).

Summary WP12 – Impact and communication (IC)

This work package aimed to:

• Develop an internal and external communication platform for the project to ensure effective information exchange within the consortium and with the wider neuro-field and establish the project’s profile and visibility
• Maintain close links with stakeholders, in particular patient organisations, industry and regulators, building on the already highly advanced existing stakeholder integration mechanisms developed by the consortium partners
• Deal with ethical issues raised by the project through a Project Ethics Council in close collaboration with a Patient Association Council, and ensuring the implementation of ethical and consent standards generated within RD-Connect
• Develop a training plan to educate additional sites in cutting-edge techniques to broaden the impact base of the project results to reach as many parts of Europe as possible (including E. Europe)

The NeurOmics project website has regularly been updated and functions as a highly demanded information tool for people from all over the world. > 10,000 users visited the website within the last year. The integrated intranet is password protected and provided a secure area for project partners to store and share key resources.

Impact and communication activities have been joined with RD-Connect to make use of available resources and to disseminate NeurOmics aims and achievements more widely. NeurOmics updates have been a regularly component of the RD-Connect newsletter. This newsletter is released once a month and reaches more than 1000 subscribers.

An outreach day has been organised jointly by NeurOmics, EURenOmics and RD-Connect at the final meeting in Berlin in May 2017 with multiple stakeholders invited. This outreach day was well received among the approximately 300 participants. The participants included researchers, politicians and representatives from patient organisations, health care organisations and pharma industry.

Ethics and the patient voice were combined within the RD-PEC (Project Ethics Council) and PAC (Patient Association Council). This brought together representatives and interests of NeurOmics, EURenOmics
and RD-Connect. The RD-PEC group is chaired by Pauline McCormack (UNEW) and it responds to any question about ethics, social issues and patient participation related to the work of RD-Connect, NeurOmics and EURenOmics. Anyone with an interest in the work can pose a question to the RD-PEC. In addition, project partners can seek the advice of this group.

The RD-PEC also includes members of the cross-cutting PAC (Patient Association Committee). The PAC consists of patients and patient representatives representing the diversity of diseases covered by RD-Connect, EURenOmics and NeurOmics projects. This group is chaired by Virginie Bros-Facer (EURORDIS) and regularly joined and contributed to the annual project meeting. One additional activity of this group has been to provide information on the RD-Connect website for patients and families. Since this group grew together over the course of the project and enjoyed discussing ethical issues and recent advantages in research, we aim to maintain this structure and keep them involved in further rare disease projects.

Regular training workshops to inform additional sites about progress and newly available data and tools were jointly organized with RD-Connect and arranged to coincide with annual meetings.

Summary WP13 – Research infrastructure
This work package aimed to:

• Integrate and utilise facilities and tools that have been developed over recent years in the field of neuromuscular and neurodegenerative diseases with the aim of maximising the output and impact of the project
• Develop the interface with the RD-Connect (including exchange of reference data and standards and harmonisation of resources) and the Support IRDiRC (for IRDiRC links and information exchange)
• Develop a plan for the integrated scientists and clinicians to improve clinical interpretation of Omics results

The Care and Trial Site Registry was expanded and holds now 350 sites in 52 countries of which 99 sites are registered for NDD. A ‘phenotype search facility’ has been implemented previously. Previously developed clinical data sheets (CDS) have been mapped to the Human Phenotype Ontology (HPO). The standardised terms have been used to create a phenotypic database at PhenoTips. Clinical data have been entered and stored at PhenoTips. Mapping of terms specific for the diseases studied in NeurOmics have been improved in a workshop in December 2014 with Peter Robinson (HPO, Berlin) and Mike Brudno (PhenoTips, Toronto). 16 NeurOmics partners attended the workshop. The PhenoTips development team in Barcelona and Newcastle is currently working on a solution to transfer SPATAX data directly into PhenoTips.

NeurOmics has deposited all WES, WGS and RNAseq data generated by deCODE, as well as sequencing data from targeted sequencing into the EGA archive that it can be securely stored and, importantly, shared via the RD-Connect platform. Annotation of data at EGA to lead to full submission of all NeurOmics-generated data is ongoing. First data sets have been created.

Summary WP14 – Project Management
This work package aimed to:

• Ensure that all budgetary actions are performed correctly and according to the rules and regulations established by the European Commission and the consortium agreement; ensure that the received funds are correctly distributed, and accounted for, including independent auditing.
• Provide the administrative and coordinative measures that help to ensure that the work and tasks are completed on time and within the budget and satisfy the high quality requirements as laid out in the work plan.

• Ensure that reporting is performed on a regular basis, in the most efficient and pragmatic way, according to EU Commission guidelines. The objective is to provide all consortium members with all important, relevant and impacting information that can influence the project’s outcome.

• Ensure appropriate links and coordination with Support IRDiRC, EURenOmics and RD-Connect projects and future related IRDiRC projects

• Ensure that the knowledge required for the work is accessible

• Ensure that the newly generated knowledge will be adequately used and/or exploited

• Ensure that all ethical requirements needed for the research are in place

Project coordination, execution of the Steering Committee’s decision: In order to deal with the scientific and administrative matter of the NeurOmics project we established a management structure which has proved its efficiency. The established structure at the coordination level comprises the co-coordinators, the Project Board, the Steering Committee and the management office at the University of Tübingen. The Steering Committee was being supported by several advisory committees i.e. the Scientific Advisory Board (SAB), the Rare Disease Patient and Ethics Council (RD-PEC), the Patients Advisory Committee (PAC) and the Innovation Council (IC).

At the operational level, an effective and successful operational workflow relied on the subproject leaders, the work package leaders and the disease coordinators. Disease coordinators were assigned to a major part of the project. They oversaw the different projects of their disease group and decided upon samples to be sent to deCODE for sequencing.

All issues relevant for the scientific progress of NeurOmics were regularly discussed in the Project Board which met at least twice per year and was led by the NeurOmics coordinator Prof. Olaf Riess. In case of urgent issues advice from the Project Board was requested by Email or at telephone conferences. The project management office was in charge of the day-to-day coordination and the administrative management of the consortium. A project manual was created, regularly updated and shared with the consortium.

Administrative and coordinative aspects of progress monitoring: Annual project meetings were held and organised as joint meetings or as back-to-back meetings with RD-Connect and EURenOmics. These meetings contained joint oral and poster sessions of all three projects. Most importantly, a joint outreach day focussing on key stakeholders in the rare disease field has been organised as part of the final meeting in Berlin in May 2017.

The implementation and monitoring of the work packages was performed in accordance with the established management structures. According to the reporting guidelines of the EC, annual periodic reports and deliverable reports have been created using specific templates, internally reviewed and submitted to the EC via the project management office.

Coordination with Support-IRDiRC, EURenOmics and RD-Connect projects: vivid exchange took place over the whole course of the project and resulted in several close links with RD-Connect and EURenOmics and IRDiRC projects. Highlights of these collaborations are:

• Joint meetings or back to back meetings with RD-Connect and EURenOmics,

• Regular coordinator calls, involving management and project coordinators of all three projects took place to discuss cross-project issues.
• Establishment of a joint Rare Disease Patient and Ethics Council (RD-PEC) and Patients Advisory Committee (PAC).
• Regular contributions from NeurOmics partners to the RD-Connect newsletter.
• NeurOmics partners contribute to the development and beta-testing of the bioinformatics pipelines to analyse genomic as well as metabolomics/ lipidomics data of the RD-Connect platform.
• Genomic data has been transferred to the European Genome-phenome archive (EGA; part of the EBI and partner in RD-Connect) and RD-Connect partner CNAG has re-analysed the available raw data. A beta-version of the RD-Connect platform is accessible for NeurOmics partners for further analysis of the identified variants.
• Bio-Prodict developed 3DM pathogenicity prediction tools which were integrated in the RD-Connect platform.
• NeurOmics adhered to the general IRDiRC policies i.e. collaboration in rare diseases research, the involvement of patients and their representatives in all relevant aspects of research, as well as the sharing of data and resources.
• NeurOmics partners are involved in IRDiRC activities as members of the scientific committees and of the different task forces which have replaced the working groups:

Knowledge management and exploitation of results: A data sharing policy has been developed and provides a framework for data sharing within and outside the consortium. It includes i) process for data sharing, ii) managed access, iii) applications for managed data access, iv) publication, and v) processing of data access requests. In order to manage access requests to data stored at EGA, a Data Access Agreement has been generated.

All kind of data generated within NeurOmics i.e. WES-, WGS-, RNAseq- and NGS panel-based genetic data, clinical as well as metabolomics and lipidomics data have and will be transferred and stored at EGA or MetaboLights. Clinical data sheets have been developed for each of the 10 neuromuscular and neurodegenerative diseases studied within NeurOmics by the respective disease coordinators to collect phenotype information of patients. Phenotypic data was collected in the online database PhenoTips. A use and exploitation plan was created and updated for all key project outcomes. The purpose of the task was to make sure that partners are following the rules stated in the Consortium Agreement.

Potential Impact:
Impact on clinical utility and patient health and well being
Improved diagnosis of rare NMD and NDD: The NeurOmics project widely contributed to the field of rare diseases at a time point when big pharma industries dramatically reduced their contribution to basic research of brain disease and neurodegeneration. It bridged the gap between the new biomedical research approaches and clinical practice by establishing and using advanced research infrastructures (cohorts, registries, biobanks, clinical outcome measures etc.). This enabled rapid and efficient transfer of NeurOmics outcomes into clinical practice, embracing improved diagnosis and personalised treatment of NMD/NDD for 500,000 – 600,000 patients in Europe.

Main achievements already found their way into standard diagnostics and clinical routine. A prominent example is the discovery and validation of disease causing mutations in almost 100 new genes. 81 modifications have been functionally validated and published by end of the project. Additional 25 publications describe expanded phenotypes for several diseases investigated in NeurOmics and a
A platform for standardised collection of phenotypical data has been established and filled with data from more than 1500 patients. In addition, several disease specific diagnostic panels developed within NeurOmics were successfully integrated into diagnostic routine and guidelines for clinical assessment and diagnosing rare diseases by WES/WGS or disease specific panel diagnostic have been published. The data sharing policy of the NeurOmics project facilitated the availability of huge amounts of WES/WGS, panel, metabolomics, lipidomics and transcriptomics data sets at EGA (European Genome-Phenome Archive), the RD-Connect platform and other platforms. Bio-Prodict, a NeurOmics partner, developed a 3DM mutation prediction systems which is now integrated into the RD-Connect platform and freely accessible. This availability of -omics data in combination with detailed phenotypic information and the tools provided by the RD-Connect platform will help to identify further disease causing gene mutations and rare diseases and to classify disease subtypes.

This clear focus on transferring NeurOmics outcomes into clinics already helped and will further help to diagnose rare disease patients and to make this process faster and more cost-efficient. It also enabled physicians to apply the most appropriate treatment as well as prevent possible complications and unnecessary or invasive test procedures. Beyond these benefits, it also allowed affected individuals and families to establish a supportive treatment plan and to make informed family planning decisions. The increase in availability of targeted genetic testing for familial NMD/NDD mutations linked with a high predictive value for positive and negative test results decreased and will further decrease the uncertainty and fear for patients which are often associated with an undiagnosed malady. Considering that correct diagnosis of a genetic disease does not only affect the tested person but also his close relatives we can multiply the number of people on which improved diagnosis has an impact by a factor between 2 and 5. Individualized treatments: Through its combination of Omics based diagnostics and Omics assisted therapy development, NeurOmics outcomes allow significant progress towards full implementation of individualised medicine in NMD/NDD.

To promote clinical trials and therapy development, a NDD/NMD care and trial site registry (CTSR) for collecting high quality, well annotated biospecimens (through harmonized biobanks and registries) was established and continuously expanded. The CTSR currently collects data on 350 sites in 52 countries which shows that the CTSR is known worldwide. 99 sites for neurodegenerative diseases are now registered in the CTSR and 74,368 patients are treated at all registered sites. This registry facilitated and will further facilitate statistically significant Omics studies for discovery and verification for rapidly developing new interventions in molecularly and clinically well characterized and stratified patient cohorts. It also allows to reduce time-to-trial significantly, to reach most appropriate to form specific cohorts and to ensure standards of care throughout the entire care and trial registry.

Several cell and mouse models facilitating basic research and therapeutic trials have been developed in the course of NeurOmics. hiPSCs (human induced pluripotent stem cells) which can be personalized and provide the opportunity to compare responses of tissue cells from different individuals were established for SPG5, SMA, HD and HMN patients. The corresponding NeurOmics researchers are interested in future collaborations and sharing of their hiPSCs. These cell lines provide the means that are appropriate and necessary for adjusting therapy protocols to stratified patient groups, thus increasing the efficiency of therapies and reducing the risks. In addition, 12 new mouse models covering SPG, SCA, LGMD, HMN and general neuropathy have been developed and characterized. The efforts in development and utilization of new disease models, combined with information gained from Omics experiments lead to the discovery of several druggable targets for HD, CMS, HSP and SMA. A potential drug for SPG treatment has been identified and will be further validated and tested.
The NeurOmics project also discovered a variety of biomarkers (biomarker panel for DMD, HSP and CMT) which allow monitoring and predicting disease progressions as well as efficiency of treatment before classical symptoms appear. These findings will facilitate treatment studies in the future. In addition, new exon skipping approaches have been developed for HD, MD and SCA3. Patents have been filed and both promising approaches will be further developed and validated in cooperation with pharmaceutical companies.

Social Impact
Reducing health costs: Neuromuscular diseases (NMDs) and neurodegenerative diseases (NDDs) include a range of highly heterogeneous, frequently devastating rare and ultra-rare conditions, which affect all age groups across the world. There are no cures and few effective treatments. Very few drugs have been licensed specifically for the conditions included in the NeurOmics project and the number of clinical trials is low.

Because of the lack of industry involvement in the past there is a real and unmet need for treatment options amongst the patient community. A collaborative project such as NeurOmics, which brought leading experts together with one common aim, helped to raise awareness for the needs of patients with NMDs and NDDs and accelerate trial readiness of the neuromuscular and neurodegenerative community.

At the beginning of the NeurOmics project, increasing life expectancy and improving quality of life for NMD and NDD patients required a complex diagnostic approach and a multidisciplinary care team. This means that the cost of the conditions, in terms of health economics, was enormous. Taking NMDs alone, a report in 2010 (Gustavsson et al., 2011) estimated that across 30 European countries, €8 billion was either spent on health care or lost due to reduced productivity. That equates to around €30,000 per patient per year. This is likely to be a conservative estimate, in part because a limited range of NMDs were included and because the estimate of the number of NMD patients was low. A study by the Muscular Dystrophy Campaign (Pohlschmidt and Meadowcroft, 2010) put the number of people living with a NMD in the UK at 71,000 compared to the European report’s figure for the UK of just 31,000.

Health economics data for the NDDs have been established for spinocerebellar ataxias (SCA) in Spain (Lopez-Bastida et al., 2008). The mean annual cost per patient with SCA was €18,776. The most important categories of costs were informal care, early retirement (permanent disability), medications, and orthopaedic devises. Moreover, a paper from 2011 (McCrone et al., 2011) placed a conservative estimate on the cost of medical services alone for 2 similar diseases, supranuclear palsy and multiple system atrophy, at up to €5.7 billion per year in the 27 EU countries.

Both the above mentioned figures allowed conclusions to be drawn for Huntington’s disease (HD), since HD and MSA have a similar prevalence and patients manifest with symptoms significantly overlapping those of SCA patients.

It is hard to estimate the benefit on the above mentioned costs gained by NeurOmics. However, NeurOmics significantly improved diagnosis of rare diseases resulting in lower costs for genetic testing, faster availability of profound results and minimisation of false treatment strategies. In combination with the identification of novel biomarkers, treatment approaches, druggable targets and potential drugs and the establishment of huge, stratified patient cohorts, we believe that NeurOmics outcomes will step by step reduce overall health costs.

Impact on awareness for the needs of patients with NMDs and NDDs:
NeurOmics put great efforts in keeping patients and stakeholders from politics and industry updated and informed, to enhance communication within these groups and to raise general awareness. In detail this
was achieved by:

- Establishing a Rare Disease Patients Advisory Council and Ethics Council (PAC and RD-PEC) together with RD-Connect and EURenOmics and providing a communication platform for this councils (online platform, newsletters and regular meetings)
- Keeping researchers, clinicians, pharma industry and other stakeholders updated by a regular newsletter (combined with RD-Connect)
- Organising a joint Outreach Day together with RD-Connect and EURenOmics in 2017 which was attended by approximately 300 researchers, patients and stakeholders.

Especially the joint Outreach Day reached a huge audience and generated very positive feedback from all invited stakeholder groups. To reach an even broader community contents of the Outreach Day have been combined in a report authored by members of NeurOmics, EURenOmics and RD-Connect which is currently being reviewed (Lochmüller et al., 2017). Following topics were discussed at the outreach day and are the main topics of the above mentioned publication:

Data sharing: This session explored the current trends in data sharing, as rare disease data is often fragmented, siloed and inaccessible for research. The session focused on the integrated genomics analysis platform, and other data sharing mechanisms, tools and requirements developed by RD-Connect and how this has been utilised to improve diagnostics and therapy. Discussion looked towards future challenges, both the technical and the legal, ethical and social implications of increased data sharing.

Diagnostics: Genetic testing has an increasing role in diagnosing rare disorders and is important for genetic counselling, carrier testing, prenatal diagnosis, preimplantation genetic diagnosis (PGD), identifying risk factors and may be useful for determining appropriate treatment. With the availability of Next Generation Sequencing (NGS) techniques (such as disease-specific multi-gene panels, whole exome sequencing [WES] and whole genome sequencing [WGS]) the diagnostic yield of genetic testing has significantly improved. The discussion focused on gene discovery, as yet undiagnosed patients and the next frontiers in rare disease diagnostics.

Therapies: This discussed the recent advances made in Omics research into the development of genetic and other targeted therapies. Recent years have seen new drugs and approaches making their way from the lab to the patient in rare neurological and renal diseases. This is an effort requiring patients, academics and industry. In this session we reflected on past efforts to identify success factors but also our mistakes, for the benefit of future therapy development in rare diseases.

These three sessions consisted of an introductory talk and a panel discussion. Panel members were equally recruited from all three projects and the audience lively contributed to the discussion.

Impact on European and international policies: The European Commission, in its White Paper ‘Together for Health: A Strategic Approach for the EU 2008-2013’ of 23 October 2007, which develops the EU Health Strategy, identified rare diseases as a priority for action. Taking up this priority, The NeurOmics project supported the European Commission communication of 11 November 2008 on “Rare disease: Europe’s challenge” (COM(2008) 679 final) and the Proposal for a Council recommendation of 11 November 2008 on a European action in the field of rare diseases (COM(2008) 726 final) which were calling for (among other things):

- Cooperation and knowledge sharing between centres of expertise: NeurOmics brought together leading experts in the field of NMD/NDD. Sharing of data and knowledge within and outside the consortium was a main goal of the project. All WGS/NGS and panel sequencing data (and corresponding meta data) has been transferred to the RD-Connect platform and EGA and will be available to a broad community. All
other –omics data will also be stored at appropriate databases (e.g. MetaboLights) and will be available at
the RD-Connect platform after last developmental steps have been completed by RD-Connect. Data and
knowledge sharing lead to several collaborations and shared publications within the NeurOmics
consortium and within NeurOmics and RD-Connect.

• Identify needs and priorities for basic, clinical, translational and social research in the field of rare
diseases and modes of fostering them, and promote interdisciplinary cooperative approaches
(NEUROMICS implements Omics research in NMD/NDD)

• Development of European guidelines on diagnostic tests: Several guidelines for genetic testing of
NMD/NDD have been published by the NeurOmics consortium. A paper comparing all disease specific
diagnostic panels developed in NeurOmics focusing on cost-effectiveness and reduction of time for
diagnosis is in preparation.

• Foster the participation of national researchers in research projects on rare diseases: The NeurOmics
consortium consisted of 19 partners and 7 associated partners from 8 European countries, Canada, USA
and Australia.

Most importantly, NeurOmics strongly contribute to the International Rare Disease Research Consortium
(IRDiRC), international initiative, with its goals of 200 therapies for Rare Diseases by 2020 and the
development of diagnostic tests for most rare diseases. Indeed, NeurOmics may be viewed as one of the
first genuine IRDiRC enterprises as it brought together leading groups from the vast majority of countries
that participate in IRDiRC in order to apply, transfer and utilize research driven by Omics technologies for
personalized medicine approaches in NMD/NDD. In this way NeurOmics was exploiting two of the major
opportunities that have been emphasised at the IRDiRC meeting in Montreal, Canada, 08-09 October
2011.

Main dissemination activities and the exploitation of results

In the course of NeurOmics at least 353 articles have been published in peer reviewed journals, 4 article
sections have been contributed to edited books and a minimum of 9 PhD theses have been submitted.
Since evaluation of results is not yet completed for some approaches, analysis tool provided at the RD-
Connect platform have been significantly improved and interesting, unexpected findings were obtained
which needed further validation in bigger cohorts or different disease models, we are expecting several
additional publications within the next months.

More than 400 times NeurOmics researchers presented their work at national or international conferences
(poster or oral presentation) and more than 35 interviews were given and articles were published in
popular press. In addition, 3 patents have been filed. A list of all dissemination activities can be found
attached to this report.

A Plan for Using and Exploitation of Foreground, with contributions from every partner, was prepared at
the beginning and continuously updated throughout the project. One goal of this document was to describe
the analytical and comprehensive approach and concrete basis for the strategies of visibility,
dissemination, implementation and exploitation of the research results. The table for use and exploitation
of results can be found attached to this report.

List of Websites:

http://www.rd-neuromics.eu

Address of the Coordinator: Prof. Dr. Olaf Riess, Institute of Medical Genetics and Applied Genomics,