Joint Programming Neurodegenerative Disease:
European research projects for the optimisation of biomarkers and harmonisation of their use between clinical centres

Acronym: SOPHIA

Title of the transnational collaborative project:
Sampling and biomarker **OP**timization and **H**armonization **I**n **A**LS and other motor neuron diseases

Runtime of the project: 3 years: 1 June 2012 – 1 June 2015

Report year: Year 1

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1. Short progress summary of the collaboration, major achievements

SOPHIA aims to develop optimally informative biomarkers for ALS, and establish stakeholder agreement regarding their use. This will be achieved by defining, validating and harmonizing optimal methodologies that can be reliably implemented within a pan-European framework program.

The provision of a common European strategy for the prioritization and selection of candidate biomarker domains for optimization and harmonization will in turn provide a long term platform by which existing collaborative structures that are relevant to neurodegenerative disease biomarkers (including academic initiatives, co-funding strategies, biobanks, industrial efforts, private-public alliances) are integrated within an inclusive web-based virtual biobank. The established platform will act as an important communication channel between this consortium and the broader international ALS/Neurodegenerative Diseases field, to ensure that the optimization efforts are consistently applied.

The work to be done for SOPHIA is subdivided in work packages (WPs). WP 1-3 relate to the optimization and harmonization of data management (WP1), sampling and molecular biomarkers (WP2) and imaging and neurophysiologic biomarkers (WP3a – Imaging Biomarkers; WP3b - Neurophysiological Biomarkers). WP4 is dedicated to the two-way communication with the entire ALS/ND field, to ensure that all input is acquired for efficient and effective optimization, and that all results are effectively disseminated for use within the wider neurodegenerative diseases field. WP5 entails project management and clinical coordination.

In general the SOPHIA project is well underway and each WPs is on track with its tasks and deliverables.

- The web-based data repository for SOPHIA (based on the Euro-MOTOR Progeny database) is being finalised and core clinical datasets have been defined by WP1. SOPs for collection and storing of biological material including serum, urine, CSF, DNA, RNA and fibroblast have been generated as well.

- WP2 is collecting a common clinical dataset from patients including optimally processed CSF and blood samples. Blood, DNA and CSF of 50 patients with ALS were sampled, as well as 100 samples of reference material (CSF and serum samples) of control patients. A first integration into a round-robin system has been implemented, and first results are in, showing that measurement of neurofilaments seem to be most promising with regard to differential diagnostic use.

- WP3 is developing quantitative markers of ALS-related tissue damage using MRI and MUNIX measurements.

  In WP3a the central repository system for MRI data has been commissioned and access for SOPHIA centres has been enabled through OpenSSH secure connections on the backbone of the NISALS MRI repository developed by the WP leader. Quality control algorithms are being developed and implemented to automatically identify systematic and centre specific deviations in T1 images. Currently, a feedback system is being established to inform SOPHIA centres about the status of the centre specific MRI data analyses in the repository. The interface to the central clinical data management system is being established.
  A comprehensive toolset to automatically estimate specific sources of quality loss is in use for T1 images. A first recommendation for the acquisition of T1 and DTI images in SOPHIA centres has been delivered. Data gathering for the first cross-sectional SOPHIA T1 quality check and deviations analysis is at 60%.

  WP3b has booked progress by providing MUNIX investigator training to 11 participating centres over 2 courses in 2012. The detailed MUNIX protocol including SOPs has been disseminated to all participating centres, and ethical approval has been issued in three centres already (St. Gallen, Warsaw, Lisbon). These centres have started collecting longitudinal data to meet the other tasks in this WP.

- Work on the interactive European ALS biomarker website is progressing within WP4. WP4 is setting up the basis for the website together with the project coordinators of SOPHIA (NET) and in collaboration with WP5. The website should go live in April 2013.

- Six months into the project, the major achievement in overall project management (WP5) is the implementation of the SOPHIA Consortium Agreement among project partners and the start of a clear communication system through building of a project website (including logo) and distribution of (bi-annual) newsletters. The new logo can be seen below:

All in all the SOPHIA consortium is well on its way to provide an interactive European ALS biomarker website with an open innovation platform accessible to the general public, patient organisations, industry and policy makers, etc., and a restricted section containing a virtual biobank for the scientific ALS and ND community. The members aim to have the full website including all its functionalities up and running by the end of the first project year, in June 2013.

2. Short progress summary of the respective subprojects

WP1 Data & Sampling Infrastructure

WP1 aims to ultimately provide optimized and harmonized SOPs of patient biomarker assays, analytic methods and neuropathological techniques mapped to defined pathological and genetic subtypes of disease. Utilizing existing technologies, including a web-based data repository provided by the Euro-MOTOR consortium, this work will provide a fully integrated virtual biobank from which biological samples and clinical/imaging/neurophysiologic and neuropathological datasets provided by participating members can be utilized to enable state of the art collaborative analyses. The virtual biobank will be accessible though the project website, which is being set up by members of WP4 and WP5.

To establish the virtual biobank within WP1, the web-based data repository provided by the Euro-MOTOR consortium (Progeny) is used. A structure for the SOPHIA part of the database system is being developed and scheduled to go live by Q2 2013 (T2 / deliverable 2). For SOPHIA, this biobank will enable integration of core clinical variables with biomarker datasets from all patients from the participating centres.

Definition of a core clinical dataset is on track and CDEs have been defined and shared (T1 / deliverable 1). Relevant clinical variables have been defined and chronological-progression markers based on validated functional scores have been included (deliverable 1.1).

The WP1-members have completed the generation of SOPs for the collection and storing of biological material including serum, urine, CSF, DNA, RNA and fibroblasts (T3 / deliverable 4). These SOPs will be uploaded to the project website once it is live (T8).

All other tasks / deliverables within WP1 are on track. Genetic screening of existing donor cohorts is underway and GWAS data on a number of cases is already available. Early protocols for a consolidated database of existing brain donor cohorts are available (T7 / deliverable 3).

WP2 Molecular Biomarkers

WP2 interacts directly with WP1, ensuring collection of a common clinical dataset from patients for whom optimally processed CSF and blood samples are also available, with the ultimate goal of generating well defined patient cohorts for scientific interrogation of the biological data.

The first task (T1) of this WP was to come to a consensus regarding which protocols to use for biosampling and which biomarkers to measure. Extensive work in this area had been done beforehand already (Otto et al. 2011). Within the first months of the SOPHIA project, blood, DNA and CSF of 50 patients with ALS were sampled as high quality reference material (deliverable 2). The most recent SOPs were applied (Otto et al. 2012) (deliverable 1.1). Additionally 100 samples of reference material (CSF and serum samples) of control patients were collected (deliverable 1.2). Sampling protocols for stem cells and for hair cells are still under investigation.

The next step (T2) was to implement a first integration into a round-robin system, an interlaboratory quality control program which involves sending the predefined standardized samples out as an external quality control of local test performance. First results of this round-robin system have been obtained. It turned out that measurement of neurofilaments seem to be most promising with regard to differential diagnostic use, whereas markers like MCP-1, progranulin, and sAPP only have a minimal differential diagnostic use, see Table 1.

For the teaching initiative (T2) the group used existing protocols of the German Society of Neurochemistry and CSF Diagnostics. This protocol was adapted for the SOPHIA group. However, thus far no teaching has been given to the whole group. There have been local meetings and two hands-on courses in English at the University of Ulm. The first hands-on course for the entire SOPHIA group will be given in 2013, which is still within the timeframe set for this deliverable (deliverable 2.1).

Table 1: Results of the first interlaboratory quality control round

	Lab 1		**Lab 2**		**Lab 3**		**Lab 4**		**Lab 5**		**Lab 6**	
	CON	ALS	CON	ALS	CON	ALS	CON	ALS	CON	ALS	CON	ALS
Tau [pg/ml)	223.6 ±178.7	223.4 ±230.1	99.8 ±24.0	228.4 ±73.4	89.9 ±33.4	143.8 ±44.1	78.2 ±75.8	54.0 ±26.2	114.4 ±41.4	180.2 ±99.8	118.2 ±22.3	166.0 ±99.9
Abeta [pg/ml]	700.0 ±375.5	705.0 ±222.3	844.0 ±607.5	880.2 ±317.0	845.0 ±184.2	825.8 ±136.5	492.0 ±121.7	488.0 ±345.4	519.2 ±107.9	582.8 ±375.5	992.6 ±679.9	980.6 ±607.5
Cystatin C [ng/ml]	3234 ±607	3553 ±997	2028 ±1133	2223 ±376	1724 ±680	1928 ±608	2462 ±3170	2152 ±1842	1820 ±1365	2006 ±1217	1794 ±345	2038 ±1079
NFH [pg/ml]	832 ±779	3503 ±1601	287 ±249	5130 ±4603	176 ±74	4876 ±7043	238 ±281	1372 ±1248	565 ±442	1972 ±748	146 ±44	3288 ±3866
S100 [ng/ml]	2.28 ±2.62	1.14 ±0.37	1.22 ±0.56	1.72 ±0.64	0.44 ±0.25	0.30 ±0.10	0.60 ±0.64	0.54 ±0.50	1.22 ±0.70	1.16 ±0.31	1.18 ±0.49	1.12 ±0.38
Progranulin [ng/ml]	2.12 ±0.78	4.7 ±2.86	2.62 ±1.17	2.66 ±1,76	2.18 ±1.09	1.84 ±0.64	1.78 ±1.48	1.74 ±0.86	1.36 ±0.17	1.50 ±0.27	1.74 ±0.50	1.64 ±0.11
MCP-1 [pg/ml]	3691 ±2547	2923 ±763	2153 ±996	3127 ±605	2667 ±948	2286 ±595	4276 ±649	2223 ±865	2017 ±337	3171 ±1406	2079 ±966	1940 ±644

WP3

WP3 extends the clinical data in WP1 with standardized quantification of brain (MRI; upper motor neuron) and spinal cord (MUNIX; lower motor neuron) motor neuron involvement, the hallmarks of ALS. Computational MRI analyses are inherently investigator-independent but contain numerous potential error sources (i.e. protocol, scanner type and analysis tool). Validated SOPs for MUNIX measurements exist, but investigator training is essential to establish cross-centre reliability. Thus, to implement optimization and harmonization, MRI requires continuous multicentre comparative analyses feeding identified sources of quality loss back to ALS centres. MUNIX demands analysis of factors influencing test-retest reliability across centres.

WP3a Imaging Biomarkers (MRI)

WP3a is finalising deliverable 1 of its list: The Jena-based MRI database has been set up by GER2 and UK2 through a secure root repository server at sftp://nedigs03.med.uni-jena.de. Access is enabled in a centre specific way through an OpenSSH sftp protocol only without shell access. All participating SOPHIA centres have been registered to this MRI database and are currently uploading initial datasets.

The members of WP3a are also establishing the bidirectional interface to the core clinical data management in WP1 (T2) to obtain harmonization of the MRI acquisition with the clinical dataset: The interface is being defined as an API and its content tested to ensure the subjects identity is preserved in a pseudomized fashion across databases. A core translation table in the MRI repository and the central clinical database has been achieved by GER2, NET, IRE and UK2 (deliverable 2).

Finally, the workgroup is performing the assessment of acquisition parameters driving variability in T1W and DTI datasets (GER1, GER2 and UK2) (T3). A T1 quality control toolbox is available in an alpha version and it is being tested in a subgroup of ten centres. This resulted in a positive automated identification of grossly aberrant datasets. Quantification of the amount of noise, geometric distortion, contrast variability and overall quality is on-going. The group aims to publish results of the detailed study of variability in acquisition and analysis parameters across multiple centres within 1.5 year after the SOPHIA project started.

WP3a is on track with its deliverables and tasks.

Note: Some SOPHIA centres are newly establishing ALS MRI scanning with high resolution imaging and require scanner and centre specific recommendations which cannot be validated at this stage. For a first approximation, best guess recommendations are given.

WP3b Neurophysiological Biomarkers

Investigator training in using MUNIX is one of the key tasks (T1) within WP3b, to establish cross-centre reliability in those measurements. Two training courses for participating centres have been organised within SOPHIA so far. The first course took place in St. Gallen on 23 March 2012, the second course took place during the ENCALS meeting in Dublin, on 25 May 2012. During these courses theoretical lectures were given by Professor E. Stalberg and S. Nandedkar. After the presentations participants were instructed and could familiarize themselves with the MUNIX technique during hands-on sessions. At the end of the course participants were able to independently perform MUNIX measurements on various muscles relevant for the SOPHIA project. The following project partners were present during the courses: NET, GER1, GER2, IRE, ITA1, POL1, POR1, SWI, UK1, FRA, BEL.

The detailed MUNIX protocol including SOPs has been disseminated to all participating centres. Ethical approval has been issued in three centres (St. Gallen, Warsaw, Lisbon), the other centres are in the process of obtaining ethical approval (~ deliverable 1 of WP3b – *Implementation of SOPs MUNIX measurements*).

The three centres that obtained ethical approval are in the process of collecting longitudinal data to meet T2 and T3 of WP3b: Each investigator is required to study 4 patients at their home institution twice. Variability will be analysed centrally, and the results will be fed back to the investigators. Longitudinal measurements can be proceeded if variability is less than 20%.

All other tasks and deliverables for WP3b are on track.

WP4 Open Innovation Platform

WP4 aims to set up an interactive European ALS biomarker website with an open innovation platform accessible to the general public, patient organisations, industry and policy makers, etc., and a restricted section containing a virtual biobank and data retrieval and deposition features for the scientific ALS/ND community. WP4 will set-up the basis for the website together with the project managers of SOPHIA (WP5). Design and implementation of the virtual biobank will be performed in close cooperation with WP1-members.

Since the project kick-off meeting in September work on website design has started by project partner NET. The website, which will serve as a vehicle to support implementation of the pan-European methodology on ALS biomarker optimization and harmonization, should be up and running by April 2013, which is slightly later than scheduled (deliverable 1). Integration with the virtual biobank (WP1) will take place once the website is live. Work on the other tasks and deliverables within WP1 is on track.

WP5 Management, Clinical Coordination & Ethics

WP5 entails overall management of the project and the consortium, focussing on communication among members but also externally to JPND and the wider ND research community, arrangement of meetings, management of budget, scheduling and project objectives, etc.

The following actions have been taken since project start in June 2012, fulfilling several WP5 tasks and taking care of the WP deliverable regarding annual progress reporting to JPND and national funding agencies:

- After the official project start on 1 June 2012, the Project Coordinator (PC) organised a SOPHIA kick-off meeting on 12 and 13 September 2012, at which representatives of all project partners were present. The 2-day meeting was held in Sheffield, UK. The agenda and minutes of the kick-off meeting were afterwards provided to all members by the PC.
- The PC has drafted a Consortium Agreement (CA) to define and implement technical, managerial, financial and IPR related provisions to enable partners to carry out their work. The CA has been reviewed by all project partners and will be send out for signing by all by 28 February 2013.
- Fulfilling its communication task within the consortium, the PC released the first SOPHIA newsletter in January 2013. At the same time, the PC checked up on project progress, looking at schedules, deliverables and milestones for each WP.

- The PC is currently setting up the next meeting, which will be held in September 2013.
- The PC is working on a project website, in collaboration with WP4 (more details in paragraph *WP4 Open Innovation Platform*). A clear SOPHIA logo to facilitate communications has been designed and approved by the consortium. See below a screenshot of the first draft of the website:

3. Amendments to the original work plan (if applicable) and its rationale
Currently not (yet) applicable

4. Problems and their solutions (if applicable)
Currently not (yet) applicable

5. Publications (please state only direct outcome of the funded project)
Currently not (yet) applicable

6. Patents, PhD thesis and other outcomes (if applicable)
Currently not (yet) applicable

7. Exchange of researchers, students etc. (if applicable)
Currently not (yet) applicable