

BIORESOURCE PAPER

Biobank of Cells, Tissues and DNA from Patients with Neuromuscular Diseases: An Indispensable link between Clinical Centers and the Scientific Community

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The Biobank was established in 1986 as part of the routine diagnostic activity of the Division of Neuromuscular Diseases and Neuroimmunology, of the Carlo Besta Neurological Institute. It stores muscle tissue, cells and DNA from patients with neuromuscular diseases.

The biobank provides samples as a service to the scientific community conducting research on neuromuscular disorders. Samples are from patients affected by different forms of muscular dystrophy, including the severe congenital and Duchenne muscular dystrophies, as well as limb girdle muscular dystrophies, congenital myopathies, distal and myofibrillar myopathies, inflammatory myopathies, and metabolic myopathies. Different types of biomaterials are frequently available from a single patient.

The Biobank is founding partner of the EuroBioBank network, the first operating network of biobanks for rare diseases in Europe, and of the Italian Telethon Network of Genetic Biobanks. The involvement of the biobank into both networks has been instrumental for standardization of procedures and activities, implementation of sample access policies, and compliance with ELSI requirements. The biobank, with about 13000 biospecimens stored in total at the time of writing, constitutes a key source of biological samples for researchers worldwide.

Keywords: Biobank; neuromuscular diseases; muscle tissue; cell cultures; DNA

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(1) Bioresource Overview

Project description

The biobank of “Cells, tissues and DNA from patients with neuromuscular diseases” (NeuMD) was established in 1986 based on the routine diagnostic activity of the Neuromuscular Diseases and Neuroimmunology Unit of the Carlo Besta Neurological Institute in Milan. This is a leading center for the diagnosis and treatment of neurological disorders and for pre-clinical and clinical research. The biobank was started as collection of muscle tissue samples; muscle cells and DNA were subsequently added. Very recently the biobank has started collecting also serum, fibroblasts and lymphoblasts from selected cases.

The NeuMD biobank has received funds from the Italian Telethon almost uninterruptedly since 1999 and was founding partner of the Telethon network of genetic disease biobanks (TNGB) (<http://biobanknetwork.telethon.it/Pages/View/Catalogue>), a non-profit association of

Italian repositories, created in 2008 with the aim of developing a virtually unique catalogue of biological samples and associated data [1]. Such catalogue lists, at present, more than 750 rare genetic defects.

The biobank is founding partner of the EuroBioBank (EBB) network (www.eurobiobank.org) [2], the first operating network of biobanks in Europe, providing human biological samples as a service to the scientific community conducting research on rare diseases (RDs).

The involvement of the NeuMD biobank in the TNGB and EBB networks has been instrumental for improving the quality control process, for standardization of biobanking procedures and activities, for implementation of sample access policies and for compliance with ELSI requirements.

In 2014, the NeuMD biobank joined the Italian node of the BBMRI-ERIC consortium (<http://www.bbMRI.it/home>), and, in 2015, the NeuMD ID card has been included in the biobank catalogue of the RD-Connect

platform (http://catalogue.rd-connect.eu/web/bank-of-the-muscle-cell-biology-laboratory-fondazione-i.r.c.c.s-istituto-neurologico-carlo-besta/bb_home), a European global infrastructure project, funded under the FP7 program, whose main aim is to link up databases, registries, biobanks and clinical bioinformatics data for rare disease research (www.rd-connect.eu) [3].

To date, the contribution of the NeuMD biobank has been acknowledged in 68 scientific publications.

Classification (1)

Human

Species

Human

Classification (2)

Biological samples and associated data.

Context

Spatial coverage

Via Giovanni Celoria, 20133 Milan, Lombardy, Italy.
Latitude: 45 degrees, 28 minutes, 38.856 seconds.
Longitude: 9 degrees, 13 minutes, 50.1054 seconds.

Temporal coverage

Active since 1986.

Temporal coverage for accessibility

N/A

(2) Methods

NeuMD follows the Standard Operating Procedures (SOPs) of EBB (<http://www.eurobiobank.org/en/documents/sops.htm>) and TNGB (http://biobanknetwork.org/documents/0-TNGB_SOPs_Aug2012_online.pdf).

Steps

Compliance with ELSI requirements

Samples are collected for diagnosis by the laboratory of the Unit of Neuromuscular Diseases and Neuroimmunology. After signing an informed consent form for minimal surgery, patients undergo a muscle biopsy, which can either be performed as open surgery or as needle biopsy [4]. In addition, patients are informed about depositing samples in the biobank and about their possible use for research, and asked to sign a biobank specific consent form.

Sample Collection

For all biopsies:

- two samples are snap frozen in isopentane precooled in liquid nitrogen for histology and biochemistry;

for most biopsies:

- a small tissue fragment is fixed in glutaraldehyde for electron microscopy;
- another fragment is collected in sterile medium for myoblast culture and processed as described [5];

- a blood sample is also withdrawn for DNA extraction.

The samples are assigned a laboratory code and, those to be stored in liquid nitrogen, also a unique bar code.

Data Collection

Patient personal data, bar code and laboratory code, are registered in the biobank database. Data and sample codes are also recorded in a database of the Unit containing the muscle biopsy reports. The sample data set of this database includes personal data (name, date of birth, address, ethnic origin), sex, phenotype (affected/not affected), essential anamnestic data, presence of consanguinity, presence of familiarity, diagnostic tests performed on the muscle biopsy and, when available, DNA molecular tests and diagnosis, and internal unit or external center sending the patient.

Sample storage

DNA samples are stored in freezers with protection against electrical failure.

Muscle biopsies and cell cultures, after receiving a bar code uniquely identifying each sample, are transferred to a company in charge for the management of all Institutional biosample collections that are to be stored in liquid nitrogen (www.sol.it) (certified UNI CEI EN ISO 13485:2002).

The company ensures sample storage conditions by continuous presence of personnel during working hours, alert systems during non-working hours, restricted access to the bank facility; and guarantees traceability of samples and security of data by use of dedicated software and computers, use of passwords enabling different levels of access to data, updated antivirus and security software.

Sample access

Applications for accessing biological samples normally reach the biobank director either through the TNGB website or the EuroBioBank website. In both cases the biobank manages all sample requests through the TNGB website, upon users' registration and description of the project. All submitted requests are collected in a unique Request Control Panel, which is visible to all members of the network. After evaluation and approval of the submitted project (see access criteria), a "Material Transfer Agreement" (MTA) form and an order form for invoicing the service costs, are sent to the researcher. The website keeps records of all requests for biological samples, including those requests refused for any reason.

Stabilization/preservation

Glycerol or DMSO (cryopreservation) for cells; none for tissues.

Type of long-term preservation

Liquid nitrogen for tissues and cells; freezers with protection against electrical failure for DNA.

Storage temperature

From $-20\text{ }^{\circ}\text{C}$ to $-180\text{ }^{\circ}\text{C}$ (liquid nitrogen).

Shipping temperature from patient/source to preservation or research use

Flask of cell cultures and DNA samples are shipped at room temperature; frozen tissues, frozen cell lines, and RNA samples are shipped in dry ice. Tissue sampling for shipping is done inside a cryostat chamber at -25°C using pre-cooled tools.

Quality assurance measures

The quality of the material is ensured by compliance with standard operating procedures (available for each step of the process), established in collaboration with the Eurobiobank (<http://www.eurobiobank.org/en/documents/sops.htm>) and TNGB (http://biobanknetwork.org/documents/0-TNGB_SOPs_Aug2012_online.pdf) networks. In addition, regular updating of the control quality process for the annual renewal of UNI EN ISO9000 certification, maintains good quality practices in the laboratories and in the biobank of the Neuromuscular Diseases and Neuroimmunology Unit.

Main measures

- Regular inspections to guarantee that the cell viability and absence of mycoplasma, bacteria, and fungi;
- Systematic detection/exclusion of mycoplasma by appropriate tests before distribution service;
- Assessment of DNA integrity by agarose gel electrophoresis;
- Adoption of procedures for correct sample authentication, based on bar code;
- Controlled access to the cryocontainer room and regular replenishment of liquid nitrogen by the assigned staff of SOL Company;
- Alarm system in cell culture incubators to control variations in temperature and % of CO_2 ;
- Periodic clinical revision of the patients' data chart by clinicians experts in the field.

Source of associated data

Most samples stored derive from the diagnostic activity of the Division of which the bank is part; therefore data derive from medical records, instrumental tests and laboratory reports.

Ethics statement

Muscle biopsies are obtained after informed consent of patients or parents (in the case of children). Patients or their parents sign a written informed consent, which has been approved by the local Ethics Committee. The biobank follows confidentiality and data protection rules in compliance with principles or guidelines delineated in the following documents:

- Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine (Oviedo Convention, issued on 04 April 1997) and the Additional Protocol to the Convention on Human Rights and Biomedicine, concerning Biomedical Research (CETS n. 195) (Strasbourg, 25 January 2005) and concerning

Genetic Testing for Health Purposes (CETS n. 203), 2008 (Strasbourg, 27 November 2008).

- European Recommendation CM/Rec(2016)6, of the Committee of Ministers to member States on research on biological materials of human origin (Adopted by the Committee of Ministers on 11 May 2016).
- International Declaration on Human Genetic Data, 2003 (16 October 2003, 32nd session, the General Conference of UNESCO).
- Telethon-SIGU (Società Italiana di Genetica Umana) guidelines for genetic biobanks, 2003.
- Italian Data Protection Authority (Garante della Privacy), Personal Data Protection Code, Legislative Decree no. 196, 30 June 2003, published in the Official Gazette of the Italian Republic, No. 174, 29 July 2003.
- Italian Data Protection Authority (Garante della Privacy), General Authorisation for the processing of genetic data, 12 December 2013, published in the Official Gazette of the Italian Republic, No. 302, 27 December 2013.
- Italian Data Protection Authority (Garante della Privacy), General Authorisation to process personal data for scientific research purposes, 01 March 2012, published in the Official Gazette of the Italian Republic, No. 72, 26 March 2012.

Constraints

N/A

(3) Bioresource description**Object name**

Neuromuscular Disease oriented biobank.

Bioresource name

Cells, tissues and DNA from patients with neuromuscular diseases.

Acronym: NeuMD.

Bioresource location

IRCCS Foundation Carlo Besta Neurological Institute, via Libero Temolo 4, 20126 Milan, Italy and Via Giovanni Celoria 11, 20133 Milan, Italy.

Bioresource contact

- marina.mora@istituto-besta.it
- Ph. +39 02 2394 2632/2625
- Fax +39 02 2394 2619

Bioresource URL

<http://www.eurobiobank.org/en/partners/partners.htm>
http://catalogue.rd-connect.eu/web/bank-of-the-muscle-cell-biology-laboratory-fondazione-i.r.c.c.s-istituto-neurologico-carlo-besta/bb_home
<http://www.istituto-besta.it/biobank>

Identifier used

Double code: one internal for the staff and one external published in the catalogue.

Bioresource type

Rare Disease and genetic biobank.

Type of sampling

Disease based, sampled in clinical care.

Anatomical site

Predominantly quadriceps femoris, but also deltoid, biceps brachii, tibialis anterior, gastrocnemius and intercostal muscles; skin; blood.

Disease status of patients/source

Patients affected (muscle tissue) and healthy/carrier relatives (DNA).

Clinical characteristics of patients/source

General data (age, gender, disease) and all patient related information that is stored in the clinical information system of the Institute (upon request).

Size of the bioresource

- To date: 5896 individuals/13,380 samples.
- Average of the incoming samples per year: approx. 250 muscle samples, approx. 100 cell lines and 300–400 DNA.
- Average of the outgoing samples per year: approx. 170 samples.

Vital state of patients/source

Alive at the sampling.

Clinical diagnosis of patients/source

Neuromuscular disorders: muscular dystrophies, metabolic myopathies, mitochondrial myopathies, congenital muscular dystrophies, congenital myopathies, myotonic dystrophies, muscle channelopathies, spinal muscular atrophies, motor neuron diseases, inflammatory myopathies, undetermined myopathies (endocrine, dysmetabolic, toxic) and peripheral neuropathies.

Pathology diagnosis

Histological for all patients, with immunochemical tests (immunohistochemistry and Western blot) and/or genetic diagnosis for most of them.

Control samples

Individuals that have undergone a muscle biopsy, but resulted unaffected by clinical and histological criteria.

Biospecimen type

Frozen muscle tissue; frozen stocks of myoblasts, fibroblasts, and lymphoblasts; DNA; resin-embedded muscle samples. Sera are being collected in a few cases.

Release date

N/A

Access criteria

To access the Biobank service, rules are applied to all researchers, including Biobank staff, according to criteria agreed among the TNGB directors, as follows:

- The biobank must guarantee the storage of an adequate aliquot of patient samples in order to offer the possibility of retrospective analysis on the basis of more recent scientific acquisitions;
- Sample distribution should be only to qualified professionals working at research or medical institutions involved in health-related research or health care;
- Project employing the samples must be compliant with the biobank mission;
- Justification for use must be convincing (description of the research project and expected results should be sound, the PI scientific track record should be adequate, and a grant sponsor be present);
- Results obtained using the samples including results relevant for patients' health should be made available in confidence to the biobank director and the biobank should be acknowledged in the resulting scientific publication;
- Shipping costs and partial coverage of the cost of some basic procedures related to the Biobank distribution service (cost recovery) should be paid to the biobank.

(4) Reuse potential

Reuse is possible for diagnosis and research in the field of the disease for which the samples have been acquired, with the permission of the biobank director, who must be contacted directly by the applicant. If necessary, a new specific consent is sought and a new MTA issued.

Acknowledgements

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Competing Interests

The authors have no competing interests to declare.

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 Zanotti S., Cell lines processing, Quality control
 Curcio M., Cell lines processing, Annotation of data
 Canioni E., DNA processing, Quality control
 Salerno F., Muscle biopsy processing, Annotation of data
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 Saredi S., Cell lines processing, Annotation of data
 Ruggieri A., Cell lines processing, Annotation of data

Pasanisi M.B., Patient clinical assessment

Bernasconi P., Genetic diagnosis

Mantegazza R., Patient clinical assessment, Director of the Unit hosting the Bioresource.

Maggi L., Muscle biopsy diagnosis and patient clinical assessment

Andreetta F., Cryobank sample flow management

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