Lombardia GENS: a collaborative registry for monogenic diseases associated with stroke

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Summary

The Italian region of Lombardy, with its existing stroke centers and high-technology laboratories, provides a favorable context for studying monogenic diseases associated with stroke. The Lombardia GENS project was set up to create a regional network for the diagnosis of six monogenic diseases associated with stroke: CADASIL, Fabry disease, MELAS, familial and sporadic hemiplegic migraine, hereditary cerebral amyloid angiopathy and Marfan syndrome. The network comprises 36 stroke centers and seven high-technology laboratories, performing molecular analysis. In this context, all stroke/TIA patients fulfilling clinical criteria for monogenic diseases are currently being included in an ongoing study. Demographic, clinical and family data and diagnostic criteria are collected using standardized forms. On the basis of stroke incidence in Lombardy and the reported prevalence of the diseases considered, we expect, during the course of the study, to collect datasets and DNA samples from more than 200 stroke patients suspected of having monogenic diseases. This will allow evaluation of the regional burden and better phenotype characterization of monogenic diseases associated with stroke.

KEY WORDS: cerebrovascular disease, genetics, monogenic disorders, stroke

Introduction

Stroke is a leading cause of mortality and long-term disability. To relieve the heavy burden of stroke, there is a need for better understanding of its pathogenetic mechanisms, in order to improve its prevention and treatment. The genetic contribution to stroke is well established and there is evidence that genetic factors influence stroke occurrence and outcome (1,2). Underlying monogenic diseases account for about 1% to 5% of all strokes, although their incidence is believed to be underestimated. In fact, monogenic disorders can be misdiagnosed simply because physicians fail to include them in the differential diagnosis. Moreover, even when there are elements that may support a genetic cause, such as young age at onset, positive family history, presence of specific associated clinical features, and absence of conventional vascular risk factors, the wide phenotypic spectrum makes it difficult to select patients and decide which disorders to screen for. However, these disorders, although rare, are usually life-threatening or chronically debilitating diseases; because of their low prevalence and high complexity they are also difficult to manage. The diagnosis of these disorders is important both for genetic counseling and therapeutic decision-making (3-5). In this context, the identification of a large number of patients affected by monogenic diseases may help to better clarify stroke pathogenesis and lead to the development of potential new drugs and therapeutic targets. Also, careful selection of phenotypes is considered an essential requirement for all genetic studies (6).
In the Italian region of Lombardy (about 6 million inhabitants) there is a clinical network of neurological centers specialized in stroke diagnosis and care, including 29 stroke units; the region also has seven high-technology

laboratories with well-established expertise in stroke genetics. The presence of these centers prompted the creation of a regional stroke genetics network (Lombardia GENS) designed to exploit the databases of the existing clinical stroke network and also benefit from collaboration with laboratories interested in stroke genetics.
The Lombardia GENS project is a prospective multicenter cohort study aimed at organizing a structured service for complete diagnosis of six single-gene disorders associated with stroke [CADASIL, Fabry disease, MELAS, familial and sporadic hemiplegic migraine (FHM/SHM), hereditary cerebral amyloid angiopathy (H-CAA), and Marfan syndrome] through the setting up of a specific diagnostic work-up for patients with clinically suspected genetic diseases. The aim of the project is to create a regional database and DNA biobank of well-phenotyped stroke patients for studies on regional incidence of monogenic diseases in stroke, phenotypic characterization, and therapeutic trials.

Study design and population

The study population consists of a continuous series of patients with stroke or transient ischemic attack (TIA), in whom there is clinical suspicion of one of the following monogenic diseases: CADASIL, Fabry disease, MELAS, FHM/SHM, H-CAA, or Marfan syndrome, referred during the study period to the clinical units participating in the project. Both ischemic and hemorrhagic strokes are included. The coordinating center managing the project is the Neurology Unit at the Ca' Granda Foundation, Maggiore Policlinico Hospital, IRCCS, University of Milan, Italy, which is supported by a scientific steering committee (SC). The SC comprises members from the Neurology Unit, Ca' Granda Foundation, Maggiore Policlinico Hospital, IRCCS, University of Milan; the Cerebrovascular Unit, Carlo Besta Institute of Neurology Foundation, IRCCS, Milan; the Emergency Unit, C. Mondino National Institute of Neurology Foundation, IRCCS, Pavia; the Stroke Unit, Niguarda Ca' Granda Hospital, Milan; the Stroke Unit, Division of Neuroscience & Institute of Experimental Neurology (INSPE), San Raffaele Scientific Foundation, Milan, the CNS Inflammatory Unit, Division of Neuroscience & Institute of Experimental Neurology (INSPE), San Raffaele Scientific Foundation, Milan, the Stroke Unit, San Gerardo Hospital, University of Milan-Bicocca, Monza, and the Department of Clinical and Experimental Sciences, Neurology Clinic, University of Brescia, Italy.
Clinical centers that hospitalize more than 100 patients for stroke annually are included. The project envisages a two-step diagnostic pathway. Through a standardized form, stroke physicians in the participating units collect the demographic and clinical data of all stroke patients with a suspected genetic cause ('probable cases'). The form includes demographic data, family antecedents, racial descent, current pathologies, and stroke risk factors. Strokes are classified according to established standardized criteria (hemorrhagic, ischemic, TOAST, and Oxfordshire classification). Findings from neuroimaging and instrumental examinations are also recorded, as are other associated neurological or systemic clinical features. It is also envisaged that physicians fill in clinical and radiological diagnostic algorithms, developed from literature data and specific for each monogenic disease in order to address the disease suspicion ('suspected case').
The use of these standardized forms and a centralized training procedure for all clinicians participating in the patient data collection ensures standardized and homogeneous data collection across the participating centers. Furthermore, the validity of the screening procedure is periodically validated at meetings of representatives from the centers and laboratories and the group of experts belonging to the scientific steering committee. All data are stored in a computer database (Access format). On receipt of forms from the centers and laboratories, data are entered centrally by the staff at the coordinating center, who also apply quality evaluation processes. Two advisors (Prof. Hugh Markus, Centre for Clinical Neuroscience, St George's University of London, and Dr Caspar Grond Ginsbach, Neurologische Klinik Universität, Heidelberg) collaborated on the project design and thus provided a further guarantee of the quality of the project. Nine trained clinical monitors, who visit all the participating clinical centers every two weeks, are working to guarantee the recruitment of a continuous series of suspected cases, as well as the accuracy and completeness of the data. Periodic monitoring of the participating centers reduces the risk of missed cases.

Genetic analysis

Blood samples for DNA analysis are collected during each patient's hospital stay as well as during outpatient activity, after obtaining patient informed consent according to the local ethics regulations. All centers guarantee correct acquisition of informed consent; this includes providing patients with a full description of the project's goals as well as informing them of the possibility that biological material collected may be used for future analyses still to be defined at the time of the acquisition of consent. The use and sharing of biospecimens and associated clinical data complies with all applicable privacy and human subjects' protection regulations. All samples are processed by automatic extraction to obtain high purity DNA and stored locally at -80°C. Blood samples are sent together with the clinical and criteria forms to the specific laboratories for the genetic diagnosis. The genetic diagnosis is obtained by performing the following evaluations:
• for CADASIL, screening of exons 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 of the *NOTCH3* gene on chromosome 19;
• for Fabry disease, α-galactosidase activity initially in all suspected men; in suspected women and in men with reduced α-galactosidase activity, a genetic screening of the seven most frequently mutated exons of the α-*GAL A* (Xq 22.1) gene;
• for MELAS, screening for point mutations in the *tRNALeu* gene on mtDNA; in highly suspected cases or in cases with a muscular biopsy consistent with mitochondrial myopathy, screening for other mtDNA mutations, e.g. in the *MTTL1* gene and other tRNA genes (*MTTF*, *MTTv*, *MTTQ*) as well as in other subunits of complex 1 such as *MTND1*, *MTND5*, and *MTND6*;
• for FHM/SHM, screening of all exons of the *ATP1A2* gene; exons 3, 4, 5, 6, 11, 13, 14, 16, 17, 19, 20, 22, 23,

24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 41, 42, and 47 of the *CACNA1A* gene; and exons 23 and 26 of the *SCN1A* gene;

• for H-CAA, sequencing in suspected cases of all exons of *TRANSTHYRETIN, CYSTATIN C*, and *AMYLOID PRECURSOR PROTEIN gene*; and

• for Marfan syndrome, because the diagnosis is established on clinical grounds (5), molecular analysis consisting of whole-exon screening of the *FB1* and *TGFβ-R2* genes will be performed only in highly suspected cases.

The project involves the use of clinical and personal data and of blood samples for DNA extraction. The source of genetic material is venous blood collected from the patients by standard blood-drawing methods. No personal risks are envisaged for the patients involved since they are not exposed to any treatment or challenge. Italian national rules on data protection are complied with, and patient anonymity is guaranteed. The results of genetic tests are communicated to physicians in charge of the cases, who are responsible for informing the patient, or next of kin, of diagnostic test results and of the resulting diagnosis, according to local ethics regulations.

For data analysis, *a* database encryption strategy has been developed. Since results from single individuals would not be informative, information is extracted from group data. Thus, all published data will be at group, not individual, level.

Statistical analysis

Before the planned results analysis, a quality evaluation will be performed and the validity of the screening procedures assessed. The analysis will be carried out centrally, by the coordinating center, using the full dataset. Correlation analysis of phenotype and genotype characteristics will be performed on the total population as well as on different subgroups. A subgroup analysis of stroke subtypes is also planned. An analysis of monogenic disease incidence in the regional stroke population, stratified by sex and age, will also be performed.

Expected results

Through the present study, which involves the creation of an integrated regional network of clinical centers and laboratories and the coordination of their activity, and its results, we expect to provide a good practical example for the implementation of similar projects and for the diagnosis of other monogenic disorders. In addition, our network is expected to be a useful tool for supporting research excellence in stroke genetics. A cohort of well-phenotyped and well-characterized stroke patients, homogeneously collected, is expected to provide good-quality results in terms of: i) better knowledge of the natural history of monogenic disorders; ii) more accurate definitions of phenotypes associated with monogenic disorders; iii) identification of possible genotype-phenotype correlations; iv) identification of additional environmental and risk factors modulating the disease process or variably contributing to the full disease phenotype; and v) the creation of a proven network for future collaboration in therapeutic clinical trials targeting prevention of disease progression or symptom relief.

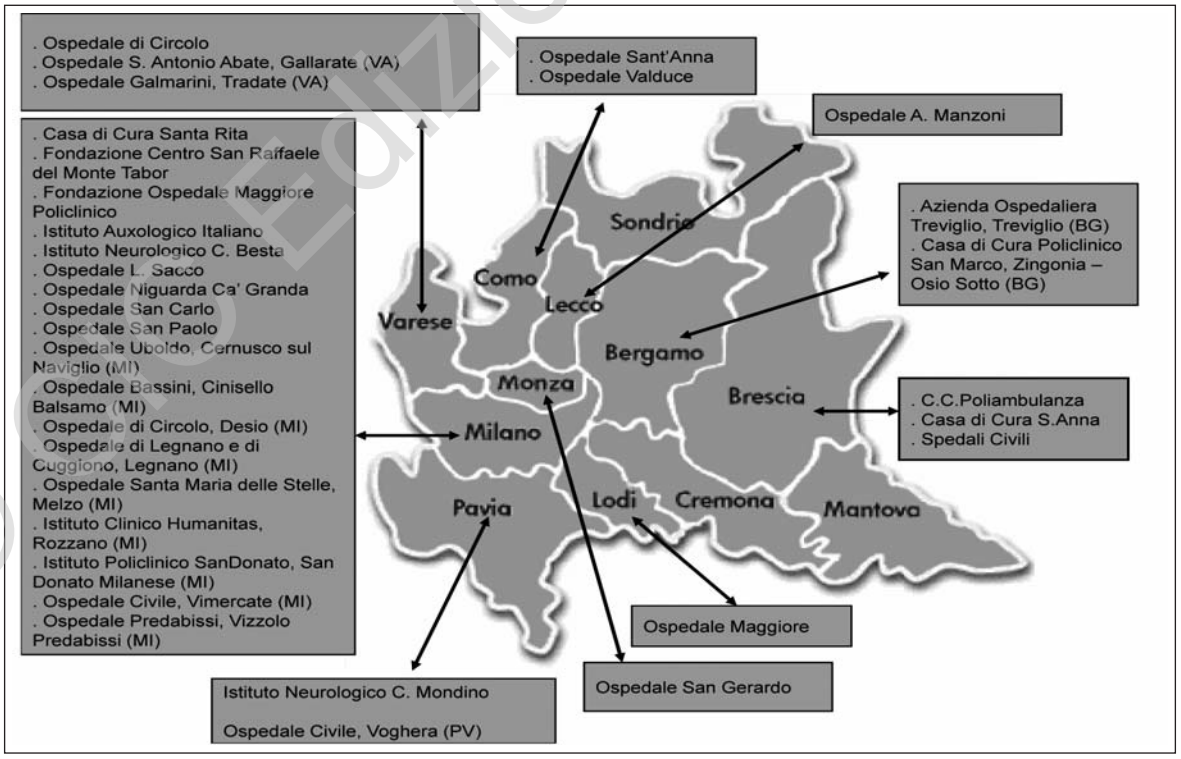

Figure 1 - Lombardia GENS recruiting centers

Preliminary results

Thirty-three clinical units among the region's centers hospitalizing more than 100 stroke patients each year are participating in the project (Fig. 1). These centers cumulatively hospitalize about 10,000 stroke patients annually, representing about 90% of all stroke cases in Lombardy. Although the recruiting period was intended to start in January 2008, patient data collection began later than the anticipated date because most centers were waiting for local ethics committee approval. Moreover, clinicians required a start-up period, which meant that the initial recruitment phase moved slowly. After April, 2008 recruitment became steady and improved further following the introduction of clinical monitors in December 2008.

In about three years of the project, data for about 200 patients with a suspected monogenic disease have been collected. Two diagnostic steps provide first for the selection of suspected phenotypes and then for the identification of patients with a positive genetic test for each specific disease. Of the patients included so far, 39% were screened for CADASIL, 13% for Fabry disease, 27% for H-CAA, 6% for MELAS, 11% for FHM/SHM and 3% for Marfan syndrome. The final expectation is that physicians will be able to apply, in their everyday clinical practice, the diagnostic tools for monogenic disease acquired through their participation in this project.

Discussion

The Lombardia GENS project will provide a database of well-phenotyped stroke patients with diagnoses of monogenic disorders. Despite the fact that stroke-associated monogenic diseases are rare and most strokes are believed to be polygenic, diagnosis of single-gene disorders is needed because some have proven amenable to disease-specific treatments. Moreover, a correct diagnosis may provide a concrete benefit to affected individuals in terms of prognostic evaluation, genetic counseling, and specific management measures (3,4).

Most of the available studies on single-gene disorders are underpowered because they included only a small number of cases and were conducted at individual centers. Moreover, given ongoing results in terms of the genetic factors underlying polygenic stroke pathogenesis, careful phenotype characterization and accurate genotype-phenotype correlations, in addition to further insight into the pathogenesis of monogenic disorders, may help to clarify some pathogenic bases of heritable stroke (3,7-9).

Unlike previous studies in this field, which focused on only one monogenic disease (10-18), Lombardia GENS is, to our knowledge, the largest genetic network, allowing the recruitment of a high number of patients suspected of having monogenic disorders, in which the cerebrovascular event is the mandatory inclusion criterion. It has a high probability of success as it favors the collaboration of people (physicians, geneticists, biologists) involved in stroke genetics research. The participation of clinicians in the project facilitates collection of well-characterized populations and the translation of research outcomes into clinical practice, with the aim of identifying possible etiological factors involved in stroke pathogenesis and new treatment modalities for all stroke subtypes. Through Lombardia GENS, stroke genetics laboratories are offered an opportunity to standardize procedures and to increase their research potential, avoiding the risk of diluting effort and delaying outcome. Moreover, the project will help to promote adequate education and training across the health professions, raising awareness of the existence of these diseases and of the resources available for their diagnosis and treatment.

The project will also give stroke patients with rare diseases increased access to care, resources, and expertise, reducing misdiagnosis and non-diagnosis of monogenic diseases and improving their quality of life. In addition, it will lead to the identification of centers of expertise throughout the country and organized healthcare pathways for patients suffering from monogenic diseases. The large patient dataset planned for this project may help to make the diagnosis of monogenic diseases more straightforward, increasing the accuracy of epidemiologic data on the incidence of rare diseases in stroke and knowledge of disease phenotypes, and harnessing the power needed to make significant genotype-phenotype correlations.

The project does, however, present some methodological limits. First, although the participating clinical centers hospitalize most stroke patients in Lombardy, the cases are recruited through selected hospitals which do not provide full coverage of the geographical area. Moreover, hospital-based sampling frames do not allow a true measure of population incidence. Second, the cases are recruited through stroke centers and the presence of stroke or TIA is an inclusion criterion; the data are biased by these criteria which allow the inclusion only of symptomatic patients. The objective of recruiting about 300 cases overall across all subgroups will also limit multiple risk factor analysis studies.

Nevertheless, the project could continue in future years and yield a network model that could be extended to other regions and countries. Because rare diseases continue to be a priority of the European Community, mostly with regard to developing new diagnostics and treatments, as well as performing epidemiological research into these disorders, multicountry or multicenter comprehensive approaches to increase the number of patients included for study are considered to be highly valuable.

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Appendix 1 - Characteristics of patients with suspected monogenic disease

LOMBARDIA GENS

1. Demographics

◆ Hospital name .....

◆ First name and Surname .....

◆ Date of birth ___/___/____ ◆ Place of birth .....

◆ Sex • Male ❑ • Female ❑

◆ Region of origin .....

◆ Phone.....

◆ Ethnic group:

• Caucasian ❑ • Asian ❑

• Hispanic ❑ • Black ❑

◆ Date of examination ___/___/____

◆ Date of blood extraction ___/___/____

◆ Type of examination:

• Emergency dept ❑

• inpatient ❑

• outpatient ❑

2. Stroke event

◆ Date and time of event ___/___/____ ____ : ____

◆ Type:

• TIA ❑ • Stroke ❑

◆ Subtype

• Ischemic ❑ • Hemorrhagic ❑

◆ First event:

• Yes ❑ No ❑

If no, type and number of previous events:

❑ TIA n. ___ ❑ Stroke n. ___

◆ NIHSS (first available) _____ Date and time ___/___/____ _____:____

• Direct examination ❑ • Derived from clinical notes ❑

◆ Pre-stroke Rankin score _____

• Direct examination ❑ • Derived from clinical notes ❑

3. Neurological examination

• Direct examination ❑ • Derived from clinical notes ❑

• Strength deficit (paresis/plegia)	Left ❑	Right ❑	Both ❑
• Sensory deficit	Left ❑	Right ❑	Both ❑
• Aphasia	Yes ❑		No ❑
• Visual defects	Yes ❑		No ❑
• Heminattention	Yes ❑		No ❑
• Consciousness impairment	Yes ❑		No ❑
• Dysarthria	Yes ❑		No ❑
• Ataxia	Yes ❑		No ❑

• Other .....

4. Diagnosis *(Please attach copy of report)*

◆ Neuroimaging:

• CT ❑ • MRI ❑

◆ Cerebral vascular imaging:

• Doppler US ❑ • MRA/CTA/cerebral angiography ❑

5. Risk factors

◆ High blood pressure (≥160/95 mmHg and/or previous history of high BP):

• Yes ❑ • No ❑

◆ Diabetes (glycemia ≥126 mg/dl and/or previous history of diabetes):

• Yes ❑ • No ❑

◆ Dyslipidemia (cholesterol≥200 mg/dl and/or tryglicerides≥180 mg/dl and/or previous history of dyslipidemia):

• Yes ❑ No ❑

◆ Ischemic cardiopathy (previous MI and/or angina and/or endovascular treatment of coronary arteries):

• Yes ❑ No ❑

◆ AF (on the basis of the ECG and/or previous detection):

• Yes ❑ No ❑

◆ Peripheral vasculopathy (stenosis/occlusion of peripheral vessel and/or previous history of intermittent claudication):

• Yes ❑ No ❑

◆ Smoking:

• never ❑
• in the past ❑ date of stopping: ___/___/____
• current ❑ number of cigarettes /day _____

◆ Weight (kg)_____ ◆ Height (cm) _____

◆ Physical inactivity (<60 min/d):

• Yes ❑ No ❑

◆ Hormone therapy (HRT or contraceptive):

• Yes ❑ No ❑

◆ Alcool abuse (>32 g for ♂ and ≥16 g for ♀):

• Yes ❑ No ❑

◆ Hyperhomocysteinemia (homocysteine >15 units):

• Yes ❑ No ❑

◆ Other

.....

6. Associated Symptoms and Signs (previous or current)

◆ Migraine/headache	Yes ❑	No ❑
◆ Seizures	Yes ❑	No ❑
◆ Psychiatric disturbances	Yes ❑	No ❑

If yes specify.....

◆ Cognitive impairment	Yes ❑	No ❑
◆ Deafness	Yes ❑	No ❑
◆ Short/tall stature	Yes ❑	No ❑
◆ Renal failure	Yes ❑	No ❑
◆ Spontaneous abortion	Yes ❑	No ❑
◆ Cardiopathy	Yes ❑	No ❑
◆ Skin lesions (neurofibromas, angiokeratomas)	Yes ❑	No ❑
◆ Dysmorphisms	Yes ❑	No ❑
◆ Joint flexibility	Yes ❑	No ❑
◆ Acroparesthesias	Yes ❑	No ❑
◆ Myopathy with or without exhaustibility	Yes ❑	No ❑
◆ Lactic acidosis	Yes ❑	No ❑
◆ Sickle cell disease	Yes ❑	No ❑

7. Family History

◆ Consanguineous parents Yes ❑ No ❑

◆ Father:

• Date of birth ___/___/____ • Place of birth _____ (____)

• Diseases:

Stroke ❑ Psychiatric disturbances ❑

Migraine	❑	Coagulopathies	❑
Renal failure	❑	Epilepsy	❑
Dementia	❑	Myopathies	❑

• Alive Yes ❑ No ❑

If no: Age at death _____
Cause of death .....
.....

◆ Mother:

• Date of birth ___/___/____ • Place of birth _____ (____)

• Diseases:

Stroke	❑	Psychiatric disturbances	❑
Migraine	❑	Coagulopathies	❑
Renal failure	❑	Epilepsy	❑
Dementia	❑	Myopathies	❑

• Alive Yes ❑ No ❑

If no: Age at death _____
Cause of death .....
.....

◆ Brothers/sisters:

• Date of birth ___/___/____ • Place of birth _____ (____)

• Diseases:

Stroke	❑	Psychiatric disturbances	❑
Migraine	❑	Coagulopathies	❑
Renal failure	❑	Epilepsy	❑
Dementia	❑	Myopathies	❑

• Alive Yes ❑ No ❑

If no: Age at death _____
Cause of death .....
.....

◆ Sons/daughters:

• Date of birth ___/___/____ • Place of birth _____ (____)

• Diseases:

Stroke	❑	Psychiatric disturbances	❑
Migraine	❑	Coagulopathies	❑
Renal failure	❑	Epilepsy	❑
Dementia	❑	Myopathies	❑

• Alive Yes ❑ No ❑

If no: Age at death _____
Cause of death .....
.....

Appendix 2 - Characteristics of patients with suspected monogenic disease

LOMBARDIA GENS

CADASIL

Diagnosis

1) Subcortical lacunar T2 sequence lesions at MRI.
2) At least one of the following signs/symptoms
❑ History of recurrent stroke /TIA
❑ Migraine with aura
❑ Dementia
❑ Major mood disorders
❑ Family history of stroke/mood disorders and/or migraine and/or dementia

Laboratory diagnosis
1) Genetic analysis

- ➢ Genetics Laboratory, Niguarda Ca' Granda Hospital (Dr Penco)
  Screening exons: **2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23**
- ➢ Biochemistry and Genetics Laboratory, Carlo Besta Institute of Neurology Foundation, IRCCS (Dr Taroni / Dr Gellera)
  Screening exons: **3, 4, 11; if high suspicion 2, 5, 6, 8, 19; 7, 9, 10, 14, 18, 20, 22, 23; 1, 12, 13, 15, 16,17,21**
- ➢ Molecular Biology Laboratory, San Raffaele del Monte Tabor Foundation, IRCCS, Università Vita-Salute, San Raffaele Hospital, IRCCS (Dr Carrera / Dr Calzavara)
  Screening exons: **2, 3, 4, 5, 6, 8, 11, 12, 14, 18, 19, 20, 22, 23**

2) Skin biopsy

- ➢ Neuropathology Unit, Carlo Besta Institute of Neurology Foundation, IRCCS (Dr Tagliavini / Dr Morbin)
- ➢ Pediatric Dermatology Laboratory, Ca' Granda Foundation, Maggiore Policlinico Hospital, IRCCS, University of Milan (Dr Tadini)

MELAS

Diagnosis

1) Stroke-like episodes (mostly cortical and not related to a vascular territory) in patients younger than 45 years
2) At least one of the following signs/symptoms:
❑ Myopathy with lactic acidosis
❑ Seizures
❑ Migraine
❑ Typical lesions on MRI (bitemporal and basal ganglia calcification)
❑ Cardiomyopathy
❑ Progressive dementia
❑ Metal retardation
❑ Short stature
❑ Diabetes
❑ Deafness

Laboratory diagnosis

Search for A3243G mutation in *tRNALeu* gene:

- ➢ Biochemistry and Genetics Laboratory, Department of Neurological Sciences, Ca' Granda Foundation, Maggiore Policlinico Hospital, IRCCS, University of Milan (Prof. Comi)

If cardiomyopathy:
Whole MtDNA sequencing:

- ➢ Center of Genetic Cardiovascular Diseases (Director: Prof. Arbustini) - Molecular Genetics Laboratory (Dr Grasso / Dr Marziliano / Dr Diegoli) San Matteo Hospital Foundation, IRCCS, Pavia

FAMILIAL OR SPORADIC HEMIPLEGIC MIGRAINE (FHM/SHM)

Diagnosis

1) At least two migraine aura attacks associated with motor weakness (hemiparesis, paresis, plegia) lasting >5 min and <24 h

2) At least one of the following signs/symptoms:
❑ Fully reversible visual symptoms including positive features (e.g., flickering lights, spots or lines) and/or negative features (i.e., loss of vision)
❑ Fully reversible sensory symptoms including positive features (i.e., pins and needles) and/or negative features (i.e., numbness)
❑ Fully reversible dysphasic speech disturbance
❑ Supplementary clinical features:
• Migraine following aura.
• Childhood onset migraine (<30 years)
• At least one first- or second-degree relative has had attacks fulfilling these criteria
• Progressive ataxia and/or nystagmus
• Attacks triggered by fever, trauma, pleocytosis

Laboratory diagnosis
Screening *CACNA1A* gene (FHM1) (exons 3, 4, 5, 6, 11, 13, 14, 16, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 41, 42, 47), *ATP 1A2* gene (FHM2) (all exons) and *SCN1A* gene (FHM3) (exons 23, 26):
Genetics Laboratory, Eugenio Medea Institute, Bosisio Parini, Lecco (Dr Bassi)
➢ Molecular Biology Laboratory, San Raffaele del Monte Tabor Foundation, IRCCS, Università Vita-Salute, San Raffaele Hospital, IRCCS (Dr Carrera), Milan

CEREBRAL HERITABLE AMYLOID ANGIOPATHY (H-CAA)

Diagnosis

1) At least one of the following signs:
❑ Recurrent atypical hemorrhage (mostly cortical and subcortical)
❑ Ischemic/hemorrhagic lesions not attributable to a different disorder
❑ Cerebral MRI consistent with the suspicion of amyloid angiopathy

2) At least one of the following signs/symptoms:
❑ Lack of hypertension or well treated hypertension)
❑ Lack of coagulation abnormalities
❑ Absence of aneurysms or arteriovenous malformations
❑ Positive family history of hemorrhagic and ischemic stroke
❑ Cognitive impairment
❑ Occipital calcifications
❑ Sensory-motor peripheral neuropathies

Laboratory diagnosis
Genetic screening of *TTR, APP, CYSTATIN C* genes:
➢ Biotechnology and Biomedical Technology Laboratory, Systemic Amyloidosis Study Center, San Matteo Hospital Foundation, IRCCS, Pavia (Dr Obici / Prof. Merlini), Pavia

MARFAN SYNDROME

Clinical diagnosis

1) Ischemic or hemorrhagic stroke due to arterial dissection, rupture of intracranial aneurysms or cardioembolic source
2) At least two of the following criteria:
❑ At least two skeletal abnormalities (tall stature, pectus excavatum or carinatum, high-arched palate, arachnodactyly, laxity of ligaments with scoliosis, or joint hyperextensibility)
❑ At least two ocular manifestations (strabismus, amblyopia, ectopia lentis, cataract)
❑ At least one cardiovascular manifestation (dissection or dilatation of ascending aorta, mitral valve prolapse, cardiac arrhythmias)
❑ At least one cutaneous manifestation (cutaneous striae, recurrent hernias)
❑ At least one pulmonary manifestation (spontaneous pneumothorax, apical bubbles)
❑ Positive familial history of arterial dissections, skeletal abnormalities, typical cardiovascular and ocular manifestations)

Laboratory diagnosis
Screening of *FBN1* and *TGFβ-R2* genes:
➢ Center of Genetic Cardiovascular Diseases (Director: Prof. Arbustini) - Molecular Genetics Laboratory (Dr Grasso / Dr Marziliano / Dr Diegoli) San Matteo Hospital Foundation, IRCCS, Pavia