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ORIGINAL ARTICLE

GEN-O-MA project: an Italian network studying clinical course and pathogenic pathways of moyamoya disease—study protocol and preliminary results

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Abstract

Background GENetics of mOyaMoyA (GEN-O-MA) project is a multicenter observational study implemented in Italy aimed at creating a network of centers involved in moyamoya angiopathy (MA) care and research and at collecting a large series and bio-repository of MA patients, finally aimed at describing the disease phenotype and clinical course as well as at identifying biological or cellular markers for disease progression. The present paper resumes the most important study methodological issues and preliminary results.

Methods Nineteen centers are participating to the study. Patients with both bilateral and unilateral radiologically defined MA are included in the study. For each patient, detailed demographic and clinical as well as neuroimaging data are being collected. When available, biological samples (blood, DNA, CSF, middle cerebral artery samples) are being also collected for biological and cellular studies.

Results Ninety-eight patients (age of onset mean ± SD 35.5 ± 19.6 years; 68.4% females) have been collected so far. 65.3% of patients presented ischemic (50%) and haemorrhagic (15.3%) stroke. A higher female predominance concomitantly with a similar age of onset and clinical features to what was reported in previous studies on Western patients has been confirmed.

Conclusion An accurate and detailed clinical and neuroimaging classification represents the best strategy to provide the characterization of the disease phenotype and clinical course. The collection of a large number of biological samples will permit the identification of biological markers and genetic factors associated with the disease susceptibility in Italy.

Keywords Moyamoya disease · Network · Markers · Endothelial progenitor cells · Genetics

Introduction

Moyamoya angiopathy (MA) is a chronic cerebrovascular condition characterized by a progressive stenosis of the terminal part of the supraclinoid internal carotid arteries (ICA) and their proximal branches associated with the compensatory development of a fragile collateral vessels network at the base of the brain (*moyamoya vessels*). MA

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is sub-classed in moyamoya disease (MMD) when MA is bilateral and it is the sole disease manifestation, or moyamoya syndrome (MMS) when MA is associated with a well-defined acquired or genetic condition. MA occurring unilaterally has been recently defined as unilateral moyamoya disease (uMMD) [1].

The pathogenesis of MA is unknown. Anomalies in angiogenesis and vasculogenesis, due to the detection of increased cytokine and growth factor concentrations have been invoked as potential disease mechanisms. Also genetic factors, given the high familial rate and the ethnic differences, are believed to be involved [1, 2].

The disease is rare with a prevalence being close to 3/100000 in East Asia and ten times less in Europe [3, 4]. MA mainly present intracranial hemorrhage or ischemic events in adults, whereas transient ischemic attacks (TIAs) or ischemic strokes occur in most symptomatic MA children [5]. Cerebral magnetic resonance imaging (MRI) can show ischemic and haemorrhagic lesions even in the absence of symptoms. The compensatory arterial moyamoya network and the intracranial and extracranial collaterals are best visualized on cerebral conventional angiography, which is still considered as the gold standard for MA diagnosis, according to established criteria [6, 7]. Besides, non-invasive imaging, such as MRI-angiography (MRA) and computed tomography-angiography (CTA) allowing visualization of steno-occlusive lesions, are used for disease suspicion and follow-up monitoring [1]. Although no treatment of proven efficacy for reversing the primary stenotic disease process in MA is currently available, surgical revascularization has been shown to improve cerebral blood flow to the affected hemisphere(s) and prevent stroke [8–10]. A major issue is that the natural history of MA remains in most patients unpredictable. In addition, factors influencing the variable severity of the clinical course are still unknown due to the limited prospective literature data in non-operated cases [1, 11]. These data are much more limited in western populations [12–15].

GENetics of mOyaMoyA (GEN-O-MA) project is a retrospective and prospective multicenter cohort study implemented in Italy aimed at creating a network of centers involved in MA care and research, and at collecting a large series of well characterized patients. This network integrates the experience of neurologists, neuroradiologists, neurosurgeons, biologists, and geneticists in MA study and care. An accurate and detailed clinical and neuroimaging classification will allow to describe the disease clinical features and disease course in Italy. Moreover, the collection of a large bio-repository of biological samples will allow the identification potential biological markers and genetic factors associated with the disease susceptibility. The present paper reports the most important methodological issues of the network and preliminary results.

Patients and methods

Study design

The study was designed as a multicenter (prospective and retrospective) observational cohort study across Italy. The project is coordinated by the Cerebrovascular Unit of the Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy. A strong collaborative relation with the Neuroradiological, Neurosurgical, and Child Neuropsychiatric Unit and Developmental Neurology Division of the same hospital and with other participating centers allows a multidisciplinary collaboration leading to an improved result achievement.

Two independent advisory boards (Prof. Med. Peter Vajkoczy - Department of Neurosurgery, Charite Universitätsmedizin, Berlin, Germany, and Prof. Med. E. Tournier Lasserve - Department of Genetics, INSERM and University Paris Diderot, Paris, France) with an extensive expertise in the field of MA have been announced to monitor the study design and its reliability.

Ethical issues

The study design was approved by the Ethical Committee of the Fondazione IRCCS Istituto Neurologico Carlo Besta Milan, Italy (report no. 12, 10/01/2014), and of each participating center. The study was also performed in accordance with the World Medical Association Declaration of Helsinki.

Since it was designed as a pure observational study, patients underwent diagnostic procedures and received therapy according to local practice and disease guidelines. Informed written consent for study participation (for data and biological samples collection) from all patients or their legal representatives was mandatory for study inclusion.

Study population

The study population consists of a series of MA patients including MMD, MMS, and uMMD (aged between 0 and 75 years), collected both retrospectively and prospectively by the referring physicians of the centers participating in the study. MA was diagnosed according to established criteria: (1) stenosis or occlusion at the terminal portion of the ICA and/or the proximal portion of the anterior and/or middle cerebral arteries (MCA), (2) abnormal vascular networks in the vicinity of the stenotic lesions, and (3) a bilateral presence of these findings [6, 16].

A first sample of 100 MA patients has been planned for clinical and neuroradiological assessment. Patients are being followed up for 2 years.

Table 1 Clinical and neuroradiological data collected

Collected data of GEN-O-MA project
Demographic (age, age at onset, sex, ethnicity, region of origin)
Clinical symptoms of qualifying event (TIA, stroke, ICH)
Stroke severity and disability scales (NIHSS, mRS, Karnofsky Scale)
Disease clinical manifestations (migraine, seizures, cognitive impairment, psychiatric disorders, ophthalmic disease)
Vascular and lifestyle risk factors (hypertension, diabetes, smoke, atrial fibrillation, ischemic cardiopathy, alcohol intake, BMI > 25, radiotherapy, traumatic brain injury)
Autoimmune disorders and history of associated conditions or syndromic features (i.e., hormone disorders, Down syndrome, Neurofibromatosis, Marfan syndrome, sickle cell disease, mental retardation, dysmorphisms, artery dissections, aneurisms, coagulation disorders)
Familial history for MA, cerebrovascular events, and other neurological manifestations (migraine, cognitive impairments, psychiatric disorders, seizures, artery dissections, aneurisms)
Diagnostic workup (MRI, MRA, CTA, cerebral angiography, MRI perfusion, TCD)
Neuroimaging features (size and location of infarctions, degree of vessel stenosis-occlusion, regional relative CBV, MTT, and TTP, unilateral/bilateral disease, modified Suzuki scoring, external carotid arteries collaterals, leptomeningeal collaterals, dilatation of the AChorA, PcomA/ICA ratio, cortical and basal neovascularization, TCD mean flow in the middle cerebral arteries
Medical therapy (anti-aggregants, antiepileptic drugs, statins, other)
Surgical intervention (date, type, and side effects)
Follow-up (cerebrovascular events, neuroradiological assessment)

BMI, body mass index; *CTA*, computed tomography angiography; *ICH*, intracerebral hemorrhage; *MA*, moyamoya angiopathy; *MRA*, magnetic resonance angiography; *MRI*, magnetic resonance imaging; *mRS,* modified Ranking Scale; *NIHSS*, National Istitute of Health Stroke Scale/Score; *TIA*, transient ischemic attack; *TCD*, Transcranial Doppler ultrasound, *CBV,* cerebral blood flow; *MTT,* mean transit time; *TTP*, time to peak; *AChorA*, Anteriori choroidal artery, *PcomA*, posterior communicating artery, *ICA*, internal carotid artery

Clinical data and biological samples collection

All included patients underwent a comprehensive work-up including routine and autoimmunity blood tests, neuro-ophtalmologic and electrophysiological evaluation, Doppler/transcranial ultrasound, and neuroimaging examinations. A specific form implemented from current literature has been developed for standardized data collection. For each patient demographic and clinical data, cerebrovascular risk factors, familial antecedents, racial descents, current pathologies including immune diseases, genetic syndromes, and pharmacological treatment have been registered. Cerebrovascular events are classified according to established standardized criteria (i.e., hemorrhagic, ischemic, lesion site) based on clinical features and MRI findings. Also, other neurological clinical features associated with MA including cognitive deficits, seizures, and psychiatric disorders have been reported. The use of these standardized forms, the centralized data managing, and the control of quality assures a harmonized and homogeneous data collection across the participating centers. Clinical and neuroradiological data collected within the GEN-O-MA project are summarized in Table 1.

Blood and plasma samples from a subgroup of MA patients and age-sex matched healthy and unrelated (atherosclerotic patients) controls (HC and UC respectively) are being collected. Cerebrospinal fluid CSF samples and arterial specimens (MCA) of MA patients and UC are being also gathered in a subset of patients undergoing revascularization surgery.

Central neuroimaging analysis

The study provides for a centralized analysis of brain imaging studies, including contrast-enhanced magnetic resonance imaging (MRI), MR angiography, perfusion weighted imaging (PWI), digital subtraction angiography (DSA), and transcranial doppler (TCD) ultrasound. For patients who underwent brain MRI, sagittal T1-weighted spin echo, axial T2-weighted turbo spin echo, coronal T2-weighted FLAIR, diffusion weighted images (DWI), three-dimensional time of flight phase contrast angiography, and three-dimensional T1-weighted gradient echo sequences after gadolinium injection were recommended. Two neuroradiologists (G.F and M.G.), with more than 7 years experience in neuroimaging visual rating and blinded to clinical and biological/genetic data are carried out quantitative and qualitative analyses, with reference to the shape, size and location of infarctions, and the degree of vessels stenosis-occlusion in the corresponding areas. Perfusion weighted images (PWI) were acquired on a 1.5 T or 3 T units, to evaluate the regional relative cerebral blood volume (CBV), relative mean transit time (MTT), and time to peak (TTP) maps. A comprehensive digital subtraction cerebral angiography (DSA) with selective

catheterization of both internal and external carotid arteries and the dominant vertebral artery was mandatory to evaluate collateral circulation for MA diagnosis for study inclusion. MA was classified into the bilateral or unilateral type according to the number of the involved distal internal carotid arteries, observed on DSA. Disease severity was evaluated on DSA by applying a modified Suzuki scoring [16] on cerebral angiography findings, as follows: (0) no evidence of disease, (1) mild-to-moderate stenosis around internal carotid artery (ICA) bifurcation with absent or slightly developed ICA MMD, (2) severe stenosis around the ICA bifurcation or occlusion of either proximal anterior or MCA branches with well-developed ICA MMD, (3) occlusion of both anterior and MCA branches with well-developed ICA MMD (only a few of anterior or MCA branches or both are faintly opacified in antegrade fashion through meshwork of ICA MMD), and (4) complete occlusion of both anterior and MCA branches with absent or small amount of ICA MMD (without opacification of either anterior or MCA branches in antegrade fashion). [17] The external carotid artery collaterals were graded by using a 4-point scale: (0) no collateral distribution; (1) slight collateral distribution, often with dilution; (2) small but definite collateral supply; and (3) full collateral filling [18, 19].

In addition, we investigated the presence of leptomeningeal collaterals (LMC) coursing from the posterior cerebral artery (PCA) to the frontal, temporal or parietal lobes, the dilatation of the anterior choroidal artery (AChorA), and the posterior communicating artery (PcomA)/ICA ratio*.Cortical neovascularization was defined as an enlarged winding of the distal cortical arteries observed in the arterial DSA phase and categorized into anterior or posterior cortical neovascularization depending on their sources (anterior vs. posterior circulation). The degree of basal collaterals (BCs) was measured as the ratio of the height from the carotid T-junction to the end of the BC vessels and the height from the carotid T-junction to the highest point of the imaginary superior sagittal sinus in the antero-posterior projection × 100.

Transcranial Doppler (TCD) was performed using a 2-MHz transducer to acquire a continuous measurement of mean flow velocities of the middle (M1-M2 segments whenever available), anterior (A1 segment, whenever available) and posterior (P1-P2 segments) cerebral arteries, basilar artery, and intracranial vertebral arteries. Moreover, an evaluation of vasomotor reactivity through breath-holding technique from the middle cerebral artery territory was bilaterally obtained in case of adequate bone temporal windows [20, 21].

Experimental and genetic analysis

Blood, DNA, and CSF samples are being collected, when available, to evaluate (1) number and function of circulating endothelial progenitor cells (cEPCs), (2) levels of growth factor within plasma and CSF, (3) MCA gene expression profile of angiogenic and growth factors coding genes, and (4) genetic factors associated with sporadic and familial MA cases.

Fig. 1 Clinical center participating to the GEN-O-MA project: (1) Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano; (2) ASST– Spedali Civili Di Brescia, Brescia; (3) ASST Papa Giovanni XXIII, Bergamo; (4) Fondazione IRCCS Ca′ Granda – Ospedale Maggiore Policlinico, Milano; (5) ASST Grande Ospedale Metropolitano Niguarda, Milano; (6) Ospedale ASST Settelaghi Varese, Varese; (7) ASST Ovest Milanese, Ospedale di Legnano; (8) Ospedale Santa Maria Della Misericordia di Udine, Udine; (9) Arcispedale Santa Maria Nuova, Reggio Emilia; (10) Nocsae Modena, Modena; (11) Azienda Ospedaliera Universita' Degli Studi di Padova, Padova; (12) Clinica Neurologica Padova, Padova; (13) IRCCS Istituto Giannina Gaslini, Genova; (14) Azienda Ospedaliera Universitaria Careggi, Firenze; (15) Umberto I– Policlinico di Roma, Roma; (16) Policlinico Universitario Campus Bio-Medico, Roma; (17) Ospedale Santa Maria Della Misericordia, Perugia; (18) Ospedale Regionale San Salvatore, L'aquila; (19) Ospedale di San Benedeto Del Tronto, San Benedetto Del Tronto.

Levels of cEPCs are being evaluated in whole blood samples by flow cytometry [22] and by flow-count fluorospheres (Beckman Coulter s.r.l., Cassina De' Pecchi, Italy). CD45dimCD34 + CD133 + mononuclear cells will be considered as cEPCs. Moreover, in order to normalize the absolute number of cEPCs on the number of circulating leukocytes, a complete white blood cell (WBC) count is performed applying the Advia 120 cell counter (Bayer, Leverkusen, Germany). The % of cEPCs will be calculated as follows: % cEPCs = no. cEPC × 100/(no. WBC/μl). The EPC function will be assessed in vitro by isolating peripheral blood mononuclear cells (PB-MNCs) as previously reported [23]. The cell number for mm^2 in vitro (cells/mm^2) are being evaluated at day 3, 7, 10, 17, and 31. Conditioned medium (CM) will be used as stimulus for tube formation assays at day 7, and the angiogenic EPC activity will be tested at day 17. Finally, real-time (RT)-PCR experiments is being performed to evaluate the gene expression of endothelial markers and angiogenic growth factors in PB-MNCs and in EPC in vitro cultures (HC versus MA).

MA plasma and available CSF samples will be tested for cytokine and growth factor levels by using enzyme-linked immunosorbent assays (ELISA; R&D Systems, Minnesota, USA) and Bio-Plex Assays (Bio-Rad Laboratories, Segrate, Italy).

Total RNA will be extracted from affected MCA and the respective UC arteries followed by RT-PCR experiments using the 96-Well RT^2 Profiler™ Human Angiogenic Growth Factors PCR Array (PAHS-072ZD) (Qiagen, Hilden, Germany).

For genetic analysis, genomic DNA are being obtained from peripheral blood of patients and HCs using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). A two-step analysis has been planned including the genetic screening of RNF213 gene of all patients, whereas exome sequencing analysis was intended for familial cases.

Statistical data analysis

Before starting the analysis, a quality evaluation of all collected data will be performed and the validity of screening procedures will be assessed. Data analysis will be carried out centrally by the Coordinating Center using the full dataset. The chi^2 test will be applied to assess the significant differences between patients subgroups (i.e., MMD, MMS,

uMMD, or MA grading) with respect to the clinical and neurological features as well as to the results of biological experiments and genetic analysis. The independence of single predictive factors will be assessed by logistic regression analysis. All analyses will be calculated using STATA 8.0 (StataCorp LP, College Station, TX) and S-PLUS (Suite 44, Level 9, 88 Pitt Street Sydney New South Wales, Australia).

Results

Study centers

Nineteen centers effectively participated in MA patients recruitment (Fig. 1). Although the recruiting period was intended to start in January 2015, patient data collection began almost 6 months later than the anticipated date due to awaiting the local ethics committee approval.

Patients' characteristics

Between January 2015 and July 2017, 98 MA patients (MMD, MMS) mean ± SD age 35.5 ± 19.6 years were collected. Seventy-three (74.5%) were adults and 25 (25.5%) were children. Mean age ±SD of the onset was 33.5 ± 19.8 years and 67 (68.4%) were females. Mean NIHSS ±SD and mRS ±SD were 2.95 ± 3.24 and 0.70 ± 1, respectively.

Twenty-six cases (26.5%) presented high blood pressure, 25 (25.5%) hypercholesterolemia, 7 (7.1%) had diabetes, while as expected, atrial fibrillation and cardiopathy were present in 3.1% of our MA cases.

The index event was stroke in 64 (65.3%) cases (76.5% ischemic and 23.4% hemorrhagic) and TIA in (12.2%) cases. The number of cases for center is represented in Fig. 2. Sixty-four (65.3%) patients exhibited only one cerebrovascular event at the inclusion time. Only 20 patients referred $\geq$ 2 cerebrovascular event in the past.

Forty-five patients (45.9%) presented headache/migraine, 29 (29.6%) seizures, 21 (21.4%) cognitive impairments, 20 (20.4%) psychiatric disorders, and 10 (10.2%) ophthalmic disorders.

Bilateral involvement was identified in 60 (61.2%) patients, and 41 (41.8%) patients underwent surgical revascularization.

Familial history for MA was present in 4 (4.1%) of the patients whereas familial history for stroke/TIA was present in 33 (33.7%) of the cases. Preliminary GEN-O-MA clinical data and reported literature from Caucasian and Asian MA patients are outlined in Table 2.

Moyamoya biorepository

For cEPC analysis whole blood of 16 MA, 20 HC, and 8 UC was collected till date, whereas 8 MA versus 5 UC affected arteries were analyzed for RT^2 Profiler™ Human Angiogenic Growth Factors PCR Array.

To date, 27 plasma and 15 CSF samples along with 30 MA, 20 HC, and 8 UC DNA samples have been collected.

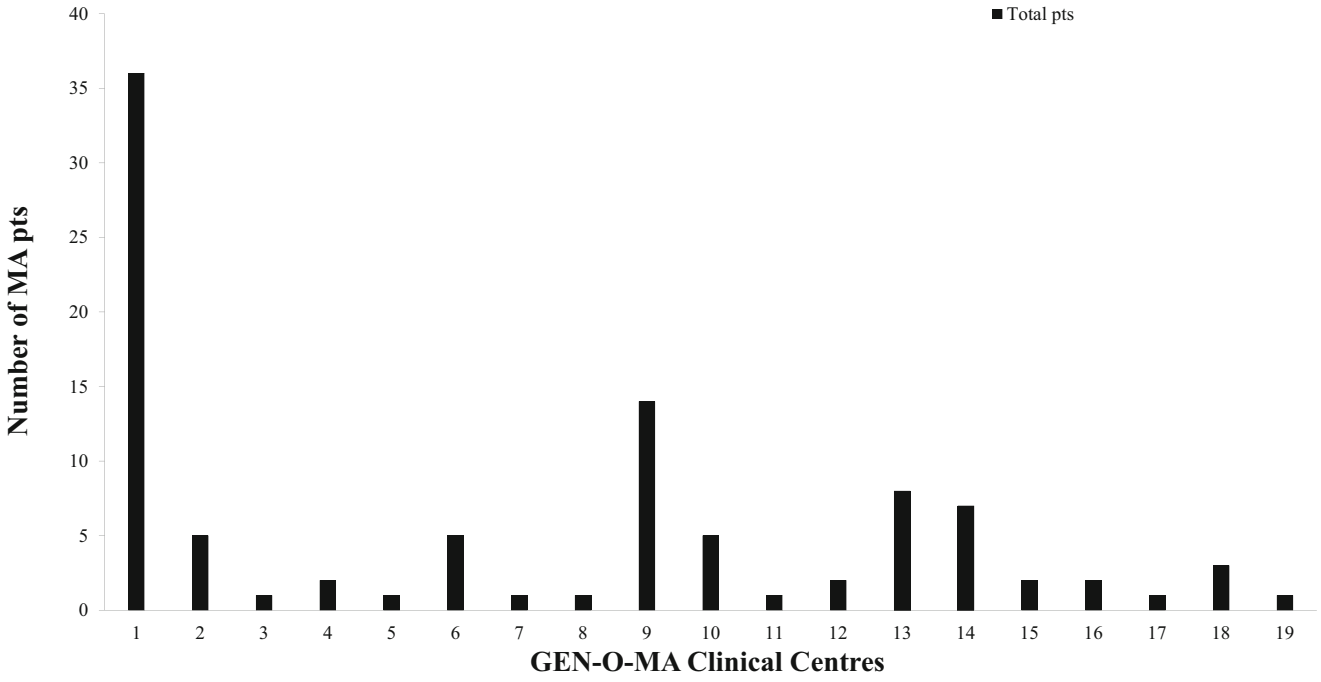

Fig. 2 Number of patients for center participating to the GEN-O-MA project

Table 2 Preliminary clinical data of GEN-O-MA vs. European and Asian MA patients

	Caucasian patients					Asian patients		
Demographic characteristics								
Clinical features	GEN-O-MA	European [12, 15]		American [24, 25]		Chinese [5]	Japanese [26]	Korean [27]
	($n = 98$)	($n = 153$)	($n = 61$)	($n = 7473$)	($n = 94$)	($n = 470$)	($n = 941$)	($n = 175$)
Age at onset (mean ± SD; year)	33.5 ± 19.8	35.8 ± 14.86	31.5 ± 17.9	–	34.5	36.8	–	42.2 ± 11.3
Female:male	2.2:1	2.9:1	4.5:1	2.2:1	2.6:1	1:1	1.98:1	1.57:1
Adults	74.5%	83.7%	83.6%	70.1%	100%	100%	–	100%
Children	25.5%	16.3%	16.4%	29.9%	0%	0%	–	0%
Index event characteristics								
Stroke	65.3%	–	63.9%	18.1%	73.4%	26.8%	41%	94.3%
Ischemic	50%	81%	50.8%	11.7%	57.4%	–	20%	45.1%
Hemorrhagic	15.3%	8.5%	13.1%	6.4%	16.0%	20.4%	21%	54.9%
TIA	11.2%	–	6.6%	34.0%	34%	41.1%	46%	–
Other symptoms/signs								
Headache	45.9%	3.9%	11.5%	–	21.3%	9.6%	6%	29.7%
Seizures	29.6%	1.3%	4.9%	–	–	0.9%	4%	2.9%
Psychiatric disorders	20.1%	–	–	–	–	–	–	–
Cognitive impairment	22.4%	1.3%	–	–	5.3%	–	–	5.1%
Ophthalmic diseases	36.4%	–	–	–	–	–	–	9.1%
Autoimmune diseases	16.3%	–	–	–	22.3%	–	–	–
Anatomic extent of moyamoya side								
Bilateral	61.2%	77.8%	–	–	86.2%	88.9%	–	–

BMI, body mass index; *MA*, moyamoya angiopathy; *NIHSS*, National Institute of Health Stroke Scale/Score; *TIA*, transient ischemic attack

Discussion

The exact phenotype and clinical history of MA are still poorly defined. The remarkable disease variability and the small size reported in studies addressing the disease pathogenesis along with inadequate data on brain imaging follow-up limit a clear definition of the disease course and the identification of factors influencing MA progression [28]. Particularly, data on MA in Western countries, hereby mostly in Europe are lacking. Although a similar phenotype between the US and European patients has been described, the rarity of the disease in Europe, the lack of systematic studies, the multi-ethnic origin, and the heterogeneity of the studied cohorts further impair the acquisition of clear information on clinical feature and disease progression in Europeans [12–15, 28–32].

The identification of clinical and imaging predictors is necessary to improve a prognostic evaluation and develop innovative therapeutic approaches. Moreover, pathophysiological mechanisms driving MA are largely unknown. Based on the existence of familial cases and the observation of a pronounced ethnicity effect, a genetic contribution is likely presumed [1, 11, 14, 28]. Strong association between MA and a RNF213 variant (R4810K) has been found in the East Asian population in around 90% of familial and 70% of sporadic cases. Despite preliminary reports excluded an association between this gene and MA in Caucasians [30]. Recently, rare RNF213 variants have been reported also in some European cases, particularly with early onset and familial MA [31]. Additional familial cases of MA associated with facial dimorphisms and achalasia respectively were recently associated mutations in BRCC3 deubiquitinase and GUCY1A3 gene, encoding the major nitric oxide receptor in vascular smooth muscle cells [32, 33]. However, these genes are not able to completely explain MA pathogenesis, and the genetic background of MA remains still not identified.

Several studies reported an overexpression of pro-angiogenic factors in the CSF, blood, or tissue samples of MA patients supporting the hypothesis that an unbalance of these factors may contribute to an impaired angiogenic and vasculogenic mechanisms leading to the characteristic vessel fragility [2, 34].

Poor understanding of the disease determinants limited so far advances in the clinical care and the development of innovative and personalized treatment. Until date, no

treatment has been identified to impair or slow the development of stenotic process and fragile collaterals. The direct or indirect revascularization surgery is considered as the best therapeutic option to reduce the occurrence of cerebrovascular events [11]. Although a recent meta-analysis has confirmed the surgical revascularization to prevent recurrent strokes in adult MMD patients, the risks, benefits, and indications for surgery are still uncertain and influenced by the retrospective design of most included studies [10]. Moreover, the differences in the selection of patient cohorts, diagnostic steps, surgical techniques, related to the clinical experience of single center limit the relevance of the available data. In summary, controlled trials prospectively analyzing the efficacy of different treatment strategies, by applying standardized clinical assessments, are largely needed [11].

The GEN-O-MA project is, to our knowledge, the first Italian Network and one of the largest Europeans studies [12–15] published so far. The objective of this network, which is integrated with other large European clinical centers involved in MA study, is to describe the clinical phenotype and identify disease progression markers of MA by applying a careful clinical and neuroradiological evaluation. Secondarily it is aimed at understanding the pathophysiology of MA by applying a translational biological approach.

At a preliminary analysis of our MA series, although the comparison with previous European and American series is difficult, due to the lack and incompleteness of previously collected data, we confirmed a similar age of onset and the higher female predominance, with a female/male ratio more comparable to the German and American [12, 24, 25] rather than to the Finnish population. The occurrence of ischemic and hemorrhagic stroke and clinical features frequency was alike, except for a modest increase rate of headache and seizures [12, 15, 25] (Table 2).

Our study has some important strength points: (1) it is a multicenter study with a systematic and standardized recruitment, (2) it permits a careful phenotyping by applying a detailed questionnaire and a standardized qualitative and quantitative characterization based on imaging data, and (3) it is characterized by an innovative experimental approach, including the evaluation of biological and genetic markers by analyzing affected tissue samples. The correlation between clinical and biological data as well as the direct analysis of affected vessel specimens makes our approach highly promising [34].

The principal limitation of the study may be the insufficient sample size, due to the high analysis costs, which may impair the understanding the significance of disease progression markers and the role of rare variants with very small effect sizes and weak gene–environment interactions.

However, a collaborative effort allowing to collect a large clinical dataset as well as DNA of MA patients, the GEN-O-MA project is expected to provide novel data on genetic susceptibility of MA within the Italian and/or European population. Due to the large cohort of Caucasian patients, stratified by specific subclasses, our study allows the identification of individuals with a potential risk for the development of MA. [23]. The identification of phenotypes at-risk and/or specific biological players driving the disease may improve our ability to specify the disease prognosis for individual patients and to develop personalized intervention/treatment options.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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