

# Fabry's disease: A prospective multicenter cohort study in young adults with cryptogenic stroke

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**Background** Stroke in young adults is etiologically diverse and may represent a diagnostic challenge remaining cryptogenic in one-fourth of cases. Limited information is available on the prevalence of Fabry's disease, a treatable multisystem inherited lysosomal storage disorder, and disability in young patients with cryptogenic stroke.

**Design and methods** The Canadian Fabry Stroke Screening Initiative (CFSSI) is a prospective multicenter cohort study of young adults (age 18–55) presenting with an ischemic stroke, transient ischemic attack, or intracerebral haemorrhage of unknown etiology to stroke centres across Canada. Diagno-

sis of Fabry's disease is made by direct DNA analysis of blood samples for  $\alpha$ -galactosidase gene mutations or polymorphisms. Demographics, clinical information, and investigations including brain Magnetic Resonance Imaging (MRI) are collected. Functional neurological assessment includes neurological examination, the National Institutes of Health (NIH) stroke scale, modified Rankin scale, and the Barthel index. A follow-up interview is conducted by telephone or in person approximately six-months after the index stroke/transient ischemic attack/intracerebral haemorrhage to determine patient outcomes, quality of life, and patient use of medications.

**Main outcome** Prevalence of positive DNA mutation or single nucleotide polymorphism screens for Fabry's disease as a proportion of total cryptogenic stroke. Secondary outcomes include incident risk of new or recurrent vascular event at six-months, discharge disposition, disability at six-months as measured by the modified Rankin scale, mean time from symptoms onset to the definite etiological diagnosis, and length of hospital stay.

**Conclusion** This study constitutes the first initiative to determine the prevalence of a positive screen for Fabry's disease in young adults with stroke in Canada. Moreover, the Canadian Fabry Stroke Screening Initiative will provide information on recurrent vascular events, disability at six-months (modified Rankin scale), and disposition in this understudied population.

Key words: cryptogenic, disability, Fabry disease, prospective, stroke, stroke in the young

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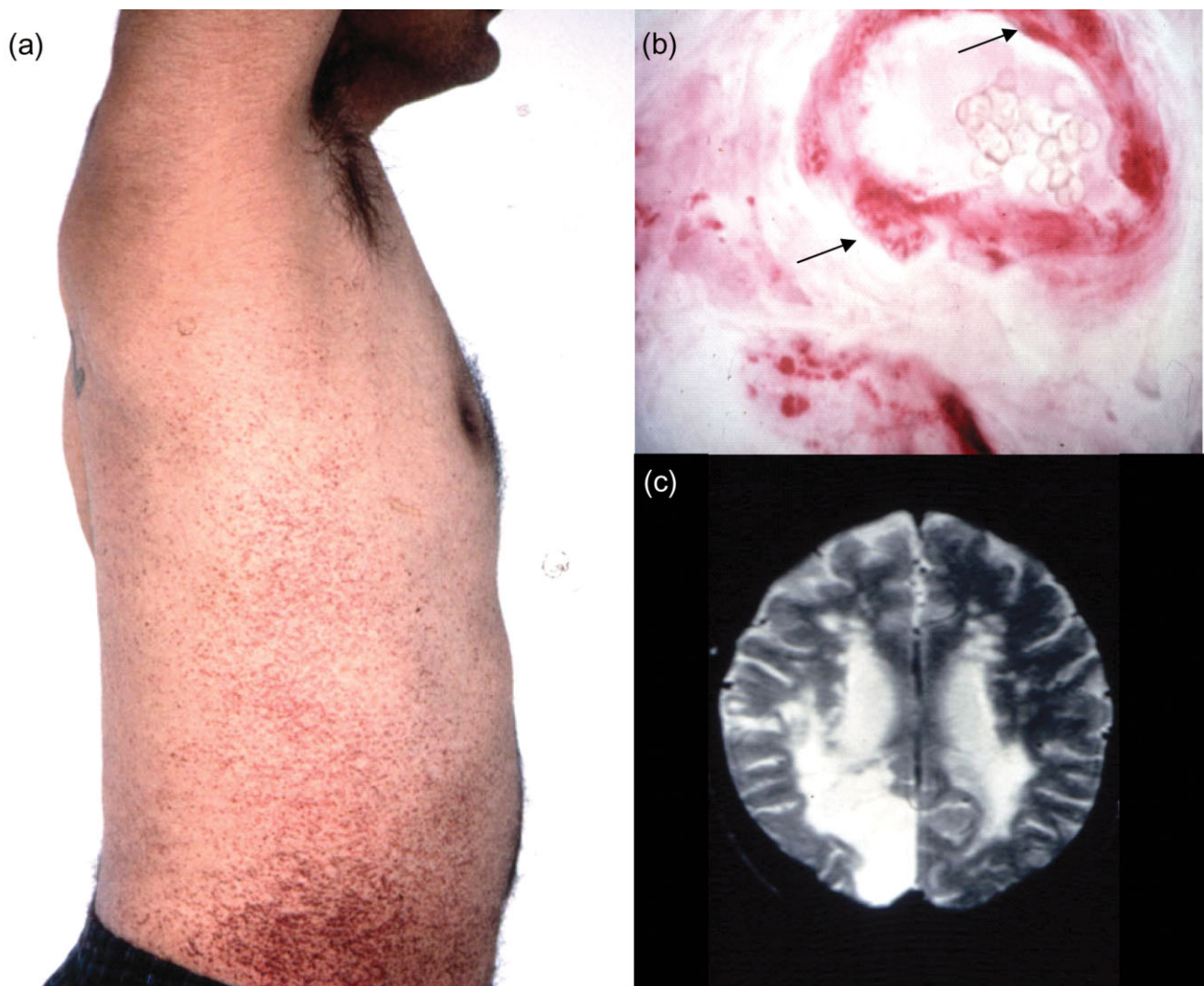
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## Background

Fabry's disease is an X-linked lysosomal disease that is caused by deficiency of lysosomal enzyme  $\alpha$ -galactosidase A ( $\alpha$ -Gal A) (1). In most homozygote males and females with <1%  $\alpha$ -Gal A activity, there is progressive tissue accumulation of upstream glycosphingolipids (globotriaosylceramide, Gb3). Multiple tissues and organ systems are usually affected includ-



**Fig. 1** Clinical features of Fabry disease. Angiokeratoma (Panel a), Oil red O stain showing glycolipid accumulation in a small vessel smooth muscle and endothelium (arrows, Panel b), T2-weighted MRI showing cortical and subcortical ischemic strokes in a patient with Fabry disease (Panel c). Pictures were kindly provided by Dr Raphael Schiffmann.

ing the skin, eyes, kidneys, and heart together with vascular beds particularly the cerebral with hallmark dolichoectasia. The initial clinical manifestations may develop in childhood and include episodes of extremity pain, acroparesthesias, hypohidrosis, cold and warmth intolerance, recurrent fever, corneal (cornea verticillata) and lenticular changes (posterior capsular cataract), and angiokeratoma (Fig. 1a) (1). Deposition of glycosphingolipids in vascular endothelial and smooth muscle cells (Fig. 1b) may result in progressive vascular dysfunction of both small and large arteries (2). As a result of the enzyme deficiency, young adults may develop renal dysfunction, cardiovascular disease, and stroke (Fig. 1c) (the major causes of morbidity and mortality in the developed nations) but earlier in onset compared with the general population (1,3). In addition, accumulation of glycosphingolipids in the myocardium causes a hypertrophic cardiomyopathy and myo-

cardial dysfunction with delayed posterior wall enhancement on magnetic resonance (MR), a potential source of embolic stroke. Atypical variants are described in heterozygote females with 1–30%  $\alpha$ -Gal A activity and either no symptoms or symptoms limited to a single or a few organs. Recent randomized clinical trials showed a clinical improvement and reduction of vascular events by the intravenous administration of  $\alpha$ -Gal A (4–8).

Stroke in young adults (age range ~18–55 years) is etiologically diverse and surprisingly common (Table 1), accounting for 15–20% of all ischemic strokes (9–11). After extensive investigation, ischemic stroke remains unexplained in approximately 25% of young patients. Some epidemiological studies suggest that Fabry's disease, as a rare cause of stroke, accounts for approximately 4% of patients with cryptogenic stroke (12). Unfortunately, limited information is available

**Table 1** Etiological differential diagnosis of stroke in young adults**Ischemic stroke***Affecting large or small vessels*

- Premature atherosclerosis
- Dissection (spontaneous or traumatic)
- Inherited metabolic diseases (Fabry's, homocystinuria, pseudoxanthoma elasticum, Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke (MELAS) syndrome)
- Inherited due to gene mutation (e.g. Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CEDASIL))
- Fibromuscular dysplasia
- Infection (Zoster, tuberculosis, syphilis, Lyme)
- Moyamoya disease
- Toxic of illicit drugs (e.g. cocaine, heroin, phencyclidine) or therapeutic drugs (e.g. L-asparaginase, cytosine arabinoside)
- Vasculitis (systemic lupus erythematosus, Takayasu's disease, Wegener's syndrome, etc.)
- Radiation vasculopathy
- Iatrogenic/Reversible vasoconstriction syndrome

*Cardiac source*

- High risk: atrial fibrillation, recent anterior myocardial infarction, rheumatic valve disease, mechanical valve, endocardial thrombus, tumor, endocarditis
- Medium risk: left ventricular hypokinesis/aneurysm, bioprosthetic valve, congestive heart failure, myxomatous mitral valve prolapse, etc.
- Low/unclear risk: patent foramen oval (PFO), spontaneous echo contrast

*Prothrombotic states*

- Thrombophilias\*:

High risk: antiphospholipid syndrome, antithrombin III, protein C or S deficiency

Moderate risk: Factor V Leiden, Prothrombin 20210A mutation, High FVIII

- Sickle cell, thalassemia major
- Myeloproliferative syndromes (e.g. polycythemia vera, essential thrombocythemia, etc.) Other (e.g. cancer, MTHFR methylenetetrahydrofolate reductase (NAD(P)H) mutation)

**Haemorrhagic stroke**

- Aneurysmal
- Arteriovenous malformation
- Neoplasm (e.g. primary central nervous system, metastatic, leukemia)
- Hematologic (e.g. neoplasm, thrombocytopenia)
- Moyamoya disease
- Inherited metabolic diseases (e.g. Fabry disease)
- Drug use (e.g. warfarin, amphetamines, cocaine, phenylpropanolamine, etc.)
- Hereditary (CADASIL)
- Iatrogenic (peri-procedural)

\*Adapted from Makris M, *Blood* 2009; **113**:5314–532.

from different countries and populations on the prevalence of this treatable and systemic genetic disorder.

We designed a prospective cohort study to determine the prevalence of DNA positive screens for Fabry's disease in young individuals with cryptogenic stroke in Canada.

The present manuscript summarizes the design of the study, protocol, and specific challenges in the study of this specific group of patients.

## Methods

### Study design

The Canadian Fabry Stroke Screening Initiative (CFSSI) is a prospective multicenter cohort study of patients presenting with an ischemic stroke, transient ischemic attack (TIA), or

intracerebral haemorrhage (ICH) of unknown etiology to stroke centers across Canada.

### Study objectives

The primary objective is to estimate the prevalence of positive DNA mutation or single nucleotide polymorphism screens for Fabry's disease in an unselected group of young patients with stroke of undetermined etiology. Fabry's disease is caused by mutations in the *GLA* (galactosidase, alpha) gene, which codes for the  $\alpha$ -Gal A enzyme (1,13). A participant will be considered to have a positive screen for Fabry's disease if:

- a known (i.e. previously identified and linked to Fabry's disease) *GLA* mutation or polymorphism is found and
- a novel (i.e. *not* previously identified) *GLA* mutation or polymorphism is found, and it is unclear whether the genetic changes result in Fabry's disease. This is because many *GLA*

mutations in Fabry patients are either individual mutations that have not been described previously or, in some cases, intronic mutations, which might influence the biological activity of the gene.

Secondary objectives include:

- to assess the clinical presentation (risk factors, diagnosis, and neuro-imaging features) among patients with premature stroke of undetermined cause and
- to determine the level of disability at six-months postindex event.

### Participants

All patients with stroke between the ages of 18 and 55 at facilities enrolled as participating centers and working within the broad guidance of the Canadian Best Practice for Stroke Care (14) will be considered for inclusion into the study. A list of participating centers and investigators is included in the appendix. Inclusion and exclusion criteria following diagnosis of a cryptogenic stroke are summarized in Table 2. After patient consent and following institutional standard stroke practice such as a routine diagnostic Computed Tomography/Magnetic Resonance Imaging (CT/MRI), a blood sample will be drawn for DNA analysis of the  $\alpha$ -Gal A gene (a genetic screen for Fabry's disease). Patients can be enrolled up to six-months following the index ischemic stroke, TIA, or ICH event. Patients with a TIA will be eligible if:

- there is clear evidence of motor and/or speech involvement as a clinically isolated syndrome;
- the duration of motor and/or speech involvement is greater than 10 mins; and
- the only possible etiological explanation of the TIA is vascular in nature. All patients not enrolled at the time of their initial presentation with stroke/TIA/ICH will have peristroke demographic and clinical data obtained from retrospective chart review.

### Physical examination

Details of the required investigations and time lines are provided in Figure 2. Systolic and diastolic pressure will be ascertained 24 h after admission or 24 h after reintroduction of preadmission hypertensive medication.

Neurological assessment includes neurological examination performed by a qualified neurologist, including National Institutes of Health (NIH) stroke scale, modified Rankin scale (mRS), and Barthel index. In those cases where a specific neurological assessment is not performed within seven-days of the index event, a score for that event may be inferred on the basis of the patient medical records and/or direct input from the patient.

Participants in the study are to be treated with any necessary drugs or medical interventions according to local institutional standards of clinical care for stroke.

Concomitant medications (e.g. antiplatelet agents, anti-coagulants, antihypertensive therapy, such as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockade), and lipid lowering therapy are specifically documented in the clinical report form (CRF).

### Blood tests

Total white blood cell count, hemoglobin, platelet count, partial thromboplastin time (PTT), International Normalized Ratio (INR), glucose, creatinine, AST (aspartate aminotransferase), ALT (alanine aminotransferase), and baseline lipid profile are determined from serum samples according to local laboratory routine and will be recorded in the CRF. Similarly, C-reactive protein (CRP), antinuclear antibodies (ANA) titer, Rheumatoid factor (RF), anticardiolipin antibodies, lupus anticoagulants, anti-beta2 glycoprotein 1, and total urinary protein will also be recorded, allowing description of the patient population and confirmation of the inclusion/exclusion criteria.

Participants in the current study (CFSSI) will also be approached and consented according to institutional guidelines and practice to provide blood for DNA banking at the Centre Hospitalier de l'Université de Montréal (CHUM) DNA bank. Samples at the CHUM DNA bank will be used in future studies on the genetics of stroke in the young following the approval of the relevant study protocols by the CHUM research ethics board.

### Diagnosis of Fabry

Screening for Fabry's disease will occur by direct DNA analysis of blood samples for Fabry gene mutations or polymorphisms (i.e. mutations or polymorphisms in the *GLA* gene that codes for the enzyme  $\alpha$ -Gal A) as reported in other studies (1,12,15). Ethylenediaminetetraacetic acid-anticoagulated blood samples will be couriered following the standard operating procedures for biological specimens using preprepared storage and shipping materials to the designated central analysis laboratory at the University of Rostock, Germany. Confirmation of a Fabry diagnosis may require further assessments and tests that fall outside the immediate mandate of this study.

### Follow-up interview

A follow-up interview will be conducted by telephone or in person approximately six-months after the index stroke/TIA/ICH in order to track patient outcomes, quality of life, and patient medication usage. There is a one-month window, from 5.5 months to 6.5 months postindex event, in which to conduct the follow-up interview. Interview questions were generated from the validated phone version of the mRS (16,17). Questions regarding motor function and quality of life included in the stroke impact scale were also included as part of the follow-up questionnaire (18). The interview (by



**Table 2** Inclusion and exclusion criteria**Inclusion criteria**

1. Written consent has been obtained prior to patient enrollment in the study.
2. Patient is between the ages of 18 and 55 years of age inclusive.
3. Patient has experienced ONE of the following within six-months prior to enrollment in the study and was diagnosed by a neurologist:
  - a. Ischemic stroke defined by an acute, focal, neurological deficit which either i) persists >24 hours, or ii) lasts <24 hours with clear evidence of a recent relevant infarct documented by MRI with positive diffusion weighted imaging or by CT. A transient ischemic attack *with positive diffusion weighted imaging* shall be considered equivalent to a stroke and should be included in this category.
  - b. Transient ischemic attack (TIA) defined by an acute, focal, neurological deficit that includes motor and/or speech deficits and which lasts >10 minutes but <24 hours without evidence of a recent relevant infarct documented by MRI with diffusion weighted imaging or by CT.
  - c. Intracerebral haemorrhage (ICH) defined by an acute, focal or global, neurological deficit, secondary to an intra-cerebral haemorrhage documented by brain CT or MRI, provided other causes of intra-cerebral haemorrhage are excluded (e.g., no evidence of pre-existing hypertension or diabetes, no history of trauma, no evidence with appropriate investigations of intra-cranial aneurysm or any arterial or venous malformation).
4. Brain imaging, either by CT or MRI (with DWI and ADC map, axial FLAIR) is achieved within six months of the clinical event (i.e., as soon as possible following the clinical event, but not later than six months).
5. Cerebrovascular imaging, either CT or MR angiography (including the circle of Willis), is achieved within 6 months of the clinical event (i.e., as soon as possible following the clinical event, but not later than six months).
6. Brain and cerebrovascular imaging excludes diagnoses other than ischemic stroke, TIA, or ICH to explain the neurological deficit.

**Exclusion criteria**

1. Patient has evidence of *either*:
  - a. Atherosclerosis (with extra-cranial stenosis greater than 70% or intra-cranial stenosis greater than 50% on ipsilateral side).
  - b. Other large artery disease determined by vascular imaging (carotid ultrasound, CTA, or MRA) that is well explained by standard atherosclerotic risk factors or other cause.
2. Patient has *one or more* obvious cardiac cause of stroke as per the list below (determined by transthoracic echocardiogram or Holter equivalents):
  - a. Chronic or paroxysmal atrial fibrillation.
  - b. Valvular heart disease including endocarditis of any type (mitral or severe aortic stenosis).
  - c. Evidence of congenital heart disease (PFO alone is eligible for inclusion but not PFO combined with an atrial secundum aneurysm)
  - d. Evidence of paradoxical embolus (i.e. proven DVT with right to left shunt – e.g. PFO).
3. Patient has *any* of the following on BOTH of two tests performed 12 weeks apart:
  - a. IgG anticardiolipin antibodies >40 GPL units or IgM anticardiolipin antibodies >40 GPM units
  - b. Positive lupus anticoagulants

NOTE: Patients with any of the above on an *initial* test can still be enrolled and have blood drawn and shipped for DNA analysis of Fabry disease. However, a *second* positive test *must* be recorded on the CRF so that these patients can be excluded from later data analyses. Cases of two positive tests are expected to be rare.
4. Imaging appearance is consistent with a lacunar mechanism of stroke (<15 mm) AND patient has evidence of *either* of the following (NOTE: Patients with a lacunar mechanism and no evidence of any of the following *are* eligible):
  - a. Chronic hypertension (>140 mmHg systolic with >90 mmHg diastolic 24 h postadmission or community value)
  - b. Diabetes mellitus (two-hour glucose tolerance test or a random blood glucose  $\geq 11.1$  mmol/l ( $\geq 200$  mg/dl); fasting glucose  $\geq 7.0$  mmol/l ( $\geq 126$  mg/dl))
5. Diagnosis of CADASIL established by genetic testing in patients with headaches, cognitive decline, or brain imaging showing CADASIL changes (leucoencephalopathy that is maximal at the temporal lobes and extreme capsules). NOTE: Genetic analysis for CADASIL is *not a requirement* of this study, but it is anticipated that it will be done as part of the standard of care whenever there is a strong suspicion of CADASIL.

DWI diffusion weight imaging; CT, computed tomography; MRI, magnetic resonance imaging; ADC, apparent diffusion coefficient; FLAIR, Fluid Attenuated Inversion Recovery; ECG, electrocardiogram; PFO, patent foramen oval; DVT, Deep Vein Thrombosis; IgG, Immunoglobulin G; GPL IgG phospholipid units; IgM, Immunoglobulin M; GPM IgM phospholipid units; CADASIL, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy; CRF, clinical report form.

phone or in person) is estimated to take approximately 15 mins and will be conducted by site level staff. Patients enrolled six-months postindex event (i.e. within the 5-5 to 6-5-month follow-up window) may have the follow-up interview conducted at the time of enrollment.

**Outcome measures**

The primary end-point is to determine the prevalence of positive DNA screens for Fabry's disease in young individuals

presenting with stroke to primary stroke care centers across Canada.

Secondary outcomes include: incident risk of recurrent vascular events at six-months postindex event, discharge disposition, disability at six-months (mRS), mean time from symptoms onset to the definite etiological diagnosis, and length of hospital stay.

**Withdrawal of patients**

A participant may choose to withdraw from the study at any time and no further individualized data will be collected.

However, any data already obtained from DNA sample analysis or collected from their charts up to the point of withdrawal will be used to keep the scientific integrity of the study.

### Procedure for patients with a positive diagnosis for Fabry's disease

Genetic counseling is not part of the CFSSI study but is 'standard of care' in Canada for all patients undergoing evaluation for Fabry's disease and will be recommended to those patients in our study with a positive screen diagnosis.

Patients who receive a positive screen for Fabry's disease will be referred to the Canadian Fabry Disease Initiative (CFDI) for extended evaluation, treatment, and/or follow-up. In some patients, additional assessments may be required in order to obtain a final diagnosis. The treatment for Fabry's disease, known as enzyme replacement therapy (ERT), is expensive and is not yet covered by provincial health plans in Canada. However, these drugs have been available since 2006 at no cost through the CFDI, a government, provincial and industry-sponsored study. As per CFDI protocols, all patients aged 5–85 who meet the criteria for ERT established by a Canadian expert committee are offered the treatment. There are currently two ERT treatments available (agalsidase alfa by Shire HGT and agalsidase beta by Genzyme Corporation), and CFDI ERT patients are randomly assigned to one of these two treatment options (5,6,13). ERT can be administered in the patient's home or at a local hospital. Those not meeting CFDI ERT criteria are followed in the natural history cohort with a proportion of these patients requiring eventual treatment.

### Statistical methods

Patients' characteristics will be evaluated by descriptive parameters. Observed frequencies together with 95% confidence intervals (CIs) will be given for categorical data. Means with 95% CIs, medians, standard deviations, and selected percentiles will be given for continuous variables.

The primary end-point, prevalence of positive screens for Fabry's disease in young patients, will be evaluated by the relative frequency together with the Wilson CI (19). Differences between stroke patients with and without positive screens with respect to demographical or clinical characteristics and to stroke subtype and severity will be analyzed in an explorative manner. Analyses will be conducted as two-sample comparisons according to the distribution of data (*t*-test, Mann–Whitney rank sum test, and chi-square test). Finally, a multivariable logistic regression analysis will be performed to examine factors associated with a positive screen for Fabry's disease.

The analysis will be performed stratified by event type (ischemic stroke, haemorrhagic stroke, and TIA) depending on the prevalence of Fabry's disease.

### Determination of sample size and feasibility of patient enrolment

For the sample size calculation, two estimates of prevalence were chosen that describe a potential prevalence range of 2% and 5%. The statistical precision (95% CI) of the observed prevalence with  $n = 400$  and observed frequency 2% would be approximately 1.0–3.9%, while for the observed frequency of 5%, it would be approximately 3.3–7.6% (calculations based on the Wilson approximate interval for binomial probabilities (19)). Given the required sample size of 400, the reported prevalence of stroke in the young, and the number of potential participating stroke centres across Canada, we estimate that an 18-month recruitment and accrual window will be sufficient.

### Data quality and quality assurance

A sophisticated web-based database system (Medidata RAVE™) was created specifically to capture data for this study in a prospective manner. Research staff at each center will collect data and enter it directly into the secure, web-based database (Medidata RAVE) managed by the Applied Health Research Centre (AHRC) and housed onsite at St. Michael's Hospital. Edit checks, control checks, range checks, and system checks were programmed into the database to minimize data entry errors. Automated edit checks within the RAVE database will notify data entry personnel when data is out of the expected range or is nonconformant. Weekly quality assurance checks will be performed by the coordinating center's data management team to ensure that the data are as accurate as possible prior to locking data within the database. Outstanding data and/or data anomalies are communicated to the site(s) for clarification/resolution. In addition, screening logs, training, and guidelines were developed to assist site staff with study procedures. Posters and pocket cards were developed to educate and create study and rare disease awareness.

### Ethical and regulatory considerations

The conduct of this study conforms to the International Conference for Harmonization Good Clinical Practice guidelines. The current version of the protocol (including protocol amendments) has been approved by the Research Ethics Boards at St. Michael's Hospital in Toronto and CHUM and at 13 other participating sites.

### Informed consent

All participants will be given detailed oral and written information about the study. Participants must sign informed consent documents that have been approved by a participating center's Research Ethics Board (REB)/Institutional Review Board (IRB) prior to the collection of any blood samples specifically for this trial and/or storage of such samples for future studies. Two consent forms will be used; one for the main CFSSI study, including collection of blood for DNA analysis to

Assessment	Schedule of assessments					
	Visit 1	Visit 1 + 7 days	Visit 1 + 4 weeks	Visit 1 + 12 weeks	Visit 2a (six-month outcomes)	Visit 2b (follow-up interview)
Inclusion / exclusion	X					
Consent / enrollment	X					
Blood draw for Fabry diagnosis (shipped to Germany)	X					
Blood draw for DNA bank (if applicable)	X					
Demographics	X					
Medical history	X					
Family history	X					
Physical examination*		X				
Neurological assessments*		X				
Presenting clinical symptoms*		X				
ECG*		X				
Imaging (if applicable; MRI/MRA, CT/CTA)**		X				
Lab tests (lipid profile, hematology, clinical chemistry)*		X				
Ultrasound (if done; not required)*		X				
IgG and IgM anticardiolipin antibodies, lupus anticoagulants***		X		X		
Input results of Fabry genetic test upon receipt from German lab			X			
Telephone / in-person interview (6-month follow-up)						
Outcomes / study termination					X	
Medication use, recurrent events, modified Rankin scale						X

**Fig. 2** Patient flow chart. ECG, electrocardiogram; MRI, Magnetic Resonance Imaging; MRA, magnetic resonance angiography; CT, computed tomography; CTA, CT angiography; IgM, Immunoglobulin M; IgG, Immunoglobulin G. \*These assessments are to be done as soon as possible following the cerebrovascular event, but no later than 7 days post-event (results may be obtained retrospectively using patient medical records). \*\*Imaging is to be done as soon as possible following the cerebrovascular event, but no later than 6 months post-event. \*\*\*Anticardiolipin antibodies and lupus anticoagulants should be tested as soon as possible following the cerebrovascular event, but no later than 6 months post-event. A second test (12 weeks later) is required only if an initial test is positive.

screen for Fabry's disease, and one for the DNA banking sub-study that includes collection and storage of blood at the CHUM DNA bank in Montreal. Participants who consent to the main study may choose whether they want to participate in the DNA banking sub-study and are free to decline.

### Study organization and data management

Study coordination and data management services are being provided by the AHRC of the Li Ka Shing Knowledge Institute at St. Michael's Hospital, Toronto. Participating stroke centers will be managed by the AHRC to ensure that all regulatory requirements are met and to monitor the quality of the data collected. Copies of Research Ethics Board approvals will be sent to AHRC prior to study initiation at any given center. Each center must maintain appropriate study files and meet regulatory/institutional requirements for the protection of confidentiality of study participants. (Fig. 2).

### Discussion

Stroke is a devastating condition for patients and families (20). Previous studies suggest the prevalence of stroke of uncertain etiology is higher in individuals younger than 55

years of age (21). Both premature stroke and the uncertainty of the etiology aggravate the distress caused by an acute stroke. This situation is not uncommon considering that approximately 15–20% of ischemic strokes occur in young individuals, and in one-quarter of these cases, the cause of stroke will remain undetermined (22). Moreover, commonly used stroke classifications (e.g. TOAST (Trial of Org 10172 in Acute Stroke Treatment)) are not useful in this group. For example, the TOAST classification was originally designed for patients older than 55 with ischemic stroke largely due to large and small artery disease or a cardiac origin (23). However, small and large artery disease due to arteriosclerosis and atherosclerosis are uncommon in the young (24,25). Finally, several etiologies for stroke in the young affect arteries of all sizes.

There are several challenges in the diagnosis and management of young patients with stroke (26). First, testing for specific genetic conditions, such as Fabry's disease, is not routinely available. Second, testing for Fabry's disease may not be ordered (even in stroke centers) unless there is a high grade of suspicion for the disease. Third, limited information is available on prognosis and disability in this age group, the most productive/active segment of the population.

The present prospective cohort study targets stroke care centers across Canada. This strategy is intended to optimize recruitment as the great majority of young patients with stroke are usually referred to stroke care centers. The results of the study will help determine the prevalence of Fabry’s disease in a consecutive series of young patients with stroke. Moreover, it will reveal information regarding the underlying etiology among those individuals where the cause of stroke is not initially clear. Additionally, the CFSSI will provide data on selected outcome measures, notably disability at six-months, in this understudied group of patients.

The results of the CFSSI may have practical implications. First, it may identify patients amenable to specific ERT (e.g. alpha-galactosidase ERT). Second, it may confirm (e.g. vertebrobasilar location) or delineate other clinical manifestations suggesting the diagnosis of Fabry among patients with otherwise unexplained stroke. Third, it may provide equal opportunities for early cardiovascular prevention (e.g. dyslipidemia and hypertension) in young individuals. Fourth, it will provide a snapshot of the short-term disability and incident risk of recurrent cerebrovascular events in young individuals. Finally, this study may serve as the initial step to evaluate the time to the etiological diagnosis and the proportion of patients that remain with undetermined etiology after completed investigations.

There are not many studies addressing the aforementioned questions in a young population with stroke. A German study (12) that included 721 patients aged between 18 and 55 years with cryptogenic stroke found that Fabry’s disease was prevalent in 4% of the patients ( $n = 28$ ). The authors stated that Fabry’s disease must be considered in all cases of unexplained stroke in young patients, especially in cases with the combination of infarction in the vertebrobasilar artery system and

proteinuria. In contrast, in a retrospective analysis of 103 patients aged 18–60 with cryptogenic stroke, the authors found no patients with Fabry’s disease following appropriate genetic testing (27). One of the clinical challenges for identifying patients with this medical condition is that both the small and large vessels are affected causing either ischemic or haemorrhagic strokes (28). Another limitation is the variability in the definition of ‘cryptogenic stroke’ in clinical practice (29). More importantly, none of these studies provided information on disability, time to the diagnosis, or the proportion of patients that remained with an undetermined etiology after completed investigations in stroke care centers. As such, the CFSSI is a multicenter prospective cohort study including an innovative design aimed at addressing these specific questions. We believe our study constitutes an important initial step in the understanding of the prevalence of Fabry’s disease in one of the most ethnically diverse populations in North America. The results of the CFSSI will not only help direct patients and families toward the appropriate treatment but will also provide guidance to clinicians and policymakers in order to optimize resources and facilitate access to specialized stroke care.

**Author’s contribution statement**

We declare that we have participated in the conception, design, drafting of the manuscript, and made a critical revision of the final manuscript.

Drs Lanthier, Saposnik, and Selchen are the Co-PI’s for the study. Dr Moore is the senior investigator of the CFSSI.

**Statistical expertise**

Professor Kevin Thorpe (statistician).

**Appendix: CFSSI Centres**

Site Investigator(s)	Participating Centre
Dr. Daniel Selchen & Dr. Gustavo Saposnik	St. Michael’s Hospital
Dr. Sylvain Lanthier	Hôpital Notre-Dame, Centre hospitalier de l’université de Montréal (CHUM)
Dr. David Spence	Stroke Prevention and Atherosclerosis Research Centre (SPARC), Robarts Research Institute
Dr. Ariane Mackey & Dr. Steve Verreault	Hôpital de l’Enfant-Jésus, Centre hospitalier affilié universitaire de Québec (CHA)
Dr. Michael Hill	Foothills Hospital
Dr. Ken Butcher	Aberhart Centre One
Dr. Brian Buck	Grey Nuns Community Hospital
Dr. Jean-Martin Boulanger	Hôpital Charles LeMoine
Dr. Vladimir Hachinski	University Hospital
Dr. Martin del Campo	Toronto Western Hospital
Dr. Rick Swartz	Sunnybrook Health Sciences Centre
Dr. Michael West, Dr. Stephen Phillips & Dr. Gordon Gubitz	Queen Elizabeth II Health Sciences Centre
Dr. Grant Stotts	Ottawa Hospital – Civic Campus
Dr. Sylvie Gosselin	Centre hospitalier de l’université de Sherbrooke (CHUS)
Dr. Stefan Pacin, Dr. Curry Grant	St. Boniface Hospital, Quinte Health Care

Other centers are currently awaiting ethical approval (Dr. Manu Mehdiratta at Trillium Health Centre and Dr. Chidam Yegappan at St. Joseph’s Health Care).



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