

Protocol and Methodology of the Stroke in Young Fabry Patients (sifap1) Study: A Prospective Multicenter European Study of 5,024 Young Stroke Patients Aged 18–55 Years

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Key Words

Stroke, young patients · Fabry disease · Multicenter
epidemiological study

Abstract

Background: Stroke in the young has not been thoroughly investigated with most previous studies based on a small number of patients from single centers. Furthermore, recent reports indicate that Fabry disease may be a significant cause for young stroke. The primary aim of our study was to determine the prevalence of Fabry disease in young stroke patients, while the secondary aim was to describe patterns of stroke in young patients. **Methods:** We initiated the Stroke in Young Fabry Patients (sifap1) study as a multinational prospective European study of stroke patients aged 18–55 years and collected a broad range of clinical, laboratory, and radiological data using stringent standardized methods. All pa-

tients were tested for Fabry disease and blood was stored for future genetic testing. **Results:** We managed to enroll 5,024 eligible young stroke patients in 15 countries and 47 centers across Europe between April 2007 and January 2010. The median number of patients included per center was 98 with a range between 8 and 315. The average duration of patient recruitment per center was 22 months, ranging between 5 and 33 months. The database was closed in July 2010. This paper describes protocol and methodology of the sifap1 study. **Conclusion:** The sifap1 study included the largest series of young stroke patients so far and will allow for analyses on a large number of aspects of stroke in the young.

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A.R., P.M., and P.U.H. contributed equally to this study. sifap1 Investigators are listed in Appendix 1.

Introduction

Stroke is often perceived as a disease of the elderly since the median age at first stroke onset in Europe is 73 years [1]. However, about one third of first-ever strokes occurs in patients under the age of 65 years [1] and about 10% of hospitalized stroke patients in Europe are aged 55 years or younger [2]. Despite these figures, only limited information is available on causes and consequences of stroke in young adults. The etiology of stroke seems to differ between young and elderly stroke patients; in young patients, rare stroke conditions, such as hematological diseases, migraine or genetic disorders, might be of particular importance [3, 4]. The societal impact of stroke in the young is substantial. It is estimated that currently up to 24% of all expenditures for stroke are caused by indirect costs attributed to loss of income or productivity in working age stroke patients. Previous studies on young stroke patients based mainly on a limited number of patients were often single-center case series, and included only a limited number of clinical and diagnostic information.

There is growing evidence of an association between Fabry disease and higher stroke risk, mainly in young patients. In a cohort of young adults with cryptogenic stroke aged between 18 and 55 years Fabry disease was found in about 4% [5]. The true prevalence of Fabry disease in the general population of young stroke patients remains unknown. Thus, the multicenter European Stroke in Young Fabry Patients study (sifap1) was recently initiated. The main aim of sifap1 was to establish the prevalence of Fabry disease in an unselected group of young stroke patients aged between 18 and 55 years. As secondary objectives, young stroke patients had been classified according to underlying etiological stroke subtype and severity of stroke using standardized instruments, such as demographics and clinical characteristics. This paper describes protocols and key methodology used in the sifap1 study.

Patients and Methods

The study was designed as a multicenter multinational prospective observational study of young stroke patients across Europe (Appendix 1). The project was coordinated by the Albrecht Kossel Institute for Neuroregeneration (AKos), Center for Mental Health, University of Rostock. An independent steering committee (Appendix 1) was established before the start of the study including experts in the field of stroke medicine, Fabry disease, neurology and epidemiology for overseeing the design and conduct of the study. Publication activities were coordinated during several meetings bringing together the members of the scientific

committees, external experts as well as representatives from the 6 best recruiting study centers.

Ethical Issues

The design of the study was approved by the ethics committee of the Medical Association Mecklenburg-Vorpommern (board 2), Medical Faculty, University of Rostock. Approval of the local ethics committees was obtained in all participating centers. Since it was designed as a purely observational study, patients received therapy and diagnostic procedures according to local practice. Patients or patients' legal representatives provided written informed consent to be included in the study. The study was registered in www.clinicaltrials.gov and www.strokecenter.org/trials (No. NCT00414583).

Study Centers

European clinical centers, whose expertise in stroke care was well recognized, were invited to participate in the study provided they could obtain ethical and other approval necessary for conducting the study according to their local rules; they also had to agree to recruit a minimum of 50 patients per year, to have a state-of-the-art infrastructure for the management of stroke patients, and to implement European [6] or similar compatible institutional written stroke guidelines. All study centers and the number of eligible patients recruited by each center are listed in Appendix 1.

Participating Centers

In the participating centers the local investigator was responsible for recruiting consecutive young stroke patients to the study. As it was designed as a purely observational study, patients received therapy and diagnostic procedures according to local practice not influenced in any way by the study protocol.

Ensuring High Inclusion Rates

To ensure high inclusion rates, the number of patients included per center was monitored by the coordinating center (University of Rostock) on a monthly basis during the whole study period. If within a center for more than 6 weeks no patient was included, standardized procedures were applied to increase inclusion rates of the respective center, including direct contact with the principal investigator and visiting of the center by members of the coordinating team. Centers not including any single patient during an 8-week time period were excluded from further patient inclusion.

Estimation of Completeness of Patient Selection across Centers

To estimate completeness of case ascertainment and potential selection biases of patients included in the study, the 7 best recruiting centers (Giessen, Berlin, Ulm, Munich, Heidelberg, Helsinki, Rostock), contributing about one third of all patients to the project, provided cross-checks between the number and demographic characteristics of patients treated in their center and patients included in the sifap1 study for the year 2008. For this purpose, collected data on discharge ICD diagnosis or routine audit documentation (in German centers only [7]) was provided, including total number of patients between 18 and 55 years with a discharge diagnosis of transient ischemic attack (TIA) or stroke, sex distribution, total and age stratified mean and median age within this group.

Data Collection within the Centers

The participating centers were responsible for collecting data for all patients meeting the predefined inclusion criteria (table 1). A detailed manual was developed for the centers giving specific advice on how to assess patients and how to collect required data. External monitoring was undertaken regularly in a subset of a mean of 35% of all patients by the coordinating center in Rostock for verification of validity and reliability of collected data in agreement with source documents. The centers received a modest case payment for each completed patient.

Central Laboratory Analyses

Blood samples were taken from participants to establish an α -galactosidase deficit; this was done in a central laboratory structure (Albrecht Kossel Institute for Neuroregeneration, University of Rostock) by direct dideoxy sequencing of the entire α -galactosidase exonic structures as well as the intron-exon boundaries; systematic MLPA for the exclusion of large deletions of the gene – especially in females – has not been done. In those cases where we had detected new mutations or those with unclear biological consequences additional biomarkers were analyzed in central laboratory structures (Gb3 in the blood, Karl Lackner/Mainz; Gb3 isoforms in the urine, Eduard Paschke/Graz; lyso-Gb3, Hermann Mascher/Baden); also gluteal skin biopsy with consecutive electron microscopy (Michael Laue/Ludwig Jonas/Rostock) and partly immunohistochemistry for Gb3 accumulation (Uwe Hillen and Thomas Jansen/Essen) was done in these cases.

Central Imaging Analyses

Cerebral magnetic resonance imaging (MRI) was a mandatory procedure. It was recommended that all participating sites should use standardized MRI sequences. As a minimal requirement it was recommended that they should obtain T₂/PD-weighted and/or FLAIR images and a diffusion-weighted imaging sequence. Investigators were also asked to use T₁- and T₂*-weighted sequences, if possible. Magnetic resonance images were analyzed centrally at the Department of Neurology, Medical University of Graz, Graz, Austria, blinded to clinical and demographic data. Following electronic data transfer all images were converted into 'Analyze' format to be available for reading by three stroke imaging experts (C.E., F.F. and R.S.). Besides a global rating of image quality as 'poor', 'sufficient', or 'good' these readers recorded all imaging findings according to prespecified characteristics. For ischemic infarcts, infarct types [8, 9] were noted as well as number, infarct location, size, and vascular territories involved; for this procedure the templates by Tatu et al. [10] were used. Diffusion-weighted imaging sequences served to identify areas of acute ischemia. For cerebral hemorrhages, we noted their number and location. So-called 'white matter hyperintensities' were defined as lesions with high-signal intensity on T₂-weighted images in the absence of evidence for complete tissue destruction [11] and were rated according to the Fazekas scale as deep white matter hyperintensities (0 = absent; 1 = punctuate; 2 = early confluent; 3 = confluent) and periventricular white matter hyperintensities (0 = absent; 1 = pencil-thin lining; 2 = halo of ≥ 5 mm thickness; 3 = irregular WMH extending into deep white matter) [12]. This rating has been shown to have high intra- and interrater reliability [13]. Diffuse signal abnormalities in the pons were also rated as absent, punctuate, early confluent or confluent. Microbleeds were defined on gradient-echo T₂*-weighted images as areas of signal

Table 1. Inclusion criteria of patients in sifap1

Age	18–55 years
Diagnosis	Acute CVE of any etiology (ischemic stroke, TIA, intracranial hemorrhage)
Time since event	Less than 3 months before inclusion into the study
Verification of diagnosis	Verification of brain infarction or hemorrhage by MRI scan In case of negative MRI diagnosis confirmed by stroke-experienced neurologist (more than 2 years of experience in stroke and at least 6 years of experience in general neurology)
Diagnostic information	MRI documentation available Diagnostic procedures according to EUSI/ESO recommendations [6]
Ethics	Written informed consent from patient or legal representative according to local ethics committee regulations

loss within the brain parenchyma not exceeding 5 mm in diameter [14, 15] and we recorded the total number and location of these abnormalities as previously reported [16]. To specifically look for changes that have been associated with Fabry disease, we also searched for signal abnormalities of the pulvinar thalami, i.e. hyperintensity on T₁-weighted images, if available [17]. Likewise we also looked for tortuosity and/or ectasia of the vertebrobasilar vessels [18], which was recorded as none, mild (some tortuosity of basilar artery with deviation from midline ≤ 1 cm), moderate (deviation of basilar artery from midline >1 cm and/or vessel diameter >5 mm) and severe (tortuosity with impression of brainstem and/or diameter >10 mm). The diameter of the basilar artery was also measured on T₂-weighted images in the axial plain at the largest diameter. Brain atrophy was rated according to prepared templates with scores from 0 to 8, separately for ventricular widening and sulcal widening, as recently reported [19, 20].

Variable Definitions and Consistency of Data

A core dataset to be collected by the participating centers for all patients was defined, including e.g. sociodemographics, clinical symptoms, comorbidities, results of diagnostic workup, stroke severity, and stroke etiology. In addition, a number of optional data items were also collected by the centers, including e.g. laboratory blood tests, vascular and cardiac imaging, and stroke-associated comorbidities (depression, pain, and headache). Compulsory and optional items documented in the sifap1 study are presented in table 2.

A series of key variables and key definitions were prespecified by the steering committee on the first publication meetings in Lund (7–9 November 2009) and Helsinki (8–9 February 2010) including the following items.

Classification of Qualifying Cerebrovascular Event. The qualifying event was classified into TIA and completed stroke based on the information provided by the participating center. Following

Table 2. Clinical data collected within the sifap1 study

Data items
<i>Mandatory core data set (collected by centers)</i>
Demographics (age, sex, ethnicity)
Family history for cerebrovascular diseases
Clinical symptoms of qualifying event
Classification of qualifying event
Diagnostic workup (including MRI results from local centers)
Acute treatment (tPA use)
Current medication
Medical history (vascular risk factors)
Lifestyle factors (physical activity, sleeping behavior, alcohol and tobacco use)
Instrumental stroke scales (NIHSS, modified Rankin Scale, Barthel Index) at time of maximum impairment
TOAST classification [21]
Total protein in urine
Adverse event
<i>Optional data set (collected by centers)</i>
Laboratory blood tests by participating centers
Vascular imaging
Electrocardiogram
Echocardiography
Cerebral CT
Depression at inclusion (Beck Depression Inventory II)
Pain at inclusion (Brief Pain Inventory)
Headache at inclusion (RoKoKo, Austrian and German centers only)
<i>Central data analyses (centrally analyzed in Graz, Mainz, Essen and Rostock)</i>
MRI documentation
Fabry analyses including genetics, biochemistry, Gb3 (blood and urine), lyso-Gb3 (blood and urine)

the study instructions TIA was defined as a cerebrovascular event (CVE) with duration of symptoms of less than 24 h. Based on the information from MRI, patients with evidence of intracerebral hemorrhage were classified as primary intracerebral hemorrhage; this information based on centrally analyzed MRI data or, if no central MRI was available, on imaging information provided by the local centers. Patients with completed stroke without evidence of intracerebral hemorrhage in brain imaging were classified as ischemic stroke. All cases receiving tPA were defined as ischemic stroke.

Classification of Underlying Etiology. The original TOAST criteria [21] were used to classify etiology of ischemic strokes. A subset of a priori defined criteria for implausible cases according to TOAST criteria was prespecified, including present AF (atrial fibrillation) = YES and TOAST = LAA (large artery etiology) or TOAST = SAO (small artery etiology); stenosis in supplying artery >50% = YES and TOAST = CE (cardioembolic etiology) or TOAST = SAO; AF = YES and stenosis in supplying arteries >50% = YES and TOAST = LAA or TOAST = CE or TOAST =

SAO; dissection = YES and TOAST = CE or TOAST = LAA or TOAST = SAO. The TOAST classification was also used for estimating underlying etiology in TIA patients; in these patients, the definition of the size of the lesion was not taken into account.

Classification of History of Stroke. History of previous stroke was defined based on the information on previous CVE and TIA; stroke patients with no documented history of prior CVE were classified as first-ever stroke.

Classification of Early Stroke Outcome. For assessing severity of stroke, NIH Stroke Scale documented at the time of maximum impairment was used as gold standard.

Categorization of Age Groups. In future analyses the following age categories will be used: 18–<25, 25–<35, 35–<45, and 45–55 years.

Data Management

All data were entered by the local investigators in an electronic Case Report Form. The central database was held at the University of Rostock and administered by Anfomed GmbH/Möhrendorf, Germany. Within the database, automatic range checks of entered data were done to check completeness of data entry.

Statistical Analyses

The primary endpoint of the sifap1 project is the estimation of the Fabry prevalence in the study population of young stroke patients. This will be done by calculating an exact two-sided 95% confidence interval based on the binomial distribution. However, as this is an observational study with focus on basic research concerning the spectrum of young stroke patients there are several ‘secondary’ research questions with focus on stroke. Univariate analysis will be done primarily using two-sided 95% confidence limits for proportions and mean values of continuous measurements. Explorative cluster analyses will be done in order to define subtypes of stroke in the young population and to identify covariables which make the classification possible.

Results

The data collection for the study took place between April 2007 and January 2010.

Study Centers

Patients were recruited in 47 centers from 15 European countries (fig. 1). During the study period, data collection was terminated in 3 of these centers because of a failure to achieve prespecified recruitment rates of at least 1 patient during 8 consecutive weeks. A detailed description of participating centers is presented in table 3.

Patient Characteristics

In total, 5,024 eligible patients were enrolled. Mean age was 44.6 years (8.2), in men 45.6 (7.5) and in women 43.3 (8.9). Age groups and age distribution by sex is presented in table 4 and figure 2. There is a clear overrepresentation

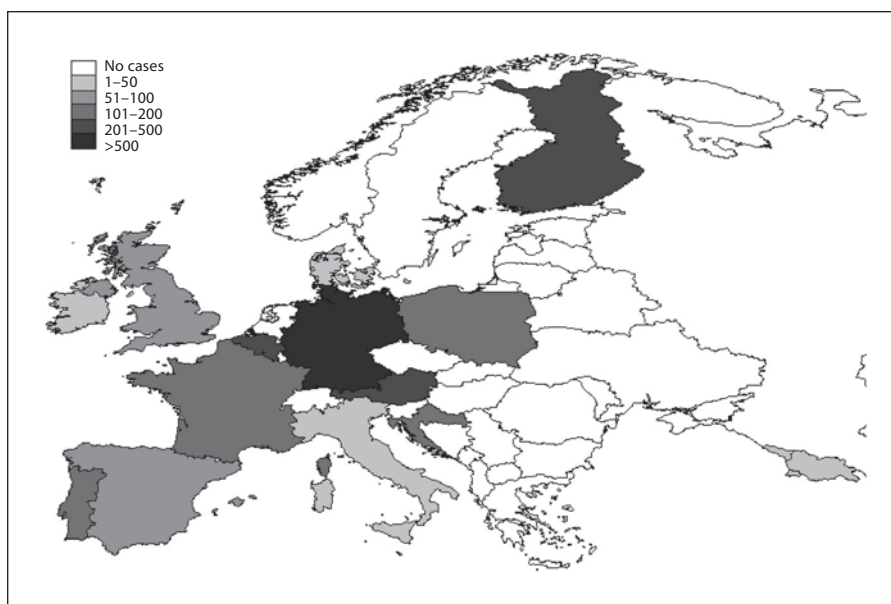


Fig. 1. Distribution of sifap patients over Europe.

of young female stroke patients with 65.6% females versus 34.4% males in the age class of 18–24 years and 53.9% females versus 46.1% males in the age class of 25–34 years. We could demonstrate a high frequency of risk factors in the young population (atrial fibrillation 2.4%; hypertension 46.7%; diabetes 10.0%), which is much higher than so far reported in the literature.

Estimation of Completeness of Case Ascertainment

In the 7 best recruiting centers 52.9% of eligible patients with stroke or TIA aged between 18 and 55 years were included. In these centers the difference in female patients recruited in the sifap1 project as compared to those patients not included ranged between –5.7 and +13.2% with one center reaching statistically significant difference. Overall, 7.0% (95% CI 1.0; 13.0) more female patients were recruited in the sifap1 cohort as compared to those patients not recruited in the study. The age difference of sifap1 patients compared to patients not included in sifap1 ranged between –3.7 and +0.4 years with only one center reaching statistically significant difference. Overall the age difference between sifap1 patients and non-sifap1 patients was –1.8 years (significant, 95% CI –3.1; –0.6). For male patients these differences were between –3.4 and +0.5 years with significantly younger patients in 1 center. Overall male patients were –2.1 years (95% CI –3.3; –0.9) younger in the sifap1 study compared to patients not included in the study. For female patients these differences ranged between –4.5 and +3.4 years,

Table 3. Description of centers participating in the sifap1 project

Total number of patients included in the study	5,024
Total number of centers included in the study	47
Total number of patients included in the study per center ¹	
<50 patients	11 (7.5%)
50–99 patients	13 (20.0%)
100–149 patients	14 (33.6%)
≥150 patients	9 (38.9%)
Average number of patients included in the study per center ²	
Total since inclusion	98 (8–315)
Per month since inclusion	4 (1–21)
Average duration of patient recruitment per center ²	
Duration of recruitment in the study, months	22 (5–33)

¹ Values represent number of centers with the percentage of all patients in parentheses.

² Values represent median with the range in parentheses.

with only 1 significant difference again. Overall in female patients age difference was –1.6 years (95% CI –3.6; 0.4), which was not statistically significant.

Discussion

Stroke in the young is currently an underresearched area. This might be caused by the fact that only limited data are available on etiology and natural course of stroke

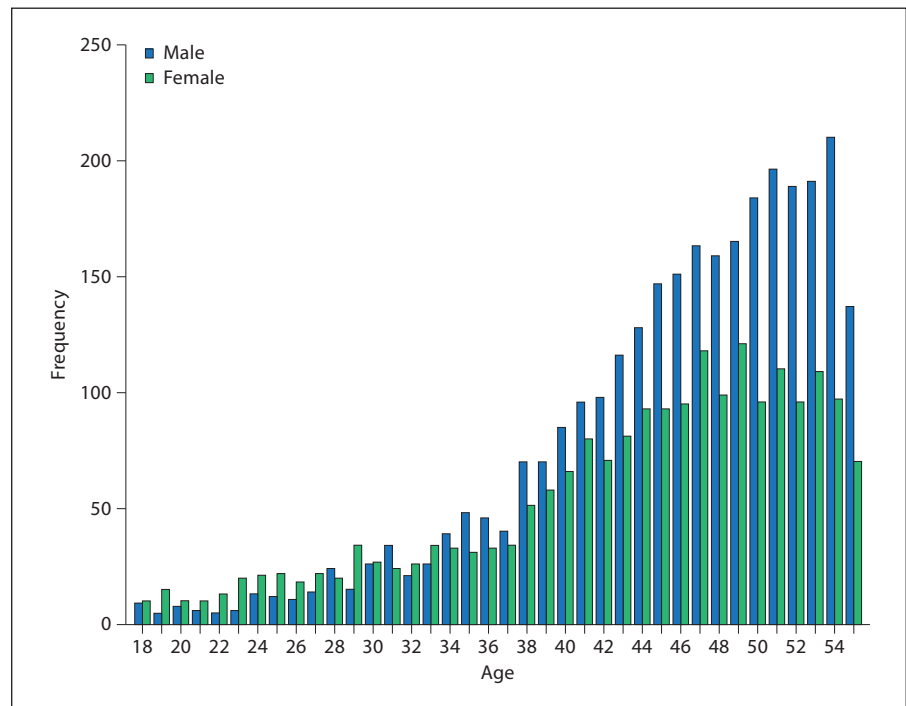


Fig. 2. Distribution of age by sex of sifap patients.

Table 4. Demographic characteristics according to age group

	All (n = 5,024)	Age groups			
		18–24 (n = 151)	25–34 (n = 482)	35–44 (n = 1,395)	45–55 (n = 2,996)
Sex					
Males	2,963 (59.0%)	52 (34.4%)	222 (46.1%)	797 (57.1%)	1,892 (63.2%)
Females	2,061 (41.0%)	99 (65.6%)	260 (53.9%)	598 (42.9%)	1,104 (36.8%)
Qualifying CVE					
Stroke	3,396 (70.6%)	106 (74.6%)	335 (71.6%)	918 (69.4%)	2,037 (70.9%)
TIA	1,071 (22.3%)	21 (14.8%)	102 (21.8%)	308 (23.3%)	640 (22.3%)
Primary hemorrhage	272 (5.7%)	5 (3.5%)	25 (5.3%)	71 (5.4%)	171 (5.9%)
Other (sinus venous thrombosis, etc.)	68 (1.4%)	10 (7.0%)	6 (1.3%)	26 (2.0%)	26 (0.9%)

in young adults. As stroke in young patients is a rare disease, previous studies were able to include only a small number of patients, which affected the validity of derived results and lacked the potential of detecting rare diseases or small effects of underlying risk factors. The only investigation with a large number of patients is the monocenter Helsinki Young Stroke Registry which studied over 1,000 patients retrospectively, including their follow-up [4, 22]. To increase the number of young stroke patients included, previous monocenter case series often expanded their recruitment over a long period of time and, thus, compa-

rability between different observation periods might be not given due to changes in diagnostics and treatment or management strategies. Therefore, information collected might not specifically meet needs of detection of causes and consequences of young stroke patients. Thus, large-scale studies that are specifically designed for this patient group are required to clarify the etiology of stroke in young patients.

The sifap1 study provides the largest prospective case series of young stroke patients reported so far. Overall 5,024 patients from 47 centers in 15 European countries

were included. High standards were applied during data collection to ensure reliability and validity of derived data. Quality measures such as a regular and frequent monitoring with an external audit of collected information and data collection within the participating centers were implemented. This project offers a number of unique features for comprehensively addressing causes and consequences of stroke in young adults, including carefully conducted phenotyping, a detailed medical history, diagnostic information, such as cardiac workup, carotid ultrasound, and MRI data, as well as a standardized laboratory protocol, including genetic analyses.

For several reasons it is important to investigate stroke in the young: (1) etiology of stroke in the young is extremely diverse with some causes which are common in the elderly population being only rarely observed in young individuals, (2) risk factors might differ considerably between the young and the elderly, (3) young stroke patients are usually at their working age; thus, stroke has significant socioeconomic consequences, including disabilities leading to premature retirement due to illness and enormous indirect costs, (4) genetic causes may be more frequently underlying stroke in the young, and (5) efficient acute treatment and prevention strategies in a young adult might be more effective in terms of life years gained than in an elderly individual.

Currently, the original TOAST classification is used as gold standard for classifying ischemic stroke according to its underlying etiology [21]. However, the TOAST classification came recently under criticism due to its high proportion of undetermined or concurrent etiology as well as due to its lacking consideration of new pathophysiological evidence [23]. Thus, a number of modifications of the TOAST classification and new classification algorithms were proposed [9, 24, 25]. This discussion might be of special interest for young patients as these patients were often classified to be of undetermined etiology [4]. The sifap1 project will contribute to better understand underlying etiology in young patients.

There are major research outputs expected from this data set to clarify etiology of stroke in young adults, including the areas of MRI/CT imaging and ultrasound, risk factor prevalence, genetics, outcome, epidemiology and gender. New pathophysiological concepts for occurrence of stroke in the young can be developed such as establishing the role of subclinical disease activity, e.g. white matter lesions (WML). WML are a frequent phenomenon in stroke patients associated with cognitive decline, impairment in activities of daily living and increased risk of recurrent stroke [26, 27]. However, as most

previous studies on frequency and impact of WML are based on older age populations, data on the frequency of WML in young adults are scarce as well as on their potential association with underlying stroke etiology, prevalence of specific risk factors (e.g. diabetes or hypertension) and outcome.

The study has strengths and limitations. sifap1 was designed as a cross-sectional study. As the collection of a number of data items was optional, some of the information is not available for all patients. The high number of patients included in sifap1 ensures a sufficient power for analyzing also specific subgroups of stroke patients for whom complete information is available. Due to the design of the study which allowed including patients within the first 3 months after the qualifying event (this was the case in less than 10% of all cases), it was not feasible to measure neurological deficits of individual patients at a defined time period after the event. Neurological deficits and functional outcome were measured at the time of maximum impairment which is in line with previous hospital-based stroke registers [2]. Case fatality in this age group in European countries is low with about 8% dying in the first 3 months after the event [2] and the proportion of patients being recruited within the main centers is high minimizing the possibility of potential selection biases. Since this is a hospital-based sample, we are not able to extrapolate our findings to calculate stroke prevalence or incidence rates for the general population.

Previous studies on young stroke patients used different age limits of 45 or 49 years [3, 22, 28, 29]. We decided to select an age limit of 55 years to explore when risk factor and etiology profiles start resembling those found in elderly stroke patients. A recent paper from Pakistan [30] describing risk factors for young Asian women with ischemic stroke from 12 centers in 8 Asian countries recruited women aged 15–45 years. Interestingly, unlike among Caucasians, the authors were able to show a high frequency of cerebral venous thrombosis and cardioembolism among young Asian women with stroke.

Conclusions

The sifap1 project so far managed to collect the largest case series of young stroke patients worldwide and offers a unique research environment to address various questions regarding etiology, clinical features, imaging and laboratory findings, and genetics of stroke as well as frequency and features of Fabry disease in young adults.

Acknowledgments

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Role of the Funding Body

The sponsors of the study had no role in the study design, data collection, data analysis, interpretation, writing of the manuscript, or the decision to submit the manuscript for publication. The corresponding author had full access to all of the data in the study and takes full responsibility for the integrity of the data and the accuracy of the data analysis, and had the final responsibility for the decision to submit for publication.

Appendix 1

sifap1 Investigators

Numbers in parentheses refer to the number of patients included to the study.

Executive Study Chairman: A. Rolfs, University of Rostock, Germany.

Steering Committee: M. Ginsberg, Miami, Fla., USA; M. Hennerici, Mannheim, Germany; C. Kessler, Greifswald, Germany; E. Kolodny, New York, N.Y., USA; P. Martus, Berlin, Germany; B. Norrving, Lund, Sweden; E.B. Ringelstein, Münster, Germany; P.M. Rothwell, Oxford, UK; G. Venables, Sheffield, UK.

Biobanking Committee: N. Bornstein, Tel Aviv, Israel; P. de Deyn, Middelheim, Belgium; M. Dichgans, Munich, Germany; F. Fazekas, Graz, Austria; H. Markus, London, UK; O. Riess, Tübingen, Germany; A. Rolfs, Rostock, Germany.

Study Administration Center: University of Rostock, Germany (T. Böttcher, K. Brüderlein, F. König, G. Makowei, D. Niemann, A. Rolfs, S. Rösner, S. Zielke).

Biostatistical Center: Department of Biostatistics and Clinical Epidemiology, Charité – Universitätsmedizin Berlin, Germany (P. Martus, U. Grittner, M. Holzhausen).

MRI Analysis Center: Department of Neurology, University of Graz, Austria (F. Fazekas, C. Enzinger, R. Schmidt, S. Ropele); JSW Research Ltd., Graz, Austria (M. Windisch, E. Sterner).

Consultants: O. Bodamer (Salzburg, Austria; biochemistry), A. Fellgiebel (Mainz, Germany; imaging), U. Hillen and Th. Jansen (Essen, Germany; immunohistochemistry), L. Jonas (Rostock, Germany; electromicroscopy), C. Kampmann (Mainz, Germany; cardiology), P. Kropp (Rostock, Germany; headache and

pain), K. Lackner (Mainz, Germany; Gb3 in blood), M. Laue (Rostock, Germany; electromicroscopy), H. Mascher (Baden, Austria; lyso-Gb3), E. Paschke (Graz, Austria; Gb3 in urine), F. Weidemann (Würzburg, Germany; cardiology).

Participating Centers

Numbers in parentheses refer to the number of patients included to the study (PI = principal investigator).

Austria (418): Medical University of Graz (123): F. Fazekas (PI), G. Schrotter, U. Krenn, S. Horner, B. Pendl, A. Pluta-Fuerst, U. Trummer. University of Innsbruck (64): J. Willeit (PI), M. Furtner, M. Spiegel, M.H. Knoflach, B. Prantl. University of Klagenfurt (8): J.R. Weber (PI), S.M. Marecek. University of Linz (73): F. Aichner (PI), H.P. Haring, E. Bach. University of Salzburg (104): G. Lardurner (PI), C. Sulzer, A. Zerbs, S. Lilek, A.M. Walleczek, D. Sinadinowska. University of Vienna (46): W. Lang (PI), W. Kristoferitsch, J. Ferrari, E. Ulrich, A. Flamm-Horak, A. Lischka-Lindner, W. Schreiber.

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Croatia (100): University of Zagreb (100): V. Demarin (PI), Z. Tranjec, M. Bosner-Puretic, M.J. Jurašić, V. Basic Kes, M. Budisic, L. Kopacevic.

Denmark (14): University of Copenhagen (14): D. Krieger (PI), G. Boysen, L. Leth Jeppesen, A. Petersen.

Finland (224): Helsinki University Central Hospital (224): T. Tatlisumak (PI), J. Putaala, S. Curtze, M. Metso.

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