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Acute Cerebrovascular Disease in the Young
The Stroke in Young Fabry Patients Study

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Background and Purpose—Strokes have especially devastating implications if they occur early in life; however, only limited information exists on the characteristics of acute cerebrovascular disease in young adults. Although risk factors and manifestation of atherosclerosis are commonly associated with stroke in the elderly, recent data suggests different causes for stroke in the young. We initiated the prospective, multinational European study Stroke in Young Fabry Patients (sifap) to characterize a cohort of young stroke patients.

Methods—Overall, 5023 patients aged 18 to 55 years with the diagnosis of ischemic stroke (3396), hemorrhagic stroke (271), transient ischemic attack (1071) were enrolled in 15 European countries and 47 centers between April 2007 and January 2010 undergoing a detailed, standardized, clinical, laboratory, and radiological protocol.

Results—Median age in the overall cohort was 46 years. Definite Fabry disease was diagnosed in 0.5% (95% confidence interval, 0.4%–0.8%; n=27) of all patients; and probable Fabry disease in additional 18 patients. Males dominated the study population (2962/59%) whereas females outnumbered men (65.3%) among the youngest patients (18–24 years). About 80.5% of the patients had a first stroke. Silent infarcts on magnetic resonance imaging were seen in 20% of patients

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with a first-ever stroke, and in 11.4% of patients with transient ischemic attack and no history of a previous cerebrovascular event. The most common causes of ischemic stroke were large artery atherosclerosis (18.6%) and dissection (9.9%).

Conclusions—Definite Fabry disease occurs in 0.5% and probable Fabry disease in further 0.4% of young stroke patients. Silent infarcts, white matter intensities, and classical risk factors were highly prevalent, emphasizing the need for new early preventive strategies.

Clinical Trial Registration Information—URL: http://www.clinicaltrials.gov. Unique identifier: NCT00414583   (***Stroke**. 2013;44:340-349.)*

The risk of stroke increases exponentially with age.[1] Whereas the majority of stroke patients are elderly (about half of all are >75 years of age), recent European data show that up to one third of first-ever strokes occur in patients <65 years of age[1] and about 10% of hospitalized stroke patients are aged ≤55 years.[2]

Strokes in adults <45 years of age accounted for about 2% of all first-ever strokes in a community-based Italian study,[3] 6% of all ischemic strokes admitted to hospital,[4] and 11% of all consecutive ischemic stroke patients in 2 centers in Switzerland.[5] In a hospital-based study in Finland, the yearly incidence of stroke increased from 2.4 per 100 000 for people aged 20 to 24 years to 4.5 per 100 000 for people aged 30 to 34 years with a further increase to 32.9 per 100 000 for people aged 45 to 59 years.[6]

As long-term survival is much more likely in younger than elderly stroke patients,[6] stroke in the young accounts for a high proportion of the total disability burden and the total costs of strokes for society.[7]

Only limited information is available on risk factors and causes of stroke in young adults, mainly because most previous studies have been small, lacked a prospectively defined protocol for the investigation of causes, or have been compiled over long periods during which changes in clinical practice have occurred. Such data, however, are needed to optimize the management of these patients. Whereas stroke in the elderly is firmly linked to classical risk factors and atherosclerosis, the spectrum of causes for the young is much broader and often a clear cause cannot be defined. In young patients nonatherosclerotic vasculopathies (dissection), hematologic diseases, migraine, or genetic disorders might be of particular importance.[4,8] In this context, some recent studies suggest that the role of Fabry disease—an α-galactosidase A gene defect resulting in the accumulation of glycosphingolipids—in stroke in the young might have been underestimated.[9–12] However, the true prevalence of Fabry disease in this population remains unknown and controversial.[13]

The multicenter European Stroke In Young Fabry Patients study (sifap)[14] was set up with the primary aim to investigate more precisely the frequency of Fabry disease in a large prospective sample of unselected patients with an acute cerebrovascular event (CVE), aged between 18 and 55 years. In addition, we aimed to determine the overall causes, clinical characteristics, and imaging findings of stroke in the young based on a standardized comprehensive study protocol including validated rating scales, cardiac and vascular diagnostics, magnetic resonance imaging (MRI), and laboratory analyses including molecular genetics for Fabry disease.

Methods

This was a prospective European multicenter observational study of young adults with an acute CVE. The study was registered in the http://www.clinicaltrials.gov registry with the identifier number NCT004414583 and its methodology has already been published elsewhere.[14]

Participating Centers

The study was conducted in 47 centers across 15 European countries.[14] All patients underwent a detailed clinical evaluation. High inclusion rates were ensured through continuous monitoring by the Coordinating Center (University of Rostock) on a monthly basis. Four centers that failed to identify patients for >8 weeks were removed from further recruitment by standardized procedures for quality reasons.

Ethics

All patients or their legal representatives provided written informed consent. The study has been approved by all local ethical committees of participating centers.

Participants

Initially, 5111 patients were recruited, 88 withdrew consent, including 1 patient with Fabry disease. Thus, 5023 patients aged between 18 and 55 years and presenting with an acute CVE of any cause within the last 3 months before recruitment were included in the study. Seventy-eight percent of all patients had been enrolled in the study within 10 days after the qualifying stroke event. The diagnosis of stroke had to be verified by brain MRI analysis (82%), or in case of negative or missing MRI, the clinical diagnosis of CVE had to be confirmed by a qualified stroke neurologist, who had at least 5 years of experience in treating stroke (18%). Patients not meeting these criteria had to be excluded from the study.

All patients underwent thorough evaluation including brain and vascular imaging, extensive laboratory testing according to the local laboratory routine (hemogram, blood fat, glucose, HbA_{1C}, liver transaminases, creatinine, electrolytes, total albumin in serum, C reactive protein, antinuclear antibodies, antineutrophil cytoplasmic antibodies, rheumatoid factor, Factor V mutation, prothrombin mutation, antiphospholipid antibodies), Fabry disease diagnostics, cardiac ultrasound examination, and ECG. Data on comorbidities and vascular risk factors were collected in a standardized prespecified case report form from medical records and self-reported from the patient.[14] In addition, centers provided vascular and cardiac imaging, as well as stroke associated comorbidities like depression, pain, or headache.[14] Ancillary tests were performed, if deemed necessary by the individual study centers on a case-to-case basis.

Classification of Qualifying Events

The qualifying CVE was classified into transient ischemic attack (TIA), ischemic stroke, primary intracerebral hemorrhage, and other causes (eg, cerebral venous thrombosis, subarachnoid hemorrhage, cerebral vasculitis). TIA was defined as a CVE with clinical symptoms lasting <24 hours; patients with stroke were classified as ischemic

stroke or primary intracerebral hemorrhage based on imaging results; patients receiving rtPA were classified as ischemic strokes.

Central Laboratory Analyses

Genetic identification of Fabry patients was performed by staff blinded to clinical data by direct di-deoxy-sequencing of the entire α-galactosidase exonic structures as well as the intron-exon boundaries to detect mutations in the α-galactosidase gene. Sequencing analyses of the blood samples were undertaken centrally at the Albrecht-Kossel-Institute, Medical Faculty of the University Rostock. Definite Fabry disease was diagnosed if a given mutation significantly reduced the enzyme activity, and was a known causative mutation, and a significant increase in at least 2 independent biochemical markers was detected (Gb3 in blood [>4.0 mg/L], lyso-Gb3 [>0.5 ng/mL], Gb3-C24 in urine [>35 mg/L]). Probable Fabry disease was diagnosed in patients carrying either the mutation D313Y or a complex intronic haplotype[15] in conjunction with an increase of at least 2 of the above mentioned biochemical markers.

Central Imaging Analyses

Cerebral MRI was a mandatory procedure with obligatory standardized T_2/PD-weighted and fluid-attenuated inversion recovery and diffusion-weighted imaging. MR images were analyzed centrally at the Department of Neurology, Medical University of Graz, Austria, whose staff was blinded to clinical and demographic data. The ratings covered ischemic infarcts, cerebral hemorrhages, white matter hyperintensities (WMHs), microbleedings, pulvinar abnormalities, brain atrophy, and tortuosity and ectasia of the vertebrobasilar vessels (including measurement of the diameter of the basilar artery) whenever appropriate sequences were provided by the centers.

Data Management and Quality Assurance

All variables analyzed were checked for completeness, ranges, and plausibility. Especially, the results of the TOAST classification were cross checked against findings from patients' history, ultrasound, and MRI.[14] In the case of inconsistencies not being clarified by queries, TOAST criteria were assigned missing values (127 of 5024 patients), no TOAST classification was changed when only based on patient findings. In the case of missing or inconsistent information from the patients' history for diabetes mellitus and arterial hypertension, decisions were based on current medication.

Statistical Analyses

The methodology, including statistical planning and analyses, has been presented in detail elsewhere. All analyses were calculated using commercially available software: PASW Statistics 18, release version 18.0.2 (© SPSS, Inc., 2009, Chicago, IL, http://www.spss.com) and SAS software, version 9.2 of the SAS System for Windows. (© 2008 SAS Institute Inc., Cary, NC). To determine the frequency of Fabry disease, a point estimate and an exact 2-sided 95% confidence interval based on the binomial distribution was calculated. Estimates of risk factors including patients and family history, comorbidities, and lifestyle were analyzed by univariate analysis. Furthermore, type (TOAST criteria) and severity of stroke (National Institutes of Health Stroke Scale scores, modified Rankin Scale), presenting symptoms and specific findings (dissections, patent foramen ovale) were analyzed descriptively using absolute and relative frequencies, or medians and quartiles, depending on the scaling of variables. Analyses were stratified according to gender and age (10 years strata). The ratio between male and female patients in different age groups was compared using Poisson regression. Other findings were compared between the strata using multiple logistic regression analysis and linear models.

Results

Fabry Disease

Among a total of 5023 patients (2962 [59.0%] males and 2061 [41.0%] females), definite diagnosis of Fabry disease was established in 27 patients (0.5%; 95% confidence interval, 0.4%–0.8%), which required a significantly reduced biochemical activity of AGLA (n=4) and the presence of a causative mutation (R118C, V315I, S126G (3×), A143T (4×), D83N, L415F, S102L, E418G), and in those cases with massive increase of at least 2 independent biochemical markers (Gb3 in blood [>4.0 mg/L], lyso-Gb3 (>0.5 ng/mL), Gb3-C24 in urine [>35 mg/L]). Fabry disease was more common among females (16/2061 [0.8%]) versus males (11/2962 [0.4%]; *P*=0.076).

Besides the group of the definitive Fabry disease, there was a group of additional 18 patients (4 males, 14 females) highly suspect of carrying this X-linked disease. This definition was based on the detection of either the mutation D313Y or the presence of a complex intronic haplotype[15] associated with an increase of at least 2 independent biochemical markers (Gb3 in blood [>4.0 mg/L], lyso-Gb3 [>0.5 ng/mL], Gb3-C24 in urine [>35 mg/L]). This constellation was found in 16 of 18 cases for Gb3 in blood, 10 of 18 cases for lyso-Gb3, and 12 of 16 cases for the Gb3-C24 isoform. Four patients with the mutation D313Y revealed a significant increase of Gb3 in blood (5.1/5.6/4.8/4.5) and an increase of Gb3-C24 isoform (56.6/50.9/44.50/58.2).

The diagnosis of Fabry disease remained problematic in females with up to now unpublished mutations or where the mutations are being missed, but where pathological biochemical markers were measured: Gb3-C24 >35 mg/L seems to have the highest sensitivity because it could be detected in 13 of 17 females.

The main characteristics of all definite and probable Fabry cases are summarized in Table 1. Both definite and probable cases were more common among younger patients (Table 2). At 76.0% for definite Fabry disease and 72.2% for probable Fabry disease, ischemic stroke is the most common type of CVE in Fabry patients, followed by TIA. Territory infarctions could be detected in 40.7% of all definite and in 33.3% of all probable Fabry disease patients. A previous TIA or stroke has been reported in 20.0% of all cases, however we found a similar distribution in the non-Fabry cohort. In none of the cases were we able to detect signs for congestive heart failure or arrhythmia. Surprisingly, we have not been able to demonstrate any association between Fabry disease and a family history for cardiac disease, renal disease, or cerebrovascular disease. Further clinical details concerning stroke subtypes, presenting clinical symptoms, comorbidities, detailed MRI, and follow-up data will be reported soon.

Type of Cerebrovascular Event and Clinical Characteristics of Stroke in the Young

The secondary aim of sifap was to investigate a young stroke cohort. The median age of the entire study population was 46 years (interquartile range, 40–51; median age, 47 for males and 45 for females) (Table 3). About 80.5% had a first-ever stroke. Three thousand three hundred ninety-seven patients had an ischemic stroke, 1071 had a TIA, and 271 had a primary intracerebral hemorrhage (Table 4, 4.3% of the patients could not be classified). Sixty-eight (1.4%) patients were classified as having other CVEs, most commonly cerebral venous thrombosis or subarachnoid hemorrhage. The number of

Table 1. Characteristics of Patients With Definite and Probable Fabry Disease (Prevalences Are Related to Valid Numbers of the Specific Characteristic)

	Definite Fabry Disease	Probable Fabry Disease	All Fabry Cases
Type of CVE			
TIA	16.0% (4/25)	27.8% (5/18)	22.3% (9/43)
Primary hemorrhage	8.0% (2/25)	0.0% (0/18)	4.7% (2/43)
Ischemic stroke	76.0% (19/25)	72.2% (13/18)	74.45% (32/43)
Other	1.4% (0/25)	0.0% (0/18)	0.0% (0/43)
Recurrent stroke	22.2% (6/27)	16.7% (3/18)	20.0% (9/45)
MRI			
Pulvinar hyperintensities	0.0% (0/16)	0.0% (0/10)	0.0% (0/10)
Territory infarction	40.7% (11/27)	33.3% (6/18)	37.8% (17/45)
Deep WMH*			
Fazekas score 0	48.0% (12/25)	72.2% (13/18)	58.1% (25/43)
Fazekas score 1	28.0% (7/25)	16.7% (3/18)	23.3% (10/43)
Fazekas score 2	8.0% (2/25)	11.1% (2/18)	9.3% (4/43)
Fazekas score 3	16.0% (4/25)	0.0% (0/18)	9.3% (4/43)
Congestive heart failure	0.0% (0/26)	0.0% (0/18)	0.0% (0/44)
Atrial fibrillation	0.0% (0/27)	0.0% (0/18)	0.0% (0/45)
Family history			
CVEs	28.0% (7/25)	50.8% (8/16)	36.6% (15/41)
Cardiac disease	30.8% (8/26)	43.8% (7/16)	35.7% (15/42)

CVE indicates cerebrovascular event; MRI, magnetic resonance imaging; TIA, transient ischemic attack; and WMH, white matter hyperintensity.
*Deep WMH were analyzed using the Fazekas score.[16]

patients was higher in older age groups, and there was also a significant higher ratio of male to female patients in older patients ($P<0.001$).

In the strata from 18 to 24 and 25 to 34 years, there was a preponderance of females suffering from an acute CVE (65.3% and 54.1% of females) whereas this proportion was reversed in subsequent age groups (35–44 and 45–55 years) where males were in the majority with 57.1% and 63.2%, respectively (Table 3).

The most frequent symptoms and signs were hemiparesis, somatosensory deficit (>50%), dysarthria, headache (>30%), vertigo, dysphasia, nausea/vomiting, and ataxia (>20%).

Table 2. Stroke Subtypes and Causes of Stroke According to Sex and Age

	n	Sex		Age, y				Sex	Age Groups
		Males	Females	18–24	25–34	35–44	45–55	P* Value	P† Value
Ischemic stroke	3291‡	1999	1291	103	331	897	1959		
Stroke subtypes (TOAST classification system)§									
Atherosclerosis	613 (18.6%)	434 (21.7%)	179 (13.9%)	2 (1.9%)	18 (5.4%)	107 (11.9%)	486 (24.8%)		
Cardiac embolic source	549 (16.7%)	335 (16.8%)	214 (16.6%)	24 (23.3%)	63 (19.0%)	178 (19.8%)	284 (14.5%)		
Small artery occlusion	443 (13.5%)	324 (16.2%)	119 (9.2%)	9 (8.7%)	16 (4.8%)	86 (9.6%)	332 (16.9%)	0.020	0.024
Other determined cause	585 (17.8%)	297 (14.9%)	288 (22.3%)	24 (23.3%)	94 (28.4%)	200 (22.3%)	267 (13.6%)		
Dissection (in this group)	321 (54.9%‖)	175 (58.9%‖)	146 (50.7%‖)	7 (29.2%‖)	55 (58.5%‖)	123 (61.5%‖)	136 (50.9%‖)		
Undetermined cause	1100 (33.4%)	609 (30.5%)	491 (38.0%)	44 (42.7%)	140 (42.3%)	326 (36.3%)	590 (30.1%)		
All cases	5024	2962	2061	150	482	1394	2997		
Fabry disease									
Definite	27 (0.5%)	11 (0.4%)	16 (0.8%)	1 (0.7%)	4 (0.8%)	6 (0.4%)	16 (0.5%)		
Probable	18 (0.4%)	4 (0.1%)	14 (0.7%)	3 (2.0%)	5 (1.0%)	3 (0.2%)	7 (0.2%)		
No	4978 (99.1%)	2947 (99.5%)	2031 (98.5%)	146 (97.3%)	473 (98.1%)	1385 (99.4%)	2974 (99.2%)		

*Multiple regressions adjusted for age groups and center heterogeneity, †Multiple regressions adjusted for sex and center heterogeneity.
‡One hundred five cases were not classified because of implausible TOAST classification (Rolfs et al., 2010)[14]
§Classification of stroke subtypes was done locally by the study physicians.
‖Percentages with regard to other determined cause.

Table 3. Demographic Characteristics (Including Risk Factors) According to Sex and Age (Prevalences Are Related to Valid Numbers of the Specific Characteristic)

Items	All (N=5023)	Sex		Age, y				Sex	Age groups
		Males (N=2962)	Females (N=2061)	18–24 (N=150)	25–34 (N=482)	35–44 (N=1394)	45–55 (N=2997)	P* Value	P† Value
Sex (% males)	59.0%			(52/98) 34.7%	(221/261) 45.9%	(796/598) 57.1%	(1893/1104) 63.2%	<0.001	<0.001
Family history									
Cardiovascular history	1947 (41.0%)	1053 (37.8%)	894 (45.6%)	37 (26.2%)	163 (35.9%)	536 (40.2%)	1211 (43.0%)	<0.001	<0.001
Cerebrovascular history	1763 (37.1%)	989 (35.3%)	774 (39.6%)	49 (34.8%)	147 (32.5%)	462 (34.7%)	1105 (39.0%)	<0.001	0.001
Previous vascular events									
Cerebrovascular events	942 (18.9%)	562 (19.1%)	380 (18.6%)	20 (13.3%)	67 (14.0%)	241 (17.3%)	614 (20.7%)	0.987	<0.001
Cardiovascular events	243 (4.8%)	191 (6.4%)	52 (2.5%)	0 (0.0%)	2 (0.4%)	44 (3.2%)	197 (6.6%)	<0.001[3]	<0.001[3]
Comorbidities									
Hypertension	2347 (47.0%)	1532 (52.1%)	815 (39.7%)	16 (10.7%)	67 (14.1%)	513 (36.9%)	1751 (58.8%)	<0.001	<0.001
Diabetes	502 (10.0%)	360 (12.2%)	142 (6.9%)	1 (0.7%)	11 (2.3%)	103 (7.4%)	387 (13.0%)	<0.001	<0.001
Hyperlipidemia	1642 (34.1%)	1095 (38.7%)	547 (27.5%)	8 (5.5%)	66 (14.3%)	384 (28.6%)	1183 (41.3%)	<0.001	<0.001
Lifestyle									
Current cigarette smoking	2079 (41.4%)	1307 (44.1%)	772 (37.5%)	56 (37.3%)	173 (35.9%)	570 (40.9%)	1280 (42.7%)	<0.001	0.141

*Multiple regressions adjusted for age groups and center heterogeneity, †multiple regressions adjusted for gender and center heterogeneity, not adjusted for center heterogeneity because of low frequencies in some categories.

Cerebellar dysarthria and cerebellar ataxia were significantly more frequent in males, whereas headache and nausea/vomiting were more frequent in females. Median neurological scores at the time of enrollment were 3, (interquartile range, 1–6) based on the National Institutes of Health Stroke Scale, and 2, (interquartile range, 1–3) based on the modified Rankin Scale scores (Table 4).

Risk Factors

Classical risk factors of atherosclerosis were common among the patients (Table 3), with higher prevalences in older patients and in males. In brief, arterial hypertension was seen in 47.0% (52.1% in males and 39.7% in females), hyperlipidemia in 34.1% (38.7% in males and 27.5% in females), and diabetes mellitus in 10.0% (12.2% in males and 6.9% in females). The frequency of hypertension was higher in older patients ranging between 10.7% in the youngest and 58.7% in the oldest patients, diabetes from 0.7% to 13.0% and hyperlipidemia from 5.5% to 41.3% (Table 3). The proportion of current smokers was high across all age groups (37.3%–42.7%; P=0.141).

There was a high overall frequency of family history for cardiovascular events (41%) with a significantly higher frequency in females (45.6% versus 37.8%, P<0.001). The same effect could be demonstrated for cerebrovascular history with an overall frequency of 37.1% and significantly higher frequency in females compared with males (39.6% versus 35.3%; Table 3). Use of contraceptive pills was recorded in 30.5% of the 637 women in our study for whom details of this risk factor was reported.

Imaging Findings

In the whole cohort, 4920 patients underwent MRI and 4716 MRIs were reported from the evaluation center in Graz, Austria. A proper MRI analysis was not possible because of technical problems in 234 MRIs leaving 4482 scans and patients were analyzed centrally. In 154 (3.4%) of these patients, an intracerebral hemorrhage was demonstrated without a difference between both genders. Of the remaining 4328 patients, 2849 (65.8%) revealed a (sub)acute infarction in the MRI analysis (69.2% in males, 61.2% in females). Of those, a territorial infarct was detected in 2212 patients (77.6% total; 76.5% in males, 79.6% in females), a lacunar infarct in 591 (20.7%), and a watershed infarct in 154 participants (5.4%). The proportion of lacunar infarcts was highest in the oldest age group (P<0.001 after adjusting for gender; 16.1% versus 13.4% versus 15.1% versus 24.8%), and they were more prevalent in males (22.4% versus 18.1% in females; P=0.047). Infarcts in the vertebrobasilar area were less frequent in the youngest age group, (14.9% versus 28.7% versus 27.0% versus 22.9%; P=0.007) and predominant in males (26.2% versus 21.4%; P=0.002). In general, 1149 patients (25.6%) had old ischemic infarcts (27.9% males, 22.4% females) with higher prevalences in older patients (11.6% versus 15.5% versus 19.5% versus 30.8%, respectively; P=0.001). Overall, 30.8% of the patients centrally analyzed were MRI negative.

It is remarkable that already 20% of patients with the first-ever ischemic stroke were demonstrating preexisting old, that is, clinically silent infarcts on the MRI. About 44.4% of those patients had territorial, 9.1% watershed, and 57.3% lacunar lesions. Patients with first-ever stroke and silent infarcts had the same frequencies of cortical lesions and of cerebellar lesions (25.2%) in contrast to those with recurrent stroke and silent infarcts (41.6% cerebellar lesions versus 21.5% cortical lesions; P<0.001). No significant differences appeared in the vascular territories in these 2 groups. Of the 938 patients with TIA and centrally analyzed MRI data, 17.8% already demonstrated preexisting infarcts. When restricting this analysis to those 682 patients with first-ever TIA, we found old infarcts

Table 4. Clinical Characteristics According to Sex and Age (Prevalences Are Related to Valid Numbers of the Specific Characteristic)

		Sex		Age, y				Sex	Age Groups
	All (N=5023)	Males (N=2962)	Females (N=2061)	18–24 (N=150)	25–34 (N=482)	35–44 (N=1394)	45–55 (N=2997)	P* Value	P† Value
Qualifying cerebrovascular event (n=4806)									
TIA	1071 (22.3%)	589 (20.8%)	482 (24.5%)	21 (14.9%)	102 (21.8%)	307 (23.2%)	641 (22.3%)	0.079	0.003
Ischemic stroke	3396 (70.7%)	2064 (72.8%)	1332 (67.6%)	105 (74.5%)	335 (71.6%)	917 (69.4%)	2039 (70.9%)		
Primary hemorrhage	271 (5.6%)	160 (5.6%)	111 (5.6%)	5 (3.5%)	25 (5.3%)	72 (5.4%)	169 (5.9%)		
Other	68 (1.4%)	22 (0.8%)	46 (2.3%)	10 (7.1%)	6 (1.3%)	26 (2.0%)	26 (0.9%)		
Presenting clinical symptoms‡									
Paresis	2714 (54.0%)	1634 (59.9%)	1080 (57.6%)	68 (49.6%)	259 (57.7%)	705 (56.0%)	1682 (61.0%)	0.244	0.002
Somatosensory deficit	2455 (55.6%)	1416 (54.0%)	1039 (57.8%)	84 (62.7%)	249 (58.5%)	695 (58.3%)	1427 (53.5%)	0.066	0.013
Dysarthria	1856 (37.2%)	1182 (40.1%)	674 (32.9%)	41 (27.3%)	148 (31.0%)	487 (35.1%)	1180 (39.6%)	<0.001	<0.001
Vertigo	1449 (29.1%)	858 (29.3%)	591 (28.9%)	41 (27.5%)	148 (31.2%)	412 (29.8%)	848 (28.6%)	0.620	0.581
Aphasia/dysphasia	1230 (24.6%)	702 (23.8%)	528 (25.7%)	38 (25.3%)	137 (28.6%)	362 (26.0%)	693 (23.2%)	0.122	0.073
Ataxia	1032 (20.8%)	661 (22.5%)	371 (18.2%)	23 (15.3%)	91 (19.2%)	279 (20.2%)	639 (21.5%)	<0.001	0.150
Nausea/vomiting	1014 (20.3%)	541 (18.4%)	473 (23.1%)	32 (21.5%)	117 (24.4%)	304 (22.0%)	561 (18.8%)	<0.001	0.036
Hemianopsia	577 (11.6%)	332 (11.3%)	245 (12.0%)	30 (20.0%)	70 (14.6%)	173 (12.5%)	304 (10.2%)	0.851	<0.001
Diplopia	518 (10.4%)	323 (11.0%)	195 (9.5%)	6 (4.1%)	54 (11.3%)	146 (10.5%)	312 (10.5%)	0.132	0.110
Loss of consciousness	257 (5.1%)	151 (5.1%)	106 (5.2%)	7 (4.7%)	26 (5.4%)	79 (5.7%)	145 (4.9%)	0.999	0.674
Stupor or coma	124 (2.5%)	73 (2.5%)	51 (2.5%)	4 (2.7%)	18 (3.7%)	34 (2.4%)	68 (2.3%)	0.894	0.317
Amaurosis fugax	113 (2.3%)	59 (2.0%)	54 (2.6%)	4 (2.7%)	11 (2.3%)	34 (2.5%)	64 (2.1%)	0.156	0.954
Headache	1665 (33.4%)	846 (28.8%)	819 (40.1%)	77 (51.3%)	222 (47.1%)	502 (36.4%)	864 (29.0%)	<0.001	<0.001
Clinical scales		Median (25th–75th percentile)							
NIH Stroke Scale score§ (n=5022)	3 (1–6)	3 (1–6)	3 (1–5)	3 (1–5)	3 (1–6)	3 (1–6)	3 (1–6)	0.035	0.385
Modified Rankin Scale score§ (n=5023)	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)	0.088	0.236

NIH indicates National Institutes of Health; and TIA, transient ischemic attack.

*Multiple regressions adjusted for age groups and center heterogeneity, †multiple regressions adjusted for sex and center heterogeneity, not adjusted for center heterogeneity because of low frequencies in some categories; ‡in conjunction with the qualifying cardiovascular event; §assessed within the first 48 h.

still in 11.4%. Of these 682 patients, 5.4% had territorial infarctions and 6.5% had lacunar infarctions. Two thousand ninety-nine patients (46.8%) showed signal hyperintensities in the deep and periventricular white matter (WMHs) of varying severity, and the prevalence of WMHs was higher in older age groups (19.6%–56.8%; *P*<0.001).

The frequency of old silent infarcts on the MRI was higher in Fabry patients than non-Fabry stroke patients (mean number of lesions 1.7±2.2 versus 1.0±2.2; *P*=0.025). Male Fabry patients had a higher frequency of detection of WMHs compared with male non-Fabry patients (71.4% versus 47.1%; *P*=0.07), but between female Fabry and female non-Fabry patients this difference was not detectable.

Stroke Subtypes and Causes of Stroke

The cause of ischemic stroke (n=3396) was classified by the investigators according to the TOAST criteria (Figure, Table 2). The TOAST classification was implausible in 105 patients and missing for 1 patient (for details see Rolfs et al, 2011[14]) resulting in 3291 eligible patients with TOAST classification. Overall, there were significant differences in the TOAST classification between male and female patients

(*P*=0.020) and between the different age groups (*P*=0.024). The largest group was undetermined cause comprising 33.4% of all patients, being more common among females than males (38.0% versus 30.5%) and becoming less frequent in older patients. Other causes were in similar ranges (13.5%–18.7%) of which large artery atherosclerosis and small vessel disease were more common among males and both more prevalent in older patients, whereas cardioembolism was equally common between genders. Other determined cause (TOAST 4) was substantially more common among females (22.3% versus 14.9%) and less prevalent in older age groups. Dissection (n=321, 54.9%) was the dominant cause in this group for both genders and being most common in age groups of 25 to 34 and 35 to 44 years (Figure, Table 2).

Detailed echocardiographic data were documented in 3756 patients (74.8% of the total cohort; 75.6% males, 73.6% females). Patent foramen ovale was described in 24.7% of these patients (24.4% in males, 25.1% in females) with a grading of severe shunting in 79 of 537 cases (14.7%), moderate shunting in 214 of 537 cases (39.9%), and minimal shunting in 244 of 537 cases (45.4%; details are given in Rolfs et al, 2011[14]).
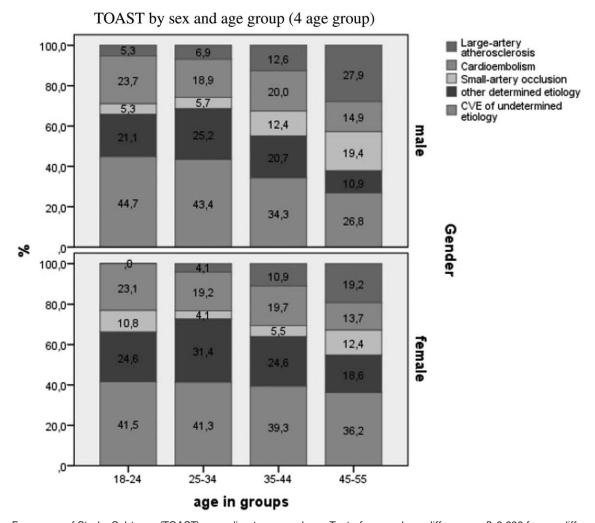
TOAST by sex and age group (4 age group)

Figure. Frequency of Stroke Subtypes (TOAST) according to age and sex. Test of age and sex differences: *P*=0.020 for sex differences and *P*=0.024 for differences between age groups in multivariate multinomial regression with random effects. CVE indicates cerebrovascular event.

Discussion

The Stroke in Young Fabry Patients (aged 18–55 years; sifap) study comprising 5023 patients is, by far, the largest prospective study of its kind. Moreover, it uses a standardized protocol including advanced imaging and molecular testing for rare causes.

The primary aim was to determine the prevalence of Fabry disease in young stroke patients. In a previous retrospective analysis of clinical data, Fabry disease was demonstrated in 4% of patients with cryptogenic stroke (18–55 years of age).[9] We estimated that this value might correspond to a frequency of about 1.2% in the general stroke population aged 18 to 55 years. The current prospective sifap study arrives at somewhat smaller numbers with 0.5% definite Fabry diagnosis and an additional 0.4% probable diagnosis (Table 2) supported by biochemical findings (including at least 2 independent biochemical markers, Gb3, lyso-Gb3, or Gb3-24 in the urine).

The Belgian Fabry stroke study (BEFAS), including 1000 patients with stroke,[10] diagnosed missense mutations in 8 unrelated female patients (D313Y [n=5], A143T [n=2], and S126G [n=1]). Our data supports the hypothesis that the mutations S126G and A143T are associated with a stroke-only

phenotype in Fabry patients. Up to now, we have never seen both mutations in other Fabry cohorts without stroke (data not shown). Interestingly, diagnosed Fabry patients in the BEFAS cohort had no signs of organ involvement besides stroke, TIAs, and headache. This is in line with our data and is further supported by the data from Sims and colleagues.[17] There is obviously a larger cohort of patients with a mono-symptomatic course of Fabry disease presenting initially as a stroke. The role of D313Y, which we considered as probable Fabry disease, if at least 2 independent biomarkers were significantly increased, still remains uncertain. Not only was it detected both in the BEFAS and the sifap study, but also in a recent study by Marquardt and colleagues.[18] Within a subcohort of the OXVASC study, 5 stroke patients with D313Y were detected. However, 3 of the 4 females identified were >90 years.[15] Taking into account the latest findings, it is still not clear which pathophysiological consequences arise from this sequence variant.

Of note, old MRI lesions were significantly more often detected in Fabry patients than in the non-Fabry sifap cohort even though the self-reported incidence of previous CVEs did

not differ between the 2 groups. Thus, silent infarctions are more common in Fabry disease.

Because Fabry disease is an x-linked disorder, in female patients Fabry disease may be suspected but not established, even in those with exonic mutations in catalytic center of the enzyme. There is a need to demonstrate the biochemical consequences of the detected mutation; by using biochemical parameters, such as Gb3, lyso-Gb3, or Gb3-C24.[15] Even with an extensive diagnostic portfolio, some patients, mostly females, remain uncertain for a final Fabry diagnosis.

Summarizing, sifap data support the presence of Fabry disease in about 1% of unselected young stroke patients. Moreover, a significant number of patients with probable Fabry disease exist among those, in whom the final diagnosis could not be ascertained despite extensive state-of-the-art diagnostic procedures.

The second aim of sifap was to determine more precisely the causes and risk factors for strokes in the young in a large European cohort. There has been the general perception that there are major differences between stroke in the elderly and in the young. Whereas classical risk factors account for the majority of strokes in the elderly, traditional risk factors manifest less often in the young. In contrast to what has been expected, our study demonstrates the high prevalence of risk factors of atherosclerosis in young stroke patients as well. In all age groups, the prevalence of hypertension and diabetes was 2 to 3 times more common than in the general population, and in the 45 to 55 year age group almost 60% of all patients were hypertensive. Remarkably, in all age groups at least one third of all patients were current smokers, a documented risk factor in a dose-dependent relation also for strokes in the young (Table 3).[19,20] Our findings corroborate with the Helsinki study[6] in which dyslipidemia, smoking, hypertension, and type II diabetes mellitus were the most frequent well-documented vascular risk factors. Likewise, an Australian case-control study[21] demonstrated that diabetes, hypertension, heart disease, cigarette smoking, and long-term heavy alcohol consumption are major risk factors in young stroke patients. Our findings regarding the gender disparity of these vascular risk factors are in line with other studies.[6,22,23]

The importance of classical vascular risk factors for strokes in the young is further substantiated by our finding of a high prevalence of silent infarcts and WMHs on MRI: silent infarcts were present in 20% of patients with a first-ever ischemic stroke and in 11% of patients with first-ever TIA (and no previous stroke), and WMHs of any degree were present in almost half of all patients. Whereas silent infarcts and WMHs are quite common in the elderly general population, they are distinctly uncommon in persons <55 years: in the Framingham Offspring Study <8% of persons aged 30 to 49 years had a silent brain infarct, and other studies have reported even lower rates.[24,25] From the Helsinki Young Stroke Registry, Putaala and coworkers[26] reported a 13% prevalence of silent infarcts and a 7% prevalence of WMHs among 669 of their patients examined with MRI. The higher proportions in sifap may be related to a slightly higher upper age limit (55 years in sifap compared with 49 years in the Helsinki

study) and the use of more modern and uniform MRI equipments. Use of higher MRI field strengths has been associated with a higher sensitivity in detecting silent small vessel disease in the brain.[27] Like in the Helsinki study, most silent infarcts were small and deep, similar to silent brain infarcts in the elderly healthy population and in elderly patients with stroke.[25] Putaala et al, 2009,[26] showed that increasing age, obesity, and type I diabetes mellitus were associated with the risk of silent brain infarctions and WMHs. A finding of silent small vessel disease in the brain in young stroke patients is of concern because it has been linked to increased risks of future vascular events as well as cognitive decline and dementia.[25] However, there was also a large proportion of patients included in sifap that were MRI negative. Because only stroke-experienced neurologist have enrolled patients for the sifap-project, we conclude that this high percentage of MRI-negative patients reflect the importance of TIA in the young population.

There is a well-recognized predominance of females among young stroke patients <35 years of age.[3,6,26] This finding has been hypothesized to be related to higher frequencies of migraine, oral contraceptive use, and hormonal factors in young females.[28] The risk of ischemic stroke in people with migraine with aura is doubled compared with people without migraine[29,30]: age of <45 years, smoking, and oral contraceptive use further raised the risk. In the sifap patients, the prevalence of migraine was 38.9% for women and 19.7% for men. Oral contraceptives have been reported to be a risk factor for stroke, with an increased risk by about 4 times for pills with a high estrogen content, twice for pills with low estrogen content, and no increased risk for pills with progestagen alone.[31] However, stroke risks with oral contraceptives also appear to be modified by the presence of migraine and prothrombotic genetic variants.[8] Data on oral contraceptive use were only available for about one third of the female patients in sifap, in whom a prevalence of 30.5% was found. Whereas this proportion is higher than the average use in 5 European countries (22.7% of women aged 25–44 years),[32] comparison with the population prevalence is uncertain because proportions vary from 5.9% (Poland) to 35% (Germany), both countries represented in the sifap study.

The most prevalent TOAST category for patients with ischemic stroke was undetermined stroke (TOAST 5), overall (33.4%) and through all age groups (Figure, Table 2). However, the proportion of undetermined stroke was lower in older patients, from 42.7% in the very young compared with 30.1% in the 45 to 55 years old. This could indicate more rare and less easily recognizable causes of stroke in the young. Otherwise, it could also be speculated that the rate of undetermined strokes decreases with ageing, in part, because of a higher likelihood of findings that can be implicated in cause of stroke such as the increasing proportion of risk factors and vascular changes with age, that is, because of an erroneous assumption of causality. These considerations point to the unfortunate situation of a still limited pathophysiologic understanding of stroke mechanisms.

Sifap has several strengths but also several limitations that should be recognized. Its strengths include the large sample

size, the prespecified investigation protocol, the relatively short time interval for inclusion of the 5023 patients, and the very high quality requirements for acceptance as a study site. Local private investigators in sifap were qualified stroke neurologists with >5 years of practice. The requirements on diagnostic facilities were high including modern MRI and ultrasound equipment. Careful monitoring and controls of data quality were conducted throughout the entire study. Although not all patients were consecutively included at all centers, the external validity of the cohort appeared high. Although the proportions of patients with TIA and intracerebral hemorrhage appear to have varied between the centers and patients and the most severe strokes causing decreased levels of consciousness were underrepresented in the sifap cohort, we found no indication of referral biases or selection of particular types of cases with respect to cause.

Conclusion

Our study expands current concepts of stroke in young adults and has several important implications for clinical practice and future directions of research.

First, there has previously been little focus on the preventive aspects of stroke in the young, presumably based on the common perception of the wide diversity of possible causes, a limited role of risk factors, and important roles of nonatherosclerotic and genetic causes. However, sifap demonstrates that the main preventable causes for strokes in the young are largely similar to strokes in the elderly, with the exception of atrial fibrillation, which is rare in the young. Population-based primary stroke prevention should target young as well as older persons and a focus on shared risk factors.

Second, despite extensive investigations about one third of all strokes in the young remained unexplained: further research is warranted, for example, with respect to genetic and currently unknown mechanisms.

Third, there is a need to establish a consensus on a common diagnostic algorithm for stroke in the young that can be applied across regions and effectively capture common as well as uncommon mechanisms of stroke.

References

1. The European Registers of Stroke (EROS) Investigators. Incidence of stroke in Europe at the beginning of the 21st century. *Stroke*. 2009;40:1557–1563.
2. Bhalla A, Grieve R, Rudd AG, Wolfe CD; BIOMED II European Study of Stroke Care. Stroke in the young: access to care and outcome; a Western versus eastern European perspective. *J Stroke Cerebrovasc Dis*. 2008;17:360–365.
3. Marini C, Totaro R, De Santis F, Ciancarelli I, Baldassarre M, Carolei A. Stroke in young adults in the community-based L'Aquila registry: incidence and prognosis. *Stroke*. 2001;32:52–56.
4. Grau AJ, Weimar C, Buggle F, Heinrich A, Goertler M, Neumaier S, et al. Risk factors, outcome, and treatment in subtypes of ischemic stroke: the German stroke data bank. *Stroke*. 2001;32:2559–2566.
5. Nedeltchev K, der Maur TA, Georgiadis D, Arnold M, Caso V, Mattle HP, et al. Ischaemic stroke in young adults: predictors of outcome and recurrence. *J Neurol Neurosurg Psychiatr*. 2005;76:191–195.
6. Putaala J, Metso AJ, Metso TM, Konkola N, Kraemer Y, Haapaniemi E, et al. Analysis of 1008 consecutive patients aged 15 to 49 with first-ever ischemic stroke: the Helsinki young stroke registry. *Stroke*. 2009;40:1195–1203.
7. Katzenellenbogen JM, Begg S, Somerford P, Anderson CS, Semmens JB, Codde JP, et al. Disability burden due to stroke in Western Australia: new insights from linked data sources. *Int J Stroke*. 2010;5:269–277.
8. Ferro JM, Massaro AR, Mas JL. Aetiological diagnosis of ischaemic stroke in young adults. *Lancet Neurol*. 2010;9:1085–1096.
9. Rolfs A, Böttcher T, Zschiesche M, Morris P, Winchester B, Bauer P, et al. Prevalence of Fabry disease in patients with cryptogenic stroke: a prospective study. *Lancet*. 2005;366:1794–1796.
10. Brouns R, Thijs V, Eyskens F, Van den Broeck M, Belachew S, Van Broeckhoven C, et al.; BeFaS Investigators. Belgian Fabry study: prevalence of Fabry disease in a cohort of 1000 young patients with cerebrovascular disease. *Stroke*. 2010;41:863–868.
11. Baptista MV, Ferreira S, Pinho-E-Melo T, Carvalho M, Cruz VT, Carmona C, et al.; PORTuguese Young STROKE Investigators. Mutations of the GLA gene in young patients with stroke: the PORTYSTROKE study–screening genetic conditions in Portuguese young stroke patients. *Stroke*. 2010;41:431–436.
12. Wozniak MA, Kittner SJ, Tuhrim S, Cole JW, Stern B, Dobbins M, et al. Frequency of unrecognized Fabry disease among young European-American and African-American men with first ischemic stroke. *Stroke*. 2010;41:78–81.
13. Lidove O, Joly D, Touzé E. Letter regarding Brouns et Al, Baptista et Al, and Wozniak et Al. *Stroke*. 2011;42:e4–e5; author reply e6.
14. Rolfs A, Martus P, Heuschmann PU, Grittner U, Holzhausen M, Tatlisumak T, et al.; sifap1 Investigators. 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. *Cerebrovasc Dis*. 2011;31:253–262.
15. Tanislav C, Kaps M, Rolfs A, Böttcher T, Lackner K, Paschke E, et al. Frequency of Fabry disease in patients with small-fibre neuropathy of unknown aetiology: a pilot study. *Eur J Neurol*. 2011;18:631–636.
16. Fazekas F, Chawluk JB, Alavi A, Hurtig HI, Zimmerman RA. MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging. *AJR Am J Roentgenol*. 1987;149:351–356.
17. Sims K, Politei J, Banikazemi M, Lee P. Stroke in Fabry disease frequently occurs before diagnosis and in the absence of other clinical events: natural history data from the Fabry Registry. *Stroke*. 2009;40:788–794.
18. Marquardt L, Baker R, Segal H, Burgess AI, Poole D, Hughes DA, et al. Fabry disease in unselected patients with TIA or stroke: population-based study. *Eur J Neurol*. 2012;19:1427–1432.
19. Love BB, Biller J, Jones MP, Adams HP Jr, Bruno A. Cigarette smoking. A risk factor for cerebral infarction in young adults. *Arch Neurol*. 1990;47:693–698.
20. Bhat VM, Cole JW, Sorkin JD, Wozniak MA, Malarcher AM, Giles WH, et al. Dose-response relationship between cigarette smoking and risk of ischemic stroke in young women. *Stroke*. 2008;39:2439–2443.
21. You RX, McNeil JJ, O'Malley HM, Davis SM, Thrift AG, Donnan GA. Risk factors for stroke due to cerebral infarction in young adults. *Stroke*. 1997;28:1913–1918.
22. Naess H, Nyland HI, Thomassen L, Aarseth J, Myhr KM. Etiology of and risk factors for cerebral infarction in young adults in western

Norway: a population-based case-control study. *Eur J Neurol*. 2004;11:25–30.

23. Haapaniemi H, Hillbom M, Juvela S. Lifestyle-associated risk factors for acute brain infarction among persons of working age. *Stroke*. 1997;28:26–30.

24. Das RR, Seshadri S, Beiser AS, Kelly-Hayes M, Au R, Himali JJ, et al. Prevalence and correlates of silent cerebral infarcts in the Framingham offspring study. *Stroke*. 2008;39:2929–2935.

25. Vermeer SE, Longstreth WT Jr, Koudstaal PJ. Silent brain infarcts: a systematic review. *Lancet Neurol*. 2007;6:611–619.

26. Putaala J, Kurkinen M, Tarvos V, Salonen O, Kaste M, Tatlisumak T. Silent brain infarcts and leukoaraiosis in young adults with first-ever ischemic stroke. *Neurology*. 2009;72:1823–1829.

27. Zhu YC, Dufouil C, Tzourio C, Chabriat H. Silent brain infarcts: a review of MRI diagnostic criteria. *Stroke*. 2011;42:1140–1145.

28. Rasura M, Spalloni A, Ferrari M, De Castro S, Patella R, Lisi F, et al. A case series of young stroke in Rome. *Eur J Neurol*. 2006;13:146–152.

29. Schürks M, Rist PM, Bigal ME, Buring JE, Lipton RB, Kurth T. Migraine and cardiovascular disease: systematic review and meta-analysis. *BMJ*. 2009;339:b3914.

30. Wolf ME, Szabo K, Griebe M, Förster A, Gass A, Hennerici MG, et al. Clinical and MRI characteristics of acute migrainous infarction. *Neurology*. 2011;76:1911–1917.

31. Chakhtoura Z, Canonico M, Gompel A, Thalabard JC, Scarabin PY, Plu-Bureau G. Progestogen-only contraceptives and the risk of stroke: a meta-analysis. *Stroke*. 2009;40:1059–1062.

32. Spinelli A, Talamanca IF, Lauria L. Patterns of contraceptive use in 5 European countries. European Study Group on Infertility and Subfecundity. *Am J Public Health*. 2000;90:1403–1408.

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Appendix 2:

Fabry disease diagnosis

1) Genomic DNA preparation, PCR, DNA fragment isolation and sequencing; biochemical assay for alpha-galactosidease A (AGLA) For the genetic testing, DNA was extracted from peripheral blood and exons 1–7 of the GLA gene were amplified by PCR and sequenced. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, GERMANY). 50ng of the DNA samples were used for PCR. The following PCR standard conditions were used**:** 15 min 95°C 13 x [30 s 94°C, 30 s 62°C, 30 s 72°C]; 8 x [30 s 94°C, 30 s 46,5°C, 30 s 72°C]; 16 x [30 s 94°C, 30 s 54,5°C, 30 s 72°C]; 5 min 72°C; ∞ 4°C. The following reaction buffer was used: RNase-free H2O [μl] 2,25; DMSO [μl] 0,5; Qiagen HotStarTaq MM [μl] 6,25; Primer (10 μM) [μl] je 1; DNA (20 ng/μl) [μl] 2,5. AGLA was determined according to (15).

2) Gb3 in blood and urine

Plasma Gb3 was determined by a HPLC/MS-MS method as described in detail recently. The 95th percentile for healthy individuals with this method has been determined to be 3.6 mg/L. The interassay coefficient of variation is approx. 10%. (Krüger R, Bruns K, Grünhage S et al. Determination of globotriaosylceramide in plasma and urine by mass spectrometry. Clin Chem Lab Med 2010; 48: 189-98.) Gb3 isoforms have been determined according to reference (Paschke E, Fauler G, Winkler H, et al. Urinary total globotriaosylceramide and isoforms to identify women with Fabry disease: a diagnostic test study. Am J Kidney Dis. 2011, 57, 673-81). In short a high-throughput method for the quantitative analysis of globotriaosylceramide isoforms using stable-isotope-dilution/internal standardization and electrospray ionization mass spectrometry (ESI-MS) was used. Urinary trihexosyl- and some of the di- and monohexosylceramide isoforms have been quantified within a single

experiment. With this method, prominent elevations of tetracosanoyl-(C24:0 plus C24:1)-globotriaosylceramides were found in urines, but not among controls.

3) Determination of Lyso-Gb3 in human plasma

As reference standard (Matreya LLC, USA; purity >98%) we used lyso-ceramide trihexoside. Lyso-lactosylceramid was used as internal standard. We used a gradient HPLC method on a reversed phase column (ACE 3 C8, 50 x 2.1 mm) for the determination of Lyso-Gb3. The two HPLC pumps and the column oven PE Series 200 were provided by Perkin Elmer, USA. The mass spectrometer used was an API 4000 Q-Trap supplied by Applied Biosystems, USA. The following experimental conditions were used: column temperature 60°C, flow at 0.9 mL/min, injection volume 10 μL, mobile phase with 50 mM formic acid in water (A) and 50 mM formic acid in acetonitrile / acetone (1/1=v/v; B), gradient at 5 % B from 0 to 0.3 minutes, followed by a linear gradient up to 73 % B (0.3 to 2.6 minutes) and further on to 100 % B (2.6 to 5.7 minutes). From 5.7 to 6.7 minutes 100 % B was used. Re-equilibration was done from 6.7 to 7.5 minutes at 5 % B. ESI in positive mode was used for peak detection. The detection mode was MRM, the vaporizer temperature was set at 500°C, ionisation voltage was 5.5 kV, and curtain gas pressure was 40 psi. Lyso-Gb3 quantifier was 786.6 to 282.2 m/z for the sample analysis; 50 μL aliquots were used. 100 μL of Internal Standard working solution (in ethanol) were added. Samples were mixed for about 30 seconds and centrifuged at 4,000 rpm for 2 minutes. The clear supernatant was transferred into appropriate auto sampler vials which were closed thereafter with crimp caps.