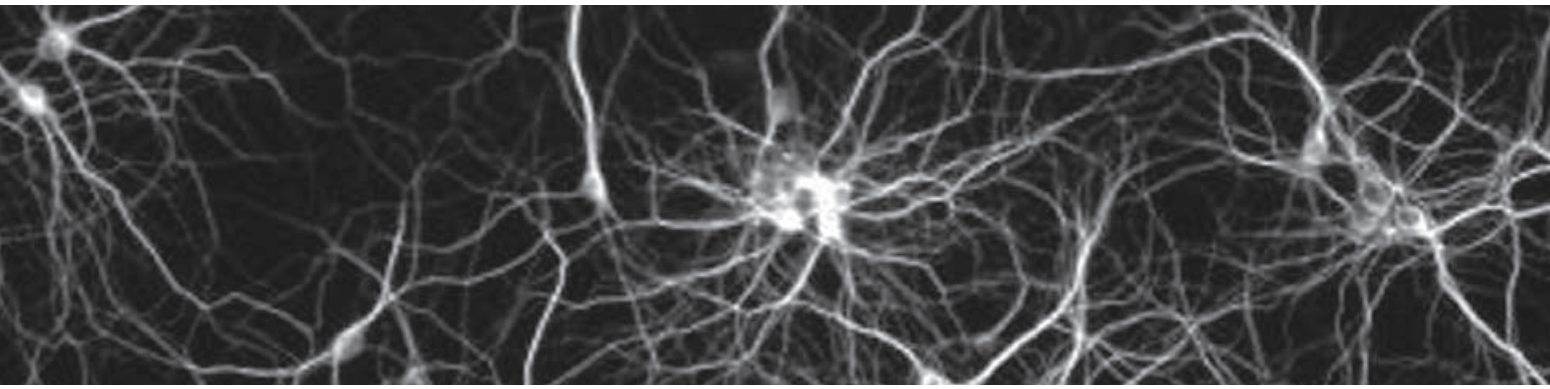

High level of education, healthy diet and moderate consumption of alcohol are associated with lower odds for first-ever ischemic stroke in hospital based case-control study in Varaždin County, Croatia

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ABSTRACT – Croatia, a Central European middle-income country, has the highest incidence of first-ever stroke in Europe. This prompted us to search for preventable and/or treatable risk factors for ischemic stroke. We performed a case-control study of first-ever ischemic stroke. Cases were patients with first-ever ischemic stroke. Controls were free from stroke and were matched to patients. All participants or their proxies were asked to fill in a questionnaire. Biometrics and laboratory values were collected. Odds ratios (ORs) were calculated for the association of stroke with selected risk factors. We enrolled 219 stroke cases from Varaždin General Hospital and 144 hospital and community controls. The risk factors significantly associated with higher odds for stroke were atrial fibrillation (OR 10.35, 95% CI 3.96-27.06) and current smoking (OR 4.53, 95% CI 1.45-14.17). Arterial hypertension was not associated with higher odds for stroke. Protective factors

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were education higher than high school (OR 0.36, 95% CI 0.15-0.89), healthy diet (OR 0.40, 95% CI 0.18-0.89), high HDL cholesterol (OR 0.14, 95% CI 0.06-0.33) and in the second model without adjustment for laboratory values alcohol intake of 1-30 drinks *per* month (OR 0.51, 95% CI 0.29-0.89). Anticoagulant therapy for atrial fibrillation, promotion of more healthy diet patterns and smoking cessation seem to be the targets for prevention of ischemic stroke in the population of Varaždin County, Croatia.

Key words: acute ischemic stroke, case-control study, risk factors, atrial fibrillation

INTRODUCTION

Stroke is the leading cause of death and disability worldwide (1,2), and low to middle income countries have the largest burden of stroke (1). There are ten risk factors associated with 90% of stroke risk (3), among them arterial hypertension, current smoking and cardiac causes found to be most relevant (3).

In our previous study, we established an unusually high incidence of stroke in the population of Varaždin County in Croatia (a Central European middle income country) compared to other European countries (4). Results from our population-based study have prompted us to seek for stroke risk factors, so we conducted this hospital based prospective case-control study of first-ever ischemic stroke in Varaždin County in order to elucidate the pattern of risk factors for ischemic stroke in our population. We believe that the results will highlight the problematic issues that we could improve and develop specific preventive strategies for lowering this unusually high burden of stroke in Varaždin County.

METHODS

We recruited consecutive patients with first-ever stroke (FES) admitted to Department of Neurology, Varaždin General Hospital (VGH) in the period from January 2010 to September 2010 (9 months). The VGH Neurology Department was the only source of stroke patients. This approach was reasonable, since in our previous study (4) we showed that virtually all stroke patients came from this source. Inclusion criteria for the case branch of the study were first-ever ischemic stroke in Varaždin County resident (VCR) confirmed by clinical examination and/or computed tomography/magnetic resonance imaging (CT/MRI) scan. In the same time window, we formed a control branch: age- and sex-matched controls (all VCRs, without prior cerebrovascular events) were recruited from departments of Internal Medicine, Urology, Neurosurgery (all in VGH), one urban general practitioner office, one rural general practitioner office, and one retirement home. In control subjects, the same questionnaire was used except for stroke-related data.

All subjects were asked to sign the informed consent form.

In the nine-month period, we recorded a total of 259 FESs: 220 (84.9%) ischemic strokes, 30 (11.6%) intracerebral hemorrhages, 7 (2.7%) subarachnoid hemorrhages, and 2 (0.8%) of unknown type.

All patients underwent standard neurologic investigative workup according to consensus guidelines (5). Neuroimaging was performed in 98.8% of patients (CT or MRI), while electrocardiography (ECG) was performed in every patient. Duplex ultrasonography (US) of neck vessels was done in 58.3% of patients (67.3% of ischemic strokes). The etiology of stroke was determined by the ASCO classification scheme (6), which categorizes strokes into four major etiologies divided by the grade of severity: A – Atherosclerosis, S – Small vessel disease, C – Cardiac source and O – Other cause. Each of these 4 categories is graded 0, 1, 2, 3 or 9, where 1 denotes definitive cause of stroke, 2 causality uncertain, and 3 disease present; 0 denotes no disease present and 9 not sufficient diagnostic workup.

The Scandinavian Stroke Scale (SSS) was performed on admission (7). Along with clinical workup, we measured height, weight, and waist circumference. Patients and/or caregivers were asked to provide comprehensive demographic and sociologic information and risk factor history: we recorded academic degree, years of education, place of residence (rural/urban), and history of standard risk factors along with year and month of the onset of exposure to the risk factor. Extensive smoking history was sought and the following variables were recorded: ever smoking, number of years smoking, current smoker, cigarettes/pipes/cigars *per* day, age when patient stopped smoking, whether patient

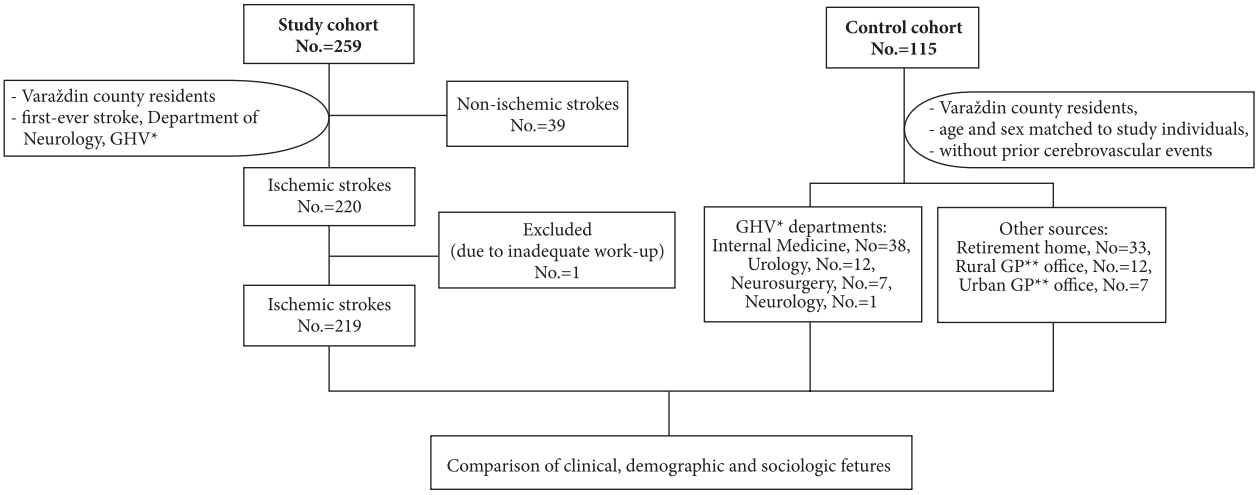

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Fig. 1. *Patient flow chart.*

stopped smoking in the last 6 months, and whether patient is willing to cease smoking (if current smoker). Also, environmental exposure to cigarette smoke (if not smoker themselves) in various settings was noted. Extensive history of diet and drinking was recorded. Specifically, we asked about drinking habits in the last year and how many drinks (white/red wine, beer and short drinks) the patient used *per* day on average. Data were categorized as "Not taking", "Less than one unit *per* day", "One to two units *per* day", "More than two and less than five units *per* day" and "More than 5 units *per* day". Recorded units were glasses in case of wine, bottles of beer, and 0.3 dL of drink for each of the short drinks. When describing dietary habits, patients were expected to report (an average) number of servings of fruit and vegetables *per* day, and usage of lard in eating habits in detail. Regarding individual eating habits, we constructed a variable "Healthy diet" that referred to consumption of more than two servings *per* day of fruit and vegetables, together with eating fried food less than once *per* week, preparing food without lard, and removing most or all of the fat from meat.

For each risk factor we calculated odds ratio (OR) and 95% confidence interval (95% CI). Univariate analysis with the usage of categorical (χ^2) test was performed. Multivariate model for stroke risk odds included age, gender, arterial hypertension, education dichotomized into high school and less and more than high school, currently smoking status, body mass index (BMI), healthy diet pattern, presence of atrial fibrillation (history and detected *de novo*), and alcohol intake (never/former *vs*. 1-30 drinks *per* month and never/former *vs*. more than 30 drinks *per* month). Variables were selected based on previously published studies that showed associations with stroke or $p<0.1$ in univariate analysis. The first model included uric acid, HDL and LDL cholesterol, while the second model was without laboratory values. Two-sided tests were used. Stata SE 11.2 was used for statistical calculations.

RESULTS

During the study period, 259 first-ever stroke patients were admitted to VGH Department of Neurology. Of them, 220 were classified as ischemic stroke, one patient had inadequate workup, and so 219 ischemic FES were left for further analysis. Control subjects were recruited from the following sources: 38 (26.4%) from VGH Internal Medicine Department, 33 (22.9%) from retirement home, 33 (22.9%) from rural general practitioner (GP) office, 20 (13.9%) from urban GP office, 12 (8.3%) from VGH Department of Urology, 7 (4.9%) from VGH Department of Neurosurgery and 1 (0.7%) from VGH Department of Neurology (see Fig. 1). Questionnaires were completed by patients or by proxy responders. At 3-month follow up, 64 (29.2%) stroke patients died.

The ASCO phenotypic classification is given in Table 1.

Lower educational background was strongly associated with the risk of stroke. Specifically, those with education less than high school had a greater risk of stroke (Table 2). The effect was most pronounced when elementary school (less than 9 years

Table 1. *Demographics and clinical characteristics of cases and controls*

	Cases (219)	*Controls (144)*
Age (yrs)	73 (68-80)	73 (65-78)
Women	122 (55.7)	78 (54.2)
ASCO classification		
A1 (large artery atherosclerotic)	23 (10.5)	-
S1 (small vessel disease)	45 (20.6)	-
C1 (cardiac causes)	88 (40.2)	-
O1 (other causes)	1 (4.6)	-
Strokes with some sign of atherosclerosis (A1, A2, A3)	122 (55.7)	-
Academic degree**		
Less than high school	140 (65.7)	74 (51.4)
High school and more	73 (34.3)	70 (48.6)
Arterial hypertension, self- reported	122 (55.7)	92 (63.9)
Currently smoking	36 (16.4)	8 (5.6)
Healthy diet‡	27 (12.3)	34 (23.6)
Atrial fibrillation, self-reported	43 (19.6)	13 (9.0)
Atrial fibrillation, self-reported and detected *de novo*	87 (39.7)	-
History of diabetes mellitus	45 (20.5)	28 (19.4)
Alcohol intake†		
Never/former	98 (44.7)	49 (34.0)
1-30 drinks *per* month	71 (32.4)	70 (48.6)
>30 drinks *per* month	36 (16.4)	15 (10.4)
Waist circumference*	100 (92-110)	100.5 (93-109)
Body mass index	27.3 (24.5-30.9)	28.9 (25.4-31.5)
Uric acid	296.5 (231.5-382.5)	332.5 (266.0-411.0)
Total cholesterol	4.87 (4.17-5.74)	5.30 (4.70-6.22)
HDL cholesterol	1.01 (0.85-1.23)	1.25 (1.1-1.47)
LDL cholesterol	3.08 (2.5-3.82)	3.20 (2.8-4.0)
Total triglycerides	1.39 (1.03-1.89)	1.47 (1.07-1.96)
Blood sugar	5.78 (5.09-7.08)	5.61 (5.1-6.67)

*Waist circumference: unknown 16 patients, 4 controls; †Alcohol intake: unknown 14 patients, 10 controls; ‡Diet pattern: unknown 16 patients, 5 controls; **Academic degree unknown 6 patients; Laboratory values unavailable in 16 patients, 30 controls.

of education) was compared with college or university degree (more than 12 years of education) and was preserved even when adjusted to all variables (Fig. 2). Also, usage of lard was consistently associated with the level of education, as lower educated people used lard more often (p=0.027).

We found no self-reported history of hypertension and/or blood pressure >160/90 mm Hg to be associated with odds for stroke.

In univariate analysis, lower HDL cholesterol was associated with a higher risk of stroke (1.35±0.51 *vs*. 1.07±0.37 mmol/L) and (unexpectedly) lower cholesterol levels (5.51±1.43 *vs*. 4.99±1.26 mmol/L) were associated with a higher risk of stroke (p<0.001 both). Smoking status, i.e. current smokers, had a significantly higher risk of stroke (Table 2). Biometric measurements showed that BMI and waist-circumference ratio were not associated with

Table 2. *Risk of stroke (multivariate analysis)*

	Odds ratio (95% CI)	*p*
Model 1 (N=265)		
Arterial hypertension, self-reported	0.53 (0.26-1.09)	0.087
Education – more than high school	0.36 (0.15-0.89)	0.027
Currently smoking	4.53 (1.45-14.17)	0.009
Body mass index	0.97 (0.91-1.04)	0.336
Healthy diet	0.40 (0.18-0.89)	0.025
Atrial fibrillation	10.35 (3.96-27.06)	0.000
Alcohol intake		
Never/former vs. 1-30 drinks per month	0.58 (0.28-1.12)	0.140
Never/former *vs*. >30 drinks *per* month	0.71 (0.24-2.03)	0.519
Uric acid	0.99 (0.99-1.00)	0.086
HDL cholesterol	0.14 (0.06-0.33)	0.000
LDL cholesterol	1.10 (0.79-1.53)	0.578
Model 2 (N=329)		
Arterial hypertension, self-reported	0.74 (0.43-1.27)	0.278
Education – more than high school	0.38 (0.19-0.72)	0.003
Currently smoking	6.29 (2.41-16.46)	0.000
Body mass index	0.98 (0.93-1.04)	0.573
Healthy diet	0.37 (0.19-0.71)	0.003
Atrial fibrillation	8.00 (3.97-16.1)	0.000
Alcohol intake		
Never/former *vs*. 1-30 drinks *per* month	0.51 (0.29-0.89)	0.019
Never/former *vs*. >30 drinks *per* month	0.82 (0.34-1.96)	0.066

Model 1: accounting for laboratory values; Model 2: without laboratory values.
All models were adjusted for age and sex.

stroke risk. The lack of association was observed across both genders.

Healthy diet was associated with a reduced risk when adjusted to other variables. Cooking with lard in univariate analysis was strongly associated with stroke risk; this association remained also when adjusted for age, sex and educational level (OR, 95% CI: 2.52, 1.59-4.00).

Diabetes mellitus was not associated with the risk of stroke. Elevated fasting blood sugar levels in univariate analysis showed an association with stroke (OR, 95% CI: 1.12, 1.01-1.25) and this association persisted after adjustment to other variables (OR, 95% CI: 1.12, 1.00-1.24).

Alcohol intake in the amount of 1-30 drinks *per* month *vs*. never (white wine most prevalent) was associated with a reduced risk of stroke in univariate analysis (OR, 95% CI: 0.53, 0.33-0.84), and that association persisted after adjustments to age, sex and educational level (OR, 95% CI: 0.51, 0.29-0.89) (Fig. 3). However, this association was no longer evident with adjustment to laboratory values.

Atrial fibrillation, self-reported or detected *de novo* in hospital, was associated with an increased risk of ischemic stroke. There were no gender differences, but individuals with atrial fibrillation were significantly older than those without it (75.5 *vs*. 69.3 years, p<0.001).

Laboratory values were available for 88.9% of controls and 98.2% of patients. Cholesterol and triglycerides were not associated with an increased stroke risk. HDL cholesterol was associated with a decreased risk of stroke.

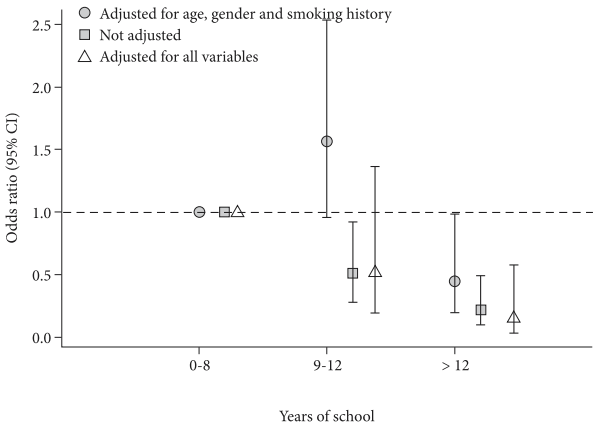

Fig. 2. *Risk of stroke associated with years of school.*

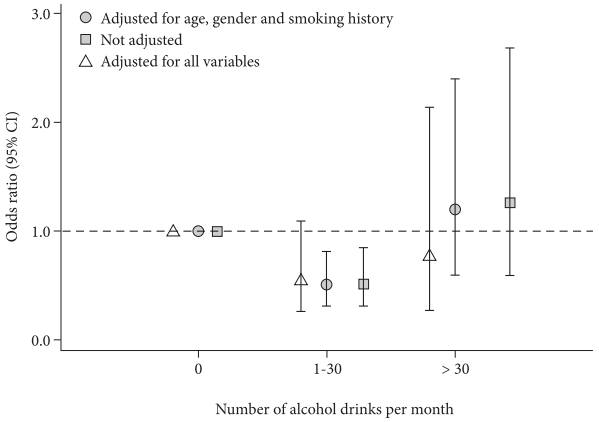

Fig. 3. *Risk of ischemic stroke associated with number of drinks per month.*

DISCUSSION

This was the first hospital-based case-control study of first-ever stroke in Croatia, a European middle-income country. The study recruited patients from a single acute stroke center in the well-defined geographical region and controls from community and hospital-based sources. We have shown that lower educational level along with some better known risk factors (such as atrial fibrillation, current smoking, HDL cholesterol and unhealthy diet) poses significant risk of stroke in our County.

Low educational attainment certainly is correlated with multiple risk factors. Ten years ago, Qureshi *et al*. showed that less than 12 years of education conferred higher odds for fatal stroke independently of other standard variables (8). In their study, this association was more pronounced in persons less than 50 years of age, while socioeconomic disparities as a risk factor for mortality decreased in advanced age (9,10). However, the results of this study showed that this may not be the case: in this study, the age of patients with FES ranged from 68 to 80, and FES was still associated with low level of education. Although we did not consider other socioeconomic factors such as occupational position, income and parental socioeconomic status, we believe that our data corroborate the notion that persons with lower educational status have greater odds for stroke.

Healthy dietary pattern consisting of high consumption of fruit and vegetables, avoidance of lard in cooking, and removing visible fat from meat, along with rare eating fried foods was demonstrated to reduce the risk of stroke. In this part of Croatia, older generations are traditionally accustomed to cooking with lard. So, we feel that stroke prevention campaigns in Varaždin County should be targeted to lowering the usage of lard in cooking, especially in the elderly.

As previously described (11,12), this study also showed moderate alcohol consumption (1-30 drinks *per* month) to be protective for stroke. Our data showed that white wine was the most frequent beverage among the people in Varaždin, while the frequencies of other beverages were too small for separate analysis. Heavy alcohol usage was not significantly associated with stroke risk, although we had a rather small sample of those patients. Since our population is rather old, we can speculate that the competing risk effect is in place in heavy alcohol group.

Compared to never smoking, current smoking and heavy smoking were associated with higher odds for stroke. Ever smoking status or status of former smoker was not associated with stroke, which is in concordance with previously published studies (3), so smoking cessation should be a mandatory agenda for stroke prevention programs.

The association of BMI and stroke is rather controversial; various studies produced different results, from positive (whether raised or lowered risk of stroke), U-shaped or negative association patterns (13-16). Biometrics, such as BMI, did not show greater odds for stroke in our study. BMI influences other risk factors such is arterial hypertension, diabetes mellitus and high cholesterol. Since none of these three risk factors showed association with stroke risk, the lack of BMI association is expected. Increased waist circumference was associated with the risk of stroke in men (13). Such a gender specific risk was not found in our study.

Atrial fibrillation, which was highly prevalent in ischemic stroke population in a previous study (4),

was also a predominant cause of stroke in our patients. Atrial fibrillation, either reported by the patients or newly detected by ECG, was present in nearly 40% of our patients, which makes the highest odds ratio of all risk factors. Such a high prevalence is in direct contradiction to recently published results showing that cardiac cause is responsible for 14% of stroke cases (3). However, a study that used ASCO classification showed that cardiac source was responsible for 36.9% of strokes (17). Since elderly people do have a higher prevalence of atrial fibrillation (18), respectable age of our population could explain such a high prevalence of atrial fibrillation among our study subjects. Furthermore, according to our results, atrial fibrillation in Varaždin County is highly unrecognized: we found 20% of previously undetected atrial fibrillation, which is more than reported in previous studies showing only 0.95% of atrial fibrillation in general population (19), 9.0% in those over 80 years of age (18), or 4.7% in population over 65 years of age. Therefore, the early (prior to stroke) detection of atrial fibrillation by routine ECG (e.g., on annual basis) and appropriate treatment, especially in elderly population, should be one of the key targets for future preventive strategies. As suggested by one study, nearly 61% of those with atrial fibrillation could benefit from some kind of anticoagulation (19). With the advent of new convenient anticoagulants, we could possibly turn the tide of atrial fibrillation associated (cardioembolic) stroke.

Unexpectedly, arterial hypertension was not associated with the risk of ischemic stroke. This is in direct contrast to the study by O'Donnell *et al*.; they found direct relationship of arterial hypertension and stroke in more than 20 countries worldwide (3). The reason for the lack of association is not clear. One of the reasons is age of the investigated population. Our case cohort was more than 10 years older than the INTERSTROKE cases (73.0 *vs*. 61.1). Since the effect of arterial hypertension is more pronounced in younger patients, it could be that we missed this relationship in our study, since patients in our study were rather old, the youngest patient being 68 years old. Moreover, in our study, there were only few (n=18, 8.2%) cases of *de novo* diagnosed arterial hypertension.

There were few limitations of this study. The number of control subjects was much lower than the number of study subjects. Furthermore, the unusual lack of stroke association with traditional risk factors of hypertension and diabetes most probably was due to the selection bias, since controls were also recruited from hospital setting with a presumably high incidence of comorbidity. Also, medical history was taken mostly from proxy respondents, but we believe that credibility of our data is similar as in other studies, since most of stroke studies recruit patients that are unable to communicate, and medical history is actually acquired by proxy. Finally, we could have done a more profound risk factor search, but the lack of funding prevented us from doing so. Therefore, we focused on the search for risk factors that are easily prevented, such as atrial fibrillation.

To conclude, we identified some risk factors, such as unhealthy diet, smoking and undetected atrial fibrillation, with a high preventive potential that could in the future lead to effective preventive strategies to reduce the high incidence of stroke in Varaždin County. Arterial hypertension and diabetes did not contribute to the higher odds for stroke in our population, most probably due to the selection bias. So, smoking, undetected atrial fibrillation and unhealthy diet are "the big three" modifiable factors for stroke.

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