Author's response to reviews

Title: Observational Dutch Young Symptomatic StrokE studY (ODYSSEY): study rationale and protocol of a multicentre prospective cohort study.

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Author's response to reviews: see over
Dear Dr. Shipley,

Thank you for giving us the opportunity to revise our manuscript MS-1317074744101977, which is entitled: “Observational Dutch Young Symptomatic StrokE studY (ODYSSEY): study rationale and protocol of a multicentre prospective cohort study”.

We would like to thank the reviewers for your valuable comments which allowed us to improve our manuscript. We have studied these comments in detail and addressed each one individually. We have made every attempt to incorporate these suggestions as thoroughly as possible.

On the attached pages we described in detail how we addressed each of the items. Changes in the manuscript have been marked.

We feel that the manuscript has improved substantially and hope that you will find it acceptable for publication in *BMC Neurology* in its current form. If there are any remaining issues, please do not hesitate to contact us.

Yours sincerely,

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Response to decision letter

Reviewer 1 (Adria Arboix)
We thank the reviewer for his/her kind remarks on our paper and included his/her suggestions as follows:

1. It is not clear whether intracranial angio-MRI study will be performed.
All patients will undergo neuro-imaging according to standard clinical care angio-MRI, angio-CT or ultrasound will be performed. We added this to our manuscript as follows:

Page 11, Classification of TIA or stroke: etiology and neuro-imaging
“All patients will undergo neuro-imaging and additionally CT-angiography, MR-angiography or ultrasound will be performed according to standard clinical care.”

2. It would be interesting to add a comment on the relevance of in-hospital mortality of the site of bleeding in intracerebral hemorrhages (Acta Neurol Scand 2002; 105: 282-8)
We thank the reviewer for this interesting suggesting. As both in-hospital mortality and characteristics of intracerebral hemorrhage will be documented, we can very well take this into account for our future analysis. We added a remark to our ‘outcome section’ on page 14 to clarify that in-hospital mortality will be documented as well:

“Primary outcome will be all cause mortality. Depending on date and location of death information on cause of death will be available either from the hospital (in-hospital mortality) or the general practitioner.”

3. Previous studies have suggested that obstructive sleep apnea (OSA) may be a risk factor for stroke. It may be interesting to investigate if OSA is an independent risk factor for stroke in young stroke patients.
We agree with Dr. Arboix that it may be very interesting to investigate if obstructive sleep apnea may be a risk factor for stroke in young patients. However as our protocol already is approved by the regional medical ethical commission we are not able to add additional questionnaires or investigations to our study.

4. It would be interesting to include a comment regarding that small subcortical infarcts have remote consequences on gray matter volume. Using MRI scans acquired before and after an incident subcortical infarct, they are able to show that the appearance of a new subcortical infarct is associated with cortical thinning in connected brain regions (Neurology 2012; 79: 2025-2028; Neurology 2012; 79: 2016-2017). Such an eventuality may affect the cognitive performance in young patients after stroke.
Thank you for this very interesting suggestion. Duering et al were able to show that subcortical infarcts were associated with cortical thinning. We agree that cortical thinning may be an explanation for a possible poor cognitive performance after stroke in young patients. However as we obviously only are able to perform neuro-imaging after the stroke, it is not possible to document changes over time and correlate cognitive performance with cortical thinning in our study. It would be very interesting in future studies to correlate cortical thinning to cognitive performance.
5. Typo errors in reference 10 should be corrected

We changed the typo errors in reference 10.
Reviewer 2 (Jukka Putaala)

We thank the reviewer for his/her kind remarks on our paper and included his/her suggestions as follows:

Major Compulsory Revisions

1. Objectives: Considering the relative rarity of the stroke event in this age group, extensive baseline data collection, and efforts being invested, the study could not only aim at estimating the risks for the endpoints, but also try to identify baseline factors associated with these endpoints. There are very scarce prospective data in this field.

   Primary and secondary outcome of our study will be the risk of mortality, vascular events, post-stroke epilepsy and cognitive performance. We agree with Dr. Putaala that it is very important to try to identify baseline factors associated with these endpoints. We adjusted this at pages 6 in the ‘objective’ of our manuscript as follows:

   “In addition we will identify baseline characteristics that are associated with our primary and secondary outcomes”

2. Sample size: The aim is to recruit 1500 patients. Please present rationale. Is this based on assumptions on the risk of the primary endpoint, all-cause mortality? Please provide power and sample size calculations and refer to publications on which these estimates may be based on.

   The sample size is determined to be able to detect the occurrence of the least frequent endpoint after young stroke. According to previous literature cumulative incidence of post-stroke dementia is approximately 1% per year. To adjust for the most important confounders (age, sex, stroke severity, education and depression) in our cox proportional hazard analysis, at least 40 cases are needed. A cumulative incidence per year and a follow-up of 3 years, will lead to a sample size of at least 1500 patients (with a power of 80%)

   We added this to our manuscripts on page 8 as follows:

   “Sample size is based on the least frequent endpoint after young stroke. According to previous literature cumulative incidence of post-stroke dementia is approximately 1% per year. To adjust for the most important confounders (age, sex, stroke severity, education and depression) in our cox proportional hazard analysis, at least 40 cases are needed. To detect a cumulative incidence of 1% per year and a follow-up of 3 years with a power of 80%, results in a sample size of at least 1500 patients.”

3. Healthy control subjects (pages 7-8): Why 250 subjects? Give rationale. How is the verification of stroke-free status planned to be done?

   Control subjects are included to compare performance on cognitive tasks between patients and healthy subjects. Our pilot studies showed that ‘attention’ is the cognitive task which differs the least between patients and healthy subjects (impaired in 14% versus 8% respectively). To identify this difference with a power of 80% and alfa 0.05 in previous mentioned 1500 patients, 265 healthy control subjects need to be included.

   We have added this to the ‘sample size calculation’ on page 8:

   “Control subjects are included to compare cognitive tasks between patients and healthy subjects. Our pilot studies showed that ‘attention’ is the cognitive task which differs the least between patients and healthy subjects (impaired in 14% versus 8% respectively). To identify this difference with a power of 80% and alfa 0.05 in previous mentioned 1500 patients, 265 healthy control subjects need to be included.”
The verification of stroke-free status will be done by questionnaires in which possible control persons will be asked about a history focal neurological deficits and/or treatment by a neurologist because of a stroke. We have added this to our manuscript (controls, page 7):

“A history of TIA, ischemic stroke or intracerebral hemorrhage (based on standardized structured questionnaires) is an exclusion criterion.”

4. Related to objectives, is the planned sample size and minimum follow-up sufficient to detect meaningful number of endpoint events (primary and secondary) with respect to covariates that can be entered in a Cox regression model? I would suggest doing power calculations separately for all endpoints stratified at least by primary event subtype, even by subtypes of ischemic and hemorrhagic events.

Thank you for this good suggestion. We agree that sample size calculation should be done separately for all endpoints stratified by primary outcome and by subtypes of ischemic and hemorrhagic events. Consequently, sample size is based on the least frequent endpoint. Which, in our opinion, is post-stroke dementia. In addition we made the assumption that at least 6 covariates should enter the cox proportional hazard model. See remark number 2.

5. Related again to sample size, but also to generalizability of the results: Consider leaving the protocol open for an international collaboration. I believe there is a lot of interest in this type of study and international collaboration would allow a substantially higher number of subjects recruited in a shorter time.

Thank you for this suggestion. As young stroke is a rare disease we would be more than happy to collaborate with any researcher in the field. The data can and will be easily shared with other researchers in the field.

6. Physical examination and additional investigations (page 10): “Additional DNA will be stored for future genetic analysis.” Will an investigational blood sample obtained for genetic analysis? There is no mention about this elsewhere in the manuscript. Must the patient consent on this or is the sample taken as part of routine care?

An additional investigational blood sample will be obtained for DNA storage and possible future genetic analysis. Patients must consent for DNA storage and possible future analysis.

We have added this to our manuscript on page 11 as follows:

“Additional investigational DNA will be stored for future genetic analysis. Patients must consent for storage of the DNA and future analysis.”

7. Endpoint events (page 11) and their validation (page 13): Consider using harmonized definitions for each event and at least two blinded reviewers. Particularly, in case of ischemic events, reports on recurrent events may be heterogeneous and careful assessment is warranted to judge an event as TIA or ischemic stroke.

Thank you very much for your suggestion. We have added definitions of our follow-up events to the outcome on page 14. In addition the outcome will be evaluated by 2 neurologists:

“Secondary measures of outcome will be the composite endpoint of any recurrent vascular event. Vascular events will include TIA, fatal or non-fatal stroke (ischemic or hemorrhagic), myocardial infarction, CABG, PTCA and other revascularization procedures, whichever occurs first. Stroke and TIA will be defined similar as the index event. Myocardial infarction will be defined by ischemic..."
symptoms with electrocardiographic, cardiac biomarker, or pathological evidence of infarction according to the universal definition of myocardial infarction.[54]

In addition, the occurrence of post-stroke epilepsy and dementia will be noted as secondary outcome. Epilepsy will be classified and defined according to the International League Against Epilepsy, in which patients with a single seizure associated with an enduring condition that could cause epilepsy, meet the criteria of epilepsy[55, 56]. Dementia will be defined according to DSM-5. Finally, tertiary outcome measures will include functional outcome, quality of life and mood disorders.

Whenever an outcome event is suspected with the aid of standardized structured questionnaires, information from the treating physician will be retrieved. This information will be verified and adjudicated by two independent experienced neurologists or, in case of a myocardial infarction, by a cardiologist, who will be blinded for the index event.”

8. Endpoint events (page 11): Is information on medication at the time/prior to event being obtained?

Information on medication at baseline at the time/prior to the event and information on medication during follow-up will be obtained. This is mentioned in ‘Measures- baseline, medical history’ page 9 as follows:

“In addition to the assessment of risk factors, patients will be asked about a history of epilepsy, pregnancies and complications, pulmonary embolism, deep vein thrombosis and current medication use.”

9. Neuropsychological assessment (table 2): Despite very comprehensive set of tests, consider adding serial Color-Word Test to assess adaptation capacity to stressful situations (André-Pettersson L et al. Stroke 2001;32:1712-1720). This may be relevant especially in the young working-aged individuals.

Thank you for this suggestion, but the medical ethics committee already approved the study and did not approve any amendments on cognitive testing. But we feel we have covered the domain with The Stroop Color-Word Test (page 12 neuropsychological assessment and table 2):

“To assess speed of information processing, Parts I and II of the Stroop Color Word test [36] and the Letter-Digit Substitution Task (an adaptation of the Digit-Symbol Substitution Test[37]) will be used”

Minor Essential Revisions

1. Medical history (page 8-9): Given that migraine frequency among young stroke cohorts is relatively high and there is a rationale to hypothesize migraine may play a role in a substantial number of young stroke, please consider screening and classifying migraine with a structured questionnaire.

We agree that migraine frequency may be very high. Therefore we added the ‘MISS questionnaire’, a screening instrument regularly used in Dutch hospitals containing questions about migraine in terms of diagnosis, frequency, aura and concomitant symptoms. We will classify migraine according to the ‘international classifications of headache disorders’.

We have added this to ‘medical history’ (page 9) and the additional files as follows:

“Patients will be screened for migraine with the MISS questionnaire, a screening instrument regularly used in Dutch hospitals containing questions about migraine in terms of diagnosis, frequency, aura and concomitant symptoms.”
Additional files
Furthermore migraine will be defined according to the international classification of headache disorders [64].

2. Potential trigger factors (page 9): Infections preceding stroke in the young are common.
   Considering screening also infections, not only fever. What is the criterion for significant fever and how it is verified?
   Questionnaires will be used to ask patients about fever (temperature > 38.5 degrees) and flu. In addition standard medical care will include laboratory investigations (CRP and leucocytes) whenever an infection is suspected. This will be documented as well as infections during the hospital stay.

3. Hazard and control periods for triggers (page 9): It is not clear whether hazard and control periods can be uniformly applied to all triggers in stroke, related to very heterogeneous underlying pathology. Therefore, consider using several hazard periods, e.g. hours 1 and 2 for physical triggers, and several control periods, e.g. hours 25 and 26 preceding the stroke, same day 1 week ago, in addition to the usual frequency. Furthermore, accuracy and timing of asking about triggers is crucial for data reliability. Aphasic patients, those with poor memory around the stroke or cognitive deficits most likely cannot provide trigger information.
   Thank you for your suggestion; maybe we have not been entirely clear but by the use of the standard questionnaire on trigger factors we think that these differences in hazard periods are taken into account. We will not only ask the patients on exposure to the potential trigger factors during the predefined period, but in addition we will ask them about the last time they were exposed to the potential trigger as well. Furthermore we will ask them on several control periods (last day, week and year ago) on the usual exposure/frequency.

   All questionnaires will be filled-out within 14 days after stroke to ensure reliability. In case of an aphasic patients, when possible the questionnaires will be filled out by family member.

   We have added this to potential trigger factors (pages 10):

   “For each trigger factor, patients will be asked to report their usual exposure during the past year and week and the exposure during a predefined period before the onset of stroke; the hazard period. Hazard periods are based on the estimated duration of the effect of each potential trigger factor as used in previous studies investigating trigger factors of cardiovascular events[13, 14, 17]. In addition to exposure in the hazard period, patients will be asked about the last exposure before stroke.”

4. Etiologic classification (page 10): Regarding the shortcomings of the original TOAST classification, would consider using CCS or ASCO to better characterize patient phenotype.
   Thank you for the suggestion, we have added both the CCS and ASCO to our classification systems to determine stroke etiology. This is changed in our manuscript as follows (page 11):

   “Etiology of ischemic stroke and TIA will be classified according to the TOAST criteria[26], Causative Classification System of ischemic stroke (CCS)[27] and ASCO[28].”

5. Classification of ICH (page 10): According to Boston criteria, amyloid angiopathy is nonexistent in patients aged <55. What criteria are then used? Some sporadic reports exist that amyloid pathology might lead to ICH also in younger patients. There are also recent attempts to create a classification system for ICH (Meretoja A et a. Stroke. 2012 Oct;43(10):2592-7).
We agree that amyloid angiopathy is nonexistent in patients under 55. Therefore we have deleted this in our classification.

Unfortunately there is not yet a universally used etiological classification system for intracerebral hemorrhage. We will collect all information that is needed to classify ICH according to recently developed classification systems (like Meretoja) or the systems that will be developed.

6. Course of the disease (page 11): modified Rankin Scale during admission does not likely provide meaningful information. Consider leaving just admission NIHSS score to depict symptom severity.
   mRS prior to index event in those with disabilities, in turn, is meaningful.
   Thank you very much for your suggestion. We have added mRS to the baseline medial history to determine functional performance prior to the index event. Pages 9:

   “Furthermore functional performance prior to the index event will be assessed by modified Rankin scale.”

7. Discussion (page 15, 4th paragraph): There is an ongoing study (NCT01934725) investigating potential triggering factors among young adults with cryptogenic ischemic stroke. A better statement would read e.g.: “In addition, this study is the first investigating potential acute trigger factors preceding a stroke or TIA in non-selected young patient population.”
   We have changed this sentence in the discussion.

8. Discussion (page 15): Many trials had minimum age criterion of >18 years, so perhaps you should state that “…stroke trials in which young adult patients have been underrepresented or excluded.”
   We have changed this sentence in the discussion as follows (page 17):

   “The estimates of recurrent vascular events risks may be used to design future intervention studies on start and withdrawal of secondary prevention in these young patients, as the current prescription of these drugs is based on extrapolated findings from stroke trials in which young patients have been underrepresented or excluded.”
Reviewer 3 (Sami Curtze)

We thank the reviewer for his/her kind remarks on our paper and included his/her suggestions as follows:

1. I would just like to encourage the study team to register the trial f.e. at clinicaltrials.gov or similar space. A similar study, the SECRETO study “Searching for Explanations for Cryptogenic Stroke in the Young: Revealing the Etiology, Triggers, and Outcome” has been registered there. The SECRETO trial focuses on “cryptogenic” strokes in the young. There would be possibility for cooperation. Advantage of registering the “ODYSSEY” trial would increase awareness of the study and new opportunities to join forces will appear. If recruitment is as expected this study has the possibility to succeed. Details provided are sufficient to allow replication of the work or comparison with related analyses. The manuscript adheres to the relevant standards for reporting and data. The writing is acceptable. There might be a few flaws, f.e.

Thank you very much for your suggestion. We will definitely consider registering ODYSSEY at clinicaltrials.gov.

2) On page 4 last sentence: “of great important as well”. Shouldn’t it be “of great importance as well”?

We have changed the sentence.

Specific comments:

3) Classification of intracerebral haemorrhage: Unfortunately there is no good “etiological classification” comparable to TOAST for ICH at present. I would prefer if “hypertensive” would not be used as a etiologic entity. Hypertension is a risk factor for ischemic and for hemorrhagic stroke but it is not the etiology. Would you mind to call it f.e. microangiopathy? Additionally I think the etiology of “bleeding disorder” a little bit dangerous, as f.e. people with anticoagulation can do well without bleeding even with high INR levels and then suddenly get an large ICH while INR is between 2.0-3.0. Etiology might then as well be microangiopathy or something else but bleeding disorder just prevents the bleeding from stopping to bleed or makes it just clinically significant while otherwise it would have been “silent” microbleed. I agree that your suggested classification seems to be fine right now, but hopefully we will get a new classification before your study results are published and therefore it would be better to be warranted not to use “bleeding disorder” too liberally.

We agree that whenever patients have a bleeding disorder it is very important to still document possible other etiologies. Therefore we will document both etiologic entities whenever there are multiple possible causes.

In addition we have changed the etiologic entity hypertensive intracerebral hemorrhage to ‘hypertensive microangiopathy’. The etiologic section is changed as follows (page 11):

“Etiology of intracerebral hemorrhage will be classified as hypertensive microangiopathy [deep or infratentorial hemorrhage in combination with hypertension], arteriovenous malformation, cavernous angioma, coagulopathy (iatrogenic or bleeding disorder), central nervous system infection, septic embolism, vasculitis, substance abuse or unknown (cryptogenic, multiple causes and incomplete evaluation)[28]. Etiology will be based on neuro-imaging, medical history and the use of medication”

4) Additional files/ risk factors: “Hypertension will be defined as a systolic blood pressure #140 mmHg or diastolic blood pressure #90 [56, 57] or the use of antihypertensive drugs. “Can you specify how many repeated measurements and in which timeframe of RR > 140/90 are needed for the diagnosis of hypertension.
Blood pressure will be measured at least 24 hours after the stroke or TIA during the in hospital stay. According to national guidelines at least two independent measures are necessary for the diagnosis hypertension. We have changed this in the additional files as follows:

“Hypertension will be defined as a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 [measured at least 24 hours after stroke, with 2 independent measures] [62, 63] or the use of antihypertensive drugs.”

5) table 1. Can you specify “recreational drugs”? Well you specifically question i.v. drugs, cocain, amphetamine etc?

*We will specifically question the use of cocaine, heroin, methadone, amphetamine, cannabis, XTC, anabolic steroids, hallucinogens and EPO. We have added this to our manuscript as follows (page 10 potential trigger factors):

“Potential trigger factors that will be investigated include smoking, consumption of alcohol, recreational drugs (cocaine, heroin, methadone, amphetamine, cannabis, XTC, anabolic steroids, hallucinogens and EPO), caffeine containing drinks (coffee and cola),...”
Reviewer 4 (Alessandro Pezzini)

We thank the reviewer for his/her kind remarks on our paper and included his/her suggestions as follows:

Because of its characteristics of methodological paper, the Authors should provide more detailed definitions.

1. Patients

TIA: “... (symptoms) lasting less than 24 hours” and “... with corresponding DWI-positive lesion on MRI-scan”. What about patients with the same clinical presentation and DWI-negative scan? What about the diagnostic work-up for these patients: will all subjects undergo a standard (imaging) protocol? If this is the case, what imaging procedures are included (for patients with ischemic stroke and for those with a TIA)?

In order to avoid misclassification we will only include TIA patients with a DWI-positive lesion on the MRI-scan. Patients without corresponding lesion on neuro-imaging will be excluded.

ODYSSEY has a very pragmatic design to allow participation of many centers and to increase generalizability. Therefore patients will not undergo a standard imaging protocol, but will undergo neuro-imaging (CT or MRI) according to standard clinical care. In addition according to standard clinical care angio-CT, angio-MRI or ultrasound will be performed.

We have changed this in our manuscript as follows (Classification of TIA or stroke: etiology and neuro-imaging, page 11):

“All patients will undergo neuro-imaging and additionally angio-CT, angio-MRI or ultrasound will be performed according to standard clinical care”

2. Exclusion criteria

Intracerebral hemorrhage due to a known ruptured aneurysm: what about the other (true) vascular malformations (i.e, arteriovenous malformations)?

Intracerebral hemorrhage due to vascular malformations (other than aneurysms) will be included.

3. What about intracerebral hemorrhages due to transformation of an infarct (is this classified as infarction or hemorrhage)?

Hemorrhagic transformation of an ischemic stroke will be classified as an ischemic stroke. However when reviewing the neuro-imaging, this will be documented.

We have added this to the inclusion criteria (page 7) and description of neuro-imaging (page 11) as follows:

“All acute stroke is defined similar, but with symptoms persisting for more than 24 hours. On the basis of neuro-imaging stroke is further divided into intracerebral hemorrhage and ischemic stroke. Hemorrhagic transformation of an ischemic stroke will be classified as an ischemic stroke.”

“CT- and MRI-scans will be reviewed centrally in the Radboud University Medical Centre. TIA and ischemic strokes will be classified according to arterial territory and addition will be classified as lacunar or territorial. Whenever hemorrhagic transformation of an ischemic stroke has occurred this will be documented.”

4. Controls

Please, explain how these subjects will be selected: in particular, how 250 stroke-free subjects are planned to match 1,500 patients? It is likely to be 1 control subject per 6 stroke patients: if this is
the case, Authors should exactly report how they will match the two groups (i.e, by sex and mean age? other?)

Thank you for giving us the possibility to clarify this. The ODYSSEY study is NOT a case-control study. Controls will only be used as a reference for cognitive performance. Controls will be recruited among patients’ spouses, relatives or social environment to obtain a comparable social status to the social status of the patients. In addition control subjects and patients will be matched by sex, mean age and education.

We have added this to our manuscript as follows (page 7):

“Controls will be recruited among patients’ spouses, relatives or social environment. Controls have to be aged 18 through 49 years and will be matched for mean age, sex and level of education.”

Classification of TIA or stroke: etiology...

5. Please, provide the exact criteria for the etiologic classification of intracerebral hemorrhage

Unfortunately there is no universally used good classification system for intracerebral hemorrhage. Therefore we will use this classification system, which we have used in previous studies. Classification of intracerebral hemorrhage is based on neuro-imaging, including angio-CT or angio-MR, medication use and medical history (hypertension and bleeding disorders).

We have changed this in our manuscript as follows (page 11):

“Etiology of intracerebral hemorrhage will be classified as hypertensive microangiopathy (deep or infratentorial hemorrhage in combination with hypertension), arteriovenous malformation, cavernous angioma, coagulopathy (iatrogenic or bleeding disorder), central nervous system infection, septic embolism, vasculitis, substance abuse or unknown (cryptogenic, multiple causes and incomplete evaluation)[28]. Etiology will be based on neuro-imaging, medical history and the use of medication.”

6. “In addition, hematoma volume will be calculated”: what criteria for volume calculation?

Hematoma volume of an intracerebral hemorrhage will be calculated according to the well established A*B*C/2 method. We have added this to neuro-imaging (page 11) as follows:

“In addition hematoma volume will be calculated according to the A*B*C/2 method [29],”

Course of the disease

7. Myocardial infarction: please, define

We will define myocardial infarction according to the universal definition. We have added this to the outcome section, page 14 as follows:

“Vascular events will include TIA, fatal or non-fatal stroke (ischemic or hemorrhagic), myocardial infarction, CABG, PTCA and other revascularization procedures, whichever occurs first. Stroke and TIA will be defined similar as the index event. Myocardial infarction will be defined by ischemic symptoms with electrocardiographic, cardiac biomarker, or pathological evidence of infarction according to the universal definition of myocardial infarction [52].”

8. Measures – Follow up

Occupation: how will the Authors sub-categorize this variable?

First of patients will be asked about their profession and education. Occupation will be categorized into 4 skills levels (ranging from first=primary education only to fourth=tertiary education with
university degree or equivalent), according to the ISCO-88 (International Standard Classification of Occupations).

We have added this to our manuscript as follows (page 12):

“As measure of functional outcome mRS and Barthel Index[29] will be administered and patients will be asked about their occupation or education. Occupation will be categorized into 4 skills levels (ranging from first=primary education only to fourth=tertiary education with university degree or equivalent), according to the ISCO-88 (International Standard Classification of Occupations).”

Additional files
- “Furthermore migraine...[58]”: I suggest to adopt the most recent criteria for migraine definition

Thank you for your careful reading, we have adopted the most recent definition of migraine.

“As furthermore migraine will be defined according to the international classification of headache disorders.”

Minor:
- Background: “Young stroke is generally...of 50 years”. Please, delete this sentence. The cut-off of age is arbitrary.

We have deleted this sentence.

- Ref 26 is no more “in press”.

We have changed the reference.

Overall, it is a bit difficult for the reader to follow the study description in the way the Authors present their protocol. I would suggest to report the different phases of the study in the text and to summarize all the procedures and variable definitions in a separate (supplemental?) part of the manuscript. A paragraph should be included in which all the diagnostic (in particular, imaging) procedures are described, in which it is explicitly stated whether all the patients will undergo a standard diagnostic protocol (if this is the case, this should be reported in details) or not, and who will be in charge with clinical and follow up evaluations (physicians, study nurses, others...).

Thank you very much for your suggestion. We have added a table to the additional files showing an overview of all investigations. Furthermore additional file 1 shows the definitions of risk factors and outcome measures during follow-up:

Additional files
Definition of well-documented and less-well documented modifiable risk factors

Well-documented potentially modifiable risk factors: Myocardial infarction will be defined by ischemic symptoms with electrocardiographic, cardiac biomarker, or pathological evidence of infarction according to the universal definition of myocardial infarction.[54] Smoking will be defined as at least 1 cigarette in the 6 months prior to the event. Overweight will be defined as Body Mass Index=25-29 and obesity as Body Mass Index≥30[59]. On the basis of laboratory findings diabetes mellitus will be defined as a random blood glucose level ≥ 200 mg/dL (11.1mmol/L) or 2 consecutive fasting venous plasma glucose levels of ≥ 126 mg/dL (7.0 mmol/L)[60, 61] or the use of antidiabetics. Dyslipidemia will be defined by either total cholesterol level ≥5.0 mmol/L or low-density lipoprotein level ≥2.5 mmol/L or high-density lipoprotein level <1.0 mmol/L or the use of lipid-lowering drugs. Hypertension will be defined as a systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90.
(measured at least 24 hours after stroke, with 2 independent measures) [62, 63] or the use of antihypertensive drugs.

Less-well documented potentially modifiable risk factors: Excess alcohol consumption will be defined as consuming more than 200 g of alcohol per week. Furthermore migraine will be defined according to the international classification of headache disorders [64].

Outcome measures
TIA is defined as a rapidly evolving focal neurological deficit, without positive phenomena such as twitches, jerks or myoclonus, with no other than vascular cause lasting less than 24 hours. Acute stroke is defined similar, but with symptoms persisting for more than 24 hours. Both with corresponding lesions on neuro-imaging.

Myocardial infarction will be defined by ischemic symptoms with electrocardiographic, cardiac biomarker, or pathological evidence of infarction according to the universal definition of myocardial infarction [54].

Table 1: Overview of investigations

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<tr>
<td>Cholesterol levels</td>
<td>X</td>
</tr>
<tr>
<td>Blood count</td>
<td>X</td>
</tr>
<tr>
<td>Sedimentation rate</td>
<td>X</td>
</tr>
<tr>
<td>DNA storage</td>
<td>X</td>
</tr>
<tr>
<td>Other specific laboratory measures on indication</td>
<td></td>
</tr>
</tbody>
</table>

(young stroke laboratory measures)

<table>
<thead>
<tr>
<th>Imaging</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuro-imaging¹</td>
<td>X</td>
</tr>
<tr>
<td>CT</td>
<td></td>
</tr>
<tr>
<td>MRI</td>
<td></td>
</tr>
</tbody>
</table>

Vascular imaging² | X

¹ All patients will undergo neuro-imaging, either CT or MRI scanning.
² All patients will undergo angio-CT, angio-MRI or ultrasound according to standard clinical care