



# Bio-repository of DNA in stroke: a study protocol of three ancestral populations

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## DECLARATIONS

### Competing interests

The authors declare that they have no competing interests

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## Summary

Stroke is a leading cause of death and disability in the world. Identifying the genes underlying stroke risk may help us to improve our understanding of the mechanisms that cause stroke and also identify novel therapeutic targets. To have sufficient power to disentangle the genetic component of stroke, large-scale highly phenotyped DNA repositories are necessary. The BRAINS (Bio-repository of DNA in stroke) study aims to recruit subjects with all subtypes of stroke as well as controls from UK, India, Sri Lanka and Qatar. BRAINS-UK will include 1500 stroke patients of European ancestry as well as British South Asians. BRAINS-South Asia aims to recruit 3000 stroke subjects and 3000 controls from across India and Sri Lanka. BRAINS-Middle East aims to enrol 1500 stroke patients from Qatar. The controls for BRAINS-Middle East will be recruited from a population-based Qatari Biobank. With the addition of new recruitment centres in India and Qatar, we present an updated version of the BRAINS study protocol. This is the first international DNA biobank for stroke patients and controls from the Middle East. By investigating the influence of genetic factors on stroke risk in European, South Asian and Middle Eastern populations, BRAINS has the potential to improve our understanding of genetic differences between these groups and may lead to new population-specific therapeutic targets.

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#### Ethical approval

BRAINS study meets  
all ethical standards  
set by local  
institutional review  
boards for the UK,  
Indian and Qatari  
research sites

#### Guarantor

N/A

#### Contributorship

IC is a postdoctoral  
fellow at Imperial  
College London and  
designed and co-  
written the final  
protocol. MSK is the  
project administrator  
for BRAINS at  
Imperial College  
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AM is the BRAINS-  
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the Sri Lankan site  
investigator. FYK and  
HAH are principal  
investigators for

## Introduction

Stroke is the third most common cause of death in the world and is expected to become the third most common cause of disease burden by 2020.<sup>1</sup> In South Asian countries (India, Pakistan, Bangladesh and Sri Lanka), which contain approximately 20% of the world's population, stroke incidence rates increased by more than 100% during the last four decades, while in developed European countries they decreased by 42% over the same time period.<sup>2</sup> The incidence rates for stroke in South Asians have increased from 52 per 100,000 person-years (1970–1979) to 117 per 100,000 person-years (2000–2008) making stroke one of the major causes of death in South Asia.<sup>2</sup> This could be partly explained due to socioeconomic changes in South Asian countries in the recent years.<sup>3</sup> However, stroke can occur without any obvious aetiological cause, suggesting that genetic factors may play an important role in stroke pathology in South Asians.<sup>4</sup>

The Arab population, which forms about 5% of the total world population, remains largely understudied for stroke epidemiology. In Arab states, the incidence of stroke varies widely from 27.5 to 63 per 100,000 with a prevalence of 42–68 per 100,000.<sup>5</sup> In Qatar, the overall incidence rate is 41 per 100,000 inhabitants per year (95% confidence interval, 30.2–52.4/100,000/year) and 238/100,000/year for the population over 45 years old.<sup>6</sup> For native Qataris, who represent approximately 35% of the total population, a crude incidence of 73/100,000 inhabitants per year has been reported as well as an overall patient fatality rate at 30 days of 16%, suggesting that stroke represents a major cause of morbidity and mortality in Qatar.<sup>6</sup> The lower stroke incidence in Qatar compared with stroke incidence in developed countries is likely to reflect its younger population; however as the age of the population is likely to rise, the burden of stroke to its population is anticipated to increase.<sup>5</sup>

Conventional risk factors such as hypertension, diabetes, dyslipidaemia and smoking account for a large proportion of stroke risk,<sup>7</sup> but as some individuals are more susceptible to these risk factors compared with others, genetic factors may explain part of the increased stroke risk. To-date,

several genome-wide association (GWA) studies have investigated the genetic contribution to stroke onset in Europeans<sup>8–11</sup> while none have been conducted in South Asian or Arab populations.

Identifying genetic risk factors for stroke in understudied populations such as those from South Asia and the Middle Eastern may improve risk prediction, enhance our understanding of the pathophysiological mechanisms underlying stroke in different populations and lead to novel population-specific targeted drugs. However, to have sufficient power to disentangle the genetic component of stroke a large study cohort is necessary.

We have established an ongoing prospective recruitment study in Europeans (BRAINS [Bio-repository of DNA in stroke]-UK) which aims to recruit 1500 stroke patients from 12 sites across the UK.<sup>12</sup> These sites were specifically chosen in areas with a high South Asian density allowing them to not only recruit stroke patients of European ancestry but also those of British South Asian ethnicity ([www.brainsgenetics.com](http://www.brainsgenetics.com)). Similarly we aim to establish a large high-quality stroke repository of Indian stroke patients in the subcontinent, BRAINS-South Asia. This effort complements our current Indian and Sri Lankan repository described previously.<sup>12</sup> BRAINS-South Asia will include a total of 3000 highly phenotyped ischaemic stroke patients and 3000 controls, of which approximately 1500 cases and 1500 controls have already been recruited.<sup>12</sup>

In addition to the South Asian repository we aim to develop the largest repository of DNA for stroke patients in the Arab countries, BRAINS-Middle East. BRAINS-Middle East will recruit 1500 highly characterized Qatari ischaemic stroke patients, while 3000 controls will be obtained from a separate ongoing national population-based Qatari Biobank.

This large and comprehensive international collaboration – which includes three multicentre studies Brains-UK (European and British Asian), Brains-SA (India and Sri Lanka) and BRAINS-Middle East (Qatar) – aims to investigate the influence of genetic factors on stroke risk in European, South Asian and Qatari population, and may contribute to a better understanding of the genetic differences between these ethnicities in terms of stroke risk.

BRAINS Middle East (Qatar site). SPG, SG, SRS and PNS are site investigators at four medical centers across India located in Delhi, Pune, Shillong and Trivandrum, respectively. PS is the principal investigator of BRAINS (UK, South Asia and Middle East) and has developed the protocol from its inception to the final version

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None

## Methods and study design

### Ethical consideration

The BRAINS study meets all ethical standards set by local institutional review boards for UK, Indian, Sri Lankan and Qatari research sites. Written informed consent will be obtained for every proband and control. For intubated patients or those rendered incompetent by stroke surrogate consent will be obtained. Patient confidentiality will be protected and patient details will be encrypted. BRAINS investigators who have access to genetic data will be blinded to individual personal identifiers such as names, addresses, phone numbers and email addresses. Investigators who have access to the personal identifiers will be blinded to the genetic data. The participants will not be told the results of genetic testing as the individual risk profile in polygenic disorders cannot be accurately measured. Future project applications wanting to access BRAINS DNA data will be assessed and will have to follow strict Medical Research Council ethical guidelines.<sup>13</sup>

### Study populations

Patients and spouse/partner or age-matched controls will be screened at the participating clinical centres in the UK (Imperial College NHS Trust, Barts and the London NHS Trust, Queen's Park Hospital/Blackburn, Luton and Dunstable Hospital, Birmingham Heartlands Hospital, Manor Hospital Walsall, New Cross Hospital/Wolverhampton, Bradford Teaching Hospital, Airedale General Hospital, Leeds General Infirmary, Northwick Park Hospital, Newham University Hospital, Hillingdon Hospital, City Hospital Birmingham and West Middlesex University), India (All India Institute of Medical Sciences [AIIMS], New Delhi [North India]; Research and Referral Army Hospital, New Delhi [North India]; Sree Chitra Tirunal Institute for Medical Sciences and Technology [SCTIMST], Thiruvananthapuram [South India]; Armed Forces Medical College, Pune [West India]; and North Eastern Indira Gandhi Regional Institute for Health and Medical Sciences, Shillong, Meghalaya [East India]), Sri Lanka (University of Sri Jayewardenepura and National Hospital of Sri Lanka) and Qatar (Hamad Medical Hospital and Al Khor General Hospital).

Phenotype data will be collected in detail in a specially designed data collection form including baseline clinical and demographic data. Stroke will be confirmed with computerized tomography (CT) or magnetic resonance imaging (MRI) and subtyped according to the Trial of ORG10172 in Acute Stroke Treatment (TOAST) classification.<sup>14</sup> Blood samples from all consenting patients and controls will be collected in ethylenediaminetetraacetic acid (EDTA)-coated plastic serological tubes by means of a single venepuncture and DNA extracted from peripheral lymphocytes using standard procedure and stored at  $-20^{\circ}\text{C}$ .

### Proband (cases)

BRAINS-UK will recruit 1500 cases of ischaemic and haemorrhagic stroke patients >18 years of age from specialist stroke centres around the UK. BRAINS-South Asia will recruit 3000 cases of ischaemic and haemorrhagic stroke from four specialist stroke centres throughout India. BRAINS-Middle East will recruit 1500 ischaemic stroke patients from two hospital centres in Qatar: Hamad Medical Hospital and Al Khor General Hospital.

Each patient will be evaluated by a stroke specialist from the specific site according to institutional patient care guidelines that are based on current international standards of patient care.<sup>15,16</sup> The patient evaluation includes recording patient history (medical, socioeconomic and financial status), physical examination (blood pressure and pulse rate), CT or MRI and laboratory testing (including lipid profile, C-reactive protein, erythrocyte sedimentation rate and glucose). Information on the exposure to environmental risk factors such as smoking and alcohol will also be recorded.

All probands will be identified using the following inclusion criteria: (1) patients should be aged >18 years at the time of enrolment; (2) diagnosis of haemorrhagic or ischaemic stroke using World Health Organization (WHO) guidelines<sup>17</sup> confirmed by clinical examination and brain imaging (CT or MRI); (3) all patients with cerebral arteriovascular malformations and aneurysms; (4) European, South Asian or Qatari ethnic background; and (5) patient or relative written informed consent. Exclusion criteria

include: (1) inability to provide consent themselves or through surrogate and (2) stroke not defined by CT or MRI. Patients with old and recurrent stroke as well as seriously ill and/or intubated patients will be included in the study to avoid bias against more severe forms of stroke.

### Controls

BRAINS-UK will recruit spouses/partners or unrelated unaffected relatives of South Asian ancestry as controls. The cases of European descent will not require recruitment of controls as we have access to 3000 controls from the 1958 British Birth Cohort portion of the Wellcome Trust Case Control Consortium (WTCCC).<sup>18,19</sup>

BRAINS-South Asia will recruit 3000 spouses/partners or unrelated unaffected relatives as control subjects, of whom 1500 have already been recruited as part of our initial single site study at AIIMS, New Delhi previously described.<sup>12</sup> Spouse/partner controls will preferably be recruited, as they tend to arise from the same geographical population and usually have similar exposure to environmental determinants.<sup>20</sup> When spouses/partners are unavailable, unrelated friends or unrelated blood donors will be recruited.<sup>21</sup>

BRAINS-Middle East will recruit 1500 control subjects from the ongoing Qatari Biobank, a general population-based biobank modelled on the UK Biobank, which aims to collect medical data from up to 100,000 volunteers.

The inclusion criteria for the controls are: (1) aged >18 years at the time of enrolment; (2) no previous history of stroke; and (3) ability to provide written consent. Spouse/partners will be identified on the inpatient acute stroke unit or outpatient stroke clinic, and stroke-free status will be confirmed using the BRAINS questionnaire.

Hospitalized patients with any medical condition are not eligible as controls for BRAINS. Gender differences will be balanced by using large numbers of sex-mixed cases and/or sex-stratification during genetic analysis.

### Data collection

#### Interview

A detailed interview will be conducted for each participant proband and control subject by the

local stroke trial co-ordinator/recruiter, and the aim of the study and role of their participation will be explained. Written consent will be obtained and baseline clinical and demographic data such as clinical diagnosis, CT or MRI results, age, sex, ethnicity and other information will be collected. Medical records and family history will also be recorded from each participant.

#### Medical records

The medical records of patients whose primary diagnosis is stroke will be reviewed by stroke research practitioners before assessing eligibility for enrolment. To ensure consistency of data across centres demographic, clinical and imaging/laboratory data will be collected from all patients using a standardized preforma. The case report forms will include information on: (1) cardiovascular risk factors including vital signs (height, weight, blood pressure and temperature), age, sex, ethnic origin, past history of hypertension and other cardiovascular disease, diabetes, smoking status, alcohol intake (units/week) and family history; (2) biomedical data including fasting glucose, lipids (total and high-density lipoprotein-cholesterol and triglycerides), prothrombin time, proteins C and S, fibrinogen, plasma homocysteine concentration, lipoprotein analysis along with other biochemical markers; and (3) imaging results including CT or MRI of the head, size and location of the symptomatic cerebral infarct as seen on head imaging. Carotid Doppler will be undertaken when patients present with anterior circulation lesions. Emergency room blood pressure and biochemistry test results will be recorded. For South Asian patients in the subcontinent, data on social status will be collected by questionnaire.

#### Stroke characterization

The clinical diagnosis of stroke will be confirmed by a study-appointed neurologist or stroke physician. The time of onset will be determined by talking to the patient and/or relatives present at the time of its occurrence. The neurologist will determine severity of neurological deficits within 48 hours of admission based on the National Institute of Health Stroke Scale (NIHSS).<sup>22</sup> Diagnosis of stroke will be confirmed using a CT scan or MRI of the brain, performed after the onset of symptoms.

Poststroke functional status will be assessed using the Barthel Index<sup>23</sup> within 48 hours of admission. The TOAST classification system will be used to subtype stroke by a neurologist who is blinded to the genetic and phenotypic data.

#### Patient follow-up

Patients will be followed up by the study co-ordinators after one year of recruitment to determine if they have had another vascular event. If patients are suffering from speech or cognition deficits, then history will be recorded from the partner or relative. In the event of mortality, cause of death will be ascertained.

#### Biological samples

Samples of 10 mL peripheral blood from patients and controls are collected in EDTA-coated vials using a single venepuncture. Each sample is assigned a unique BRAINS repository ID number and immediately stored at  $-20^{\circ}\text{C}$ . Archive-quality high-molecular-weight genomic DNA is isolated from the peripheral lymphocytes using commercially available Qiagen DNA isolation kits (Qiagen Genra Puregene Blood Kit; Qiagen, Venlo, the Netherlands). As a quality control, OD260/OD280 ratio is measured and accepted if it is  $> 1.8$ . For lower OD260/OD280 ratios DNA samples will be re-purified.

#### Adverse events

All adverse events and other vascular events will be recorded by the study co-ordinators and forwarded to the principle investigator.

#### Outcome measures

The primary outcome of the study is to establish a large DNA repository of highly phenotyped stroke patients in the South Asian (British, Indian and Sri Lankan) and Middle Eastern (Qatari) populations as well as a comparative European-descended population from the UK.

We aim to recruit 3000 cases and 3000 control subjects from BRAINS-South Asia and 1500 stroke patients from BRAINS-Middle East. The secondary outcome is to identify new common and rare genetic variants associated with ischaemic stroke. Other outcome measures include association of genetic variants with stroke subtypes,

ethnic groups and different environmental factors such as hypertension, diabetes and smoking. The large database will also allow epidemiological analysis about cerebrovascular disease in these three distinct ethnic populations.

#### Data analysis

We aim to apply a combined GWA approach and an exome sequencing approach by using the HumanOmni2.5Exome BeadChip developed recently by Illumina (Illumina Inc., San Diego, CA, USA).<sup>24</sup>

Illumina HumanOmni2.5Exome BeadChip has the advantage of having a high coverage of putative functional exonic variants selected from over 12,000 individual exome and whole-genome sequences. This microarray combines  $> 2.5$  million tag single-nucleotide polymorphisms (SNPs) (from the HapMap and 1000 Genomes Project that provide coverage of common genetic variants at  $> 2.5\%$  minor allele frequency) with  $> 240,000$  functional exonic markers.<sup>24</sup> The functional exonic markers include non-synonymous variants, stop altering variants, splice coding variants and variants located in promoter regions.

The case-control analyses will consider allele, dominant, recessive and additive genetic models by using logistic regression analyses. The significance of association will be determined using the Chi-square or Fisher-exact test for allele, dominant and recessive genetic models.

#### Sample size and power

Power calculations were performed using the genetic power calculator CaTS described by Skol *et al*<sup>25</sup>. The BRAINS-UK study, which will include 1500 cases and a similar number of controls, has 90% power to detect a relative risk (RR) of 1.3 at  $P < 0.001$  with a population allele frequency of 0.3. The BRAINS-South Asia study, which will include 3000 cases and a similar number of controls, has 90% power to detect a RR of 1.3 at a genome-wide significant  $P$  value of  $5 \times 10^{-8}$  with a population allele frequency of 0.3. The BRAINS-Middle East study, which will include a sample of 1500 cases and 3000 controls, has 90% power to detect a RR of 1.3 at  $P < 5 \times 10^{-5}$  with a population allele frequency of 0.3. At a genome-wide significant  $P$  value of  $5 \times 10^{-8}$  it

has 45% power to detect a RR of 1.3 for polymorphisms with an allele frequency of 0.3.

## Discussion

Stroke is one of the major causes of death and disability in the world. The WHO estimates that by 2050, 80% of the global burden of 15 million new strokes will occur in low- and middle-income countries such as India and China.<sup>3,26,27</sup>

The BRAINS project, which includes three large multicentre prospective recruitment studies BRAINS-UK, BRAINS-South Asia and BRAINS-Middle East, aims to gain a much better understanding of the genetics of stroke by investigating the influence of genetic factors on stroke risk in European, South Asian and Qatari populations.

In the past several years, the GWA approach facilitated by technological developments of high-density genome-wide genotyping arrays has been applied for many complex diseases and has been successful in identifying thousands of novel common genetic variants associated with the risk of disease.<sup>28</sup> However, for stroke, GWA has been applied so far mainly on European and Japanese populations.<sup>8–11,29–30</sup>

Currently there are few treatments for acute stroke, comprising mainly aspirin (which needs to be prescribed to ~80 patients to benefit one individual) and clot-busting drugs (which have very poor availability in the Middle East<sup>31</sup> and South Asia<sup>32</sup>), suggesting a clear need to identify new drug targets. Enhancing our understanding of the genetics of stroke is likely to contribute to developing population-specific drugs to combat this devastating, complex disease. Furthermore, performing whole-genome sequencing, which in the near future may become more affordable, and investigating gene–gene and gene–environment interactions may contribute to enhancing the understanding of the genetic and molecular underpinnings of stroke.

The BRAINS study will enable us to compare and contrast genetic variants between native Indians and Indians living in UK, as well as Europeans. The largest South Asian diaspora outside of the Indian subcontinent exists in the UK, representing a significant burden on the national health resources. Understanding why stroke occurs more frequently in the South Asian

population may help us understand its mechanisms and may lead to identification of population-specific targeted drugs. In addition, our stroke disease-based repository in Qatar complements the existing Qatari Cardiac Biobank which, when combined with our stroke biobank, will greatly enhance the ability to answer a larger number of questions relating to the genetics of vascular disease in its totality than would otherwise be possible.

This large well phenotyped clinical and DNA repository of ischaemic stroke on three populations has great potential of identifying novel common genetic variants associated with stroke by using a genome-wide approach, and rare variants with potentially higher effects by using recently developed exome sequencing approaches. This combined approach will allow us to gain insight into whole-genome variation while maximizing coverage of functional exonic SNPs.

## BRAINS collaborators

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