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STUDY PROTOCOL

Bangladeshi Atherosclerosis Biobank and Hub: The BANGABANDHU Study

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Background: Genetic factors contribute significantly to the risk of ischaemic heart disease (IHD), which is the leading cause of mortality in Bangladesh. The BANGABANDHU (Bangladeshi Atherosclerosis Biobank AND Hub) study will allow a hypothesis-free genome-wide association study (GWAS) to identify genetic risk factors associated with ischaemic heart disease patients undergoing coronary artery bypass graft (CABG) surgery in Bangladesh.

Methods: This is a multi-centre population-based case–control study aimed to evaluate 1500 (Fifteen Hundred) adult (≥18 years of age) people divided into 2 study groups: Case/Proband (750 IHD patients undergoing CABG surgery) and Control (750 healthy people). Spouses or family members are preferred as healthy control subjects due to their shared geographic location and similar environmental exposure.

Results: This will be the first largest DNA repository of CABG patients in Bangladesh, and identifying novel gene loci among CABG patients might help to discover novel therapeutic targets for Bangladeshi IHD patients. Further, identifying and comparing novel gene loci among CABG patients with other ancestry might help devise national guidelines for treating coronary artery disease.

Conclusion: Promising current study results will encourage Bangladeshi researchers and pharmaceutical companies to conduct further studies into the genetic basis of Bangladeshi complex coronary artery disease, which might identify novel genes for therapeutic targets for Bangladeshi patients and strengthen the healthcare standards in Bangladesh.

Keywords: Bangabandhu, Bangladesh, biobank, IHD, CABG

Introduction

Ischaemic heart disease (IHD) is the leading cause of mortality in Bangladesh, characterised by the development of atherosclerotic plaque occluding in the coronary arteries that supply oxygenated blood to the myocardium.[1,2] Over the past decade, there has been a decrease in age-adjusted cardiovascular death rates in high-income countries, while developing countries like Bangladesh have seen a substantial increase in mortality rates.[3–5] While studies from developed countries provide a general understanding of the modifiable risk factors for IHD, there is an apparent disproportionality in the frequency of these IHD risk factors among the Bangladeshi population, which is more severe and higher in prevalence.[5–7] Moreover, Bangladeshi IHD patients may have risk factors that have yet to be observed in high-income settings, whereby the pathobiology of IHD may be different in contexts with additional yet undefined risk factors.[6–8]

In Europe and the UK, genome-wide association studies (GWAS) have identified multiple genetic variants (SNPs) associated with IHD risk and associated clinical phenotypes like hypertension and dyslipidaemia.[8–10] IHD has critical genetic underpinnings considered equivalent to environmental factors, estimated at 40%–60%. Efforts are underway to identify genetic risk factors for ischemic heart disease (IHD) that are specific to certain populations.[7–9] By combining these findings with knowledge of the causal risk factors for IHD, researchers hope to uncover new opportunities to improve public health efforts to reduce the global burden of IHD, including in Bangladesh. Furthermore, recent studies of IHD prevalence in diverse ancestry populations have suggested that some populations are more susceptible to complex IHD risk.[10–12]

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Advancements in genetic studies have provided a more profound knowledge of the pathophysiology involved in ischemic heart disease and helped identify new treatment targets.[10–15] Further, genetic risk scores may improve risk prediction in the future and lead to the development of individualised treatment strategies, which are the foundation of precision medicine.[12–16] Polygenic risk scores (PRS) for IHD can improve cardiovascular risk prediction by correlating with plaque burden and advanced atherosclerosis, making them useful for IHD risk stratification, especially in younger subjects.[15] However, the interplay between genetic factors and environmental atherosclerotic risk factors is the subject of extensive research within and between ancestries.[15–18]

This study aimed to understand the association of genetic and environmental risk factors among IHD patients undergoing CABG surgery by genome-wide association study (GWAS) on ischemic heart disease in Bangladesh.

Research Gap

IHD has a genetic basis, and identifying the genes involved may help us define the mechanisms that cause disease and identify novel therapeutic targets to improve life expectancy among the Bangladeshi population. Although genome-wide association studies (GWAS) have implicated 58 loci in ischaemic heart disease, the biological mechanism of the causal associations between the relevant genes and IHD remains uncertain, specifically in the South Asian region. Although genetic studies on IHD patients are well established in developed countries like Europe, the UK, and the USA, we have yet to conduct a single GWAS study on IHD patients undergoing CABG surgery in Bangladesh. We defined the study population according to the PICO framework as follows-

P (Population): Bangladeshi IHD patients and spouses or age-matched adult healthy population.

I (Intervention): IHD patients undergoing coronary artery bypass graft surgery.

C (Control): Healthy population, specifically spouse or age-matched relative of IHD patients.

O (Outcome): Identify gene loci associated with IHD among Bangladeshi population.

Patients and Methods

This multi-centre study involves the Department of Cardiac Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU), the National Institute of Cardiovascular Diseases (NICVD), and Impulse Hospital & Research Centre, Bangladesh. We aimed to evaluate 1500 (Fifteen Hundred) people divided into 2 study groups: Case/Proband, Consisting of 750 IHD patients undergoing CABG surgery, and Control, consisting of 750 healthy people (Spouses or relatives of IHD patients). This study will comply with the Declaration of Helsinki.

Study Procedure

The participating clinical centres will screen ischaemic heart disease (IHD) patients and their spouses or age and gender-matched controls. Detailed phenotype data will be recorded in a data collection form. IHD will be confirmed with a coronary angiogram (CAG), and baseline clinical and demographic data will also be collected.

Proband (Cases)

The BANGABANDHU study will recruit 750 cases of ischemic heart disease patient's ≥18 years of age from specialist heart centres in Dhaka, Bangladesh. Although there are likely to be gender differences in the overall cohort, any slight differences can be adjusted for. Per standard guidelines, each IHD patient will be admitted and evaluated by a cardiac surgeon at the participating centre. Proband evaluation includes recording patient history (medical and socio-economic status), physical examination (BP and pulse rate), CAG and Doppler echocardiography, and laboratory testing. Exposure to environmental modifiable risk factors like alcohol, smoking, and dietary habits will also be noted in the datasheet. All proband will be identified using the following inclusion criteria:

1. Patients should be adults aged ≥18 years at the time of enrolment;
2. Diagnosis of IHD using 2023 American Heart Association (AHA) guidelines[17] confirmed by clinical examination and coronary angiogram imaging;
3. Patient or relative written informed consent, if patients unconscious or unable to provide consent.

4. To minimise the bias, we also include patients with old and recurrent MI and seriously moribund patients.

Controls

Spouses or family members are preferred as control subjects since they usually belong to the same geographic population and are exposed to similar environmental factors. Although spouses/partners may occasionally be unavailable, the BANGABANDHU study sample is large enough to accommodate this. Further, unaffected siblings could also act as suitable controls should there be any shortfall. The BANGABANDHU study will recruit 750 spouses, partners, or healthy relatives as controls. Controls will be asked to attend a hospital appointment for a direct venepuncture to collect blood samples. Alternatively, blood collection packages will be sent to control subjects identified by the probands or their relatives. Controls will meet the following inclusion criteria:[10–13]

1. Should be aged ≥18 years at the time of enrolment and able to provide written consent.
2. No previous history of IHD.
3. Hospitalised patients are not eligible as controls for the study.
4. Recruiting age and sex-matched volunteers will fill any lag in spouses as controls.

Study Exclusion Criteria:

1. IHD Patients with concomitant valvular and congenital heart defects.
2. Control subjects with known heart disease.
3. Control subjects with known significant comorbidities like renal failure and DM.
4. Patients wish or are unable to provide consent themselves or through a surrogate.

Sample Size and Power

All power estimates are based on recent Indian study findings in coronary artery disease conducted by Bhat et al[19] that showed a risk ratio [RR] of 2.1. For the study, we have ~1000 cases with an identical number of control subjects, providing 90% power to observe a relative risk (RR) of 2.0 at P <0.0001 with a sample allele frequency of 0.2 that would be seizable for 100 candidate genes. For an allele frequency of 0.2 and an RR of 2.1, a more stringent P-value of <0.00001 is achieved at the same 90% power.

Data Collection Technique

Phenotypic Data

Cardiac surgeons will review the patient's medical records, whose primary diagnosis is IHD, before assessing eligibility for enrolment. The institutional coordinator/recruiter will conduct a detailed interview with the probands and controls to explain the aim of the study and the role of their participation. Informed written consent will be obtained from all participants. Baseline clinical and sociodemographic data such as clinical diagnosis, coronary angiogram findings, and sociodemographic variables like age, sex, past medical history, family history and other comorbidity information will be collected. The following information will be recorded on the case report forms:

1. **Demographic data**: Risk factors include vital signs (age, sex, height, weight, BMI, blood pressure, diabetes, smoking status, alcohol intake (units per week), and family history).
2. **Biomedical data**: Fasting glucose, fasting lipids (total and HDL-cholesterol and triglycerides), troponin-I, and CK-MB.
3. **Imaging results**: Coronary angiogram (CAG) findings: number of coronary vessel involvement, left main disease, calcifications, diffuse lesions, previous angioplasty, stent restenosis; and Doppler echocardiography findings: Left ventricular internal diameter end diastole (LVIDd) and end-systole (LVIDs), Ejection fraction, hypokinesia/akinesia, associated ischaemic valvular disease and intracardiac thrombus.

Genetic Data

Trained nurses and healthcare personnel will collect 10 mL peripheral blood samples from IHD patients and control subjects in EDTA-coated vials using a single venipuncture. Each sample is assigned a unique BANGABANDHU repository ID number immediately stored at −20 °C. Archive-quality, high-molecular-weight genomic DNA will be isolated utilising commercially available Qiagen DNA isolation kits from the peripheral lymphocytes. As a quality control, the OD260/OD280 ratio is measured and accepted if above 1.8; DNA samples will be re-purified for a lower OD260/OD280 ratio. After genotyping, the genetic data will be merged with phenotype data, including environmental risk factors and CAG results, for analysis.

Data Management Plan

All data will be encrypted and collected during follow-up interviews with the patient. The data will be recorded in the Excel data collection sheet, and appropriate statistical tools will be used for data editing and analysis. The data and results will be presented as tables and diagrams where applicable. If data is missing randomly, the datasets will be evaluated statistically using the Little Missing Completely At Random (MCAR) test.

Quality Assurance

This BANGABANDHU study is unbiased towards discovering genetic risk factors across the entire range of IHD, including multivessel CAD and low <30% EF cases. Patient confidentiality will be protected, and data will be encrypted. The BANGABANDHU investigators with access to genetic data will be blinded to individual personal identifiers (eg names, addresses, and phone numbers), and investigators with access to personal identifiers will be blinded to genetic data. Further, no individual will be informed of their genetic testing results as the precise individual risk profile in polygenic disorders cannot be accurately determined. Data collection sheets will be stored confidentially at participating centres, and research data and genetic test results will not be recorded in patients' clinical notes. Any future project applications from investigators to access the BANGABANDHU study will be assessed and will have to follow strict BSMMU ethical guidelines. A future use agreement will be created based on guidelines to ensure the appropriate use and transparency of BANGABANDHU study DNA in collaborative efforts by investigative groups.

Ethical Considerations

Ethical clearance will be obtained from the BSMMU Institutional Review Board. Further, informed written consent will be obtained for every Proband (IHD case) and Control (Healthy subjects) and data will be encrypted.

Statistical Analysis

We will utilise R-4.3.2 and PLINK version 2.0 software to analyse the study variables, and appropriate statistical tests will be applied to test the significance level between study groups. An isolated Hardy-Weinberg equilibrium test will be conducted for probands and controls. Nevertheless, genotypic analyses will consider allele, dominant, recessive and additive genetic models. Further, the significance of association will be determined using appropriate statistical tests, either Chi-square or Fisher exact test, for allele, dominant and recessive genetic models and odds ratios (OR) will be observed utilising logistic regression analyses. As appropriate, data will be presented as tables and graphs, such as Manhattan plots and Quantile-Quantile (QQ) plots. A P value <0.05 will be considered as statistical significance.

Discussion

The first genome-wide association study (GWAS) on ischemic heart disease (IHD) was published in 2007.[1] It identified several genetic loci, including a locus on chromosome 9p21, which is still the most consistently associated with IHD. Nikpay et al conducted a thorough analysis of the genetic architecture of IHD and concluded that common SNPs of small effect size play a significant role in determining genetic susceptibility to IHD.[7] Among European populations, the SNP in the PDGFD locus associated with IHD showed tissue-specific cis expression quantitative trait locus effects, implicating

new pathways for CAD susceptibility.[10] Further, a recent study on genetic analysis identifies over 250 risk loci for IHD, which can inform the experimental investigation of causal mechanisms for coronary artery disease.[12]

In Pakistan, genome-wide association studies of coronary artery disease have identified five significant SNPs previously reported in European biobanks.[13] However, Sasidhar et al performed GWAS that recognised about 50 gene loci, determining approximately 6% of the heritability in coronary artery disease among the Indian population.[14] Furthermore, a recent Indian study by Bhat et al identified novel variants in three genes with odds ratios for variant rs1869592, rs1059091, and rs7247159 were 2.6 (1.4–4.8 95% CI), 1.9 (95% CI 1.2–3.1) and 2.1 (1.2–3.7 95% CI), respectively, with significant P value <0.01.[19]

In multi-ethnic populations from Southeast Asia, Han et al discovered a genetic variant, rs2075291, in APOA5 that is significantly associated with IHD and provides new insights into the pathways contributing to IHD susceptibility.[20] Furthermore, Natriuretic peptide receptor-C gene SNPs (rs700926, rs1833529, rs2270915, rs17541471, rs3792758, and rs696831) significantly contribute to susceptibility to ischemic heart disease (IHD) in the Chinese Han population.[21] Nevertheless, a GWAS of coronary artery disease incorporating nearly a quarter million cases found near equivalent heritability of CAD across multiple ancestral groups, identifying 95 novel loci, including the first nine to be identified on the X-chromosome.[22]

In this study, the authors aim to evaluate a better understanding of the environmental and genetic differences[23,24] in ischaemic health disease patients undergoing coronary artery bypass graft surgery in Bangladesh. Further, it seeks to establish the most extensive DNA repository of highly phenotype IHD patients and identify novel gene loci associated with complex IHD patients undergoing CABG in Bangladesh.

Significance of the Study

This will be the first Bangladeshi Biobank on CABG patients, which will open new horizons for future research to find better treatment options and healthcare research comparable to those of developed countries.

Concluding Remarks

Identifying specific genes of Bangladeshi IHD patients and comparing GWAS findings with different ancestry might help determine specific therapeutic targets for these groups of patients.

Data Sharing Statement

This is an ongoing project, and the data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. Professor Dr. Asit Baran Adhikary had full access to all of the data in this study and took complete responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgments

Professor Dr Asit Baran Adhikary affirms that this manuscript is an honest, accurate, and transparent account of the study being reported, that no important aspects of the study have been omitted and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest in this work.

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