Investigation of genetic and environmental factors underlying cardiovascular disease – the London Life Sciences Population (LOLIPOP) Study

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BACKGROUND

Indian Asians are at increased risk of cardiovascular disease

Indian Asians represent the largest ethnic minority group in the UK. National mortality statistics suggest that mortality from coronary heart disease (CHD) and stroke are both ~70% higher amongst UK Indian Asians,[1] and that this increase in cardiovascular disease (CVD) risk is evident in each of the major Asian cultural subgroups. Furthermore, admission rates with myocardial infarction are two-fold higher compared to Northern Europeans.[2, 3] The high CVD morbidity and mortality amongst Indian Asians compared to Europeans represents an important health inequality, which needs to be explained and addressed.

Mechanisms underlying increased CVD in UK Indian Asians

Traditional risk factors (cigarette smoking, hypertension and hypercholesterolaemia) make an important contribution to CVD in all populations, including Indian Asians.[4, 5] However numerous studies show that traditional CVD risk factors are not more prevalent in Indian Asians, and therefore cannot explain the increased risk of CVD in Indian Asians, compared with other ethnic groups.[6-8]

In contrast, insulin resistance and associated metabolic disturbances are more common in Indian Asians.[6-8] Compared to European whites, Indian Asians have impaired glucose tolerance, fasting and post glucose load hyperinsulinemia, and raised Homeostasis Model Assessment (HOMA) insulin resistance.[6, 9-11] Additional defects are increased fasting and postprandial triglyceride levels,[6-8] increased small dense low-density lipoprotein (LDL) particles,[12] decreased high-density lipoprotein (HDL),[6-8] abdominal obesity, and a pro-inflammatory state.[13] T2D is also two to four fold more prevalent in Indian Asians than European whites.

Insulin resistance and related disturbances are widely considered to be major contributors to the increased risk of CVD amongst UK Indian Asians. However, the evidence for this view is based on the results of a small number of cross-sectional studies.[6, 14-16] Of these, the largest included 1712 Indian Asians, and examined the relationship of glucose intolerance and hyperinsulinaemia with prevalence of pathological Q waves on the 12 lead ECG, a surrogate marker for CHD, amongst Indian Asians and European whites.[14] Although the study found an association of markers of insulin resistance with prevalent Q waves amongst Indian Asians, there remained a 1.5 fold unexplained increased risk of Q-waves amongst Indian Asians after adjustment for insulin, waist-hip ratio, glucose intolerance, and other factors. The mechanisms underlying increased CVD in Indian Asians therefore remain to be established.

Improving risk stratification for CVD among Indian Asians

Effective prevention of CVD requires robust methods for the identification of people at increased risk. Framingham and other prospective studies provide multivariate equations to predict risk of CVD.[17, 18] The Joint British Societies recommend use of the Framingham function for risk prediction in the UK.[19] However, this function has not been validated in UK ethnic minorities.

Cross-sectional data indicate that Framingham risk functions underestimate CVD risk in Indian Asians.[20, 21] One possible explanation is that the Framingham risk functions do not adequately incorporate insulin resistance, and related metabolic disturbances, which are more prevalent in Indian Asians. The failing of the Framingham functions to predict CVD mortality accurately in ethnic minority groups has been repeatedly recognised as a major limitation, with consequences for risk prediction among Indian Asians.[19] Underestimation of risk will lead to under-treatment, and widen the inequalities in CVD outcomes for this vulnerable population. The National Institute for Clinical Excellence, and the Joint British Societies (British Cardiac Society, British Hypertension Society, British Diabetic Association) have all called for urgent research to develop and validate new risk models that accurately predict CVD risk amongst Indian Asians.

Work of the investigators leading up to this proposal

This proposal builds on the ongoing collection of ~30,000 men and women in the London Life Science Prospective Population (LOLIPOP) study. The primary aims of the LOLIPOP study are to:

- Obtain prospective estimates of CVD incidence amongst UK Indian Asians and European whites in a contemporary, population based cohort.
- Identify novel environmental and genetic factors that contribute to incident CVD in UK Indian Asians and European whites.
- Develop and validate novel tools for the identification of Indian Asians at increased risk of CVD for use in daily clinical practice.

Screening for CHD risk factors in West London

We have established a collaboration with 58 GPs in West London to undertake systematic assessment of CHD risk factors amongst all ~67K persons aged 35-75 years registered with practices, as well as additional patients outside these age ranges at the GPs request. This collaboration is based within the NHS, started in 2003 and seeks to deliver standards 3 and 4 of the National Service Framework for CHD. Assessments are carried out by trained nurses. An interviewer administered questionnaire is used to collect data on medical history, current prescribed medication, cardiovascular risk factors, alcohol intake, and physical activity. Physical measurements include blood pressure, height, weight, Waist-hip ratio and fat mass (bio-impedance), urinalysis, and 12 lead ECG. Blood is collected after an 8 hour fast for biochemical analysis, including glucose, total and HDL cholesterol and triglycerides. Full clinical reports are sent to the patient and GP detailing results and identifying any clinical problems. To date, we have invited ~62 and ~35K have attended the CHD risk assessment program. We anticipate completing the screening program by June 2008, by which time almost ~40K persons will have been clinically assessed.

The LOLIPOP study

When persons attend for clinical CHD risk factor assessment, they are invited additionally to take part in the LOLIPOP research study. Participation provides consent for follow-up for incident cardiovascular events, as well as for storage of blood for future biochemical and genetic studies. The LOLIPOP study is approved by the Ealing Hospital LREC (reference 02/32: An investigation of hereditary and environmental factors in cardiovascular disease), and all participants give written informed consent. To date, ~75% of persons attending for CHD risk factor assessment have agreed to participate in the LOLIPOP study. There are currently ~27K persons in LOLIPOP. We will continue to recruit until completion of CHD screening in June 2008, by which time we expect ~30K persons to have consented to participate in the study. In keeping with the demographics of the local population, these LOLIPOP participants are ~60% Indian Asian, ~30% European white, and ~10% other ethnic group (predominantly African). Research samples are labelled with a unique, linked anonymous study identifier using a barcode. Research data are stored in a central database at Ealing Hospital, and data management systems are in place to ensure security and confidentiality.

LOLIPOP follow-up

The existing LREC approval and participant consent form allow for ascertainment of cardiovascular and health endpoints from hospital notes and primary care records. Although the use of routine collected data is a widely accepted method for identification of cardiovascular and other health endpoints, this approach is not without limitation. Up to 25% of diagnoses are miscoded at discharge. Furthermore, myocardial infarction may occur silently, particularly in the presence of type 2 diabetes. Such misclassification will weaken and distort the relationships between risk factors and incident CVD events, thereby limiting the validity of the research.

In his proposal we therefore propose to invite the ~30K participants of the LOLIPOP for clinical follow-up, to enhance identification and validation of CVD endpoints in this prospective cohort study.

RESEARCH PLAN

The baseline characterisation of participants in the LOLIPOP study provides a unique opportunity for the first well-powered, prospective evaluation of the genetic and environmental mechanisms underlying increased CVD mortality amongst Indian Asians. We will deliver this through clinical follow-up of the cohort, supplemented by routine sources of information.

Clinical follow up of the men and women participating in the LOLIPOP study

All 30K participants in the LOLIPOP study will be re-invited for assessment over the five years between Q1 2008 and Q4 2012. Participants will be invited by post to attend an appointment at specially convened LOLIPOP research clinics, located at Ealing Hospital.

Invitations will start with those recruited earliest and work sequentially through the cohort. Up to three invitations will be sent by post, at monthly intervals. NHS number is recorded for all but 13 of the LOLIPOP participants and will be used for tracking. The local Primary Care Trusts provide regular updates of the addresses of participants still resident in the area, and 96% of participants are still registered with one of the collaborating LOLIPOP GPs. Persons who have moved out of area will be located through the NHS tracing service, and invited to attend the LOLIPOP follow-up clinic.

Consenting participants will attend a 45 minute appointment with a trained LOLIPOP research nurse. All participants will complete a structured focussed on history of possible cardiovascular events (non-fatal myocardial infarction, stroke) and cardiovascular procedures (coronary angiography, percutaneous or surgical revascularisation). Additional questions will be included on current drug treatment, medical history, alcohol and tobacco consumption, current health, somatic symptoms and cognitive function. Multilingual research facilitators fluent in the major Indian Asian languages (Punjabi, Hindi, Urdu) will be available if necessary. Physical assessment will include blood pressure, anthropometric measurements (height, weight, WHR), fat mass (bio-impedance), urinalysis, and a 12 lead ECG.

Fasting (8h hours) blood samples will be collected for biochemical analysis, including glucose, total and HDL cholesterol and triglycerides. This will enable reassessment of biochemical cardiovascular risk factors and new onset diabetes. 10mls of urine, **a single saliva sample** and an additional **60ml** of blood will be stored as aliquots of plasma (EDTA, heparin, and citrated), whole blood, for future assays including genetic markers. **In addition study participants will be asked to provide two separate stool samples, collected on separate days, using a sample swab kit to take home and return by post.** All samples will be labelled with a unique study identifier using a barcode. Clinical data will be stored in an encrypted, password protected central secure database held on an Ealing Hospital NHS Trust server.

Analysis of 12 lead ECGs to identify the presence of new pathological Q waves.

Follow-up ECGs will be analysed to identify new cases of myocardial infarction, including silent myocardial infarction, considered to be more common amongst Indian Asians than other populations as a result of the high prevalence of type 2 diabetes in Asians.

ECGs will be recorded using a Burdett Atria 6100 ECG machine, and transmitted electronically to the University of Glasgow ECG Core Lab for analysis (as a collaboration with Professor P. MacFarlane). All follow-up ECGs will be computer analysed using customised software, and coded using Minnesota criteria. Those reported as showing 'definite' or 'highly probable' pathological Q waves (codes 1-1, 1-2) will be reviewed by an experienced cardiologist. If the presence of pathological Q waves is confirmed, the baseline ECG will be re-analysed to ascertain that the Q wave abnormality at follow-up is new.

The Glasgow ECG Core Lab has substantial experience in undertaking ECG analysis for cardiovascular clinical and epidemiological studies (e.g. WOSCOPS, British Regional Heart Study, Whitehall II Study and Airwave Study).[22-24] The methodology used shows high reproducibility, a low false positive rate, and reaches international quality assurance standard ISO 9001.

Ascertainment of CVD events using routine and electronic data sources

To maximise the identification of CVD events, we will supplement clinical follow-up with extraction of information from routine and electronic resources, including the Office for National Statistics (ONS), primary care records, Hospital Episode Statistics (HES) and local cardiovascular databases. To improve sensitivity for CVD event ascertainment, we have selected a broad set of ICD10 diagnosis and OPCS4 procedure codes. To increase specificity, source data will be reviewed to validate possible CVD events identified.

Validation of CVD cases through review of source data

Possible CVD events identified through follow-up, death certification, routine and clinical databases will be validated against source data, including coroner's reports, hospital and primary care records where available. Events will initially be coded by the clinical research fellow, according to written, internationally accepted criteria. All events will then be independently reviewed by the events validation.

The primary endpoint is defined as a composite of fatal and non-fatal myocardial infarction, fatal and non-fatal stroke, and advanced coronary artery disease. Endpoint definitions are as follows:

- Fatal and non-fatal myocardial infarction

Myocardial infarction is defined as i. ischaemic cardiac symptoms combined with abnormal ECG and/or raised cardiac markers (WHO definition), or ii. the development of pathological Q waves on serial 12 lead ECG (Minnesota codes 1-1 and 1-2, in 2 adjacent leads).[25] For fatal events, acute myocardial infarction can be additionally diagnosed from pathological findings of recent myocardial infarction or the presence of intracoronary thrombus.

- Fatal and non-fatal stroke

Stroke is defined as: rapidly developing clinical signs of focal or global neurological disturbance, lasting 24 hours or longer or leading to death with no apparent cause other than of vascular origin. Where neuroimaging or post-mortem have been performed, appropriate findings of cerebral infarction, intracerebral or subarachnoid haemorrhage to support the diagnosis.

- Advanced coronary artery disease

Coronary revascularisation by percutaneous coronary intervention or coronary artery by-pass surgery, or angiographically confirmed coronary artery disease (>70% stenosis in major epicardial vessel).

Study power and statistical analysis

Assuming a start date of Q1 2008 for follow-up, with events censored at Dec 31st 2012, we estimate 150,000 person years of follow-up, (average of 5 years for each of 30,000 persons). Based on national data ([www.heartstats.org] and the HES database), and taking into account loss-to-follow-up, withdrawal and healthy cohort effect, we estimate ~660 fatal CVD events and ~1420 non-fatal events, giving a total of ~2080 events. This affords 95% power to detect with odds ratio for CVD of 1.4, 1.3 and 1.25 for biomarkers markers with 10%, 20% and 30% population prevalence respectively, $P<0.05$. These estimates are similar to the initial power calculations for the LOLIPOP study: the recent decline in population CVD event rate is offset by 20% increased sample size.

Descriptive data and individual biomarkers will be compared between Indian Asians and Europeans. Cox-regression analysis will be used to examine the associations of ethnicity with CVD events. Age and sex will be included as covariates in all models. Adjustment will then be made for the traditional cardiovascular risk factors, as well as for insulin resistance and related disturbances, to identify the extent to which measured risk factors account for the increased risk of CVD in Indian Asians compared to European whites.

The results will be used to derive a new model for CVD prediction amongst UK Indian Asians. Using cox-regression we will add CVD risk factors individually and then as a set, to identify a parsimonious risk prediction model for CVD, that gives "best fit" to the data. Discrimination will be examined using appropriate methodology including ROC curves, c statistics, and predictive values. Based on these analyses, the best performing model for CVD risk prediction in Indian Asians will be selected for dissemination into routine clinical care.

PEOPLE AND PROJECT MANAGEMENT

This proposal brings together an internationally recognised team of research leaders with the necessary multi-disciplinary skills, experience, and expertise to realise the objectives of this research. The Principal Investigator (Kooner) is a Professor of Clinical Cardiology at Imperial College London. Kooner leads a large research programme on cardiovascular health in Indian Asians, and is Director of the LOLIPOP programme. Kooner takes overall responsibility for the direction, implementation and delivery of the research. Chambers is a Senior Lecturer in Cardiovascular Epidemiology. He is a co-PI of the LOLIPOP cohort, with particular responsibility for overseeing data collection, maintenance of the study databases and data analysis.

FEASIBILITY

We provide a comprehensive clinical service (based at Ealing, St Mary's and the Hammersmith Hospitals) covering ~75% of cardiovascular admissions for the local population from which the LOLIPOP cohort was recruited. We have maintained close links with the participating primary care

physicians. This places us in an ideal position to ascertain locally occurring incident health events, both through the hospitals and through primary care. A LOLIPOP office is established at Ealing Hospital and will provide a working environment, and administrative base from which the study nurses will operate.

We have extensive experience in the design, conduct and analysis of large-scale population studies. Furthermore we have close collaborations with the Department of Epidemiology and Public Health at Imperial College London (where Chambers is Senior Lecturer). This brings access to additional expertise in cohorts (including EPIC, National study of the UK Police Force [Airwave], Finnish birth cohort 1966 and 1986, INTERMAP and INTERSALT), primary care data collection (including the MRC funded VOTES project), and Hospital Episodes Statistics data (held in conjunction with the Dr Foster Unit at Imperial College). Core funding for the project has been secured through an MRC grant, and additional funding is available through unrestricted grants from Industry.

EXPECTED VALUE OF RESULTS

This is the first well-powered prospective study investigating contemporaneous cardiovascular outcomes amongst Indian Asians and European whites. It provides a unique opportunity to identify risk factors which explain the excess risk of CVD in UK Indian Asians. Identification of these risk factors will enable development of key preventative measures, and treatments to reduce the burden of CVD in the population. The results of this study will help to inform changes in healthcare delivery locally, nationally and globally.

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APPENDIX 1: Additional investigation of eye diseases amongst Indian Asians and European whites.

Background

The first wave of recruitment for the LOLIPOP study[1] was completed in Q4 2008. To date, ~30K men and women of Indian Asian and European descent have been recruited from 58 GP practices in West London. Follow-up of LOLIPOP participants began in Q1 2009; this will provide important insights into the mechanisms underlying cardiovascular disease as well as other major medical disorders amongst persons of Indian Asian origin.

In the UK, refractive error, cataracts, glaucoma, and age-related macular disease are important causes of uncorrected visual disturbance.[2] These ophthalmic disorders are associated with considerable morbidity and contribute significantly to social and healthcare costs. However, available epidemiological data is derived primarily from studies comprising European white participants; little is known of the prevalence of these ocular diseases amongst persons of Indian Asian ancestry.

We therefore propose that the LOLIPOP follow-up study provides a unique opportunity to investigate causes of visual disturbance amongst persons of Indian Asian origin. Data generated from this proposal will help inform future strategies geared towards early detection and prevention of these disorders.

Methodology

Inclusion criteria

- Participant in the LOLIPOP study.
- Willing to undergo ophthalmological assessment, including pupil dilatation. Patients will be warned not to drive following pupil dilatation

Exclusion criteria

- Contraindication to pupil dilatation, e.g. untreated acute angle closure. This will be detectable by the ophthalmologist on initial examination.

A 30 minute ophthalmic assessment will be carried out by a trained ophthalmologist in a dedicated eye room equipped with state of the art instruments. The eye room will be located within the Cardiology Department at Ealing hospital where characterization of LOLIPOP participants currently takes place.

A full ophthalmic history will be taken from the participant. An interviewer-administered questionnaire will be used to collect data on current and past ophthalmic history, previous eye surgery, family history of ocular disease, and current medications.

Visual acuity will be assessed at 3 metres using standardised charts with best available refractive correction. In addition more objective measurements of visual acuity will be made using an autorefractor. A slit lamp examination of the front of the eye will be conducted and intraocular pressures will be measured in both eyes using a Goldmann tonometer.

Following this initial assessment, both pupils will be dilated using standard drops – 1% tropicamide, 2.5% phenylephrine. The participant will be asked to wait for 10 minutes to allow for adequate dilatation. Following this, the lens will be examined and biomicroscopy at the retina, optic nerve and macula will be conducted. Retinal photographs will also be taken using a standard diabetic retinopathy screening camera. On completion of the eye tests, participants will be advised not to drive for at least two hours to allow the effects of the eye drops to wear off.

Data handling

Collected data will be stored on the secure LOLIPOP database. The GP will be informed in writing that an eye examination has taken place, and major findings will be reported along with recommendations for future action.

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APPENDIX 2: Identification of genetic variation underlying diabetes, coronary heart disease and related disorders amongst Indian Asians.

Aim

We propose to carry out whole-genome sequencing of people of Indian Asian ancestry to discover the genetic architecture, haplotype structure and variants that are specific to Asians, and to identify genetic variants underlying increased risk of type-2 diabetes (T2D), coronary heart disease (CHD) and related metabolic disturbances in this population.

Background

Indian Asians are at increased risk of T2D, CHD and related metabolic disturbances. Family studies show that T2D, CHD and related metabolic disturbances are highly heritable in Indian Asians, indicating that genetic factors contribute to the increased risks of these disorders in Asians.

We have completed genome-wide association scans amongst Indian Asian participants of the LOLIPOP study to investigate genetic factors underlying T2D and CHD in Asians. The results reveal that there are major differences in SNP allele frequencies, and in haplotype structure between Indian Asians and Europeans. These findings indicate that the Indian and European populations have become genetically divergent, and raise the possibility of genetic variation that is specific to Indian Asians and which contributes to the diseases that are more common in this population. Strong support for Indian specific variation comes from the discovery of a common variant in *MYBPC3* that is closely (odds ratio 7.0) associated with cardiomyopathy and heart failure, and which is specific to populations from the Indian subcontinent.

Methods

Participants

We will invite Indian Asians from the LOLIPOP study to participate in the study. Participants will be self reported Indian Asian, have all 4 grandparents born on the Indian subcontinent, and will be selected to represent the major cultural groups and geographic regions of the Indian subcontinent.

Whole-genome sequencing

Sequencing will be carried out using established platforms such as Illumina GA2 or Roche 454. Each genome sequenced to an expected average >8-fold sequence coverage, followed by sequence alignment to reference genome. SNP and small InDel detection will be done with SAM (Sequence Alignment/Map) Tools. SNP identification will be strengthened by integrating results of base-calling and QC metrics across all the individuals sequenced, in particular to improve calling of heterozygous SNPs. Detection and annotation of large InDels, inversions, translocation, and other Copy Number Variants (CNVs) will be carried out using BreakDancer and equivalent tools. Haplotype reconstruction will be carried out using fastPHASE. Quality control measures will include comparisons to the results of whole-genome genotyping (Illumina 610 array), and by regenotyping a random sample of novel SNPs and InDels detected.

Data analysis

The analysis will focus on the identification of coding and regulatory SNPs and InDels that are common amongst Indian Asians, but that have not been previously reported, or which are rare in other populations. Based on sequencing of just 100 individuals, we would expect to identify 80% of variants with MAF 2%, and 99% of variants with MAF 5% or higher in Indian Asians.

The haplotype map generated through sequencing of Indian Asians in this research will then be used to impute genotypes amongst the LOLIPOP Indian Asian participants with GWA data. This will enable us to test the Indian Asian specific variants as genetic susceptibility factors underlying increased risk of T2D, CHD and related disorders in Asians. Genetic variants which are not amenable to imputation, for example because of low LD with GWA tag-SNPs, will be tested for association with phenotype by direct genotyping.

Data sharing

Genotype data will be made available to other researchers, including through publicly accessible scientific data-sharing internet sites, in a manner similar to that offered by the International HapMap project (www.Hapmap.org). All genetic data will be fully anonymised before sharing.

APPENDIX 3: Investigation of physical activity levels and their contribution to the increased risk of central obesity, insulin resistance and type-2 diabetes amongst Indian Asians.

Aim

The general aim of this study is to investigate the contribution of physical inactivity to the increased risk of central obesity, insulin resistance and Type 2 diabetes in Indian Asians compared to Europeans.

Background:

Indian Asians are at increased risk of central obesity, insulin resistance and type 2 diabetes (T2D). Increased risk of these disorders amongst Indian Asians is not explained by known dietary or genetic factors. Several studies have identified physical inactivity as an important risk factor for T2D and related metabolic abnormalities. Low Physical activity (PA) levels have been documented amongst Indian Asians, but the contribution of PA levels to obesity, insulin resistance and T2D amongst Indian Asians remains to be determined.

METHODS:

PARTICIPANTS: We will study 1000 Indian Asian and 1000 European men and women aged 35-75 years, selected from amongst the ~30,000 Indian Asian and European men and women taking part in the London Life Science Prospective Population (LOLIPOP) study.

DATA COLLECTION: Measurement of PA will be carried out as an additional phenotypic measure when LOLIPOP participants attend for follow-up assessment. PA will be measured using a validated accelerometer (GeneActiv) designed for quantification of PA in population studies. The geneactive device weighs just 16g, and is worn on the wrist. It is similar in size to a wrist watch. PA measurements will be carried out over a full 7 days, with participants asked to wear the device continuously (except when in water). PA will be quantified using the appropriate software. The GeneActiv monitor has a CE mark and is being used within its licensed indication.

DATA ANALYSIS: we will compare PA levels among Indian Asians and Europeans. We will examine the association of PA with central obesity, insulin resistance and T2D in the two populations. We will quantify the possible contribution of low PA to the increased risk of these disturbances amongst Indian Asians.

EXPECTED VALUE OF RESULTS: this research will quantify for the first time the possible contribution of PA to central obesity, insulin resistance and T2D amongst Indian Asians. Our findings may enable the development of new approaches to reduce the burden of these disorders through lifestyle modification.