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ORIGINAL ARTICLE

An overview of the Guangzhou Biobank Cohort Study–Cardiovascular Disease Subcohort (GBCS-CVD): a platform for multidisciplinary collaboration

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The Guangzhou Biobank Cohort Study (GBCS, $n = 30\,519$, age $\geqslant 50$ years) was established to examine the effects of genetic and environmental influences on health problems and chronic disease development. Guangzhou is undergoing massive economic development, but from a baseline that had remained unchanged for millennia. The Cardiovascular Disease Subcohort (GBCS-CVD) consists of 2000 participants who have been intensively phenotyped including a range of surrogate markers of vascular disease, including carotid artery intima-media thickness, cerebral artery stenoses, arterial stiffness, ankle-to-brachial blood pressure index and albuminuria, as well as coagulatory and inflammatory markers. Plasma and leukocytes are stored in liquid nitrogen for future studies. Preliminary demographic data show the female volunteers are younger than the male ones, but present with greater levels of adiposity including central obesity (31 vs 16%). Women had more body fat (33 vs 24%) and associated levels of adipokines. Despite this, body mass index and hip circumferences were similar, which contrasts with Caucasian populations. Men had more physician-diagnosed vascular disease (6.1 vs 2.5%), hypertension (42 vs 34%) and hyperglycaemia (36.6 vs 29.6%) than the women, but were less insulin resistant. In men, smoking (40 vs 2%) and drinking alcohol (67 vs 50%) was more common and they also had lower energy expenditures. The genotype distributions of the 15 typed single nucleotide polymorphisms were all in Hardy–Weinberg equilibrium. This article describes the rationale and methodology for the study. Given the comprehensive characterization of demographic and psychosocial determinants and biochemistry, the study provides a unique platform for multidisciplinary collaboration in a highly dynamic setting.

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Background

The Guangzhou Biobank Cohort Study

The Cohort was established to examine the effects of genetic and environmental influences on health problems and chronic disease development. The study is a collaborative project between the Guangzhou Occupational Disease Prevention and Treatment Centre based in the Guangzhou No. 12 People's Hospital and the Universities of Hong Kong and Birmingham, UK. An outline of this cohort has been published previously.[1] Recruitment of 30 000 older Chinese subjects aged over 50 years for the study began in September 2003 and was completed in early 2008. We are currently initiating the first follow-up of the subjects to collect data on morbidity and mortality, and are also recalling them to the hospital for a repeat of the measures taken 5 years earlier. However, the main cohort, in part due to financial limitations, had minimal endpoints for the development of testable hypotheses. The Cardiovascular Disease Subcohort was therefore developed to address this issue. The study enabled a thorough evaluation of the volunteers' current cardiovascular disease status through the measurement of a range of

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surrogate markers of vascular disease/risk. Additionally, we chose to further characterize potential risk factors such as diabetes and intermediary parameters including a range of adipokines, cytokines and procoagulatory markers.

Guangzhou and the population

Guangzhou, formerly known as Canton, has a metropolitan population of 9.8 million making it the third most populous centre in China, of whom 7.6 million are located within the city boundary[2] with 84% being permanent residents (Figure 1).[1] Guangzhou is the provincial capital of Guangdong Province which has a population of 83 million permanent residents and 30 million migrants.[2]

Guangdong, and in particular Guangzhou, is undergoing massive economic development. Most of the study population were born into a China that was essentially agrarian with a per capita GDP of a current equivalent of about US$400 in 1950 being essentially unchanged over the previous 2 millennia.[3] There have been several periods of major social turmoil with the Japanese occupation resulting in 20 million dead, and following the establishment of the People's Republic of China in 1949, the Great Leap Forward (1958–1962) and The Cultural Revolution (1966–1976) led to significant mortality and an entire generation without schooling.[4,5] However, a shift in economic policy in 1978 has resulted in the current economic transformation.

The permanent resident population is generally from Guangzhou or Guangdong and represent a homogeneous Cantonese group, which, despite the turmoil and extensive economic transition, have retained traditions and cultural norms. These include lifestyle factors such as the maintenance of traditional cuisine and minimal consumption of tobacco products and alcohol in women, and psychosocial factors such as traditional belief in the need for a diet that balances Yin (cold/dark) and Yang (hot/bright) foods and offsets such imbalance in the body or the health benefits of obesity, which in developing countries is advantageous to help overcome childhood infections, including diarrhoeal disease.[1,6] Such a legacy means this population is an ideal setting to assess, both cross-sectionally and longitudinally, the impact of rapid economic development on health determinants.

When prevalence rates vary or differential associations exist between different settings this provides the opportunity to reassess potential relationships, particularly those of a potentially controversial nature. This cohort can therefore help not only to assess determinants of disease within the Chinese setting, but also to provide supportive data in Western settings where a hypothesis may not be adequately assessed. We have already utilized this in the assessment of passive smoking on chronic obstructive pulmonary disease[6] and napping on diabetes and the metabolic syndrome. The low rate of smoking in women, yet high prevalence in men, enabled a much clearer picture of the contribution of passive smoking,[6] which can often be confounded by the residual effects of active smoking in Caucasian populations. Likewise, in China napping is a very common and widely accepted practice and not, as in many Western settings, a response to tiredness resulting from disease or ageing that might otherwise confound the observation.

Figure 1 Major urban centres in China, with populations exceeding approximately 10 million.

Ethnic differences in cardio- and cerebrovascular disease
Globally, there are marked ethnic differences in mortality rates resulting from cardio- and cerebrovascular disease. Although stroke mortality has decreased in Japanese and many Caucasian populations over the last 30 years,[7] rates are still high in some populations, particularly in the People's Republic of China. In Caucasian populations, deaths rates for coronary heart disease (CHD) are generally 2- to 3-fold greater than those for stroke, whereas the reverse is true in Chinese populations.[8] For instance, in the early 1990s in China age-adjusted mortality rates of 56 and 129 per 100 000 for CHD and stroke have been reported which contrast with those from US/UK Caucasians of 124/284 and 28/51 per 100 000, respectively.[8,9] The prevalence of CHD is lower in Chinese populations, and Chinese, in particular in the rural setting, may be less susceptible to vascular disease risk factors than Caucasian populations.[8–10] Similarly, the prevalence of peripheral vascular disease (PVD) is less common in Orientals relative to most Western populations, and there are few published studies of PVD in Oriental populations.[11–13]

Differences between populations in the type of stroke have been observed. We have been leading studies distinguishing differences in cerebrovascular disease between Chinese and Caucasian populations. Comparison of our data in Hong Kong from 777 incident stroke cases to those from five stroke registries from the West, found the incidence of intracerebral haemorrhage to be two to three times higher in Chinese, accounting for 20–30% of strokes.[14–16] Additionally, small subcortical or lacunar infarcts are common in Chinese and are often associated with intracranial large artery occlusive disease, in which up to 59% of patients present with lacunar syndrome.[14] In Caucasians, carotid stenosis is a major cause of cerebral ischaemia, which is infrequent in Asian, African or Hispanic populations where intracranial large vessel stenosis is the predominant vascular lesion.[14,17–19] Cerebrovascular lesions tended to be more diffuse (rather than discrete, as in Caucasians), with over 50% of patients having two or more lesions. Stenosis of the middle cerebral artery (MCA) was the most common lesion, found in 35.9% of the patients.[18] In a population-based study of 590 rural Chinese subjects, 7% of the participants over 40 years of age had evidence of intracranial stenosis.[17] In addition to symptomatic disease or family history, the major conventional risk factors for stenosis were glycosuria (odds ratio (OR) 3.00) and hypertension (OR 2.53).[17]

In addition to large differences in stroke rates between populations, there are inter-regional differences in rates in both Japan and the United States. Depending on the region studied, the difference in stroke rates can be up to 2.5-fold greater. A well-documented study examined changes in Japanese mortality rates following migration from Hiroshima, Japan to either Honolulu in Hawaii or the San Francisco Bay Area of the United States of America.[20] Rates of intracranial haemorrhage and thromboembolic stroke werethree3 times higher in Japan than in Honolulu, whereas CHD was lower in all the Japanese groups compared to equivalent Caucasian groups, but were highest in those living in the United States of America.[20] This study supports the importance of environmental factors in the aetiology of vascular disease, and shows that modification of lifestyle to remove detrimental risk factors may be an important approach to reduce morbidity and mortality.

This cohort provides an ideal opportunity to further cross-sectionally and longitudinally elucidate the parameters involved in the aetiology of vascular disease and its determinants. This will also allow us to identify potentially susceptible individuals before developing the associated morbidity and mortality in whom preventive and active treatment strategies can be implemented to prevent or delay the impact of these diseases.

Methodology

Population
In developed countries, physician registrations are often used as the sampling frame for studies similar to the present one. However, in developing countries, the infrastructure to facilitate such studies is not available. We therefore utilized a community social and welfare association 'The Guangzhou Health and Happiness Association for the Respectable Elders' that is aligned with the provincial government as a sampling frame. It is a large association with branches throughout Guangzhou and its membership is open to anyone for a nominal discretionary fee of 48 Yuan (US$6) per year. It has a citywide network with around 107 000 members of whom 95 000 are permanent residents, approximately 7% of the Guangzhou population of that age strata. The 1996 male (49.7%) and female subjects were randomly recruited from the association's membership list of eligible subjects during phase III of the main cohort. We stratified recruitment by gender to ensure a similar proportion of men and women in the group.

Overall about 5% of eligible subjects refused to participate, with fewer than 1% of women and about 10% of men refusing. The men generally refused because of a cultural unwillingness of Chinese men to give blood due to an associated loss of 'shung qi' or 'life energy', and because of job commitments. Most of the volunteers were keen to participate because they could receive free health examinations. However, we only included those who were ambulatory and not receiving treatment for serious life-threatening diseases, such as cancer. The study has received approval from the Guangzhou Medical Ethics Committee of the Chinese Medical Association, Guangzhou, China. All subjects gave written informed consent before participating in the study.

Overview of study measures

Physical examination
To maximize participation, safety and standardization, all subjects came to a specialized centre at the Guangzhou 12th Hospital, where full-time trained nurses and technicians carried out the examinations and interviews to characterize the volunteers. To prevent contamination, all subjects received a unique identifier, which was used to barcode all items including the questionnaires, blood tubes and cryovials. The 1996 volunteers received a full medical checkup including measurement of blood pressure, obesity indices (body mass index (BMI), waist and hip circumferences, percentage body fat),[1] lung function (forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC),[6] physical function measures (2.5 m get-up-and-go, left and right hand grip strength), chest X-ray and a range of vascular disease surrogate markers as described in Table 1.

Biochemical parameters
After an overnight fast, each subject was screened for a range of fasting biochemical parameters

Table 1 Summary of clinical measures collected at baseline

Variables	*Equipment used and method*
Anthropometry	
Standing height	Manufactured equipment
Sitting height	
Hip circumference	Standard tape measure. Measured around the maximal girth of the hips
Waist circumference	Standard tape measure. Measured horizontally around the smallest circumference between ribs and iliac crest, or at the navel, if no natural waistline
Weight	RGZ-120-RT; Wuxi Weighing Apparatus Factory, Wuxi, China
Percentage body fat	Tanita BF-350; Tanita, Tokyo, Japan
Blood pressure	
Resting blood pressure	Omron 705CP; Omron, Tokyo, Japan. Measured three times. Seated blood pressure, one minute apart, after a 3-min rest.
Vascular disease measures	
12-Lead electrocardiography	CAM14; GE Medical Systems, WI, USA
Cerebral artery transcranial Doppler	Explorer CVS; Diagnostic Medical Systems, Perois, France
Carotid intima-media thickness ultrasound	5–12-MHz high-resolution linear phased array ultrasound transducer, ATL HDI-3000 mainframe, Tokyo, Japan
Ankle-to-brachial systolic blood pressure ratio	Colin VP1000; Colin Medical Technology, Komaki, Japan
Pulse wave velocity	
Echocardiography	GEVivid7; GE Medical Systems ($n = 399$)
Respiratory function	
Forced expiratory volume in 1 sec (FEV1)	COSMED microQuark, Rome, Italy. As per American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force recommendations (No reversibility testing). Best 3 of up to 8 used.
Forced vital capacity (FVC)	
Chest X-ray	Toshiba KSO-15R, Tokyo, Japan
Physical function	
2.5 m get-up-and-go	Time taken for seated volunteer to stand, walk 2.5 m, turn around a point and return to the seat.
Grip strength	Left and right hand grip strength measured. J00105 JAMAR Hydraulic Hand Dynamometer; Lafayette Instruments, Lafayette, IN, USA
Fasting biochemistry	
Glucose	Shimadzu CL-8000 Clinical Chemistry Analyzer, Shimadzu, Kyoto, Japan
Lipid profile[a]	Shimadzu CL-8000 Clinical Chemistry Analyzer
Markers of liver[b], renal[c] and cardiac[d] function	Shimadzu CL-8000 Clinical Chemistry Analyzer
Glycosylated haemoglobin A1c (HbA1c)	Shimadzu CL-8000 Clinical Chemistry Analyzer
C-reactive protein	Orion Diagnostica, Espoo, Finland
Insulin	Mercodia AB, Uppsala, Sweden
Adiponectin	Human ELISA; BioVendor, Modrice, Czech Republic
Leptin	Human ELISA; BioVendor
Plasminogen activator inhibitor-1 (PAI-1)	Human ELISA; BioVendor
Interleukin-6 (IL-6)	Quantikine HS human immunoassay; R&D Systems, Minneapolis, MN, USA
Complete blood count[e]	SYSMEX K-21 Hematology Analyzer; Sysmex, Mundelein, IN, USA

[a]Total, LDL cholesterol and HDL cholesterol, triglycerides, apolipoprotein A1 and B.
[b]Alanine aminotransferase, aspartate transaminase.
[c]Total protein, albumin, globulin, total bilirubin, urea, creatinine, uric acid.
[d]Creatine kinase, lactate dehydrogenase, hydroxybutyrate dehydrogenase.
[e]White (and subtypes) and red blood cell counts, haemoglobin levels, and platelet counts OGTT = 75 g oral glucose tolerance test (2 h glucose $n = 1303$, 2 h insulin $n = 1146$). All procedures are performed once unless otherwise stated.

including the cardiovascular risk factors, lipids (total, LDL cholesterol, HDL cholesterol, triglycerides, ApoA1, ApoB), glycaemia (glucose, glycosylated haemoglobin A1c), renal (total protein, albumin, globulin, urea, creatinine, urate), liver function (aspartate aminotransferase, alanine aminotransferase, bilirubin, γ-glytamyl transpeptidase), cardiac enzymes (α-hydroxybutyrate dehydrogenase, alkaline phosphatase, creatine kinase, lactate dehydrogenase), fibrinogen, C-reactive protein and a detailed blood cell count. Spot urinary sodium, potassium, albumin and creatinine were also measured. Additionally a subgroup of 1303 also received a 75 g anhydrous oral glucose tolerance test (OGTT) with the measurement of 2 h glucose levels. Baseline and 2 h plasma insulin levels were measured in 1150 volunteers. A number of adipokines (leptin, adiponectin), additional cytokines (interleukin-6) and procoagulant factors (plasminogen activator inhibitor-1) were also measured in those receiving the OGTT using standardized methodologies as described in Table 1. Samples were taken after an overnight fast, and aliquots were stored in liquid nitrogen for later analyses.

Demographic and lifestyle parameters

A detailed questionnaire was applied by six full-time trained nurses who interviewed the participants face to face to assess exposure to air pollutants, occupational exposures, family and personal disease histories, cognitive function and lifestyle including dietary and physical activity habits (International Physical Activity Questionnaire (IPAQ);[21] Table 2). Validation of the questionnaire has been performed 6 months into recruitment by recalling 200 randomly selected subjects for reinterview.[1] Kappa values were self-reported vascular disease (0.66), smoking (0.96 and 0.88 for the two questions on smoking status), drinking (0.60), education (0.90) and occupation (0.80).

Genetic markers

DNA has been extracted and to date 15 single nucleotide polymorphisms (Table 3) have been genotyped using commercial Taqman allelic discrimination assay kits that utilize a 5′ nuclease assay with fluorogenic allele-specific TaqMan MBG probes with the ABI 7900HT real-time PCR system (Applied Biosystems, Foster City, CA, USA; Figure 2).[22] A multicomponent algorithm was used to calculate distinct allele signal contributions from fluorescence measurements for each sample well during the assay plate read with the ABI single nucleotide polymorphism (SNP) auto-caller which then automatically determines sample genotypes.

Plasma and live leukocytes are stored under liquid nitrogen for long-term preservation. The leukocytes will subsequently be immortalized to produce a long-term source of genetic material and cells for further investigations.

Table 2 Summary of questionnaire data collected at baseline

Exposure category	*Variable / exposure*
Demographics and socio-economics	Occupation Income Education Housing type Household size Health insurance coverage
Personal health behaviour	Smoking Alcohol Tea Afternoon nap Physical activity (at work and leisure) Diet (66-item semi quantitative food frequency questionnaire)
Environment	Occupational exposures Cooking and heating fuels Passive smoking exposure
Family history	Family history of coronary heart disease, diabetes, cancers, other chronic diseases Parental age, age of death and cause of death
Specific health questions/ disability/ impairment	Rose angina screening questionnaire MRC respiratory health questionnaire Self-reported general health Arthritis screening/activities of daily living (ADL) Dementia screening (delayed word recall test) Mini mental state examination Geriatric depression scale SF-12 Snoring/sleep disturbance Weight/waist changes Eyesight Hearing Dental health
Past medical history	Diagnosed medical conditions (specifically itemised) Use of health services Use of Chinese medicines Western and other medication use
Reproductive history	Age of menarche Parity and breastfeeding history Menopause status and associated timing Contraceptive history History of hormone-replacement therapy use Hysterectomy or other gynaecological interventions

Measurement of the cardio- and cerebrovascular disease surrogates

Transcranial doppler assessment of cerebral artery stenoses

All patients were examined by transcranial doppler (Explorer CVS, Diagnostic Medical Systems, Perois,

Table 3 Single nucleotide polymorphism (SNP) allele and genotype frequencies in 15 cardiovascular-related genes

Gene (other names; abbreviation)	*SNP rs no.*	*Chromosomal location*	*SNP Type*	*Alleles*	*Minor allele frequencies*				*Genotypes*	*Frequencies*
					Current	*Caucasian*	*Yoruba*†	*Chinese*		
Adiponectin (C1Q and collagen domain containing; ADIPOQ)	rs2241766	3q27.3a	Silent	T/G*	0.316	—	—	0.27	TT/GT/GG	0.462:0.444:0.094
Apolipoprotein A-V (zinc finger protein 259; APOA5)	rs662799	11q23.3c	Promoter	A/G*	0.293	0.017 (G)	0.133 (G)	0.267	AA/AG/GG	0.501:0.411:0.088
C-reactive protein (pentraxin-related; CRP)	rs1130864§	1q23.2b	3′ UTR	C/T*	0.043	0.31 (T)	0.07 (T)	0.03 (T)	CC/CT/TT	0.916:0.082:0.002
Dopamine receptor D2 (ankyrin repeat and kinase domain containing 1; DRD2)	rs1800497§	11q23.2a	3′ UTR	C/T*	0.397	0.23 (A)	0.45 (A)	0.48 (A)	CC/CT/TT	0.372:0.462:0.166
Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1)	rs1409181§	6q23.2a	Intron	C/G*	0.453	0.48 (G)	0.30 (G)	0.46	GG/CG/CC	0.215:0.477:0.308
Plasminogen activator inhibitor type 1 (serpin peptidase inhibitor, clade E (nexin), member 1; PAI1)	rs7242	7q22.1d	3′ UTR	G*/T	0.439	0.48 (G)	0.45 (T)	0.37 (G)	GG/GT/TT	0.190:0.499:0.311
Transforming growth factor, beta 1 (hypothetical protein MGC4093; TGFB1)	rs4803455	19q13.2c	Intron	C/A*	0.426	0.48 (A)	0.43 (C)	0.46 (A)	CC/CA/AA	0.341:0.466:0.193
Fibrinogen beta chain (FGB)	rs4220	4q31.3d	Mis-sense	A*/G	0.278	0.21 (A)	0.08 (A)	0.18 (A)	AA/AG/GG	0.081:0.396:0.523
E-selectin (endothelial adhesion molecule 1; SELE)	rs5368§	1q24.2c	Mis-sense	C/T*	0.221	0.08 (T)	0.04 (T)	0.32 (T)	CC/CT/TT	0.605:0.348:0.047
Hepatic lipase (LIPC)	rs6083	15q21.3d	Mis-sense	A*/G	0.213	0.42 (G)	0.36 (A)	0.28 (A)	AA/AG/GG	0.045:0.337:0.618
Interleukin-6 (interferon, beta 2; IL6)	rs1524107	7p15.3c	Intron	C*/T	0.218	0.04 (T)	0.07 (T)	0.25 (C)	CC/CT/TT	0.048:0.339:0.613
Interleukin-6 receptor (IL6R)	rs8192284/ rs2228145	1q21.3e	Intron	A/C*	0.344	0.35 (C)	0.04 (C)	0.43 (C)	AA/AC/CC	0.431:0.449:0.120
Lipoprotein lipase (LPL)	rs285	8p21.3c	Intron	C/T*	0.314	0.50 (T)	0.01 (C)	0.31 (T)	CC/CT/TT	0.483:0.407:0.110
Paraoxonase 1 (PON1)	rs662§	7q21.3b	Mis-sense	A*/G	0.346	0.36 (G)	0.22 (A)	0.43 (A)	AA/AG/GG	0.116:0.459:0.425
Peroxisome proliferative activated receptor, gamma (PPARG)	rs1797912	3p25.2b	Intron	C*/A	0.447	0.40 (C)	0.08 (C)	0.41 (C)	CC/CA/AA	0.198:0.497:0.305

All SNPs were in Hardy–Weinberg equilibrium; *minor allele; †Yoruba, Black Nigerian; §detected using reverse chain by TAQMAN genotyping kits; Allele frequencies from the ethnic groups are from the HapMap study.

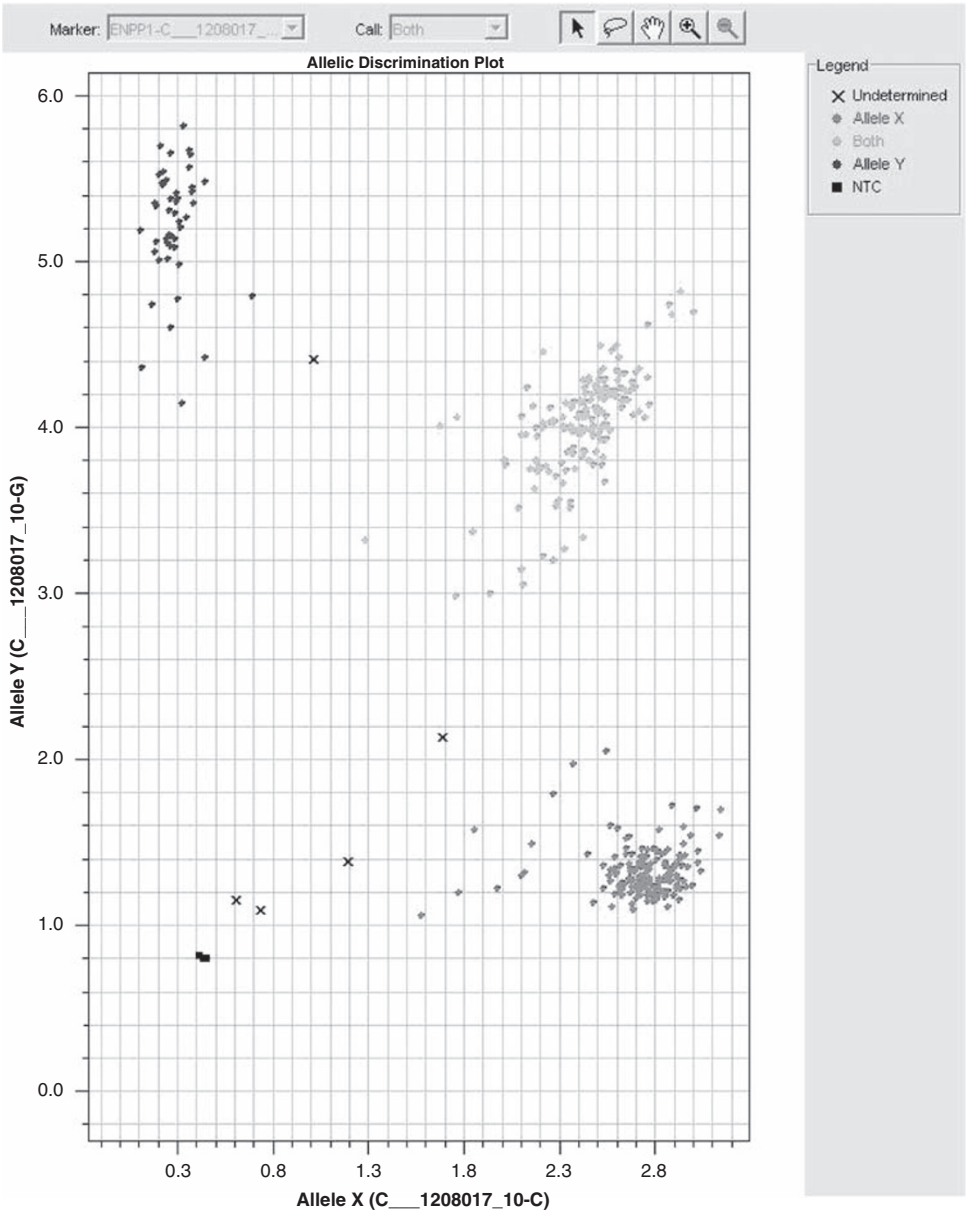

Figure 2 Example of an allelic discrimination plot for the ectonucleotide pyrophosphatase/ phosphodiesterase 1 (ENPP1) rs1409181 polymorphism.

France). The intracranial large arteries were assessed through the temporal, occipital and orbital windows. Briefly, we examined the following arteries with 4 cm increments: MCA (temporal window, 52–64 mm), anterior cerebral artery (ACA, temporal window, 68–72 mm), posterior cerebral artery (PCA, temporal window, 56–64 mm), intracranial internal carotid artery (orbital window, 60–68 mm) and vertebrobasilar artery (VA, occipital window, 56–106 mm).[18,23–25] Patients were classified as having occlusive disease if at least one of the studied arteries showed evidence of stenosis or occlusion. The criteria for occlusive arteries were defined by the peak systolic flow velocity as follows: $\geqslant 140\,cm\,s^{-1}$ for the MCA, $\geqslant 120\,cm\,s^{-1}$ for the ACA, $\geqslant 100\,cm\,s^{-1}$ for the PCA and VA, and $\geqslant 120\,cm\,s^{-1}$ for the siphon internal carotid artery and extracranial carotid artery. Apart from the above velocity criteria, we took account of age, presence of turbulence or musical sound and whether the abnormal velocity was segmental. Cerebral arteries that could not be insonated because of poor temporal acoustic windows are regarded as non-occlusive.[18,23–25]

Intima-media thickness measurement

B-mode ultrasound examinations were made using an ATL HDI-3000 mainframe with a high-resolution, linear phased array ultrasound transducer (frequency 5–12 MHz).[10,26] All ultrasound systems

used similar gain and depth settings, as previously described. All scans were performed by operators following a predetermined, standardized scanning protocol pressure for the right and left carotid arteries using images of the far wall of the distal 10 mm of the common carotid arteries.[10,26] Three scanning angles were used, with the image focused on the posterior wall, and recorded from the angle showing the greatest distance between the lumen-intima interface and the media-adventitia interface. All scans were analysed by the same investigator, blinded to subject identity. The ultrasound assessment of atheroma has been previously validated.[10]

Pulse wave velocity and ankle-to-brachial blood pressure index

Ankle-to-brachial blood pressure index (ABI) and pulse wave velocity (baPWV) were measured using a volume-plethysmographic apparatus with an automatic waveform analyser (Colin VP1000; Colin Medical Technology, Komaki, Japan, Figure 3). Measurements were taken with patients lying in a supine position after 5 min of rest. Occlusion and monitoring cuffs were placed around both sites of the lower legs of patients. Pressure waveforms of the brachial and tibial arteries were then recorded simultaneously using semiconductor plethysmographic and oscillometric pressure sensors.

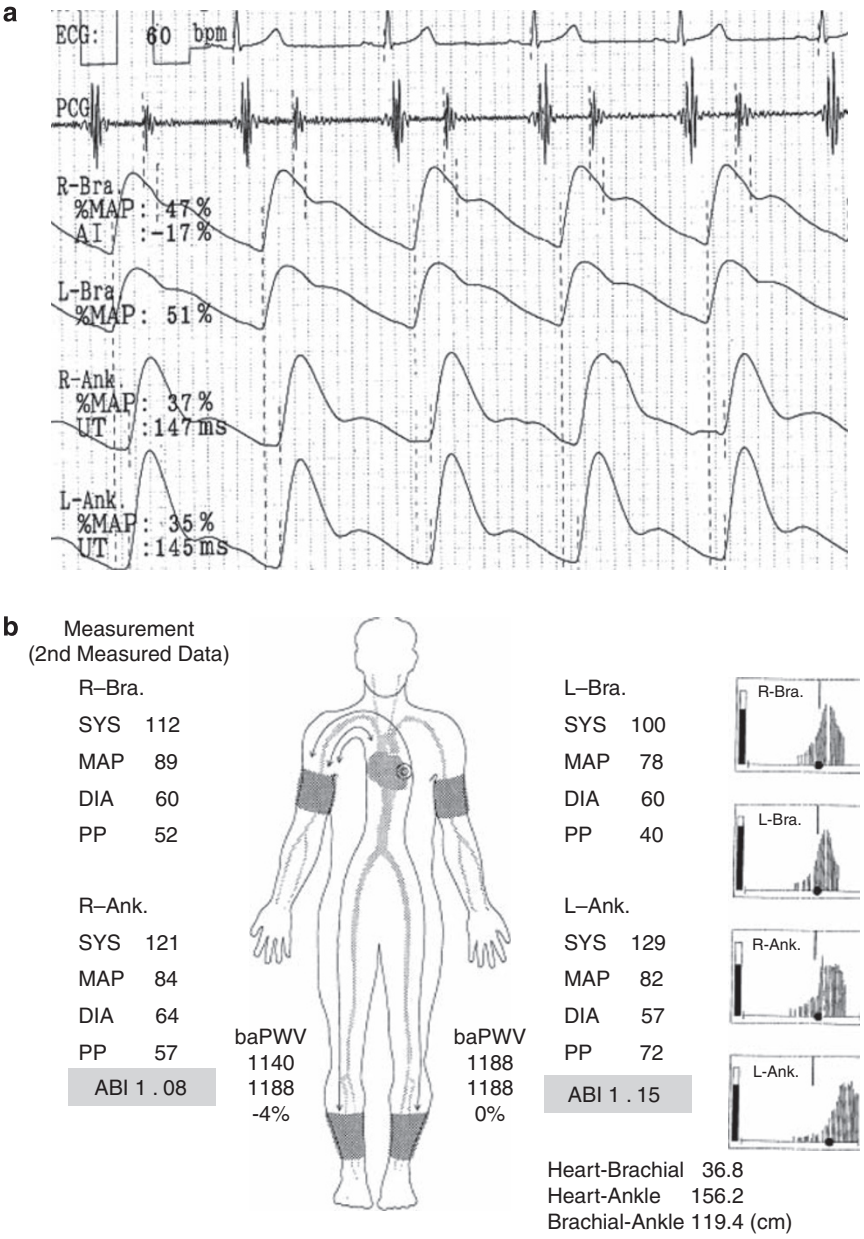

Figure 3 (**a**) Mechanocardiogram and pulse volume of a healthy male volunteer; (**b**) limb blood pressure, ankle-to-brachial systolic blood pressure index and pulse wave velocity output from the Colin VP1000.

These waveforms as observed in Figure 3 allow the determination of the time interval between the initial rise in the brachial and tibial pressure waveforms (T). The path length from the suprasternal notch to the elbow (L_a) and also from the suprasternal notch to the ankle (L_b) were automatically statistically determined from the patient's height. baPWV was calculated using the formula $baPWV = (L_b - L_a)/T$. Measurements of the right and left baPWV were obtained for an average of 10 s. The method validity has been reported previously, with an interobserver coefficient of variation of 8.4% and intraobserver coefficient of variation of 10.0%.[27] The average of the left and right baPWV will be used for subsequent analyses. The ABI was calculated using the formula, ABI = ankle systolic BP/brachial systolic BP. The lowest values of the left and right sides will be used in the analyses. The output of the systolic, diastolic, mean arterial and pulse pressures are recorded for each limb and the PWV and ABI described (Figure 3).

Ocular examination

All ophthalmological examinations were performed by four ophthalmologists and a registered nurse, specialized in ocular complications of older patients. Visual acuity was assessed using an 'E-type' international standard visual acuity chart for the illiterate. Most participants were unable to use of western characters. The distance between the chart and volunteer was 5 m and standard lighting conditions. If the examinee wore corrective lenses, an aided visual acuity was also measured.

External eye examination, assessment of pupillary reaction and anterior segment examination were inspected with a slit lamp bimicroscope (YZ5EII Photo Slit Lamp Microscope, Suzhou Medical Instrument Factory, China). The anterior chamber depth was assessed to identify glaucoma. Lens opacity was described based on types (cortical, nuclear or subcapsular) and stages of cataract progression (incipient, intumescent, mature or hypermature), rather than using the Lens Opacities Classification System III (LOCS III) as the pupils were not dilated.

Vitreous and fundus were checked by direct ophthalmoscopy (YZ6EI ophthalmoscope, Suzhou Medical Instrument Factory, China). The colour and rim of the optic disk, ratio of optic cup and the disk, central light reflex of the fovea and retinal vessel were described to support the diagnosis of both hypertensive and diabetic retinopathy.

Intraocular pressure (IOP) was measured using Schiotz tonometry (YZ7A Tonometer; Suzhou Medical Instrument Factory, China). Two drops of 0.5% dicarine solution as a local anaesthetic were applied before the IOP measurement.

Proposed study follow-up

Integrating the GBCS-CVD within the main GBCS enables the subjects to be followed up indefinitely for health service utilization and disease-specific mortality utilizing the study infrastructure on an ongoing basis. We are currently aiming to recall the subjects every 5 years as per the main cohort.[1] Certain disease groups including those with diabetes will be recalled for more intensive phenotyping and monitoring of disease progression. A pilot follow-up study 1 year after initial recruitment of the first 1100 subjects achieved reattendance of 72.2% and telephone interview with a further 22.4%. Information on the remaining subjects was sought from family members, close friends and the elderly association, after which only 0.4% remained unaccounted for.[1]

Observations

The database is currently being cleaned, but preliminary demographic data can be found in Table 4. On average, the female volunteers were almost 6 years younger than the male ones. However, the women presented with greater levels of adiposity, with almost twice as much central obesity, although the men, as expected, had larger waist circumferences by 5.3 cm. Women also had a greater proportion of body fat and associated levels of adipokines. Despite this, BMI was similar between the male and female subjects, as has been reported in other Chinese populations.[28] Similarly, there was also no difference in hip circumferences between the male and female subjects, which again contrasts with Caucasian populations. There was a high prevalence of hypertension in both sexes with one-third of the women having this condition, and over 40% of the men. Both groups had similar glycaemia indices, but the women were generally more insulin resistant, as suggested by having higher insulin levels in both the fasting and postprandial states. Apart from LDL-cholesterol levels, which were higher in the women, the other lipid parameters were generally worse in the men. The men also had more associated vascular disease risk factor levels than the women, which were associated with the higher prevalence of physician-diagnosed vascular disease events. For the lifestyle parameters, large differences were seen between the genders. In men, smoking and drinking alcohol was much more common, and also they had lower physical activity-related energy expenditures.

The genotype distribution of the 15 typed SNPs were all in Hardy–Weinberg equilibrium (Table 3), although 2, E-selectin and fibrinogen β-chain, differed significantly from previously reported HapMap frequencies in Chinese (fibrinogen minor allele frequency 0.278 vs 0.178, $P = 0.028$; E-selectin 0.221 vs 0.322, $P = 0.024$). However, the Chinese subjects recruited for the HapMap study were of northern Chinese origin which may contribute to the observed differences.[29]

Table 4 Summary demographics of the 1996 older Chinese subjects

Parameters	*Females* (n = *1004*)	*Males* (n = *992*)	P-*value*
Age (years)	56.7 ± 5.7 (range 50–81)	62.2 ± 7.0 (range 50–96)	<0.001
Body mass index (kg m^{-2})	23.8 ± 3.0	23.7 ± 3.0	NS
Waist circumference (cm)	75.9 ± 8.2	81.2 ± 8.9	<0.001
Hip circumference (cm)	90.2 ± 5.9	89.2 ± 5.9	NS
Percentage body fat (%)	33.0 ± 7.4	23.5 ± 6.8	<0.001
Systolic blood pressure (mm Hg)	124 ± 21	130 ± 20	<0.001
Diastolic blood pressure (mm Hg)	72.4 ± 10.7	75.7 ± 10.6	<0.001
Glucose (mmol l^{-1})	5.54 ± 1.50	5.62 ± 1.37	NS
2 h OGTT glucose (mmol l^{-1})	7.9 ± 3.1	7.8 ± 3.1	NS
Insulin (μU ml^{-1})	4.8 (4.2–5.4)	3.4 (3.0–4.0)	0.001
2 h OGTT insulin (μU ml^{-1})	26.7 (24.6–29.0)	22.7 (20.9–24.8)	0.008
Insulin glucose product	25.4 (22.3–28.8)	18.5 (16.0–21.4)	0.002
Glycosylated haemoglobin A1c (%)	6.0 ± 0.9	6.0 ± 0.9	NS
Total cholesterol (mmol l^{-1})	6.1 ± 1.1	5.6 ± 1.0	<0.001
LDL-cholesterol (mmol l^{-1})	3.51 ± 0.70	3.23 ± 0.62	<0.001
ApoB (mmol l^{-1})	1.09 ± 0.28	1.00 ± 0.24	<0.001
HDL-cholesterol (mmol l^{-1})	1.72 ± 0.40	1.45 ± 0.36	<0.001
ApoA1 (mmol l^{-1})	1.55 ± 0.31	1.38 ± 0.29	<0.001
Triglycerides (mmol l^{-1})	1.48 (1.43–1.52)	1.60 (1.55–1.66)	0.001
Fibrinogen (mg per 100 ml)	300 ± 77	301 ± 90	NS
Plasminogen activator inhibitor-1 (ng ml^{-1})	117 (111–123)	135 (127–144)	<0.001
C-reactive protein (mg l^{-1})	1.43 (1.34–1.52)	1.46 (1.36–1.56)	NS
Interleukin-6 (ng ml^{-1})	10.2 (9.7–10.8)	11.7 (11.2–12.3)	<0.001
Leptin (ng ml^{-1})	12.2 (11.6–12.8)	3.9 (3.7–4.1)	<0.001
Adiponectin (ng ml^{-1})	6864 (6346–7425)	5168 (4772–5596)	<0.001
Hypertension (%)	34.2	41.6	0.001
IFG/Diabetes (%)	25.0/4.6	30.6/6.0	0.001
Generally overweight/obese (%)	26.5/30.6	27.1/32.1	NS
Centrally obese (%)	30.9	16.3	<0.001
AHA metabolic syndrome (%)	21.6	23.8	NS
Vascular disease (%)	2.5	6.1	<0.001
Physical activity (METS)	3317 (3099–3551)	2666 (2480–2866)	<0.001
Alcohol (ever, %)	49.7	66.5	<0.001
Smoker (current, %)	2.4	40.2	<0.001

Hypertension, S/DBP 140/90 or Rx; IFG/Diabetes, FBS 5.6–<7.0/⩾7.0 or Rx, vascular disease, self-reported MI, stroke, PVD, CHD, angina. AHA, American Heart Association;[32] General overweight/obesity, BMI ⩾23/25 kg m^{-2}; Central obesity, ⩾80 or 90 cm waist circumferences in females and males, respectively using the AHA guidelines for the metabolic syndrome.[32]

Strengths and weaknesses

This cohort with detailed baseline characterization was designed to investigate the impact and aetiology of vascular diseases in a population undergoing rapid economic transition. The strengths and weaknesses of the cohort have been described in detail previously.[1] Briefly, the study participants are permanent residents of Guangzhou and members of a well-organized association. They were recruited through a well-equipped general hospital, which efficiently facilitates follow-up at low cost with a high response rate. Standardized and mostly automated collection of detailed baseline and follow-up data in a single centre by a well-trained team maximizes reliability and validity. Furthermore, the higher rate of incident events in this older cohort means pertinent data can be expected within a relatively short period of time.

There are also a number of limitations to the study. Our subjects are unlikely to be completely representative of the older population of Guangzhou although they are an unselected sample of the elderly association. However, we have previously shown the cohort has similar levels of diabetes and hypertension to nationally representative samples of urban Chinese.[30,31] There remains the chance of a 'healthy volunteer bias' in those who joined the association and also given our exclusion of those with serious existing disease. This could result in the potential for a type of 'reverse Berkson's bias', particularly in nested case–control studies, where the prevalence of exposure to a health risk would expectedly be artefactually lower in our sample than the general population, possibly leading to an overestimation of effect size. In common with all cohorts in developing countries, our subjects are with age increasingly survivors. However, if survivorship were an issue, we would expect different relationships in the older compared with the younger subjects, which we will check for routinely, although in the main cohort we have yet to encounter this issue.

Further information
For further information regarding the GBCS-CVD or for specific proposals for collaboration please contact the corresponding author and co-PI Dr GN Thomas (gneilthomas@yahoo.co.uk) as the first point of contact.

What is known about this topic:
- China is a rapidly developing country that a few decades ago had almost no obesity or diabetes, the prevalences of which are now increasing dramatically.
- Vascular disease presentation has been traditionally the result of hypertensive stroke, but how these changes will modify the presence of atherosclerotic disease is unknown.

What this Study adds:
- This study provides a well-characterised group of volunteers for the investigation of determinants of the surrogate markers of vascular disease and subsequently assessing their relationship with events.
- This study provides a platform for potential collaborative work investigating a wide-range of aspects pertaining to vascular disease in this older population.

Conflict of interest

The authors declare no conflict of interest.

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