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# The UK Adult Twin Registry (TwinsUK Resource)

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## Abstract

TwinsUK is a nation-wide registry of volunteer twins in the UK, with about 12,000 registered twins (83% female, equal number of monozygotic and dizygotic twins, predominantly middle-aged and older). Over the last 20 years, questionnaire and blood/urine/tissue samples have been collected on over 7,000 subjects, as well as three comprehensive phenotyping assessments in the clinical facilities of the Department of Twin Research and Genetic Epidemiology, King's College London. The primary focus of study has been the genetic basis of healthy ageing process and complex diseases including cardiovascular, metabolic, musculoskeletal, and ophthalmologic disorders. Alongside the detailed clinical, biochemical, behavioural, and socio-economic characterisation of the study population, the major strength of TwinsUK is availability of several 'omics' technologies for the participants. These include genome-wide scans of single nucleotide variants, next-generation sequencing, exome sequencing, epigenetic markers (MeDIP sequencing), gene expression arrays and RNA sequencing, telomere length measures, metabolomic profiles, and gut flora microbiomics. The scientific community now can freely access parts of the phenotype data from the 'TwinsUK Resource' and interested researchers are encouraged to contact us via our website (www.twinsuk.ac.uk) for future collaborations.

The UK Adult Twin Registry (TwinsUK) is a cohort of volunteer adult twins from all over the United Kingdom. The Department of Twin Research and Genetic Epidemiology at St. Thomas' Hospital, King's College London hosts the registry, which started in 1992 via media campaigns targeted at middle-aged women. The success of early studies led to rapid evolution of the registry and it now incorporates twins, both male and female, from other sources such as the Aberdeen Twin Registry and Institute of Psychiatry Adult Registry. The primary focus of study has been the genetic basis of complex diseases (cardiovascular, metabolic, musculoskeletal, and ophthalmologic diseases), which has broadened to include the complex healthy ageing process. The third health check of the volunteer twins has provided longitudinal data that, alongside with the state-of-the-art 'omics' technologies data, can significantly advance the field of genetic and clinical epidemiology of ageing. We have previously described the design of the original twin registry, facilities and procedures for data collection, clinical and biological assessments, and main findings in 2006 (Spector & Williams, 2006). The current paper presents a brief update on the study procedures and recent technological applications in our cohort. More detailed description of phenotypes, research projects and collaborations, papers and study findings can be accessed through our updated study website (http://www.twinsuk.ac.uk).

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## The collection

The TwinsUK registry now consists of about 12,000 monozygotic (MZ) and dizygotic (DZ) twins aged 18 to 103 years (Table 1). About 83% of the registry is female (mean age of 55 years). The registry now contains 51% MZ and 49% DZ twins. Between 1992 and 2004, twins were invited for a full comprehensive visit and several project-led studies. More than 7,000 twins responded to some of the annual questionnaires and 5,725 attended a comprehensive visit. Apart from a life-long lower weight in MZ twins of around 1kg, all other age-matched characteristics of these volunteer twins were found not to differ from a singleton population-based cohort of British women (Chingford study) (Andrew et al., 2001). Between April 2004 and May 2007, all the 6,740 active twins on the registry were invited for a 1-day clinical visit, of whom 3,725 twins attended and 1,299 twins posted their blood DNA samples via their GPs. The age of participants ranged between 18 and 82 years (mean 52.5 ± 13 years) and 3,299 of the clinic attendants (89%) were female.

The second follow-up visit, also known as the HATS (Healthy Ageing Twin Study) visit, started in August 2007. Only women aged ≥40 years with at least one previous clinical visit (n=4,610) were invited for this visit. In total, 3,125 women (mean age 59.6 ± 9 years, 48% MZ twins) attended the clinic (response rate = 68%). Follow-up time between first and last visits ranged between 6.1 and 17.4 years (mean 11.2 ± 2 years). Six hundred of the participants in this visit had 4 or more previous clinical visits. Compared to the baseline and first follow-up visits, participants in the HATS visit appeared to have higher socio-economic status, lower self-rated health status, and be more health aware given their level of alcohol intake and smoking. Comparison of the respondents and non-respondents to the HATS invitation showed that the non-respondents were generally younger and of lower socio-economic status. However, no significant clinical differences were observed between attendants and non-attendants to the HATS visit (Moayyeri et al., 2012b), suggesting that the participants in this visit can be considered as representative of the original population in the study. Zygosity status was assessed for all twins at the time of registration by a ‘peas in a pod’ questionnaire and confirmed via subsequent genotyping or genome-wide association studies.

## Longitudinal data

Longitudinal data is available for a wide range of phenotypes based on different questionnaires, clinical tests, and biochemical assays performed at different stages of the study (Moayyeri et al., 2012b). Table 2 summarises some of these clinical measures showing the breadth of the available data and the potential for studies on various aspects of healthy ageing in this population. For instance, longitudinal changes in bone mineral density in more than 4,000 twins over an average of 17.5 years serves as a valuable endpoint for clinical and genetic epidemiological studies (Moayyeri et al., 2012a). During the study follow-up, care has been taken to perform key clinical tests with similar protocols across all visits. Incident clinical endpoints (e.g. cardiovascular events, stroke, fractures, osteoarthritis, and different cancers) have been assessed over the course of study using questionnaires. Twins are all now registered using their National Health Services (NHS) numbers with the Office for National Statistics (ONS) of England for retrospective analysis and future follow-up regarding their cancer and mortality status.

## Novel molecular and genetic phenotypes

Alongside with the conventional epidemiological phenotypes assessed by questionnaires and clinical visits, the TwinsUK registry benefits from generous and continued donation of biological samples by its volunteering participants. The methods for collection of these biological samples have been described previously and the updated figures are presented in

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Table 1. Recently, a wide array of the latest ‘omics’ advances has been applied to subsections of these samples that makes TwinsUK one of the most uniquely phenotyped and deeply genotyped populations in the world. Here we describe some of these advances.

## Genome-wide association studies

TwinsUK has contributed to many international consortia for genome-wide association analysis of various phenotypes. Genome-wide scan data using two chips (Illumina HumanHap300 BeadChip and Illumina HumanHap610 QuadChip) is available for 5,710 twins. The data has been fully imputed using ‘HapMap II’ and ‘1000 Genomes’ reference panels (containing ~2.5 and ~ 16 million single nucleotide polymorphisms, respectively). TwinsUK is a member of many ongoing international consortia for meta-analysis of various traits (such as GIANT, CHARGE, ENGAGE, GEFOS, SUNLIGHT, MolPAGE, VisiGEN, TreatOA, and SpiroMeta). Some of the main publications from these consortia are listed in the bottom of this paper and the full list can be viewed in the TwinsUK website.

## Next-Generation Sequencing

The UK10K study is an ongoing collaboration between TwinsUK study, the Wellcome Trust Sanger Institute and several other collaborators for using the state-of-the-art next-generation sequencing methods to uncover rare genetic variants associated with health and disease. The study involves whole-genome sequencing of 4,000 healthy people with well-documented physical characteristics (2,000 twins from TwinsUK and 2,000 children from ALSPAC study) and whole-exome (protein-coding regions of DNA) sequencing of 6,000 people with extreme health problems (obesity, neurological problems, and rare diseases). At present, all twin samples have been sequenced (6× depth) and both phenotype and sequence data will be publically available soon. More details about the study can be accessed at: www.uk10k.org. Moreover, about 1000 exome sequences at 30-60× depth have been performed for twin participants as part of projects with Pfizer and the GoT2D consortium. Over 2,000 Exome chips are currently being performed mainly for control purposes in other consortia.

## Epigenetic Markers

The first epigenetic assessment in TwinsUK was performed on DNA methylation patterns using Illumina HumanMethylation27 BeadChip in a sample of 172 female twins. This array examines 27,578 promoter CpG-sites that map uniquely across the genome and some of these sites were found to be associated with age and age-related phenotypes (Bell et al., 2012). Currently, the Infinium HumanMethylation450 BeadChip (Illumina) is being applied to 500 additional MZ and DZ twin pairs to generate higher-resolution genome-wide DNA methylation profiles. This array includes 485,764 cytosine positions (CpG dinucleotides and CNG sites) across the human genome. Meanwhile, the major ongoing epigenetic project using the TwinsUK population is the EpiTwin study (http://www.epitwin.eu), which uses MeDIP (Methylated DNA immunoprecipitation) sequencing in whole blood samples (Bell & Spector, 2011). This is the largest epigenetic project of its kind, in collaboration with the Beijing Genomics Institute (BGI), aiming to assay epigenomic differences in 5,000 adult UK twins aged 16–85 years, discordant and concordant for a wide variety of diseases and environments. Next-generation sequencing has the potential to prove powerful in detecting disease-related methylation differences at a high level of resolution in a sample of this size. The initial targets of the study include obesity, diabetes, allergy, heart disease, osteoporosis, depression and longevity, but the method can be applied to every common trait or disease.

## Gene expression measures

Eight hundred fifty six twins with detailed clinical profiles have been biopsied during the HATS clinical visit. This has been done in the context of the MuTHER (Multiple Tissue

Human Expression Resource) project, which is a Wellcome Trust funded study designed to understand the mechanisms involved in common trait susceptibility via gene expression across multiple tissues (Nica et al., 2011). Gene expression in three tissues of skin, fat and lymphoblastoid cell lines (LCL) have been measured using Illumina's whole genome expression array (HumanHT-12 version 3) containing 48,803 probes in three technical replicates. Results for expression quantitative trait loci (eQTL) analysis in 856 twins are freely available in the website (http://www.muther.ac.uk) and the main paper has recently been published (Grundberg et al, Nature Genetics, in press). All of these tissues are now being RNA sequenced as part of the EuroBATS project (Biomarkers of Ageing using whole Transcriptome Sequencing), which is a 3-year European (EU-FP7) project started in January 2011. EuroBATS aims to discover novel biomarkers of ageing by incorporating novel RNA sequencing, telomere measurement and bioinformatics techniques (http://www.eurobats.eu). Sequencing is being performed using Illumina GAIIx platform and the standard protocol of RNAseq (expecting 10-20 million reads per sample).

## Telomere length

Telomere length, as a marker of cellular senescence and subsequent cell death, was firstly measured in 3,256 twins with available genome-wide scans. These measures were derived from the mean of the terminal restriction fragment length by using the Southern blot method on DNA extracted from peripheral leukocytes. This data has contributed to detection of several genes implicated to affect biological age (Codd et al., 2010; Mangino et al., 2009). Recently, a larger sample (4,899 twins aged 16-99 years) has been assessed for telomere length using an established and validated quantitative polymerase chain reaction (qPCR) technique. Quality control has now been finalised and the data is available for collaborations.

## Metabolomic Profiles

In 2009, fasting serum concentrations of 163 metabolites were measured for 1,270 twins using electrospray ionization tandem mass spectrometry (Biocrates AbsoluteIDQ technology). This targeted panel of metabolites covers a wide range of known lipids, amino acids, sugars, acylcarnitines and phospholipids. This data has been used in several outstanding studies (Zhai et al., 2010). More recently, a larger sample of 6,055 twins has been assessed using a new method of non-targeted metabolomic analysis. This new platform (Metabolon Inc., Durham, USA) incorporates two separate ultra-high performance liquid chromatography/tandem mass spectrometry injections (optimised for basic and acidic species) and one gas chromatography/mass spectrometry (GC/MS) injection per sample. The platform has detected and quantified concentration of 510 small molecules (299 known and 211 unknown molecules) including amino-acids, lipids, carbohydrates, vitamins, nucleotides, peptides, xenobiotics and steroids. Genome-wide association studies of ~37,000 traits from 60 biochemical pathways in a sub-sample of this population has identified several genes involved in metabolic individuality in humans and promises significant advances in future functional studies (Suhre et al., 2011).

## Microbiomics

We have recently started a collaborative NIH-funded study with Cornell University, aiming to collect gut flora DNA for analysis with 16S sequencing technology in 5,000 twins. In addition, pilot data assaying microbiome diversity from other human body sites, such as skin, oral and nasal cavities, as well as gut flora metagenomics are currently underway.

## Future directions and collaborations

Frequent data collection with detailed clinical, biochemical, behavioural, and socio-economic characterisation of participants for about 2 decades provides the opportunity to look at the prospective single-measure and repeated-measure associations for complex diseases and domains of healthy ageing in TwinsUK population. This also offers a unique opportunity to explore personalised medicine. Data collection, database management, biological sample storage, and statistical quality control have been carried out to a high standard. Blood, urine, and DNA sample aliquots from all visits are available for future measurements. We currently use online questionnaires and are actively engaging with our twin participants via using email and social networking websites. Our 'Volunteer Advisory Panel' helps informed decisions about the ethics, practicalities and appropriateness of potential studies.

The TwinsUK registry has a history of numerous successful scientific collaborations, and we remain committed to providing the scientific community with access to the phenotype data from the 'TwinsUK Resource'. A recent Biomedical Resource Grant from the Wellcome Trust is continuing to fund the core functions of TwinsUK. This will enable access to our publicly-funded data from the wider scientific community (the 'resource'), which will be separate from the individual research projects performed by academics within the Department of Twin Research at KCL. The TwinsUK Resource is opening up access of data, currently harmonizing and standardizing phenotypic data collected over the last 20 years; a subset of the data is already available for full open access via our website (http://www.twinsuk.ac.uk/data-access) and a search engine for the available phenotypes has been provided. We have an access committee which meets weekly and reviews about 20 requests a month (www.twinsuk.ac.uk/data-access/submission-procedure). Researchers are encouraged to find out if TwinsUK resource can help them answer their research questions and get in contact for future collaborations.

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## Table 1

### The UK Adult Twin Registry Update

| | |
|---|---|
| Name of register | UK Adult Twin Registry (TwinsUK) |
| Country | United Kingdom |
| Kind of ascertainment | Volunteers unselected |
| Opposite-sex twins (yes or no) | Yes |
| Number of pairs (separated by birth range and sex) | 1900–1920: 22 FF; 2 MM; 2 FM<br>1920-1930: 390 FF; 70 MM; 4 FM<br>1930–1940: 1,518 FF; 189 MM; 32 FM<br>1940–1950: 2,480 FF; 308 MM; 58 FM<br>1950–1960: 2,172 FF; 429 MM; 88 FM<br>1960–1970: 1,889 FF; 486 MM; 62 FM<br>1970–1980: 1,356 FF; 394 MM; 42 FM<br>1980–2000: 558 FF; 124 MM; 40 FM |
| Grand total | 10393 FF; 2002 MM; 328 FM (6369 pairs) |
| Major interests | Common complex diseases and ageing traits |
| Traits measured | Full questionnaires and clinical examinations on majority of twins for wide range of over 1,000 clinical and biochemical traits including: cardiovascular diseases, obesity, metabolic syndrome, respiratory diseases, dermatology, osteoarthritis, osteoporosis, eye diseases, back diseases, coagulation system, immune function, cognitive function, gastro-intestinal system, pain thresholds, allergy, atopy, sexuality, pitch perception, and various aspects of personality. |
| DNA samples | 13,458 aliquots from 7548 twins<br>9,321 aliquots from 5,965 twins stored as back up<br>DNA samples from 995 parents and 1,227 siblings taken and stored |
| Other samples | 119511 blood samples (65,980 serum, 43,527 plasma EDTA, 10004 plasma Li heparin) from 7,681 twins (16,677 back up samples)<br>28,276 urine samples from 7681 twins (16,677 back up samples)<br>Range of 2 -76 aliquots of various specimens at multiple time points |
| Comments | Monozygotic: Dizygotic ratio is approximately 1:1<br>Majority of twins are female with mean age of 55 years<br>5,710 twins with genome-wide association data<br>5,000 twins with DNA methylation data by the end of 2012<br>2,000 twins with next-generation sequencing data by the end of 2012<br>Data available for transcriptome across multiple tissues, telomere length, and metabolomic profile in different sub-samples |
| Main sources of funding | Wellcome Trust, UK Medical Research Council (MRC), British Heart Foundation, NIHR Biomedical Research Centre, Pfizer, Chronic Disease Research Foundation, and the European Union framework programmes |
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FF = female–female; MM = male–male; FM = female–male

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## Table 2
### Longitudinal data available in the TwinsUK registry participants

| Phenotype | N of measurements | N of participants | N of participants with ≥ 2 measurements | Maximum number of visits | Duration of follow-up (yr) | Maximum duration of follow-up (yr) |
|---|---|---|---|---|---|---|
| **Blood Pressure** | 13180 | 7189 | 3836 | 5 | 7.3 ± 3.3 | 13.7 |
| **Lipid Profile** | 12652 | 6881 | 3585 | 7 | 8.5 ± 3.9 | 16.4 |
| **Electrocardiography** | 8083 | 5533 | 1864 | 5 | 8.0 ± 3.1 | 12.6 |
| **Fasting Glucose** | 16305 | 7731 | 4344 | 11 | 8.4 ± 3.6 | 16.8 |
| **Blood Insulin** | 13650 | 6953 | 3810 | 11 | 8.4 ± 3.6 | 16.8 |
| **Respiratory function** | 12245 | 7128 | 3326 | 5 | 8.7 ± 3.5 | 16.2 |
| **Hip BMD** | 15367 | 7025 | 4098 | 12 | 8.5 ± 3.8 | 17.5 |
| **Spine BMD** | 15489 | 7046 | 4115 | 13 | 8.5 ± 3.8 | 17.5 |
| **Whole body DXA** | 13850 | 7292 | 3882 | 7 | 8.5 ± 3.7 | 17.5 |
| **Heel QUS** | 8299 | 5419 | 1753 | 7 | 3.4 ± 1.1 | 7.7 |
| **Grip Strength** | 6861 | 4840 | 1999 | 3 | 3.1 ± 0.7 | 5.5 |
| **Sexual Hormones** | 5779 | 5350 | 428 | 3 | 5.3 ± 3.0 | 10.3 |

BMD: bone mineral density, DXA: dual-energy X-ray absorptiometry, QUS: quantitative ultrasound