Twin Research and Human Genetics

**Aging Trajectories in Different Body Systems Share Common Environmental Etiology: The Healthy Ageing Twin Study (HATS)**

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**Supplementary Methods**

***Study Population***

The TwinsUK study has enrolled 12,000 volunteer twins for assessment of the genetic and environmental aetiology of age-related complex traits (Moayyeri et al., 2013a). The cohort, started in 1992 through national media campaigns, has extensive demographic, physiological, behavioural and lifestyle data available and is one of the most deeply phenotyped and genotyped cohorts in the world. The majority of participating twins are female since the study originally recruited only middle-aged women. The primary focus of study has been the genetic basis of healthy ageing process and complex diseases including cardiovascular, metabolic, musculoskeletal, and ophthalmologic disorders. All registered twins have been invited for 1-day clinical follow-up visits on an approximately four yearly basis, in 1998–2003, and again 2004–2007. Over 3,000 twins participated in the third major follow-up of the study in 2007–2010, also known as the Healthy Ageing Twin Study (HATS), which has focused on the age-related longitudinal phenotypes associated with ‘healthy ageing’ (Moayyeri et al., 2013b). Another wave of visits is now underway. The study was approved by St. Thomas’ Hospital Research Ethics Committee, and all twins provided informed written consent. For this study, all female twins with at least 2 clinical visits were included. Male twins were excluded due to low numbers (<5%).

***Aging Systems***

We investigated longitudinal changes in 5 body systems. These included cardiovascular, respiratory, skeletal, morphometric, and metabolic systems (*Supplementary* *Table 1*). For the cardiovascular system, we included systolic and diastolic blood pressure, serum lipid profile, electrocardiography measures, and six serum cardiovascular biomarkers (emerging risk factors). Blood pressure was measured using Omron Mx3 Digital Blood Pressure Monitors. Three measurements were taken and the mean of second and third measures was used. People treated for hypertension were excluded from this analysis. Respiratory function was assessed in each visit using the Vitalograph 2150 volumetric spirometer (Vitalograph Ltd, Buckingham, UK). Morphometric measures including total body fat mass, lean mass and total body bone mineral content as well as skeletal measures of bone mineral density (BMD) at hip and spine regions were assessed using dual-energy X-ray absorptiometry (Hologic Discovery W, Hologic, Bedford, MA, USA) (Moayyeri et al., 2013b). Grip strength of the dominant hand was measured (to the nearest kilogram) using a Jamar hand dynamometer (Sammons Preston Inc., Bolingbrook, IL). Participants were advised to exert maximal force and one test trial was allowed, followed by three test measurements. The best measure recorded was taken for the final analysis. We included grip strength in the morphometric system since it is recommended as a reliable surrogate for muscle function (Cruz-Jentoft et al., 2010). Heel quantitative ultrasound measurements, broadband ultrasound attenuation (BUA) and velocity of sound (VOS), were measured at the left heel using a McCue Cuba clinical scanner (McCue Ultrasonics, Hampshire, UK). For the metabolic system, we included serum urea levels and estimated glomerular filtration rate (as markers of renal function) and serum dehydroepiandrosterone sulphate (a known biomarker of ageing; Kroboth et al., 1999).

***Statistical Analysis***

Database management and statistical modelling for estimation of longitudinal ‘ageing trajectories’ were performed using Stata software, version 13 (StataCorp LP., College Station, TX, USA). Results of a simpler model (based on estimation of change in variables per year) are presented in *Supplementary Table 6*.

***Structural Equation Modelling (SEM)***

We used MX open-source software (Neale & Cardon, 1992) to separate the observed variance in ‘ageing trajectories’ into their genetic and environmental components. This approach is based on comparing the covariances of measured traits between monozygotic (MZ) and dizygotic (DZ) twin pairs. The observed phenotypic variance can be divided into additive genetic (A), common environmental (C), and unique environmental (E) components. The common environmental component (C) estimates the contribution of family environment, which is assumed to be equal in both MZ and DZ twin pairs (Derks et al., 2006; Kendler et al., 1993; Scarr, 1968), whereas the unique environmental component (E) estimates the effects that apply only to each individual including measurement error. Any greater similarity between MZ twins than DZ twins is attributed to greater sharing of genetic influences. Heritability is defined as the proportion of the phenotypic variation attributable to genetic factors, and is given by the equation, h2 = (A)/(A + C + E). After running univariate models for all body systems, and in order to investigate whether the same genetic and environmental factors influence ageing in different body systems, we used a multivariate Cholesky decomposition model with correlated factors solution (Gillespie & Martin, 2005). In multivariate twin analysis, MZ and DZ correlations are compared across traits. If cross-trait twin correlations are greater for MZ than for DZ twins, this implies that genetic factors contribute to the covariation across traits. Correlated factor solution of the Cholesky model is mathematically equivalent to the original Cholesky model but avoids giving precedence to any latent variable over others (Gillespie & Martin, 2005). In order to avoid inflation of estimates of C due to shared age of twins, we entered the ‘ageing trajectories’, adjusted using the residual approach for chronological age into the models.

We also fitted a common latent factor model which hypothesised a single common factor underpinning slopes of biological age predicted by all five organ systems (*Supplementary Figure 3*). Akaike Information Criteria were used to compare the Cholesky model and the common factor solution according to Burnham and Anderson (Burnham & Anderson, 2002).

**Supplementary Table 1**: Details of clinical measures and their weights used for estimation of ageing trajectories in 5 body systems.

|  |  |  |
| --- | --- | --- |
| **Body system** | **Measure** | **Weight** |
| Cardiovascular | * Blood pressure:   + Systolic   + Diastolic * Lipid profile:   + Total cholesterol   + High-density lipoprotein (HDL)   + Low-density lipoprotein (LDL)   + Triglyceride * ECG measures: * PR interval * QRS axis * QT interval * Serum risk factors: * Adiponectin * Homocysteine * Fibrinogen * Leptin * Apolipoprotein A-I * Apolipoprotein B | * 1   + 0.5\*   + 0.5\* * 1   + 0.25\*   + 0.25\*   + 0.25\*   + 0.25\* * 1   + 0.33\*   + 0.33\*   + 0.33\* * 1   + 0.16\*   + 0.16\*   + 0.16\*   + 0.16\*   + 0.16\*   + 0.16\* |
| Respiratory | * Forced expiratory volume in 1 sec (FEV1) * Forced vital capacity (FVC) * FEV1/FVC ratio | * 1 * 1 * 1 |
| Morphometric | * Total body fat mass * Total body lean mass * Total body bone mineral content * Grip strength | * 1 * 1 * 1 * 1 |
| Skeletal | * Hip bone mineral density (BMD):   + Femoral neck   + Total hip * Lumbar spine BMD * Heel quantitative ultrasound measures:   + Broadband ultrasound attenuation (BUA)   + Velocity of sound (VOS) | * 1 * 0.5\* * 0.5\* * 1 * 1 * 0.5\* * 0.5\* |
| Metabolic | * Serum urea levels * Dehydroepiandrosterone sulphate (DHEAS) * Estimated glomerular filtration rate (eGFR) | * 1 * 1 * 1 |

Note: \* For individuals with missing data, scalars for each components was 1/*n*, where *n* = number of non-missing components.

**Supplementary Table 2**: Correlation between ageing trajectories in 5 body systems for shared and unique environmental latent factors. The top-right side of the matrix depicts the correlation coefficients in shared environmental factors (C) and the bottom-left side shows the correlation coefficients for unique environmental factors (E).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  | ***Shared environmental factors (C)*** | | |
|  | **Cardiovascular** | **Respiratory** | **Morphometric** | **Skeletal** | **Metabolic** |
| **Cardiovascular** |  | 0.57  (0.28, 0.92) | 0.02  (-0.12, 0.14) | 0.12  (-0.10, 0.27) | -0.24  (-0.40, -0.11) |
| **Respiratory** | 0.43  (0.35, 0.50) |  | 0.32  (-0.02, 0.74) | 0.58  (0.18, 0.94) | 0.0  (-0.39, 0.43) |
| **Morphometric** | 0.27  (0.20, 0.33) | 0.18  (0.09, 0.25) |  | 0.39  (0.24, 0.53) | 0.13  (-0.01, 0.27) |
| **Skeletal** | 0.57  (0.52, 0.62) | 0.43  (0.35, 0.50) | 0.41  (0.35, 0.46) |  | 0.11  (-0.07, 0.28) |
| **Metabolic** | 0.12  (0.04, 0.20) | 0.11  (0.03, 0.20) | 0.07  (-0.01, 0.14) | 0.12  (0.04, 0.19) |  |
|  | ***Unique environmental factors (E)*** | | |  |  |

**Supplementary Table 3:** Estimated variance components from the ‘Common Factor Model’ This model had 14909 degrees of freedom; -2 Log-Likelihood of data -1862.760; Akaike’s Information Criterion -31680. ΔAIC compared to Cholesky model = 146 indicating that this model is very unlikely.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Genetic** | | | **Shared Environment** | | | **Unique Environment** | | |  |
|  | **General** | **Specific** | **Total** | **General** | **Specific** | **Total** | **General** | **Specific** | **Total** | **Total variance**  **accounted for** |
| **Cardiovascular** | 0.03 | 0.04 | 0.07 | 0.07 | 0.51 | 0.57 | 0.17 | 0.19 | 0.35 | 1.00 |
| **Respiratory** | 0.03 | 0.04 | 0.07 | 0.07 | 0.06 | 0.13 | 0.18 | 0.62 | 0.80 | 1.00 |
| **Morphometric** | 0.02 | 0.02 | 0.04 | 0.05 | 0.49 | 0.53 | 0.11 | 0.31 | 0.42 | 1.00 |
| **Skeletal** | 0.07 | 0.00 | 0.07 | 0.16 | 0.19 | 0.35 | 0.40 | 0.18 | 0.58 | 1.00 |
| **Metabolic** | 0.00 | 0.00 | 0.00 | 0.00 | 0.54 | 0.55 | 0.01 | 0.44 | 0.45 | 1.00 |

**Supplementary Table 4**: Summary measures of chronological and predicted age based on various clinical measures, domains or body systems and derived ‘ageing trajectories’ (slope of change) in monozygotic (MZ) and dizygotic (DZ) twins. All the values are mean (standard deviation). The columns on correlation and covariance show the pairwise Pearson correlations (rho values) and covariance for ‘ageing trajectories’ between twin pairs from the same family (MZ or DZ).



Note: ageing trajectories are the main final outcomes from this table used for structural equation modelling. Pairwise correlations and covariance are reported for illustrative purposes. Ageing trajectories (or slopes of change) should be estimated separately for each row. Comparing the difference between baseline and last chronological age and the difference between baseline and last predicted age are indicative, but slopes are estimated for each participant separately and using, in some cases, multiple visits. The averaging across measures and domains has been done for the *predicted age values*, therefore, ageing trajectories for a domain or system are not necessarily average of the slopes for different measures in that domain/system.

**SupplementaryTable 5**: Distribution of missing values in various body systems among study participants. Nearly 3,250 of our participants contributed to 3 or more ageing trajectory measures in various body systems. The Table indicates that there are no significant trends or differences between participants with different number of missing values.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Number of body systems contributed by participants | | | | |  |
|  | 1 | 2 | 3 | 4 | 5 | Total |
| N | 93 | 170 | 380 | 958 | 1,907 | 3,508 |
| Age at baseline | 50.4 (13.3) | 52.5 (11.3) | 51.9 (10.5) | 48.8 (10.8) | 47.9 (9.9) | 48.9 (10.5) |
| Duration of follow-up | 5.9 (2) | 6.7 (2.6) | 7.3 (2.9) | 9.8 (3) | 11.1 (2.2) | 10 (3) |
| BMI at baseline | 25.9 (5.1) | 25.8 (5.3) | 25.6 (4.6) | 24.7 (4.3) | 24.9 (4.3) | 25 (4.4) |
| Average systolic blood pressure | 121.2 (14.5) | 124.4 (15.6) | 125.5 (13.4) | 125 (14.5) | 124.4 (14.2) | 124.6 (14.3) |
| Average Total cholesterol | 5.5 (1.2) | 5.8 (1.3) | 5.6 (1.1) | 5.5 (1) | 5.6 (0.9) | 5.6 (1) |
| Average FEV1 | 2.6 (0.5) | 2.4 (0.6) | 2.5 (0.6) | 2.6 (0.6) | 2.6 (0.5) | 2.6 (0.6) |
| Average femoral neck BMD | 759.7 (133.9) | 763.8 (131.5) | 776.9 (116.3) | 780.4 (119.5) | 791.6 (121.5) | 784.9 (121.5) |
| Average DHEAS | 4.1 (3.1) | 3.4 (2) | 3.3 (2) | 3.5 (2.3) | 3.1 (2) | 3.2 (2.1) |

**Supplementary Figure 1**: Age trajectories (or slope of change in predicted biological age) illustrated for a single exemplar participant. The first and last clinical visits of this participant were in ages 50 and 60 years, respectively. Cardiovascular system shows a higher than expected increase in predicted age (from 50 years in first visit to 68 years in last visit), which indicates fast-ageing. However, respiratory system shows a small change in predicted biological age (range between 51 and 54 years) indicating slow-ageing. Morphometric system shows a normal ageing (predicted age between 52 and 62 years).



**Supplementary Figure 2**: Relative contribution of genetic, shared environmental and unique environmental factors to variance in ageing trajectories in 5 body systems



**Supplementary Figure 3**: Structure and estimated variance components resulting from the ‘Common Factor Model’. Level I denotes the general genetic (A), shared environmental (C), and unique environmental (E) influences on the common ageing factor. Level II denotes the factor structure of 5 ageing domains combined into a single factor; the path coefficients represent factor loadings. Level III denotes the specific genetic (a), shared environmental (c), and unique environmental (e) influences on the individual ageing domains. Detailed estimates are shown in *Supplementary Table 3.*



**References**

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* (2nd ed.). New York: Springer-Verlag.

Cruz-Jentoft, A. J., Baeyens, J. P., Bauer, J. M., Boirie, Y., Cederholm, T., Landi, F., … Zamboni, M. (2010). Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing*, *39*, 412-423.

Derks, E. M., Dolan, C. V., & Boomsma, D. I. (2006). A test of the equal environment assumption (EEA) in multivariate twin studies. *Twin Research and Human Genetics*, *9*, 403-411.

Gillespie, N. A., & Martin, N. G. (2005). Multivariate genetic analysis*.* In B. S. Everitt & D. C. Howell (Eds.), *Encyclopedia of statistics in behavioral science* (pp. 1363–1370)*.* Hoboken, NJ: John Wiley & Sons.

Kendler, K. S., Neale, M. C., Kessler, R. C., Heath, A. C., & Eaves, L. J. (1993). A test of the equal-environment assumption in twin studies of psychiatric illness. *Behavior Genetics*, *23*, 21-27.

Kroboth, P. D., Salek, F. S., Pittenger, A. L., Fabian, T. J., & Frye, R. F. (1999). DHEA and DHEA-S: a review. *Journal of Clinical Pharmacology*, *39*, 327-348.

Moayyeri, A., Hammond, C. J., Hart, D. J., & Spector, T. D. (2013a). The UK Adult Twin Registry (TwinsUK Resource). *Twin Research and Human Genetics*, *16*, 144-149.

Moayyeri, A., Hammond, C. J., Valdes, A. M., & Spector, T. D. (2013b). Cohort profile: TwinsUK and healthy ageing twin study. *International Journal of Epidemiology*, *42*, 76-85.

Neale, M. & Cardon, L. (1992). *Methodology for genetic studies of twins and families*. Dordrecht: Kluwer Academic Publishers.

Scarr, S. (1968). Environmental bias in twin studies. *Eugenics Quarterley*, *15*, 34-40.