Telomere Length in Circulating Leukocytes Is Associated with Lung Function and Disease

Online Data Supplement

Eva Albrecht, Elina Sillanpää, Stefan Karrasch, Alexessander Couto Alves, Veryan Codd, Iiris Hovatta, Jessica L Buxton, Christopher P Nelson, Linda Broer, Sara Hägg, Massimo Mangino, Gonneke Willemsen, Ida Surakka, Manuel AR Ferreira, Najaf Amin, Ben A Oostra, Heli M Bäckmand, Markku Peltonen, Seppo Sarna, Taina Rantanen, Sarianna Sipilä, Tellervo Korhonen, Pamela AF Madden, Christian Gieger, Rudolf A Jörres, Joachim Heinrich, Jürgen Behr, Rudolf M Huber, Annette Peters, Konstantin Strauch, H.-Erich Wichmann, Melanie Waldenberger, Alexandra IF Blakemore, Eco JC de Geus, Dale R Nyholt, Anjali K Henders, Päivi L Piirilä, Aila Rissanen, Patrik KE Magnusson, Ana Viñuela, Kirsi H Pietiläinen, Nicholas G Martin, Nancy L Pedersen, Dorret I Boomsma, Tim D Spector, Cornelia M van Duijn, Jaakko Kaprio, Nilesh J Samani, Marjo-Riitta Jarvelin, and Holger Schulz

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ERF

The Erasmus Rucphen Family (ERF) study is a cross-sectional cohort including 3,000 living descendants of 22 couples who had at least 6 children baptized in the community church around 1850-1900. The participants are not selected on any disease or other outcome. Details about the genealogy of the population have been described elsewhere [1, 2]. The study protocol was approved by the medical ethics board of the Erasmus MC Rotterdam, the Netherlands. Asthma was defined by medication intake considering combinations of beta agonists, leucotriene antagonits, and inhaled steroids. The following ACT codes were used, R03AC* and R03DC* or R03AK61/71/72 or R03AK61/71/72 and R03DC* or R03AC* and R03BA* or R03AC* and R03BA* and R03DC*. Individuals were taken as controls if they were free of R03* medication.

EX-ATHLETES

Former elite Finnish male athletes who had represented Finland in international events from 1920 through 1965 and the controls who were classified healthy at the age of 20 years participated in this population-based cohort study [3]. The initial data collection was carried out in 1978-1979 and cohort members were sent questionnaires in 1985, 1995 and 2001. The original cohort of study athletes included 2448 men and 1712 controls [3].

A large epidemiological and clinical research study was carried out in the year 2008, using the same protocols as the FINRISK 2007 [4]. The FINRISK study by the National Institute for Health and Welfare is a large and detailed health, clinical and lifestyle study done every five years on a population sample of Finnish adults. The study protocol for the FINRISK surveys included a postal questionnaire on health behaviour (such as smoking, use of alcohol and physical exercise) and other health related data, and a personal health examination including physical measurements and blood sampling. Details of the FINRISK 2007 survey have been published earlier [4].

The survey in 2008 were offered to 747 former elite athletes and 436 control subjects (N = 1183). 665 (56.2%) men participated in the health survey and/or filled in the questionnaires, of which 425 (63.9%) were former athletes and 240 (36.1%) were control subjects. Due to health reasons, some could not participate in the health survey and filled only in the questionnaire (N = 33 former athletes and N = 33 control subjects). The study subjects

comprised of participants who both participated in the health survey and filled in the questionnaire (N = 599). Among those who participated in the health survey, there were a total of 392 athletes and 207 control subjects, of which endurance athletes were 64 (10.7%), strength athletes 107 (17.9%), team athletes 221 (36.8%), and 207 control subjects (34.6%). Doctor diagnosed/treated asthma and COPD (chronic bronchitis and emphysema) were obtained by questionnaire. Telomere length was determined from peripheral blood DNA. DNA was extracted from venous blood samples taken at local health centre laboratories or other survey sites by specially trained nurses and shipped to the National Institute for Public Health (currently National Institute for Health and Welfare). Some missing data in the phenotypes and/or DNA samples yielded the final sample used in the analysis.

FITSA

The Finnish Twin Study on Aging (FITSA) was set up to investigate the genetic and environmental effects on the disablement process in older women [5, 6]. The participants were recruited from the Finnish Twin Cohort, which comprises all the same-sex twin pairs born before 1958 and with both co-twins alive in 1975 [7, 8]. In August 2000, there were 1260 female twin pairs in the age group of 63–76 years who had participated in the Finnish Twin Cohort in 1975. In this group an invitation to participate in the FITSA study was sent on the basis of age and zygosity to a subsample of 414 twin pairs aged 63–76 years. Inclusion criteria were willingness to participate and the ability to travel to the laboratory. 103 monozygotic and 114 dizygotic twin pairs (434 individuals) participated to the laboratory measurements. Spirometry measurements were performed according to international guidelines (American Thoracic Society, 1995) using an electronic spirometer (Spiro 2000, Medikro Oy). Reasons for non-attendance in spirometry (n = 25) were lack of time, physician's recommendation, and communication problems. In addition, 10 subjects provided incomplete spirometry performances. Telomere length was determined from peripheral blood DNA as described below.

GRAPHIC

The GRAPHIC Study comprises individuals from 520 white nuclear families of European descent recruited from the general population in Leicestershire UK, for the purpose of investigating the genetic determinants of blood pressure and related cardiovascular traits. Families were included if both parents aged 40-60 years and two offspring ≥18 years wished

to participate. Families were recruited through participating family practitioners in Leicestershire, UK, between 2003 and 2005. Further details are provided elsewhere [9].

KORA

The KORA studies (Cooperative Health Research in the Region of Augsburg) is a series of independent population based studies from the general population living in the region of Augsburg, southern Germany [10, 11]. Here, data of KORA F3, KORA F4 and KORA-Age was used. The KORA F3 study (2004/05) is a follow-up study to KORA S3 (1994/95), including 3,184 individuals. The KORA F4 study (2006/08) is a follow-up study to KORA S4 (1999/2001), including 3,080 individuals. KORA-Age is follow up study of all four KORA surveys S1-S4 and was conducted in 2008/09, only including participants born until 1943 [12]. Telomere length of all three studies was measured by qPCR in Leicester as described below.

In the KORA S3/F3 survey, spirometry was measured in 1997/98 for all participants younger than 60 years, who did not smoke or use inhalers one hour before the test. All spirometric tests were performed strictly adhering to the ECRHS protocol [13]. Tests were accounted valid if at least two technically satisfactory manoeuvres could be obtained throughout a maximum of nine trials. FEV_1 and FVC were defined as the maximum value within all valid maneuvers. COPD was defined as $z(FEV_1/FVC) < -1.6445$. Doctor diagnosed asthma was obtained by questionnaire in the KORA F3 survey.

In KORA F4, lung function tests were performed in random subsample of subjects born between 1946 and 1965 (age range 41 – 63 years). Spirometry was performed in line with the ATS/ERS recommendations [14] using a pneumotachograph-type spirometer (Masterscreen PC, CardinalHealth, Würzburg, Germany) before and after inhalation of 200 µg salbutamol. The present study is based on maximum values of FEV_1 and FVC measured before bronchodilation. The spirometer was calibrated daily using a calibration pump (CardinalHealth, Würzburg, Germany), and additionally, an internal control was used to ensure constant instrumental conditions. The presence of acute or chronic respiratory diseases as well as medication was assessed by a standardized questionnaire. COPD was defined as $z(FEV_1/FVC) < -1.6445$ for individuals with available spirometry or by questionnaire if no spirometry was available. Doctor diagnosed asthma was obtained by questionnaire.

In KORA Age, 935 randomly selected subjects, aged 65–90 years underwent spirometry in 2009. Measurement conditions including the examiner were the same as in KORA F4 except that inhalation of salbutamol was not performed due to the high number of contraindications

anticipated in this aged population. Acute and chronic respiratory diseases, medication and doctor diagnosed asthma were obtained by standardized questionnaire. COPD was defined as $z(FEV_1/FVC) < -1.6445$. Doctor diagnosed asthma was obtained by questionnaire.

NAG-FIN

The Nicotine Addiction Genetics (NAG) is a multisite consortium study of nicotine dependence genetics including Finland, Australia, and USA (PI Pamela Madden). As part of the consortium, the NAG-FIN (Nicotine Addiction Genetics – Finland) study sample was ascertained from the Finnish Twin Cohort consisting of adult twins born between 1938 and 1957. Based on earlier health questionnaires, the twin pairs concordant for ever-smoking were identified and recruited along with their family members (mainly siblings), totaling 2,265 individuals. Data collection took place between 2001 and 2005. The subjects were interviewed and they also completed additional questionnaires, in which a history of asthma and COPD (chronic bronchitis and emphysema) diagnosed by a physician was identified. DNA was extracted from the venous blood samples taken at local health centre laboratories and shipped to the National Public Health Institute (currently National Institute for Health and Welfare). DNA was extracted by standard methods.

NFBC1966

The Northern Finland Birth Cohort (NFBC) study programme was initiated in the 1960s. The cohort was established in the provinces of Oulu and Lapland and consists of 12231 children that had an expected date of birth in 1966 (NFBC1966). Spirometry and other measurements were done at the age of 31 year. Spirometry was done using a Vitalograph Pmodel spirometer (Vitalograph Ltd., Buckingham, UK), with a volumetric accuracy of ±2% or ±50 mL whichever was greater. The spirometer was calibrated regularly using a 1-Litre precision syringe. The spirometric manoeuvre was performed three times but was repeated if the coefficient of variation between two maximal readings was >4%. We defined the asthma cases as those who answered yes to both questions: 1) “have you ever had asthma during the last 12 months or more than a year ago?” and 2) “has this been verified or treated by doctor?”.

NTR

The Netherlands Twin Register (NTR, http://www.tweelingenregister.org/) recruits twins and their family members to study the causes of individual differences in health, behavior and lifestyle. Participants are followed longitudinally through survey studies, while some also

participate in experimental and biobanking studies [15, 16]. Telomere length was measured in individuals who participated in a large biobank study [17] and for most of these participants asthma data were available through one or more surveys. Individuals were asked to answer “Yes” or “No” to the question “Have you ever had asthma diagnosed by a doctor?”. A diagnosis for asthma was obtained by combining the answers across one to seven questionnaires. This score was based on consensus in the answers: if all answers were the same, the composite score was that answer. If participants answered ‘Yes’ in some questionnaires, but ‘No’ in others, then the composite score was the answer that was given most often, but only if this was done two times more than the other answer [18].

QIMR

The Queensland Institute of Medical Research (QIMR) adolescent study comprised twins and their non-twin siblings living in south-east Queensland, Australia [19]. Most (98% by self-report) are of mixed European ancestry, mainly from the British Isles. The participants are not selected on the basis of any disease or other outcome. Blood samples were collected at the end of testing sessions from participants and, if possible, from their parents. Pedigree relationships and zygosity were confirmed by genotype data. Further details are provided elsewhere [20].

TwinFat

The TwinFat study population was recruited from two population-based longitudinal studies, FinnTwin16 (birth cohorts 1975-1979) and FinnTwin12 (birth cohorts 1983-1987) [21]. The spirometric examinations were performed by Vmax encore, Sensormedics (Palm Springs, CA, USA) device’s mass flow sensor. The flow calibration was performed with a 3 liter pump, and the temperature was checked and corrected if needed. The flow device was cleaned before calibration by the device’s automatic cleaning program, after which 0 calibration was performed. Flow calibration was then performed between flow values 0.5 l/s and 9 l/s, after which a volume calibration was performed with an accuracy of ± 3%. The spirometric measurements were measured according to ERS/ATS recommendation from 2005. During spirometry, patient was sitting with the nose closed with a clip, and at least 3 maximal flow volume curves were measured, the difference of best two FEV_1 values or FVC values had to be less than 150 ml, and the expiration should last at least 6 seconds. If this was not fulfilled, additional measurements were performed.

TwinGene

In the TwinGene project, which is part of the Swedish Twin Registry (STR) [22], twins born before 1958 were contacted to participate in a simple health check-up, with measurement of height, weight, waist and hip circumference and blood pressure. Health and medication data were collected from self-reported questionnaires, and blood sampling materials were mailed to the subjects who then went to a local health care center for blood sampling for subsequent DNA extraction, serum collection and clinical chemistry tests. COPD and asthma diagnoses were collected from patient registries and self-reported questionnaires. For the purpose of this study a subset of 300 female MZ twin pairs (600 individuals) were used for telomere length assessments.

TwinsUK

The TwinsUK cohort consisted of a group of twins ascertained to study the heritability and genetics of age-related diseases (www.twinsUK.ac.uk). These unselected twins were recruited from the general population through national media campaigns in the UK and shown to be comparable to age-matched population singletons in terms of disease related and lifestyle characteristics [23]. Spirometry (Vitalograph model 2150, Buckingham, England) was conducted at the clinical centre during a visit. Twins were instructed before the test and forced vital capacity (FVC) manoeuvres were performed in a standing position, without the use of nose clips. Three manoeuvres were performed and the maximum obtained values for FEV_1 were obtained. Smoking status, acute and chronic respiratory diseases, medication and doctor diagnosed asthma were obtained by standardized questionnaire. COPD was defined as $z(FEV_1/FVC) < -1.6445$.

Telomere length measurements

Leukocyte telomere length measurements were made using a quantitative PCR assay comparing a TL PCR product (T) against a PCR product of a reference (S) gene to produce a T/S ratio, but with some modifications in relation to the reference gene and/or the calibrator samples or method used to enable inter-plate comparisons.

For 9 of the 14 studies (**ERF**, **GRAPHIC**, **KORA Age**, **KORA F3**, **KORA F4**, **NTR**, **QIMR**, **TwinGene** and **TwinsUK**) telomere length was determined in one central laboratory in Leicester according to a common protocol described in detail elsewhere [24]. In brief, DNA samples were run in duplicate in 25µL reactions using a CAS-1200 liquid handling system (Qiagen, UK) and run on a Rotorgene-Q Real Time Thermal Cycler (Qiagen, UK). The single copy gene used was *36B4*. Alongside the samples, each run also contained a Calibrator sample (DNA from the K562 cell line) in duplicate and a no template control. Analysis of the PCR output was performed using Comparative Quantification (Qiagen Rotorgene analysis software, Qiagen, UK) and quantification is relative to the calibrator DNA. Samples were checked for concordance between duplicate measurements and to ensure that they ran within the established linear range of the assay. In addition, to ensure reproducibility of the assay, samples were re-run at random on different days. Inter-run coefficients of variation were between 2.7% and 3.9% for the cohorts measured using this method.

In the **NFBC1966**, telomere lengths measurements were performed using a multiplex quantitative realtime PCR method, with minor modifications as described previously [25]. Hgb was used as the single copy reference gene. Five serial dilutions of a single common reference sample (leukocyte DNA from a 42 year-old female) spanning 5-50ng were run in triplicate on each plate. Any samples found to have an input DNA amount outside of this range were diluted and run again. The overall mean coefficient of variation for duplicate test samples on the same plate was 5%, and the mean inter-run CV for selected samples was 6.2%.

Leukocyte telomere length for **EX-ATHLETES**, **FITSA**, **NAG-FIN**, and **TwinFat** were performed by qPCR as described in Ahola et al. 2012 [26]. Briefly, all samples were assayed in triplicate using the BioRad CFX384 system, and Hgb was used as the single copy reference gene.

Supplementary Figures

Figure E1: Sex-stratified association results for telomere length and COPD in women (left panel) and men (right panel). 95% confidence intervals are given for all estimates. COPD was defined by GLI criteria.

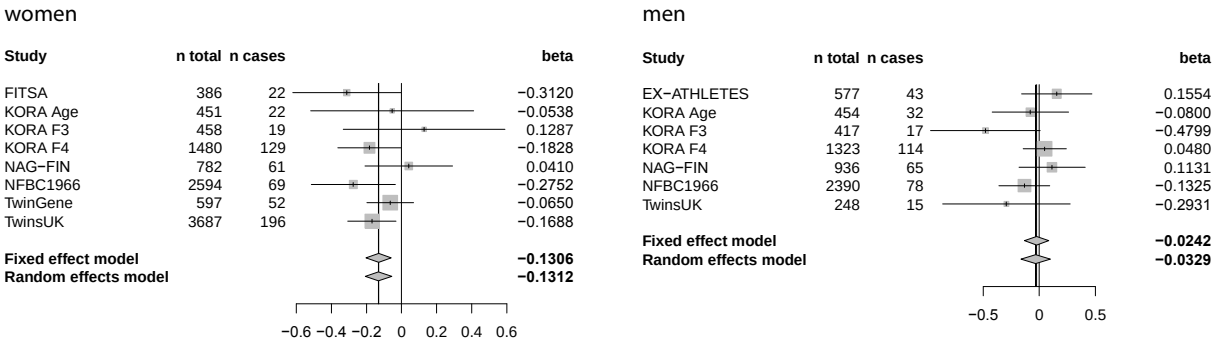


Figure E2: Association results for telomere length and COPD in the overall sample, in all subjects <70 years, all subjects <60 years and further stratified by sex. 95% confidence intervals are given for all estimates. COPD was defined by GLI criteria.

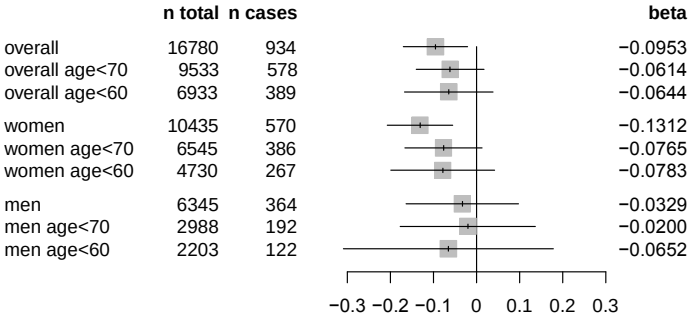


Figure E3: Sex-stratified association results for telomere length and asthma in women (left panel) and men (right panel). 95% confidence intervals are given for all estimates.

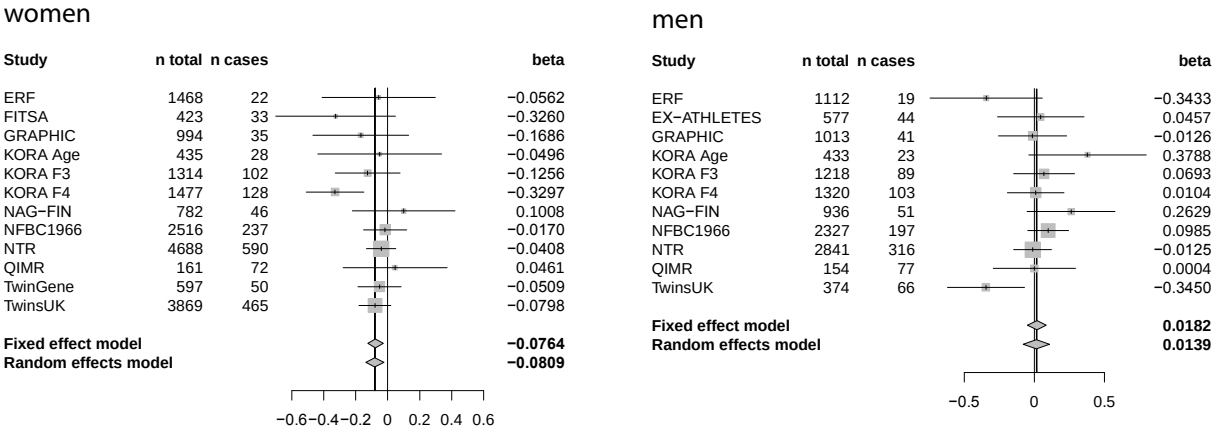


Figure E4: Sex-stratified association results for telomere length and FEV_1, FVC, and FEV_1/FVC in women (left) and men (right). 95% confidence intervals are given for all estimates.

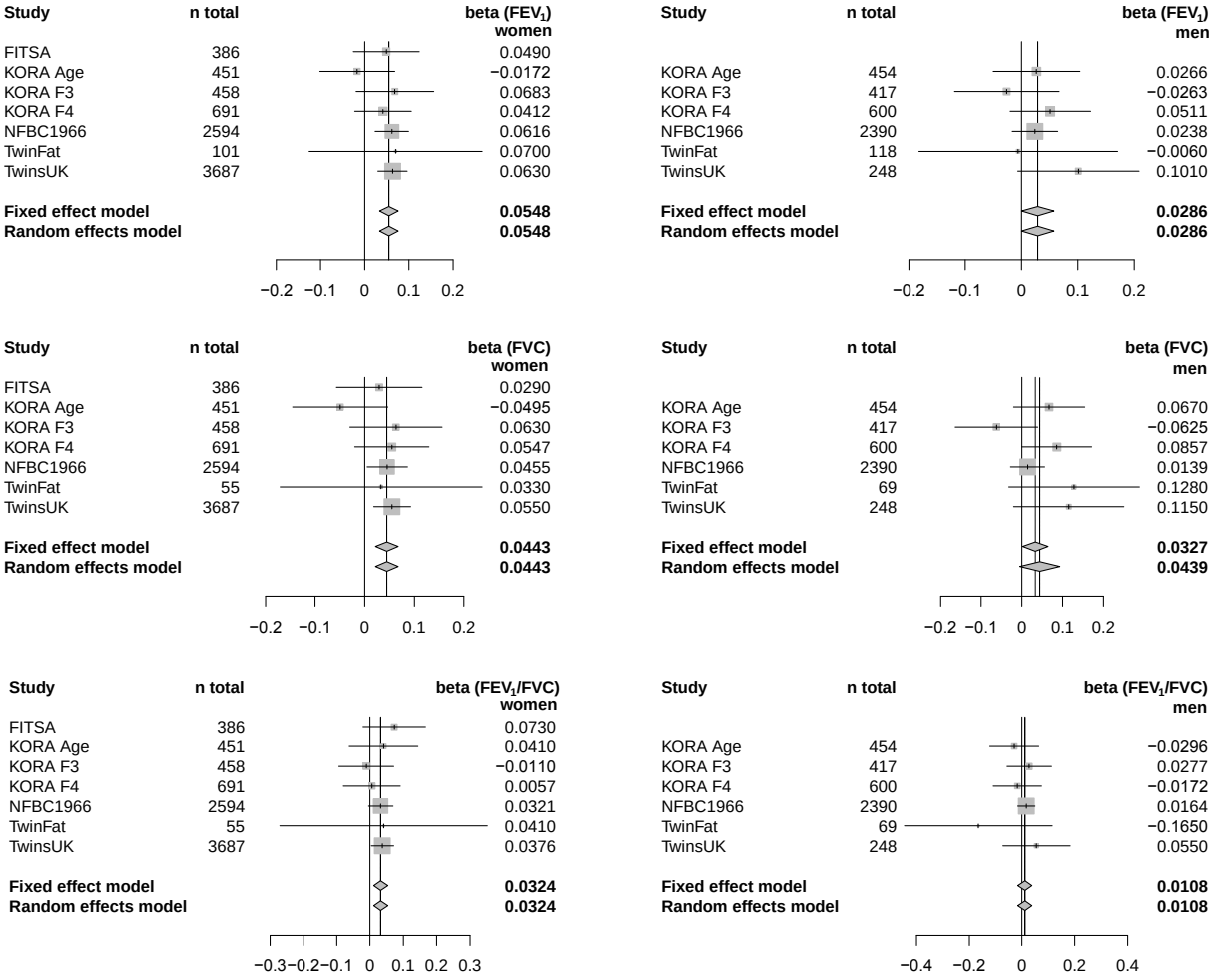


Figure E5: Sex- and health status-stratified association results for telomere length and FEV_1, FVC, and FEV_1/FVC. 95% confidence intervals are given for all estimates. COPD was defined by GLI criteria.

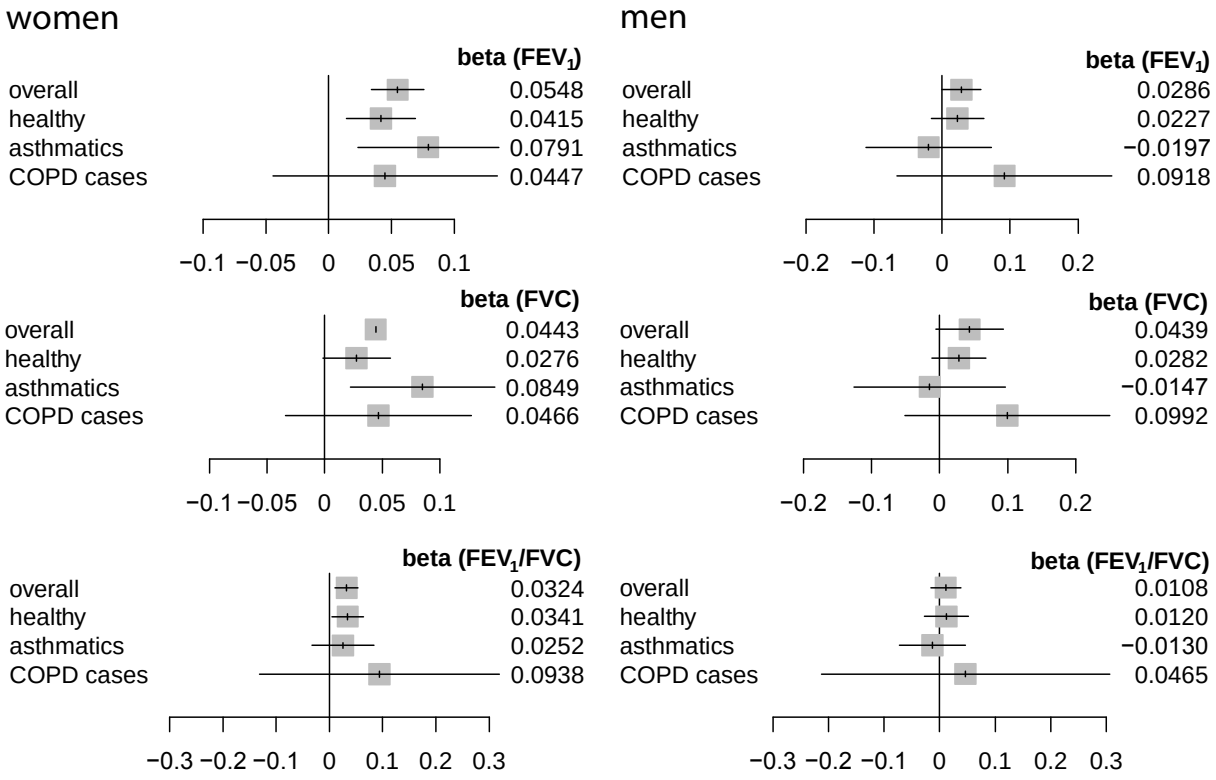
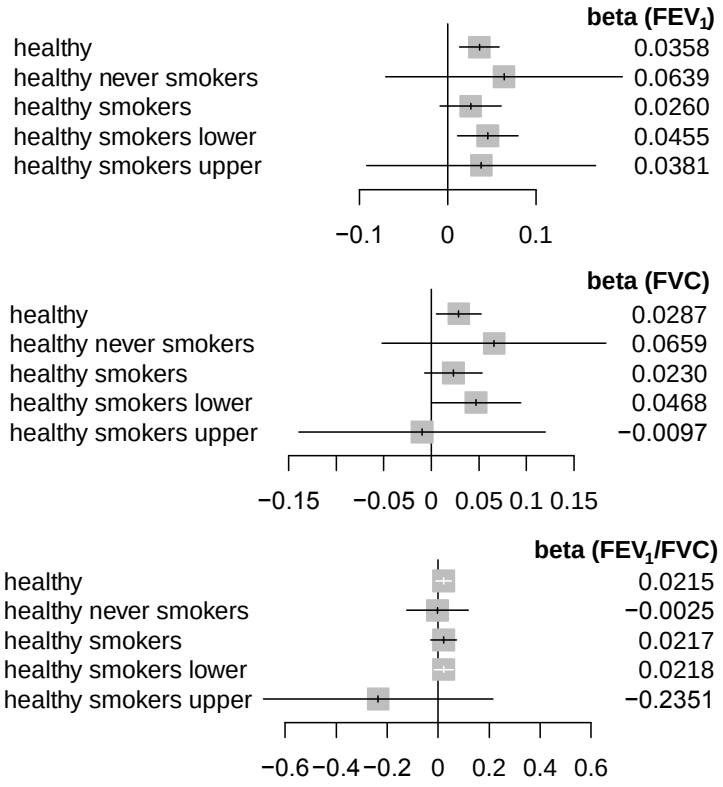


Figure E6: Smoking status-stratified association results for telomere length and FEV_1, FVC, and FEV_1/FVC. 95% confidence intervals are given for all estimates. The group of apparently healthy subjects was divided into never smokers and smokers. In the group of healthy smokers we additionally investigated to lower 25%-quantile of FEV_1, FVC, and FEV_1/FVC respectively, as well as the upper 25%-quantile.



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References

1. Aulchenko YS, Heutink P, Mackay I, Bertoli-Avella AM, Pullen J, Vaessen N, Rademaker TAM, Sandkuijl LA, Cardon L, Oostra B, van Duijn CM. Linkage disequilibrium in young genetically isolated Dutch population. *Eur J Hum Genet* 2004: 12(7): 527-534.
2. Pardo LM, MacKay I, Oostra B, van Duijn CM, Aulchenko YS. The effect of genetic drift in a young genetically isolated population. *Ann Hum Genet* 2005: 69(Pt 3): 288-295.
3. Sarna S, Sahi T, Koskenvuo M, Kaprio J. Increased life expectancy of world class male athletes. *Medicine and science in sports and exercise* 1993: 25(2): 237-244.
4. Vartiainen E, Laatikainen T, Peltonen M, Juolevi A, Mannisto S, Sundvall J, Jousilahti P, Salomaa V, Valsta L, Puska P. Thirty-five-year trends in cardiovascular risk factors in Finland. *International journal of epidemiology* 2010: 39(2): 504-518.
5. Tiainen K, Sipila S, Alen M, Heikkinen E, Kaprio J, Koskenvuo M, Tolvanen A, Pajala S, Rantanen T. Heritability of maximal isometric muscle strength in older female twins. *Journal of applied physiology* 2004: 96(1): 173-180.
6. Tiainen K, Pajala S, Sipila S, Kaprio J, Koskenvuo M, Alen M, Heikkinen E, Tolvanen A, Rantanen T. Genetic effects in common on maximal walking speed and muscle performance in older women. *Scandinavian journal of medicine & science in sports* 2007: 17(3): 274-280.
7. Kaprio J, Sarna S, Koskenvuo M, Rantasalo I. The Finnish Twin Registry: formation and compilation, questionnaire study, zygosity determination procedures, and research program. *Progress in clinical and biological research* 1978: 24 Pt B: 179-184.
8. Kaprio J, Koskenvuo M. Genetic and environmental factors in complex diseases: the older Finnish Twin Cohort. *Twin research : the official journal of the International Society for Twin Studies* 2002: 5(5): 358-365.
9. Tobin MD, Tomaszewski M, Braund PS, Hajat C, Raleigh SM, Palmer TM, Caulfield M, Burton PR, Samani NJ. Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. *Hypertension* 2008: 51(6): 1658-1664.
10. Holle R, Happich M, Löwel H, Wichmann HE, Group MONICAKORAS. KORA--a research platform for population based health research. *Gesundheitswesen* 2005: 67 Suppl 1: S19-S25.
11. Wichmann H-E, Gieger C, Illig T, Group MONICAKORAS. KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* 2005: 67 Suppl 1: S26-S30.

12. Peters A, Doring A, Ladwig KH, Meisinger C, Linkohr B, Autenrieth C, Baumeister SE, Behr J, Bergner A, Bickel H, Bidlingmaier M, Dias A, Emeny RT, Fischer B, Grill E, Gorzelniak L, Hansch H, Heidbreder S, Heier M, Horsch A, Huber D, Huber RM, Jorres RA, Kaab S, Karrasch S, Kirchberger I, Klug G, Kranz B, Kuch B, Lacruz ME, Lang O, Mielck A, Nowak D, Perz S, Schneider A, Schulz H, Muller M, Seidl H, Strobl R, Thorand B, Wende R, Weidenhammer W, Zimmermann AK, Wichmann HE, Holle R. [Multimorbidity and successful aging: the population-based KORA-Age study]. *Zeitschrift fur Gerontologie und Geriatrie* 2011: 44 Suppl 2: 41-54.

13. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994: 7(5): 954-960.

14. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J, Force ATSERST. Standardisation of spirometry. *Eur Respir J* 2005: 26(2): 319-338.

15. Boomsma DI, de Geus EJ, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TC, Hudziak JJ, Bartels M, Willemsen G. Netherlands Twin Register: from twins to twin families. *Twin research and human genetics : the official journal of the International Society for Twin Studies* 2006: 9(6): 849-857.

16. Willemsen G, Vink JM, Abdellaoui A, den Braber A, van Beek JH, Draisma HH, van Dongen J, van 't Ent D, Geels LM, van Lien R, Ligthart L, Kattenberg M, Mbarek H, de Moor MH, Neijts M, Pool R, Stroo N, Kluft C, Suchiman HE, Slagboom PE, de Geus EJ, Boomsma DI. The Adult Netherlands Twin Register: Twenty-Five Years of Survey and Biological Data Collection. *Twin research and human genetics : the official journal of the International Society for Twin Studies* 2013: 1-11.

17. Willemsen G, de Geus EJ, Bartels M, van Beijsterveldt CE, Brooks AI, Estourgie-van Burk GF, Fugman DA, Hoekstra C, Hottenga JJ, Kluft K, Meijer P, Montgomery GW, Rizzu P, Sondervan D, Smit AB, Spijker S, Suchiman HE, Tischfield JA, Lehner T, Slagboom PE, Boomsma DI. The Netherlands Twin Register biobank: a resource for genetic epidemiological studies. *Twin research and human genetics : the official journal of the International Society for Twin Studies* 2010: 13(3): 231-245.

18. Willemsen G, van Beijsterveldt TC, van Baal CG, Postma D, Boomsma DI. Heritability of self-reported asthma and allergy: a study in adult Dutch twins, siblings and parents. *Twin research and human genetics : the official journal of the International Society for Twin Studies* 2008: 11(2): 132-142.

19. Wright MJ, Martin NG. Brisbane Adolescent Twin Study: Outline of study methods and research projects. *Brisbane Adolescent Twin Study: Outline of study methods and research projects* 2004: 56(2): 65-78.

20. Medland SE, Nyholt DR, Painter JN, McEvoy BP, McRae AF, Zhu G, Gordon SD, Ferreira MA, Wright MJ, Henders AK, Campbell MJ, Duffy DL, Hansell NK, Macgregor S, Slutske WS, Heath AC, Montgomery GW, Martin NG. Common variants in the trichohyalin gene are associated with straight hair in Europeans. *Am J Hum Genet* 2009: 85(5): 750-755.

21. Tyynismaa H, Raivio T, Hakkarainen A, Ortega-Alonso A, Lundbom N, Kaprio J, Rissanen A, Suomalainen A, Pietilainen KH. Liver fat but not other adiposity measures influence circulating FGF21 levels in healthy young adult twins. *The Journal of clinical endocrinology and metabolism* 2011: 96(2): E351-355.

22. Magnusson PK, Almqvist C, Rahman I, Ganna A, Viktorin A, Walum H, Halldner L, Lundstrom S, Ullen F, Langstrom N, Larsson H, Nyman A, Gumpert CH, Rastam M, Anckarsater H, Cnattingius S, Johannesson M, Ingelsson E, Klareskog L, de Faire U, Pedersen NL, Lichtenstein P. The Swedish Twin Registry: Establishment of a Biobank and Other Recent Developments. *Twin research and human genetics : the official journal of the International Society for Twin Studies* 2012: 1-13.

23. Moayyeri A, Hammond CJ, Hart DJ, Spector TD. The UK Adult Twin Registry (TwinsUK Resource). *Twin research and human genetics : the official journal of the International Society for Twin Studies* 2012: 1-6.

24. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res* 2002: 30(10): e47.

25. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. *Nucleic Acids Res* 2009: 37(3): e21.

26. Ahola K, Siren I, Kivimaki M, Ripatti S, Aromaa A, Lonnqvist J, Hovatta I. Work-related exhaustion and telomere length: a population-based study. *PLoS One* 2012: 7(7): e40186.