Supplemental Material - Methods

Subject recruitment and sample handling

All the participating twins were self reported healthy volunteers. All participants gave written informed consent before entering the study and the St Thomas' Hospital research ethics committee approved the UK part of the project, while all the Danish regional Scientific-Ethical Committees approved the Danish part. DNA-based microsatellite markers with the PE Applied Biosystems AmpFISTR Profiler Plus Kit were used to determine zygosity of the Danish twins.

Phenotyping

In brief, 25μl of citrated plasma was added to 75μl assay buffer (0.05M Tris-HCl, 0.1M NaCl, pH 7.4), and 50μl of activation mix (final concentrations: 0.03 U/ml thrombin [Calbiochem], and 7.5 mmol/l calcium in assay buffer) was added to each column of the 96-well plate using a multichannel pipette at 10 sec intervals. Plates were shaken and read at 340 nm every 12 sec for 1h in a BIO-TEK ELx-808 microplate reader. The turbidimetric lysis assay was carried out as above with the addition of 12.5 ng of tPA (Technoclone) to the 75μl assay buffer (83 ng/ml final concentration) prior to addition of activation mix. Plates were read at 340 nm every 12 sec for 1h and subsequently every 2 min for up to 9h.

Genotyping

For the UK sample, genotyping was performed using 2231 polymorphic genetic markers - 737 microsatellite markers from the ABI Prism set (Applied Biosystems, Foster City, CA) and 1494 SNP markers from the HuSNP GeneChip linkage mapping set (Affymetrix Inc. Santa Clara, CA), as described previously[1]. The estimated genotyping error rate was < 1%. Allele frequencies were estimated from the whole sample of genotyped subjects. The map positions were taken from Rutgers combined linkage physical map (MAP-O-MAT). The

genetic locations of markers not on the Rutgers maps were interpolated from their physical position. The Danish twins were genotyped as part of the GenomEUtwin consortium as described by Perola et al[2].

Heritability

Data were modelled using structural equation models implemented in Mx[3] to obtain estimates for the parameters of the ACE model and its sub-models AE, CE and E. The full model (ACE) was compared to submodels AE, CE and E to find the best fitting model with comparison of the fit made using a chi-square difference test. If this test statistic is not significant, the reduced model is accepted as the more parsimonious explanation of the data, otherwise the full model is retained. In all analyses, age was included as a covariate (Table 2).

Joint Linkage Analysis

To evaluate jointly the partially overlapping UK and DK linkage results, multipoint identity-by-descent (IBD) probabilities were calculated on a 1 cM grid using Merlin[4] for each sample separately, using their specific genetic map and allele frequencies. The IBD estimates were collected in a single IBD file and the two samples were pooled together in a joint linkage analysis using the variance component engine implemented in QTDT[5]. Age, sex and country of origin were considered the most important vascular risk factors in this study so they were included as covariates in the linkage analysis. Approximate support intervals (SI) were generated using a -1 LOD approach.

Reference List

(1) Wilson SG, Reed PW, Bansal A, Chiano M, Lindersson M, Langdown M, Prince RL, Thompson D, Thompson E, Bailey M, Kleyn PW, Sambrook P, Shi MM, Spector TD. Comparison of genome screens for two independent cohorts provides replication of

suggestive linkage of bone mineral density to 3p21 and 1p36. *Am J Hum Genet* 2003 January;72(1):144-155.

(2) Perola M, Sammalisto S, Hiekkalinna T, Martin NG, Visscher PM, Montgomery GW, Benyamin B, Harris JR, Boomsma D, Willemsen G, Hottenga JJ, Christensen K, Kyvik KO, Sorensen TI, Pedersen NL, Magnusson PK, Spector TD, Widen E, Silventoinen K, Kaprio J, Palotie A, Peltonen L. Combined genome scans for body stature in 6,602 European twins: evidence for common Caucasian loci. *PLoS Genet* 2007 June;3(6):e97.

(3) Neale MC, Boker SM, Xie G, Maes HH. Mx: Statistical Modeling. [7th Edition]. 2006. VCU Box 900126, Richmond VA23298, Department of Psychiatry.

(4) Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet* 2002 January;30(1):97-101.

(5) Abecasis GR, Cardon LR, Cookson WO. A general test of association for quantitative traits in nuclear families. *Am J Hum Genet* 2000 January;66(1):279-292.