Genetic Dominance Influences Blood Biomarker Levels in a Sample of 12,000 Swedish Elderly Twins

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In twin studies of cardiovascular disease biomarkers the dizygotic correlations are often estimated to be less than half of monozygotic correlations indicating a potential influence of nonadditive genetic factors. Using a large and homogenous sample, we estimated the additive and dominance genetic influences on levels of high density lipoprotein, low density lipoprotein, apolipoprotein A-I, apolipoprotein B, total cholesterol, triglycerides, glucose, hemoglobin Alc and c-reactive protein, all of which are biomarkers associated with cardiovascular disease. The blood biomarkers were measured on 12,000 Swedish twins born between 1911 and 1958. The large sample allowed us to obtain heritability estimates with considerable precision and provided adequate statistical power for estimation of dominance genetic components. Our study showed complete absence of the shared environment component for the investigated traits. Dominant genetic component was shown to be significant for low density lipoprotein (0.18), glucose (0.31), Hemoglobin Alc (0.55), and c-reactive protein (0.27). To our knowledge, this is the first statistically significant evidence for dominance genetic variance found for low density lipoprotein, glucose, hemoglobin Alc, and c-reactive protein in a population based twin sample. The study highlights the importance of acknowledging nonadditive genes underlying the risk of developing cardiovascular diseases.

Keywords: Swedish twins, heritability, CVD biomarkers

Cardiovascular diseases (CVD) caused by atherosclerosis are the major contributors to premature death in Europe (De Backer et al., 2004). Most CVDs are complex disorders and it is well known that genetic factors play important role in the disease development. Atherosclerosis is a central process for development of CVD and is caused by dysfunction in cholesterol and lipid metabolism in conjunction with inflammation (Steinberg, 2002). Several established biomarkers of atherosclerosis are also, to a varying extent, genetically regulated. These biomarkers are components of cholesterol and lipid metabolism, inflammatory pathways, and involved in the metabolic syndrome.

It is now evident that cholesterol and lipoproteins such as low density lipoprotein (LDL), high density lipoprotein (HDL), apolipoprotein B (apoB), and apolipoprotein A-I (apoA-I), play a substantial role in the progression of atherosclerosis (Krauss & Kesaniemi, 1994; Lauer & Fontanarosa, 2001; Steinberg, 2002). Elevated levels of LDL in the blood have been demonstrated to associate with higher risk of cardiovascular diseases, LDL levels above 6 mmol/l is considered to be a markedly high risk factor (Chan & Watts, 2006; De Backer et al., 2004; Krauss & Kesaniemi, 1994; Steinberg, 2002). Contrary to LDL, there is for HDL an inverse relation between concentration and risk of developing CVD. This is due to the opposing roles of these two different lipoproteins, while LDL transports cholesterol and lipids from the intestine to the tissues, excess cholesterol in peripheral tissues is transported to the liver by HDL. Apolipoprotein B (apoB) and apoA-I are the major protein components in LDL and HDL, respectively (Dastani et al., 2006). ApoB-containing particles in excess can lead to atherogenecity since apoB is the molecule mainly responsible for the oxidization of LDL, a key step in the atherogenic process. ApoA-I is crucial for the reverse cholesterol transport, since it enables esterification of cholesterol and promotes the externalization of excess cholesterol in peripheral cells to HDL (Andrikoula & McDowell, 2008; Walldius & Jungner, 2004).

The pathogenesis of atherosclerosis is also influenced by inflammatory reactions. The serum acute

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Table 1

Published Additive Heritability for Investigated CVD Biomarkers

Phenotype	Heritability	References
HDL	0.44–0.76	a,b,c,d,e,f,g,h,i,j,k
LDL	0.36–0.98	b,c,d,e,j,l,,m
apoA-I	0.24–0.71	d,i,l,n,o,p,q
apoB	0.25–0.78	d,i,l,n,o,p,q
Total Cholesterol	0.30–0.74	a,b,c,d,e,i,j,p,q
TG	0.03–0.72	a,d,g,i,n,o,p,q
Glucose	0.17–0.67	a,c,j,r
HbA1c	0.62–0.75	s,t
CRP	0.00–0.62	t,u,v,w,x,y,z

Note: [a](Bayoumi et al., 2007) [b](Chen et al., 2004) [c](de Oliveira et. al., 2008) [d](Fenger et al., 2007) [e](Goode et al.,2007) [f](Henneman et al., 2008) [g](Isaacs et al., 2007) [h](McQueen et al., 2003) [i](Snieder et al., 1999) [j](Souren et al., 2007) [k](Velasquez-Melendez et al., 2007) [l](Chien et al., 2007) [m](Iliadou et al.,2005) [n](Hokanson et al., 2003) [o](Beekman et al., 2002) [p](Iliadou et al., 2001) [q](Middelberg et al., 2002) [r](Santos et al., 2006) [s](Snieder et al., 2001) [t](Simonis-Bik et al., 2008) [u](Berrahmoune et al., 2007) [v](Fox et al., 2008) [w](Lange et al., 2006) [x](Su et al., 2008) [y](Wessel et al., 2007) [z](Worns et al., 2006)

phase protein, C-reactive protein (CRP), is an indicator of the inflammatory state. CRP is considered a contributor to the initiation and progression of atherosclerosis (Paffen & DeMaat, 2006; Steinberg, 2002). Hemoglobin Alc (HbAlc) is commonly used to assess long-term blood glucose control in patients with diabetes mellitus. Former epidemiological studies show that HbAlc is a predictor for incident coronary heart disease and is frequently associated with increased CVD risk (Golden et al., 2007).

Table 1 summarizes previously published heritability estimates for the various biomarkers. A substantial portion of these estimates are from family (i.e., non-twin) based materials, furthermore the sizes of the studies vary but have generally been small, that is, less than 1000 twin pairs in total. As a consequence the precision of the heritability estimates have been limited. Some studies have indicated the possibility of nonadditive (i.e., dominance) genetic influences for the biomarkers mentioned above. Using a family based design, significant dominance genetic variance component has previously been reported for LDL in a founder population (Abney et al., 2001). No population based studies have, however, been able to provide significant statistical evidence for nonadditive genetic variance for the remaining traits. One of the reasons for this might be insufficient power due to small sample sizes (Martin et al., 1978).

In this study we aim to estimate additive and nonadditive genetic influences for HDL, LDL, total cholesterol, apoA-I, apoB, triglycerides, glucose, HbA1c, and CRP in a large sample of participants belonging to the Swedish Twin Registry. This is the largest twin study to date for several of the investigated biomarkers, providing data with better power than has previously been possible. Furthermore, since all the biomarkers have been assessed at the same occasion for all participants, direct comparisons between the estimates are facilitated.

Materials and Methods

The participants were obtained from the TwinGene project which is a population-based study of Swedish twins born between 1911 and 1958, who were contacted and tested between 2004 and 2007. The zygosity of the twins was based on self-reported childhood resemblance, or by using DNA markers (for 5% of the total sample). Informed consent was obtained from all participants. To be included both twins within a pair had to be alive. Eligible participants had to have participated in a previous computer assisted telephone interview called SALT (Screening Across the Lifespan Twin Study), which was conducted between 1998 and 2002. Those who had donated DNA for previous Swedish Twin Registry studies, who had a record of hepatitis, or who had declined participation in further studies were excluded from the study. In total, 11991 individuals participated by donating blood to the study.

Sampling

The participants were asked to make an appointment at their local health-care facility on Monday to Thursday mornings (not the day before a national holiday), to ensure that the sample would reach the KI Biobank in Stockholm the following day by over night mail. The participants were instructed to fast from 8 PM (20:00) the previous night. A total volume of 50 ml of blood was drawn from each individual by venipuncture. Tubes with serum for clinical chemistry assays and whole blood for DNA extraction were sent to Karolinska University Laboratory by overnight post.

Clinical Blood Tests

Clinical blood chemistry assessments were performed by the Karolinska University Hospital Laboratory. Levels of HbA1c were measured by a high-liquid performance chromatography separation technique. Levels of the other biomarkers were determined by Synchron LX systems (Beckman Coulter).

Twin Contact and Age at Separation

Through the SALT-interview performed between 1998 and 2002, data on self-reported intra-pair contact frequency (i.e., the frequency by which the twins in a pair met in person) and age at separation from the co-twin was available for a majority of the study participants. Data on contact frequency from at least one of the twins within a pair was available for 11225 of the TwinGene participants (94%). The replies to how often the participants usually met with their co-twin were divided into 4 levels; (1) less than once a year; (2) on a yearly basis; (3) on a monthly basis; or (4) on a weekly basis. There was a high level of intra-pair agreement on contact frequency for the 4317 pairs in which both twins responded (rho = 0.81). The average level of contact frequency within the pair was used for

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these pairs. Data on age at separation from co-twin (in whole years) was available from 10028 (83%) study participants. If both twins had reported age at separation, the average value within the twin pair was used. There was a moderate correlation in self-reported age at separation between twins (rho = 0.72).

Statistical Procedure

Data handling and calculation of descriptive statistics as well as correlation coefficients were performed in SAS version 9.1 (SAS Institute, Cary, NC, USA). The distributions for HDL, triglycerides, glucose, HbA1c, and CRP were skewed and therefore log transformed to yield an approximately normal distribution. The PROC *t* test procedure was used to investigate the statistical differences in means and variances between twins belonging to MZ or DZ pairs. Before conducting further analysis on twin similarity, each trait was adjusted for age and sex by linear regression models. Values of LDL, HDL, total cholesterol, TG, and CRP from individuals reporting to be taking Atorvastatin, Simvastatin or Bezafibrate treatment were adjusted to resemble the original lipid values before the normalizing effect of the medications. These adjustments were applied on values of LDL, HDL, triglycerides, total cholesterol and CRP values. Previously published average effect sizes of the medications suggest that Atorvastatin reduces the levels of total cholesterol, LDL, triglycerides, and CRP with 27%, 36%, 17%, and 35.1% respectively (Betteridge et al., 2007; Edwards & Moore, 2003; Gomez-Gerique et al., 2002; Jialal et al., 2001) while it increases HDL levels with 7%. Simvastatin reduces the levels of total cholesterol, LDL, TG, and CRP with 25%, 34%, 17%, and 22.8% respectively, and raises the levels of HDL with 6% (Edwards & Moore, 2003; Jialal et al., 2001). Bezafibrate lowers the levels of total cholesterol with 4.7%, the levels of LDL with 5.2% and the levels of TG with approximately 24.6%, and increase HDL levels with 14.4% (Gould et al., 2007; Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the Bezafibrate Infarction Prevention (BIP) study, 2000). CRP values above 10 mg/L are considered to reflect infection induced inflammation and were thus removed from the CRP dataset (Clyne & Olshaker, 1999). After these adjustments all values were transformed into *z* scores. To restrain influence from outliers, trait values above or below 4 standard deviations from the mean were set to missing. Consequently, 0.14 % of apoA-I values, 0.05% of apoB values, 0.03% of HDL values, 0.6% of LDL values, 0.03% of total cholesterol values, 0.09% of TG values, 1% of Glucose values, 1.18% of HbA1c values, and 4.4% of all CRP values were set to missing. For each trait the intra-pair correlation coefficients were estimated for MZ, SSDZ (same-sexed dizygotic) and OSDZ (opposite-sexed dizygotic) twin pairs separately.

A variance component maximum likelihood method was implemented for estimation of variance components for each phenotype, using the Mx statistical program (Neale et al., 2006). Univariate twin analyses were conducted in which the variance of the adjusted phenotypic values was divided into additive genetic effects (A), dominant genetic effects (D), shared environmental effects (C), and unique environmental effects (E). Scripts downloaded from the GenomEUtwin Mx-script library (http://www.psy.vu.nl/mxbib/) were used after appropriate modification. Akaike's information criterion was used to compare goodness of fit between non-nested model (e.g., ACE and ADE). The principle of parsimony was implemented to determine which nested model was to be preferred (e.g., ACE or AE) when the χ^2 test was not significant ($p < .05$).

Table 2

General Characteristics of Study Participants

	Men	Women
N[a]	5327	6421
Age (mean ± sd)	66.6 ± 8.7	65.9 ± 8.9
Weight (Kg, mean ± sd)	81.3 ± 12	68.3 ± 12
BMI (Kg/m^2, mean ± sd)	26.2 ± 3.5	25.7 ± 4.3
Individuals receiving statins (N[a])	744 (14%)	601 (9%)
Individuals receiving fibrates (N[a])	9 (0.2%)	3 (0.05%)
MZ[b] (N[a])	1281	1656
SSDZ[c] (N[a])	1817	2376
OSDZ[d] (N[a])	2175	2346
Unknown zygosity (N[a])	54	43

Note: [a]Number of individuals, [b]Monozygotic, [c]Same-sexed dizygotic, [d]Opposite-sexed dizygotic

Results

The final results are obtained from 1285 complete monozygotic twin pairs, 1627 complete same-sexed dizygotic twin pairs, and 1635 complete opposite-sexed dizygotic twin pairs. General characteristics of the study population are summarized in Table 2.

Descriptive statistics on raw values and values after log transformation, adjustments and *z* score standardization, stratified by zygosity are shown in Table 3. There is a statistically significant difference in mean adjusted apoB value between MZ and DZ twins. The standard deviations of adjusted glucose and HbA1c values differ significantly between MZ and DZ twins. However, the absolute differences are small in magnitude. The adjusted means and standard deviations for the Z-score values never differ more than 0.05 and 0.1, respectively. We are aware that these differences may result in slight reduction of fit of the models for these phenotypes.

Intra-pair correlation coefficients for each phenotype are presented in Table 3 for MZ twin pairs, same-sexed DZ twin pairs, and opposite-sexed DZ twin pairs separately. For all traits the MZ twins showed greater correlation coefficients than the DZ

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Table 3

Descriptive Statistics of Raw Values and Values After Log Transformation, Adjustment and *z*-Score Standardization, Stratified by Zygosity

Phenotype	Class	*N*	Mean	*SD*	*P*(mean)	*P*(variance)
apoA-I						
Unadjusted raw values	DZ	6576	1.6453	0.2976		
	MZ	2556	1.6475	0.2987	.7588	.8153
Adjusted, z-transformed	DZ	6576	–0.008	0.9843		
	MZ	2556	–0.044	0.9837	.1144	.9732
apoB						
Unadjusted raw values	DZ	6580	1.0849	0.2429		
	MZ	2568	1.0697	0.2414	.0071	.7154
Adjusted, z-transformed	DZ	6580	0.0079	0.9993		
	MZ	2568	–0.05	0.991	.0064	.6131
HDL						
Unadjusted raw values	DZ	6592	1.4106	0.4191		
	MZ	2564	1.4311	0.4261	.0370	.3142
Adjusted, z-transformed	DZ	6592	–0.006	0.9979		
	MZ	2564	0.0149	0.9914	.3740	.6927
LDL						
Unadjusted raw values	DZ	6592	1.4012	0.4203		
	MZ	2564	1.4222	0.4272	.0327	.3196
Adjusted, z-transformed	DZ	6592	–0.013	0.9647		
	MZ	2564	0.0187	0.9808	.1589	.2000
Total cholesterol						
Unadjusted raw values	DZ	6568	5.9453	1.0741		
	MZ	2568	5.9819	1.0892	.1450	.3935
Adjusted, z-transformed	DZ	6568	–0.008	0.978		
	MZ	2568	0.0149	0.995	.3127	.2912
HbA1c						
Unadjusted raw values	DZ	6404	4.7736	0.5199		
	MZ	2524	4.7477	0.4635	.0222	< .0001
Adjusted, z-transformed	DZ	6404	–0.064	0.8401		
	MZ	2524	–0.082	0.7507	.3486	< .0001
TG						
Unadjusted raw values	DZ	6574	1.3879	0.7949		
	MZ	2568	1.3652	0.7945	.2201	.9837
Adjusted, z-transformed	DZ	6574	–0.002	0.986		
	MZ	2568	–0.016	0.9841	.5210	.9097
CRP						
Unadjusted raw values	DZ	5440	2.4434	2.0974		
	MZ	2132	2.3369	2.0017	.0400	.0104
Adjusted, z-transformed	DZ	5440	–0.087	0.8913		
	MZ	2132	–0.095	0.8714	.7195	.2160
Glucose						
Unadjusted raw values	DZ	6442	5.4961	0.9417		
	MZ	2534	5.4465	0.8564	.0578	<.0001
Adjusted, z-transformed	DZ	6442	–0.039	0.8321		
	MZ	2534	–0.047	0.7657	.6591	.0001

Note: All values ± 4 SD from the mean and all CRP values > 10 mg/L have been excluded.

twins. The strongest correlation was observed for levels of HbA1c in MZ twins. We note that, for each trait, the correlation coefficient for MZ twins was more than twice as large as the average correlation coefficient for SSDZ and OSDZ twins. The results from analysis from ACE models (data not shown) showed that additive genetic component and the nonshared environmental component are significant ($p < .05$) for all traits. No evidence for influences of shared-environment was obtained for any of the biomarkers. Since the correlation coefficient for same-sexed and opposite-sexed dizygotic twins were on average less than half of the respective correlation coefficients for MZ twins for all phenotypes, analyses of ADE models were carried out. A dominant genetic effect was significant for HbA1c, LDL, glucose, CRP and HbA1c. According to Akaike's

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Table 4

Intra-Class Correlations and Parameter Estimates With 95% Confidence Intervals for Additive Genetic (a^2), Dominant Genetic (d^2) and Non-Shared Environmental (e^2) Variance Components of Age and Sex Adjusted Trait Levels in AE and ADE Models

Phenotype	*r*MZ[a] (*N*)	*r*SSDZ[b] (*N*)	*r*OSDZ[c] (*N*)	Model	a^2(95%CI)	d^2(95%CI)	e^2(95%CI)
HbA1c	0.67 (1262)	0.24 (1591)	0.22 (1576)	ADE	0.16 (0.03–0.29)	0.55 (0.55–0.68)	0.29 (0.27–0.32)
apoA-I	0.65 (1278)	0.33 (1621)	0.27 (1632)	AE	0.64 (0.64–0.67)	—	0.36(0.33–0.37)
apoB	0.50 (1284)	0.21 (1623)	0.22 (1632)	AE	0.48 (0.44–0.51)	—	0.52 (0.49–0.56)
HDL	0.66 (1282)	0.34 (1626)	0.29 (1635)	AE	0.66 (0.63–0.68)	—	0.34 (0.32–0.37)
LDL	0.54 (1254)	0.22 (1577)	0.21 (1582)	ADE	0.35 (0.21–0.49)	0.18 (0.03- 0.33)	0.47 (0.44–0.51)
TG	0.55 (1284)	0.28 (1624)	0.21 (1628)	AE	0.54 (0.51–0.57)	—	0.46 (0.43–0.49)
Total cholesterol	0.52 (1284)	0.23 (1621)	0.24 (1628)	AE	0.50 (0.46–0.53)	—	0.50 (0.47–0.54)
Glucose	0.49 (1267)	0.22 (1591)	0.17 (1596)	ADE	0.22 (0.08–0.35)	0.31 (0.16–0.46)	0.47 (0.44–0.56)
CRP	0.42 (1066)	0.15 (1340)	0.13 (1352)	ADE	0.13 (0.00–0.28)	0.30 (0.12–0.46)	0.57 (0.53–0.62)

Note: [a]Pearson's correlation coefficient for MZ twins, [b]Pearson's correlation coefficient for SSDZ twins, [c]Pearson's correlation coefficient for OSDZ twins.

information criterion, the ADE model should be favored over the ACE model for these traits. The AE model was preferable for the remaining phenotypic traits. Table 4 shows the variance components from ADE and AE models.

In order to examine the extent to which adjusting for lipid lowering therapy influenced the results, each analysis was repeated without this adjustment. The correlation coefficients and heritability estimates for LDL and total cholesterol decreased slightly, while the estimates for the remaining phenotypes subject to this adjustment were unaffected.

We also investigated the effect of additionally adjusting for body mass index (BMI) by including BMI in the linear regression together with age and sex before conducting *z* score transformation. The heritability estimates for all traits remained virtually unaltered (data not shown).

In summary, additive genetic variance component was estimated to 0.66 for HDL, 0.64 for apoA-I, 0.48 for apoB, 0.50 for total cholesterol and 0.54 for TG. For these traits the dominant genetic component was not significant. Both additive and dominant genetic component were significant for LDL, glucose, and HbA1c. For LDL, additive and dominant genetic effects were estimated to 0.35 and 0.18, respectively. For glucose the corresponding estimates were 0.22 and 0.31, while for HbA1c additive genetic component was 0.16 and dominant genetic component was 0.55. In the ADE model the additive genetic component for CRP was not statistically significant, 0.13 (95% CI 0.00–0.28) while the dominant genetic component was estimated to 0.30 (0.12–0.46). Effect of nonshared environment was significant for all phenotypic traits.

Since violation of the assumption of equal shared environment between MZ and DZ (i.e., MZ twins are exposed to more trait-relevant shared-environmental influences than DZ) would mimic genetic dominance, we investigated discrepancies in reported contact frequency between MZ and DZ twins (see material and methods for definition of contact levels). MZ twins reported greater contact frequency than DZ twins, mean contact level was 3.00 for MZ twins while it was 2.57 for DZ twins (*t* test, $p < .0001$). Next, we investigated if contact frequency also was correlated with similarity in trait levels by computing the rank-order correlation (Spearman) between contact frequency and the absolute intra-pair difference in adjusted trait-levels. None of the zygosity specific correlations of trait difference and twin contact frequency reached significance except for difference in HDL among MZ twin pairs for which marginal significance was observed, $r = -0.058$, $p = .04$ (Table 5a).

The age at separation from co-twin (i.e., time shared same household environment) is also a measure of the degree of shared-environmental influences. Results showed that mean age at separation was significantly higher for MZ than for DZ twins, 19.7 years and 18.4 years, respectively (*t* test, $p < .0001$). For each separate zygosity strata the relation between absolute intra-pair difference in adjusted trait levels and age at separation was insignificant for all traits except apoA-I in MZ twins ($r = -0.061$, $p = .04$).

Discussion

We here investigate the relative importance of genes and environment for variation in lipoproteins, apolipoproteins, total cholesterol, triglycerides, glucose, and CRP levels by using a large and homogenous sample consisting of nearly 12,000 Swedish twins. Our results for additive genetic effects for HDL, apoA-I, total cholesterol, and TG are consistent with what have been demonstrated in previous publications (Table 1). It is interesting to note that a former variance partitioning analysis conducted on Swedish twins by Heller et al. showed considerably higher heritability estimates for apoB (0.78) and TG (0.72), however these results were obtained from a subgroup of younger twins and their study sample was much smaller compared to our sample. Their results on apoA-I, HDL and total cholesterol are similar to our

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Table 5

Correlation Between Absolute Intra-Pair Difference of Adjusted Trait Values and (a) Co-Twin Contact Frequency and (b) Age at Separation From Co-Twin

	MZ			DZ		
Phenotype	r^a	*p* value	*N*	r^a	*p* value	*N*
(a) Contact frequency						
HbA1c	–0.038	.19	1222	–0.021	.23	3156
CRP	–0.013	.67	1013	–0.02	.31	2613
Glucose	–0.026	.36	1226	–0.019	.29	3166
LDL	–0.015	.59	1199	–0.028	.12	3089
HDL	–0.058	.04	1226	–0.008	.63	3170
Total cholesterol	–0.006	.84	1226	–0.017	.33	3170
TG	–0.037	.19	1226	–0.006	.75	3169
ApoA-I	–0.028	.33	1225	–0.022	.22	3168
ApoB	–0.026	.36	1225	–0.023	.20	3168
(b) Age at separation						
HbA1c	–0.041	.16	1166	0	.93	3087
CRP	-0.047	.14	965	–0.001	.94	2557
Glucose	–0.007	.8	1170	0.023	.21	3095
LDL	0	.99	1144	0.013	.46	3021
HDL	–0.053	.07	1170	–0.031	.08	3101
Total cholesterol	0.012	.67	1170	0.008	.64	3101
TG	–0.055	.06	1170	–0.011	.55	3100
apoA-I	–0.061	.04	1169	–0.017	.34	3099
apoB	0.018	.52	1169	0.004	.82	3099

Note: [a]Spearman correlation coefficient.

findings (Heller et al., 1993). In addition, the contribution of nonshared environment was significant for all traits, which is also in agreement with what has previously been found. Here, we show for the first time significant effects of genetic dominance for LDL, CRP, glucose, and HbA1c in a population based twin sample. The Akaike's information criterion clearly showed that ADE is the preferred model (over ACE) for these traits. The reason for the novel findings of dominant genetic effects may be due to the enhanced power of the large and homogenous sample in our study compared to previous studies, enabling us to detect weaker variance components underlying the phenotypic traits. Another contributing factor may be the high age of the study participants, possibly leading to decreased influences from shared familial environment. In the case of CRP, additive genetic effect was found insignificant. This should not be taken as evidence for an absence of influences from additive genes (which appears biologically implausible for a quantitative trait) but indicates insufficient statistical power. Since variance component estimates are specific to the studied population, it is important to bear in mind that the obtained results not necessarily are representative of other populations or ethnic groups. Both genetic and environmental factors may be specific to particular populations that in turn may cause the differences observed between studies.

In the present study, adjustments were carried out to obtain biomarker values more similar to the original pre-drug therapy values. Even though it did not have a large impact on our heritability estimates, it did increase the genetic variance component of LDL and total cholesterol. Some preceding studies have measured the change in results after excluding all observations from individuals on lipid lowering therapy, resulting in slightly increased heritability estimates (Isaacs et al., 2007). We also performed analyses excluding all individuals on lipid lowering therapy from the dataset. The subsequent heritability estimates increased slightly (data not shown) although the confidence intervals were broader since observations were lost from approximately 1381 individuals. We decided that this was not the best approach due to the considerable loss of power. Even though the estimation on average effect sizes from medication was rough and only included the most common types of lipid lowering therapy, we argue that conducting adjustments to account for the effect of lipid lowering drugs is more preferable than ignoring the effects these medications have on the biomarker levels.

For all investigated traits the point estimate of the shared environment component is 0. Thus, there appears to be no influence from shared environmental factors in this population. In previous studies based on younger twins, the estimated effect of shared-envi-

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ronment has generally been small and rarely been significant. Beekman et al. for example, found no significant effect of shared environments when investigating lipid levels in three different populations (Beekman et al., 2002).

In the classical twin design comparing variance/covariance structures in MZ and DZ twins reared together it is not possible to model the effect of shared environment and dominance genetics simultaneously. Therefore, both influences may co-exist but their influences are not estimable in the same model. Also, the estimated additive/dominance genetic effects could potentially consist of epistasis or gene–environment interactions, consequently our results on additive and dominance genetic variance could be biased.

Another source of bias could come from violations of the assumption of equal importance of shared environmental influences between MZ and DZ twins. By using data on contact frequency and age at separation available for a majority of the study participants, we demonstrated that there was evidence for differences in two proxy variables (self-reported contact frequency and age at separation) for amount of shared environment between the zygosity classes. We found contact frequency and age at separation to differ significantly between MZ and DZ twins ($p < .0001$ for both) in this material. However, for these differences to be a problem in the variance component models, there must be an association between degree of sharing environments (e.g., contact frequency) and trait similarity (i.e., twins meeting more often, and twins with higher age at separation should exhibit more similar traits). We investigated this by testing the correlation between absolute intra-pair difference in adjusted trait values and the available proxies for amount of shared environment. For intra-pair difference in HDL, the association with contact frequency was significant in MZ twins but not in DZ twins. Intra-pair difference in HDL also showed borderline significance with age at separation in both MZ ($p = .07$) and DZ ($p = .08$) twins. This is in agreement with an earlier report based on Swedish twins reared apart where contact with twin partner post separation was found only important for HDL levels in the younger twins (age 50–65) (Heller et al., 1993). Furthermore, intra-pair difference in apoA-I, which is a component of HDL, showed an association with age at separation in MZ ($p = .04$) but not in DZ ($p = .34$) twins. It is interesting to note that for all the zygosity specific estimates of the relation between contact frequency and trait similarity, the direction of the effect is consistent, which speaks in favor of a general (albeit very weak) effect of contact frequency and similarity in levels of blood biomarkers. The consistency in direction of effect was less pronounced for age at separation. This might be expected since the average time from when the twins moved apart to the time of measurements in this study generally is close to 50 years. In conclusion, even if MZ twins report significantly higher contact frequency and higher age at separation compared to DZ twins, we only found weak evidence for this to have an impact on twin trait similarity and more so this was only shown for HDL and apoA-I. Possible mechanisms underlying the association between twin trait similarity and used proxies for shared environment could be diet or other lifestyle habits.

The study emphasizes the importance of acknowledging nonadditive genetic contribution to the risk panorama of cardiovascular diseases. Here, we show for the first time significant effects of genetic dominance for LDL, CRP, glucose, and HbA1c in a population based twin sample. The results suggest that dominant genetic models also should be considered when molecular studies are conducted in order to identify genes associated or linked to these phenotypes.

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