Title

Association of height and pubertal timing with lipoprotein subclass profile: Exploring the role of genetic and environmental effects

Authors´s name

Aline Jelenkovic[1,2,3], Leonie H. Bogl[3], Richard J. Rose [4], Antti J Kangas[5], Pasi Soininen[5,6], Mika Ala-Korpela[5,6,7,8], Jaakko Kaprio[3,9,10], Karri Silventoinen[3,11]

Affiliations

[1]Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country UPV/EHU, Leioa 48940, Spain.
[2]IKERBASQUE, Basque Foundation for Science, Bilbao 48011, Spain.
[3]Department of Public Health, Hjelt Institute, University of Helsinki, Helsinki 00014, Finland.
[4]Department of Psychological and Brain Sciences, Indiana University, Bloomington 47405, United States of America.
[5]Computational Medicine, Institute of Health Sciences, Faculty of Medicine, University of Oulu 90014, Finland.
[6]NMR Metabolomics Laboratory, School of Pharmacy, University of Eastern Finland, Kuopio 70211, Finland.
[7]Oulu University Hospital, Oulu, Finland.
[8]Computational Medicine, School of Social and Community Medicine, University of Bristol, Bristol, UK.
[9]Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki 00271, Finland.
[10]Institute for Molecular Medicine FIMM, Helsinki 00014, Finland.
[11]Population Research Unit, Department of Social Research, University of Helsinki, Helsinki 00014, Finland.

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Corresponding author's information: Aline Jelenkovic : tel: +358 9 191 27 607; fax: +358 9 191 27 600; e-mail: aline.jelenkovic@helsinki.fi

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ABSTRACT

Objectives: Little is known about the relationship between growth and lipoprotein profile. We aimed to analyze common genetic and environmental factors in the association of height from late childhood to adulthood and pubertal timing with serum lipid and lipoprotein subclass profile.

Methods: A longitudinal cohort of Finnish twin pairs (FinnTwin12) was analysed using self-reported height at 11-12, 14, 17 years and measured stature at adult age (21-24 years). Data were available for 719 individual twins including 298 complete pairs. Serum lipids and lipoprotein subclasses were measured by proton nuclear magnetic resonance spectroscopy. Multivariate variance component models for twin data were fitted. Cholesky decomposition was used to partition the phenotypic covariation among traits into additive genetic and unique environmental correlations.

Results: In men, the strongest associations for both adult height and puberty were observed with total cholesterol, low-density lipoprotein cholesterol, intermediate-density lipoprotein cholesterol and low-density lipoprotein particle subclasses (max. r = -0.19). In women, the magnitude of the correlations was weaker (max. r = -0.13). Few associations were detected between height during adolescence and adult lipid profile. Early onset of puberty was related to an adverse lipid profile, but delayed pubertal development in girls was associated with an unfavorable profile, as well. All associations were mediated mainly by additive genetic factors, but unique environmental effects cannot be disregarded.

Conclusions: Early puberty and shorter adult height relate to higher concentrations of atherogenic lipids and lipoprotein particles in early adulthood. Common genetic effects behind these phenotypes substantially contribute to the observed associations.

Keywords: Height, genetic pleiotropy, lipoproteins, puberty, twins.

Height is an important trait defining human morphology, and is determined by the genetic background, nutrition and other life conditions during childhood and adolescence (Batty et al., 2009; Silventoinen et al., 2003). Adult stature has also shown to be affected by pubertal timing both in men and women (Biro et al., 2001; Silventoinen et al., 2008). Accumulating evidence suggests that both height and timing of puberty are related to the lipid profile. Short stature in childhood and adulthood is associated with higher levels of total cholesterol (total-C) and low-density lipoprotein cholesterol (LDL-C), whereas its relationship with high-density lipoprotein cholesterol (HDL-C) and triglycerides is more divergent (Benetou et al., 2006; Fujita et al., 2011; Henriksson et al., 2001; Kouda et al., 2003; La Batide-Alanore et al., 2003; Orchard et al., 1980; Ranasinghe et al., 2011). Likewise, early pubertal timing has been related generally to an unfavourable lipid profile (Feng et al., 2008; Frontini et al., 2003; Widen et al., 2012). However the biological mechanisms of these associations are poorly understood. Several studies have demonstrated that height, pubertal timing and multiple metabolites are quite highly heritable (Benyamin et al., 2007; Jelenkovic et al., 2011; Kettunen et al., 2012; Silventoinen et al., 2003; Wehkalampi et al., 2008), but to our knowledge, no study has utilized a multivariate genetic analysis to analyse the relationship of height and puberty with lipid profile. Therefore, whether these associations are attributable to genetic factors remain largely unknown.

Circulating metabolites have key roles in numerous biological pathways. Until recently, the search for metabolic risk factors had focused on only relatively few metabolites, but recent investigations suggest that alternative lipoprotein measures may improve risk prediction (Bertram et al., 2009; Chasman et al., 2009; Tukiainen et al., 2012). This is relevant because alterations in lipid and lipoprotein levels represent one of the major risk factors for coronary heart disease (CHD) (Kuulasmaa et al., 2000; Levy, 1987), which in turn has been inversely associated with

height during childhood and adolescence (Silventoinen et al., 2012), pubertal timing (Lakshman et al., 2009) and adult height (Paajanen et al., 2010). The concentration of distinct lipoprotein subclasses, as well as the average particle size, can be measured by nuclear magnetic resonance (NMR) spectroscopy (Ala-Korpela, 2008; Ala-Korpela et al., 2009; Jeyarajah et al., 2006).

Because the association between adult height and lipid profile is shaped over the life course, detailed knowledge of links with critical developmental events may highlight important biological mechanisms. Major limitations of existing studies include the restriction of the analyses to basic serum lipids and the lack of exploration of common genetic and environmental factors between growth and lipid profile. The present data offer us an opportunity to analyse the association of height and puberty with a large set of serum lipids and lipoproteins in a population-based longitudinal sample of Finnish twins. Accordingly, the specific aims of this study are as follows: 1) to assess the relationship between height and lipid profile in young adulthood, 2) to ascertain whether this association is due to common genetic and/or environmental factors, 3) to analyse the effect of earlier physical development (height during adolescence and pubertal timing) on the lipid profile later in life and 4) to explore possible sex differences in these associations.

MATERIALS AND METHODS

Data sources

The data were derived from the FinnTwin12 study, described in detail elsewhere (Kaprio et al., 2002). This cohort includes five consecutive birth cohorts of Finnish twins (2,600 families with twins) born in 1983-1987. Questionnaires were used to collect data on height at ages 11-12 (mean age: 11.4 years), 14, 17.5 years and adult age (19-24 years) and pubertal development scale

(PDS). Zygosity was determined by well-validated items on physical similarity during school age. DNA analysis confirmed questionnaire assignment of zygosity in 97% of these same-sex adolescent Finnish twins (n=395 pairs). During the fourth wave of the data collection (years 2006-2009), 780 of these young adult twins visited the study clinic and gave a blood sample for the nuclear magnetic resonance (NMR) spectroscopy analysis, and their height and weight were measured. Self-reported height from a questionnaire filled out at home prior to the clinic visit was found to be highly correlated with measured height (r=0.99, N=797). The exclusion criteria for this study were lipid-lowering medication, pregnancy and missing NMR data, which left 719 participants available for the analyses. Finally, our sample included 298 complete twin pairs: 123 monozygotic (MZ) (46 brother-brother and 77 sister-sister), 98 same-sex dizygotic (DZ) (47 male and 51 female) and 77 opposite sex dizygotic (OSDZ) pairs. Data collection and analysis were approved by the ethics committee of the Department of Public Health of the University of Helsinki and the Institutional Review Board (IRB) of Indiana University. Written informed consent was obtained from all participants.

Assessment of pubertal timing

Pubertal timing was estimated from PDS score and pubertal height growth. The five-item PDS questionnaire is described in detail elsewhere (Dick et al., 2001; Petersen et al., 1983). PDS score is large for early maturers and small for late maturers. Pubertal timing based on pubertal height growth was assessed using a previously evaluated quantitative estimate of the timing of the pubertal height growth spurt (Wehkalampi et al., 2008). At age when height growth peaks in the general population, relative height (height SD) is influenced by pubertal timing, as well as by genetic height potential (Tanner, 1986). By calculating the difference between height SD at pubertal peak height velocity (phv) age and at adulthood, the influence of genetic height potential

can be excluded. Therefore, the change in height SDs between these ages reflects timing of puberty (HD:SDS). The age of 12 in girls and 14 in boys represent ages of phv in the general population (Tanner, 1976). In the present study, height SD at age 11-12 and 14 years was subtracted from height SD at adult age creating the difference: HD:SDS12 and HD:SDS14.

NMR-derived serum lipids and lipoprotein subclass profile

Serum lipid and lipoprotein subclass concentrations were measured by proton NMR spectroscopy as previously described in detail (Soininen et al., 2009; Tukiainen et al., 2012). This NMR platform has recently been applied in various extensive epidemiological and genetics studies (Chambers et al., 2011; Inouye et al., 2010; Kettunen et al., 2012). In the present study, concentrations of the lipoprotein subclasses were grouped in 3 subclasses (large, medium and small) of HDL, LDL and VLDL (see Table 1). The mean particle size for HDL, LDL and VLDL particles was calculated by weighting the corresponding subclass diameters with their particle concentrations. IDL particles were included in the LDL measure. Apolipoprotein B (apoB) and apolipoprotein A-1 (apoA-1) were estimated from an extended version of the Friedewald formula (Niemi et al., 2009).

Statistical methods

We conducted the statistical analyses using the Stata statistical software package (release 12.0; Stata Corporation, College Station, Texas). Since NMR derived particle concentrations were not normally distributed, logarithmic transformation was applied to these variables. Height, pubertal timing and metabolite values were adjusted for age by linear regression model, including sex as a covariate. In the analyses where twins were treated as individuals, the standard errors were corrected for clustering of twin pairs by survey methods (Rao and Scott, 1984). The resulting

residuals were used as input phenotypes for the following analyses. Principal components analysis was used to determine the number of principal components that explain most part of the variance of the studied lipids and lipoproteins. Since the strong correlation among these metabolites makes the traditional Bonferroni correction for multiple testing too conservative, the number of principal components provides a more permissive p-value threshold. In this study, the first five principal components explained more than 97% of the variance, allowing associations to be significant at $p < 0.01$ after the Bonferroni correction.

In genetic analyses we used quantitative genetic modeling for twin and family data (Neale et al., 2003). Briefly, the analysis is based on the fact that MZ twins share the same gene sequence, whereas DZ twins share, on average, 50% of their genes identical-by-descent. Variance of a trait can be partitioned into additive genetic effects (A: correlated 1.0 for MZ and 0.5 for DZ pairs), common (shared) environmental effects (C: by definition, correlated 1.0 for all pairs), unique (nonshared) environmental effects (E: by definition, uncorrelated in all pairs) and dominance effects (D: 1.0 for MZ and 0.25 for DZ pairs). Because pubertal timing and growth differ between sexes, we did not test whether the same sets of genes influence the variation of height in boys and girls and we present parameter estimates for each sex separately. This decision was also based on our previous results which showed that parameters estimated for height during adolescence could not be set equal between males and females in this cohort (Jelenkovic et al., 2011). The genetic models were carried out by the Mx statistical package (Neale and Maes, 2003) for each phenotype. We continued the analyses by studying the associations of height and pubertal timing with lipid profile using multivariate correlation model based on reparametrization of Cholesky decomposition. This procedure decomposes the variation and covariation in the data into a series of uncorrelated genetic and environmental factors. According to this model, the trait correlation

between two phenotypes is due to additive genetic correlation (r_A) indicating the same or closely linked genes, and unique environmental correlation (r_E) indicating same or correlated environmental factors unique to each twin individual.

RESULTS

Characteristics of the participants

The characteristics of the sample are reported separately by sex in Table 1. Mean values for height were expectedly higher in boys than in girls over the study period; only at 11-12 years girls were slightly taller. Men also presented a significantly greater weight and body mass index (BMI) than women at adult age. As expected, the PDS score was greater for females both at 11-12 and 14 years of age. Women had generally a more favourable lipid profile than men characterized by higher HDL-C, increased HDL-C/ LDL-C, larger HDL and LDL particle size, higher HDL particles concentration, higher apoA-1 and a lower apoB/apoA-1 ratio. However, women had higher total-C, IDL-C and LDL particle concentration.

(Table 1 here)

Association between height and lipid profile

The number and magnitude of significant phenotypic correlations between adult height and lipid profile was greater for men than for women (Table 2). Taller individuals tend to present lower levels of serum lipids and lipoproteins than shorter individuals. Since very small changes were detected when additionally adjusted for BMI (Supplementary Table 1), changes that were non-existent in women, we consider that the non-adjusted correlations offer greater clarity in interpreting the data. Very few correlations were consistent between height during growth and

lipid profile in young adulthood (Supplementary Table 2). Among men, stature at 17 years was inversely associated with medium HDL and apoA-1, whereas shorter women tended to have a worse lipid profile. No evidence for C or D effect was found in this study (data not shown). Accordingly, we chose to use the additive/specific environmental (AE) model for all phenotypes in further multivariate analyses. Genetic correlations followed the pattern of phenotypic ones, but specific environmental correlations between height and lipid profile were non-significant (Table 2 and Supplementary Table 2).

(Table 2 here)

Associations between pubertal timing and lipid profile

None of the correlations were significant for PDS at 11-12 years (max. $r = 0.07$). For PDS14, only those that are significantly associated are presented (Table 3). Although detectable only in females, correlations between PDS14 and HDL variables were positive, suggesting that very late puberty was related to an unfavourable HDL particle profile; this was confirmed by the results from HD:SDS14 (Table 4). HD:SDS12 was inversely associated with small LDL in females and with several unfavourable serum lipids and lipoproteins in males.

(Table 3 here)

(Table 4 here)

DISCUSSION

In this longitudinal population-based twin study, detailed information is provided on the relationship of height and pubertal timing with lipid profile. We report several associations that have not been described before, as well as novel findings on the genetic background determining these relationships. The results highlight that being taller is associated with the propensity to have lower concentrations of atherogenic lipids and lipoproteins. Likewise, early onset of puberty is related to an overall adverse lipid profile, but a delayed puberty can also have unfavourable effects. The revealed associations are mainly mediated by genetic factors, although unique environmental factors shared by traits cannot be disregarded.

The general pattern across populations (Bertram et al., 2009; La Batide-Alanore et al., 2003; Pierce et al., 2010; Widen et al., 2012) in determining a more favourable lipid profile in women than in men was observed in our analyses, as well. It has been suggested that the significantly higher HDL-C content in females could be due to a higher lipid synthesis (Bertram et al., 2009). In this study of Finnish young adult twins, height and puberty were more closely related to the studied lipid and lipoprotein levels in males than in females, which justifies analyzing these relationships separately by sex. The small change observed in the associations when additionally adjusted for BMI is in agreement with the findings of Henriksson et al. (2001), and may be explained by the correlation of height with BMI (0.12 and 0.05 in men and women, respectively). That is, the adjustment for BMI, which is positively correlated with a worse lipid profile, would be increasing the associations between height and unfavourable lipids.

According to the research literature, taller children and adults tend to have lower total-C and LDL-C content but the pattern is not so well established for HDL-C, for which associations vary from non-significant to negative and positive (Benetou et al., 2006; Fujita et al., 2011; Gunnell et

al., 2003; Henriksson et al., 2001; Kouda et al., 2003; La Batide-Alanore et al., 2003; Orchard et al., 1980; Ranasinghe et al., 2011). It has been reported that LDL-C concentration tracks better from childhood to adulthood than do HDL-C levels (Uiterwaal et al., 1997; Webber et al., 1991) suggesting that HDL-C level could be affected by additional sources of variability, especially during the pubertal stage where a change in central pattern of body fat occurs (van Lenthe et al., 1998). The relationship of height with triglycerides has been less studied, and the results are divergent (La Batide-Alanore et al., 2003; Orchard et al., 1980; Ranasinghe et al., 2011).

Concerning lipoprotein subclasses, the pattern for large HDL is very similar to HDL-C in our sample. This is consistent with the findings of Mora et al. (2009), who reported a high correlation between HDL-C and large HDL in comparison to that with small and medium HDL, thus suggesting that the potentially protective effects of HDL-C may be due to the large HDL particles. The strongest (negative) associations between adult height and lipoproteins were found for LDL particles in both sexes. Recent studies have suggested that LDL particle concentration is a significant predictor of future CHD risk (El Harchaoui et al., 2007; Mora et al., 2009) and that CVD risk may be more closely related to atherogenic lipoprotein particle concentration than to LDL-C (Barter et al., 2006). In the study of Mora et al. (2009), HDL and VLDL particle size were significantly correlated (inversely and directly, respectively) with CVD even after adjustment for the respective concentrations; however, we only observed a significant association between HDL particle size and PDS14 in women.

Earlier developing boys had more adverse lipid and lipoprotein levels. In women, pubertal timing (at 11-12 years) correlated only with small LDL particle concentration, which has been associated with more than a three-fold increase in the risk of CHD (Lamarche et al., 1999). In

contrast to the findings from the Northern Finland Birth Cohort 1966 (Widen et al., 2012), in which an inverse relationship was found between puberty and HDL-C both in men and women at the age of 31 years, associations with "good" lipids levels were not significant in our study. These authors also observed that triglycerides in men remain significant after adjustment for several covariates (Widen et al., 2012); however, Pierce et al. (2010) reported that the association between pubertal timing and lipids were completely explained by BMI or waist circumference. A noteworthy finding of the present study is the consistent inverse association between pubertal development at 14 years (both HD:SDS14 and PDS14) and a favorable lipid profile in women. As pubertal development is normatively in an advanced stage at 14 years in girls, these results suggest that very late puberty is related to an unfavorable lipid profile.

Our results showed that the associations of height and pubertal timing with the lipid profile are explained to a great extent by pleiotropic effects, that is, genes acting to increase the value of one trait also increase (positive association) or decrease (negative association) the magnitude of the other. It is important to note that in the previous studies (Jelenkovic et al., 2011; Kettunen et al., 2012; Wehkalampi et al., 2008) the effect of the common environmental component was much smaller than that of the genetic component, and thus even if it exists, the associations appear to be mainly because of common genetic factors. Common genetic factors between height and lipid profile could be supporting the hypothesis of "thrifty genotype" (Neel,1962). Based on this hypothesis, some genes could prompt the body to store up fat for later times of starvation and therefore were, advantageous in times of alternating food abundance and scarcity. Thus, if height is inversely related to serum lipid and lipoprotein concentration, we can speculate that natural selection might have led to shorter individuals, who would have higher probability to survive until the reproductive age because they need to use less energy for growth. However, it should be

mentioned that there is not necessarily any association between total energy intake and serum lipid levels. For example, it has been observed that total-C, LDL-C concentrations are higher in subjects with an apolipoprotein E (apoE) e4 allele (Bennet et al., 2007; Mahley et al., 2000; Ordovas, 1999) and, in turn, e4 allele frequencies follow a curvilinear relationship with absolute latitude (Eisenberg et al., 2010). Since mean stature is greater in northern than in southern part of Europe, apoE e4 allele frequency is related to height, but to our knowledge apoE is not a height gene. However, other height genes have shown to be associated with lipid metabolism. That is, genome-wide association studies confirmed that NCAPG/LCORL locus has an effect on human height (Gudbjartsson et al., 2008; Weedon et al., 2008) and that NCAPG 1442M mutation is related to postnatal growth (especially with the onset of puberty) and lipid deposition in bovine cattle (Weikard et al., 2010).Therefore, similar physiological mechanisms seems to operate in different mammalian species. Finally, the influence of unique environmental factors on the relationship of height at 11.5 years with HDL-C and total HDL in boys might be due to the effect of nutrition before puberty, that is, favorable nutrition would result in taller individuals with a better lipid profile. To our knowledge, no study has focused on common genetic and environmental factors between pubertal timing and lipid profile, and the scarce data available for height were inconsistent (Bastarrachea et al., 2007; Butte et al., 2006); that is, height showed negative genetic correlation with total-C and HDL-C (-0.33 and -0.27, respectively) in childhood (Butte et al., 2006) and non-significant with total-C and total-TG in adulthood (Bastarrachea et al., 2007).

Our study has several strengths. It is unique in that it provides comprehensive information on the pleiotropic effects determining the relationship of height and puberty with a detailed lipid profile in males and females. The four measures of height available from this longitudinal study allow us

also to detect changes in these associations during the growth process. Furthermore, we have information on puberty measured by PDS at two time points in both sexes. But our study has potential limitations, as well. The main limitation of the study is our relatively small sample size, creating challenges in evaluating statistical significance for some of the associations of interest. Reliance on self-estimated assessments (height at 11-12, 14 and 17 years) may cause some inaccuracy, but self-reported final height was highly correlated with measured height. Finally, another limitation is the issue of multiple testing, with potentially false positive associations among the correlations evaluated. Although the Bonferroni correction based on principal components showed a more permissive threshold ($p<0.01$) than the traditional correction, we still believe that associations with $p <0.05$ cannot be disregarded because many related lipids and lipoproteins showed the same trend and thus provides considerable robustness to the study. However, these results need to be replicated in other cohorts.

To summarize, adult height and pubertal timing are related to several serum lipids and lipoproteins in young adulthood, both in males and females. Although unique environmental factors shared by traits cannot be disregarded, these associations are in great part determined by common genetic factors. Shorter stature, early pubertal timing in boys and delayed puberty in girls are associated with a worse lipid profile. Despite of the relative small magnitude, the pattern of these associations is consistent. Thus, both being shorter and develop early/very late might be considered as risk factors for adult lipid abnormalities.

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