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THE EFFECT OF GENETICAL AND ENVIRONMENTAL FACTORS ON LIPIDS: A TWIN STUDY

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ABSTRACT

To assess the relative contribution of genetic and environmental factors (particularly androgens) on circulating levels of lipid fractions, serum androgen and lipid fractions were measured in 34 pairs of female-female twins aged from 15-45 years, some of whom were discordant for polycystic ovary syndrome (PCOS) diagnosed by ultrasound. Nineteen pairs were monozygotic twins (MZ) and 15 pairs were dizygotic twins (DZ). Five pairs of MZ and 6 pairs of DZ were discordant for scan-PCO. We measured serum concentrations of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TRIG), lipoprotein (a) [LP(a)], and apolipoprotein B (apo B). Also, testosterone (T), dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG) and 3α-androstanediol glucuronide (3α-diol G) levels were measured. Transabdominal ultrasound was performed. Serum levels of TC, HDL-C, TRIG, LP(a) and apo B in the twins with PCO were not significantly different from the levels in their matched co-twins with normal ovaries. There were no significant correlations between androgen-hormones including T, DHEAS and 3α-diol G with any of the lipid measurements. Body mass index (BMI) was positively correlated with TRIG, LP(a) (both $p<0.05$) and negatively correlated with HDL-C ($p<0.001$). SHBG was negatively correlated with TRIG and LP(a) and positively associated with HDL-C ($p<0.05$). Insulin was significantly correlated with TRIG ($p<0.001$) and negatively with HDL-C ($p<0.01$). The MZ intraclass correlation exceeded that of the DZ for all the lipid variables measured. The heritability estimates for LP(a), apo B, TC and HDL-C were 0.95, 0.56, 0.48 and 0.54, respectively. However, the intraclass correlation coefficient for TRIG was not significantly different between MZ and DZ but maximum likelihood analysis indicated that at least 10% of the variance of circulating TRIG concentration is determined by genetic factors. We conclude that twins discordant for PCOS do not

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have significantly different lipid profiles. We were unable to show any significant effect of androgens on the lipid fractions measured. The results confirm that levels of LP(a), HDL-C, TC and apo B are under significant genetic control and that this is particularly so for LP(a). However, only 10% of the variation in TRIG levels could be attributed to genetic influences after controlling for age and obesity. Increase in BMI and insulin had a significant adverse effect on the lipid profile in these female twins, effects which may enhance coronary risk.

Keywords: Polycystic ovary syndrome, androgens. lipids, twin study.

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INTRODUCTION

There have been many studies of heritability factors in the regulation of circulating lipid levels. In general, these have shown that LP(a), an atherogenic lipoprotein, is largely genetically regulated[1] and that this is mediated by its unique apolipoprotein apo(a). On the other hand apo B, the carrier protein for the atherogenic low density lipoprotein cholesterol (LDL-C), which is also a carrier protein for LP(a), is generally subject to much less genetic regulation. So too is the anti-atherogenic HDL-C and its principal carrier protein, apo A1 (apo A1); and the same is true for the level of TC.[2-4] It is also known that sex hormones may influence lipoprotein levels.[5-6] Serum androgen levels are increased in PCOS[7] and some have expressed concern about the coronary risk factors in women with PCOS.[8]

The availability of female twins of reproductive age, some of whom were discordant for the presence of PCOS, provided us with an opportunity to compare lipid and sex hormone levels in MZ and DZ twins who were discordant for PCOS. Since the variables we measured may be affected by obesity,[9] results were corrected for BMI.

MATERIAL AND METHODS

Two hundred and fifty female-female twin pairs aged 15-45, living in the Sydney metropolitan area were identified from the National Health and Medical Research Council (NH & MRC) twin register and were invited to take part in the study. Replies were received from 366 individuals, but in some cases the co-twin was unwilling to participate in the study. We excluded those twins who were taking contraceptive pills. So finally 34 twin pairs (68 individuals) were included in the study.

There were 19 pairs of MZ and 15 pairs of DZ twins. Subjects completed the consent form approved by the ethics committee of Royal Hospital for Women. All the subjects were examined during their menstrual cycle (day 5-7). Height and weight were measured and after an overnight fasting, blood was sampled. No subjects had taken hormonal medications including contraceptive pills for at least 3 months preceeding sampling. The zygosity of twins were determined by asking about their physical similarity, and frequency of confusion as children by parents, teachers, and strangers. A recent study in the Garvan Institute has shown that comparing the subject's stated zygosity with DNA matching has over 99% accuracy.[10]

All the subjects had undergone transabdominal ultrasound using an ACUSON 128 machine with a 3.5 MHz linear transducer. Polycystic ovaries were diagnosed using Adam's criteria,[11] namely the existence of 10 or more peripheral small (2-6 mm) follicles. Ultrasound is a highly reliable method for diagnosing PCO.[12] The precision of ultrasound diagnosis of PCO in our department has been estimated by a prospective study.[13] Using laparoscopy as the gold standard, ultrasound was 95% sensitive and 100% specific for diagnosing PCO, with no false positives. Clinical examination and laboratory measurements have also been used to confirm the ultrasound diagnosis.

Venous blood was drawn from the subjects after overnight fasting. The plasma was collected by centrifugation and immediately stored at -20°C before it was transported in dry ice to the central laboratory for lipid measurements. The samples were then stored at -70°C until analysis. Sex hormones were measured in the Endocrine Laboratory of the Royal Hospital for Women. Plasma TC, HDL-C and TRIG levels were measured in the Department of Clinical Chemistry, Prince of Wales Hospital, Sydney using a Cobas Bio analyzer (Hoffman-LaRoche, Basel, Switzerland). Plasma apo B and Lp(a) levels were measured by an enzyme linked immunosorbent assay (ELISA) developed in the Department of Cardiovascular Medicine, Prince Henry Hospital.[14-15] The intra- and inter-assay coefficients of variation (CV)s were 4.7% and 8.6% for the apo B assay and for the LP(a) assay

Table I. The mean values (±SD) for lipids, according to zygosity.

Parameters		MZ (n= 38)	DZ (n= 30)	P value
LP(a)	mg/L	707.95±3.67	524.81±3.28	0.911
HDL-C	mmol/L	1.02±0.47	1.12±0.42	0.578
TC	mmol/L	4.40±1.01	4.83±0.85	0.796
apo B	g/L	0.99±0.37	0.82±0.21	0.073
TRIG	mmol/L	1.02±0.47	1.01±1.02	0.934

Table II. Intraclass correlation coefficient and model fitting analysis of lipid values in MZ and DZ twins.

Parameters	r_{MZ}	r_{DZ}	H^2 value[a]	GE vs GCE	CE vs GCE	%G
LP(a)	0.95	0.47	(0.96)	0.00	0.96	96
HDL-C	0.74	0.39	(0.54)	0.80	0.26	55
TC	0.59	0.21	(0.48)	0.96	0.46	49
apo B	0.56	0.004	(0.56)	0.33	0.67	90
TRIG	0.55	0.49	(0.12)	0.28	0.85	10

a: Heritability estimates or H^2 were calculated according to Falconer's formula: $H^2 = (r_{MZ}-r_{DZ}) / (1-r_{DZ})$ after adjustment for BMI.

4.5% and 4.5%. The precision profiles of these ELISAs were constantly monitored by analyzing three internal quality control samples with different concentrations. All the lipid measurements were CDC standardized. Our methodology and precision data for the measurements of T, DHEAS and SHBG have been published elsewhere.[10] Radioimmunoassay was employed to measure 3α-diol (DSL, Texas) with a 10% CV for inter-assay.

The data was analyzed by using a conventional twin statistical model which reasons that the observed quantitative variable's trait is an additive function of the genetic effect (G), the environmental effect (C) and the effect of specific factors related to a particular individual within the twin pair (not shared by member of a twin pair), including measurement error (E). A full description of twin statistical analysis has been given previously.[13] All computations were done by the SAS statistical analysis system (1988). Body mass index (BMI) was calculated using the following formula: Weight (kg)/Height (m)^2. All the measured parameters were adjusted for BMI and age.

RESULTS

Sample analysis

In the study sample as a whole, the median value for Lp(a) was 168 mg/L with a range from 2 to 1600 mg/L. The HDL-C levels ranged from 0.5-2.5 mmol/L with a median level of 1 mmol/L. TC levels were from 2.1 to 7.7 mmol/L, with an overall median of 4.70 mmol/L. The median value for TRIG was 0.9 mmol/L (range 0.5-4.6), and for apo B level was 0.92 g/L (range 0.35-1.83). The mean value for age and BMI of the participants was about 28.32 (±1.16) and 23.13 (±0.51), respectively. Also, the mean level of the sex hormones measured were as follows: T 1.14 (±0.9), DHEAS 5.78 (±0.4), SHBG 67.61 (±1.07), 3α-diol 3.82 (±0.25).

Scan-discordant twins

Eleven pairs of twins were scan-discordant (i.e., one twin had scan-PCO and the co-twin was scan-normal); 5 MZ and 6 DZ pairs. Seven out of 11 affected discordant

twins had at least one abnormal biochemical result (a high level of T, DHEAS, LH or a low level of SHBG) to support the ultrasound diagnosis. Using the Wilcoxon matched pairs test to compare parameters of all 11 scan-discordant twin pairs, no significant difference was found for any of the lipid measurements. Our results could not be explained by differences in BMI since each trait was adjusted for BMI. Thus we were unable to demonstrate a significant difference for any measured lipid fraction between scan discordant twins.

Correlations between various assays of lipids and sex hormones, BMI and age

Overall, in this group of 68 women, there were no correlations between lipids and T, DHEAS or 3α-diol. However, insulin was significantly positively correlated with TRIG ($r= 0.57$, $p<0.001$) and negatively with HDL-C ($r= -0.30$, $p<0.01$). BMI had significant correlation with TRIG ($r= 0.40$, $p<0.001$), HDL-C ($r= -0.48$, $p<0.0001$), Lp(a) ($r= 0.32$, $p<0.01$) and apo B ($r= 0.37$, $p<0.01$). SHBG levels were also correlated with TRIG ($r= -0.33$, $p<0.05$), and HDL-C ($r= 0.35$, $p<0.05$). Also, SHBG was negatively correlated with BMI ($r= -0.50$, $p<0.0001$). Age was not significantly correlated with any lipid fractions.

Genetic analysis

The mean lipid levels were calculated according to zygosity of twins (Table I). There was no significant difference between MZ and DZ twins for mean levels of lipids. The pairwise correlation coefficients for all the measured lipids are shown in Table II. For all the variables, the MZ within-pair correlation (r_{MZ}) exceeded that of the DZ pairs (r_{DZ}). This difference was found to be significant for LP(a) where $r_{MZ}= 0.95$ and $r_{DZ}= 0.47$ ($p<0.0001$, $h^2= 0.96$). The heritability estimates were high for HDL-C ($h^2= 0.54$), TC ($h^2= 0.48$) and apo B ($h^2= 0.56$). The r_{MZ} of TRIG exceeded the r_{DZ}; however, its heritability value was low ($h^2= 0.12$) (Table II). To clarify these results the genetic effect was tested for each variable using the model fitting approach. A p-value was obtained by comparing model 2 (y= m+C+E) with model 1 (y= m+C+G+E). The lower this value, the greater is the genetic significance. For example, as shown in Table II, the p-value for Lp(a) is very low ($p<0.000$) suggesting that 96% of Lp(a) profile is genetically determined. On the other hand, the environmental effect was assessed from a comparison of model 3 (y= m+G+E) with model 1. The p-value for Lp(a) was then not significant, suggesting that environmental elements have a minor role in the regulation of Lp(a). A genetic effect could also be calculated for TRIG. This showed that only 10% of the variation in the level of this lipid is genetically determined and that environmental factors have a major influence on the variation of TRIG serum concentrations. The genetic contribution of the levels of the other measured lipids are also summarized in Table II. The values (%G) for the other lipids were around 50% or more and was very high for Lp(a) and apo B.

DISCUSSION

We conducted this study in a defined twin population. All were female, of reproductive age (15-45) and on no medication including the contraceptive pill. All had lived their childhood and early adulthood together, at least until the age of 16 years, so that demographic and lifestyle factors were shared. Some were discordant for PCOS. Thus if the difference in the androgenic hormone concentrations between the discordant twins is relevant to circulating lipid levels in this age and gender group, it is reasonable to anticipate that these differences would have emerged in our study. No such differences were found.

The analysis of our discordant twins indicated that PCOS and its associated altered androgenic hormone profile do not affect lipid levels in this age group. There were no significant correlations between T, DHEAS, SHBG, 3α-diol and any of the lipids measured. However, a strong negative correlation between BMI and HDL-C ($p<0.0001$), and positive correlations with TRIG ($p<0.001$), LP(a) and apo B ($p<0.01$ for each) were found. SHBG and BMI were significantly correlated.[16] SHBG was also negatively correlated with TRIG and positively with HDL-C. These results are consistent with these lipid levels being more influenced by obesity than by androgenic hormones in women of reproductive age. Also, the association of insulin, TRIG and HDL-C confirms that insulin may play an important role in the regulation of some lipid fractions.

The availability of MZ twins who share identical gene types and DZ twins who share 50% of the segregating genes enabled us to assess the contributions of heredity and environment to the circulating levels of the lipid variables we measured. For example, plasma LP(a) appeared to be largely controlled by genetic factors. The correlation between MZ twins was 0.95 and for DZ co-twins 0.47, resulting in a heritability value of 0.96. This finding is consistent with previous studies using different methodology,[17-19] although a few exceptional pairs have been reported in which the co-twin's difference in LP(a) levels was large.[19]

The heritability estimates of HDL-C and TC in our study were also similar to those reported in other twin studies.[4,20,21] The intraclass correlations for MZ twins were much higher than for DZ twins reflecting the genetic influence on the regulation of the levels of these lipid variables. For apo B the intraclass correlation in MZ twins was also significantly higher than in DZ twins consistent with a heritability value of 0.54, a result similar to that

found in a group of American twins.[17] However, our results did not show a major genetic effect for the regulation of TRIG levels. The heritability estimate for this lipid has usually been reported as high whereas in our study it was low–approximately 10%–after adjustment for age and BMI.

In summary, our study of female twins demonstrates that discordant twins with scan-proven PCOS have similar levels of lipids as their matched co-twins with normal ovaries. Our study also shows highly significant genetic contributions to circulating levels of LP(a), HDL-C, TC and apo B and that this is particularly so for LP(a) which is almost entirely regulated by genetic factors. However, environmental factors are more important than genetic in the regulation of circulating TRIG levels.

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