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RAPID COMMUNICATION

Monosomy for the X-Chromosome Is Associated with an Atherogenic Lipid Profile

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Context and Objective: Men typically have a more atherogenic lipid profile than women characterized by higher low-density lipoprotein (LDL) cholesterol and triglyceride levels and reduced lipid particle size, contributing to a greater risk for coronary disease. To determine whether X-chromosomal gene dosage affects lipid metabolism independent of sex steroid effects, we compared lipid profiles in age- and body mass-matched young women with ovarian failure, differing only in X-chromosome dosage.

Design, Setting, and Patients: Women with premature ovarian failure associated with monosomy X or Turner syndrome (TS, n = 118) were compared with women with 46,XX premature ovarian failure (n = 51) in an in-patient clinical research center unit at the National Institutes of Health. These women were normally on estrogen replacement treatment but discontinued the estrogen 2 wk before study.

Major Outcomes: Fasting lipid levels and nuclear magnetic resonance lipid particle profiles in the two study groups were the major outcomes.

Results: Average age and body mass were similar in the two groups of women, but LDL cholesterol ($P = 0.001$) and triglyceride levels ($P = 0.0005$) were higher in the TS group. Also among women with TS, average LDL particle size was reduced ($P < 0.0001$) and LDL particle concentration increased, with a 2-fold increase in the smallest particle categories ($P < 0.0001$). Whereas total high-density lipoprotein cholesterol levels were similar, high-density lipoprotein particle size was significantly smaller in women with TS, compared with women with premature ovarian failure ($P < 0.0001$).

Conclusions: Women with 45,X with ovarian failure exhibit a distinctly more atherogenic lipid profile than 46,XX women with ovarian failure, suggesting that the second X-chromosome contributes to a more salutary lipid profile in normal women, independent of sex steroid effects. **(*J Clin Endocrinol Metab* 91: 2867–2870, 2006)**

WOMEN ENJOY A more salutary lipid profile than men, which is thought to contribute to their relative protection from coronary disease. Specifically, healthy young women have higher plasma high-density lipoprotein (HDL) and lower low-density lipoprotein (LDL) cholesterol levels (1, 2), larger LDL and HDL particle size (3–6), and lower triglyceride levels than men (5, 7, 8). The role of endogenous estrogens in these metabolic differences between the sexes remains controversial. Oral estrogen treatment increases HDL and reduces LDL cholesterol levels but also increases triglycerides (9). However, these changes appear due to first pass effects on the liver by high-dose, oral estrogens, with more physiological, transdermal estrogen treatments having much less pronounced and quite variable effects on lipids (10, 11). Total and LDL cholesterol levels tend to increase with age in both sexes (12), but in women it is assumed that this is due to loss of ovarian hormones at menopause, rather than, for example, a more sedentary lifestyle (12). We have explored the notion that in addition to sex steroids, differences in X-chromosome gene dosage may contribute to gender-specific differences in physiology and disease processes.

Women with monosomy X or Turner syndrome (TS) have an increased risk for ischemic heart disease (TS) (13, 14). In the past it was generally assumed that this increased risk was due to the premature ovarian failure associated with TS. However, in recent years the view of ovarian hormones as protective from ischemic heart disease has lost ground. An alternative explanation for the relative protection in women from ischemic heart disease is that the second X-chromosome may somehow be cardioprotective. This view has gained support with the recent findings that a substantial number of X-chromosome genes escape inactivation (15, 16), suggesting that normal women experience differential X-linked gene dosage effects, compared with normal men who are monosomic for the X-chromosome. To investigate the effect of X-chromosome dosage on lipid metabolism, we have evaluated lipids in women with TS in comparison with karyotypically normal women with premature ovarian failure (POF). In a preliminary study, we found that LDL cholesterol and triglyceride levels were significantly higher in women with TS (17). Because lipid particle size is an equally if not more important predictor of coronary disease independent of absolute lipid levels (6, 18, 19), in the present study, we compared lipids/profile, insulin sensitivity, and lipid particle size using nuclear magnetic resonance (NMR) spectros-

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Abbreviations: BMI, Body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance; POF, premature ovarian failure; TS, Turner syndrome.

copy in age- and body mass-matched TS and POF study groups.

Subjects and Methods

Study subjects

Study subjects were enrolled in intramural National Institute of Child Health and Human Development protocols on TS and POF at the National Institutes of Health (NIH) Clinical Research Center. All study subjects signed informed consents approved by the National Institute of Child Health and Human Development Institutional Review Board. Inclusion in the POF protocol requires a normal 46,XX karyotype, more than 4 months amenorrhea, and elevated FSH levels in independent determinations at least 2 months apart in women aged 18–40 yr. Inclusion in the TS study requires a 50-cell karyotype by G banding with 70% or more of cells showing absence or abnormality of the second sex chromosome in girls/women aged 7–70 yr. The karyotype distribution for TS subjects in the lipid profile comparison (Table 1) was 57% 45,X (66 of 116), 24% mosaic for 45,X and an abnormal cell line (including delXp, iXq, delXq, and rX: 28 of 116), 8% mosaic for 45,X with a normal cell line (10 of 116), 4% 46,XdelXp (five of 116), 5% 46,XiXq (five of 116), and 2% 46,XdelXq (two of 116). The karyotype distribution for the subjects with TS in the NMR study (Table 2) was 67% 45,X (41 of 61), 3% 46,XdelXp (two of 61), 3% 46,XiXq (two of 61), 1.6% 45,X/46, XX (one of 61), and the remainder mosaic for 45,X/46,X abnormal X (including delXp, iXq, delXq, and rX: 15 of 61). Both groups routinely take hormone replacement therapy, but all study subjects were off estrogen/progestin treatment for 2 wk before admission and were euthyroid during the evaluation. A few women with TS were taking statins before admission but discontinued use during inpatient study. No women with POF were on statins or other lipid-lowering medications. The usual estrogen therapy was similar in both groups. Among women with TS, 3% were using transdermal estradiol, 51% conjugated equine estrogens, and 41% oral contraceptive (~5% not on treatment). Among women with POF, 4% used transdermal estradiol, 49% conjugated equine estrogen, and 40% oral contraceptive (7% not on treatment). Both groups were predominantly Caucasian and sedentary in habit with no trained athletes in either group.

Experimental studies

Subjects were studied as inpatients in the NIH Clinical Research Center during 2002–2004. Fasting glucose, insulin, and lipid levels were measured by direct assays as described on the NIH Lab Directory Web site (http://cclnprod.cc.nih.gov/dlm/testguide.nsf/Index?OpenForm). Lipoprotein subclass profile was determined by measurement of plasma NMR spectrum followed by computer deconvolution of the spectral data and calculation of the subclass concentrations, as performed by LipoScience, Inc. (Raleigh, NC). Details are provided elsewhere (http://www.lipoprofile.com/control.cfm?ID=174). Data on some members of the TS group were included in a previous study (17), whereas all the subjects in the POF group are new to this study.

Statistical analysis

Data are expressed as mean with SD. Comparisons between group means were made by analysis of covariance, adjusted for age and body mass index (BMI), followed by Fisher's protected least significant difference test. Analysis was performed using StatView for Windows (version 5.0.1; SAS Institute Inc., Cary, NC).

TABLE 1. Lipid profiles in 45,X *vs.* 46,XX women with ovarian failure

	TS (n = 118)	POF (n = 51)	*P* value
Age (yr)	32.7 ± 5.0	32.1 ± 5.8	0.15
BMI (kg/m^2)	26.4 ± 4.6	26.6 ± 5.7	0.5
Cholesterol (mg/dl)	208 ± 46	196 ± 38	0.06
HDL cholesterol (mg/dl)	58 ± 14	62 ± 17	0.12
LDL cholesterol (mg/dl)	134 ± 40	114 ± 34	0.001
Triglycerides (mg/dl)	126 ± 65	92 ± 44	0.0005

Data are means ± SD. Group means were compared by analysis of covariance with age and BMI as covariates. SI conversions: to convert total cholesterol, LDL cholesterol, and HDL cholesterol to millimoles per liter, multiply by 0.0259; to convert triglycerides to millimoles per liter, multiply by 0.0113.

Results

Lipid profile in TS vs. POF

The women with TS and POF were similar in age and BMI (Table 1). The TS group has significantly higher LDL cholesterol ($P = 0.001$) and triglyceride levels ($P = 0.0005$), compared with POF controls (Table 1). Total cholesterol is slightly elevated ($P = 0.06$) and HDL cholesterol slightly decreased ($P = 0.12$) in TS. The LDL concentration *per se* may not be the sole determinant of risk for ischemic heart disease because LDL particles are heterogeneous with respect to their size, density, and lipid composition. Among LDL particles, the smaller and denser LDL particles are more atherogenic, and the small, dense LDL phenotype is strongly associated with development of ischemic heart disease. Therefore, we proceeded to examine the profile of lipoprotein particles in our unique study groups.

NMR lipid particle profile in TS vs. POF

The composition of the lipid particle classes was more atherogenic in TS than POF (Table 2). The TS group had approximately 2-fold higher levels of medium-small and very small LDL particles and a significantly smaller average LDL size ($P < 0.0001$ for all observations). The LDL particle concentration was correspondingly increased (Table 2). HDL particle size was also significantly decreased in TS ($P = 0.0002$). In contrast, very low-density lipoprotein fractions were enriched for large particles in TS (Table 2).

Due to the eligibility criteria for our TS study, only a few participants (≤8%) in the present study had karyotypes that contained a normal cell line, and in all these cases, the normal cells were less than 30%. Given these strict criteria, we have not detected any difference in phenotype between pure 45,X and mosaic karyotype groups in previous studies (17, 20) as well as in the present study (data not shown). Likewise, in the present study, there were no significant differences in lipid values within the TS group based on karyotype (*i.e.* pure 45,X *vs.* all other karyotypes, data not shown). The comparison between the pure 45,X TS group and the POF group remains highly statistically significant. For example, in the 45,X-only TS (n = 66), LDL cholesterol was 137 ± 43 mg/dl ($P = 0.002$ *vs.* POF) and triglycerides were 125 ± 57 mg/dl ($P = 0.0004$). For the NMR profile, the 45,X-only TS group (n = 40) had an average LDL particle size of 21.10 ± 0.68 nm and a total LDL particle concentration of 1333 ± 351 mmol/liter ($P < 0.0001$ *vs.* POF, for both comparisons). There were too few subjects with informative Xp or Xq deletions to attempt mapping the lipid locus. Fasting glucose (85 ± 7 for POF and 85 ± 6 mg/dl for TS) and insulin sensitivity [quantitative insulin sensitivity check index (21) 0.37/0.03 for POF and 0.37/0.05 for TS] were very similar in the two groups.

Discussion

This study shows that LDL cholesterol and triglyceride levels are significantly higher and that LDL and HDL particles are significantly smaller in 45,X women, compared

TABLE 2. NMR lipid profiles in women with TS (45,X) and POF (46,XX)

	Normal	POF (51)	TS (61)	*P* value
Age (yr)		32.1 ± 5.8	34.2 ± 10.6	0.4
BMI (kg/m^2)	20–30	26.4 ± 4.6	27.2 ± 7.2	0.4
HDL particles (μmol/liter)	24.2–43.5	30.5 ± 4.3	34.8 ± 5.4	<0.0001
Large				
HDL-H5	3.5–16.1	8.9 ± 4.2	8.1 ± 4.1	0.4
HDL-H4				
Medium				
HDL-H3	0–8.8	4.3 ± 3.7	4.0 ± 3.3	0.7
Small				
HDL-H1	13.7–28.8	17.3 ± 5.7	20.8 ± 5.9	0.003
HDL-H2				
HDL size (nm)	8.5–9.6	9.40 ± 0.48	9.06 ± 0.49	0.0002
LDL particles (nmol/liter)	949–2118	954 ± 350	1285 ± 345	<0.0001
Large				
LDL-L3	172–912	549.1 ± 184.6	488.8 ± 248	0.14
Small				
LDL-L1	242–1698	387.7 ± 408.7	777.4 ± 376.3	
LDL-L2				
Medium-small				
LDL-L2	63–371	88.9 ± 80.4	174.0 ± 83.2	<0.0001
Very small				
LDL-L1	172–1329	298.8 ± 331.0	603.2 ± 299.1	<0.0001
LDL size (nm)	19.9–22.3	21.83 ± 0.72	21.14 ± 0.67	<0.0001
Very low-density lipoprotein particles (nmol/liter)	20–60	47.0 ± 40	62.1 ± 45	0.07
Large	0–3	1.55 ± 2.3	3.65 ± 4.9	0.007
Medium	7–20	17.2 ± 2.7	27.15 ± 3.3	0.03
Small	10–60	28.5 ± 22.4	31.0 ± 19.1	0.5

Data are means ± SD or range. NMR lipoprofiles were obtained for 61 of the women with TS and all of the POF women shown in Table 1. Group means were compared by analysis of covariance with age and BMI as covariates.

with 46,XX women with ovarian failure. Because the two groups of women are so similar in lifestyle, hormonal status, body composition (17), and insulin sensitivity but differ in the presence of a second X-chromosome, their differences in lipid metabolism are likely due to previously unrecognized disparity in X-chromosome gene dosage.

The degree of difference in lipid levels and lipid particle size observed between 45,X and 46,XX women in this study is strikingly similar to the well-documented differences in these same parameters observed between normal men (also monosomic for the X-chromosome) and women (3, 5, 6). In normal men, triglycerides and LDL cholesterol are high, LDL particles smaller and more numerous, but very low-density lipoprotein particles are larger (22). One potential explanation for these observations is that X-chromosome gene or genes are involved in lipid metabolism or transport [*e.g.* MBTPS2, which encodes the site 2 protease that cleaves sterol regulatory element-binding protein (23), a key regulator of cholesterol metabolism]. If such X-chromosome gene(s) without a Y-chromosome escapes inactivation in normal women, then increased dosage effects could explain the more salutary lipid profile in women. Alternatively, disparity in X-linked gene dosage may occur from parental imprinting. For example, if a gene or genes promoting a beneficial lipid pattern is selectively expressed from paternally inherited X-chromosomes, then women would experience this effect in approximately 50% of their cells, given random X-inactivation, whereas men that possess only a single maternally inherited X-chromosome would not receive these beneficial effects at all.

Our studies in young women with ovarian failure have neutralized the confounding effects of gonadal steroids, which complicate the comparison of lipid profiles in normal men and women. The major variable distinguishing our two study groups is the presence of a second X-chromosome in the POF group. The fact that the direction and magnitude of the differences in lipid levels and lipid particle characteristics between TS and POF parallel that seen between normal men and women is consistent with the view that the second X-chromosome normally contributes to a reduced risk for ischemic heart disease in women. Further studies elucidating the mechanisms involved in differences in ischemic heart disease susceptibility between men and women may have broad implications for public health.

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P.L.V., V.K.B., and C.A.B. have nothing to declare.

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