Atherosclerosis and Lipoproteins

Association of the Human Y Chromosome with Cholesterol Levels in the General Population

Fadi J. Charchar, Maciej Tomaszewski, Beata Lacka, Jaroslaw Zakrzewski, Ewa Zukowska-Szczechowska, Wladyslaw Grzeszczak, Anna F. Dominiczak

Objective—Males are at higher risk of cardiovascular diseases than females. The aim of the study was to test whether the potential of the Y chromosome to affect cardiovascular risk could be attributed to its influence on lipids.

Methods and Results—1288 Polish men (1157 subjects from young healthy cohort and 131 individuals from middle-aged hypertensive population) were phenotyped for determinants of cardiovascular risk including BMI, blood pressures, lipids, and testosterone. Each subject was genotyped for the HindIII(+/−) polymorphism within the nonrecombining region of the Y chromosome. Men with the HindIII(−) variant exhibited significantly higher total cholesterol (TC) and low-density lipoprotein cholesterol (LDL) levels than subjects with the HindIII(+) genotype in both populations. The differences between the genotypes were 0.15 mmol/L (P=0.0107) and 0.45 mmol/L (P=0.0377) in TC and 0.15 mmol/L (P=0.0059) and 0.41 mmol/L (P=0.0436) in LDL among young apparently healthy men and middle-aged hypertensive men, respectively. The HindIII(+) was associated with a significant increase in blood pressure of the middle-aged men. Testosterone serum concentrations correlated positively with HDL-cholesterol levels, and this association was independent of the Y chromosome.

Conclusions—The results indicate that a locus/loci on the Y chromosome may influence LDL levels, independent of testosterone levels. (Arterioscler Thromb Vasc Biol. 2004;24:308-312.)

Key Words: lipids • genetics • blood pressure • gender • male

Males tend to show higher prevalence of lipids disturbances, elevated blood pressure, and atherosclerosis than females. This leads to an increased cardiovascular mortality in men, as compared with age-matched women.

The presence of the Y chromosome is a factor distinguishing male and female genome and may therefore contribute to the sexual dimorphism in cardiovascular risk. In support of this hypothesis, associations of the Y chromosome with blood pressure were shown in European and Australian populations of middle-aged men. Furthermore, breeding experiments in the spontaneously hypertensive rat and the stroke-prone spontaneously hypertensive rat have linked the Y chromosome not only to blood pressure but also to lipid levels. This mechanism is thought to be in part mediated by the hypertensive rat Y chromosome relationship to steroids, specifically to testosterone. However, relationships between the Y chromosome, testosterone, and lipid profile in humans have not been investigated. Furthermore, it is not known whether there is an association between the human Y chromosome and other markers of atherosclerosis, such as the B phenotype of low-density lipoprotein (LDL) cholesterol (high number of small LDL particles), which is closely associated with coronary heart disease (CHD).

Therefore, we performed a genetic association study of lipids and testosterone with a HindIII(+/−) biallelic polymorphism contained in the nonrecombining region of the Y chromosome (NRY). Most of the male-determining Y chromosome NRY does not recombine with the X chromosome and is inherited intact from fathers to sons, therefore we can utilize one marker to determine Y chromosome genes that may contribute to metabolic phenotypes.

Methods

Subjects

The Young Men Cardiovascular Association (YMCA) Study

The YMCA study was designed to investigate genetic predisposition to cardiovascular disorders and was based on collecting healthy men. One thousand, one hundred eighty-eight unrelated young males were recruited from randomly selected secondary schools in Silesia in the south of Poland.

All male individuals aged over 17 years attending these secondary schools were invited to participate. Included were men who gave informed consent, which was obtained from the subjects, or the subjects parents if an individual was younger than 18. Clinical history was obtained from standardized questionnaires validated previously and coded anonymously. Anthropometric measure-
Characteristics of the Participants

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>YMCA Study</th>
<th>Silesian Hypertension Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All</td>
<td>HindIII(−)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>HindIII(−)</td>
</tr>
<tr>
<td>N (%)</td>
<td>1157 (100%)</td>
<td>836 (72.3%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>19.07±3.56</td>
<td>19.04±3.55</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.84±3.04</td>
<td>22.87±3.05</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>117.93±13.18</td>
<td>118.15±13.12</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>74.20</td>
<td>74.40</td>
</tr>
<tr>
<td>Hypertensive subjects (%)</td>
<td>120 (10.4%)</td>
<td>86 (10.3%)</td>
</tr>
<tr>
<td>Antihypertensive therapy (%)</td>
<td>18 (1.6%)</td>
<td>14 (1.7%)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>376 (32.5%)</td>
<td>271 (32.4%)</td>
</tr>
<tr>
<td>Alcohol intake (g/week)</td>
<td>30.79±29.24</td>
<td>30.29±29.03</td>
</tr>
<tr>
<td>Current alcohol drinkers (%)</td>
<td>735 (63.5%)</td>
<td>525 (62.8%)</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>26.55±12.95</td>
<td>26.46±11.82</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.81±0.72</td>
<td>4.82±0.73</td>
</tr>
</tbody>
</table>

The data are presented as mean±SD unless stated otherwise. Unpaired t-test (with Welch’s correction where appropriate) and Mann–Whitney test were used to compare quantitative traits between the two different genotypes. Fisher’s exact test was used in case of categorical variables. All P-values refer to the differences between groups representing 2 HindIII (+/−) genotypes.

Biochemical Analysis

Fasting lipoprotein profile [total cholesterol (TC), high-density lipoprotein cholesterol (HDL), triglycerides (TG)] and plasma glucose levels were determined by enzymatic methods on a Cobas Bio-AutoAnalyzer. LDL was calculated using the Friedewald equation. Free testosterone was measured by solid-phase radioimmunoassay (Diagnostic Products).

Molecular Analysis

Genotype analysis of the HindIII(+/−) polymorphism was performed as described earlier.9

Statistical Analysis

Data are presented as mean±SD unless stated otherwise. Unpaired t-test (with Welch’s correction where appropriate), Mann–Whitney test, and Kruskal–Wallis test were used to compare quantitative traits among the groups. Fisher’s exact test was used in case of categorical variables. Pearson’s linear correlation was used to examine associations between quantitative phenotypes in the entire population. A multiple regression analysis was performed to evaluate the relationship of the HindIII(+/−) polymorphism/testosterone with LDL and HDL serum concentrations, controlling for factors known as potential confounders such as age, BMI, blood pressure, plasma glucose concentrations, smoking status, and alcohol consumption. χ² test for trend was used to test for a linear trend among ordered categories (defined as quartiles of LDL or HDL) and the proportion of subjects representing either two different genetic variants [HindIII(+/+) versus HindIII(−)] or two contrasting paternal cardiovascular histories (fathers with myocardial infarction before age of 55 versus fathers with low cardiovascular risk, ie, without history of hypertension, coronary heart disease, and diabetes). A value of P<0.05 in a 2-sided test was considered significant.

At α=0.05, the study had sufficient power to detect a clinically significant difference (10% difference between mean values of a quantitative variable) in lipid levels between groups representing opposite genetic variants of the HindIII(+/−) polymorphism (the power indicators were 100%, 95%, 99%, and 72% for TC, LDL, HDL, and TG, respectively).

Results

Demographic, Clinical, and Biochemical Characteristics

The general characteristics of the participants are presented in the Table.
There was no association between the Y chromosome polymorphism and serum concentrations of testosterone (Table), and adjustment for testosterone levels did not attenuate the difference in TC and LDL between the two variants of the HindIII (+/−) polymorphism ($P=0.01$ and $P=0.0016$ for TC and LDL, respectively).

HDL was the only fraction of lipids remaining in a linear correlation with testosterone ($r=0.1$, $P=0.002$). In the multiple regression analysis that explained 5.4% of variance in HDL levels, testosterone, but not HindIII (+/−) polymorphism, was a significant determinant of HDL levels ($P=0.001$), controlling for age, BMI, smoking status, blood pressures, glycemia, alcohol consumption, and the HindIII (+/−) polymorphism. There was a significant difference in circulating concentrations of HDL among men representing four quartiles of testosterone levels (1.11 mmol/L versus 1.13 mmol/L versus 1.18 mmol/L versus 1.19 mmol/L, from the lowest to the highest quartile of testosterone distribution, respectively, $P=0.0038$).

**The HindIII (+/−) Polymorphism and Lipids in the Silesian Hypertension Study (SHS)**

TC and LDL levels were significantly higher in men with the HindIII (−) genotype (Figure 1B). There was no significant difference in use of antihypertensive drugs that could affect lipid metabolism (nonselective β-blockers and diuretics) between HindIII (−) [15 men (17.4%)] or HindIII (+) [8 men (17.7%)] genotypes. Both SBP and DBP were significantly higher in subjects with the HindIII (+) variant (Table). After exclusion of men on antihypertensive treatment, the difference in SBP was still significant (137.8 ± 9.8 mm Hg versus 145 ± 9.8 mm Hg in the HindIII (−) and HindIII (+) groups, respectively, $P=0.04$).

**Paternal History of Myocardial Infarction and Lipids**

We identified a representative number of subjects with documented paternal history of myocardial infarction only in the YMCA Study. There were 4.7% (54) subjects whose fathers have had a documented history of myocardial infarction before the age of 55, while 36.8% (426) men had fathers with a low cardiovascular risk profile (no history of hypertension, CHD, or diabetes mellitus).

There was a stepwise increase in the percentage of subjects with paternal history of myocardial infarction and a gradual decrease in the percentage of men having fathers with low cardiovascular risk, from the lowest to the highest quartile of LDL ($P=0.039$) (Figure 2).

Without taking into account the HindIII (+/−) genotype, men having fathers with history of myocardial infarction had significantly higher LDL levels than individuals with fathers representing low cardiovascular risk (2.62 ± 1.01 mmol/L versus 2.35 ± 0.83 mmol/L, respectively, $P=0.026$) (Figure 1C).

**Discussion**

This is the first study to reveal an association between a marker of the Y chromosome with circulating concentrations of LDL-cholesterol in males. The magnitude of the difference in TC and LDL between the genetic variants of the HindIII (+/−) poly-
morphism was similar in both populations. Interestingly, this association, independent of several powerful determinants of lipid profile (as seen in multiple regression analysis), was evident in young adulthood, when most individuals have low cardiovascular risk. However, early pathobiological markers of atherosclerosis are present in the arterial system in the teenage years,14 and circulating concentrations of LDL are important determinants of atherosclerosis in young men, 20 to 30 years before manifestation of CHD.15

It is too early to speculate whether the genes within the Y chromosome may influence atherosclerosis; however, data from the current study seem to indirectly support this notion. The significant association between the \( \text{HindIII}(+)/H11002 \) and TG/HDL ratio, a good marker of pro-atherogenic B phenotype of LDL,16 suggests that gene/genes within the NRY may affect not only the LDL concentration but also its propensities to promote CHD. Moreover, the magnitude of the difference in the mean level of LDL between men having fathers with a history of myocardial infarction and individuals with fathers representing low cardiovascular risk followed the difference in LDL between the \( \text{HindIII}(+)/H11001 \) and \( \text{HindIII}(+)/H11002 \) genotypes. Finally, the distribution of paternal history of cardiovascular risk across the quartiles of LDL followed the \( \text{HindIII}(+/−) \) pattern.

The data presented here support our previous observations regarding the genetics of blood pressure in middle-aged men.5 The association of the \( \text{HindIII}(+) \) with elevated SBP remained significant even after exclusion of subjects on antihypertensive treatment. No association of the \( \text{HindIII}(+/−) \) with either SBP or DBP in the cohort of young men suggests that the association between the Y chromosome and blood pressure becomes apparent at later stages of life and thus may be determined by the NRY locus different from the NRY gene(s) related to LDL. In support of this assumption, the allele associated with a favorable LDL level \( [\text{HindIII}(+)] \) was different to the variant related to lower blood pressure in SHS \( [\text{HindIII}(−)] \). In addition, the influence on LDL remained significant after adjusting for blood pressure in multiple linear regression. In light of these data, blood pressure and LDL may be regulated by independent loci within the NRY.

Circulating concentrations of testosterone and alcohol consumption are the major factors that could potentially affect associations between the Y chromosome and lipids. Evidence for such a confounding influence of alcohol have been provided by investigations showing associations between alcoholism and certain variants of the human Y chromosome,17 as well as studies suggesting correlation between alcohol consumption and lipid levels.18,19 However, the current analysis does not seem to be affected by this confounder. First, alcohol consumption treated either as a qualitative or quantitative variable did not differ between men representing two different variants of the Y chromosome. Second, adjustment for alcohol consumption in multiple regression analysis did not influence the relationship between the \( \text{HindIII}(+/−) \) and LDL. Finally, the association between HDL levels and circulating concentrations of testosterone remained significant after controlling for alcohol consumption.

A significant association between endogenous testosterone levels and coronary events in men and women has not been established in large prospective studies, although cross-sectional data have suggested that coronary heart disease can be associated with low testosterone in men.20 Our study confirms the previous results that testosterone is associated with HDL levels21–23 and that this relationship is independent of classical cardiovascular risk factors. Testosterone has also

![Figure 2. A, HindIII(+)/−) genotypes across increasing quartiles of LDL in YMCA study. B, Paternal history of cardiovascular risk (MI, fathers with history of myocardial infarction; LOW RISK, fathers with low cardiovascular risk, without a history of hypertension, CHD and diabetes) across increasing quartiles of LDL in YMCA study.](http://atvb.ahajournals.org/)

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been implicated as a possible hormonal component in the development and maintenance of essential hypertension in the rat model.\textsuperscript{24} This testosterone-related mechanism is thought to be in part mediated by the hypertensive rat Y chromosome.\textsuperscript{25} It may be that the genes encoded on the sex chromosomes also contribute directly (independent of gonadal secretions) to sex differences in cardiovascular phenotypes such as recently observed in the brain.\textsuperscript{26}

Localization of causative genes/mutations of the Y chromosome will not be achieved by conventional linkage methods, because the NRY does not recombine at meiosis.\textsuperscript{27} Use of the candidate gene strategy also remains uncertain because most of the known roles of the NRY genes have not been assigned to cardiovascular functions. The recent publication of the complete Y chromosome genetic content\textsuperscript{28} will be of great benefit to the search and crucial understanding of genes that contribute to cardiovascular phenotypes. Expression profiling along with direct sequencing will be needed to identify the loci influencing BP and lipid metabolism.

In summary, this study demonstrates an association between the human Y chromosome and circulating concentrations of LDL. The presence of such an association suggests that the Y chromosome may harbor genes that influence cardiovascular risk profiles in males. Future studies are required to identify the causative gene(s) in males.

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