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Male-Specific Region of the Y Chromosome and Cardiovascular Risk

Phylogenetic Analysis and Gene Expression Studies

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Objective—Haplogroup I of male-specific region of the human Y chromosome is associated with 50% increased risk of coronary artery disease. It is not clear to what extent conventional cardiovascular risk factors and genes of the male-specific region may explain this association.

Approach and Results—A total of 1988 biologically unrelated men from 4 white European populations were genotyped using 11 Y chromosome single nucleotide polymorphisms and classified into 13 most common European haplogroups. Approximately 75% to 93% of the haplotypic variation of the Y chromosome in all cohorts was attributable to I, R1a, and R1b1b2 lineages. None of traditional cardiovascular risk factors, including body mass index, blood pressures, lipids, glucose, C-reactive protein, creatinine, and insulin resistance, was associated with haplogroup I of the Y chromosome in the joint inverse variance meta-analysis. Fourteen of 15 ubiquitous single-copy genes of the male-specific region were expressed in human macrophages. When compared with men with other haplogroups, carriers of haplogroup I had ≈ 0.61 - and 0.64 -fold lower expression of ubiquitously transcribed tetratricopeptide repeat, Y-linked gene (*UTY*) and protein kinase, Y-linked, pseudogene (*PRKY*) in macrophages ($P=0.0001$ and $P=0.002$, respectively).

Conclusions—Coronary artery disease predisposing haplogroup I of the Y chromosome is associated with downregulation of *UTY* and *PRKY* genes in macrophages but not with conventional cardiovascular risk factors. (*Arterioscler Thromb Vasc Biol.* 2013;33:1722-1727.)

Key Words: association ■ cardiovascular risk ■ DNA ■ gene ■ genetics ■ mRNA

The human Y chromosome is one of the smallest in the genome. Its major part, the male-specific region (MSY), constitutes $\approx 95\%$ of its length, does not recombine with the X chromosome during meiosis, and is inherited as an indivisible unit from fathers to sons.¹ Because of its haploid nature, the MSY has been routinely excluded from large-scale genome-wide association studies and is one of the most underexplored portions of the human DNA.

A growing body of evidence suggests that the human MSY may contribute to cardiovascular risk in men. Several studies conducted in the general population revealed associations between single nucleotide polymorphisms of the MSY and blood pressure,²⁻⁴ low-density lipoprotein-cholesterol,⁵ a surrogate of proatherogenic B phenotype of low-density lipoprotein-cholesterol particles, and paternal history of myocardial infarction.⁵ Our recent phylogenetic analysis showed that one of the most common European lineages of the Y chromosome (haplogroup I) was associated with increased risk of coronary

artery disease (CAD) among British men.⁶ Indeed, the analysis of ≈ 3000 men from 2 British cohorts revealed that compared with other lineages of the Y chromosome, haplogroup I increased risk of CAD by $\approx 50\%$.⁶ This makes haplogroup I of the Y chromosome one of the strongest common genetic risk factors of CAD known to date.⁶

The biological mechanisms underlying the association between CAD and human Y chromosome are not fully understood. Our previous transcriptome-wide analysis of human macrophages revealed that compared with other Y chromosome lineages, haplogroup I was associated with suppression of adaptive immunity and increased response to inflammation.⁶ Specifically, 19 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways connected by genes of immunity and inflammation showed differential regulation in macrophages between men with haplogroup I and carriers of the other MSY lineages.⁶ However, it is not clear which genes of the MSY may drive the association between haplogroup I

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and predisposition to CAD. Of the 27 distinct protein-coding genes of the MSY, 15 single-copy genes with homologs on the X chromosome (so-called X-degenerate genes) show mostly ubiquitous tissue expression (including cells and organs of cardiovascular system) and thus are the strongest biological candidates for further functional studies on MSY and cardiovascular disorders. So far little is known about their function and specific roles in male health and disease.

It is also not clear whether the altered immune response may entirely explain the increased susceptibility to CAD in men with haplogroup I. The effect of the MSY on CAD risk could be mediated, at least to some extent, by traditional cardiovascular and metabolic risk factors, such as adiposity, blood pressure, lipids, glucose, or insulin resistance. Taken together these risk factors explain <50% individual variation in susceptibility to CAD. Although in our previous study the association between haplogroup I and CAD in the West of Scotland Coronary Prevention Study was independent of some sociodemographic and conventional cardiovascular risk factors, this analysis was limited only to 1 cohort of middle-age Scottish men, many of whom were on pharmacological treatment with a potential to affect measurements of several key phenotypes.⁶ Thus, it is difficult to extrapolate these data into apparently healthy general population of European men.

Here, we examined whether CAD-related haplogroup I of the Y chromosome is associated with either previously investigated or new traditional cardiovascular risk factors in young men of white European ethnicity recruited from the general population. This was followed by a systematic comparative expression analysis of 15 ubiquitous X-degenerate genes of the MSY in macrophages from carriers of haplogroup I and men from other MSY lineages.

Materials and Methods

Materials and Methods are available in the online-only Supplement. In brief, 1940 biologically unrelated, apparently healthy men from 3 populations of white European ethnicity (Young Men Cardiovascular

Association Study 1-YMCA1,⁵ Young Men Cardiovascular Association Study 2-YMCA2⁷ and Genetic Regulation of Arterial Pressure in Humans in the Community-GRAPHIC⁸) were included in the genetic association analysis. Macrophage RNA from 48 white British men with a history of premature myocardial infarction (recruited into Cardiogenics Transcriptome Project)⁶ was used in MSY genes expression studies. Reconstruction of the MSY phylogenetic tree was based on information provided by 11 bi-allelic polymorphisms (M9, M35, M45, M89, M170, M173, M201, M207, M269, M304, and SRY10831) genotyped in all cohorts, as reported before (Table I in the online-only Data Supplement).⁶ This set of polymorphic variants allows classification of >95% of European Y chromosomes into major haplogroups (Y[xBR], BR[xE1b1b1,F], E1b1b1, F*, G, I, J, K*, P*, R*, R1*, R1a, R1b1b2).⁹⁻¹¹ The presence of population stratification/population admixture was examined using information provided by autosomal SNPs from previous array-based experiments (GRAPHIC⁸, Cardiogenics⁶) or a panel of 34 ancestrally informative SNPs (YMCA1 and YMCA2).¹²⁻¹⁵ Gene expression analysis of all ubiquitous single-copy genes of MSY was conducted using quantitative real-time PCR.

Results

Clinical and Demographic Characteristics

A total of 1068 and 509 unrelated men in Young Men Cardiovascular Association Study 1 (YMCA1) and YMCA2, respectively, were informative in the Y chromosome phylogenetic analysis. In the Genetic Regulation of Arterial Pressure in Humans in the Community (GRAPHIC) Study, 363 biologically unrelated men from the offspring generation provided full genetic information for haplogroup resolution. In the Cardiogenics cohort, 48 men had full DNA and mRNA information necessary for further haplogroup I-stratified gene expression analysis. The clinical characteristics of YMCA1, YMCA2, and GRAPHIC sons are shown in Table 1 and are largely consistent with the phenotypic characteristics of young, apparently healthy males from general population. The basic phenotypic information on men from the Cardiogenics Study included in this project is shown in Table II in the online-only Data Supplement.

Table 1. Demographic and Clinical Characteristics of Men From YMCA1, YMCA2, and the GRAPHIC Study

Phenotype	YMCA1	YMCA2	GRAPHIC Sons
n	1068	509	363
Age, y	19.3 (3.6)	18.9 (3.3)	25.7 (5.0)
BMI, kg/m ²	22.9 (3.0)	22.6 (3.0)	25.1 (4.1)
Clinic SBP, mm Hg	118.1 (13.2)	118.8 (13.2)	128.4 (13.0)
Clinic DBP, mm Hg	74.2 (7.9)	74.1 (7.9)	76.4 (9.6)
TC, mmol/L	4.3 (0.9)	3.7 (1.0)	4.6 (0.9)
HDL-C, mmol/L	1.2 (0.3)	1.1 (0.3)	1.3 (0.3)
Triglycerides, mmol/L	1.1 (1.0-1.1)	0.9 (0.8-0.9)	...
LDL-C, mmol/L	2.6 (0.9)	2.2 (0.8)	2.5 (0.7)
Glucose, mmol/L	4.8 (0.7)	4.4 (0.8)	4.9 (0.8)
Creatinine, μmol/L	82.8 (11.1)	74.9 (9.8)	90.8 (9.1)
CRP, mg/L	0.08 (0.07-0.08)
HOMA-IR	1.6 (1.6-1.7)	1.6 (1.6-1.7)	...

Data are mean and SD or geometric mean and 95% confidence intervals (triglycerides, CRP, and HOMA-IR). BMI indicates body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; GRAPHIC, Genetic Regulation of Arterial Pressure in Humans in the Community; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment insulin resistance index; LDL-C, low-density lipoprotein-cholesterol; n, No. of subjects; SBP, systolic blood pressure; TC, total cholesterol; and YMCA, Young Men Cardiovascular Association Study.

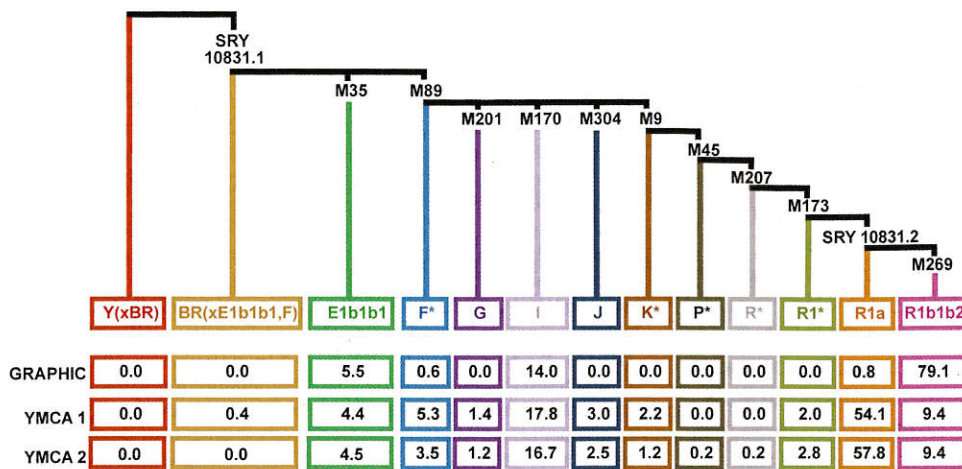


Figure 1. Frequency of Y chromosome haplogroups in each cohort. Colored bars and letters represent each haplogroup running from the evolutionarily oldest on the left to the youngest of the right (youngest haplogroup is shown in pink [R1b1b2]); M and SRY markers indicate the terminal marker of each haplogroup; data are percentages of individuals in each haplogroup. GRAPHIC sons indicates Genetic Regulation of Arterial Pressure in Humans in the Community; and YMCA, Young Men Cardiovascular Association Study.

Phylogenetic Analysis of the Y Chromosome

A total of 1988 individuals from YMCA1, YMCA2, GRAPHIC, and Cardiogenics with sufficient genetic/phenotypic information were classified into 1 of 13 major European MSY haplogroups. Approximately 75% to 93% of the haplotypic variation in these cohorts was attributable to 3 lineages (I, R1a, and R1b1b2; Figure 1). As expected, haplogroup R1a was the most common in Polish populations, whereas R1b1b2 predominated in the British men (Figure 1). Haplogroup I (previously associated with increased risk of CAD) was the second most common haplogroup in each cohort.

There was no evidence of population stratification/admixture in any of the cohorts (Figures I–III in the online-only Data Supplement).

Association of Haplogroup I and Major Cardiovascular Risk Factors: Individual and Joint Analysis

The cohort-specific associations between haplogroup I and conventional risk factors are shown in Tables III–V in the

online-only Data Supplement. None of traditional cardiovascular risk factors was associated with haplogroup I in the meta-analysis of 1944 men from 3 populations (Table 2). There was no evidence of heterogeneity in the joint analysis (Table 2). Sensitivity analyses showed that adjustment for blood pressure-lowering effect of antihypertensive treatment did not affect the association findings from analysis of systolic blood pressure and diastolic blood pressure (data not shown).

Association Between Haplogroup I and Expression of Single-Copy MSY Genes in Human Macrophages

Of 15 MSY genes, 14 were expressed in human macrophages. Two genes (ubiquitously transcribed tetratricopeptide repeat, Y-linked gene [UTY] and protein kinase, Y-linked, pseudo-gene [PRKY]) showed statistically significant difference in expression between men with haplogroup I and those with other lineages of the Y chromosome after correction for multiple testing (Table VI in the online-only Data Supplement). Specifically, carriers of haplogroup I had ≈0.61- and 0.64-fold

Table 2. The Association Between Haplogroup I and Cardiovascular Risk Factors in YMCA1, YMCA2, and GRPAHIC Sons: Fixed-Effect Age-Adjusted Inverse Variance Meta-Analysis

Phenotype	β-Coefficient (SE)	P Value	Heterogeneity
BMI, kg/m ²	-0.07 (0.19)	0.709	0.590
Clinic SBP, mm Hg	0.14 (0.81)	0.865	0.555
Clinic DBP, mm Hg	-0.32 (0.49)	0.524	0.356
TC, mmol/L	-0.01 (0.06)	0.862	0.131
HDL-C, mmol/L	0.00 (0.02)	0.798	0.113
Triglycerides, mmol/L*	0.04 (0.03)	0.161	0.823
LDL-C, mmol/L	-0.01 (0.05)	0.775	0.017
Glucose, mmol/L	-0.01 (0.05)	0.820	0.445
Creatinine, μmol/L	-0.02 (0.62)	0.972	0.937
CRP, mg/dL**	0.18 (0.17)	0.290	—
HOMA-IR*	-0.11 (0.10)	0.263	0.624

Data are expressed as β-coefficients, SE, and levels of statistical significance from meta-analysis; heterogeneity, level of statistical significance from χ² test for heterogeneity. BMI indicates body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; GRAPHIC, Genetic Regulation of Arterial Pressure in Humans in the Community; HDL-C, high-density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment insulin resistance index; LDL-C, low-density lipoprotein-cholesterol; SBP, systolic blood pressure; TC, total cholesterol; and YMCA, Young Men Cardiovascular Association Study.

*YMCA1 and YMCA2 only; and **GRPAHIC sons only.

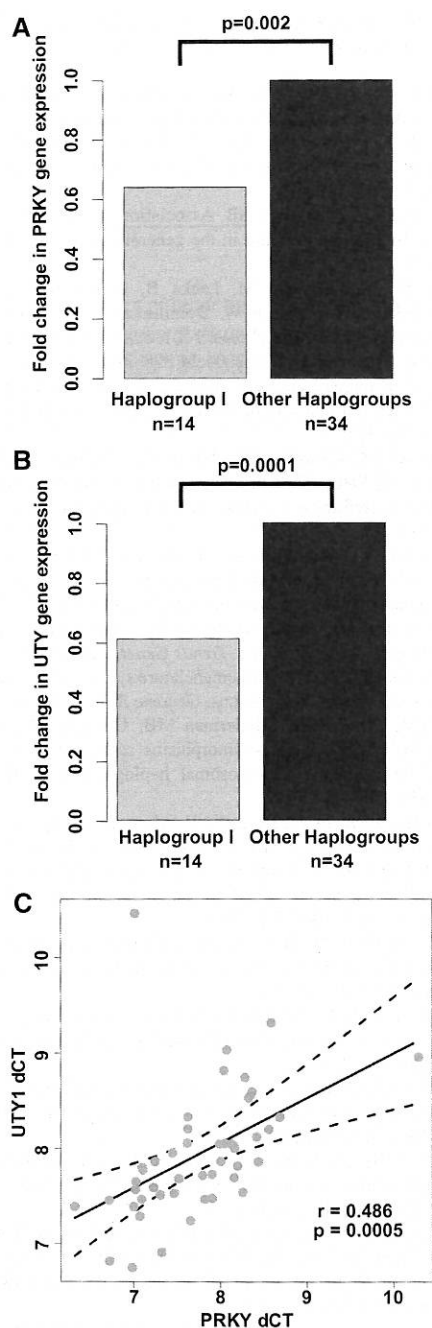


Figure 2. Macrophage expression of *UTY* and *PRKY* mRNA in British men from Cardiogenics cohort. **A**, Differences in relative (β -actin normalized) expression of *PRKY* between men with haplogroup I and carriers of other haplogroups. **B**, Differences in relative (β -actin normalized) expression of *UTY* between men with haplogroup I and carriers of other haplogroups. **C**, Linear correlation in macrophage expression of *UTY* and *PRKY*.

lower age-adjusted levels of *UTY* and *PRKY* in macrophages than men with non-I haplogroups ($P=0.0001$ and $P=0.002$, respectively; Figure 2). There was a significant linear correlation in mRNA levels of both genes in human macrophages ($r=0.486$; $P=0.0005$; Figure 2).

Discussion

Our data revealed that CAD-predisposing haplogroup I of the Y chromosome⁶ was not associated with any major

conventional cardiovascular risk factors in apparently healthy men from the general population of white European ancestry. The systematic analysis of all ubiquitous single-copy MSY X-degenerate genes revealed that a vast majority of them are indeed expressed in human macrophages. We also uncovered a significant downregulation of 2 MSY genes (*UTY* and *PRKY*) in macrophages from men with haplogroup I when compared with carriers of other lineages.

Our data from YMCA studies and the GRPAHIC cohort provide important support for previously proposed notion that the association between haplogroup I of the Y chromosome and CAD is independent of traditional cardiovascular risk factors.⁶ Indeed, using continuous measures of cardiovascular risk (essentially un-confounded by medications), we showed that neither blood pressures nor metabolic phenotypes were associated with haplogroup I of the Y chromosome among apparently healthy men. The absence of association between the Y chromosome and C-reactive protein in this analysis is particularly important given that CAD-related haplogroup I was linked to immunity and inflammation in the recent transcriptome-wide analysis of human macrophages.⁶ The data collected here show that C-reactive protein, a simple measure of low-grade inflammation^{16,17} and increasingly recognized surrogate of cardiovascular risk,¹⁶ is unlikely to explain the reported associations between haplogroup I and increased susceptibility to CAD⁶ or possibly higher risk of AIDS progression or failure of retroviral therapy.¹⁸

Our data also reveal that other correlates of CAD not included in the previous observation,⁶ such as creatinine levels (a measure of renal function) and insulin resistance, do not track with haplogroup I of the Y chromosome among young apparently healthy men. Insulin resistance is a recognized contributor to metabolic syndrome, type 2 diabetes mellitus, as well as CAD¹⁹ and data from experimental models suggested a linkage between the Y chromosome and measures of insulin sensitivity.²⁰ Estimated glomerular filtration rate, a measure of renal function, is also associated with the risk of CAD and cardiovascular mortality in the general population.^{21,22} In this study, instead of estimated glomerular filtration rate we used creatinine levels because a significant proportion of YMCA men was aged <18 years, and The Modification of Diet in Renal Disease-estimated glomerular filtration rate equation is simply not validated as a measure of renal function in this age category. The inclusion of these additional cardiovascular risk factors in our analysis extended the range of conventional correlates of CAD that are unlikely to account for its association with the haplogroup I of the Y chromosome.

Our gene expression analysis provided the first insights into MSY transcriptome in human macrophages. This cell type is a recognized player in the development of atherosclerosis.²³ Perhaps more importantly, macrophages from men with haplogroup I showed suppression of adaptive immunity and upregulation of proinflammatory response pathways when compared with other haplogroups.⁶ Indeed, we hypothesized that changes in activity/expression of these pathways in human macrophages could possibly mediate the effect of MSY on CAD.⁶ However, it was not known which MSY genes and transcripts may be the biological drivers of the identified findings. In this context, association between

CAD-predisposing haplogroup I and 2 mRNAs of MSY (*PRKY* and *UTY*) reported here is an important step forward to elucidate the role of the Y chromosome in atherosclerosis. *PRKY* encodes one of cAMP-dependent serine/threonine protein kinases. These enzymes are key messengers in the cellular responses to cAMP and act through phosphorylation of proteins to activate (or deactivate) receptors in different cells and tissues. *PRKY* is classified as a transcribed pseudogene; it lost its exon 6 and a part of exon 5 (encoding functionally active domains of the kinase) and is prone to nonsense-mediated decay. The biological role of *PRKY* is not clear. Its fully functional homolog on the X chromosome (*PRKX*) is involved in macrophage maturation and kidney development.^{24,25} The protein product of *UTY* belongs to one of the histocompatibility antigens recognized by T cells.²⁶ Expressed in a wide variety of tissues,²⁶ *UTY* elicits T-cell response in allogeneic transplantation and is hypothesized to play a major role in graft-versus-host disease and male grafts rejections.²⁷ Interestingly, both allograft rejection and graft-versus-host-disease KEGG pathways were associated with haplogroup I in macrophage transcriptome profiling in our previous study.⁶ The results shown here together with previously published data suggest that of 2 genes, *UTY* is a biologically more likely mediator of the association between haplogroup I and immune system, and possibly CAD. However, further studies are warranted to confirm the direct association between the *UTY* (*PRKY*) and CAD and elucidate the biological mechanisms of these findings.

In summary, our results show that haplogroup I of the Y chromosome previously linked to increased risk of CAD does not track with conventional cardiovascular and metabolic risk factors in young men from the general white European population. We also show for the first time that haplogroup I is associated with expression of ≥ 2 MSY genes, of which 1 is a strong biological candidate linked to immune system. Further studies should focus on functional characterization of biological underpinnings of the association between haplogroup I and *UTY/PRKY* expression to fully elucidate the mechanism of increased susceptibility to CAD among men with haplogroup I of the Y chromosome.

Disclosures

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Significance

Compared with carriers of other haplogroups, men with haplogroup I of the Y chromosome exhibit increased susceptibility to coronary artery disease, possibly because of downregulation of adaptive immunity and upregulation of response to inflammation pathways in macrophages. Traditional cardiovascular risk factors, including body weight, blood pressure, lipids, glucose, C-reactive protein, measures of renal function, and insulin resistance, are unlikely mediators of the association between haplogroup I and coronary artery disease. Fourteen of 15 ubiquitous single-copy genes of the male-specific region of the Y chromosome are expressed in human macrophages. Two of these genes (UTU and PRKY) are downregulated in macrophages from carriers of haplogroup I when compared with men with other haplogroups of the Y chromosome. Future functional studies will clarify contributions of these genes to haplogroup I-driven risk of coronary artery disease.