

# ***HindIII*(+/-) Polymorphism of the Y Chromosome, Blood Pressure, and Serum Lipids: No Evidence of Association in Three White Populations**

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**Background:** Male sex is associated with elevated levels of cardiovascular risk factors, including higher blood pressure (BP). Genetic variants on the Y chromosome may contribute to explain the sexual dimorphism in cardiovascular diseases. Among them, the *HindIII*(+/-) polymorphism of the male-specific region of the Y chromosome has been associated with BP and serum cholesterol levels, with conflicting results. We evaluated the association between the *HindIII*(+/-) polymorphism, prevalence of hypertension, BP, and serum lipid levels in a large sample of white men and the previously reported epistatic interaction between *HindIII*(+/-) and the -344C/T polymorphism of the aldosterone synthase gene (*CYP11B2*) on BP.

**Methods:** From three European populations (UK  $n = 422$ ; Belgium  $n = 313$ ; Italy  $n = 1248$ ) 1983 white men were phenotyped for BP and serum lipids and genotyped for *HindIII*(+/-) site and for -344C/T polymorphism in the promoter of *CYP11B2* by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).

**Results:** A higher frequency of the *HindIII* (+) was found in Italians (63%) as compared to both British (31%) and Belgians (28%) ( $P < .0001$ ). We found no evidence of association of the *HindIII*(+/-) site with prevalence of hypertension, BP, and serum lipids in any of the three European populations examined and in the entire sample. Finally, we did not observe any interaction between the *HindIII*(+/-) polymorphism and the -344C/T variant of *CYP11B2* on BP.

**Conclusions:** Our data do not support the hypothesis that the *HindIII*(+/-) site of the Y chromosome is a marker of cardiovascular risk in white men, highlighting the need for replication in genetic association studies. Am J Hypertens 2006;19:331–338 © 2006 American Journal of Hypertension, Ltd.

**Key Words:** Y chromosome, blood pressure, lipids, cardiovascular diseases, genetic association.

**M**en are at higher risk of developing cardiovascular diseases than are age-matched, premenopausal women. In particular, it is well known that men have a significantly higher blood pressure (BP) as compared with premenopausal women.<sup>1</sup> The gender-associated differences in BP observed in humans have also been documented in various animal models.<sup>2–4</sup>

Differences in sex hormones, environmental and social factors, as well as genetic variants and chromosomal re-

gions could contribute to the sexual dimorphism observed for hypertension and related phenotypes. The Y chromosome seems to be an obvious candidate for gender-related differences in the development of cardiovascular diseases.<sup>5</sup> The male-specific region of the Y chromosome (MSY), which comprises 95% of the length of the chromosome in humans, is functionally active and inherited in block from father to male offspring.<sup>6</sup>

Although most genes in the MSY region are involved in

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male-specific function, such as male sex determination and spermatogenesis, this region also contains genes probably involved in other cellular functions and possibly responsible for the sexual dimorphism of cardiovascular diseases.<sup>5–7</sup> Studies on consomic strains of rats supported the role of the Y chromosome not only in BP control,<sup>8–10</sup> but also in the expression of lipid phenotypes.<sup>10</sup> Indirect evidence for an effect of the Y chromosome on BP in humans was given by Uehara et al,<sup>11</sup> suggesting that BP in male offspring was influenced by paternal, but not maternal, hypertension.

More recently, population genetic studies explored the association between polymorphisms of the Y chromosome and BP<sup>12–16</sup> or lipid levels,<sup>14,17</sup> also analyzing the possible interaction of Y chromosome markers with autosomal genes involved in BP regulation, such as the aldosterone synthase gene (*CYP11B2*).<sup>13</sup>

In light of the contrasting results among the different studies published so far and taking into account the paramount issue of replication in genetic association studies,<sup>18,19</sup> we evaluated the association between the bi-allelic *HindIII*(+/-) polymorphism of MSY and prevalence of hypertension, BP, and serum lipid levels in a large and well-characterized sample of 1983 white men belonging to three European countries (Italy, UK, Belgium), recruited in three independent cardiovascular risk factor surveys: the IMMIDIET project (IP), the Wandsworth Heart & Stroke Study (WHSS), and the Olivetti Prospective Heart Study (OPHS). The interaction of the *HindIII*(+/-) site with the -344C/T variant of the aldosterone synthase gene (*CYP11B2*) was also investigated.

## Methods

Methods and populations of each survey have been described in detail elsewhere.<sup>20–23</sup> The studies were performed according to the Principles of the Declaration of Helsinki and were approved by the Ethics Committees of all the participating institutions. All study participants agreed to give blood samples for DNA analysis and biochemical measurements, by informed consent.

## Population Samples

**IMMIDIET Project** The IP was a cross-sectional, ecologic study of cardiovascular risk factors on primary care-based population samples.<sup>20</sup> The study involved about 1000 male/female couples living together, recruited randomly in the 2001 to 2002 period from general practice in the Flemish area of Belgium, in the Abruzzo region of Italy, and in South London and Surrey in England. Participating couples attended their general practitioner's clinic for the screening visit performed by physicians and nurses of the IP team.

**Wandsworth Heart and Stroke Study** The WHSS was a population-based survey carried out in South London from 1994 to 1996 to estimate the prevalence of the

major cardiovascular risk factors in both men and women of different ethnic background.<sup>21</sup> The general practice sample was drawn from within the former Wandsworth Health Authority in South London and included 1578 men and women, aged 40 to 59 years (524 white, 549 of African descent, and 505 of South Asian origin). Only white men were considered in the present analysis. The visits were performed at a dedicated screening unit at St George's Hospital in London.

**Olivetti Prospective Heart Study** The OPHS was an epidemiologic investigation of cardiovascular risk factors in men working at the Olivetti factories in southern Italy.<sup>22</sup> Between May 1994 and December 1995, 1075 men 25 to 75 years of age were examined. The participants were seen in the morning at the medical centre of the Olivetti factories in Pozzuoli and Marcianise, suburbs of Naples, Italy.

## Anthropometric Measurements

In all studies, body weight and height were measured on a standard beam balance scale with an attached ruler. The body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters ( $\text{kg}/\text{m}^2$ ).

## Blood Pressure Measurement

In all studies, BP measurements were performed during the screening visit before blood sampling, with similar protocols. In the IP, BP was measured with an automatic sphygmomanometer (OMRON - HEM-705CPOmron Health Care, Inc, Bannockburn, IL)<sup>23</sup> after the subject had been sitting for 10 min. In the WHSS, BP was measured using an automatic ultrasound sphygmomanometer (Arteriosonde, Roche Products, Welwyn Garden City, UK)<sup>24</sup> after the subject had been resting for at least 10 min in the supine position. In the OPHS, BP was measured with a random-zero sphygmomanometer (Gelman Hawksley Ltd., Sussex, UK) after the participant had been sitting upright for at least 10 min. In all studies, systolic and diastolic BPs were taken three times, with 2-min intervals between measurements and the average of the last two readings was used for the analyses. For the present analysis, hypertension was defined as a systolic BP  $\geq 140$  mm Hg, a diastolic BP  $\geq 90$  mm Hg, or both, or as current use of antihypertensive drugs.

## Biochemical Measurements

In all studies, blood samples for biochemical measurements and DNA extraction were obtained between 7 and 9 AM after overnight fasting. In all studies, serum lipid levels were measured by automated methods (Cobas Mira, Roche, Milan, Italy). The coefficient of variation between assays ranged from 1.70% for serum triglycerides to 1.97% for total cholesterol.

**Table 1.** Characteristics of the participants and distribution of the *HindIII*(+) and *HindIII*(-) alleles in each European population and in the whole sample

	UK (n = 422)	Belgium (n = 313)	Italy (n = 1248)	All (n = 1983)
Age (y)	49.3 ± 6.8‡	46.5 ± 8.6	50.0 ± 7.8‡	49.3 ± 7.8
BMI (kg/m <sup>2</sup> )	26.5 ± 4.0§	26.6 ± 3.8§	27.2 ± 3.2	27.0 ± 3.5
SBP (mm Hg)	127.1 ± 16.8 <sup>5</sup>	127.1 ± 14.8 <sup>5</sup>	129.8 ± 17.0	128.8 ± 16.6
DBP (mm Hg)	81.7 ± 9.8§	80.4 ± 9.3§	83.4 ± 10.0	82.6 ± 9.9
TC (mmol/L)	5.93 ± 1.08§	5.90 ± 1.11	5.74 ± 1.04	5.81 ± 1.06
TG (mmol/L)	1.45 ± 0.82¶	1.41 ± 0.88¶	1.71 ± 1.10	1.60 ± 1.09
Hypertensives*,†	122 (28.9)	86 (27.5)	479 (38.6)	687 (34.8)
Antihypertensive therapy*	46 (10.9)	21 (6.7)	184 (14.8)	251 (12.7)
Hypolipidemic treatment*	37 (8.8)	62 (21.8)	157 (12.7)	256 (13.2)
Smokers (%)	28	27	41	
Alcohol (% of total calories)	4.0 ± 0.6#	4.6 ± 4.0	6.7 ± 6.5	
<i>HindIII</i> (+)*	119 (28.2)	96 (30.7)	782 (62.7)	997 (50.3)
<i>HindIII</i> (-)*	303 (71.8)	217 (69.3)	466 (37.3)	986 (49.7)

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; TC = total cholesterol; TG = triglycerides.

Values are mean ± SD. \* Values are n (%). † Hypertension defined as SBP >140 mm Hg or DBP >90 mm Hg or pharmacologic antihypertensive treatment. ‡ P < .001 v Belgium; § P < .01 v Italy; || P < .05 v Italy; ¶ P < .001 v Italy. # Data on alcohol intake for the UK group refers only to the IMMIDIET study.

## Genotyping

The DNA samples were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) for *HindIII* (+/–) polymorphism according to Charchar et al.<sup>13</sup> and for –344C/T variant of *CYP11B2* according to Russo et al.<sup>25</sup>

For each polymorphism, a 10% random sample was genotyped twice in a blinded fashion with concordant results.

## Statistical Analysis

Data are presented as mean ± SD. As the distribution of HDL-cholesterol and triglyceride levels deviated significantly from normality, they were normalized by log transformation; log-transformed values were used in the analysis, as appropriate. Differences in allele frequency and prevalence of hypertension between populations were determined by  $\chi^2$  statistics. Differences in phenotypic variables between populations were assessed by multiple comparison test with Bonferroni's correction. Differences in the prevalence of hypertension (both within-populations and in the entire sample) were compared by logistic regression analysis adjusting for covariates (age, BMI, smoking habits, alcohol intake, and degree of physical activity). Odds ratios (OR) and their 95% confidence intervals (CI) were also calculated. Differences in continuous phenotypic variables between quantitative genotypes were compared by analysis of covariance (ANCOVA), adjusting for covariates. Individuals taking antihypertensive or hypolipidemic agents were excluded from the analysis. A small number of individuals for whom information on antihypertensive or hypolipidemic treatment was unavailable were also excluded from this analysis (n = 19 for antihyperten-

sive treatment; n = 64 for hypolipidemic treatment). To exclude between-countries differences in phenotypic variables as well as in allelic distribution, which may affect the results, in the whole sample analysis, two different ANCOVA were performed, by including or excluding in the model a factor "country" weighted for the size of each national sample.

The entire population sample provided at least 90% power to detect at P < .05 a 7% difference in the prevalence of hypertension, a 3/2 mm Hg difference for systolic and diastolic BP, respectively, and a 5% difference in serum lipid levels between genotypes. Statistical analyses were performed with the SPSS statistical software package (SPSS 11.0; Chicago, IL).

## Results

All white male participants of the three surveys genotyped for the *HindIII*(+/–) site (n = 1983) were included in the present analysis (UK n = 422, 240 from IP and 182 from WHSS; Belgium n = 313, from IP; Italy n = 1248, 387 from IP and 861 from OPHS). The characteristics of the population samples are described in Table 1. Italian men showed a significantly higher frequency of *HindIII*(+) allele as compared to both British and Belgian men, in whom allele frequency was similar to that found in previous studies in white populations<sup>12,13</sup> ( $\chi^2$  distribution of the alleles in the three populations = 206.9, P < .0001). Significant differences among countries were also found for phenotypic variables. In particular, Italian men had higher BMI, systolic and diastolic BP, prevalence of hypertension, serum triglycerides, and lower cholesterol levels as compared to both British and Belgian men. Prevalence of smokers and contribution of alcohol to total caloric intake were also higher in Italian men, whereas no

**Table 2.** Systolic and diastolic blood pressure according to the presence [*HindIII*(+)] or the absence [*HindIII*(-)] of the Y chromosome *HindIII* polymorphism in untreated individuals\* in each European population and in the whole sample

	UK (n = 375)			Belgium (n = 292)		
	<i>HindIII</i> (+) (n = 111)	<i>HindIII</i> (-) (n = 264)	P	<i>HindIII</i> (+) (n = 91)	<i>HindIII</i> (-) (n = 201)	P
SBP (mm Hg)	126.0 ± 16.1	127.0 ± 16.8	.400	127.7 ± 16.8	126.7 ± 14.3	.476
DBP (mm Hg)	81.6 ± 10.9	81.2 ± 9.3	.846	79.5 ± 9.2	80.4 ± 9.3	.572

Values are mean ± SD.

SBP = systolic blood pressure; DBP = diastolic blood pressure.

P values are derived from analysis of covariance, adjusted for age, BMI, and environmental factors.

† In the analysis of covariance on the whole sample, a factor "country" was also included taking into account the size of each national sample.

\* Data refers to individuals not treated for hypertension.

difference in the degree of physical activity was observed among the three population.

### *HindIII*(+/-) Polymorphism and Blood Pressure

No evidence for the association of the *HindIII*(+/-) variant with the prevalence of hypertension was found in each country as well as in the entire sample (logistic regression analysis, adjusted for covariates—UK: OR 1.15, 95% CI 0.70 to 1.89, *P* = .587; Belgium: OR 0.64, 95% CI 0.35 to 1.16, *P* = .138; Italy: OR 0.95, 95% CI 0.74 to 1.23, *P* = .729; all countries: OR 0.95, 95% CI 0.77 to 1.17, *P* = .632). Comparison of BP values was performed by excluding individuals taking pharmacologic treatment for hypertension. Within each country, no significant difference in systolic and diastolic BP between the *HindIII*(+) and *HindIII*(-) carriers was observed after adjustment for age, BMI, and environmental factors possibly associated to BP regulation, such as alcohol intake, physical activity, and smoking habit (Table 2). To further exclude the possibility

that the difference in allele frequency observed between British and Belgian men on one hand and Italian men on the other may affect the possible association of the *HindIII*(+/-) polymorphism with prevalence of hypertension and BP levels, we performed separate statistical analysis on the Belgium/UK group (*n* = 735, having similar allele frequency) and on the Italian group (*n* = 1248). Also in this case, no significant association in both continuous and categorical analyses was found in any of the two groups (data not shown). Finally, no significant association was found when the entire population was tested, pooling together participants by adjusting also for the size of each national sample (Table 2).

### *HindIII*(+/-) Polymorphism and Serum Lipids

Comparison of serum lipid values was performed by excluding individuals taking hypolipidemic drug treatment. Within each country, no significant difference in total, LDL- and HDL-cholesterol, and triglyceride levels between the *Hin-*

**Table 3.** Serum lipid levels according to the presence [*HindIII*(+)] or the absence [*HindIII*(-)] of the Y chromosome *HindIII* polymorphism in untreated individuals\* in each European population and in the whole sample

	UK (n = 384)			Belgium (n = 222)		
	<i>HindIII</i> (+) (n = 110)	<i>HindIII</i> (-) (n = 274)	P	<i>HindIII</i> (+) (n = 72)	<i>HindIII</i> (-) (n = 150)	P
TC (mmol/L)	5.80 ± 1.13	5.94 ± 1.06	.203	5.68 ± 0.94	5.67 ± 1.00	.886
TG (mmol/L)	1.38 ± 0.62	1.41 ± 0.85	.256	1.33 ± 1.02	1.22 ± 0.79	.274
	n = 110	n = 274		n = 70	n = 150	
HDL (mmol/L)	1.20 ± 0.28	1.28 ± 0.35	.111	1.32 ± 0.34	1.34 ± 0.31	.397
LDL (mmol/L)	3.97 ± 0.98	4.01 ± 0.98	.651	3.76 ± 0.87	3.76 ± 0.88	.908

Values are mean ± SD.

TC = total cholesterol; TG = triglycerides; HDL = high density lipoprotein-cholesterol; LDL = low density lipoprotein-cholesterol. LDL was calculated using the Friedewald equation [TC-HDL-(TG/5)].

P values are derived from analysis of covariance, including age, BMI, treatment with antihypertensive drug, and environmental factors.

† In the analysis of covariance on the whole sample, a factor "country" was also included taking into account the size of each national sample.

\* Data refers to individuals not treated for hyperlipidemia.

**Table 2.** Continued

Italy (n = 1046)			All (n = 1713)†		
HindIII(+) (n = 653)	HindIII(-) (n = 393)	P	HindIII(+) (n = 855)	HindIII(-) (n = 858)	P
127.3 ± 15.9	127.4 ± 15.3	.511	127.2 ± 16.0	127.1 ± 15.5	.359
82.1 ± 9.3	82.0 ± 9.3	.803	81.8 ± 9.5	81.4 ± 9.3	.668

dIII(+) and HindIII(-) carriers was observed after adjustment for age, BMI, antihypertensive drug treatment, and environmental factors possibly associated to lipid metabolism, such as alcohol intake, physical activity, and smoking habit (Table 3). To further exclude the possibility that the difference in allele frequency observed between British and Belgian men on one hand and Italian men on the other may affect the possible association of the HindIII(+/-) polymorphism with serum lipid levels, we performed separate statistical analysis on the Belgium/UK group and on the Italian group. Also in this case, no significant association between the HindIII(+/-) polymorphism and serum lipid levels was found in any of the two groups (data not shown). Finally, no significant association was found when the entire population was tested, pooling together participants by adjusting also for the size of each national sample (Table 3).

**Interaction Between HindIII(+/-) and the -344C/T of CYP11B2 Polymorphisms**

In the entire population sample, there was no significant association between the -344C/T polymorphisms of

CYP11B2 and BP or prevalence of hypertension. On binary logistic regression analysis adjusted for the covariates, no significant association with the prevalence of hypertension was found for any HindIII(+/-)/-344C/T genotype combination, neither in each national sample nor in the entire population (data not shown). After the exclusion of individuals taking antihypertensive drugs, both systolic and diastolic BP levels were not significantly different among all the HindIII(+/-)/-344C/T genotype combinations, neither in the entire population (Table 4), nor in each national sample (data not shown).

**Discussion**

The first report of a direct genetic association between the Y chromosome and BP was provided by Ellis et al<sup>12</sup> in a cohort of Australian men of white origin. In this population, the HindIII(-) allele was associated with higher diastolic BP. At variance with this first study, Charchar et al<sup>13</sup> reported that the same HindIII(-) allele was asso-

**Table 3.** Continued

Italy (n = 1057)			All (n = 1663)†		
HindIII(+) (n = 669)	HindIII(-) (n = 338)	P	HindIII(+) (n = 851)	HindIII(-) (n = 812)	P
5.67 ± 0.98	5.61 ± 0.95	.416	5.68 ± 1.00	5.74 ± 1.01	.977
1.62 ± 1.14	1.59 ± 0.91	.789	1.57 ± 0.96	1.47 ± 0.88	.632
n = 180	n = 147		n = 360	n = 571	
1.29 ± 0.37	1.28 ± 0.40	.542	1.27 ± 0.34	1.30 ± 0.35	.276
3.68 ± 0.82	3.58 ± 0.91	.331	3.79 ± 0.89	3.84 ± 0.95	.909

**Table 4.** Systolic and diastolic blood pressure according to the *HindIII* and *CYP11B2* genotype combinations in the whole population sample

	<i>HindIII</i> (+)				<i>HindIII</i> (-)				<i>P</i> *
	CC ( <i>n</i> = 194)	CT ( <i>n</i> = 405)	TT ( <i>n</i> = 256)	CC ( <i>n</i> = 187)	CT ( <i>n</i> = 434)	TT ( <i>n</i> = 237)	CT ( <i>n</i> = 434)	TT ( <i>n</i> = 237)	
SBP (mm Hg)	126.7 ± 16.6	127.6 ± 15.8	126.9 ± 16.0	128.2 ± 15.2	127.8 ± 15.7	125.1 ± 15.5	127.8 ± 15.7	125.1 ± 15.5	.341
DBP (mm Hg)	81.3 ± 9.8	82.2 ± 9.4	81.4 ± 9.6	82.0 ± 9.4	81.6 ± 9.1	80.5 ± 9.6	81.6 ± 9.1	80.5 ± 9.6	.612

Values are mean ± SD. SBP = systolic blood pressure; DBP = diastolic blood pressure; CC, CT, and TT are the three *CYP11B2* genotypes.

\* *P* values are derived from analysis of covariance, including age, country, and BMI.

ciated with lower systolic and diastolic BP among Scottish and Polish men. They also found a significant epistatic interaction between this variant and the -344C/T polymorphism of *CYP11B2*, an autosomal gene in position 8q21.22, often involved in BP regulation.<sup>13,25,26</sup> Finally, null association with BP was reported in a case-control study in Spanish patients who had myocardial infarctions.<sup>15</sup>

With regard to other variants of the Y chromosome, the Alu insertion polymorphism at DYS287 was not associated with BP in Japanese men.<sup>14</sup> More recently, Rodriguez et al<sup>16</sup> genotyped 2743 British white men for five SNPs (including the *HindIII* site) and two microsatellites spanning the MSY region of the Y chromosome. In that study, no significant association was found between any of the markers analyzed and BP. In particular, the previously reported association between the *HindIII* site and BP was not evident in the population.

Only two studies in humans explored the role of the Y chromosome on lipid levels.<sup>14,17</sup> Shoji et al<sup>14</sup> showed that the Alu insertion polymorphism at DYS287 was associated with minor differences in HDL-cholesterol levels in Japanese men, whereas Charchar et al<sup>17</sup> reported a significant association between the *HindIII*(+/-) variant and serum levels of LDL-cholesterol.

To our knowledge, the present study is the largest report on the association of the *HindIII*(+/-) polymorphism with BP and serum lipids in diverse white populations. The results within each country as well as in the entire sample are consistent and not supportive of the previously reported finding of the association of the *HindIII*(+/-) polymorphism with BP,<sup>12,13</sup> but are in accordance with the recent report by Rodriguez et al.<sup>16</sup> In addition, we did not observe the epistatic interaction between the *HindIII*(+/-) polymorphism and the -344C/T variant of *CYP11B2* on BP previously described by Charchar et al.<sup>13</sup> Finally, in the present analysis we did not confirm the association between the *HindIII*(+/-) site and cholesterol levels previously reported.<sup>17</sup>

The current study highlights many of the important issues in association studies of genetic variants and complex disease. As repeatedly pointed out, the issue of replication in this kind of studies is fundamental, because prior probabilities of true association are low and, thus, all genetic associations demand a high level of proof.<sup>18,19</sup> As a general consideration, in genetic association studies of complex diseases the first study often suggests a stronger genetic effect than is found by subsequent studies, perhaps overestimating the disease protection or predisposition conferred by a genetic polymorphism.<sup>27,28</sup> Among the most important factors underlying the inability to replicate first associations are publication bias, failure to attribute results to chance, and inadequate sample sizes.<sup>29</sup> As recently suggested, large studies based on multicenter collections and done

with standardized methods, as in the present study, may help to prevent replication failure.<sup>29</sup>

Nevertheless, failure to replicate can be due to other possible explanations beyond false-positive studies and type 1 error. First, lack of power to exclude modest effects and different phenotyping procedures in different collections can both contribute to apparent heterogeneity among studies.<sup>18,19</sup> With 90% power to detect a meaningful difference, we cannot exclude the possibility of inadequate power. However, inadequate power is an unlikely explanation of our results. Our combined sample was larger than that of most previously published reports on the association of the *HindIII*(+/-) polymorphism with cardiovascular phenotypes, and large enough to detect clinically and biologically meaningful differences in the phenotypic variables under study, although we recognize that a larger sample size would be needed to exclude minor differences in lipid levels between genotypes. We also recognize as a possible limitation of the present analysis that the three white populations under study may not be considered homogeneous both for cardiovascular phenotypes and for allelic distribution. In particular, the significant difference in the *HindIII*(+/-) genotypes between British and Belgian men on one hand and Italian men on the other may suggest a different Y chromosome lineage in these populations, thus raising the issue of ethnicity in determining genetic associations.<sup>30</sup> However, no trend toward biologically meaningful and statistically significant association was apparent either when single populations were explored or in the separate analysis of the two groups—combined British/Belgian and Italian—homogeneous for *HindIII*(+/-).

Another possible explanation of the conflicting results is that this polymorphism per se is not functional and does not bring any biological relevance. In different populations, changes at the *HindIII* site may have occurred at different time during the evolution,<sup>30</sup> thus placing or not this site in linkage disequilibrium with the “true” functional loci on the Y chromosome possibly affecting BP and/or lipid levels, and, finally, obscuring the association in some populations.

In conclusion, our results contrast with the hypothesis that the *HindIII*(+/-) site of the Y chromosome is a marker of cardiovascular risk in white men and reinforce the need for extensive replication studies if we are to be successful in defining true risk factors in complex diseases.<sup>29</sup> Although the presence of cardiovascular markers on the Y chromosome is still hypothetical, the biological hypothesis under investigation remains of great interest. In particular, family and father-son studies of MSY region markers and the identification in the same region of haplogroups with either protective or predisposing effects may be more informative on the role of Y chromosome in the sexual dimorphism of cardiovascular phenotypes.

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