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Association Between a Thyroid Hormone Receptor-α Gene Polymorphism and Blood Pressure but Not With Coronary Heart Disease Risk

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BACKGROUND

Thyroid hormones (THs) exert multiple biological roles including effects on the cardiovascular system (lipid profile, blood pressure (BP) and cardiac output). The lipid-lowering actions of TH are mediated by the TH receptor-β whereas the mechanisms explaining the BP variations concomitant with the thyroid disorders are less understood. As the TH receptor-α (TR-α) has been associated with many of TH actions on the cardiovascular system in mice models, we hypothesized that it could be involved in the latter. We thus tested whether polymorphisms in TR-α (*THRA* gene) could be associated with BP level variation. Secondarily, we tested for association with coronary heart disease (CHD) risk.

METHODS

We analyzed the associations between five *THRA* polymorphisms and (i) BP level in two population-based studies (MONICA Lille $n = 1,155$; MONICA Toulouse $n = 1,170$) and (ii) the risk of CHD in two case–control studies (Lille CHD $n = 558$ cases/568 controls; PRIME $n = 527$ cases/584 controls).

RESULTS

Individuals carrying the rs939348 T allele had higher systolic BP (~+1.3 mm Hg) than CC individuals in both the MONICA Lille ($P = 0.02$) and Toulouse ($P = 0.03$) studies. The odds ratio (OR) for hypertension was 1.25 ($P = 0.02$) in the combined sample. Concerning the CHD risk, no significant association could be detected.

CONCLUSIONS

For the first time, our study showed associations between the *THRA* rs939348 polymorphism and systolic BP and the risk of hypertension but not with CHD, although we admit that the statistical power available to study any relationship with CHD was very limited. Further larger association studies are needed to confirm our findings.

Keywords: *blood pressure; coronary heart disease; hypertension; polymorphism; thyroid hormone; thyroid hormone receptor*

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Thyroid hormones (THs) (thyroxine, T_4; and 3,5,3′-triiodothyronine, T_3) act on nearly every cell in the body. They modulate, among other effects, basal metabolic rate, protein synthesis, bone growth, brain development and maturation, lipid and carbohydrate metabolisms.

THs act in the nucleus via specific TH receptors (TRs). TRs are members of the nuclear receptor family. These receptors regulate target gene transcription by binding to specific DNA sequences (TH response elements) in the promoter region. TRs constitutively bind to thyroid hormone response elements as homodimers or, more commonly, as heterodimers with the retinoid X receptor (see ref. 1 for review). For positively regulated target genes, the TRs actively repress transcription in the absence of T3. Ligand binding induces structural changes, the exchange of bound cofactors and, ultimately, target gene expression. The opposite situation is true for thyroid-stimulating hormone and thyrotropin-releasing hormone, the expression of which is strongly repressed by T3.[1] TRs are encoded by the *THRA* (for TR-α) and *THRB* (for TR-β) genes, located respectively on chromosomes 17 and 3.[2] The expression of TRs is tissue-dependent and developmentally regulated.[2] In humans, alternative splicing of the primary transcripts gives rise to several TR isoforms. Although TR-α and TR-β are expressed ubiquitously, TR-β1 is more abundant in the liver, kidney, and thyroid. Expression of TR-β2 is limited to the pituitary, hypothalamus, retina, and inner ear, whereas TR-α1

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is expressed predominantly in the heart, bone, intestine, and brain.

Observational studies have suggested that hypothyroid patients have accelerated coronary atherosclerosis.[3] In the Rotterdam study, elderly women with subclinical hypothyroidism had a higher prevalence of myocardial infarction, compared with euthyroid women.[4] Although these results suggest that THs have anti-atherosclerotic effects, other studies do not, however, support the existence of a strong association between thyroid hormone levels and coronary heart disease (CHD).[5,6] Recent meta-analyses tended to suggest that subclinical hypothyroidism is associated with a greater susceptibility to CHD.[7,8] However, a large study assessing the effect of hormone replacement therapy on the CHD outcome in hypothyroid patients is currently lacking. A few studies on small numbers of patients suggest that the treatment of hypothyroidism could slow down atherogenesis[9] and even reverse the increased intima-media thickness.[10]

The link between overt hypothyroidism and atherosclerosis has been attributed to main cardiovascular risk factors such as hypercholesterolemia, high levels of diastolic (DBP) and systolic blood pressure (SBP) and endothelial dysfunction, all often observed in patients with overt hypothyroidism. It has been shown that adequate thyroid hormone replacement reduces BP and improves endothelial function in these patients.[11] Although it is established that the alterations in cholesterol metabolism in overt hypothyroidism are largely due to TR-β in liver,[12–14] and that heart rate is governed by TR-α,[15–17] the mechanisms related to changes in BP level are not perfectly understood. In fact, BP is altered across the entire spectrum of thyroid disorders (see ref. 18 for review). Thyroid hormone exerts an acute effect on BP as patients suffering from thyroidectomy have higher DBP and SBP levels.[19] Moreover, hyperthyroidism is associated with systolic hypertension in some patients, especially in the elderly.

We hypothesized that TR-α could be involved in the regulation of BP and we tested the impact of five *THRA* tagSNPs (rs868150, rs7502966, rs1568400, rs939348, and rs3744805) on BP level variation in two population-based studies.

METHODS

Subjects

The MONICA study: Participants were recruited as part of the World Health Organization-MONICA population survey performed from 1995 to 1997 in two different parts of France: the Lille Urban Community in northern France (n = 1,155) and the Haute-Garonne county in southern France (n = 1,170). Subjects (aged 35–64 years) were randomly selected from electoral rolls after stratification by town size, gender, and age in order to obtain 200 participants for each gender and each 10-year age group (World Health Organization-MONICA Project protocol).[20] Details of the study have been described elsewhere.[21]

After providing written informed consent, participants filled out a standard questionnaire and physical measurements were taken by a specially trained nurse. The questionnaire covered questions on socioeconomic factors, physical activity, alcohol consumption, smoking status, personal and family medical history, attitudes and knowledge concerning several diseases and any current medication use. Physical activity was defined as at least a 15-min walk a day, and/or lifting or carrying heavy objects at work every day and/or sport or physical exercise for more than 2 h a week. In terms of smoking exposure, individuals were categorized as never smokers, former smokers and current smokers (i.e., individuals reporting at least one cigarette per day). Alcohol intake was expressed as the total number of milliliter of alcohol per week from wine, beer, cider, and spirits. Anthropometric measurements including body weight were taken on individuals in light clothing without shoes. The body mass index was calculated according to the Quetelet equation. Blood pressure was measured on the right arm, with the subject in a sitting position and after a minimum 5-min rest, using a standard mercury sphygmomanometer. The mean value of two consecutive BP readings was taken into account. A 20 ml blood sample was drawn into a disodium EDTA tube (after the subjects had fasted for at least 10 h).

The Lille case–control study of CHD: A sample of 585 individuals with CHD were drawn from the EUROASPIRE study (European Action on Secondary Prevention by Intervention to Reduce Events), which has been described elsewhere.[22] The present report focuses on patients enrolled by hospitals in the Lille Urban Area during the first and the second EUROASPIRE surveys (performed in 1995–1996 and 1999–2000, respectively). Consecutive patients with confirmed CHD were retrospectively enrolled from hospital admission lists, with the following diagnosis: acute myocardial infarction, acute myocardial ischemia or CHD treatment with coronary bypass grafting or percutaneous transluminal coronary angioplasty. The selected patients were interviewed and examined at least 6 months after their initial admission. The major cardiovascular risk factors and treatments were collected from hospital records. The control group was composed of part of individuals from the population-based MONICA Lille study described above. We selected 582 individuals with no personal history of CHD and then matched them (by 5-year age-class and by gender) with the CHD cases.

The PRIME study: PRIME is a prospective, population-based cohort study designed to identify risk factors for CHD. Details on recruitment, baseline examination, and follow-up in the PRIME study have been described elsewhere.[23,24] Briefly, during the period 1991–1994, 10,600 European-Caucasian men aged 50–59 years (living in or around the cities of Lille, Strasbourg and Toulouse in France and Belfast in Northern Ireland) were recruited by various employment groups, health screening centers and general practitioners. Each subsample of ~2,500 men was built to broadly match the social class structure of the underlying population. Approvals from the appropriate local ethics committees were obtained and all individuals gave a written informed consent. The study entry examination included standardized questionnaires relating to medical history, medication use, the presence of CHD, various habits (including tobacco and alcohol consumption), and a clinical examination. Of the initial sample of 10,600 men, 9,779 were found free of

CHD at the baseline examination. By the end of the 10-year follow-up period, 661 men had experienced at least one coronary event. A nested, case–control study within the PRIME study was performed with 626 cases and 626 matched controls. The age-matched (±5 years) controls were study participants who had been recruited by the same center around the same day (±7 days) as their corresponding case. All were free of CHD on the date of the case's ischemic event. DNA samples were available for 539 CHD cases and 607 controls for the present study.

Genotyping. The single-nucleotide polymorphisms (SNPs) were genotyped using TaqMan (Applied Biosystems, Foster City, CA) or mass spectrometry (Sequenom, San Diego, CA) techniques. The genotyping conditions are available on request. The genotyping success rates varied between 93% and 99%.

Statistical analysis. Statistical analyses were performed with SAS 8.02 software (SAS Institute, Cary, NC). Hardy–Weinberg equilibrium was tested using the χ^2 test (1 degree of freedom). In the population-based studies, intergroup comparisons of means for SBP and DBP were performed with a general linear model for both recessive and dominant models. The adjustment variables were age, gender, body mass index, smoking habit, alcohol consumption, and level of physical activity. We calculated the association between the *THRA* genotypes and the odds of hypertension (odds ratio and 95% confidence intervals) in the combined MONICA Lille and Toulouse sample using unconditional logistical regression adjusted for age, gender, center, body mass index, smoking habit, alcohol consumption, and level of physical activity.

Haplotype frequencies were estimated using a stochastic version of the expectation-maximization algorithm, as implemented in Thesias software.[25]

In the two CHD case–control studies, the associations between the *THRA* genotypes and the risk of CHD were calculated as the odds ratio (OR) and 95% confidence interval in logistic regression analyses. The adjustment variables were age, body mass index, smoking status, and history of diabetes, with the addition of gender for the Lille CHD case–control study.

Power calculations were performed using Quanto software, version 1.1.1.[26]

RESULTS

We previously described that five tagSNPs (rs868150, rs7502966, rs1568400, rs939348, and rs3744805) capture the known common genetic variability of the *THRA* gene.[27] The population-based MONICA Lille (n = 1,155) and MONICA Toulouse (n = 1,170) samples were genotyped for these five SNPs. The genotype distributions are presented in **Table 1** and all were conformed to the Hardy–Weinberg equilibrium.

The associations between the five *THRA* SNPs and SBP and DBP were assessed separately for the MONICA Lille and Toulouse samples. Only associations that were consistently significant at $P < 0.05$ in the two independent samples were considered. Using this approach, we did not detect any association between rs868150, rs7502966, rs1568400, and rs3744805 and BP (**Table 2**). In contrast, we detected significant associations between rs939348 and SBP in the MONICA Lille (P = 0.02) and Toulouse (P = 0.03) studies (**Table 2**). Indeed, in both studies, individuals bearing the minor T allele of rs939348 had higher SBP (average difference: +1.3 mm Hg) compared with CC individuals. It is noteworthy that rs939348 was also moderately associated with DBP in the two studies with the same direction of effect as that for SBP (P = 0.03 in MONICA Lille and P = 0.14 in MONICA Toulouse) (**Table 2**). To take medication into account, we used two different models. First,

Table 1 | Genotype distribution of *THRA* polymorphisms in the MONICA Lille and MONICA Toulouse studies

		MONICA Lille			MONICA Toulouse		
		N (freq)	MAF	HWE	*N* (freq)	MAF	HWE
rs868150	GG	402 (0.36)	0.40	0.48	395 (0.35)	0.42	0.33
	GA	551 (0.49)			535 (0.47)		
	AA	173 (0.15)			204 (0.18)		
rs7502966	TT	367 (0.32)	0.43	0.67	341 (0.31)	0.44	0.95
	TC	559 (0.50)			543 (0.49)		
rs1568400	TT	613 (054)	0.27	0.71	612 (0.54)	0.27	0.26
	TC	436 (0.39)			424 (0.38)		
	CC	82 (0.07)			87 (0.08)		
rs939348	CC	593 (0.53)	0.27	0.97	604 (0.53)	0.27	0.38
	CT	437 (0.40)			461 (0.40)		
	TT	81 (0.07)			77 (0.07)		
rs3744805	CC	865 (0.77)	0.12	0.87	1,034 (0.89)	0.06	0.08
	CT	237 (0.21)			119 (0.10)		
	TT	17 (0.02)			7 (0.01)		

HWE, *P* value for Hardy–Weinberg equilibrium test; MAF, minor allele frequency. *THRA*, thyroid hormone receptor-α.

Table 2 | Association between *THRA* SNPs and blood pressure level in the MONICA Lille and MONICA Toulouse studies

	MONICA Lille						MONICA Toulouse					
rs868150 (*N*)	GG (402)	GA (551)	AA (173)	*P*	*P1*	*P2*	GG (395)	GA (535)	AA (204)	*P*	*P1*	*P2*
SBP (mm Hg)	134.1 ± 20.2	133.3 ± 19.0	133.7 ± 19.3	0.79	0.73	0.65	130.7 ± 17.5	131.0 ± 17.5	133.0 ± 19.6	0.11	0.33	0.03
DBP (mm Hg)	83.3 ± 11.8	82.7 ± 11.23	82.7 ± 11.7	0.97	0.89	0.89	79.5 ± 10.4	79.5 ± 10.4	80.1 ± 11.1	0.56	0.82	0.27
rs7502966 (*N*)	TT (367)	TC (559)	CC (202)	*P*	*P1*	*P2*	TT (341)	TC (543)	CC (218)	*P*	*P1*	*P2*
SBP (mm Hg)	134.1 ± 18.8	132.9 ± 19.0	135.1 ± 21.7	0.73	0.71	0.43	131.0 ± 17.9	130.8 ± 17.9	130.8 ± 17.2	0.93	0.65	0.61
DBP (mm Hg)	83.2 ± 11.3	82.2 ± 11.3	84.5 ± 12.4	0.23	0.90	0.12	78.9 ± 10.5	79.8 ± 10.5	79.7 ± 10.4	0.46	0.30	1.00
rs1568400 (*N*)	TT (613)	TC (436)	CC (82)	*P*	*P1*	*P2*	TT (612)	TC (424)	CC (87)	*P*	*P1*	*P2*
SBP (mm Hg)	133.5 ± 19.3	133.4 ± 19.5	135.6 ± 20.4	0.91	0.99	0.68	131.5 ± 18.3	131.1 ± 17.8	130.9 ± 17.4	0.62	0.30	0.61
DBP (mm Hg)	82.7 ± 11.4	82.9 ± 11.7	84.7 ± 11.7	0.42	0.88	0.20	79.8 ± 10.47	79.2 ± 10.8	79.7 ± 10.2	0.50	0.24	0.99
rs939348 (*N*)	CC (593)	CT (437)	TT (81)	*P*	*P1*	*P2*	CC (604)	CT (461)	TT (77)	*P*	*P1*	*P2*
SBP (mm Hg)	132.9 ± 18.9	134.5 ± 20.6	134.4 ± 17.9	0.07	**0.02**	0.78	130.6 ± 17.2	132.0 ± 18.5	131.8 ± 19.9	0.07	**0.03**	0.85
DBP (mm Hg)	82.5 ± 11.4	83.4 ± 11.8	83.9 ± 10.8	0.08	0.03	0.29	79.4 ± 10.1	80.0 ± 10.6	79.6 ± 13.3	0.31	0.14	0.85
rs3744805 (*N*)	CC (865)	CT (237)	TT (17)	*P*	*P1*	*P2*	CC (1034)	CT (119)	TT (7)	*P*	*P1*	*P2*
SBP (mm Hg)	133.2 ± 19.4	135.0 ± 19.8	135.3 ± 16.5	0.15	0.11	0.50	131.6 ± 18.2	129.0 ± 15.8	121.6 ± 11.3	0.76	0.76	0.46
DBP (mm Hg)	82.5 ± 11.4	83.9 ± 11.6	87.1 ± 11.0	0.05	0.02	0.31	79.9 ± 10.6	77.4 ± 9.4	71.7 ± 9.2	0.08	0.06	0.08

Data are expressed as means ± s.d. ***P*** values for the 3 group model (*P*), the dominant model (*P1*) or the recessive model (*P2*) were adjusted for age, gender, body mass index, smoking habit, alcohol consumption and level of physical activity. Significant *P* values are indicated in bold.
DBP, diastolic blood pressure; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism; *THRA*, thyroid hormone receptor-α.

Table 3 | Association of rs939348 with hypertension in the combined MONICA Lille and Toulouse studies.

rs939348	Normortensive	Hypertensive			
Genotype	*N* (freq)	*N* (freq)	P^a	OR[b] (95% CI)	P^b
CC	738 (0.54)	459 (0.52)		reference	
CT	529 (0.39)	368 (0.41)	0.46	1.25 (1.03–1.15)	0.02
TT	95 (0.07)	63 (0.07)			
	1,362	890			

CI, confidence interval; OR, odds ratio.
[a]*P* value for a global test of significance. [b]OR and *P* value (dominant model) are adjusted for age, gender, center, body mass index, smoking habit, alcohol consumption, and level of physical activity.

we excluded individuals on antihypertensive medication (n = 200 and n = 150 excluded individuals in MONICA Lille and MONICA Toulouse, respectively) and we observed that the association between rs939348 and SBP persisted (P = 0.05 and P = 0.02 in MONICA Lille and Toulouse, respectively) (data not shown). Second, in individuals taking antihypertensive therapies, BP was imputed by adding 15 mm Hg and 10 mm Hg to SBP and DBP, respectively, as found in previous studies[28,29] and the association between rs939348 and SBP was still detected (P = 0.03 for both studies).

We then examined the association with hypertension (SBP ≥140 mm Hg or DBP ≥90 mm Hg or antihypertensive medication use) compared to normotension (SBP<140 mm Hg and DBP<90 mm Hg and no antihypertensive medication use) in the combined sample to increase statistical power (N = 890 hypertensive/1,362 normotensive individuals). The minor allele of rs939348 was associated with higher odds of hypertension, consistent with the continuous trait effect (Table 3). The adjusted OR (95% confidence interval) for hypertension was 1.25 (1.03–1.51), P = 0.02.

Haplotype analyses performed with the five SNPs did not add any further information (data not shown).

We then searched for possible associations between the five *THRA* SNPs and the risk of CHD. To this end and by adopting the same strategy as above, we used two independent CHD case–control studies: the PRIME nested case–control study (584 controls/527 cases) and the Lille CHD case–control study (568 controls/558 cases). The genotype distributions of the five SNPs in the control groups respected the Hardy–Weinberg equilibrium. Controls and CHD cases were compared in terms of distributions of the five *THRA* SNP genotypes (Table 4); no significant difference was observed ($P \geq 0.12$). Consequently, the ORs for CHD did not vary according to the *THRA* genotype ($P \geq 0.08$) (Table 4). Even when combining the two studies, no significant association could be detected (data not shown).

DISCUSSION

Here, we report on the detection of significant and consistent associations between the minor allele of the *THRA* rs939348 SNP and elevated SBP in two population-based studies. The same direction of effect was also observed for DBP. Consequently, this allele was also associated with a 25% higher risk of hypertension.

We are the first to report an association between *THRA* SNPs and BP. These results are in accordance with the known links between thyroid dysfunction and BP. However, the underlying mechanisms are unclear. Atrial and brain natriuretic peptides seem to be directly regulated by THs and may play an important role in this process (see ref. 30 for review). Blood pressure

Table 4 | *THRA* genotype distributions among CHD controls and cases and ORs (95% CI) of CHD in the two studies

	Lille CHD study					PRIME study				
	Controls	Cases				Controls	Cases			
	N (freq)	*N* (freq)	*P*[a]	OR (95% CI)	*P*	*N* (freq)	*N* (freq)	*P*[a]	OR (95% CI)	*P*
rs868150	568	558				584	527			
GG	203 (0.36)	207 (0.37)		Reference		199 (0.34)	181 (0.34)		Reference	
AG	280 (0.49)	246 (0.44)	0.12	0.98 (0.74–1.29)	0.88	271 (0.46)	252 (0.48)	0.76	1.05 (0.78–1.41)	0.74
AA	85 (0.15)	105 (0.19)				114 (0.20)	94 (0.18)			
rs7502966	574	580				607	539			
CC	192 (0.33)	196 (0.34)		Reference		212 (0.35)	170 (0.32)		Reference	
CT	274 (0.48)	269 (0.46)	0.87	1.00 (0.76–1.31)	0.97	275 (0.45)	259 (0.48)	0.47	1.20 (0.91–1.58)	0.19
TT	108 (0.19)	115 (0.20)				120 (0.20)	110 (0.20)			
rs1568400	573	581				604	538			
TT	305 (0.53)	321 (0.55)		Reference		345 (0.57)	288 (0.53)		Reference	
TC	231 (0.40)	209 (0.36)	0.16	0.97 (0.75–1.26)	0.83	219 (0.36)	209 (0.39)	0.46	1.25 (0.96–1.62)	0.11
CC	37 (0.07)	51 (0.09)				40 (0.07)	41 (0.08)			
rs939348	559	574				590	526			
CC	306 (0.55)	320 (0.56)		Reference		306 (0.52)	281 (0.53)		Reference	
CT	220 (0.39)	223 (0.39)	0.91	0.93 (0.71–1.21)	0.59	239 (0.41)	211 (0.40)	0.72	0.89 (0.68–1.16)	0.37
TT	33 (0.06)	31 (0.05)				45 (0.08)	34 (0.07)			
rs3744805	569	581				602	536			
CC	435 (0.76)	459 (0.79)		Reference		462 (0.77)	435 (0.81)		Reference	
CT	130 (0.23)	115 (0.19)	0.32	0.89 (0.65–1.23)	0.49	132 (0.22)	92 (0.17)	0.12	0.78 (0.56–1.05)	0.10
TT	4 (0.01)	7 (0.01)				8 (0.01)	9 (0.02)			

CHD, coronary heart disease; CI, confidence interval; OR, odds ratio; *THRA*, thyroid hormone receptor-α.
[a]*P* value for a global test of significance. ORs and *P* values were calculated using a dominant model and were adjusted for age, body mass index, smoking status and history of diabetes (with the addition of gender for the Lille CHD study).

is also controlled by the autonomic nervous system, providing an alternate possible mechanism based on a central action of T3. Catecholamine secretion is normal or even reduced in hyperthyroidism,[31,32] while in hypothyroidism, plasma noradrenaline concentrations are increased.[33,34] Thyroid hormone deficiency is associated with an increased sympathetic influence on the autonomic cardiovascular system.[35] Finally, recent studies in transgenic mice also suggest that TR-α1 functions to activate parasympathetic signaling.[36]

Identification of common genetic variants influencing BP has proven to be challenging. Recently, a genome-wide association study including ~35,000 individuals identified eight loci associated with SBP or DBP.[29] The *THRA* locus was not one of these top hits. However, due to the very low *P* value threshold required in a genome-wide association study ($P < 5 \times 10^{-8}$), nominal associations with *THRA* SNPs may have gone unreported. Thus, a candidate gene approach, as in the present study, may still help to detect associations between SNPs and disorders. Our findings, however, must be interpreted with caution and replications in other population samples are necessary before a link between *THRA* gene variability and BP can definitely be established.

Hypertension, being a known risk factor for cardiovascular diseases, the presence of an association between rs939348 and higher BP levels in the MONICA studies, encouraged us to investigate a possible association between the *THRA* SNPs and the risk of CHD in two independent case–control studies. However, no significant effect of the *THRA* SNPs on CHD risk was found. It is likely that the SNP effects on BP are not substantial enough to modify the CHD risk. Previous genome-wide association studies on CHD with ~25,000 individuals did not detect either significant associations with *THRA*, at least at the genome-wide significance level,[37–40] suggesting that the risk of CHD associated with *THRA* polymorphisms is very small, if any.

Our study presents a number of limitations and advantages. One strength is that we covered the whole known genetic variability of the *THRA* gene by studying the five tagSNPs. Rather than performing a potentially over-conservative Bonferroni correction, we chose to reduce type I errors by using two independent studies for each category of phenotype (two population-based studies for BP and two case–control studies for the CHD risk. We did not assess the impact of the *THRA* SNPs on BP level in the PRIME nested CHD case–control study as individuals are, by definition, selected on their CHD status. At baseline, half of the individuals of the study are future CHD cases. Therefore, their level of BP is biased and not representative of that of the population. Similarly, control subjects are matched to the cases and therefore not representative of the

population. We admit that the statistical power available to study any relationship with CHD was very limited. Indeed, each CHD case–control study was powered (≥80%) to identify effect sizes larger than 1.47 (for the OR, using a dominant model and a minor allele frequency of 0.12), suggesting that smaller clinical effects have certainly been missed. As such an effect size is high for a multifactorial disease, no reliable conclusion on the lack of effect can be drawn. The rs939348 SNP is located in the first intron after the translation start site (see **Supplementary Figure S1** online) and it remains to evaluate whether it could modulate the level of expression of one of the transcripts encoded by the *THRA* gene in endothelial or smooth muscle cells for example, to explain the present association with BP. In mice, both TR-α1 and TR-α2 are highly expressed in the heart with no significant detection of either TRΔα1 or TRΔα2.[41] No data are available in humans. As both hyper- and hypothyroidism have been linked to systolic hypertension, variation of the TR-α1 expression is unlikely to explain the observed variation in BP. TR-α2 might thus appear as a better candidate since its activity is independent of the thyroid hormone status and its function has not been established yet. However, using Genomatix software, we did not identify any relevant transcription factor encompassing rs939348 that could generate functional hypotheses. Last, it would have been interesting to assess the association between the SNPs and thyroid hormone levels but unfortunately, this phenotype was not initially measured in any of the sample.

We previously explored the association between *THRA* SNPs and the risk of Alzheimer's disease in a sample of 5,840 individuals and we could not entirely exclude a possible role for rs939348 in the susceptibility of this disease.[27] The fact the same SNP in *THRA* may be associated with BP level and Alzheimer's disease risk are in line with the epidemiological data showing that midlife high BP increases the risk of late-life Alzheimer's disease (refs. 42–44,45 for review). These elements might provide a clue for later investigations of the mechanisms underlying the observed associations.

In conclusion, our study constitutes the first association study of *THRA* gene polymorphisms and BP or CHD risk. We observed associations between the minor allele of rs939348 and higher SBP and higher risk of hypertension. Additional larger association studies are needed to confirm our findings.

APPENDIX

The PRIME Study Group

The PRIME Study is organized under an agreement between INSERM and the Merck Sharp and Dohme-Chibret Laboratory, with the following participating laboratories:

- The Strasbourg MONICA Project, Laboratoire d'Epidémiologie et de Santé Publique, EA3430, Strasbourg, F-67085, France; Université Louis Pasteur, Faculté de Médecine, Strasbourg, F-67085, France (D.A. and B Haas).
- The Toulouse MONICA Project, INSERM U558, Departement d'Epidemiologie, Université Paul Sabatier-Toulouse Purpan, Toulouse, France (J. F. and JB Ruidavets).
- The Lille MONICA Project, INSERM U744, Lille, France; Institut Pasteur de Lille, Lille, France; Université de Lille 2, Lille, France (P.A. and M Montaye).
- The Department of Epidemiology and Public Health, Queen's University Belfast, Belfast, Northern Ireland (A Evans, J Yarnell and F.K.).
- The Department of Atherosclerosis, INSERM U545, Lille, France; Institut Pasteur de Lille, Lille, France; Université de Lille 2, Lille, France (G Luc and JM Bard).
- The Laboratory of Haematology, INSERM U626, Marseille, Hôpital La Timone, Marseille, France (I Juhan-Vague and P Morange).
- The Laboratory of Endocrinology, INSERM U563, Toulouse, France (B Perret).
- The Vitamin Research Unit, The University of Bern, Bern, Switzerland (F Gey).
- The Nutrition and Metabolism Group, Centre for Clinical and Population Sciences, Queen's University Belfast, Belfast, Northern Ireland (J Woodside and I Young).
- The DNA Bank, INSERM U525, Paris, France (F Cambien).
- The Coordinating Center, INSERM U780, Villejuif, F-94807, France; University Paris-Sud, Faculty of Medicine, Villejuif, F-94807, France (P Ducimetiere and A Bingham).

Supplementary material is linked to the online version of the paper at http://www.nature.com/ajh

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