

## ORIGINAL ARTICLE

# Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort

V. Panicker\*†, S. G. Wilson\*¶, T. D. Spector‡, S. J. Brown\*, M. Falchi‡, J. B. Richards‡, G. L. Surdulescu‡, E. M. Lim\*§, S. J. Fletcher§ and J. P. Walsh\*¶

\*Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia, †Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol, UK, ‡Twin Research and Genetic Epidemiology Unit, King's College London, London, UK, §Pathwest Laboratory Medicine WA, Nedlands, Western Australia, Australia and ¶School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia, Australia

## Summary

**Objective** Thyroid hormone action influences many metabolic and synthetic processes, but the degree of regulation attributed to genes and environmental factors affecting normal variation remains controversial.

**Design** We investigated the magnitude of the genetic and environmental determination of serum concentrations of free (f) T3, fT4, TSH and the fT4 × TSH product and their variation, in a large cohort of twin pairs. Female dizygous and monozygous twins (849 and 213 pairs, respectively) from the TwinsUK registry (mean age 45.5, range 18–80 years) were studied.

**Results** Comparison of thyroid parameters within various groups showed no differences between smoking categories, and higher serum TSH and lower fT3 in subjects with positive thyroid antibodies. Using structural equation modelling, we estimated the heritable contribution to serum thyroid parameters (with 95% confidence intervals) to be 65% (58%–71%) for TSH, 65% (58%–71%) for the fT4 × TSH product, 39% (20%–55%) for fT4 and 23% (3%–41%) for fT3.

**Conclusions** We conclude that genetic regulation is a particularly important determinant of TSH and the fT4 × TSH product, and is a less important determinant of fT4 and fT3 concentrations in Caucasian women. These data from a large well-characterized cohort suggest that while there is a strong heritable contribution to serum TSH, variation in fT4 and fT3 concentrations may be less explained by genetic factors and more driven by environmental effects than previously thought.

(Received 1 July 2007; returned for revision 1 August 2007; finally revised 9 August 2007; accepted 23 August 2007)

Correspondence: Clin A/Prof John P. Walsh, Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia 6009, Australia. E-mail: john.walsh@health.wa.gov.au

## Introduction

It has been recognized for some time that levels of thyroid function parameters in healthy subjects show considerable interindividual variability leading to wide (population-based) laboratory reference ranges, whereas intra-individual variability has a much narrower range.<sup>1–3</sup> This is important because clinical management of thyroid conditions depends very much on estimations of serum TSH, free (f) T4 and fT3 in relation to the reference range, and what is abnormal for a given individual may not necessarily be abnormal according to the designated reference range.<sup>3</sup> Furthermore, there is increasing evidence that at the population level, small differences in thyroid function are associated with differences in clinically important parameters such as body mass index (BMI),<sup>4–6</sup> blood pressure<sup>7,8</sup> and the presence of atrial fibrillation.<sup>9</sup>

It appears therefore that each individual has a unique set point of thyroid function. This is likely to be genetically determined (at least in part), but there are limited data regarding the extent of genetic influence on pituitary–thyroid axis function, and conflicting data from published studies to date. Hansen *et al.* used structural equation modelling (SEM) to determine the relative contributions of genetic and environmental effects to phenotypic variance in a large study of 690 Danish twin pairs.<sup>10</sup> They determined that heritability was fairly consistent across the thyroid parameters being responsible for 64% of the variation of plasma TSH, 65% of fT4 and 64% of fT3. In an earlier, much smaller study (30 twin pairs) Meikle *et al.* determined that heritability accounted for 44% of the variation in plasma fT4.<sup>11</sup> In this study, heritability of plasma fT3 and TSH did not reach significance and environmental influences were not examined. There have also been family studies looking at heritability of thyroid hormone traits. Martin *et al.* studied the heritability of total T4 in 378 Mennonites (112 families) from Kansas and Nebraska, using a path analysis method and determined the genetic contribution to be 32%; however, there was an environmental contribution of only 6%, leaving a very high unexplained residual component.<sup>12</sup> Samollow *et al.* examined heritability in a family study in 1011 Mexican Americans (27 families) using a pedigree-based likelihood approach to calculate residual heritability which they determined to contribute approximately 32% of the variance in TSH, 37% in fT4 and 67% in fT3.<sup>13</sup> Serum TSH and

total T4 concentrations differ significantly between Mexican Americans and white, non-Hispanic Americans,<sup>14</sup> but it is not known whether this reflects genetic or environmental differences, or both.

A key component of pituitary–thyroidal axis function is the negative feedback effect of circulating thyroid hormones on TSH secretion by pituitary thyrotrophs. In studies of individuals with resistance to thyroid hormone (caused by mutations in thyroid hormone receptor  $\beta$ ), Yagi *et al.* showed that the product of serum fT4 and TSH concentrations provides a measure of the sensitivity of the thyrotrophs to this negative feedback.<sup>15</sup> Recently, Fernández-Real *et al.* applied the fT4  $\times$  TSH product to normal subjects as a measure of thyrotroph T4 resistance, and showed a strong correlation between the fT4  $\times$  TSH product and each of fasting triglycerides, high-density lipoprotein cholesterol and endothelium-dependent vasodilatation.<sup>16</sup> There are, however, no published data on the extent to which the fT4  $\times$  TSH product is genetically determined (other than in subjects with the well-defined syndrome of thyroid hormone resistance).

Because of the uncertainty regarding the extent of the genetic influence on pituitary–thyroid axis function, we analysed the heritability of variation in serum TSH, fT4, fT3 and the fT4  $\times$  TSH product in a large twin population from the UK using a classical twin study approach.

## Subjects and methods

### Subjects

The subjects were female twin pairs from St Thomas' UK Adult Twin Registry, a volunteer sample recruited through a national media campaign in the UK without selecting for particular diseases or traits. The initial sample contained 1291 twin pairs. Twins were 18–80 years of age and were assessed for a range of clinical phenotypes related to thyroid metabolism. Clinical data collected included age, height and weight. General medical, gynaecological and lifestyle questionnaires were completed at interview. Both twins attended for interview, examination and blood sampling at the same time on the same day. To minimize the confounding effects of thyroid disease, we excluded participants (and their twins) who had a history of thyroid disease and those whose results indicated undiagnosed thyroid dysfunction with a high level of confidence: serum TSH less than 0.1 mU/l, greater than 10 mU/l or greater than 6 mU/l with positive thyroid peroxidase antibodies (TPOAb) (defined below). This resulted in exclusion of 79 twin pairs. Subjects with mildly reduced or increased serum TSH (between 0.1 and 0.4 mU/l or between 4.0 and 6.0 mU/l, respectively) were not excluded, as some healthy subjects have serum TSH levels just outside the reference range, and the pathological significance of a single measurement of TSH just outside the reference range is not well established.<sup>17,18</sup> Samollow *et al.* suggested in their study that there was increased heritability of serum TSH in females with TSH values  $\geq$  4.5 mU/l, which may have been due to an inherited autoimmune component,<sup>13</sup> however, our method of determining heritability allows us to adjust for the presence of TPOAb and hence take account of this effect. Subjects who were taking medications likely to influence serum thyroid function or assessments were also excluded from the analysis; this included subjects on thyroxine, antithyroid drugs, unspecified anticonvulsants, phenytoin, carbamazepine or oral glucocorticoid

treatment. This resulted in a further 87 twin pairs being excluded. For purposes of the analyses, twins with missing data on BMI, age or smoking category were unable to be analysed, resulting in the exclusion of a further 63 twin pairs. Therefore, the final sample on which all of the analyses could be performed was 1062 twin pairs [849 dizygotic (DZ) and 213 monozygotic (MZ)]. Zygosity was determined by a standard questionnaire and by multiplex DNA fingerprinting with variable tandem repeats. All subjects provided written informed consent, and the study was approved by the Research Ethics Committee of St Thomas' Hospital and Sir Charles Gairdner Hospital.

### Biochemistry methods

TSH, fT4, fT3 and TPOAb were measured on serum obtained from blood samples taken at interview, by chemiluminescence immunoassay on the Abbott Diagnostics Architect (Abbott Diagnostics, North Ryde, Australia). The interassay coefficients of variation were as follows: TSH, 3.8% at 0.25 mU/l, 4.7% at 5.3 mU/l; fT4, 4.8% at 10.5 pmol/l; fT3, 5.0% at 4.0 pmol/l and TPOAb 5.4% at 5.97 kU/l. Intra-assay coefficients of variation were: TSH, 3.7% and 3.4% at 0.39 and 26 mU/l, respectively; fT4, 4.3% and 3.8% at 10.5 and 32 pmol/l, respectively; fT3, 6.7% and 2.9% at 4.0 and 19.5 pmol/l, respectively; and TPOAb, 9.0% and 7.9% at 27 and 195 kU/l, respectively. A TPOAb titre of  $>6$  kU/l was considered positive.

### Statistical analyses

Thyroid measures in different groups were compared using a generalized Wilcoxon test, modified to account for the dependence between twin observations.<sup>19</sup> Serum fT4 and fT3 were normally distributed, whereas TSH and the fT4  $\times$  TSH product had skewed distributions and were log transformed before subsequent quantitative genetic analysis. Smoking status was classified as never smoked, ex-smoker or current smoker.

Prior to variance component analysis, each thyroid trait was adjusted for the remaining two thyroid variables and other known covariates using Generalized Least Squares regression, which takes into account the correlation between twins. Regression estimates were obtained for the following covariates: TPOAb status, age, BMI, menopause status, smoking status and resultant first order interactions. Covariates were assessed for collinearity, and the less significant of two collinear variables removed accordingly. This resulted in elimination of menopause status in the analysis, due to its high correlation with smoking status in this data set. Backward stepwise elimination with an exit threshold *P*-value of 0.1 was applied in the model refinement. Intraclass correlations (ICC) for fT4, fT3, lnTSH and ln(fT4  $\times$  TSH) were calculated using the adjusted residuals. Statistical analyses were performed using the R statistical computing program version R-2.4.1 (<http://CRAN.R-project.org>).

### Twin data analysis

The classical twin study is based on the assumption that MZ twins share identical genotypes while DZ twins share on average 50% of their segregating genes. Therefore, if a particular trait is more concordant in MZ than DZ twins, it is likely to be caused by genetic

**Table 1.** Comparisons of TSH, free T4, free T3 and the thyrotroph T4 resistance index within antibody status, smoking status and zygosity classifications

		n	TSH		Free T4		Free T3		fT4 × TSH	
			Median (quartiles)	P-value	Mean (SD)	P-value	Mean (SD)	P-value	Median (quartiles)	P-value
Antibodies	No	1769	1.20 (0.87, 1.62)	< 0.001	13.68 (1.70)	0.319	3.94 (0.57)	< 0.001	16.23 (11.67, 22.21)	< 0.001
	Yes	355	1.71 (1.16, 2.65)		13.49 (1.94)		3.78 (0.54)		23.43 (15.26, 34.44)	
Smoking	Never	1953	1.26 (0.89, 1.75)	0.394*	13.64 (1.74)	0.414*	3.92 (0.57)	0.577*	17.06 (11.99, 23.93)	0.226*
	Ex-	42	1.02 (0.81, 1.49)		13.70 (1.77)		3.92 (0.43)		13.85 (11.61, 19.20)	
	Current	129	1.26 (1.02, 1.82)		13.76 (1.80)		3.87 (0.50)		17.65 (13.06, 23.43)	
Zygosity	MZ	426	1.24 (0.91, 1.75)	0.938	13.61 (1.77)	0.526	3.82 (0.55)	0.001	17.02 (12.56, 22.52)	0.877
	DZ	1698	1.26 (0.89, 1.76)		13.66 (1.74)		3.94 (0.57)		17.07 (11.91, 24.12)	

\*P-value for comparison of current smokers with those who have never smoked. P-values from all tests comparing current with ex-smoker groups, or ex-smokers with never-smoked, were all greater than 0.05.

factors. Initial indication of this was obtained by looking at ICC, which should be higher in MZ than DZ twins if genetic factors are important. More extensive and quantitative analysis was obtained by SEM.<sup>20,21</sup> SEM is a method of estimating genetic and environmental contributions to phenotypic variance. The sources of variance are designated 'A' for additive genetic influences (the sum of the additive effects of all alleles that influence the trait), 'D' for non-additive genetic influences (interactions with and between loci such as dominance and epistasis), 'C' for common environmental variation (environmental influences shared by family members) and 'E' for unique environmental influences (environmental sources of difference between family members). A and D form the genetic contribution which is expected to differ between MZ and DZ twins for a trait under genetic control while the environmental contributions of C and E are expected to be the same.

The significance of these four components was tested using a maximum likelihood approach: the fully specified ACE or ADE models were compared with the nested submodels AE, CE or DE, and E, respectively, with model selection guided by the difference in  $\chi^2$  between models and the Akaike Information Criterion (AIC). The model with the lowest AIC ( $\chi^2 - 2 \times$  degrees of freedom) reflects the best balance between goodness-of-fit and parsimony (fits a model with as few parameters as possible to the data). Estimates of variance components and their 95% confidence intervals (CI) were obtained from the best fitting model.

The basic genetic model for variance components, which assumes equal means and variances in the MZ and DZ groups, was applied to the adjusted residuals for lnTSH, ln(fT4 × TSH) and fT4. A significant mean difference in the adjusted fT3 values was detected between the zygosity groups, and this was accounted for directly by fitting a zygosity-dependent means model for variance components. This analysis was performed using the 'Mx' statistical program.<sup>21</sup>

## Results

### Descriptive statistics

The mean age of the twins studied was  $45.5 \pm 12.4$  years (mean  $\pm$  SD). Mean BMI of the twins was at the upper level of normal  $25.2 \text{ kg/m}^2$ ,

while mean height ( $1.63 \pm 0.06 \text{ m}$ ) and weight ( $66.4 \pm 12.8 \text{ kg}$ ) were consistent with values expected from a population sample of UK women. The median serum TSH concentration was  $1.26 \text{ mU/l}$ , and the 2.5 and 97.5 centiles were  $0.42$  and  $3.63 \text{ mU/l}$ , respectively. Comparisons of thyroid variables within various groupings showed no differences between smoking categories and significantly higher serum TSH and lower fT3 in the antibody-positive compared with the antibody-negative group (Table 1). When the groups were compared according to zygosity, there was no difference in serum TSH or fT4; mean fT3 was significantly higher in DZ than MZ twins ( $3.94$  vs.  $3.82 \text{ pmol/l}$ ,  $P < 0.001$ ), but the magnitude of the difference was small and was adjusted for in the final heritability analysis (see above).

The Spearman rank correlations between the thyroid hormone parameters were  $0.215$  for fT3 vs. fT4 ( $P < 0.001$ ),  $-0.130$  for fT4 vs. TSH ( $P < 0.001$ ) and  $0.060$  for fT3 vs. TSH ( $P = 0.006$ ). Table 2 shows the regression coefficients for fT3, fT4, lnTSH and ln(fT4 × TSH), which were used to adjust the residuals used in calculation of heritability estimates. Regression coefficients of the interaction terms were also used for adjustment and are also shown.

### Intraclass correlations

The ICC for lnTSH, ln(fT4 × TSH), fT4 and fT3 are presented in Table 3. The ICC are all significantly different from zero ( $P < 0.001$ ). The MZ correlations were significantly higher than the DZ correlations for lnTSH ( $P < 0.001$ ), ln(fT4 × TSH) ( $P < 0.001$ ), fT4 ( $P < 0.001$ ) and fT3 ( $P = 0.015$ ). This suggests a genetic influence on each of these thyroid hormone traits, which is visually represented in scatter plots in Fig. 1.

### Biometric analysis

The results of the biometric analysis are presented in Table 4. We evaluated the genetic component of variation of each thyroid phenotype by way of fully specified ACE and ADE models, and all possible simplifications thereof. For each of fT3 and fT4, a fully specified ACE variance components model provides a significantly better fit to the data than any of the simpler models ( $P < 0.05$  for all

**Table 2.** Regression analysis results (co-efficients and *P* values) for thyroid variables free T3, free T4, lnTSH and ln(ft4 × TSH)

	Free T3	Free T4	lnTSH	ln(ft4 × TSH)
<b>Main effects</b>				
Free T3	–	2.584 (< 0.001)	ns	0.261 (< 0.001)
Free T4	0.280 (< 0.001)	–	ns	–
lnTSH	ns	ns	–	–
Antibodies	ns	2.571 (< 0.001)	1.011 (< 0.001)	0.338 (< 0.001)
Age	0.014 (0.063)	0.078 (< 0.001)	0.020 (0.004)	0.016 (< 0.001)
BMI	0.070 (< 0.001)	0.204 (< 0.001)	0.008 (< 0.001)	0.008 (0.010)
Smoking	–0.101 (0.040)*	ns	ns	ns
<b>Interactions</b>				
Free T3 × free T4	–	–	0.024 (0.022)	–
Free T3 × lnTSH	–	0.236 (0.056)	–	–
Free T3 × antibodies	–	–0.402 (0.015)	ns	ns
Free T3 × age	–	–0.013 (0.009)	ns	–0.003 (0.084)
Free T3 × BMI	–	–0.050 (< 0.001)	ns	ns
Free T4 × lnTSH	0.028 (0.009)	–	–	–
Free T4 × antibodies	–0.036 (0.019)	–	–0.049 (0.001)	–
Free T4 × age	–0.002 (< 0.001)	–	–0.001 (0.028)	–
Free T4 × BMI	–0.005 (< 0.001)	–	ns	–
lnTSH × antibodies	ns	–0.408 (0.008)	–	–
lnTSH × age	ns	–0.011 (0.040)	–	–
Antibodies × smoking	ns	ns	0.260 (0.016)*	0.262 (0.016)*
Age × smoking	ns	0.026 (0.026)*	ns	ns

ns, not significant, *P* > 0.1; –, not in the fitted model.

\*Comparison of current smokers to those never smoked. The difference between ex-smokers and never-smoked was not significant.

**Table 3.** Intraclass correlations for lnTSH, ln(ft4 × TSH), free T4 and free T3 concentrations

Phenotype	<i>r</i> <sub>MZ</sub>	95% CI	<i>r</i> <sub>DZ</sub>	95% CI	<i>P</i> -value*
ln(TSH)	0.63	0.55–0.71	0.30	0.24–0.36	< 0.001
ln(ft4 × TSH)	0.62	0.53–0.70	0.31	0.25–0.37	< 0.001
Free T4	0.61	0.52–0.69	0.43	0.37–0.48	< 0.001
Free T3	0.58	0.48–0.66	0.46	0.40–0.51	0.015

\**P*-value from the test of equality for *r*<sub>MZ</sub> and *r*<sub>DZ</sub>.

comparisons with nested models), while for lnTSH, the AE model is the optimal choice, with no significant loss of fit when compared to the ACE model (*P* > 0.1).

Additive genetic effects explained 65% (95% CI 58%–71%) of the total variation in serum TSH concentration, whereas unique environmental effects explained 35% (29%–42%). The situation was different for ft3 and ft4, where common environmental effects were significant, even after the removal of known covariate effects. Genetic effects accounted for only 23% (3%–41%) of the variation in ft3, whereas for ft4, they accounted for 39% (20%–55%). Unique environmental effects were predominant in explaining 43% (35%–52%) of the variation in ft3, and 37% (31%–45%) for ft4. Using this analysis for the ft4 × TSH product, the AE model provides the best fit, suggesting that genetic effects account for 65% (53%–71%) of variation and unique environmental effects 35% (29%–42%).

## Discussion

Using SEM in a large twin cohort, we conclude that serum TSH concentrations and the ft4 × TSH product exhibit a strong genetic influence, with an estimated heritability of 65%, whereas heritability is less for ft4 and ft3, at 39% and 23%, respectively. With regard to TSH, our results are consistent with those of Hansen *et al.* in which heritability of serum TSH was estimated at 64%<sup>10</sup> but differ from the study of Samollow *et al.*<sup>13</sup> in which it was only 32%. By contrast, our estimate of heritability of ft4 is broadly consistent with those of Samollow *et al.*<sup>13</sup> and Meikle *et al.*<sup>11</sup> (in which ft4 heritability was 37% and 44%, respectively) as well as that of Martin *et al.*<sup>12</sup> in which heritability of total T4 was estimated at 32%. This is, however, not consistent with the data of Hansen *et al.*<sup>10</sup> in which heritability of ft4 was 65%. With regards to ft3, the estimate of heritability in our study is much lower than reported either by Hansen *et al.* (64%) or Samollow *et al.* (67%).

The reasons for the conflicting results between these various studies are not clear. Differences in study design may contribute, as the analyses of Samollow *et al.*<sup>13</sup> and Marin *et al.*<sup>12</sup> were pedigree based and of Mexican Americans and Mennonites, respectively, whereas our study and that of Hansen *et al.*<sup>10</sup> were of Caucasian twin pairs. The methods we used were similar to those used by Hansen *et al.*, yet we detected a greater influence of common environmental effects on variance in serum ft3 and ft4 concentrations. There may have been greater power in our study to detect common environmental effects due to its larger size. The nature of this environmental

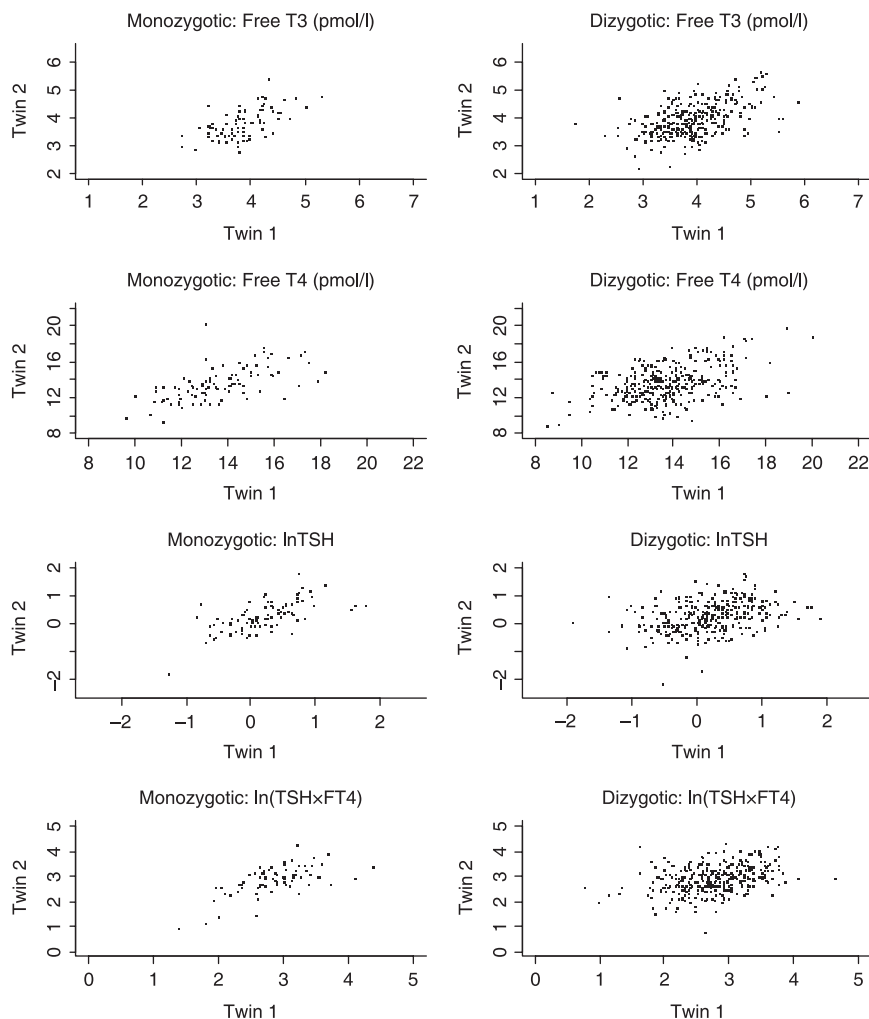


Fig. 1 Scatterplots of free T3, free T4, lnTSH and  $\ln(\text{ft4} \times \text{TSH})$  for each twin pair by zygosity.

influence is not clear, but there are several possibilities. First, Denmark (the setting of the study of Hansen *et al.*) is an area of iodine deficiency, whereas the UK is believed to be iodine sufficient. Similarly, there may be differences in selenium intake between the two populations. While the evidence for the influence of this trace element on thyroid function is not as convincing as that for iodine, there is some evidence to suggest severe deficiency may affect thyroid hormone levels.<sup>22</sup> Second, approximately half of the Danish twins were smokers compared with only approximately 6% of UK twins. Smoking has complex effects on thyroid function,<sup>23,24</sup> and differences in smoke exposure might account for some of the difference observed. Third, all of our study subjects were female, and there may be common environmental influences unique to females. There may well be other environmental influences that cannot be readily determined using this study design.

We found a strong genetic influence of the  $\text{ft4} \times \text{TSH}$  product, with heritability estimated at 65%, which was identical to TSH alone. This has been proposed as a measure of thyrotroph sensitivity to  $\text{ft4}$  negative feedback, and is considered a less complex but still meaningful way to quantify this relationship compared to correlating serum  $\text{ft4}$  with TSH,<sup>15</sup> although this may be an oversimplification of the relationship. This is a key component of pituitary–thyroid axis function, and might be expected to exhibit a strong genetic influence.

Having said that,  $\text{ft4} \times \text{TSH}$  product as a measure of thyrotroph sensitivity is derived from studies of patients with thyroid hormone resistance<sup>15</sup> and has so far received limited attention in euthyroid subjects without this syndrome;<sup>16</sup> so it may be premature to draw definitive conclusions regarding its significance. Nevertheless, our data suggest a strong heritable component to its variance.

Our results suggest that variation in serum TSH concentrations is under a stronger genetic influence and a lesser environmental influence than  $\text{ft4}$  and  $\text{ft3}$ . Physiologically, this is consistent with the observation that serum  $\text{ft4}$  and particularly  $\text{ft3}$  levels are subject to many sources of variation from many different tissues. Serum levels are affected by the action of the three iodothyronine deiodinase enzymes, two of which (D1 and D2) are activating and the other (D3) inactivating. These enzymes have tissue-specific distributions and are differentially regulated by a wide range of factors, and hence their activity reflects the needs and availability of thyroid hormone in particular tissues.<sup>25,26</sup> This is particularly the case for  $\text{ft3}$ , due to its lower serum concentration and the fact that the majority of serum  $\text{ft3}$  is produced from deiodinase action in the liver, kidney and skeletal muscle rather than by thyroidal secretion.<sup>27</sup> It is therefore, perhaps, not surprising that  $\text{ft3}$  demonstrates the lowest degree of heritability and the strongest environmental influence of the parameters examined.

**Table 4.** Variance components results for lnTSH, free T3, free T4 and ln(fT4 × TSH)

Model	Genetic components		Environmental components		Goodness-of-fit			Model fit comparison				
	A	D	C	E	$\chi^2$	df	P	AIC	Compare to	$\Delta\chi^2$	$\Delta$ df	P
<b>lnTSH</b>												
1. ACE	0.65 (0.54–0.71)	–	0.00 (0.00–0.00)	0.35 (0.29–0.42)	8.13	3	0.043	2.13	–	–	–	–
2. ADE	0.48 (0.23–0.69)	0.19 (0.00–0.46)	–	0.33 (0.27–0.40)	6.15	3	0.105	0.15	–	–	–	–
3. AE	<b>0.65 (0.58–0.71)</b>	–	–	<b>0.35 (0.29–0.42)</b>	<b>8.13</b>	<b>4</b>	<b>0.087</b>	<b>0.13</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>&gt; 0.99</b>
4. CE	–	–	0.36 (0.31–0.41)	0.64 (0.59–0.69)	52.06	4	0.000	44.06	1	43.93	1	0.000
5. E	–	–	–	1	195.10	5	0.000	185.10	1	186.96	2	0.000
6. DE	–	0.69 (0.63–0.74)	–	0.31 (0.26–0.37)	19.78	4	0.001	11.78	2	13.63	1	0.000
<b>Free T3</b> Analysis incorporating means												
7. ACE	<b>0.23 (0.03–0.41)</b>	–	<b>0.34 (0.22–0.47)</b>	<b>0.43 (0.35–0.52)</b>	<b>1.12</b>	<b>5</b>	<b>0.952</b>	<b>–8.88</b>	–	–	–	–
8. ADE	0.65 (0.60–0.70)	0.00 (0.00–0.00)	–	0.35 (0.30–0.40)	28.36	5	0.000	18.36	–	–	–	–
9. AE	0.65 (0.60–0.70)	–	–	0.35 (0.30–0.40)	28.36	6	0.000	16.36	7	27.24	1	0.000
10. CE	–	–	0.48 (0.43–0.52)	0.52 (0.48–0.57)	5.94	6	0.430	–6.06	7	4.82	1	0.028
11. E	–	–	–	1	281.33	7	0.000	267.33	7	280.21	2	0.000
12. DE	–	0.00 (0.00–0.00)	–	1.00 (1.00–1.00)	281.33	6	0.000	269.33	8	252.97	1	0.000
<b>Free T4</b>												
13. ACE	<b>0.39 (0.20–0.55)</b>	–	<b>0.24 (0.12–0.37)</b>	<b>0.37 (0.31–0.45)</b>	<b>2.01</b>	<b>3</b>	<b>0.571</b>	<b>–3.99</b>	–	–	–	–
14. ADE	0.68 (0.61–0.73)	0.00 (0.00–0.06)	–	0.32 (0.27–0.38)	16.31	3	0.001	10.31	–	–	–	–
15. AE	0.68 (0.62–0.73)	–	–	0.32 (0.27–0.38)	16.31	4	0.003	8.31	13	14.30	1	0.000
16. CE	–	–	0.47 (0.42–0.52)	0.53 (0.48–0.57)	16.74	4	0.002	8.74	13	14.73	1	0.000
17. E	–	–	–	1	285.40	5	0.000	275.40	13	283.40	2	0.000
18. DE	–	0.68 (0.62–0.73)	–	0.32 (0.27–0.38)	79.38	4	0.000	71.38	14	63.07	1	0.000
<b>ln(fT4 × TSH)</b>												
19. ACE	0.65 (0.53–0.71)	–	0.00 (0.00–0.00)	0.35 (0.29–0.42)	8.57	3	0.036	2.57	–	–	–	–
20. ADE	0.54 (0.30–0.70)	0.12 (0.00–0.39)	–	0.33 (0.27–0.41)	7.73	3	0.052	1.73	–	–	–	–
21. AE	<b>0.65 (0.58–0.71)</b>	–	–	<b>0.35 (0.29–0.42)</b>	<b>8.57</b>	<b>4</b>	<b>0.073</b>	<b>0.57</b>	<b>19</b>	<b>0</b>	<b>1</b>	<b>&gt; 0.99</b>
22. CE	–	–	0.00 (0.00–0.64)	1.00 (0.36–1.00)	198.38	4	0.000	190.38	19	191.81	1	0.000
23. E	–	–	–	1	198.38	5	0.000	188.38	19	189.81	2	0.000
24. DE	–	0.69 (0.63–0.74)	–	0.31 (0.26–0.37)	25.49	4	0.000	17.49	20	17.77	1	0.000

95% CI are given in parentheses. A small  $\chi^2$  and large *P*-value indicate goodness-of-fit to the data; a low Akaike Information Criterion (AIC) indicates the best balance between goodness-of-fit and parsimony.  $\Delta\chi^2$ , the difference in  $\chi^2$  between the full model and submodel; df, degrees of freedom. Best fitting model is given in bold.

The genes responsible for variation in pituitary–thyroid axis set points between individuals are not well established. Single nucleotide polymorphisms in the TSH receptor and type 1 deiodinase genes are reported to be associated with differences in serum TSH and the serum fT3, respectively,<sup>28</sup> and it is plausible that polymorphisms in these and other relevant genes such as thyroid hormone transporters and receptors account for some of the interindividual variability in pituitary–thyroid axis function. Hansen *et al.* investigated the influence of an identified polymorphism in the TSH receptor gene: Asp727Glu which has been shown to influence serum TSH levels, however, found its effect to be small, accounting for 0.91% of the total phenotypic variance of serum TSH levels.<sup>29</sup> Further discovery and investigation of related polymorphisms may increase our understanding of genetic regulation of pituitary–thyroid axis function.

The importance of a genetically determined individual set point is becoming clearer. There is increasing evidence that small differences in thyroid hormone levels are associated with clinically important outcomes. Large studies have shown an association between fT4 or TSH, even within the reference range and BMI<sup>4–6</sup> (although some

have not shown an association<sup>30</sup>), blood pressure,<sup>7,8</sup> cholesterol<sup>31,32</sup> and insulin sensitivity.<sup>16</sup> Subclinical hyperthyroidism has been shown to influence clinical outcomes such as occurrence of atrial fibrillation,<sup>33–35</sup> cardiovascular risk<sup>36</sup> and bone density,<sup>37,38</sup> while subclinical hypothyroidism has been shown to influence atherosclerosis and cardiovascular risk,<sup>39,40</sup> and serum cholesterol.<sup>41,42</sup> A better understanding of the genetic influences on pituitary–thyroid axis set points could potentially lead to a better understanding of subclinical thyroid dysfunction and clarify whether small differences in thyroid function between euthyroid individuals are indeed risk factors for disease.<sup>9</sup> There are few studies which show that normalization of thyroid hormone levels by treatment of subclinical thyroid disease can change these outcomes. Further research is needed before a change in clinical practice relating to subclinical disease may be prescribed.

The strengths of this study include its large sample size and detailed statistical analysis to determine estimates of heritability. The principal weakness of the study is that all participants were female. Although there is little evidence that pituitary–thyroid axis function

differs between females and males (in the absence of thyroid disease, to which females are more prone), it cannot be assumed that the results apply to males. As with all twin studies, our study relies on the assumption that MZ twins are not treated more similarly than DZ, as this may marginally overestimate the genetic influence. We are also assuming that the twin population is a reasonable reflection of the general population in terms of thyroid function; however, there is no reason to suggest this is not the case.<sup>43</sup> Certainly, the median, 2.5 and 97.5 centiles for TSH were broadly similar from those reported from large community- and population-based studies.<sup>14,44</sup>

In conclusion, we find that genetic factors are an important determinant of pituitary–thyroid axis function, particularly serum TSH and the fT4 × TSH product, but less so for fT4 and fT3 concentrations. Further research is needed to determine which genes are responsible for these important aspects of endocrine homeostasis.

### Acknowledgements

We thank Abbott Diagnostics, North Ryde, Australia, who provided support for the biochemical analysis. TwinsUK receives funding from the Wellcome Trust, the CDRF and the Biomed EU GenomEUtwin and EuroClot project (Ref: LSHM-CT-2004–005268, QLK2-CT-2002–01254) programs. Portions of this work were funded by the Australian National Health and Medical Research Council (Project Grants 343603). We gratefully acknowledge the contribution of the volunteer twins who participated in this research.

### References

- Meier, C.A., Maisey, M.N., Lowry, A., Muller, J. & Smith, M.A. (1993) Interindividual differences in the pituitary–thyroid axis influence the interpretation of thyroid function tests. *Clinical Endocrinology*, **39**, 101–107.
- Keffer, J.H. (1996) Preanalytical considerations in testing thyroid function. *Clinical Chemistry*, **42**, 125–134.
- Andersen, S., Pedersen, K.M., Bruun, N.H. & Laurberg, P. (2002) Narrow individual variations in serum T (4) and T (3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1068–1072.
- Nyrnes, A., Jorde, R. & Sundsfjord, J. (2006) Serum TSH is positively associated with BMI. *International Journal of Obesity*, **30**, 100–105.
- Lin, S.Y., Wang, Y.Y., Liu, P.H., Lai, W.A. & Sheu, W.H. (2005) Lower serum free thyroxine levels are associated with metabolic syndrome in a Chinese population. *Metabolism*, **54**, 1524–1528.
- Iacobellis, G., Ribaldo, M.C., Zappaterreno, A., Iannucci, C.V. & Leonetti, F. (2005) Relationship of thyroid function with body mass index, leptin, insulin sensitivity and adiponectin in euthyroid obese women. *Clinical Endocrinology*, **62**, 487–491.
- Gumieniak, O., Perlstein, T.S., Hopkins, P.N., Brown, N.J., Murphey, L.J., Jeunemaitre, X., Hollenberg, N.K. & Williams, G.H. (2004) Thyroid function and blood pressure homeostasis in euthyroid subjects. *Journal of Clinical Endocrinology and Metabolism*, **89**, 3455–3461.
- Asvold, B.O., Bjoro, T., Nilsen, T.I. & Vatten, L.J. (2007) Association between blood pressure and serum thyroid-stimulating hormone concentration within the reference range: a population-based study. *Journal of Clinical Endocrinology and Metabolism*, **92**, 841–845.
- Gammage, M.D., Parle, J.V., Holder, R.L., Roberts, L.M., Hobbs, F.D., Wilson, S., Sheppard, M.C. & Franklyn, J.A. (2007) Association between serum free thyroxine concentration and atrial fibrillation. *Archives of Internal Medicine*, **167**, 928–934.
- Hansen, P.S., Brix, T.H., Sorensen, T.I., Kyvik, K.O. & Hegedus, L. (2004) Major genetic influence on the regulation of the pituitary–thyroid axis: a study of healthy Danish twins. *Journal of Clinical Endocrinology and Metabolism*, **89**, 1181–1187.
- Meikle, A.W., Stringham, J.D., Woodward, M.G. & Nelson, J.C. (1988) Hereditary and environmental influences on the variation of thyroid hormones in normal male twins. *Journal of Clinical Endocrinology and Metabolism*, **66**, 588–592.
- Martin, L.J. & Crawford, M.H. (1998) Genetic and environmental components of thyroxine variation in Mennonites from Kansas and Nebraska. *Human Biology*, **70**, 745–760.
- Samollow, P.B., Perez, G., Kammerer, C.M., Finegold, D., Zwartjes, P.W., Havill, L.M., Comuzzie, A.G., Mahaney, M.C., Goring, H.H., Blangero, J., Foley, T.P. & Barmada, M.M. (2004) Genetic and environmental influences on thyroid hormone variation in Mexican Americans. *Journal of Clinical Endocrinology and Metabolism*, **89**, 3276–3284.
- Hollowell, J.G., Staehling, N.W., Flanders, W.D., Hannon, W.H., Gunter, E.W., Spencer, C.A. & Braverman, L.E. (2002) Serum TSH, T (4), and thyroid antibodies in the United States population (1988–94): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism*, **87**, 489–499.
- Yagi, H., Pohlenz, J., Hayashi, Y., Sakurai, A. & Refetoff, S. (1997) Resistance to thyroid hormone caused by two mutant thyroid hormone receptors beta, R243Q and R243W, with marked impairment of function that cannot be explained by altered *in vitro* 3,5,3′-triiodothyronine binding affinity. *Journal of Clinical Endocrinology and Metabolism*, **82**, 1608–1614.
- Fernandez-Real, J.M., Lopez-Bermejo, A., Castro, A., Casamitjana, R. & Ricart, W. (2006) Thyroid function is intrinsically linked to insulin sensitivity and endothelium-dependent vasodilation in healthy euthyroid subjects. *Journal of Clinical Endocrinology and Metabolism*, **91**, 3337–3343.
- Surks, M.I., Ortiz, E., Daniels, G.H., Sawin, C.T., Col, N.F., Cobin, R.H., Franklyn, J.A., Hershman, J.M., Burman, K.D., Denke, M.A., Gorman, C., Cooper, R.S. & Weissman, N.J. (2004) Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *Journal of the American Medical Association*, **291**, 228–238.
- Huber, G., Staub, J.J., Meier, C., Mitrache, C., Guglielmetti, M., Huber, P. & Braverman, L.E. (2002) Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. *Journal of Clinical Endocrinology and Metabolism*, **87**, 3221–3226.
- Brunner, E. (1991) A nonparametric estimator of the shift effect for repeated observations. *Biometrics*, **47**, 1149–1153.
- Neale, M.C. & Maes, H.H. (2002) *Methodology for Genetic Studies of Twins and Families*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Neale, M.C., Boker, S.M., Xie, G. & Maes, H.H. (2003) *Mx: Statistical Modeling*, 6th edn. Virginia Commonwealth University, Richmond, Virginia, USA.
- Kohrle, J. (1999) The trace element selenium and the thyroid gland. *Biochimie*, **81**, 527–533.
- Belin, R.M., Astor, B.C., Powe, N.R. & Ladenson, P.W. (2004) Smoke exposure is associated with a lower prevalence of serum thyroid

- autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism*, **89**, 6077–6086.
- 24 Jorde, R. & Sundsfjord, J. (2006) Serum TSH levels in smokers and non-smokers. The 5th Tromso study. *Experimental and Clinical Endocrinology and Diabetes*, **114**, 343–347.
- 25 Bianco, A.C. & Kim, B.W. (2006) Deiodinases: implications of the local control of thyroid hormone action. *Journal of Clinical Investigation*, **116**, 2571–2579.
- 26 Bianco, A.C., Salvatore, D., Gereben, B., Berry, M.J. & Larsen, P.R. (2002) Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews*, **23**, 38–89.
- 27 Pilo, A., Iervasi, G., Vitek, F., Ferdeghini, M., Cazzuola, F. & Bianchi, R. (1990) Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis. *American Journal of Physiology*, **258**, E715–E726.
- 28 Peeters, R.P., van der Deure, W.M. & Visser, T.J. (2006) Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. *European Journal of Endocrinology*, **155**, 655–662.
- 29 Hansen, P.S., van der Deure, W.M., Peeters, R.P., Iachine, I., Fenger, M., Sorensen, T.I., Kyvik, K.O., Visser, T.J. & Hegedus, L. (2007) The impact of a TSH receptor gene polymorphism on thyroid-related phenotypes in a healthy Danish twin population. *Clinical Endocrinology*, **66**, 827–832.
- 30 Manji, N., Boelaert, K., Sheppard, M.C., Holder, R.L., Gough, S.C. & Franklyn, J.A. (2006) Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects. *Clinical Endocrinology*, **64**, 125–128.
- 31 Petersson, U. & Kjellstrom, T. (2001) Thyroid function tests, serum lipids and gender interrelations in a middle-aged population. *Scandinavian Journal of Primary Health Care*, **19**, 183–185.
- 32 Iqbal, A., Jorde, R. & Figenschau, Y. (2006) Serum lipid levels in relation to serum thyroid-stimulating hormone and the effect of thyroxine treatment on serum lipid levels in subjects with subclinical hypothyroidism: the Tromso Study. *Journal of Internal Medicine*, **260**, 53–61.
- 33 Sawin, C.T., Geller, A., Wolf, P.A., Belanger, A.J., Baker, E., Bacharach, P., Wilson, P.W., Benjamin, E.J. & D'Agostino, R.B. (1994) Low serum thyrotropin concentrations as a risk factor for atrial fibrillation in older persons. *New England Journal of Medicine*, **331**, 1249–1252.
- 34 Auer, J., Scheibner, P., Mische, T., Langsteger, W., Eber, O. & Eber, B. (2001) Subclinical hyperthyroidism as a risk factor for atrial fibrillation. *American Heart Journal*, **142**, 838–842.
- 35 Cappola, A.R., Fried, L.P., Arnold, A.M., Danese, M.D., Kuller, L.H., Burke, G.L., Tracy, R.P. & Ladenson, P.W. (2006) Thyroid status, cardiovascular risk, and mortality in older adults. *Journal of the American Medical Association*, **295**, 1033–1041.
- 36 Parle, J.V., Maisonneuve, P., Sheppard, M.C., Boyle, P. & Franklyn, J.A. (2001) Prediction of all-cause and cardiovascular mortality in elderly people from one low serum thyrotropin result: a 10-year cohort study. *Lancet*, **358**, 861–865.
- 37 Faber, J., Jensen, I.W., Petersen, L., Nygaard, B., Hegedus, L. & Siersbaek-Nielsen, K. (1998) Normalization of serum thyrotrophin by means of radioiodine treatment in subclinical hyperthyroidism: effect on bone loss in postmenopausal women. *Clinical Endocrinology*, **48**, 285–290.
- 38 Tauchmanova, L., Nuzzo, V., Del Puente, A., Fonderico, F., Esposito-Del Puente, A., Padulla, S., Rossi, A., Bifulco, G., Lupoli, G. & Lombardi, G. (2004) Reduced bone mass detected by bone quantitative ultrasonometry and DEXA in pre- and postmenopausal women with endogenous subclinical hyperthyroidism. *Maturitas*, **48**, 299–306.
- 39 Hak, A.E., Pols, H.A., Visser, T.J., Drexhage, H.A., Hofman, A. & Witteman, J.C. (2000) Subclinical hypothyroidism is an independent risk factor for atherosclerosis and myocardial infarction in elderly women: the Rotterdam Study. *Annals of Internal Medicine*, **132**, 270–278.
- 40 Walsh, J.P., Bremner, A.P., Bulsara, M.K., O'Leary, P., Leedman, P.J., Feddema, P. & Michelangeli, V. (2005) Subclinical thyroid dysfunction as a risk factor for cardiovascular disease. *Archives of Internal Medicine*, **165**, 2467–2472.
- 41 Kanaya, A.M., Harris, F., Volpato, S., Perez-Stable, E.J., Harris, T. & Bauer, D.C. (2002) Association between thyroid dysfunction and total cholesterol level in an older biracial population: the health, aging and body composition study. *Archives of Internal Medicine*, **162**, 773–779.
- 42 Walsh, J.P., Bremner, A.P., Bulsara, M.K., O'Leary, P., Leedman, P.J., Feddema, P. & Michelangeli, V. (2005) Thyroid dysfunction and serum lipids: a community-based study. *Clinical Endocrinology*, **63**, 670–675.
- 43 Andrew, T., Hart, D.J., Snieder, H., de Lange, M., Spector, T.D. & MacGregor, A.J. (2001) Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. *Twin Research*, **4**, 464–477.
- 44 Vanderpump, M.P., Tunbridge, W.M., French, J.M., Appleton, D., Bates, D., Clark, F., Grimley Evans, J., Hasan, D.M., Rodgers, H., Tunbridge, F. & Young, E.T. (1995) The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. *Clinical Endocrinology*, **43**, 55–68.