The pituitary-thyroid axis set point in women is uninfluenced by X chromosome inactivation pattern. A twin study.

Thomas H Brix, Pia Skov Hansen, Kirsten Ohm Kyvik, Laszlo Hegedus

CLINICAL ENDOCRINOLOGY

The pituitary-thyroid axis set point in women is uninfluenced by X chromosome inactivation pattern. A twin study.

Journal:	*Clinical Endocrinology*
Manuscript ID:	CEN-2010-000235.R2
Manuscript Type/Office:	2 Original Article - Europe, excluding UK
Date Submitted by the Author:	07-Jul-2010
Complete List of Authors:	Brix, Thomas; Odense University Hospital, Endocrinology Hansen, Pia; University of Southern Denmark, Odense, The Danish Twin Registry Ohm Kyvik, Kirsten; The Danish Twin Registry, Epidemiology, Institute of Public Health, University of Southern Denmark Institute of Regional Health, - Hegedus, Laszlo; Odense University Hospital, Endocrinology and Metabolism
Key Words:	Thyroid hormones, X chromosome inactivation, Epigenetics, Twins

The pituitary-thyroid axis set point in women is uninfluenced by X chromosome inactivation pattern? A twin study.

Thomas Heiberg Brix[1], Pia Skov Hansen[1,2], Kirsten Ohm kyvik[2,3], and Laszlo Hegedüs[1].

[1] Department of Endocrinology and Metabolism, Odense University Hospital, Denmark
[2] The Danish Twin Registry, Epidemiology, Institute of Public Health, University of Southern Denmark, Odense, Denmark
[3] Institute of Regional Health Services Research, University of Southern Denmark, Odense, Denmark

Short title: X chromosome inactivation and thyroid hormones

Key words: Thyroid hormones, X chromosome inactivation, Epigenetics, Twins

Word count:
Abstract: 250
Manuscript: 2223
Number of tables: 2

Acknowledgment
This study was supported in part by grants from the Agnes and Knut Mørks foundation, The Danish Research Agency, The Foundation of 17-12-1981, The Novo Nordisk Foundation, The Danish Medical Research Council, The Danish Diabetes Association, and The Danish Hearth Foundation.

Disclosure statement
Thomas Heiberg Brix, Pia Skov Hansen, Kirsten Ohm Kyvik and Laszlo Hegedüs state that they have no conflicts of interest.

Correspondence:

Thomas Heiberg Brix, MD, Ph.d.
Department of Endocrinology
Odense University Hospital
Sdr. Boulevard 29
5000 Odense C
Denmark
Tel. +45 65 41 17 10
Fax. + 45 65 90 69 38
Email: thomas.brix@ouh.regionsyddanmark.dk

Abstract

Objective: The pituitary-thyroid axis set point is determined by a combination of genetic and environmental factors. However, despite considerable efforts to characterize the background, the causative genes as well as environmental factors are not well established. Theoretically, as shown for autoimmune thyroid disease the pattern of X chromosome inactivation (XCI) could offer a novel explanation for the observed variability of the pituitary-thyroid axis set point in women.

Design and Patients: In order to examine the impact of XCI pattern on the pituitary-thyroid axis set point, we studied whether within cohort (n = 318 subjects) and within twin pair (n = 159 pairs) differences in XCI are correlated with serum concentrations of thyrotropin (TSH), free triiodothyronine (FT3) and free thyroxine (FT4).

Methods: XCI was determined by PCR analysis of a polymorphic CAG repeat in the first exon of the androgen receptor gene. Thyroid variables were measured using a solid-phase time-resolved fluoroimmunometric assay. Zygosity was established by DNA fingerprinting.

Results: In the overall study population (within cohort), no significant correlations were found between TSH [regression coefficient (β) = -0.28 (95% confidence intervals, -0.66-0.11), p = 0.158], FT3 [β= -0.25 (-0.85-0.34), p = 0.403], FT4 [β= 0.08 (-0.91-1.07), p = 0.876] and XCI pattern. Essentially similar results were found in the within pair analysis. Controlling for confounders such as age, body mass index, smoking and zygosity did not change the findings.

Conclusions: In a sample of female twins, we found no evidence of a relationship between XCI pattern and pituitary-thyroid axis set point.

Introduction

In a population of healthy euthyroid subjects, serum levels of thyrotropin (TSH) and the thyroid hormones (free thyroxine, FT4 and free triiodothyronine, FT3) show substantial interindividual variation, whereas the intraindividual variation is generally within a narrow range [1]. It appears therefore that each individual has a unique set point of the pituitary-thyroid axis (PTA). Recently, two large twin studies have provided indisputable evidence for the contribution of both genetic and environmental factors in the regulation of these parameters [2,3]. A number of genetic loci [4] as well as polymorphisms in different thyroid hormone pathway genes, such as phosphodiesterase 8B [5], the deiodinases [6-8], the TSH receptor [9,10], the thyroid hormone receptors [11], and thyroid hormone transporters [12,13] have been associated with serum levels of TSH, FT3 and FT4. So far, however, their contribution to phenotypic variance is week to modest, indicating that there is no single gene with a major regulatory influence on the PTA set point. The same is certainly true for suggested non-genetic variables such as body mass index (BMI) [14,15], iodine intake [16] and smoking habits [17,18].

In the last several years, there has been considerable discussion and disagreement regarding gender specific differences in the PTA set point [19-24]. However, in most studies - irrespective of significant gender differences in median TSH [21], presence of thyroid autoantibodies [22] and/or thyroid ultrasound abnormalities [23,24] - the variation of the PTA variables is greater (reflected by wider reference limits) in women than in men. At present, the reason for this phenomenon is unknown.

Recent evidence indicates that some of the observed gender specific differences in a number of common traits and diseases might, at least partially, be due to sex-specific differences in genetic architecture [25]. Theoretically, as shown for

autoimmune thyroid disease [26-28], the pattern of X chromosome inactivation (XCI) could offer an explanation for the observed increased variability of the PTA set point variables in women. In female cells, one of the two X chromosomes is inactivated in early embryonic life in order to equalize the dosage of X linked genes between females and males [29]. Consequently, females are mosaics for two cell lines, cells with the paternal and cells with the maternal X chromosome as the active X. In young to middle aged females the distribution of the two cell lines is close to normal (50:50 ratio) [29, 30]. A skewed XCI pattern is a marked deviation from the 50:50 ratio (80:20 or more) and is present in approximately 15% of females below 55 years [29, 30]. The XCI process is under genetic control [30] and it has been linked to loci on the X chromosome [31]. The X chromosome may also be involved in the regulation of the PTA set point because variables with impact on the serum levels of TSH, FT3 and FT4 such as monocarboxylate transporter 8 (thyroid hormone transporter) [13] and thyroxine-binding globulin [32] are X linked. Additionally, the X chromosome may also be involved in the development of thyroid diseases biochemically characterized by skewed values of TSH, FT3 and FT4 [33].

It follows that variation in XCI pattern may allow female monozygotic (MZ) twins to express discordance for traits that are influenced by X linked genes [25]. This has been demonstrated in X linked single gene disorders such as Duchenne muscular dystrophy and X linked immunodeficiencies [29]. Recently, the potential contribution of polymorphic X linked quantitative trait loci to complex phenotypes has been evaluated by comparing correlations between female and male MZ pairs [34]. If the phenotype is influenced by polymorphic X linked loci, female MZ pairs should be more discordant than male MZ pairs as a result of skewed XCI [34]. Recently, we have

found evidence for lower correlations (reflecting more discordance) in the PTA set point variables in female MZ pairs compared with male MZ pairs [2].

Given the potential gender specific differences in the PTA set point variables together with the possible involvement of X linked genes, the aim of our study was to test whether, or not, variation in the PTA set point variables in women is influenced by XCI pattern.

Subjects

A detailed description of the ascertainment procedure has been published elsewhere [35]. In brief, in 1997 a representative sample of self-reported healthy twin pairs born between 1931 and 1982 was recruited from the Danish Twin Register on the basis of nationwide questionnaire surveys concerning health and health related behaviour performed in 1994 and 1996. In all, 1512 individuals (756 twin pairs) were examined from 1997 to 2000. Blood samples were available from 736 twin pairs. Twin pairs with self-reported thyroid disease (28 twin pairs) or overt biochemical thyroid disease (18 pairs) were excluded. Moreover, all males (688 subjects) and females from opposite sex pairs (120 subjects) were also excluded leaving 572 female subjects (286 pairs). Of these, 318 subjects (159 twin pairs, distributed in 82 MZ and 77 dizygotic (DZ) pairs) were informative regarding TSH, thyroid hormones and XCI pattern and hence suitable for data analysis. Informed consent was obtained from all participants, and the study was approved by all the Regional Scientific-Ethical Committees in Denmark.

Methods

Assays

Thyrotropin, thyroid hormones and thyroid autoantibodies

Serum concentrations of TSH and thyroid peroxidase autoantibodies (TPOAb) were measured using solid-phase time-resolved fluoroimmunometric assays (AutoDELFIA; Perkin Elmer/Wallac, Turku, Finland). Serum free T4 and free T3 were determined using the AutoDELFIA FT4 and FT3 kits (Perkin Elmer/Wallac, Turku, Finland), respectively. The reference range for TSH, FT4, FT3 and TPOAb is 0.30-4.00 mU/l, 9.9-17.7 pmol/l, 4.3-7.4 pmol/l and 2-10 KIU/l, respectively [20,36]. All blood samples were drawn between 8 and 9 a.m. hour after a 12 hour fast.

X-chromosome inactivation analysis

DNA was extracted from peripheral blood cells. The X chromosome phenotype was determined by polymerase chain reaction (PCR) analysis of a polymorphic (CAG)n repeat in the first exon of the androgen receptor gene. After digestion of the DNA with the methylation sensitive enzyme *Hpa*II, a PCR product is obtained from the inactive X chromosome only. The PCR products were separated on an ABI 3100 automated sequencer, and analysed by GeneScan software (Applied Biosystems, Foster City, California, USA). Each sample was analysed in duplicate and blinded as to the result in the corresponding co-twin. XCI was calculated as the percentage of the predominantly inactive allele to the sum of both alleles and varies between 50 and 100, where 50 reflects random XCI and 100 reflects a completely skewed XCI.

Zygosity determination

Zygosity was established by DNA fingerprinting using a PE Applied Biosystems AmpFISTER Profiles Plus Kit (Foster City, California, USA).

Statistical methods

Group frequencies were compared with the Pearson x^2 test, whereas group medians were compared with a two-sample Wilcoxon rank-sum (Mann-Whitney) test using Brunner's adjustment for clustering within twin pairs [37]. Because of deviation from normality, logarithmic transformation of TSH, TPOAb, XCI and BMI was carried out. The relationship between the thyroid traits (TSH, FT4 and FT3) and the logged values of XCI (within cohort analysis) as well as the relationship between the within twin pair differences in the thyroid traits and within pair differences in logged XCI (within twin pair analysis) were assessed by linear regression.

In the within-cohort analysis, the paired nature of the twin data was taken into account by using the cluster option in STATA (StataCorp, College Station, Texas, USA). Subsequently, the data were analysed with the thyroid traits (TSH, FT4, FT3) as the response variable and XCI, TPOAb, age, BMI, smoking and zygosity as the explanatory variables.

In the within twin-pair analyses, the regression line was constrained to pass through the origin so that the results were independent of the labelling of the twin as the first or the second. Subsequently, we stratified according to zygosity and adjusted for TPOAb, BMI and smoking by using the within pair differences of these variables as explanatory variables in the within twin-pair regression model.

Significant differences were defined as a P-value less than 0.05 using two tailed tests. All analyses were carried out using version 11 of the STATA statistical package (StataCorp, College Station, Texas, USA).

Results

Descriptive characteristics and distribution of thyroid variables and XCI measurements in the twin cohort, as a whole and stratified by zygosity, are summarized in table 1.

In the overall study population (within cohort), no significant correlations were found between TSH [regression coefficient (β) = -0.28 (95% confidence intervals, -0.66-0.11), p = 0.158], FT3 [β= -0.25 (-0.85-0.34), p = 0.403], FT4 [β= 0.08 (-0.91-1.07), p = 0.876] and XCI pattern. Controlling for potential confounders (age, TPOAb, BMI, smoking and zygosity) did not change the findings of nonsignificant regression coefficients (Table 2).

The analyses relating the within twin pair differences in TSH, FT3 and FT4 to the within pair differences in XCI pattern are outlined in table 2. Overall, irrespective of zygosity, no significant associations were found. Stratifying for zygosity, and controlling for TPOAb, BMI and smoking did not change the results (Table 2). However, the within-pair difference in the logged value of XCI pattern is rather small. Theoretically, this small variability could mask a genuine correlation. To overcome this potential limitation, we restricted the analyses to twin pairs with the highest degree of within-pair variation in lnXCI (difference of at least 0.2, n = 46 pairs). The regression coefficients were, however still nonsignificant [TSH; β = 0.25 (-0.24-0.73), p = 0.312. FT3; β = -0.17 (-1.32-0.97), p = 0.761. FT4; β = 0.42 (-1.56-2.40), p = 0.670].

Finally, since mutations in the MCT8 gene results in an increase in FT3 and a decrease in FT4, the FT3/FT4 ratio could be a more sensitive parameter for variation in XCI pattern. However, neither in the overall study population (within cohort) nor in the within-pair analyses did we find significant correlations between the FT3/FT4 ratio and XCI pattern (β = -0.22 (-0.71-0.03), p = 0.389 and β= -0.02 (-0.08-0.04), p = 0.499, respectively). Adjusting for confounders as well as stratifying for zygosity did not change the finding of non-significant correlations between FT3/FT4 ratio and XCI pattern (data not shown).

Discussion

The present study was initiated to test the hypothesis that a non-hormonal sex specific genetic mechanism related to the X chromosome could influence the set point of the PTA in women. In our sample of euthyroid Danish female twins, we did not observe a significant correlation between the serum concentrations of TSH, FT3 and FT4 and the XCI pattern. To minimize the effect of genetic and environmental confounding we also analysed the association between TSH, FT3 and FT4 and XCI within MZ and DZ twin pairs, which did not change the finding of a non-significant correlation. Essentially similar results were obtained when the analyses were restricted to twin pairs with the most pronounced within pair differences in the XCI pattern. Thus, neither in the within cohort nor in the within twin pair analysis could we demonstrate a statistically significant correlation between thyroid variables and the XCI pattern.

The strengths of this study include: a relatively large sample size (n = 159 twin pairs), ascertainment of participants from a nation-wide population based register, use of standardized and validated procedures for examination and measurement of

biochemical variables, and exclusion of subjects with evidence of thyroid disease. In addition, our study is very robust, because the twin design allows optimal control for genetic and environmental factors affecting both the pituitary-thyroid axis set point and the XCI pattern [38].

Our findings should be interpreted cautiously in the light of some methodological limitations. First, the XCI pattern was tested in the leukocytes of peripheral blood, but not in the hypothalamus, pituitary or thyroid gland, the primary tissues involved in determining the PTA set point. Clearly, there are fundamental ethical and practical limitations in obtaining brain and thyroid biopsies from otherwise healthy individuals. However, from the few studies available, it appears that the correlations of XCI profiles between peripheral blood and different human tissues, including brain tissue, are reasonably high and that XCI in blood can be extrapolated to the XCI profile in other less accessible tissues [29,39]. Second, it is important to notice that variations in TBG has a much more pronounced effect on the levels of total T3 and T4 than on FT3 and FT4 levels, making a XCI dependent variation of total T3 and T4 possible. In the present study we measured FT3 and FT4. Thus, we are not able to investigate this issue further. Finally, all the participants were twins, but there is no reason to suspect that the XCI process and the factors influencing the PTA differ between twins and singletons. Available studies have clearly shown that Danish twins are representative of the general background population as for a number of thyroid traits including distribution of thyroid hormones [2] and the XCI pattern [30]. Epidemiological surveys have repeatedly shown that the spectrum of thyroid phenotypes, including the values of TSH, FT3 and FT4, in a community changes with variations in the iodine intake [40]. In the present study, all participants were Caucasian women living in Denmark, an area with borderline iodine deficiency and with only minor regional

differences in iodine intake. Moreover, cultural background and living conditions are generally quite homogeneous in Denmark. Consequently, our results cannot uncritically be extrapolated to other populations.

In conclusion, in a sample of healthy euthyroid Danish female twins, we found no evidence of a relationship between XCI pattern and pituitary-thyroid axis set point.

Reference List

1. Andersen,S., Pedersen,K.M., Bruun,N.H. et al. (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.

2. Hansen,P.S., Brix,T.H., Iachine, I. et al. (2007) Genetic and environmental interrelations between measures of thyroid function in a healthy Danish twin population. *American Journal of Physiology Endocrinology and Meatbolism,* **292**, 765-770.

3. Panicker,V., Wilson,S.G., Spector,T.D. et al. (2008) Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. *Clinical Endocrinology,* **68**, 652-659.

4. Panicker,V., Wilson,S.G., Spector,T.D. et al. (2008) Genetic loci linked to pituitary-thyroid axis set points: A genome-wide scan of a large twin cohort. *Journal of Clinical Endocrinology and Metabolism,* **93**, 3519-3523.

5. Arnaud-Lopez,L., Usala,G., Ceresini,G. et al. (2008) Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *AmericanJournal of Human Genetics,* **82**, 1270-1280.

6. Peeters,R.P., van den Beld,A.W., Attalki,H. et al. (2005) A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. *American Journal of Physiology Endocrinology and Metabolism,* **289**, 75-81.

7. van der Deure,W.M., Hansen,P.S., Peeters,R.P. et al. (2009) The effect of genetic variation in the type 1 deiodinase gene on the interindividual variation in serum thyroid hormone levels: an investigation in healthy Danish twins. *Clinical Endocrinology,* **70**, 954-960.

8. Panicker,V., Cluett,C., Shields,B. et al. (2008) A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. *Journal of Clinical Endocrinology and Metabolism,* **93**, 3075-3081.

9. Peeters,R.P., van,T.H., Klootwijk,W. et al. (2003) Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *Journal of Clinical Endocrinology and Metabolism,* **88**, 2880-2888.

10. Hansen,P.S., van der Deure,W.M., Peeters,R.P. et al. (2007) The impact of a TSH receptor gene polymorphism on thyroid related phenotypes in a healthy Danish twin population. *Clinical Endocrinology,* **66**, 827-832.

11. Sorensen,H.G., van der Deure,W.M., Hansen,P.S. et al. (2008) Identification and consequences of polymorphisms in the thyroid hormone receptor alpha and beta genes. *Thyroid,* **18**, 1087-1094.

12. van der Deure,W.M., Hansen,P.S., Peeters,R.P. et al. (2008) Thyroid hormone transport and metabolism by organic anion transporter 1C1 and consequences of genetic variation. *Endocrinology,* **149**, 5307-5314.

13. Friesema,E.C., Visser,W.E., & Visser,T.J. (2010) Genetics and phenomics of thyroid hormone transport by MCT8. *Molecular and Cellular Endocrinology, Doi:10.1016/j.mce.2010.01.016*.

14. Knudsen,N., Laurberg,P., Rasmussen,L.B. et al. (2005) Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *Journal of Clinical Endocrinology and Metabolism,* **90**, 4019-4024.

15. Manji,N., Boelaert,K., Sheppard,M.C. et al. (2006) Lack of association between serum TSH or free T4 and body mass index in euthyroid subjects. *Clinical Endocrinology,* **64**, 125-128.

16. Knudsen,N., Bülow,I., Jørgensen,T. et al. (2000) Comparative study of thyroid function and types of thyroid dysfunction in two areas in denmark with slightly different iodine status. *European Journal of Endocrinology,* **143**, 485-491.

17. Vejbjerg,P., Knudsen,N., Perrild,H. et al. (2008) The impact of smoking on thyroid volume and function in relation to a shift towards iodine sufficiency. *European Journal of Epidemiology,* **23**, 423-429.

18. Soldin,O.P., Goughenour,B.E., Gilbert,S.Z. et al. (2009) Thyroid hormone levels associated with active and passive cigarette smoking. *Thyroid,* **19**, 817-823.

19. Franklyn,J.A., Ramsden,D.B., & Sheppard,M.C. (1985) The influence of age and sex on tests of thyroid function. *Annals of.Clinical Biochemistry,* **22**, 502-505.

20. Jensen,E., Hyltoft,P.P., Blaabjerg,O. et al. (2004) Establishment of a serum thyroid stimulating hormone (TSH) reference interval in healthy adults. The importance of environmental factors, including thyroid antibodies. *Clinical Chemistry and Laboratory Medicine,* **42**, 824-832.

21. Volzke,H., Alte,D., Kohlmann,T. et al. (2005) Reference intervals of serum thyroid function tests in a previously iodine-deficient area. *Thyroid,* **15**, 279-285.

22. Hollowell,J.G., Staehling,N.W., Flanders,W.D. et al. (2002) Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism,* **87**, 489-499.

23. Hamilton,T.E., Davis,S., Onstad,L. et al. (2008) Thyrotropin levels in a population with no clinical, autoantibody, or ultrasonographic evidence of thyroid disease:

implications for the diagnosis of subclinical hypothyroidism. *Journal of Clinical Endocrinology and Metabolism,* **93**, 1224-1230.

24. Estaquio,C., Valeix,P., Leenhardt,L. et al. (2009) Serum thyrotropin and free thyroxine reference ranges as defined in a disease-free sample of French middle-aged adults. *Clinical Chemistry and Laboratory Medicine,* **47**, 1497-1505.

25. Ober,C., Loisel,D.A., & Gilad,Y. (2008) Sex-specific genetic architecture of human disease. *Nature Review Genetics,* **9**, 911-922.

26. Brix,T.H., Knudsen,G.P., Kristiansen,M. et al. (2005) High frequency of skewed X-chromosome inactivation in females with autoimmune thyroid disease: A possible explanation for the female predisposition to thyroid autoimmunity. *Journal of Clinical Endocrinology and Metabolism,* **90**, 5949-5953.

27. Brix,T.H., Hansen,P.S., Kyvik,K.O. et al. (2010) Preliminary evidence of a noncausal association between the X-chromosome inactivation pattern and thyroid autoimmunity: a twin study. *European Journal of Human Genetics,* **18***, 254-257.*

28. Ozcelik,T., Uz,E., Akyerli,C.B. et al. (2006) Evidence from autoimmune thyroiditis of skewed X-chromosome inactivation in female predisposition to autoimmunity. *European Journal of Human Genetics,* **14**, 791-797.

29. Brown,C.J. & Robinson,W.P. (2000) The causes and consequences of random and non-random X chromosome inactivation in humans. *Clinical Genetics,* **58**, 353-363.

30. Kristiansen,M., Knudsen,G.P.S., Bathum,L. et al. (2005) Twin study of genetic and aging effects on X chromosome inactivation. *European Journal of Human Genetics* **13**, 599-606.

31. Naumova,A.K., Olien,L., Bird,L.M. et al. (1998) Genetic mapping of X-linked loci involved in skewing of X chromosome inactivation in the human. *European Journal of Human Genetics,* **6**, 552-562.

32. Refetoff,S., Murata,Y., Mori,Y. et al. (1996) Thyroxine-binding globulin: organization of the gene and variants. *Hormone Research,* **45**, 128-138.

33. Barbesino,G., Tomer,Y., Concepcion,E.S. et al. (1998) Linkage analysis of candidate genes in autoimmune thyroid disease. II. Selected gender-related genes and the X-chromosome. *Journal of Clinical Endocrinology and Metabolism,* **83**, 3290-3295.

34. Loat,C.S., Asburry,K., Glasworthy,M.J. et al. (2004) X inactivation as a source of behavioural differences in monozygotic female twins. *Twin Research,* **7,** 54-61.

35. Benyamin,B., Sørensen,T.I.A. et al. (2007) Are there common genetic and environmental factors behind the endophenotypes associated with the metabolic syndrome? *Diabetologia,* **50**, 1880-1888.

36. Jensen,E., Petersen,P.H., Blaabjerg,O. et al. (2006) Establishment of reference distributions and decision values for the antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and the thyrotropin receptor (TRAb). *Clinical Chemistry and Laboratory Medicine,* **44**, 991-998.

37. Brunner,E. (1991) A nonparametric estimator of the shift effect for repeated observations. *Biometrics,* **47**, 1149-1153.

38. MacGregor,A.J., Snieder,H., Schork,N.J. et al. (2000) Twins. Novel uses to study complex traits and genetic diseases. *Trends in Genetics,* **16**, 131-134.

39. Bittel,D.C., Theodoro,M.F., Kibiryeva,N. et al. (2008) Comparison of X-chromosome inactivation patterns in multiple tissues from human females. *Journal of Medical Genetics,* **45**, 309-313.

40. Laurberg,P., Jørgensen,T., Perrild,H. et al. (2006) The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives. *European Journal of Endocrinology,* **155**, 219-228.

Table 1

Basic characteristics

Variable	Study population		
	MZ + DZ (n=318)	MZ (n=164)	DZ (n=154)
Smoking (%)	52	48	56
Age (year)	36 (19-51)	35 (19-51)[a]	38 (21-52)
BMI (kg/m^2)	23 (19-31)	23 (19-31)	23 (19-31)
Degree of skewing	63 (51-87)	63 (51-87)	63 (51-88)
TPOAb (kIU/liter)	4.7 (2.2-93.0)	4.6 (2.3-79.6)	4.9 (2.8-156.3)
TSH (mU/liter)	1.6 (0.7-3.3)	1.7 (0.7-3.6)[b]	1.4 (0.6-3.2)
Free T3 (pmol/l)	5.8 (4.6-7.3)	5.9 (4.8-7.1)	5.8 (4.5-7.3)
Free T4 (pmol/l)	12.4 (10.1-14.8)	12.4 (10.4-14.5)	12.3 (9.8-15.0)

All values, except for smoking, are presented as medians with 5th and 95th percentiles in parentheses. MZ = monozygotic and DZ = dizygotic.

[a] MZ vs DZ, p = 0.022

[b] MZ vs DZ, p = 0.017

Table 2

Regression coefficients for correlation between different thyroid variables and X chromosome inactivation pattern.

	Regression coefficients (95% confidence intervals)					
	Unadjusted			Adjusted[a]		
Analysis	TSH	Free T3	Free T4	TSH	Free T3	Free T4
Within cohort	-0.28 (-0.66-0.11)	-0.25 (-0.85-0.34)	0.08 (-0.91-1.07)	-0.25 (-0.63-0.12)	-0.24 (-0.84-0.37)	0.21 (-0.83-1.24)
Within twin pairs	0.11 (-0.29-0.50)	-0.18 (-0.90-0.54)	0.08 (-1.32-1.48)	0.04 (-0.36-0.44)	-0.22 (-0.95-0.52)	0.35 (-1.10-1.79)
MZ pairs	-0.17 (-0.88-0.54)	0.03 (-1.05-1.11)	-0.81 (-3.10-1.48)	-0.22 (-0.93-0.49)	0.04 (-1.05-1.12)	-0.76 (-3.09-1.56)
DZ pairs	0.21 (-0.29-0.71)	-0.25 (-1.27-0.77)	0.41 (-1.49-2.30)	0.14 (-0.39-0.67)	-0.35 (-1.43-0.73)	0.87 (-1.12-2.87)

[a] Adjusted for age, TPOAb, BMI, smoking and zygosity in the within cohort analysis. In the within twin pair analysis there is adjusted for TPOAb, BMI and smoking.