Genetic Loci Linked to Pituitary-Thyroid Axis Set Points: A Genome-Wide Scan of a Large Twin Cohort

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Objective: Previous studies have shown that circulating concentrations of TSH, free T_4, and free T_3 are genetically regulated, but the genes responsible remain largely unknown. The aim of this study was to identify genetic loci associated with these parameters.

Design: We performed a multipoint, nonparametric genome-wide linkage scan of 613 female dizygotic twin pairs. All subjects were euthyroid (TSH 0.4–4.0 mU/liter) with negative thyroid peroxidase antibodies and no history of thyroid disease. The genome scan comprised 737 microsatellite markers supplemented with dinucleotide markers. Data were analyzed using residualized thyroid hormone data after adjustment for age, smoking, and body mass index.

Results: Multipoint linkage analysis gave linkage peaks for free T_4 on chromosome 14q13 and 18q21 [logarithm of odds (LOD) 2.4–3.2]; TSH on chromosomes 2q36, 4q32, and 9q34 (LOD 2.1–3.2); and free T_3 on chromosomes 7q36, 8q22, and 18q21 (LOD 2.0–2.3).

Conclusions: This study has identified eight genomic locations with linkage of LOD of 2.0 or greater. These results should enable targeted positional candidate and positional cloning studies to advance our understanding of genetic control of the pituitary-thyroid axis. **(*J Clin Endocrinol Metab* 93: 3519–3523, 2008)**

In healthy subjects, variability in circulating concentrations of TSH, free T_4, and free T_3 is greater between individuals (interindividual variation) than in the same individual sampled repeatedly over time (intraindividual variation), suggesting that different people have different set points for pituitary-thyroid axis function (1). There is greater concordance in serum TSH, free T_4, and free T_3 concentrations in monozygotic compared with dizygotic twins (2, 3), suggesting a genetic influence, and heritability estimates for these parameters range from 32 to 65% for TSH, 32 to 65% for free T_4, and 23 to 67% for free T_3 (2–4). The identity of the genes involved is, however, not well established. Polymorphisms in the genes encoding the iodothyronine deiodinase enzymes (5), TSH receptor (5, 6), and thyroid hormone transporters (7) are associated with thyroid hormone parameters, but their contribution to phenotypic variance is modest.

We performed a genome-wide screen on a large cohort of unselected dizygotic twin pairs to identify regions of the genome containing quantitative trait loci (QTL) for serum TSH, free T_4, and free T_3 concentrations.

Subjects and Methods

Subjects

The subjects were Caucasian dizygous female twin pairs aged 18–80 yr from St. Thomas' United Kingdom Adult Twin Registry (8). Each pair

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Abbreviations: BMI, Body mass index; FC, freely estimated coefficient of variation; GLM, generalized linear model; IR, iterative robust; LOD, logarithm of odds; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; TPOAb, thyroid peroxidase antibody.

of twins attended together for a medical and lifestyle interview and underwent physical examination and blood sampling in the fasting state. The initial data set contained 1078 dizygous twin pairs. We excluded 71 twin pairs in whom either twin was taking medications which affect thyroid function tests (T_4, antithyroid drugs, phenytoin, or carbamazepine). To minimize the confounding effects of thyroid disease, we excluded a further 377 twin pairs in which either twin gave a history of thyroid disease, had a serum TSH concentration outside the reference range (0.4–4.0 mU/liter), or had positive thyroid peroxidase antibodies (TPOAbs) (defined below). A further 17 twin pairs were excluded because of missing TSH or TPOAb data. The final sample therefore constituted 613 twin pairs. All subjects provided written informed consent. The study was approved by the Research Ethics Committees of St. Thomas' Hospital and Sir Charles Gairdner Hospital.

Biochemistry methods

Serum TSH, free T_4, free T_3, and TPOAbs were measured by chemiluminescence immunoassay on the Abbott Diagnostics Architect (Abbott Diagnostics, North Ryde, Australia), as previously described (3). A TPOAb titer of greater than 6 kU/liter was considered positive.

Genotyping

DNA was extracted from whole blood, and fluorescence-based genotyping methodologies were used to analyze 737 highly polymorphic microsatellite markers from the ABI Prism linkage mapping set (Applied Biosystems, Foster City, CA) as described previously (9) and 1494 single nucleotide polymorphism (SNP) markers from the HuSNP GeneChip linkage mapping set (Affymetrix Inc., Santa Clara, CA), providing average intermarker distance of 2 cM (range 0–16 cM). Allele frequencies were estimated from the whole sample of genotyped subjects. Map positions were taken from Rutgers combined linkage physical map (10, 11) or interpolated from their physical position. Twin zygosity and family relationships were rigorously investigated and discrepant pairs discarded from further analyses. The estimated genotyping error rate was less than 1%.

Statistical analyses

In previous studies, age, smoking, and body mass index (BMI) were associated with differences in circulating thyroid hormone parameters (12–14). Using generalized least squares regression analysis, in which the correlation between sibling pair phenotypes is accounted for, we tested whether these variables were significantly associated with free T_4, free T_3, or TSH in a stepwise backward elimination method, with an exit threshold $P = 0.05$. For TSH and free T_3, age and BMI were significant, whereas for free T_4, age, BMI, and smoking were significant, and the thyroid phenotypes were adjusted for these covariates. Raw and adjusted phenotype distributions were assessed for deviation from normality using the Anderson-Darling normality test and QQ plots. TSH had a skewed distribution and was log transformed in covariate adjustments and before subsequent genetic linkage analysis. Clinical data were analyzed using the program R version 2.6.1 (http://CRAN.R-project.org).

Multipoint genome-wide linkage analyses were performed using unadjusted and adjusted thyroid function data and optimal Haseman and Elston regression methods (15), implemented using a generalized linear model (GLM) (16). The approach in this analysis is to regress the square of the sibling pair phenotypic difference on genetic marker identity by descent status. This was implemented in Stata (release 7.0; Statacorp, College Station, TX) (17). This approach is equivalent to other maximum likelihood techniques (17) but has the advantage of being robust to deviations from normality by freely estimating the residual coefficient of variation (*i.e.* the mean and variance-corrected residual error) and using a Huber estimate of variance (16). Estimates obtained using this method are referred to in the text as statistics freely estimated coefficient of variation (FC). An alternative iterative robust (IR) statistic was also calculated whereby the GLM is fitted with an additional routine that effectively downweights unduly influential outliers. We adopted a conservative approach and focused on the FC (unadjusted and adjusted) values greater than or equal to 2, in regions in which FC and IR results were relatively consistent. Approximate support intervals were generated using a −1 logarithm of odds (LOD) approach based on the data for the FC statistics. Simulated *P* values were derived by permuting each GLM test 1000 times. In each permutation the phenotype data were randomly reassigned to the genotypes. Individuals in the study were considered randomly ascertained because sampling was not based on a subject's thyroid data. All twin pairs were from independent families.

Results

Descriptive statistics

The mean age (± SD) of the 1226 subjects was 45.3 ± 12.5 yr and mean BMI was 25.1 ± 4.7 kg/m^2. The mean free T_4 concentration was 13.7 ± 1.7 pmol/liter, mean free T_3 4.0 ± 0.6 pmol/liter, and median TSH (with interquartile range) 1.21 (0.88–1.34) mU/liter. There were 92% nonsmokers, 2% former smokers, and 6% current smokers.

Linkage

Multipoint analysis showed eight linkage peaks with an FC LOD score of 2.0 or greater (Fig. 1 and Table 1). Of particular interest is a region on chromosome 18q associated with a peak in the analyses for free T_4 (85 cM, LOD score 3.2 unadjusted and 2.9 adjusted), free T_3 (75 cM, adjusted LOD score 2.0), and a minor peak for unadjusted TSH (LOD score 1.7). There are also peaks for free T_4 on chromosome 14 at 40 cM (LOD 3.0 unadjusted) and lnTSH on chromosome 2 at 230 cM (LOD 3.2 unadjusted). Other linkage peaks for lnTSH are on chromosome 4 at 160 cM (LOD 2.5 adjusted) and chromosome 9 at 150 cM (LOD 2.1 adjusted) and for free T_3 are on chromosome 7 at 190 cM (LOD 2.3 unadjusted) and chromosome 8 at 100 cM (LOD 2.2 unadjusted). The support intervals for these peaks are provided in Table 1.

Discussion

This genome-wide linkage analysis identified a number of significant linkage peaks for each of serum TSH, free T_4, and free TSH. The −1 LOD approximate support intervals for these peaks contain some genes thought to be relevant to pituitary-thyroid axis physiology, including *TITF-1* (thyroid transcription factor 1), located in the support interval for the linkage peak on chromosome 14 for free T_4; *LHX3* (LIM homeobox 3) and *RXRA* (retinoid X receptor-α) in the support interval for the peak on chromosome 9 for TSH and *TRIP12* (thyroid receptor interacting protein 12), within the support interval on chromosome 2 for TSH. None of the other QTL, including the peak on 18q associated with all three phenotypes, are in regions containing genes known to affect thyroid function. It must be acknowledged that although the LOD scores associated with the identified peaks are statistically significant, they are relatively modest (between 2 and 3), suggesting that there is no single gene with a major regulatory influence on pituitary-thyroid axis set point. Rather, it may be that TSH, free T_4, and free T_3 are polygenic

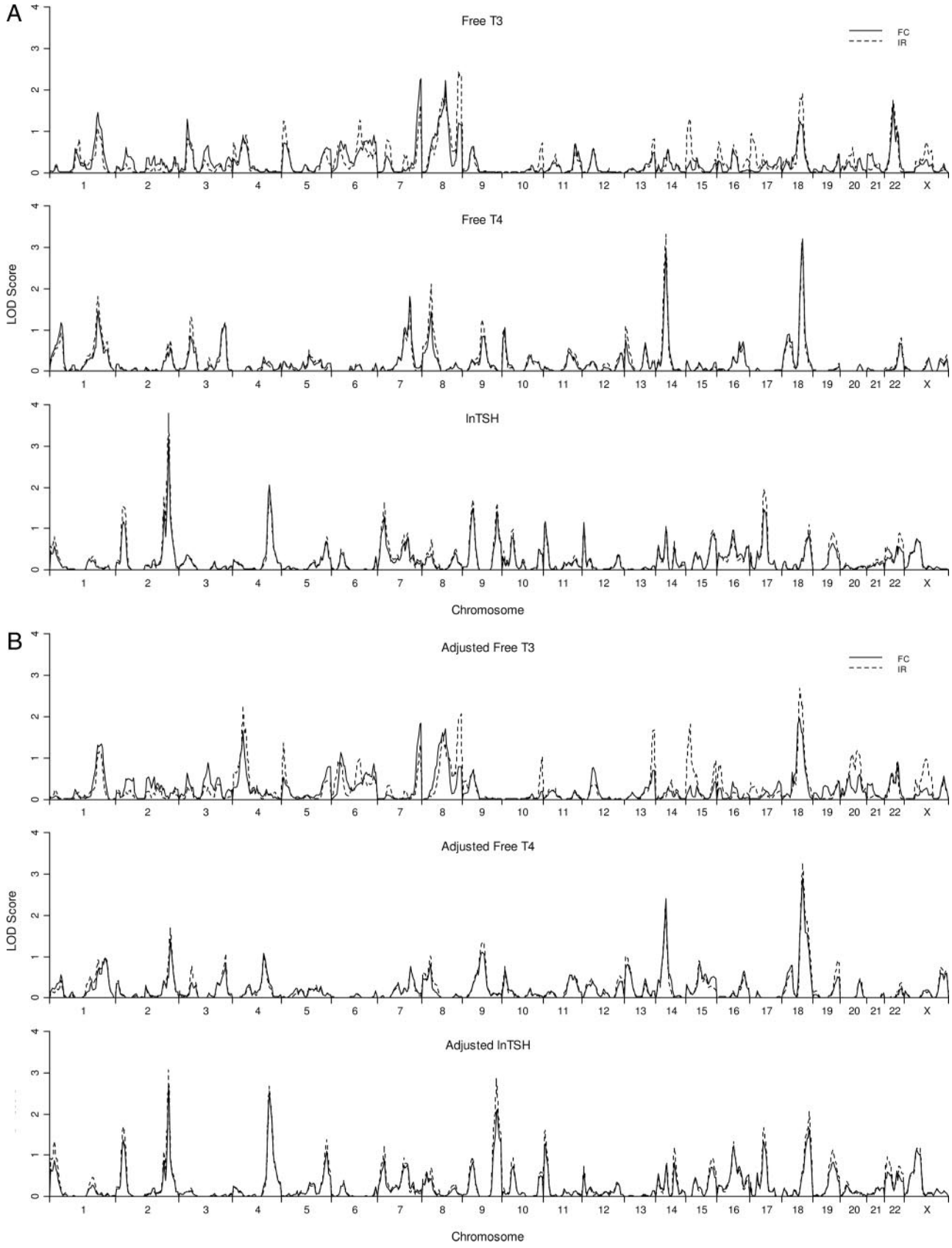

FIG. 1. Genome-wide scans for quantitative trait loci associated with pituitary-thyroid axis set points. Plots display linkage data for free T_3, free T_4, and TSH unadjusted (A) and adjusted for age, smoking, and body mass index (B).

TABLE 1. Summary of linkage data (all linkage peaks with FC LOD score ≥ 2.0 included)

Chromosome	Position (cM)	Cytogenetic band	Support interval (cM)	FC LOD	FC *P* value	IR LOD	IR *P* value	FC (adjusted) LOD	FC (adjusted) *P* value	IR (adjusted) LOD	IR (adjusted) *P* value
Free T_4											
14	40	14q 13.3	36–46	3.0	0.0002	3.3	0.00009	2.4	0.0009	2.4	0.0009
18	85	18q 21.32	77–91	3.2	0.0001	3.2	0.0001	2.9	0.0002	3.3	0.0001
Free T_3											
7	190	7q 36.3	173–190	2.3	0.001	1.6	0.006	1.8	0.004	1.3	0.014
8	100	8q 22.1	84–110	2.2	0.001	2.0	0.003	1.7	0.005	1.4	0.012
18	75	18q 21.2	57–95	1.2	0.017	1.8	0.004	2.0	0.003	2.7	0.0004
lnTSH											
2	230	2q 36.3	225–236	3.2	0.0001	3.8	0.00003	2.7	0.0004	3.1	0.0002
4	160	4q 32.3	152–172	2.1	0.002	2.0	0.002	2.5	0.0007	2.7	0.0005
9	150	9q 34.2	135–165	1.4	0.011	1.6	0.006	2.1	0.002	2.7	0.0005

FC (adjusted), FC statistic adjusted for age, smoking, and BMI; IR (adjusted), IR statistic adjusted for age, smoking, and BMI.

traits, with a number of genes each making a small contribution to phenotypic variance.

We present analyses using two metrics for assessment of linkage: FC and IR. The main value of the IR statistic is that the effect of unduly influential outliers is reduced with the application of this statistic. As might be expected, for the majority of the data, the results for the two statistics are very similar; however, it is interesting to note that a linkage peak on 8q for free T_3 achieves a LOD above 2 only when the IR statistic is considered, suggesting that outliers may be affecting the FC analysis. Further scrutiny of this locus will be necessary to determine whether a valid QTL exists at this location. Furthermore, we examined unadjusted and covariate-adjusted phenotype data. There were no instances in which the location of a peak LOD 2 or greater showed a major change between the adjusted and unadjusted data; however, the magnitude of the linkages did vary as did the precise boundaries of the support intervals. In general, the magnitude of the unadjusted linkages was greater, suggesting that contrary to what one might expect, adjustment for covariates in this analysis did not materially assist in locating the QTL.

Recently two groups have published results of genome-wide association studies for serum TSH but not free T_4 or free T_3. In the study of Hwang *et al.* (18), subjects with thyroid disease were not excluded, and none of the reported associations correspond to QTL for TSH in our study. In the study of Arnaud-Lopez *et al.* (19), however, the fourth ranked association (rs657152; $P = 2.4 \times 10^{-7}$), falls within the support interval of our linkage for TSH on chromosome 9, affirming the potential relevance of variants within the linkage disequilibrium bin tagged by rs657152 in the regulation of TSH. In addition, SNPs rs2288629 on chromosome 2 and rs2545308 on chromosome 4 lie either within or close to the boundary of the support interval for linkages to TSH on those chromosomes, thus prescribing further additional research focus on these regions.

The strengths of this study include its large sample size, detailed statistical analysis, and exclusion of subjects with evidence of thyroid disease or autoimmunity. A limitation is that all the participants were female, and it cannot be assumed that the results apply to males. There is, however, no evidence that pituitary-thyroid axis regulation is sexually dimorphic. All the participants were twins, and there is no reason to suspect that pituitary-thyroid axis regulation differs between twins and singletons; certainly previous studies have suggested that these twins are representative of the general population for a range of phenotypes (20). Because of the common age and, usually, shared upbringing for twins, a number of common important environmental influences are controlled for in a study of twins. By contrast, in sibling pair studies, there is often little reliable information on shared upbringing for siblings of different ages, on whom environmental factors may not act equally. This results in increased environmental variance, which is a potential confounder.

In conclusion, this study has identified eight genomic locations with linkage of LOD 2.0 or greater. Targeted positional candidate and positional cloning studies are now required to advance our understanding of genetic control of the pituitary-thyroid axis.

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