Genetic Linkages for Thyroxine Released in Response to Thyrotropin Stimulation in Three Sets of Recombinant Inbred Mice Provide Evidence for Shared and Novel Genes Controlling Thyroid Function

Sepehr Hamidi,^{1,2,*} Holly A. Aliesky,¹ Robert W. Williams,³ Basil Rapoport,^{1,2} and Sandra M. McLachlan^{1,2}

Background: Graves' hyperthyroidism is induced by immunizing mice with adenovirus expressing the human thyrotropin (TSH)-receptor. Using families of recombinant-inbred mice, we previously discovered that genetic susceptibility to induced thyroid-stimulating antibodies and hyperthyroidism are linked to loci on different chromosomes, indicating a fundamental genetic difference in thyroid sensitivity to ligand stimulation. An approach to assess thyroid sensitivity involves challenging genetically diverse lines of mice with TSH and measuring the genotype/strain-specific increase in serum thyroxine (T4).

Methods: We investigated genetic susceptibility and genetic control of T4 stimulation by 10 mU bovine TSH in female mice of the CXB, BXH, and AXB/BXA strain families, all previously studied for induced Graves' hyperthyroidism.

Results: Before TSH injection, T4 levels must be suppressed by inhibiting endogenous TSH secretion. Three daily intraperitoneal L-triiodothyronine injections efficiently suppressed serum T4 in females of 50 of 51 recombinant inbred strains. T4 stimulation by TSH was more strongly linked in CXB and BXH sets, derived from parental strains with divergent T4 stimulation, than in AXB/BXA strains generated from parents with similar TSH-induced responses. Genetic loci linked to the acute TSH-induced T4 response (hours) were not the same as those linked to induced hyperthyroidism (which develops over months).

Conclusions: Genetic susceptibility for thyroid sensitivity to TSH stimulation was distinct for three families of inbred mouse lines. These observations parallel the human situation with multiple genetic loci contributing to the same trait and different loci associated with the same trait in different ethnic groups. Of the genetic loci highlighted in mice, three overlap with, or are located up or downstream, of human TSH-controlling genes. Other studies show that human disease genes can be identified through cross-species gene mapping of evolutionary conserved processes. Consequently, our findings suggest that novel thyroid function genes may yet be revealed in humans.

Introduction

GRAVES'-LIKE HYPERTHYROIDISM is commonly induced by immunizing genetically susceptible strains of mice with adenovirus expressing the human thyrotropin holoreceptor (TSHR) or its isolated A-subunit (1–3). Unlike the susceptible BALB/c strain, some strains, including the C57BL/6 mice widely used by the genomics community, remain euthyroid despite developing TSHR antibodies (4). Indeed, susceptibil-

ity to induced Graves'-like hyperthyroidism is linked to a different set of chromosomes and loci from those linked to the induction of TSHR antibodies (3,5,6). A critical parameter for the development of hyperthyroidism is the induction by the human TSHR A-subunit of antibodies that cross-react with the mouse TSHR (7). Such TSH receptor antibody diversity increases the complexity of distinguishing between the parameters controlling antibody generation versus the factors that control thyroid sensitivity to stimulation.

¹Thyroid Autoimmune Disease Unit, Cedars-Sinai Research Institute, Los Angeles, California.

²The David Geffen School of Medicine, University of California, Los Angeles, California.

³Department of Anatomy and Neurobiology, University of Tennessee Health-Science Center, Memphis, Tennessee.

*Present affiliation: Department of Pathology and Laboratory Medicine, Los Angeles County and University of Southern California (LAC+USC) Health Network, Los Angeles, California.

Our previous studies of genetic susceptibility to induced hyperthyroidism were performed using three families of recombinant inbred strains: CXB, BXH, and AXB/BXA sets. Recombinant inbred lines are generated by crossing two inbred parental strains to provide the first filial generation (F1), crossing F1 progeny to generate the second generation (F2); subsequently, by repeated brother-sister F2 matings for 20 generations or more, to establish a set of stable homozygous and isogenic lines (8). High-resolution genetic maps, available for four families of recombinant inbred strains that share one parental strain (C57BL/6) provide powerful tools for mapping chromosomal loci linked with selected phenotypic traits [for example (9,10)].

An approach to assess thyroid sensitivity involves challenging different mouse lines with a defined dose of thyrotropin (TSH) and measuring the subsequent increase in serum thyroxine (T4). However, before TSH injection, it is necessary to rest the thyroid and suppress serum T4 levels by inhibiting endogenous TSH secretion. We previously have also shown that, unlike oral administration that is only effective in outbred or male mice, L-triiodothyronine (L-T3) injected intraperitoneally (i.p.) for 3 days efficiently suppresses females belonging to different inbred strains (11).

In the present study, we investigated the rise in serum T4 induced by a single dose of bovine TSH in the three sets of recombinant inbred lines that we previously investigated for induced Graves' hyperthyroidism. In addition, we tested the parental strains and the F1 hybrids of these families. Like many autoimmune conditions, Graves' disease develops predominantly in women. Consequently, our previous studies of induced Graves' disease, as well as our current investigations, were performed in female mice.

Materials and Methods

Mouse strains

Female mice of the following strains (5–9 weeks old) were obtained from the Jackson Laboratory:

1. Parental strains C3H/HeJ, A/J, BALB/cJ, and C57BL/6J; the latter two are the most closely related available to the parental strains of the CXB set (BALB/cByJ and C57BL/6ByJ). Parental strains are abbreviated C3H, A, BALB, and B6.
2. F1 hybrids: B6C3F1/J (B6 female X C3H male), CB6F1/J (BALB female X B6 male), and B6AF1/J (B6 female X A/J male). T4 levels of parental strains and F1 progeny are of great interest, but are not used in computing genetic linkage.

3. AXB/PgnJ 1, 2, 4, 5, 6, 8, 10, 12, 13, 15, 19a, 23, 24; BXA/PgnJ 1, 2, 4, 7, 8, 11, 12, 13, 14, 16, 24, 25, 26 (abbreviated A1 to A24 for AXB strains and B1 to B26 for BXA strains; as a group, they are referred to as "AXBXA").
4. CXB/ByJ 1 to 7, CXB/HiAJ 8 to 13 (abbreviated CXB1 to 13).
5. BXH TyJ 2, 4, 6–11, 14, 19, BXH KccJ 20 and 22 (abbreviated BXH2, 4 etc. to BXH22).

Mice from the Jackson Laboratory were fed LabDiet 5K52/5K67 or LabDiet 5K54 (containing 2.1 and 2.2 ppm iodine, respectively). All mice were maintained at the Cedars-Sinai Medical Center until 7–9 weeks old (on average); during this time (1–3 weeks), they were fed LabDiet Mouse Chow 5015 (1.01 ppm iodine) *ad libitum*. The mean ($\pm$ standard deviation [SD]) ages of these recombinant inbred families was 7.7 ± 1.0 weeks for CXB; 7.9 ± 0.6 for BXH; 8.9 ± 1.0 for AXB and BXA. Strains exceeding the 90th percentile for age in each set were CXB2 (9.9 weeks), BXH11 (9.1 weeks), AXB4 and AXB23 (11.3 and 11.1 weeks, respectively). All use of mice was approved by the Institutional Animal Care and Use Committee and performed with the highest standards of care in a pathogen-free facility.

L-T3-induced suppression of T4 and TSH stimulation

T4 release induced by TSH was studied in L-T3 suppressed mice based on the protocol of Maia *et al.* (12) as previously described (11). L-T3 for i.p. injections was prepared as follows: 2 mg L-T3 (T6397; Sigma-Aldrich) was dissolved in 1 mL 0.1 N NaOH, diluted 1:40 (0.2% bovine serum albumin in phosphate-buffered saline) and the pH adjusted to 7.0; 0.1 mL of this solution (containing 5 μ g L-T3) was injected.

The schedule for injections and blood drawing (Fig. 1) was as follows: Day 0, mice were weighed and bled from the tail vein to provide baseline T4 values; Days 1, 2, and 3, each morning, L-T3 (5 μ g/mouse) was injected i.p.; Day 3, afternoon, blood was drawn from the tail vein to establish the degree of L-T3-induced suppression on T4; Day 4, bovine TSH (10 mU; Sigma-Aldrich) was injected i.p. and mice were euthanized 5 hours later. Variability in the response of mice to i.p. injections has been documented (13,14). To reduce such variability to a minimum, all mice were handled and injected by the same experienced investigator (S.H.).

Serum T4

Total T4 was measured by radioimmunoassay in sera (25 μ L) from individual mice (Coat-a-Count total T4 kit; Siemens Health Diagnostics, Deerfield, IL). Standards ranged

FIG. 1. Schedule for L-triiodothyronine (L-T3) administration i.p. to suppress baseline thyroxine (T4) before injecting bovine thyrotropin (TSH; 10 mU) to induce T4 secretion in mice. The measurement parameters used for genetic linkage analysis in recombinant inbred mice are included.

from 1–24 $\mu\text{g}/\text{dL}$ T4 (sensitivity 0.25 $\mu\text{g}/\text{dL}$). For each mouse strain, sera for all time intervals (baseline, after L-T3 treatment and at postmortem after TSH injection) were tested in the same assay. T4 levels were computed from kit standards and reported as $\mu\text{g}/\text{dL}$. The following T4 measurements were examined for genetic linkage (summarized in Fig. 1): (i) Baseline T4; (ii) T4 post-L-T3 suppression; (iii) T4 release (T4 after TSH challenge minus T4 post-L-T3 suppression); (iv) percentage suppression by L-T3 calculated from (baseline T4 – T4 post-L-T3 suppression)/baseline T4 $\times$ 100%; and (v) T4 release as % baseline T4 (calculated from T4 release/baseline T4 $\times$ 100%).

Baseline TSH

Baseline TSH levels were determined by Dr. Roy Weiss (Thyroid Unit, University of Chicago, IL; fee for service) by radioimmunoassay (15). For this purpose, pooled samples from 4–5 mice were analyzed in duplicate aliquots of undiluted serum (50 μL). Sera with values $<10\text{ mU}/\text{L}$ were assigned an arbitrary value of 5.0 for linkage analysis.

Statistical analysis and linkage analysis

Comparisons between traits in different mouse strains were performed by analysis of variance (ANOVA; SigmaStat, Jandel Scientific Software). In calculating the mean and standard errors, the value 0.001 was assigned to mice with undetectable T4 levels after L-T3 and for mice in which there was no rise in serum T4 after TSH.

Quantitative trait loci (QTL) for the traits summarized in Figure 1 were mapped using genotype files for AXB/BXA, BXD, BXH, and CXB strains generated by Williams *et al.* (16) and embedded in GeneNetwork (GN) (17). The probability of linkage between T-T4 and F-T4 and previously mapped genotypes was estimated at ~ 1 centiMorgan intervals (~ 2 megabase [Mb]) along the entire genome, except for the Y chromosome. To establish criteria for suggestive and significant linkage, a permutation test was performed (24,000 permutations at 1-centiMorgan intervals) (18). This test compares the peak likelihood ratio statistics (LRS; $\text{LRS} = \text{LOD} \times 4.6$, where LOD is the logarithm of the odds) obtained for a given data set with the peak LRS score obtained for the same data set.

The phenotypes have been entered into the GN databases (18) under the following trait accession identifiers: AXB/BXA GN 10207 through 10213, 10256; CXB GN 10692 through 10698, 10742; BXH GN 10162 through 10169. These data can be found by searching the CXB, BXH or AXB/BXA databases for the name “McLachlan.” The GN trait numbers are provided to enable readers to verify and extend the data analysis. In addition, GN interval maps connect directly to ENSEMBL and to the University of California Santa Cruz Genome Browsers and it is comparatively easy to study the gene complement of the QTLs.

Results

Suppression by L-T3 and TSH-induced T4 release in parental strains and F1 offspring (all females)

We investigated serum T4 suppression by three daily i.p. injections of L-T3 (5 μg) and the release of T4 following one i.p. injection of bovine TSH (10 mU) in the parental strains and F1 offspring (all females) of three recombinant inbred sets as

follows: BXH parental strains and F1 offspring (termed B6, C3H, and B6C3F1); CXB parental strains and F1 offspring (BALB, B6, and CB6F1); AXB/A parental strains and F1 offspring (A, B6, and B6AF1). As mentioned, we tested the Jackson (J) strains of BALB/c and B6, the most closely related strains to the parental CXB Bailey (By) strains. The latter would require resuscitation and breeding from cryopreserved embryos.

Baseline T4 values were significantly lower in B6 mice than in C3H, BALB, or their F1 offspring. However, A and B6AF1 offspring had lower levels similar to B6 mice (Fig. 2A). L-T3 administration suppressed serum T4 by 80% in C3H mice and

FIG. 2. Baseline T4, L-T3-induced suppression and TSH induced release of T4 in the parental strains and F1 cross for three recombinant inbred sets: BXH (parents B6 and C3H; cross B6C3F1); CXB (parents BALB and B6; cross CB6F1); and AXB/A (parents A and B6, cross B6AF1). The data for B6 mice are repeated in each panel to provide a direct comparison with the other parents (C3H, BALB, and A). Bars are the mean $\pm$ SEM for 3–5 mice in each strain. (A) Baseline T4 ($\mu\text{g}/\text{dL}$); (B) % L-T3 suppression of T4, (C) Release of T4 ($\mu\text{g}/\text{dL}$) after TSH injection. The dotted line in (C) indicates 80% suppression. *Values significantly different from B6 (ANOVA, $p < 0.05$).

by greater than 85% in the other strains (Fig. 2B). T4 release induced by TSH was lowest in A and B6 mice compared to more robust responses in BALB/c and all three sets of F1 offspring, including those derived from parental A and B6 strains (Fig. 2C).

It should be emphasized that all our studies, including parental and F1 strains and recombinant inbred strains, were performed in female mice. Given the potential effects of the estrous cycle and sex differences in some parameters of the thyroid axis, our results may not be applicable to males.

Suppression by L-T3 and responses to TSH in three recombinant inbred sets

Previously, we observed that orally administered L-T3 only suppressed T4 levels in large outbred mice and inbred males, whereas L-T3 injected i.p. was effective for inbred female mice (11). Consequently, in the present study, L-T3 suppression was performed by three daily i.p. injections of L-T3 (5 µg). Our protocol was based on that of Maia *et al.* (12) showing that, in approximately 7-week-old male mice, serum T4 levels were reduced 24 hours after the last of three L-T3 (5 µg) injections. Moreover, to ensure that the mouse weight was not a genetic susceptibility factor influencing L-T3 suppression and/or the

subsequent response to TSH, mice were weighed at the start of the experiments.

Despite variability in body weight, L-T3 effectively suppressed T4 levels by >85% in all CXB and BXH strains (Fig. 3A) and AXB/AXA strains (hereafter called "AXBXA") with one exception (BXA16, broken arrow, Fig. 3B). However, several strains in each of the three recombinant sets did not release T4 in response to TSH (Fig. 3A, B).

Genetic linkage for baseline parameters: body weight, T4, and TSH

QTL were mapped using the CXB, BXH, and AXBXA database files (16,19) for the following parameters: body weight, serum T4, and TSH (all at baseline), T4 post-L-T3 suppression, % L-T3 suppression of serum T4, and three measurements of the response to bovine TSH in L-T3-suppressed mice (Fig. 1). The outcome of linkage analysis for baseline parameters is described next and the genetic susceptibility for L-T3 suppression and TSH-induced T4 release is reported in the subsequent section.

Body weight was suggestively linked to loci on chromosomes (Chr) 12, 17, and 10 in the BXH, AXBXA, and CXB sets, respectively (Table 1). Mice of these strains were previously studied for body weight, generally in much younger mice

FIG. 3. Weight, T4 at baseline and post-L-T3 administration, % suppression, and T4 release in response to TSH in three sets of recombinant inbred mice: CXB (13 strains) and BXH (12 strains; **A**) and AXBXA (26 strains; **B**). Bars are the mean $\pm$ SEM for 4–5 mice for all recombinant inbred strains except for BXH8, where $n=2$. Note the difference in scale for T4 values post-L-T3 administration and T4 release in response to TSH. (**A**): #Unexpectedly high baseline T4 in CXB11; arrow, BXH9 with subclinical hypothyroidism (low T4 and elevated TSH; the latter not shown); (**B**) L-T3 suppression <85% in BXA16 (broken arrow). (**A**, **B**): *Strains that developed the most severe hyperthyroidism after immunization with TSHR A-subunit adenovirus; namely, three immunizations for CXB and AXBXA strains (3,5) and two immunizations for BXH strains (6). The bracket links BXA12 and BXA13, strains that responded to bovine TSH (as illustrated), but discrepant in that BXA12, unlike BXA13, did not develop TSHR antibodies or hyperthyroidism (3).

than those used in our investigation. Also, some earlier investigations involved only six or seven CXB strains and, therefore, linkage analysis could only be performed by including data for the parental strains (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/thy). For CXB and BXH sets, there were no similarities between the Chr or loci linked to body weight in earlier studies compared with our findings, possibly because of differences in age and body weight. In the larger AXBXA recombinant inbred subset, despite size and age differences, body weight was linked to the same Chr 17 interval (68.0835–69.3988 Mb) observed previously (20) and in our present study (Supplementary Table S1). It is of interest that the closest marker gene on Chr 12 for body weight in BXH mice in our study was *Dio3* (Table 1). However, it must be emphasized that this linkage is tentative, not causal.

Baseline T4 was linked to Chr 2 in the AXBXA set and to loci on Chr 9 and Chr 19 in the CXB set (Table 2). The Chr 2 linkage for basal T4 that we previously observed in AXBXA strains (21) was located slightly downstream of the present locus (~108 Mb versus ~112 Mb; Supplementary Table S2). The major loci for baseline T4 in CXB strains in the present study differed from those reported earlier, but linkage was suggestive (LRS 7.40) for the locus on Chr 13 (~104 Mb)

highlighted previously (21). In the 12 BXH strains available (the 13th strain has been cryopreserved), we found no linkage for baseline T4 levels.

Serum TSH was undetectable in serum pools from most recombinant inbred strains, all females. These findings are in agreement with previous studies for females of other strains [for example, Ref. (15)]. Only 3 of 25 AXBXA and 2 of 13 CXB strains had TSH levels greater than 10 mU/L (the assay detection limit). Therefore, linkage analysis could not be performed for these two recombinant inbred sets. However, linkage analysis was performed for the BXH set in which 6 of 12 strains had detectable TSH levels. TSH in female BXH mice is tentatively linked to a Chr 18 locus close to *Rab18*, a member of the *Ras* oncogene family (Table 1).

Analysis of serum TSH provided other unexpectedly valuable information. In the BXH9 strain, TSH was much higher than in the other 5 BXH strains with detectable TSH levels, and far exceeded TSH levels in the CXB and AXBXA strains in which TSH was also detectable; namely, 80 mU/L in BXH9 versus 38.4 mU/L (mean + 2SD) in the other 10 strains. The BXH9 strain also had the lowest serum T4 for this set (3 µg/dL, Fig. 3A), reflecting subclinical hypothyroidism. Mice of this strain were unresponsive to endogenous TSH and, as we observed, to bovine TSH. Therefore, linkage

TABLE 1. GENETIC LINKAGE FOR BODY WEIGHT AT BASELINE, SERUM THYROXINE AT BASELINE, AND PERCENT THYROXINE SUPPRESSION BY L-TRIiodOTHYRONINE IN AXBXA, BXH, AND CXB STRAINS

Set (no. of strains)	LRS	LOD	Mouse		Human	Marker gene
			Chr	Mb	Chr	
Body weight						
BXH (<i>n</i> =12)	13.065	2.83	12	111.536	14	Dio3
				112.559	14	
AXBXA (<i>n</i> =26)	12.583	2.73	17	68.08354	18	Lama1 Epb4.113
				69.39884	18	
CXB (<i>n</i> =13)	8.507	1.85	10	73.6141	21	
				90.37886	12	
Baseline T4 (no linkage in BXH)						
AXBXA (<i>n</i> =26)	15.346	3.32	2	113.5898	15	Grem1
CXB (<i>n</i> =13)	8.976	1.94	9	105.677	3	Pik3r1
				111.095	3	Lrrfip2
	7.046	1.52	11	72.9250		
				76.4087		
				7.397	1.60	13
			103.899	5	EG621312	
7.547	1.64	19	23.6610	9		
			25.4108	9	Pip5k1b	
Baseline TSH (linkage analysis not possible in AXBXA or CXB)						
BXH (<i>n</i> =12)	20.711	4.49	18	5.04112	10	Rab18
				8.52836	10	

For each trait studied, the following information is provided: recombinant inbred set (number of strains), likelihood ratio statistic (LRS) suggestive of linkage (significant linkages in **bold**), logarithm of the odds (LOD = LRS/4.61) score equivalent, the mouse chromosome (Chr) and megabase (Mb) for linkage, the equivalent human chromosome, and the abbreviation for the closest marker gene, regardless of function, from GeneNetwork (17). CXB11 had unexpectedly high thyroxine (T4) values. GeneNetwork trait numbers for baseline values for body weight, T4, and thyrotropin (TSH): AXBXA 10207, 10213, 10256; BXH 10162, 10168, 10169; CXB 10692, 10698, 10742.

Cd180 (103.48364 Mb), CD180 antigen; *Dio3* (111.517 Mb), deiodinase type 3; *EG621312* (103.94987 Mb), predicted gene, *EG621312*; *Epb4.113* (69.50615 Mb), erythrocyte protein band 4; *Grem1* (33.01 Mb), gremlin 1 (human 15q13.3); *Pik3r1* (102.450717 Mb), phosphatidylinositol 3 kinase regulatory subunit 1; *Lama1* (68.0466 Mb), laminin alpha 1; *Lrrfip2* (111.02064 Mb), leucine-rich repeat (in FLII) interacting protein 2; *Pip5k1b* (24.36929 Mb), phosphatidylinositol-4-phosphate 5-kinase, type 1 beta; *Rab18* (6.7652 Mb), member of *Ras* oncogene family.

TABLE 2. GENETIC LINKAGE FOR PERCENT THYROXINE SUPPRESSION BY L-TRIiodOTHYRONINE AND THYROXINE RELEASE IN AXBXA, BXH, AND CXB RECOMBINANT INBRED STRAINS

Set (no. of strains)	LRS	LOD	Mouse		Human	Marker gene
			Chr	Mb	Chr	
% T4 suppression by L-T3 (no linkage for AXBXA or BXH)						
CXB (<i>n</i> = 13)	12.097	2.62	14	105.7214	13	<i>Pou4f1</i>
				107.173	5	<i>Trim52</i>
	10.395	2.55	8	125.072	16	<i>Trhr2</i>
				125.472	16	
T4 release ^a after TSH						
CXB (<i>n</i> = 13) (<i>n</i> = 12)	20.331	4.41	2	173.92	20	<i>Gnas</i>
	(18.125)			181.54	20	<i>Rgs19</i>
	20.331	4.41	13	102.223	5	<i>Pik3r1</i>
	(18.125)			103.343	5	<i>Cd180</i>
BXH (<i>n</i> = 11 ^b) (<i>n</i> = 10)	16.470	3.57	2	169.029	20	
	(15.360)			169.939	20	<i>Tshz2</i>
AXBXA (<i>n</i> = 25 ^c) (<i>n</i> = 23)	14.284	3.09	4	23.41949	6	<i>Pouf3f2</i>
	(11.848)			27.0832	6	
				29.1904	6	
				30.349	6	<i>Lasc1</i>

For each trait studied, the following information is provided: recombinant inbred set (number of strains), LRS value suggestive of linkage (significant linkages in **bold**), LOD score equivalent (LOD=LRS/4.61), the Chr and Mb location for linkage, the corresponding human chromosome, and the abbreviation for the closest known marker gene, regardless of function, from GeneNetwork. Also included are data for linkage analysis repeated after excluding older strains in the CXB and AXBXA sets, with likelihood ratio statistics values in parentheses. *Italics*: Repeat linkage analysis excluding strains with mice exceeding the 90th percentile for each set; namely, excluding CXB2 (9.9 weeks), BXH11 (9.1 weeks), AXB4, AXB23 (11.3 and 11.1 weeks), as well as BXA16. GeneNetwork trait numbers for % L-T3 suppression and T4 release after TSH: AXBXA, 10209 and 10211; BXH, 10164 and 10166; CXB, 10693 and 10694.

^aT4 release = T4 after TSH – T4 after L-T3.

^bWithout BX9 (hypothyroid; see text).

^cExcluding BXA16 (incomplete L-T3 suppression).

Cd180 (103.483634 Mb), CD180 antigen; *Gnas* (174.1089821 Mb), guanine nucleotide binding protein, alpha stimulating complex locus; *Lasc1*, uncharacterized protein 4931422A14; *Pik3r1* (102.450717 Mb), phosphatidylinositol 3 kinase; *Pou4f1* (104.86154 Mb), Pou domain class 4 transcription factor 1; *Pouf3f2* (22.4139 Mb), Pou domain class 3 transcription factor 2; *Rgs19* (181.423127 Mb), regulator of G-protein signaling 19; *Trhr2* (124.8807 Mb), thyrotropin releasing hormone receptor 2; *Trim52* (106.50541 Mb), tripartite motif-containing 52; *Tshz2* (169.45915 Mb), t-shirt zinc finger family.

analyses of the T4 response to TSH in BXH strains were performed by excluding BXH9 strain data.

Genetic basis for L-T3-induced suppression of T4- and TSH-induced release of T4

In CXB strains, the serum levels of T4 post-L-T3 suppression were linked (although with low LRS values) to loci on Chr 14 and 8 (Table 2) and the percentage suppression of T4 by L-T3 was linked to similar Chr 8 loci (Supplementary Table S3). No linkage was observed for these traits in the AXBXA and BXH sets.

Because our specific interest was in determining the genetic basis for the T4 response to a single injection of bovine TSH in L-T3-suppressed mice, we focused on the linkage analysis of the following traits: (i) T4 levels attained after TSH; (ii) T4 release (T4 after TSH minus T4 post-L-T3 suppression); and (iii) T4 release expressed as % baseline T4. The major findings are as follows:

1. CXB set (13 strains): T4 release was strongly linked (LRS 20.683) to loci on the distal ends of Chr 2 and 13 (Table 2 and Fig. 4). T4 levels after TSH were linked to the same loci as for T4 release, but with lower LRS values (Supplementary Table S3). T4 release expressed as % baseline continued to be linked to Chr 2 (but not Chr 13) and the loci involved were different (Supplementary Table S3).

2. BXH set (11 strains, excluding BXH9): T4 release and T4 after TSH were linked to loci on Chr 2 and 7 and (with a lower LRS value) to loci on the distal terminus of Chr 4 (Table 2 and Supplementary Table S3). The trait T4 release expressed as % baseline was linked to a Chr 1 locus, as well as maintaining linkage (with lower LRS values) to the same Chr 2 and Chr 7 loci (Supplementary Table S2).
3. AXBXA set (25 strains; excluding the strain BXA16, which was not effectively suppressed by L-T3): All three traits, namely, the T4 release expressed as % baseline T4, and T4 after TSH, were linked to similar loci on the proximal end of Chr 4 (Table 2 and Supplementary Table S3).

For all three recombinant inbred sets, the trait giving the highest LRS values was the T4 release. To eliminate the possible effect of increased age, the linkage analyses were repeated for this trait for each recombinant inbred set excluding the strains with ages exceeding the 90th percentile for each set; namely, CXB2 (9.9 weeks), BXH11 (9.1 weeks) and both AXB4 and AXB23 (11.3 and 11.1 weeks, respectively). After excluding these strains, the LRS values were reduced from 20.333 to 18.135 (CXB), 16.470 to 15.360 (BXH), and 14.284 to 11.848 (AXBXA). Importantly, however, linkage was maintained and the Chr and loci identified were unchanged (Table 2, italics).

FIG. 4. Interval mapping on chromosomes 2, 13, 4, and 7 for T4 released in response to bovine TSH in CXB, BXH, and AXBXA mice. Likelihood ratio statistic (LRS) values are on the vertical axis; Mb on the horizontal axis. The broken horizontal lines indicate the mean LRS value above which there is a suggestive linkage for the three recombinant inbred sets (mean 12.2 from CXB 13.3, BXH 12.6, and AXBXA 10.7). GeneNetwork (GN) identifications for CXB (GN10694), BXH (GN 10166); AXBXA (GN10211). Number of strains studied: CXB, 13; BXH, 11; AXBXA, 25.

To summarize, the genetic susceptibility to T4 release was linked to Chr 2 loci in CXB and BXH strains and Chr 4 loci in AXBXA and BXH strains. The Chr 2 loci linkage for T4 release in CXB and BXH strains, although to the same distal terminus, were discrete and nonoverlapping (Fig. 4), separated by 3.437 Mb. As already indicated, the Chr 4 linkage was to loci on the proximal end in the AXBXA set and to the distal terminus in the BXH set. Overall, distinct linkage patterns were observed for these traits in the three recombinant inbred sets with no chromosomal overlap.

Genetic susceptibility for TSH-induced T4 release compared with genetic linkage for elevated T4 induced by A-subunit adenovirus immunization

Previously, we investigated the genetic basis for hyperthyroidism in recombinant inbred mice immunized with TSHR A-subunit adenovirus to induce hyperthyroidism (3,5,6). There were no similarities, or overlap, between the loci linked to induced hyperthyroidism (Table 3) and those we observed in the present study to be linked to the response to TSH in L-T3-suppressed mice of the same three recombinant inbred sets (Table 2 and Supplementary Table S3).

Discussion

We investigated the genetic basis for the magnitude of the T4 response to a single dose of bovine TSH in three sets of L-T3-suppressed recombinant inbred mice, CXB, BXH, and AXBXA. All three sets share B6 as one parental strain. Consistent with some previous findings, baseline serum T4 values (22,23) and the response to TSH stimulation (11) were lower in parental B6 and A than in BALB and C3H mouse strains (22,23). Unexpectedly, T4 release was high in all three sets of F1, including the F1 of the low responder A and B6 parents, suggesting hybrid vigor.

An important issue is why we studied female rather than male mice. First, Graves' disease predominantly affects women rather than men. Consequently, our studies of the genetic basis for induced murine hyperthyroidism were performed in female mice (3,5,6). Second, it is advantageous to investigate mice of the same sex when comparing the genetic basis for the T4 response to TSH relative to our previous studies on induced autoantibody-mediated hyperthyroidism. Indeed, a recent study of severe murine influenza identified sex-specific candidate genes in recombinant inbred mice (23), confirming the importance of using mice of the same sex for genetic studies.

In recombinant inbred lines, we measured the mouse weight, serum T4, and TSH at baseline. In our earlier study, body weight played a critical role in the efficacy of T4 suppression using L-T3-supplemented drinking water (11), rather than L-T3 injected intraperitoneally as in the present study. In other investigations, body weight had been investigated, as one parameter, in the CXB, BXH, or AXBXA sets (20,24–28). In AXBXA strains (but not CXB or BXH sets), body weight was linked to the same Chr 17 locus identified in a previous study (20). It is important to emphasize that, although some marker genes are provided, no candidate genes for body weight were suggested by the present or earlier investigations.

The major CXB loci for baseline T4 in the current study differed from those we reported earlier, which were located on Chr 1, 2, 11, and 13 (21) although linkage was suggestive for a previously identified Chr 13 locus (21). No linkage for baseline T4 was observed for the 12 BXH strains in the present study. In the AXBXA set, baseline T4 was linked to a Chr 2 locus slightly upstream of the locus highlighted previously (21). Few previous studies examined the genetic basis for T4 levels alone rather than in relation to another trait, such as audiogenic seizures (29) or as contributing to the life span (for

TABLE 3. GENETIC LINKAGE FOR HYPERTHYROIDISM INDUCED BY IMMUNIZING RECOMBINANT INBRED SETS CXB, BXH, AND AXBXA WITH THYROTROPIN HOLORECEPTOR A-SUBUNIT ADENOVIRUS

Set	Trait	Chr	LRS	LOD	Mb (range)	Human Chr
CXB	T4 (2×)	19	12.41	2.70	43.736	[10]
	Delta T4 (3×)	10	11.18	2.56	113.886	12q15–q21
BXH	T4 (2×)	16	18.93	4.11	5.567–12.366	16p13
		2	18.23	3.96	152.351–152.723	20
	T4 (3×)	8	17.48	3.80	116.599–118.038	
		3	12.81	2.78	26.081–30.098	3
		13	12.33	2.68	112.892–114.378	5q11.2
	Delta T4 (2×)	9	15.23	3.31	24.367–25.639	[7]
	Delta T4 (3×)	13	16.83	3.65	112.892–114.378	5q11.2
		13	15.01	3.26	43.836–45.370	6
		3	14.00	3.04	31.138–31.272	[3]
AXBXA	T4 (3×)	16	14.52	3.15	60.329–64.435	3

Traits examined include serum T4 after two or three immunizations (2× or 3×) as well as the difference between T4 after immunization and baseline T4 ("delta T4"). Suggestive LRS values are provided, together with the LOD values ($LOD = LRS/4.61$), the relevant Chr, location in Mb, and the corresponding [or likely] human Chr. *References:* (3,5,6).

example (30). In UM-HET3 mice, derived by crossing the F1 of (BALBc×B6) with the F1 of (C3H/He×DBA/2), loci that affected T4 levels were located on Chr 4, 15, and 17 (30). None of these Chr or loci were similar to those we observed for baseline T4 summarized on page 7 or for the response to injected TSH (to be discussed below).

Our observations for serum TSH are limited because, as explained above (Introduction) we studied female mice in which TSH is usually undetectable [for example, Ref. (15)]. Previous studies in male rats demonstrated linkage between TSH and two chromosomal intervals; one included the genes for the TSH receptor and type 2 deiodinase and the other included the type 1 deiodinase gene (32). In a subsequent study using consomic rats, the higher TSH levels were associated with reduced expression of the TSHR, reflecting an adjustment of the feedback loop to reduced TSH sensitivity (33). In female BXH mice, the only recombinant inbred set for which analysis was possible, serum TSH was tentatively linked to a Chr 18 locus with no obvious candidate genes.

Suppression of serum T4 by i.p. injection of L-T3 was effective in all except one recombinant inbred strain (BXA16). Not surprisingly, therefore, no linkage was observed for serum T4 after L-T3 administration, or for the percentage of T4 suppression, in AXBXA or BXH strains. However, in the CXB set, the same traits were linked, with low LRS values, to loci on Chr 14 and 8. T4 suppression by L-T3 is likely associated with the hypothalamus/pituitary function. Accordingly, in the CXB set, T4 suppression by L-T3 was suggestively linked, although with a low LRS value to a Chr 8 locus near the gene for the TSH releasing hormone receptor 2 (*Trh2*).

Despite effective L-T3 suppression of serum T4 levels by >85% in virtually all CXB, BXH, AXB/AXA mice, several strains in each of the three recombinant inbred sets did not release T4 in response to bovine TSH. Of interest, in animals not subject to L-T3 suppression of endogenous TSH, recombinant human TSH readily induced T4 release from CRL:CF-1BR male mice, but not in male Sprague-Dawley rats (34).

We analyzed three traits for the response (in L-T3-suppressed mice) to TSH, namely: serum T4 after TSH, T4 release (the difference between T4 after TSH and T4 post-L-T3 suppression), and T4 release expressed as a percentage of

basal T4 levels. Of these three traits, the highest LRS values were obtained for T4 release. Moreover, the strongest linkage for T4 release was in the CXB and BXH sets (13 and 12 strains studied), not in the larger AXBXA set (25 strains studied). This finding reflects the divergence of responses to TSH in the parental strains, BALB or C3H versus B6 compared with similar responses in parental B6 and A strains. The observation also suggests that the divergent parental strain responses may sometimes have more impact than the statistical power provided by the increased size of a recombinant inbred set.

In CXB strains, T4 release was strongly linked (LRS 20.683) to loci on the distal ends of Chr 2 and 13. In the BXH set, the same trait was linked to loci on Chr 2 and 7 and (with a lower LRS value) to loci on the distal terminus of Chr 4. Although T4 release was linked to the distal end of Chr 2 in both CXB and BXH sets, the loci were discrete with no overlap. In AXBXA strains, T4 release was linked to the proximal region of Chr 4, different from the BXH Chr 4 loci at the proximal end. As already emphasized, given the potential effects of the estrous cycle and sex differences in some parameters of the thyroid axis, our results may not be applicable to males. Overall, as we previously observed for baseline T4 levels (21), the linkages for T4 release were distinct in the three sets of recombinant inbred mice. Although T4 release is conceptually simple, several parameters are involved, including baseline T4 levels and the ability to respond to TSH. The linkage variability in different sets of recombinant inbred mice parallels the findings for ethnic variations in humans.

There was no relationship between strains showing the greatest release of T4 induced by TSH (present study) and the increase in T4 after two or three immunizations with A-subunit adenovirus to induced Graves' disease (3,5,6). Some discrepancies are readily explained. For example: TSH-induced T4 release was similar in BXA12 versus BXA13 (Fig. 3B, indicated by brackets). In contrast, after immunization, BXA12 mice were euthyroid, whereas BXA13 mice developed extreme hyperthyroidism (3). This difference arises because, unlike BXA13 strains, BXA12 mice were unable to generate TSHR antibodies in response to immunization. In addition, it should be emphasized that the present study involves the acute release of T4 in response to TSH. In contrast, the

immunization protocol takes place over a period of 6 weeks and hyperthyroidism develops one to 2.5 months after the initial immunization.

A major issue is whether there is any relationship between the loci or genes we observed and those linked to the thyroid function in humans. The T4 response to TSH is a parameter associated with the thyroid gland function and the genes highlighted by our investigation are in line with those controlling the thyroid function in humans. A number of genetic loci play a role in the levels of TSH, thyroid hormones and thyroid volume and goiter in humans (35–40) (summarized in Table 4). To our knowledge, none of the murine genes we observed were the same as in humans. However, two linkages in mice are of interest. It must be emphasized that confirma-

tion of a role for any of these putative genes/loci will require further investigation. First, one of the two strongest linkages for T4 released after TSH in CXB mice is to regions on Chr 13 between the genes *PDE8B* and *PDE4D* (encoding phosphodiesterase 8B and 4D, respectively; Table 4). Accumulating evidence points to *PDE8B* as a gene responsible for TSH levels in humans (35,39). Because the LRS peaks from our study do not correspond to the locations of *PDE8B* and *PDE4D*, it is possible that other, as yet, unidentified genes in this region may play a role. Second, the other strong linkage for T4 released after TSH in CXB mice is to a Chr 2 region close to the *GNAS1* gene (guanine nucleotide-binding protein, alpha-subunit), also shown to play a role in controlling TSH levels in humans (35).

TABLE 4. GENES LINKED TO THYROID FUNCTION IN HUMANS TOGETHER WITH THE CORRESPONDING MOUSE CHROMOSOMES AND GENE LOCATIONS

Trait	Gene ID	Chr		Mouse		Gene name	Ref.
		Human	Mouse	Mb			
TSH	<i>IGFBP5</i>	2q35	1	72.900	Insulin-like growth factor-binding protein 5		(38)
TSH	<i>GNAS1</i>	20q13.32	2	174.284	Guanine nucleotide-binding protein, alpha-subunit		(35)
‡			2	173.92	<i>CXB strains T4 levels after TSH</i>		Table S3
Thyroid volume, goiter	<i>FGF7</i>	15q21	2	126.034	Fibroblast growth factor 7		(38)
F-T4	<i>LHX3</i>	9q34	2	26.060	LIM homeobox protein 3		(38)
TSH	<i>ABO</i>	9q34	2	26.690	ABO blood group		(38)
TSH, F-T3, F-T4	<i>DIO1</i>	1p33–p32	4	106.250	Deiodinase type 1		(38,39)
TSH, thyroid volume, goiter	<i>CAPZB</i>	1p36	4	138.740	Beta subunit of the barbed-end F-actin binding protein		(37–40)
F-T4	<i>FOXE1</i>	9q22	4	46.350	Forkhead box E1		(38)
TSH	<i>GNAI1</i>	7q21	5	18.261	Guanine nucleotide-binding protein, alpha inhibiting polypeptide 1		(35)
TSH	<i>GNAO1</i>	16q13	8	93.800	Guanine nucleotide-binding protein, activating polypeptide O		(35)
TSH	<i>INSR</i>	19p13.2	8	3.150	Insulin receptor		(38)
F-T4	<i>AADAT</i>	4q33	8	63.024	Aminoacidate aminotransferase		(38)
TSH	<i>NR3C2</i>	4q31.2	8	79.426	Nuclear receptor subfamily 3, group C, member 2		(38)
TSH	<i>PDE7B</i>	6q23–q24	10	20.398	Phosphodiesterase 7B		(35)
TSH	<i>TSHR</i>	14q31	12	91.401	Thyroid stimulating hormone receptor		(35)
TSH	<i>THRB</i>	3p24.2	14	18.490	Thyroid hormone receptor beta		(35)
TSH	<i>PDE8B</i>	5q13.3	13	95.794	Phosphodiesterase 8B		(37–40)
‡			13	102.223	<i>CXB strains T4 release after TSH</i>		Table 2
‡			13	103.343	<i>CXB strains T4 release after TSH</i>		Table 2
TSH	<i>PDE4D</i>	5q12	13	109.775	Phosphodiesterase 4D		(35)
TSH	<i>TG</i>	8q24	15	66.671	Thyroglobulin		(35)
TSH	<i>POU1F1</i>	3p11	16	65.206	POU domain, class 1, transcription factor 1		(35)
TSH	<i>MAF</i>	16q23	16	118.220	Avian musculoaponeurotic fibrosarcoma (v-maf)		(38,40)
TSH	<i>PDE10A</i>	6q26	17	8.990	Phosphodiesterase		(35,38)
TSH	<i>VEGFA</i>	6p12	17	46.150	Vascular endothelial growth factor A		(38)
TSH	<i>GNAQ</i>	9q21	19	16.207	Guanine nucleotide-binding protein, alpha q polypeptide;		(35)

Bold indicates linkages highlighted for (39), a review. *References:* (35–40).

‡Included in *italics* are the gene locations for the strongest linkage data for T4 levels or release in response to thyrotropin in CXB strains (mouse Chr 2 and 13; LRS values 20.331).

It should be appreciated that in humans, multiple loci may contribute to the same trait, as illustrated for TSH in Table 4. Although some loci have been replicated in additional cohorts [for example, Refs. (35,40)], the same trait may be controlled by different loci in different ethnic groups: for example, the variable role of the *THRB* gene and TSH levels [reviewed in Ref. (30)] and the well-known ethnic differences in human leucocyte antigen (HLA) associations in autoimmune thyroid disease [reviewed by Jacobson *et al.* (41)]. These ethnic variations in human genetic susceptibilities parallel the different genetic linkages in recombinant inbred mice, derived from B6 parents bred to C3H/He, BALB/c, or A strains, which we previously observed for baseline T4 levels (21), induced hyperthyroidism (3,5,6), and, as we report here, for TSH-induced T4 responses.

In conclusion, T4 release in response to TSH was more strongly linked in CXB and BXH sets, derived from parental strains with widely divergent T4 release values, than in AXBXA strains, which were generated from parents with similar TSH-induced responses. The genetic susceptibility for thyroid sensitivity, measured as T4 released in response to bovine TSH, was distinct for three sets of recombinant inbred mice, at least in females, despite the fact that they share the C57BL/6 parental strain. There was no relationship between Chr or loci linked to induced hyperthyroidism (which develops over months) versus those linked to the acute TSH-induced T4 response. In mice, as in humans, multiple loci contribute to the same trait and different loci are observed in different ethnic groups for the same trait (38). In addition, evidence supports the concept that "human disease genes can be identified through cross-species gene mapping of evolutionary conserved processes" (42). Consequently, our findings for loci that overlap with, or are located upstream or downstream of putative human TSH genes, suggest that novel loci responsible for controlling thyroid function in humans may yet be revealed.

Acknowledgments

This work was supported by the National Institutes of Health Grants DK54684 (S.M.M.), DK19289 (B.R.), U01AA13499, U24AA13513, P20-DA-21131, U01CA105417, and U24RR021760 (R.W.W.). We are also grateful for support by Boris Catz.

Disclosure Statement

No competing financial interests exist.

References

- Nagayama Y, Kita-Furuyama M, Ando T, Nakao K, Mizuguchi H, Hayakawa T, Eguchi K, Niwa M 2002 A novel murine model of Graves' hyperthyroidism with intramuscular injection of adenovirus expressing the thyrotropin receptor. *J Immunol* **168**:2789–2794.
- Chen CR, Pichurin P, Nagayama Y, Latrofa F, Rapoport B, McLachlan SM 2003 The thyrotropin receptor autoantigen in Graves' disease is the culprit as well as the victim. *J Clin Invest* **111**:1897–1904.
- McLachlan SM, Aliesky H, Chen CR, Williams RW, Rapoport B 2011 Exceptional hyperthyroidism and a role for both major histocompatibility class I and class II genes in a murine model of Graves' disease. *PLoS One* **6**:e21378.
- Chen CR, Aliesky H, Pichurin PN, Nagayama Y, McLachlan SM, Rapoport B 2004 Susceptibility rather than resistance to hyperthyroidism is dominant in a thyrotropin receptor adenovirus-induced animal model of Graves' disease as revealed by BALB/c-C57BL/6 hybrid mice. *Endocrinology* **145**:4927–4933.
- Aliesky HA, Pichurin PN, Chen CR, Williams RW, Rapoport B, McLachlan SM 2006 Probing the genetic basis for thyrotropin receptor antibodies and hyperthyroidism in immunized CXB recombinant inbred mice. *Endocrinology* **147**:2789–2800.
- McLachlan SM, Aliesky HA, Pichurin PN, Chen CR, Williams RW, Rapoport B 2008 Shared and unique susceptibility genes in a mouse model of Graves' disease determined in BXH and CXB recombinant inbred mice. *Endocrinology* **149**:2001–2009.
- Rapoport B, Williams RW, Chen CR, McLachlan SM 2010 Immunoglobulin heavy chain variable region genes contribute to the induction of thyroid stimulating antibodies in recombinant inbred mice. *Genes Immun* **3**:254–263.
- Bailey DW 1971 Recombinant-inbred strains. An aid to finding identity, linkage, and function of histocompatibility and other genes. *Transplantation* **11**:325–327.
- Airey DC, Lu L, Williams RW 2001 Genetic control of the mouse cerebellum: identification of quantitative trait loci modulating size and architecture. *J Neurosci* **21**:5099–5109.
- Mountz JD, Yang P, Wu Q, Zhou J, Tousson A, Fitzgerald A, Allen J, Wang X, Cartner S, Grizzle WE, Yi N, Lu L, Williams RW, Hsu HC 2005 Genetic segregation of spontaneous erosive arthritis and generalized autoimmune disease in the BXD2 recombinant inbred strain of mice. *Scand J Immunol* **61**:128–138.
- Hamidi S, Aliesky H, Chen CR, Rapoport B, McLachlan SM 2010 Variable suppression of serum thyroxine in female mice of different inbred strains by triiodothyronine administered in drinking water. *Thyroid* **20**:1157–1162.
- Maia AL, Kieffer JD, Harney JW, Larsen PR 1995 Effect of 3,5,3'-Triiodothyronine (T3) administration on *dio1* gene expression and T3 metabolism in normal and type 1 deiodinase-deficient mice. *Endocrinology* **136**:4842–4849.
- Steward JP, Ornellas EP, Beernink KD, Northway WH 1968 Errors in the technique of intraperitoneal injection of mice. *Appl Microbiol* **16**:1418–1419.
- Gaines DR, North D 2007 Implications of experimental technique for analysis and interpretation of data from animal experiments: outliers and increased variability resulting from failure of intraperitoneal injection procedures. *Lab Anim* **41**:312–320.
- Pohlenz J, Maqueem A, Cua K, Weiss RE, Van Sande J, Refetoff S 1999 Improved radioimmunoassay for measurement of mouse thyrotropin in serum: strain differences in thyrotropin concentration and thyrotroph sensitivity to thyroid hormone. *Thyroid* **9**:1265–1271.
- Williams RW, Gu L, Qi S, Lu L 2001 The genetic structure of recombinant inbred mice: high-resolution consensus maps for complex trait analysis. *Genome Biol* **2**:Research0046.1–0046.18.
- GeneNetwork; WWW service initiated January 1994 as The Portable Dictionary of the Mouse Genome and June 15, 2001 as WebQTL. Available at www.genenetwork.org Accessed 2011 and 2012.

18. Churchill GA, Doerge RW 1994 Empirical threshold values for quantitative trait mapping. *Genetics* **138**:963–971.
19. Shifman S, Bell JT, Copley RR, Taylor MS, Williams RW, Mott R, Flint J 2006 A high-resolution single nucleotide polymorphism genetic map of the mouse genome. *PLoS Biol* **4**:e395.
20. Zhou G, Williams RW 1999 Eye1 and Eye2: gene loci that modulate eye size, lens weight, and retinal area in the mouse. *Invest Ophthalmol Vis Sci* **40**:817–825.
21. McLachlan SM, Lu L, Aliesky HA, Williams RW, Rapoport B 2011 Distinct genetic signatures for variability in total and free serum thyroxine levels in four sets of recombinant inbred mice. *Endocrinology* **152**:1172–1179.
22. Berry MJ, Grieco D, Taylor BA, Maia AL, Kieffer JD, Beamer W, Glover E, Poland A, Larsen PR 1993 Physiological and genetic analyses of inbred mouse strains with a type I iodothyronine 5' deiodinase deficiency. *J Clin Invest* **92**:1517–1528.
23. Boivin GA, Pothlichet J, Skamene E, Brown EG, Loredó-Osti JC, Sladek R, Vidal SM 2012 Mapping of clinical and expression quantitative trait loci in a sex-dependent effect of host susceptibility to mouse-adapted influenza H3N2/HK/1/68. *J Immunol* **188**:3949–3960.
24. Blizard DA, Bailey DW 1979 Genetic correlation between open-field activity and defecation: analysis with the CXB recombinant-inbred strains. *Behav Genet* **9**:349–357.
25. Shukri NM, Shire JG 1989 Genetic variation in testicular development in mice of the C57BL/10ScSn, C57BL/6By and BALB/cBy strains and the CXB recombinant-inbred lines. *J Reprod Fertil* **87**:587–592.
26. Tankersley CG, Fitzgerald RS, Levitt RC, Mitzner WA, Ewart SL, Kleeberger SR 1997 Genetic control of differential baseline breathing pattern. *J Appl Physiol* **82**:874–881.
27. Turner CH, Hsieh YF, Muller R, Bouxsein ML, Rosen CJ, McCrann ME, Donahue LR, Beamer WG 2001 Variation in bone biomechanical properties, microstructure, and density in BXH recombinant inbred mice. *J Bone Miner Res* **16**:206–213.
28. Leamy LJ, Pomp D, Lightfoot JT 2009 Genetic variation in the pleiotropic association between physical activity and body weight in mice. *Genet Sel Evol* **41**:41.
29. Seyfried TN, Glaser GH, Yu RK 1984 Genetic analysis of serum thyroxine content and audiogenic seizures in recombinant inbred and congenic strains of mice. *Exp Neurol* **83**:423–428.
30. Harper JM, Galecki AT, Burke DT, Miller RA 2004 Body weight, hormones and T cell subsets as predictors of life span in genetically heterogeneous mice. *Mech Ageing Dev* **125**:381–390.
31. Harper JM, Galecki AT, Burke DT, Pinkosky SL, Miller RA 2003 Quantitative trait loci for insulin-like growth factor I, leptin, thyroxine, and corticosterone in genetically heterogeneous mice. *Physiol Genomics* **15**:44–51.
32. Baum AE, Solberg LC, Kopp P, Ahmadiyeh N, Churchill G, Takahashi JS, Jameson JL, Redei EE 2005 Quantitative trait loci associated with elevated thyroid-stimulating hormone in the Wistar-Kyoto rat. *Endocrinology* **146**:870–878.
33. Moeller LC, Alonso M, Liao X, Broach V, Dumitrescu A, Van Sande J, Montanelli L, Skjei S, Goodwin C, Grasberger H, Refetoff S, Weiss RE 2007 Pituitary-thyroid setpoint and thyrotropin receptor expression in consomic rats. *Endocrinology* **148**:4727–4733.
34. Colzani RM, Alex S, Fang SL, Braverman LE, Emerson CH 1998 The effect of recombinant human thyrotropin (rhTSH) on thyroid function in mice and rats. *Thyroid* **8**:797–801.
35. Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, Sestu N, Maschio A, Busonero F, Albai G, Dei M, Lai S, Mulas A, Crisponi L, Tanaka T, Bandinelli S, Guralnik JM, Loi A, Balaci L, Sole G, Prinzis A, Mariotti S, Shuldiner AR, Cao A, Schlessinger D, Uda M, Abecasis GR, Nagaraja R, Sanna S, Naitza S 2008 Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. *Am J Hum Genet* **82**:1270–1280.
36. Panicker V, Wilson SG, Walsh JP, Richards JB, Brown SJ, Beilby JP, Bremner AP, Surdulescu GL, Qweitin E, Gillham-Naseny I, Soranzo N, Lim EM, Fletcher SJ, Spector TD 2010 A locus on chromosome 1p36 is associated with thyrotropin and thyroid function as identified by genome-wide association study. *Am J Hum Genet* **87**:430–435.
37. Teumer A, Rawal R, Homuth G, Ernst F, Heier M, Evert M, Dombrowski F, Volker U, Nauck M, Radke D, Ittermann T, Biffar R, Doring A, Gieger C, Klopp N, Wichmann HE, Wallaschofski H, Meisinger C, Volzke H 2011 Genome-wide association study identifies four genetic loci associated with thyroid volume and goiter risk. *Am J Hum Genet* **88**:664–673.
38. Medici M, van der Deure WM, Verbiest M, Vermeulen SH, Hansen PS, Kiemeny LA, Hermus AR, Breteler MM, Hofman A, Hegedus L, Kyvik KO, den Heijer M, Uitterlinden AG, Visser TJ, Peeters RP 2011 A large-scale association analysis of 68 thyroid hormone pathway genes with serum TSH and FT4 levels. *Eur J Endocrinol* **164**:781–788.
39. Panicker V 2011 Genetics of thyroid function and disease. *Clin Biochem Rev* **32**:165–175.
40. Tamai H, Hirota Y, Kasagi K, Matsubayashi S, Kuma K, Iida Y, Konishi J, Okimura MC, Walter RM, Kumagai LF 1987 The mechanism of spontaneous hypothyroidism in patients with Graves' disease after antithyroid drug treatment. *J Clin Endocrinol Metab* **64**:718–722.
41. Jacobson EM, Huber A, Tomer Y 2008 The HLA gene complex in thyroid autoimmunity: from epidemiology to etiology. *J Autoimmun* **30**:58–62.
42. Poot M, Badea A, Williams RW, Kas MJ 2011 Identifying human disease genes through cross-species gene mapping of evolutionary conserved processes. *PLoS One* **6**:e18612.

Address correspondence to:

Sandra M. McLachlan, PhD
Thyroid Autoimmune Disease Unit
Cedars-Sinai Medical Center
8700 Beverly Blvd, Suite B-131
Los Angeles, CA 90048

E-mail: mclachlans@cshs.org