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Identifying Quantitative Trait Loci Affecting Resistance to Congenital Hypothyroidism in 129^{+Ter}/SvJcl Strain Mice

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Abstract

Tyrosylprotein sulfotransferase 2 (TPST2) is one of the enzymes responsible for tyrosine O-sulfation and catalyzes the sulfation of the specific tyrosine residue of thyroid stimulating hormone receptor (TSHR). Since this modification is indispensable for the activation of TSH signaling, a non-functional TPST2 mutation (Tpst2grt) in DW/J-grt mice leads to congenital hypothyroidism (CH) characterized by severe thyroid hypoplasia and dwarfism related to TSH hyporesponsiveness. Previous studies indicated that the genetic background of the 129^{+Ter}/SvJcl (129) mouse strain ameliorates Tpst2grt-induced CH. To identify loci responsible for CH resistance in 129 mice, we performed quantitative trait locus (QTL) analysis using backcross progenies from susceptible DW/J and resistant 129 mice. We used the first principal component calculated from body weights at 5, 8 and 10 weeks as an indicator of CH, and QTL analysis mapped a major QTL showing a highly significant linkage to the distal portion of chromosome (Chr) 2; between D2Mit62 and D2Mit304, particularly close to D2Mit255. In addition, two male-specific QTLs showing statistically suggestive linkage were also detected on Chrs 4 and 18, respectively. All QTL alleles derived from the 129 strain increased resistance to growth retardation. There was also a positive correlation between recovery from thyroid hypoplasia and the presence of the 129 allele at D2Mit255. Further studies suggested that the major QTL on Chr 2 is involved in thyroid development. Moreover, since DW/J congenic strain mice carrying both a Tpst2grt mutation and 129 alleles in the major QTL show resistance to dwarfism and thyroid hypoplasia, we confirmed the presence of the resistant gene in this region, and that it is involved in thyroid development. Further genetic analysis should lead to identification of genes for CH tolerance and, from a better understanding of thyroid organogenesis and function, the subsequent development of new treatments for thyroid disorders.

Introduction

Congenital hypothyroidism (CH) is one of the most frequent endocrinological disorders. It is caused by insufficient production of thyroid hormone by the thyroid gland, and about 1:3000–4000 newborns are affected by CH. If untreated, they suffer from irreversible growth delay and mental retardation. In most cases (80–90%), CH is caused by thyroid dysgenesis (TD) characterized by abnormal thyroid gland development, such as ectopic thyroid gland, athyreosis and thyroid hypoplasia [1,2]. Both environmental and genetic factors affect the development of TD [3–5]. Molecular genetic analyses have identified some TD susceptible genes in humans; thyroid stimulating hormone receptor (TSHR), thyroid transcription factor 1 (TTF1), TTF2, paired box gene 8 (PAX8), AKX2.5 and HHEX [1,6,7]. Studies of the spontaneous mutation and targeted-disruption of the above genes in mice have also provided insights into the molecular mechanisms of thyroid organogenesis and thereby formed the basis for molecular genetic studies in human patients affected by TD [1,8]. Numerous studies have used a candidate gene approach to identify genes involved in common forms of TD. However, most forms of TD appear sporadically, and no significant linkage between the TD phenotype and haplotypes surrounding TSHR, TTF1, TTF2 and PAX8 locus have been reported [9]. Moreover, the incomplete penetrance and the variable expression observed in familial cases of hypothyroidism demonstrate that TD-associated CH is a genetically heterogeneous disease [9,10]. Thus, there is little information available on the genetic factors involved in thyroid disease.

DW/J-grt is a mouse model for TD-associated CH that is characterized by autosomal recessive growth retardation, significantly lowered T3 and T4 levels, and severe thyroid hypoplasia related to TSH hyporesponsiveness [11]. Previously, we identified a point mutation (Tpst2grt) in the tyrosylprotein sulfotransferase 2 (Tpst2) gene causing a decrease in enzymatic activity [12]. TPST2 is one of the enzymes responsible for tyrosine O-sulfation and catalyzes the sulfation of tyrosine 385 of TSHR [13]. Since this modification is indispensable for the activation of TSH signaling [12–14] and, furthermore, since signal transduction via TSHR is prerequisite for the development and function of thyroid gland [15,16], DW/J-grt mice develop CH [12]. Previously, to examine the effects of genetic background on hypothyroidism, we produced congenic strains carrying this Tpst2grt mutation on the genetic...
background of standard strains, C57BL6/J (B6) and 129<sup>Ts</sup>/SvJcl (129), and analyzed growth rate and thyroid function. The B6 congenic mice show a severe hypothyroid phenotype similar to the DW/J (DW) strain. In contrast, and interestingly, the 129 congenic mice exhibit normal growth and thyroid function. This result suggests that 129 strain have resistant gene(s) against CH [17]. In order to identify the resistant gene(s) against <i>Tpst2<sup>2<sup>ov</sup></sup></i> induced CH, we carried out quantitative trait locus (QTL) analysis using backcross progeny from susceptible DW and resistant 129 mice. Because the tolerance to growth retardation in 129 strain is caused by the normal thyroid development and function in spite of TSH hyporesponsiveness [17], body weight is expected to reflect the severity of CH. We used the first principal component of body weights at 5, 8 and 10 weeks age, calculated by principal component analysis, as an indicator of CH. QTL analysis mapped a major QTL showing a highly significant linkage to the distal portion of chromosome (Chr) 2; between <i>D2Mit62</i> and <i>D2Mit504</i>, particularly close to <i>D2Mit255</i>. In addition, a further two QTLs showing suggestive statistical linkages were detected on Chr 4 and 18, respectively, in the male progenies. High percentages of the variance (63–69%) at <i>D2Mit255</i> also supported the validity of the QTL on Chr 2 as a powerful modifier for growth delay by <i>Tpst2<sup>ov</sup></i>. Statistical significant correlations between body weight and thyroid index for both sexes demonstrated that the growth retardation in backcross progeny is related to thyroid hypoplasia. Also, a significant difference in male thyroid index between genotypes at <i>D2Mit255</i> suggested the major tolerant gene on Chr 2 is involved in thyroid development. Finally, we generated DW congenic strain mice carrying both a <i>Tpst2<sup>ov</sup></i> mutation and 129 alleles in the major QTL, and confirmed the recovery from both growth retardation and thyroid hypoplasia.

**Materials and Methods**

**Ethical Statement**

All research and experimental protocols were approved by the Regulation for the Care and Use of Laboratory Animals, Hokkaido University (approval ID: No. 110226), and performed under the guidance of the Institute for Laboratory Animal Research (ILAR). All animals were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC) International.

**Animals**

129 mice were purchased from CLEA Japan (129<sup>Ts</sup>/SvJcl; Tokyo, Japan). Backcross progeny were obtained by mating DW female mice heterozygous for <i>Tpst2<sup>ov</sup></i> to DW129-F1 hybrid male mice heterozygous for <i>Tpst2<sup>ov</sup></i>. Backcross mice homozygous for <i>Tpst2<sup>ov</sup></i> (<i>BC-Tpst2<sup>ov</sup></i>) were born in accordance with Mendelian inheritance and the equal sex ratio. Backcross progeny with homozygotic wild-type alleles for <i>Tpst2</i> gene (BC-WT) were used as littermate controls. Genotyping of the <i>Tpst2<sup>ov</sup></i> allele was performed as previously described [12]. In order to confirm the effect of the main QTL on Chr 2 between <i>D2Mit62</i> and <i>D2Mit504</i> (the 129-derived locus for resistance to CH on Chr 2; <i>Lch</i>, congenic strain mice in which the <i>Lch</i> allele is introduced into the susceptible DW strain was generated by a marker-assisted speed congenic strategy [18]. In brief, among the male mice harboring the 129 alleles between <i>D2Mit62</i> and <i>D2Mit504</i>, the male mice with the highest percentage of the DW genetic background, evaluated using 72 markers (Table S1), were selected for breeding the next generation. Backcrossing was repeated six times and finally, congenic mice possessing heterozygous 129 alleles between <i>D2Mit62</i> and <i>D2Mit504</i> (DW.129-[D2Mit62-D2Mit504]; CG) were produced. Female CG mice heterozygous for both <i>Tpst2<sup>ov</sup></i> and <i>Lch</i> alleles were then crossed with DW male mice heterozygous for <i>Tpst2<sup>ov</sup></i> and their progenies were used for congenic strain analysis. The animal room was air-conditioned at 22±0.4°C, maintained at 40–60% relative humidity, and mice were maintained under a 12 hr light-dark cycle. A standard laboratory diet, CE-2 (Nihon Clea, Tokyo, Japan), and tap water were available ad libitum.

**Genotyping Analysis**

We used 49 (male = 26, female = 23) BC-<i>Tpst2<sup>ov</sup></i> mice for a genome-wide scan. Extraction of genomic DNA from tail clips was performed by a standard method. For QTL analysis, a total of 74–75 informative microsatellite markers spanning 19 autosomes were used for the genotyping analysis, as listed in Table S1. The map positions of the microsatellite markers were based on information from the Mouse Genome Informatics of Jackson Laboratory (MGI; http://www.informatics.jax.org/, MGI 4.41). PCR was carried out with a cycling sequence of 95°C for 3 min (one cycle), followed by 35 cycles consisting of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec, and extension at 72°C for 30 sec. Amplified samples were electrophoresed with 10% polyacrylamide gels and stained with ethidium bromide. The stained gels were then visualized and photographed under an ultraviolet lamp.

**QTL Analysis**

For the evaluation of resistance to TD-associated CH induced by <i>Tpst2<sup>ov</sup></i>, we measured body weights at 5, 8 and 10 weeks of age and conducted principal component analysis. The first principal component, which provides the comprehensive value of body weights at each time-point and reflects a synthetic view of growth retardation, was used as quantitative trait for CH. Principal component analysis was performed with free software program for multivariate analysis (mulvar95; http://www.vector.co.jp/sold/win95/edu/edu203904.html). QTL analysis was performed with the Map Manager QTXb20, software program that uses a maximum likelihood algorithm with “interval mapping” and “simultaneous search”, and permits better localization of loci and exclusion mapping [19]. Recombination frequencies (%) were converted into genetic distance (cM) using the Kosambi map function. This program provides linkage data as a likelihood ratio statistic (LRS) score. The LRS values were calculated by 10000 random permutations of the trait values relative to genotypes of the marker loci. Genome-wide significance thresholds were set at the 37th (“suggestive”), 95th (“significant”), and 99.9th (“highly significant”) percentiles, which correspond to genome-wide <i>p</i> values of 0.63, 0.05 and 0.001 times, respectively, based on 10000 random permutation [20]. Since there are significant sex differences in weekly body weight, the principal component analysis and QTL analysis were performed separately for each sex.

**Histological Analysis of Thyroid Glands**

Thyroid glands from 10-week-old mice were fixed with 5% neutral-buffered formalin and embedded in paraffin. Serial sections of thyroids were cut at 3 µm and stained with hematoxylin–eosin. Thyroid indices were calculated as previously reported [17]. In brief, the thyroid index indicates the average ratio of total colloid areas per thyroid cross-section area.

**Statistics**

Statistical analyses were performed using the Stat-View program (SAS Institute Inc. Cary, USA). T-tests were used to
compare the differences in body weight and thyroid index between each genotype. The correlation between body weight and thyroid index was analyzed using Pearson’s correlation test. Scheffe’s F tests were conducted for multiple comparisons between the body weights of each genotype in the congenic strain analysis. Statistical significance was set at $P<0.05$.

**Results**

**Phenotypic Characterization of BC-Tpst2grt Mice**

To verify the effect of genetic background on susceptibility to growth retardation induced by $Tpst2^{grt}$, the body weight of forty-nine (male = 26, female = 23) BC-Tpst2grt mice was measured at 5, 8 and 10 weeks of age. Figure 1 shows the plots of body weights of BC-Tpst2grt mice and mice homozygous for $Tpst2^{grt}$ in DW and 129 congenic strains (DW-Tpst2grt and 129-Tpst2grt, respectively) at 8 weeks of age [17]. The body weight of each individual BC-Tpst2grt mouse of either sex varied compared to that of DW-Tpst2grt or 129-Tpst2grt mice; i.e., some mice showed severe growth retardation similar to DW-Tpst2grt mice whereas, other mice showed growth comparable to that of resistant 129-Tpst2grt mice. This scattered distribution was also observed at 5 and 10 weeks of age (data not shown). Complete recovery from dwarfism observed on Chr 2 in both sexes. Table 1 summarizes the significant correlations between them in BC-Tpst2grt mice of both sexes (Fig. 2B), the main modifier locus is estimated to be between D2Mit62 and D2Mit304, and particularly close to D2Mit255.

**Histological Evaluation of the Thyroid Glands of BC-Tpst2grt Mice**

Since the tolerance to growth retardation in 129-Tpst2grt mice is due to their normal thyroid development and function in spite of hyperactivity to TSH signaling [17], we conducted a histological analysis of the thyroid glands. Thyroid sections of randomly selected 10-week-old BC-Tpst2grt mice showed some correlation between the severity of growth retardation and the degree of thyroid hypoplasia (Fig. 3A). Namely, the thyroid glands of BC-Tpst2grt mice with severe growth delay show more severe hypoplastic symptoms, such as a decrease in the number of collaid-filled follicles, diversity in follicle size and increased replacement of parenchyma cells with adipocytes, compared to those with milder growth retardation. Next, we carried out a quantitative evaluation of the severity of thyroid dysplasia using a “thyroid index” (average ratio of total colloid areas per thyroid cross-section area) as previously described [17]. There was no correlation between body weight and thyroid index in either sexes BC-WT (Fig. 2A). $P=0.1495$, $r=0.394$ and $n=15$ in males; $P=0.9687$, $r=-0.015$ and $n=15$ in females), although there were significant correlations between them in BC-Tpst2grt mice of both sexes (Fig. 2B). $P=0.0042$, $r=0.698$ and $n=14$ in males; $P=0.0441$, $r=0.586$ and $n=12$ in females). This result demonstrates that the growth retardation in BC-Tpst2grt mice is correlated to the severity of thyroid hypoplasia. In addition, although there was no significant difference in females, male BC-Tpst2grt mice heterozygous for D2Mit255 showed a significant increase in thyroid index compared to male BC-Tpst2grt mice homozygous for D2Mit255. There was no significant difference when male BC-Tpst2grt mice were grouped according to genotype at the weaker QTLs on Chrs 4 and 18, respectively (data not shown). These results suggest that the main CH resistant gene(s) is located on Chr 2 near D2Mit255, and that it is involved in thyroid development.

**Evaluation of the Effects of Lrch by Analysis of Congenic Mice**

In order to validate the effects of the 129-derived resistant locus (locus for resistance to CH: designated Lrch), a DW congenic mouse strain carrying 129 alleles at this locus was generated (Fig. 4A). Figure 4B shows the growth curves of each genotype of CG mice. CG mice with wild-type Tpst2 alleles were used as littermate controls. Among the CG mice homozygous for $Tpst2^{grt}$ (CG-Tpst2grt), mice with the 129-derived Lrch allele showed partial recovery from growth retardation in both sexes, compared to mice without the 129-derived Lrch allele. The Lrch allele showed no obvious influence on growth in littermate controls (data not shown). In addition, thyroid index of CG-Tpst2grt with the 129-derived Lrch allele showed a significant elevation compared to CG-Tpst2grt without the 129-derived Lrch allele for both sexes (Fig. 4C). Thus, the congenic analysis confirmed the significant impact of Lrch, located between D2Mit43 and D2Mit286, on TD-associated growth retardation.

![Figure 1. Plots of the individual body weights of DW-Tpst2grt, BC-Tpst2grt and 129-Tpst2grt mice at 8 weeks of age. Individual numbers are given in parentheses.](doi:10.1371/journal.pone.0031035.g001)
Discussion

In a previous study, we demonstrated that growth retardation by Tpst2grt-related CH is influenced by genetic background. Mice harboring homozygotic Tpst2grt in a B6 genetic background, as well as DW-Tpst2grt, show susceptibility to CH. In contrast, the thyroid function in 129-Tpst2grt mice is fully recovered irrespective of TPST2 deficiency, and the 129 strain is remarkably resistant to growth retardation by CH [17]. Most gene targeting is carried out in cultured ES cells derived from the 129 strain because of its higher efficiency of germline transmission. Our data indicated that thyroid-related gene knockout mice might sometimes appear phenotypically normal in a 129 genetic background.

In this study, to identify the resistant gene(s) for CH, we performed QTL analysis using backcross progenies from susceptible DW and resistant 129 strain mice. Although the mapping resolution is not good enough to distinguish two QTLs close to each other, QTL analysis using the first principal component

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**Table 1. Characteristics of QTLs for growth retardation.**

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<th>Sex</th>
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<th>%</th>
<th>P</th>
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<th>Heterozygous</th>
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<td>Male</td>
<td>D2mit255</td>
<td>25.5</td>
<td>63</td>
<td>$&lt;10^{-5}$</td>
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<td>23.44±2.60*</td>
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<tr>
<td></td>
<td>D4mit152</td>
<td>8.4</td>
<td>28</td>
<td>0.0037</td>
<td>17.64±3.96</td>
<td>22.13±4.21*</td>
</tr>
<tr>
<td></td>
<td>D18mit110</td>
<td>8.5</td>
<td>29</td>
<td>0.0036</td>
<td>16.80±4.98</td>
<td>21.02±3.85*</td>
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<tr>
<td>Female</td>
<td>D2mit255</td>
<td>27.0</td>
<td>69</td>
<td>$&lt;10^{-5}$</td>
<td>15.73±1.95</td>
<td>20.70±1.97*</td>
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The microsatellite markers linked to the indicator for CH with the highest LRS value on each Chr, percentage of the variance, genome-wide P value detected by marker regression analysis based on 10000 permutation replicates and body weights of each genotype at 10 weeks age are indicated. %: percentage of the variance. P: genome-wide P value as calculated by QTX software. Means ± s.d. are shown.

*: P<0.01.

doi:10.1371/journal.pone.0031035.g002
calculated from pubertal body weights identified a highly significant QTL on Chr 2 adjacent to D2Mit255, and suggestive QTLs on Chrs 4 and 18 (Fig. 2). In fact, 129-derived alleles of each QTL increased resistance to dwarfism in both BC-Tpst2grt and CG-Tpst2grt mice (Table 1 and Fig. 4B). Although body weight is a compound trait reflecting the weights of lean muscle and bones as well as fat mass, a significant correlation between the severity of growth delay and the degree of thyroid hypoplasia suggested the involvement of thyroid dysplasia to growth retardation in BC-Tpst2grt mice (Fig. 3A and B). Indeed, a significant elevation in thyroid index for both sexes was observed in CG-Tstp2grt with the 129-derived Lrch allele, compared to CG-Tsp2grt without the 129-derived Lrch allele (Fig. 4C). This result confirmed the significant impact of Lrch, located between D2Mit43 and D2Mit286, on TD-associated growth retardation. Since there were complete recovery effects on dwarfism and thyroid development in backcross population (Fig. 1A and 3B), and the resistant gene(s) to CH in 129 strain is supposed to follow dominant inheritance, we used backcross population for examination of growth and thyroid development in CG mice. The partial recovery from growth retardation in CG-Tsp2grt mice with the 129-derived Lrch (Fig. 4B; compared to littermate controls) suggests the effects of other QTLs, though dosage effects of the Lrch cannot be excluded. Further examination using intercross population will prove this problem.

Besides the major QTL on Chr 2, we detected male specific QTLs on Chrs 4 and 18 (Fig. 2). In fact, 129-derived alleles of each QTL increased resistance to dwarfism in both BC-Tsp2grt and CG-Tsp2grt mice (Table 1 and Fig. 4B). Although body weight is a compound trait reflecting the weights of lean muscle and bones as well as fat mass, a significant correlation between the severity of growth delay and the degree of thyroid hypoplasia suggested the involvement of thyroid dysplasia to growth retardation in BC-Tsp2grt mice (Fig. 3A and B). Indeed, a significant elevation in thyroid index for both sexes was observed in CG-Tsp2grt with the 129-derived Lrch allele, compared to CG-Tsp2grt without the 129-derived Lrch allele (Fig. 4C). This result confirmed the significant impact of Lrch, located between D2Mit43 and D2Mit286, on TD-associated growth retardation. Since there were complete recovery effects on dwarfism and thyroid development in backcross population (Fig. 1A and 3B), and the resistant gene(s) to CH in 129 strain is supposed to follow dominant inheritance, we used backcross population for examination of growth and thyroid development in CG mice. The partial recovery from growth retardation in CG-Tsp2grt mice with the 129-derived Lrch (Fig. 4B; compared to littermate controls) suggests the effects of other QTLs, though dosage effects of the Lrch cannot be excluded. Further examination using intercross population will prove this problem.

Besides the major QTL on Chr 2, we detected male specific QTLs on Chrs 4 and 18. This may explain the sex bias for TD in human. In fact, gender bias in TD is reported in humans, with a female: male ratio of 1.4 for entire TD, 1.9 for ectopic thyroid glands and 0.9 for athyreosis [5], although the reason for this remains unclear. It is possible that the TD resistant genes are influenced by the male sex hormone. Alternatively, QTLs detected in this study might be correlated with genes differentially expressed in a gender-specific manner.

By use of CH mice with TD caused by a double-heterozygous null mutation in genes encoding Ttf1 and Pax8 (DHTP), Amendola et al. also found that the B6 strain has a greater susceptibility to CH in comparison to 129 strain mice [10]. They mapped modifier genes for CH on Chrs 2 and 5 by genetic linkage analysis, and further localized the CH-related QTL on Chr 2 (HTRC2) from 112–121 Mb [21]. In the present study, we localized the major modifier QTL on Chr 2 from 117.9–
123.2 Mb by QTL mapping and confirmed that \( Lrch \) is located between 104.0–154.3 Mb by congenic strain analysis. This locus overlaps \( HTRC2 \). From a subsequent transcriptional assay and sequencing of candidate genes, they concluded that the amino acid substitution of highly conserved tyrosine 273 to phenylalanine in \( Dnajc17 \) (119.0 Mb), encoding for a member of the type III heat-shock protein-40 family, results in reduced thyroglobulin (\( Tg \)) transcription, leading to CH in B6 mice [21]. We also found a Y273F polymorphism in \( Dnajc17 \) in DW strain mice, which is the same as the susceptible B6 mice in the above study. However, we could not detect any deficiency in \( Tg \) transcription, synthesis, transportation or secretion in DW-\( Tpst2grt \) mice [12,22]. As the pathology of CH differs between each model, modifier genes for \( Tpst2grt \)-induced CH may differ from those for DHTP-induced CH. The fact that the peak LRS on Chr 2 is between \( D2Mit255 \) and \( D2Mit304 \) (123.2–128.2 Mb), rather than between \( D2Mit62 \) and \( D2Mit255 \) (117.9–123.2 Mb) in male (Fig. 2) supports the above idea.

Since the \( Tpst2grt \) mutation causes TSH hyporesponsiveness [12], we predict that the resistant factor(s) for CH in the 129 strain are mainly involved in signal transduction downstream of TSHR or TSH-independent activity in thyrocyte proliferation and thyroid hormone biosynthesis. The positional MEDLINE (http://omicspace.riken.jp/PosMed/) revealed that there are at least 18 genes related to thyroid development and function between \( D2Mit62 \) and \( D2Mit304 \) (Table 2). In particular, dual oxidase 2 (\( Duox2 \)) (122.1 Mb), dual oxidase maturation factor 2 (\( Duoxa2 \)) (122.1 Mb) and K\( v \) channel interacting protein 3 (\( Kcnip3 \), also known as Dream) (127.3 Mb) agree with our prediction. \( Duox2 \) and \( Duoxa2 \) attracted our interest in that these genes are involved in several forms of CH in human and mice [23,24]. DUOX2 is one of the NADPH oxidases producing \( H_2O_2 \), which is required for iodination of TG whose modification is prerequisite for thyroid hormone synthesis. In addition to its transcription, enzymatic activity of DUOX2 is also regulated by TSH signaling in human [25,26]. DUOX2 is essential for DUOX2 maturation and trafficking from the endoplasmic reticulum to the plasma membrane where DUOX2 functions [25,27]. \( Kcnip3 \) is preferentially expressed in the central nervous system and thyroid gland in mice, and binds to the downstream regulatory element where its binding to the sequence suppresses the transcription of target genes [28]. Recently, transcriptional regulation of thyroid

**Figure 4. Evaluation of the effect of \( Lrch \) on TD-associated growth retardation by congenic strain analysis.**

A) Schematic diagram of the genomic structure surrounding \( Lrch \) in CG mice. Gray bars indicate the minimum genomic regions derived from DW. A black bar indicates a genomic region into which 129-derived alleles were introduced. Dotted bars represent recombined regions between the DW and 129 genomes. The numbers to the left of the bars represent physical locations based on the MGI. B) Growth curves of CG mice. a: littermate controls, b: CG-\( Tpst2grt \) mice heterozygous for the 129-derived \( Lrch \) allele, c: CG-\( Tpst2grt \) mice without the 129-derived \( Lrch \) allele. Individual numbers of a, b, c are 5, 4 and 5 for males, and 9, 5 and 2 for females, respectively. Vertical lines indicate s.d. \( P<0.05 \); *; b versus c, †; b versus a, ‡; c versus a. C) Thyroid indices of CG-\( Tpst2grt \). Individual numbers are given in parentheses.

![Figure 4](10.1371/journal.pone.0031035.g004)
specific genes (for example, Tg, Pax8 and Foxe1) by KCNIP3 was reported [29,30]. Furthermore, constitutively active mutant KCNIP3 interferes with thyroid cell proliferation in vitro [30] and induces thyroid enlargement and nodular development in mice [31]. Moreover, the regulation of TSHR activity by KCNIP3 is suggested in thyrocytes [31]. We examined the expression of these possible candidate genes (Dnajc17, Duox2, Duoxa2 and Kcnip3) in thyroid glands of DW and 129 strain mice with semi-quantitative RT-PCR. However, there was no quantitative difference observed (data not shown). To determine the resistant genes, the generation and analysis of subcongenic mice together with further genetic study is required.

In conclusion, genome-wide analysis and a congenic mouse study identified a major modifier gene for Tpst2grt-induced CH on Chr 2, between 104.0–154.3 Mb and adjacent to D2Mit255. The major QTL identified in our study overlaps with HTRC2 which was identified in another study. It is intriguing that genetic linkage analyses using distinct CH models with different origins mapped the major QTL on the same region on Chr 2. Identification of genes responsible for resistance to hypothyroidism in 129-Tpst2grt mice will provide insights into the molecular events involved in thyroid development. Moreover, the discovery of novel causative gene(s) should lead to the development of treatment strategies for thyroid cell disorders including thyroid tumors and hyperthyroidism, as well as hypothyroidism.

### References


### Supporting Information

**Figure S1** Details of male-specific suggestive linkages on Chr 4 and Chr 18. Suggestive, significant and highly significant values are 6.9, 13.7 and 21.8, respectively. Horizontal black bars represent 95% confidence intervals. The maximum LRS is 8.4 for Chr 4, and 10.6 for Chr 18.

**Figure S2** Plots of body weight and thyroid index of BC-WT mice at 10 weeks of age. Solid triangles: BC-WT (n = 15 and 10 for male and female, respectively), blue triangles: the average of the DW mice with wild-type Tpst2grt allele (n = 5 and 3 for male and female, respectively), red triangles: the average of the 129 mice with wild-type Tpst2grt allele (n = 5 and 3 for male and female, respectively).

**Table S1** List of microsatellite markers used for the QTL analysis.

### Author Contributions

Conceived and designed the experiments: NS. Performed the experiments: YH YK NS. Analyzed the data: YH DT NS. Contributed reagents/materials/analysis tools: NS TA. Wrote the paper: YH NS.


