The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. 106, No. 5, e2191–e2202
doi:10.1210/clinem/dgaa975
Clinical Research Article

Clinical Research Article

Epigenome-Wide Association Study of Thyroid Function Traits Identifies Novel Associations of fT3 With *KLF9* and *DOT1L*

Nicole Lafontaine,[1,2] Purdey J. Campbell,[1] Juan E. Castillo-Fernandez,[3] Shelby Mullin,[1] Ee Mun Lim,[1,4] Phillip Kendrew,[4] Michelle Lewer,[4] Suzanne J. Brown,[1] Rae-Chi Huang,[5] Phillip E. Melton,[6,7,8] Trevor A. Mori,[9] Lawrence J. Beilin,[9] Frank Dudbridge,[10] Tim D. Spector,[3] Margaret J. Wright,[11,12] Nicholas G. Martin,[13] Allan F. McRae,[14] Vijay Panicker,[1] Gu Zhu,[13] John P. Walsh,[1,2] Jordana T. Bell,[3] and Scott G. Wilson[1,3,6]

[1]Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA 6009, Australia; [2]Medical School, University of Western Australia, Crawley, WA 6009, Australia; [3]Department of Twin Research & Genetic Epidemiology, King's College London, London SE5 9NU, UK; [4]Pathwest Laboratory Medicine, Nedlands, WA 6009, Australia; [5]Telethon Kids Institute, University of Western Australia, Perth 6009, Australia; [6]School of Biomedical Sciences, University of Western Australia, Perth 6009, Australia; [7]School of Pharmacy and Biomedical Sciences, Curtin University, Perth 6845, Australia; [8]Menzies Institute for Medical Research, University of Tasmania, Hobart, Tasmania 7000, Australia; [9]Medical School, Royal Perth Hospital Unit, University of Western Australia, Perth, WA 6000, Australia; [10]Department of Health Sciences, University of Leicester, Leicester, LE1 7RH, UK; [11]Queensland Brain Institute, University of Queensland, Brisbane, QLD 4072, Australia; [12]Centre for Advanced Imaging, University of Queensland, Brisbane, QLD 4072, Australia; [13]QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia; and [14]Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD 4072, Australia

ORCiD numbers: 0000-0001-7101-2058 (N. Lafontaine); 0000-0002-5727-4323 (P. J. Campbell); 0000-0002-0034-8029 (J. E. Castillo-Fernandez); 0000-0002-8464-6639 (R.-C. Huang); 0000-0003-4026-2964 (P. E. Melton); 0000-0002-5264-9229 (T. A. Mori); 0000-0002-8817-8908 (F. Dudbridge); 0000-0001-7133-4970 (M. J. Wright); 0000-0003-4069-8020 (N. G. Martin); 0000-0002-5691-1917 (G. Zhu); 0000-0002-1766-2612 (J. P. Walsh); 0000-0002-0357-1373 (S. G. Wilson).

Abbreviations: BSGS, Brisbane Systems Genetics Study; CpG, cytosine-phosphate-guanine; DMP, differentially methylated position; DMR, differentially methylated region; DNAm, DNA methylation; EWAS, epigenome-wide association study; fT3, free triiodothyronine; fT4, free thyroxine; GWAS, genome-wide association study; HCC, hepatocellular carcinoma; HPT, hypothalamus-pituitary-thyroid; T3, triiodothyronine; T4, thyroxine; TR, thyroid receptor; TSH, thyrotropin (thyroid-stimulating hormone).

Received: 14 August 2020; Editorial Decision: 24 December 2020; First Published Online: 23 January 2021; Corrected and Typeset: 23 January 2021.

Abstract

Context: Circulating concentrations of free triiodothyronine (fT3), free thyroxine (fT4), and thyrotropin (TSH) are partly heritable traits. Recent studies have advanced knowledge of their genetic architecture. Epigenetic modifications, such as DNA methylation (DNAm), may be important in pituitary-thyroid axis regulation and action, but data are limited.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Objective: To identify novel associations between fT3, fT4, and TSH and differentially methylated positions (DMPs) in the genome in subjects from 2 Australian cohorts.
Method: We performed an epigenome-wide association study (EWAS) of thyroid function parameters and DNAm using participants from: Brisbane Systems Genetics Study (median age 14.2 years, n = 563) and the Raine Study (median age 17.0 years, n = 863). Plasma fT3, fT4, and TSH were measured by immunoassay. DNAm levels in blood were assessed using Illumina HumanMethylation450 BeadChip arrays. Analyses employed generalized linear mixed models to test association between DNAm and thyroid function parameters. Data from the 2 cohorts were meta-analyzed.
Results: We identified 2 DMPs with epigenome-wide significant ($P < 2.4E{-}7$) associations with TSH and 6 with fT3, including cg00049440 in *KLF9* ($P = 2.88E{-}10$) and cg04173586 in *DOT1L* ($P = 2.09E{-}16$), both genes known to be induced by fT3. All DMPs had a positive association between DNAm and TSH and a negative association between DNAm and fT3. There were no DMPs significantly associated with fT4. We identified 23 differentially methylated regions associated with fT3, fT4, or TSH.
Conclusions: This study has demonstrated associations between blood-based DNAm and both fT3 and TSH. This may provide insight into mechanisms underlying thyroid hormone action and/or pituitary-thyroid axis function.

Key Words: epigenetics, EWAS, thyroid hormone, DNA methylation, KLF9, DOT1L

The thyroid synthesizes and secretes triiodothyronine (T3) and thyroxine (T4), hormones required for growth and the regulation of metabolism (1). Circulating levels of thyrotropin (TSH) and thyroid hormones are tightly regulated via the hypothalamus-pituitary-thyroid (HPT) axis; intraindividual variation is less than interindividual variation, suggesting that individuals have unique set points (2). Variations of thyroid function within the population-based normal range have been associated with adverse health outcomes including atrial fibrillation (3), coronary heart disease (4), stroke (5), mood (6), cognitive disorders (7), body mass index (BMI) (8), and overall mortality (4, 9). It is therefore important to improve our understanding of mechanisms behind these variations to facilitate better management of patients.

Genetic factors influence interindividual variation in free T3 (fT3), free T4 (fT4) and TSH levels. From studies comparing monozygotic and dizygotic twins, it is estimated that heritability accounts for up to 65% of variation in fT3, fT4, and TSH (10-12). Genome-wide association studies (GWAS) have advanced knowledge in this area by identifying a substantial number of genes that may contribute to thyroid function variance (13-16). However, established loci account for only 21% and 33% of genetic variance in fT4 and TSH, respectively (13). Epigenetics could provide a link between genes, environmental exposures, and differences in fT3, fT4, and TSH levels.

Epigenetics describes mechanisms that control the regulation of gene expression, including DNA methylation (DNAm) and histone modification, among others and that do not involve a change in the DNA nucleotide sequence (17). DNAm is one of the most commonly described epigenetic mechanisms (18) and involves the addition of a methyl group to a cytosine in a cytosine-phosphate-guanine (CpG) dinucleotide sequence through the action of DNA-methyltransferases (18, 19). DNAm can cause major effects on gene expression levels by influencing the binding of regulatory elements to DNA. These epigenetic features further augment transcriptional regulation dictated by the DNA nucleotide coding and are additional critical regulators of gene expression that are considered to make a significant contribution to complex disease susceptibility (20). The methylation profile of a cell changes during differentiation, as certain genes are up- and downregulated, resulting in the formation of a unique methylome in each cell type (21). The relationship between DNAm and gene expression is complex; methylation within a gene promoter typically represses transcription of the gene, whereas DNAm located within exons or introns is frequently associated with active expression and can influence splicing and activity of alternate promoters (22). In line with this, the DNAm landscape has been found to change profoundly during the process of cell differentiation (23) and across the lifespan (24-27).

Epigenome-wide association studies (EWAS) have been used to investigate variations in DNAm genome-wide and explore relationships between methylation and clinical phenotypes (19, 28, 29). EWAS can identify differentially methylated positions (DMPs), which are individual CpGs

that show differential methylation depending on the phenotype, as well as differentially methylated regions (DMRs) which are segments of adjacent CpGs that show overall differential methylation depending on the phenotype (30). Despite the increasing epigenetics literature in other fields, including autoimmune thyroid disease, there have, to our knowledge, been no published EWAS with thyroid function markers as a phenotype.

In this study, we performed an EWAS to look for associations between blood-based DNAm and circulating levels of fT3, fT4, and TSH from 2 Australian-based cohorts to provide further insight into mechanisms underlying thyroid hormone action and/or pituitary-thyroid axis function.

Methods

Study Participants

Two population-based cohorts were used in the research, the Brisbane Systems Genetics Study (BSGS) and the Raine Study. The participants from BSGS were recruited as part of a prospective study, the Brisbane Longitudinal Twin Study (BLTS), made up of healthy monozygotic and dizygotic twins and triplets, their singleton siblings, and their parents who were recruited in Brisbane, Queensland, Australia (31-33). BLTS participants were enlisted by media appeals, word of mouth, and by contacting the principals of primary schools in the greater Brisbane area. The study was approved by the Human Research Ethics committee of the Queensland Institute of Medical Research. All participants provided written informed consent.

The Raine Study (formerly known as the Western Australian Pregnancy Cohort Study) is a prospective multigenerational observation study which recruited pregnant women of 16 to 20 weeks gestation in Perth, Western Australia, between 1989 and 1991. It has followed these participants and their offspring (Generation 2) since birth, as described in detail previously (34). The study was approved by the Human Ethics Committee of the University of Western Australia. All participants and their parents or carers provided written informed consent. The present study uses plasma, clinical assessment, and questionnaire data from Generation 2 participants at age 14 (35) and DNAm data from specimens collected at age 17 (36).

In each cohort, participants were excluded if they had peroxidase antibodies (TPOAb) above the reference range (>6 IU/mL) or if plasma samples for thyroid function and DNAm were collected more than 5 years apart. In both cohorts, most participants are of self-reported European ancestry and reside in areas of iodine sufficiency (37).

Laboratory Data

In both cohorts, fT3, fT4, TSH, and peroxidase antibodies were measured on securely archived frozen plasma samples by automated immunoassay using the Abbott ARCHITECT analyzer (Abbott Diagnostic, Illinois, USA), as previously described (38). DNA methylation profiles were generated from leucocyte DNA from whole-blood samples using the Illumina Infinium HumanMethylation 450 BeadChip array (Illumina, San Diego, CA), as described previously for BSGS (32) and the Raine Study (36). This array interrogates more than 485 000 CpG sites, targeting gene regions and covering 99% of RefSeq genes and CpG islands, among other sites (32, 39).

Statistical Analysis

Linear mixed models were used to test for association between quantile normalized DNA methylation beta values and each of fT3, fT4, and TSH (natural log transformed), adjusting for sex, age, age squared, difference between ages at which thyroid function and DNAm were measured, and white blood cell composition (CD8T, CD4T, NK, Bcell, Mono, Gran). Random intercepts were fitted to account for plate number and sample position variation. A nested random effects structure with separate intercepts for each subject within zygosity group within family was used to account for relatedness (within monozygotic twins and triplets or among dizygotic twins and triplets and siblings more generally). All analyses were performed using R version 3.5.2 (including packages lme4, qqman, EasyStrata, and data.table).

Results from both cohorts were then meta-analyzed using METAL software. The epigenome-wide significance threshold was defined as a *P* value below 2.4E−7, calculated by using a permutation approach which takes into account the correlation structure of CpGs across the genome (40). The threshold for suggestive associations was defined as a *P* value below 1E−5 following previous studies which used Illumina methylation arrays to identify suggestive DMPs for preliminary consideration and to guide future hypotheses (41, 42). R package coMET was used to generate regional association plots (43).

For the detection of DMRs, association tests of fT3, fT4, and TSH were performed using the results from the meta-analysis that had directionally consistent effects. We examined the association between fT3, fT4, and TSH and DMRs using comb-p software with an analysis window of 300 base-pairs, an autocorrelation lag of 300 bases, a seed *P* value of 0.001 and a minimum of 3 probes per DMR (42). The corrected *P* values (P_{cor}) were reported after Sidak multiple testing correction.

Results

Descriptive Statistics

BSGS comprised 563 participants; 53% were male (Table 1). The median age was 14.2 years (interquartile range [IQR], 12.1-17.8) and 442 participants (79%) were in the adolescent age range (10-19 years old) (44). The Raine Study included 863 participants, 53% were male, and had a median age of 17.0 years (IQR, 16.9-17.2). All participants were adolescents.

EWAS Results

Miami plot results of the EWAS meta-analysis of the BSGS and the Raine Study for fT3 and TSH, comprising up to 483 254 probes, are shown in Fig. 1 and for fT4 in Supplemental Fig. 1 (QQ plots are presented in Supplemental Fig. 2-; λ = 1.005, 1.071 and 1.068 for FT3, fT4, and TSH respectively) (45). The meta-analysis found 6 novel epigenome-wide significant DMPs associated with fT3, and 2 associated with TSH (Table 2), while no significant associations were seen for fT4. Details of DMPs that were below the suggestive threshold for association are presented in Supplemental Tables 1-3 (45).

The epigenome-wide significant DMPs associated with fT3 were cg00024471 on chromosome 3 which lies in intron 1 of tumor protein p63 regulated 1 (*TPRG1*) (Supplemental Fig. 3A), cg02183564 located on chromosome 7 within intron 4 of coiled-coil domain–containing 146 (*CCDC146)* (Supplemental Fig. 3B), cg00049440 on chromosome 9 within intron 1 of Krüppel like factor 9 (*KLF9*) (Fig. 2A), cg04173586 on chromosome 19 within intron 1 of the disruptor of telomeric silencing 1-like (*DOT1L*) (Fig. 2B), and 2 probes in intergenic regions: cg01695994 on chromosome 17 and cg19837174 on chromosome 10 (Supplemental Fig. 3C and 3D, respectively) (45). All 6 DMPs had a negative association between DNAm and fT3.

The epigenome-wide significant DMPs associated with TSH were cg03445151 on chromosome 2 in an intergenic region (Supplemental Fig. 3E) and cg20065905 on chromosome 17 within the 3′ untranslated region of forkhead box K2 (*FOXK2)* (Supplemental Fig. 3F) (45).

Differentially Methylated Region Analysis

Data from the DMR analyses highlighted 4 regions associated with fT3, including an intergenic region in chromosome 4 nearest to Sep(O-Phosphoserine) TRNA: Selenocystine TRNA synthase (*SEPSECS)* associated with increased methylation; 11 associated with fT4; and 8 associated with TSH, including one on chromosome 1, within intron 3 of NOD-, LRR-, and pyrin domain–containing protein 3 (*NLRP3*) associated with increased methylation (Table 3).

Discussion

In this EWAS of thyroid function we identified 6 novel epigenome-wide significant DMPs associated with fT3 and reduced DNAm and 2 associated with TSH and increased DNAm in a meta-analysis of 2 independent cohorts of healthy participants. This may indicate that altered methylation at these loci plays a role in HPT axis physiology or (since we studied DNAm from blood) may reflect fT3 action on leucocyte DNAm. It is also possible that fT3 or TSH and DNAm at these sites are not causally related but have a common association with one or more as yet unidentified variables.

Of the DMPs associated with fT3, cg00049440 is within *KLF9*, previously known as basic transcription element binding protein 1, a member of the Krüppel family of zinc-finger transcription factors. These factors bind to GC-rich regions in the genome (46) and regulate proliferation, differentiation, development and programmed-cell death (47). *KLF9* is a T3 response gene. T3, via nuclear receptor activation, upregulates *KLF9* mRNA. KLF9 then acts as a transcription activator or repressor (48). It is expressed in a large number of tissues and has many roles, including in hematopoiesis (18), hippocampal neurogenesis (49), oligodendrocyte differentiation, myelin regeneration (50), and intestinal morphogenesis (47), and it is downregulated in

Table 1. Descriptive Statistics of Study Participants

	BSGS (n = 563)	Raine Study (n = 863)
Age at DNAm collection, years, median [IQR]	14.2 [12.1-17.8]	17.0 [16.9-17.2]
Male sex, %	53%	53%
fT3, pmol/L	4.92 (0.65)	5.47 (0.60)
fT4, pmol/L	12.64 (1.36)	12.25 (1.25)
TSH, mU/L	1.58 (0.88)	2.08(0.94)
Time between blood sample taken for DNA and thyroid function, years	−0.005 (0.19)	3.08 (0.51)

Data are shown as mean (SD) unless otherwise stated. Abbreviations: BSGS, Brisbane Systems Genetics Study; DNAm, DNA methylation; IQR, interquartile range.

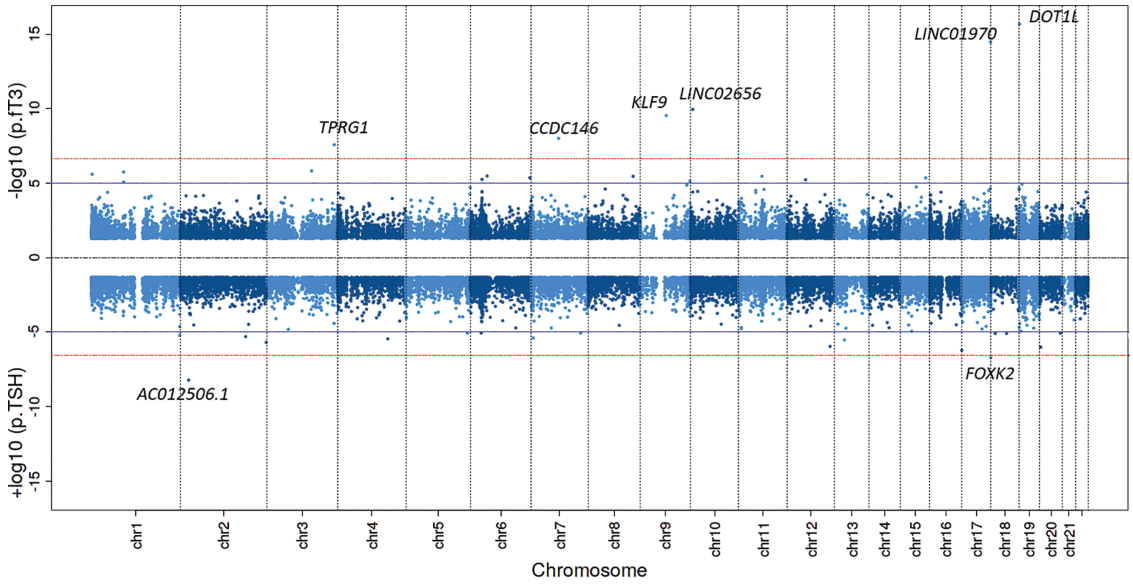

Figure 1. Miami plot of meta-analysis of EWAS for fT3 (top panel) and for TSH (bottom panel). The x-axis shows chromosome position, and the y-axis the $-\log_{10} P$ values. The epigenome-wide significance threshold is represented by the horizontal red lines ($P = 2.4E{-}7$) and the threshold for suggestive association shown by the blue horizontal lines ($P = 1.0E{-}5$).

many cancers, as discussed further below in this discussion. *KLF9* also helps mediate the neuronal protective role of T3 on neurons exposed to hypoxia (51).

KLF9 is downregulated in multiple cancers, including endometrial (52), esophageal squamous cell carcinoma (53), colorectal cancer (54), hepatocellular carcinoma (HCC) (55), breast cancer (56), and neuroblastoma (57). *KLF9* has been demonstrated to suppress neuroblastoma growth and progression (57); inhibit growth, migration, and metastasis of esophageal squamous cell carcinoma (53); inhibit breast cancer metastasis (56); and inhibit proliferation and induce apoptosis of HCC (55). Interestingly, a recent study showed that short-term treatment with T3 in rats with HCC caused a prolonged reduction in the number and burden of HCCs compared with untreated rats, by induction of genes involved in hepatocyte differentiation including *KLF9* (58). Although the authors hypothesize that this may be due to the restoration of the T3/thyroid receptor (TR) axis, effects on DNAm may be responsible for the persistent effects. This could have therapeutic implications for several cancers.

DOT1L is a methyltransferase and is an enzyme well-known to methylate H3K79, an activation histone mark. Histone methylation can alter chromatin structure and may recruit effector proteins to certain chromatin regions (59). *DOT1L*, like *KLF9*, is known to be activated by T3. *Xenopus* metamorphosis is a hormone-dependent period of development when T3 is high. During metamorphosis, T3 activates *DOT1L*, which in turn increases methylation of H3K79 in TR targets, thereby inducing chromatin remodeling and allowing gene activation by TR; it also acts as a TR coactivator (60). Functions of *DOT1L* include DNA repair and cell cycle regulation, and it has an essential role in general embryogenesis, chondrogenesis, and cardiac development in mice (61). In the present study, most participants were studied during adolescence, a period of developmental change, during which circulating fT3 levels are higher than in adults (38, 62), and it is possible that the observed association between fT3 and DNAm of *DOT1L* is relevant to pubertal development.

Both *DOT1L* and *KLF9* have been demonstrated to play an important role in hematopoiesis, and *KLF9* with T cell lymphopoiesis (51, 63). Hypothyroidism is known to be a cause of anemia and, to a lesser extent, reduced lymphocyte count (64-66). Given that DNAm is a tissue-specific process and *KLF9* and *DOT1L* are known to have a role in the formation of blood cellular components, it is possible that changes in the levels of DNAm in white blood cells form part of the mechanism by which T3 affects hematopoiesis rather than being involved in regulation of circulating T3 levels.

Probe cg19837174 on chromosome 10 is within 1.1kbp of *LINC02656*, which is associated with thyroid hormone administration (67). Other identified DMPs associated with fT3 in this EWAS have no currently known associations with thyroid hormones. Of interest, one of the DMPs that reached the suggestive threshold was cg20146909, on chromosome 1 within intron 1 of leucine-rich repeat–containing 8 family, member D (*LRRC8D*). *LRRC8D* has also been demonstrated to be directly regulated by thyroid hormone (68).

In our study, increased methylation in cg20065905, which is within *FOXK2*, was associated with higher TSH

Table 2. Details of the Epigenome-Wide Significant Differentially Methylated Positions From the Meta-Analysis

Phenotype	CpG site	Chr	Position (hg19)	Nearest gene	Location	BSGS (n)	BSGS β	BSGS *P* value	Raine Study (n)	Raine Study β	Raine Study *P* value	Meta-analysis (n)	Meta-analysis β	Meta-analysis *P* value
fT3														
	cg00024471	3	188692547	*TPRG1*	Intron 1	563	-2.26	5.93E–12	863	-0.44	4.11E–2	1426	-0.99	2.58E–8
	cg00049440	9	73026643	*KLF9*	Intron 1	563	-1.87	2.44E–7	863	-1.20	1.16E–4	1426	-1.49	2.88E–10
	cg01695994	17	80246403	*LINC01970*	Intergenic	563	-2.75	1.33E–13	863	-1.19	4.81E–5	1426	-1.81	3.31E–15
	cg02183564	7	76874892	*CCDC146*	Intron 4	563	-2.63	2.29E–13	863	-0.38	1.66E–1	1426	-1.24	9.69E–9
	cg04173586	19	2167496	*DOT1L*	Intron 1	559	-2.11	4.64E–18	863	-0.64	7.89E–4	1422	-1.22	2.09E–16
	cg19837174	10	6389707	*LINC02656*	Intergenic	563	-1.98	5.48E–10	863	-0.80	1.38E–3	1426	-1.26	1.10E–10
TSH														
	cg03445151	2	23516881	*AC012506.1*	Intergenic	559	0.21	2.33E–4	863	0.27	5.45E–6	1422	0.24	6.19E–9
	cg20065905	17	80560980	*FOXK2*	3′ UTR	562	0.23	1.81E–4	863	0.23	2.45E–4	1425	0.23	1.75E–7

Abbreviations: BSGS, Brisbane Systems Genetics Study; Chr, chromosome; fT3, free triiodothyronine; n, number; TSH, thyrotropin (thyroid-stimulating hormone); UTR, untranslated region.

concentrations; the physiological relevance of this requires further elucidation. *FOXK2* is a member of the forkhead box (FOX) family (69). Although other members of this family, including *FOXE1* and *FOXO1*, have established roles in thyroid physiology and thyroid hormone action, *FOXK2* has no known associations with TSH or thyroid hormones (70-72). *FOXK2* has physiological roles in glycolysis, lipid metabolism, and mitochondrial function, which may potentially be relevant to thyroid hormone action and has a reciprocal translocation pattern into the nucleus with the FOXO family in response to insulin (69, 73). Increased methylation was also seen in cg03445151 with higher TSH concentrations, which lies within an intergenic region with no known significance to thyroid function.

We identified 23 significant DMRs associated with fT3, fT4, or TSH. A DMR associated with fT3 and increased methylation, within an intergenic region in chromosome 4, was closest to *SEPSECS*, which is important in the selenoprotein biosynthesis pathway (74). Iodothyronine deiodinases, which are crucial for thyroid hormone metabolism, are selenoenzymes and require selenocysteine at their catalytic site (75). Selenium deficiency and mutations affecting selenoprotein synthesis are known to affect thyroid hormone levels (75, 76).

A DMR within *NLRP3* was associated with TSH and increased methylation. *NLRP3* is part of the NOD-like receptor family, which are inflammasomes that have been associated with autoimmune thyroiditis pathogenesis (77). *NLRP3* activation is also known to be involved in the pathogenesis of ischemia-reperfusion liver injury and research has demonstrated that T3 treatment prior to ischemia-reperfusion in rats reduced the expression of *NLRP3* and liver injury (78).

In the present study, we identified 6 DMPs associated with fT3 at an epigenome-wide level of significance and 2 associated with TSH, but none with fT4. We also identified 23 DMRs, 4 associated with fT3, 11 associated with fT4, and 8 with TSH. Epigenetic modifications such as DMPs are subject to both genetic and environmental factors. Heritability estimates for fT4 and TSH are higher than those for fT3 in most (10, 11) but not all studies (12), and GWAS have been more successful in identifying common genetic variants associated with TSH and fT4 than with fT3. Circulating fT3 appears more responsive than TSH or fT4 to environmental influences, such as nutritional state (79), childhood growth and pubertal development (38, 66), and nonthyroidal illness (80). It is possible that the identification of 6 DMPs for fT3, 2 for TSH, and none for fT4, indicates a greater degree of epigenetic influence from environmental factors on circulating fT3 than on TSH or fT4. Alternatively,

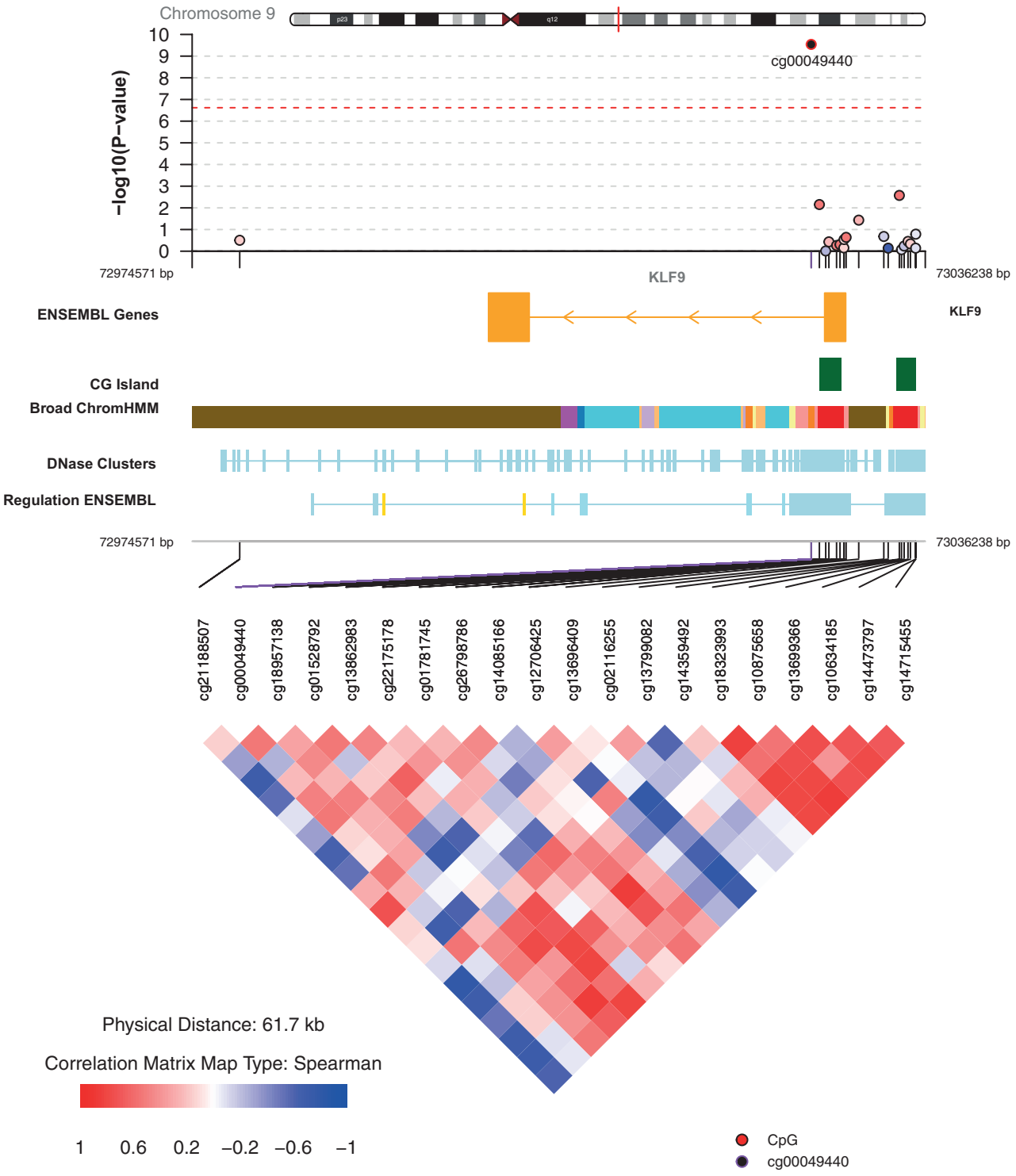

Figure 2. Local association plots describing the genomic region for each of the significant DMP (top panel), the functional annotation (middle panel), and the pattern of co-methylation at individual CpG sites at **2a,** cg00049440 and **2b,** cg04713586. Co-methylation relationships are derived from BSGS participants.

since T3 is the active thyroid hormone (whereas T4 is largely a prohormone and TSH the major trophic hormone to thyrocytes), the DMPs associated with fT3 in this study may reflect hematopoietic effects of thyroid hormone, reflected in reduced methylation of leucocyte DNA. T3 has been previously demonstrated to have effects on DNAm. Treatment with T3 in rodent primary cortical neurons exposed to hypoxia reduces hypoxia-mediated DNA hypermethylation by upregulating ten-eleven translocation (TET) genes and downregulating

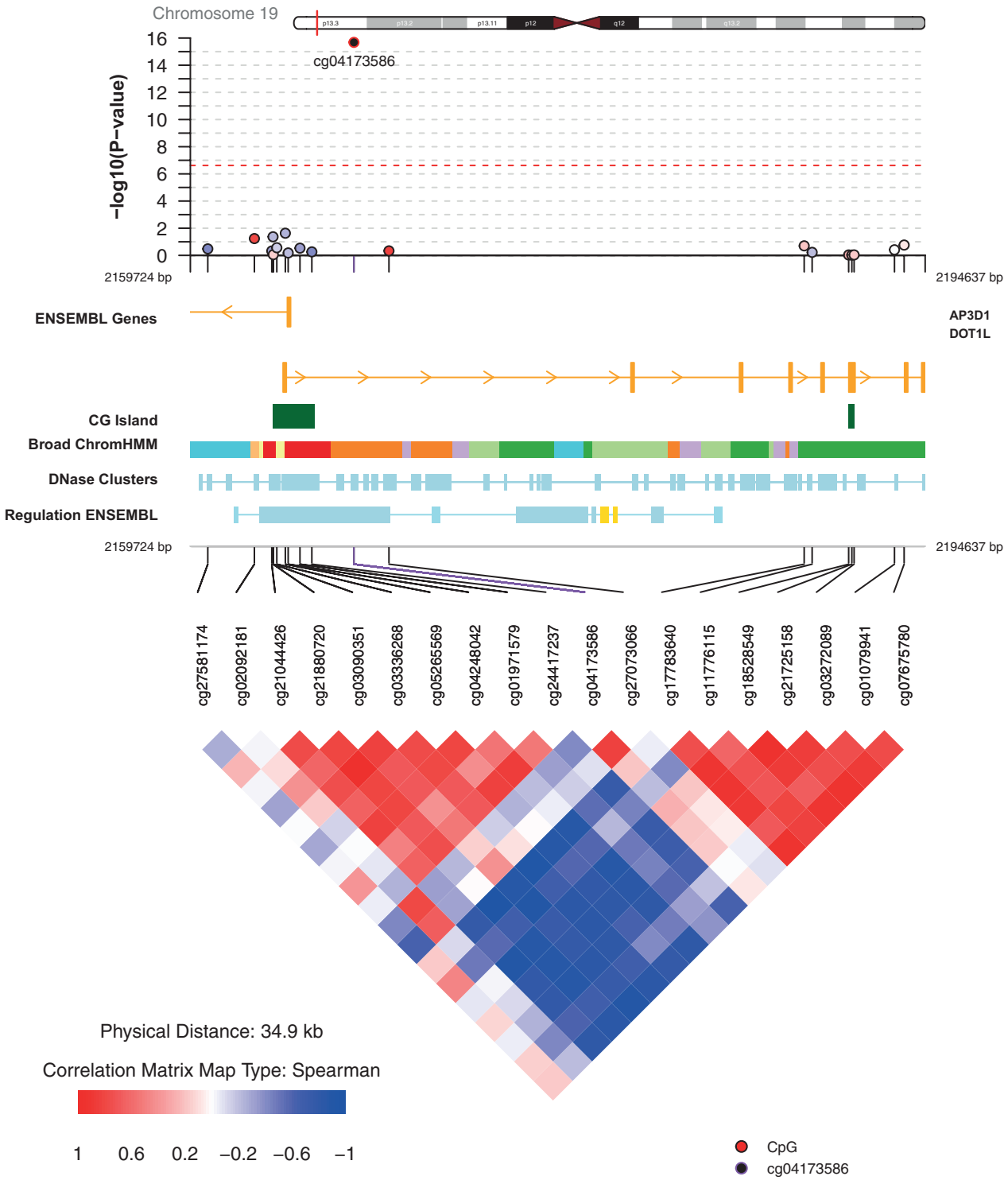

Figure 2. Continued.

DNA methyltransferase (Dnmt)3a and Dnmt3b (51), required for demethylation. Our significant DMPs showed an association of fT3 with reduced DNAm and TSH with increased DNAm, and a number of our results are within genes known to be directly regulated by thyroid hormones. It is possible that DNAm regulation plays an important role in the actions of T3 and regulation of other genes.

This EWAS of thyroid function has identified novel associations between the level of methylation and fT3 at 6 DMPs and TSH at 2 DMPs and provides a basis for further targeted studies, particularly in relation to probes

Table 3. Statistically Significant Differentially Methylated Regions (DMRs) Associated With fT3, fT4, or TSH

Phenotype	Chr	Position (hg19)	Nearest gene	Location	Probes (n)	Unadjusted *P* value	Sidak *P* value (*Pcor*)	Direction
fT3								
	10	124638874-124639167	*FAM24B*	Intron	8	1.81E−7	1.40E−4	+
	11	65546988-65547172	*AP5B1*	Exon 2	4	8.18E−9	1.01E−5	-
	3	48694451-48694673	*CELSR3*	Exon	4	2.83E−8	2.90E−5	+
	4	25090491-25090665	*SEPSECS*	Intergenic	4	1.22E−7	1.59E−4	+
fT4								
	4	186732837-186733060	*SORBS2*	Various	7	5.86E−12	5.78E−9	+
	4	206112–206442	*ZNF876P*	Exon 1	6	1.06E−8	7.08E−6	-
	22	38092643-38093079	*TRIOBP*	Intron 1	10	1.57E−9	7.90E−7	+
	20	5485144-5485294	*LINC00654*	Exon 1	5	8.51E−8	1.25E−4	-
	10	135051233-135051475	*VENTX*	Exon 1	8	2.89E−7	2.63E−4	-
	12	47225979-47226301	*SLC38A4*	Exon + Intron 1	5	2.47E−8	1.69E−5	-
	15	91473291-91473569	*UNC45A/HDDC3*	Exon	6	2.21E−7	1.75E−4	+
	17	79380493-79380585	*BAHCC1*	Intron	3	1.81E−5	4.23E−2	+
	2	239008929-239009118	*ESPNL*	Exon 1	5	1.14E−6	1.34E−3	+
	7	4848814-4848939	*RADIL*	Intron	3	3.18E−8	5.60E−5	+
	22	30476089-30476525	*HORMAD2-AS1*	Exon 1	11	3.50E−9	1.77E−6	-
TSH								
	11	7110074-7110196	*RBMXL2*	Exon 1	5	9.78E−10	1.83E−6	-
	1	247611448-247611517	*NLRP3*	Intron	3	9.90E−11	3.28E−7	+
	12	54446253-54446537	*HOXC4*	Intron 1	6	7.73E−7	6.23E−4	+
	13	36871878-36872246	CCDC169	Exon 1	9	3.39E−7	2.10E−4	-
	13	50703549-50703841	*DLEU1*	Intron	3	7.44E−8	5.83E−5	+
	20	3051954-3052345	*OXT*	Exon 1	9	1.13E−9	6.62E−7	-
	4	118006619-118006825	*TRAM1L1*	Exon 1	6	3.82E−9	4.24E−6	-
	5	150325954-150326312	*ZNF300P1*	Exon 1	8	9.70E−9	6.20E−6	-

Abbreviations: Chr, chromosome; fT3, free triiodothyronine; fT4, free thyroxine; n, number; TSH, thyrotropin (thyroid-stimulating hormone).

cg00049440 and cg04173586. Strengths of the study include use of a robust, well-characterized technology platform for detection of differential methylation of CpGs and extensive characterization of community-based cohorts. The study also has limitations. Firstly, we used whole blood to examine DNAm however methylation varies across tissue types (28); therefore, DNAm levels in the pituitary, thyroid, and peripheral tissues may differ. Secondly, we used a methylation array that targets more than 485 000 selected CpG sites; however, it does not provide the high level of coverage that would be achieved using whole-genome bisulfite sequencing. Therefore, many CpGs that exist in the genome, but which were not present on the array that we used, may be relevant to thyroid function; other approaches such as whole-genome bisulfite sequencing may be needed to fully characterize the association between thyroid hormones and DNA methylation. Thirdly, although we adjusted for major confounders in our analysis, residual confounding cannot be excluded. Finally, our study was observational; although we found significant associations between fT3 and DMPs, we cannot establish whether this reflects a causal relationship. Studies in an independent cohort are required to replicate our findings. Larger studies, with substantially increased numbers of study subjects and therefore increased statistical power are likely to identify additional sites of differential methylation associated with thyroid function, as are analytical platforms which survey more CpGs throughout the genome.

In conclusion, we describe 6 novel DMPs with reduced DNAm associated with increased levels of fT3, 2 novel DMPs with increased DNAm associated with increased levels of TSH, and 23 DMRs associated with fT3, fT4, or TSH in whole blood of healthy individuals and highlight novel candidate DMPs and genes. Further research is required to establish the roles of these loci in pituitary-thyroid axis physiology and/or thyroid hormone action and their possible relevance to health outcome and disease. Improved understanding of the relationship between methylation and thyroid function may provide therapeutic targets in the future.

Acknowledgments

The plasma samples were collected in the context of the BSGS within the Brisbane Longitudinal Twin Study 1992-2016. We thank Anjali Henders, Lisa Bowdler, and Tabatha Goncales for Biobank collection and Kerrie McAloney for collating samples for this study. We also thank Abbott Diagnostics Australia for donating immunoassay reagents. We gratefully acknowledge the participation of the twins and their families. We thank Marlene Grace, Ann Eldridge, and Kerrie McAloney for sample collection and processing; the staff of the Molecular Epidemiology Laboratory at QIMR for DNA sample processing and preparation; Harry Beeby and David Smyth for IT support; and Dale Nyholt and Scott Gordon for their substantial efforts involving the QC and preparation of the BLTS datasets.

We acknowledge the Raine Study participants and their families, the Raine Study Team for cohort co-ordination and data collection, the National Health and Medical Research Council (NHMRC) for long-term contribution to funding the study, and the Telethon Kids Institute for long-term support of the Study. We also acknowledge the University of Western Australia (UWA), Curtin University, Telethon Kids Institute, Women and Infants Research Foundation, Edith Cowan University, Murdoch University, University of Notre Dame Australia, and Raine Medical Research Foundation for providing funding for Core Management of the Raine Study.

Financial Support: This work was supported by the Australian National Health and Medical Research Council (NHMRC) (project grant 1087407). Study participants were recruited in the context of the Brisbane Longitudinal Twin Study 1992-2016, supported by grants from NHMRC (project grants 1031119, 1010374, 496667 and 1046880), the National Institutes of Health (NIH) (grants GM057091 and GM099568), Australian Research Council (A7960034, A79906588, A79801419, DP0212016, DP0343921, DP1093900) and NHMRC Medical Bioinformatics Genomics Proteomics Program (grant 389891) for building and maintaining the adolescent twin family resource through which samples were collected. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Support was also received from The Sir Charles Gairdner Osborne Park Health Care Group Research Advisory Committee (grant 2018–19/015) and the iVEC/Pawsey Supercomputing Centre (with funding from the Australian Government and the Government of Western Australia; project grants: Pawsey0260, Director2025). Abbott Diagnostics Australia donated immunoassay reagents for thyroid function tests. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

The DNA methylation work was supported by NHMRC grant 1059711. The 17-year follow-up was supported by NHMRC (program grant 353514 and project grant 403981). RCH was supported by NHMRC fellowship 14254.

Additional Information

Correspondence: Nicole Lafontaine, MBBS, BMedSci, RACP, Department of Endocrinology & Diabetes, Level 1, Building C, QEII Medical Centre, Sir Charles Gairdner Hospital, Hospital Ave, Nedlands, WA 6009, Australia. Email: nicole.lafontaine@gmail.com.

Disclosures: The authors have no disclosures.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but may be accessed through the corresponding author on reasonable request.

References

1. Siu C, Wiseman S, Gakkhar S, et al. Characterization of the human thyroid epigenome. *J Endocrinol*. 2017;**235**(2):153-165.
2. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. *J Clin Endocrinol Metab*. 2002;**87**(3):1068-1072.
3. Baumgartner C, da Costa BR, Collet TH, et al.; Thyroid Studies Collaboration. Thyroid function within the normal range, subclinical hypothyroidism, and the risk of atrial fibrillation. *Circulation*. 2017;**136**(22):2100-2116.
4. Bano A, Chaker L, Mattace-Raso FUS, et al. Thyroid function and the risk of atherosclerotic cardiovascular morbidity and mortality: the rotterdam study. *Circ Res*. 2017;**121**(12):1392-1400.
5. Chaker L, Baumgartner C, den Elzen WP, et al.; Thyroid Studies Collaboration. Thyroid function within the reference range and the risk of stroke: an individual participant data analysis. *J Clin Endocrinol Metab*. 2016;**101**(11):4270-4282.
6. Medici M, Direk N, Visser WE, et al. Thyroid function within the normal range and the risk of depression: a population-based cohort study. *J Clin Endocrinol Metab*. 2014;**99**(4):1213-1219.
7. Chaker L, Wolters FJ, Bos D, et al. Thyroid function and the risk of dementia: The Rotterdam Study. *Neurology*. 2016;**87**(16):1688-1695.
8. Nyrnes A, Jorde R, Sundsfjord J. Serum TSH is positively associated with BMI. *Int J Obes (Lond)*. 2006;**30**(1):100-105.
9. Chaker L, van den Berg ME, Niemeijer MN, et al. Thyroid function and sudden cardiac death: a prospective population-based cohort study. *Circulation*. 2016;**134**(10):713-722.
10. Panicker V, Wilson SG, Spector TD, et al. Heritability of serum TSH, free T4 and free T3 concentrations: a study of a large UK twin cohort. *Clin Endocrinol (Oxf)*. 2008;**68**(4):652-659.
11. Samollow PB, Perez G, Kammerer CM, et al. Genetic and environmental influences on thyroid hormone variation in Mexican Americans. *J Clin Endocrinol Metab*. 2004;**89**(7):3276-3284.
12. Hansen PS, Brix TH, Sørensen TI, Kyvik KO, Hegedüs L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. *J Clin Endocrinol Metab*. 2004;**89**(3):1181-1187.
13. Teumer A, Chaker L, Groeneweg S, et al.; Lifelines Cohort Study. Genome-wide analyses identify a role for SLC17A4 and AADAT in thyroid hormone regulation. *Nat Commun*. 2018;**9**(1):4455.
14. Porcu E, Medici M, Pistis G, et al. A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. *Plos Genet*. 2013;**9**(2):e1003266.
15. Taylor PN, Porcu E, Chew S, et al.; UK0K Consortium. Whole-genome sequence-based analysis of thyroid function. *Nat Commun*. 2015;**6**:5681.
16. Kuś A, Chaker L, Teumer A, Peeters RP, Medici M. The genetic basis of thyroid function: novel findings and new approaches. *J Clin Endocrinol Metab*. 2020;**105**(6):1707-1721.
17. Henikoff S, Matzke MA. Exploring and explaining epigenetic effects. *Trends Genet*. 1997;**13**(8):293-295.
18. Han L, Zhang H, Kaushal A, et al. Changes in DNA methylation from pre- to post-adolescence are associated with pubertal exposures. *Clin Epigenetics*. 2019;**11**(1):176.
19. Flanagan JM. Epigenome-wide association studies (EWAS): past, present, and future. *Methods Mol Biol*. 2015;**1238**:51-63.

20. Richard MA, Huan T, Ligthart S, et al.; BIOS Consortium. DNA methylation analysis identifies loci for blood pressure regulation. *Am J Hum Genet*. 2017;**101**(6):888-902.
21. Dor Y, Cedar H. Principles of DNA methylation and their implications for biology and medicine. *Lancet*. 2018;**392**(10149):777-786.
22. Maunakea AK, Nagarajan RP, Bilenky M, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature*. 2010;**466**(7303):253-257.
23. De La Rica L, Rodríguez-Ubreva J, García M, et al. PU.1 target genes undergo Tet2-coupled demethylation and DNMT3b-mediated methylation in monocyte-to-osteoclast differentiation. *Genome Biol*. 2013;**14**(9):R99.
24. Grönniger E, Weber B, Heil O, et al. Aging and chronic sun exposure cause distinct epigenetic changes in human skin. *Plos Genet*. 2010;**6**(5):e1000971.
25. Bjornsson HT, Sigurdsson MI, Fallin MD, et al. Intra-individual change over time in DNA methylation with familial clustering. *Jama*. 2008;**299**(24):2877-2883.
26. McGowan PO, Sasaki A, D'Alessio AC, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci*. 2009;**12**(3):342-348.
27. Tobi EW, Lumey LH, Talens RP, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum Mol Genet*. 2009;**18**(21):4046-4053.
28. Mill J, Heijmans BT. From promises to practical strategies in epigenetic epidemiology. *Nat Rev Genet*. 2013;**14**(8):585-594.
29. Michels KB. The promises and challenges of epigenetic epidemiology. *Exp Gerontol*. 2010;**45**(4):297-301.
30. Rakyan VK, Down TA, Balding DJ, Beck S. Epigenome-wide association studies for common human diseases. *Nat Rev Genet*. 2011;**12**(8):529-541.
31. Wright MJ. Brisbane adolescent twin study: outline of study methods and research projects. *Aust J Psychol*. 2004;**56**:65-78.
32. McRae AF, Powell JE, Henders AK, et al. Contribution of genetic variation to transgenerational inheritance of DNA methylation. *Genome Biol*. 2014;**15**(5):R73.
33. Powell JE, Henders AK, McRae AF, et al. The Brisbane Systems Genetics Study: genetical genomics meets complex trait genetics. *Plos One*. 2012;**7**(4):e35430.
34. Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. *Lancet*. 1993;**342**(8876):887-891.
35. Straker L, Mountain J, Jacques A, et al. Cohort profile: the Western Australian Pregnancy Cohort (Raine) Study-Generation 2. *Int J Epidemiol*. 2017;**46**(5):1384-1385j.
36. Rauschert S, Melton PE, Burdge G, et al. Maternal smoking during pregnancy induces persistent epigenetic changes into adolescence, independent of postnatal smoke exposure and is associated with cardiometabolic risk. *Front Genet*. 2019;**10**:770.
37. Li M, Eastman CJ, Waite KV, et al. Are Australian children iodine deficient? Results of the Australian National Iodine Nutrition Study. *Med J Aust*. 2006;**184**(4):165-169.
38. Campbell PJ, Brown SJ, Kendrew P, et al. Changes in thyroid function across adolescence: a longitudinal study. *J Clin Endocrinol Metab*. 2020;**105**(4):e1162-e1170.
39. illumina. Infinium® HumanMethylation450 BeadChip. Data Sheet: Epigenetics. Published 2012. Updated 09 March 2012. Accessed October 19, 2020. Pub. No. 270-2010-001. https://sapac.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_humanmethylation450.pdf
40. Saffari A, Silver MJ, Zavattari P, et al. Estimation of a significance threshold for epigenome-wide association studies. *Genet Epidemiol*. 2018;**42**(1):20-33.
41. Hannon E, Schendel D, Ladd-Acosta C, et al.; iPSYCH-Broad ASD Group. Elevated polygenic burden for autism is associated with differential DNA methylation at birth. *Genome Med*. 2018;**10**(1):19.
42. Mooney MA, Ryabinin P, Wilmot B, Bhatt P, Mill J, Nigg JT. Large epigenome-wide association study of childhood ADHD identifies peripheral DNA methylation associated with disease and polygenic risk burden. *Transl Psychiatry*. 2020;**10**(1):8.
43. Martin TC, Yet I, Tsai PC, Bell JT. coMET: visualisation of regional epigenome-wide association scan results and DNA co-methylation patterns. *BMC Bioinformatics*. 2015;**16**:131.
44. Sacks D. Age limits and adolescents. *Paediatr Child Health*. 2003;**8**(9):577-578.
45. Lafontaine Bedecarratz N. Epigenome wide association study of thyroid function traits identifies novel associations of fT3 with KLF9 and DOT1L - Supplement. The University of Western Australia 2020. Deposited November 26, 2020. doi:10.26182/2jar-e332
46. Knoedler JR, Subramani A, Denver RJ. The Krüppel-like factor 9 cistrome in mouse hippocampal neurons reveals predominant transcriptional repression via proximal promoter binding. *BMC Genomics*. 2017;**18**(1):299.
47. McConnell BB, Yang VW. Mammalian Krüppel-like factors in health and diseases. *Physiol Rev*. 2010;**90**(4):1337-1381.
48. Zhang JS, Moncrieffe MC, Kaczynski J, Ellenrieder V, Prendergast FG, Urrutia R. A conserved alpha-helical motif mediates the interaction of Sp1-like transcriptional repressors with the corepressor mSin3A. *Mol Cell Biol*. 2001;**21**(15):5041-5049.
49. Scobie KN, Hall BJ, Wilke SA, et al. Krüppel-like factor 9 is necessary for late-phase neuronal maturation in the developing dentate gyrus and during adult hippocampal neurogenesis. *J Neurosci*. 2009;**29**(31):9875-9887.
50. Dugas JC, Ibrahim A, Barres BA. The T3-induced gene KLF9 regulates oligodendrocyte differentiation and myelin regeneration. *Mol Cell Neurosci*. 2012;**50**(1):45-57.
51. Li J, Abe K, Milanesi A, Liu YY, Brent GA. Thyroid hormone protects primary cortical neurons exposed to hypoxia by reducing DNA methylation and apoptosis. *Endocrinology*. 2019;**160**(10):2243-2256.
52. Simmons CD, Pabona JM, Heard ME, et al. Krüppel-like factor 9 loss-of-expression in human endometrial carcinoma links altered expression of growth-regulatory genes with aberrant proliferative response to estrogen. *Biol Reprod*. 2011;**85**(2):378-385.
53. Qiao F, Yao F, Chen L, et al. Krüppel-like factor 9 was down-regulated in esophageal squamous cell carcinoma and negatively regulated beta-catenin/TCF signaling. *Mol Carcinog*. 2016;**55**(3):280-291.
54. Kang L, Lü B, Xu J, Hu H, Lai M. Downregulation of Krüppel-like factor 9 in human colorectal cancer. *Pathol Int*. 2008;**58**(6):334-338.

55. Sun J, Wang B, Liu Y, et al. Transcription factor KLF9 suppresses the growth of hepatocellular carcinoma cells in vivo and positively regulates p53 expression. *Cancer Lett*. 2014;**355**(1):25-33.
56. Bai XY, Li S, Wang M, et al. Krüppel-like factor 9 down-regulates matrix metalloproteinase 9 transcription and suppresses human breast cancer invasion. *Cancer Lett*. 2018;**412**:224-235.
57. Chen S, Gu S, Xu M, et al. Krüppel-like factor 9 promotes neuroblastoma differentiation via targeting the sonic hedgehog signaling pathway. *Pediatr Blood Cancer*. 2020;**67**(3):e28108.
58. Kowalik MA, Puliga E, Cabras L, et al. Thyroid hormone inhibits hepatocellular carcinoma progression via induction of differentiation and metabolic reprogramming. *J Hepatol*. 2020;**72**(6):1159-1169.
59. Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev*. 2011;**25**(13):1345-1358.
60. Wen L, Fu L, Shi YB. Histone methyltransferase Dot1L is a coactivator for thyroid hormone receptor during Xenopus development. *Faseb J*. 2017;**31**(11):4821-4831.
61. McLean CM, Karemaker ID, van Leeuwen F. The emerging roles of DOT1L in leukemia and normal development. *Leukemia*. 2014;**28**(11):2131-2138.
62. Taylor PN, Sayers A, Okosieme O, et al. Maturation in serum thyroid function parameters over childhood and puberty: results of a longitudinal study. *J Clin Endocrinol Metab*. 2017;**102**(7):2508-2515.
63. Zhang Y, Xue Y, Cao C, et al. Thyroid hormone regulates hematopoiesis via the TR-KLF9 axis. *Blood*. 2017;**130**(20):2161-2170.
64. Dorgalaleh A, Mahmoodi M, Varmaghani B, et al. Effect of thyroid dysfunctions on blood cell count and red blood cell indice. *Iran J Ped Hematol Oncol*. 2013;**3**(2):73-77.
65. Wopereis DM, Du Puy RS, van Heemst D, et al.; Thyroid Studies Collaboration. The relation between thyroid function and anemia: a pooled analysis of individual participant data. *J Clin Endocrinol Metab*. 2018;**103**(10):3658-3667.
66. Arpin C, Pihlgren M, Fraichard A, et al. Effects of T3R alpha 1 and T3R alpha 2 gene deletion on T and B lymphocyte development. *J Immunol*. 2000;**164**(1):152-160.
67. Wu Y, Byrne EM, Zheng Z, et al. Genome-wide association study of medication-use and associated disease in the UK Biobank. *Nat Commun*. 2019;**10**(1):1891.
68. Paquette MA, Dong H, Gagné R, et al. Thyroid hormone-regulated gene expression in juvenile mouse liver: identification of thyroid response elements using microarray profiling and in silico analyses. *BMC Genomics*. 2011;**12**:634.
69. Nestal De Moraes G, Carneiro L, Maia R, Lam E, Sharrocks A. FOXK2 transcription factor and its emerging roles in cancer. *Cancers (Basel)*. 2019;**11**(3):393.
70. Fernández LP, López-Márquez A, Martínez AM, Gómez-López G, Santisteban P. New insights into FoxE1 functions: identification of direct FoxE1 targets in thyroid cells. *Plos One*. 2013;**8**(5):e62849.
71. Sinha RA, Singh BK, Yen PM. Thyroid hormone regulation of hepatic lipid and carbohydrate metabolism. *Trends Endocrinol Metab*. 2014;**25**(10):538-545.
72. Ferdous A, Wang ZV, Luo Y, et al. FoxO1-Dio2 signaling axis governs cardiomyocyte thyroid hormone metabolism and hypertrophic growth. *Nat Commun*. 2020;**11**(1):2551.
73. Sakaguchi M, Cai W, Wang CH, et al. FoxK1 and FoxK2 in insulin regulation of cellular and mitochondrial metabolism. *Nat Commun*. 2019;**10**(1):1582.
74. Anttonen AK, Hilander T, Linnankivi T, et al. Selenoprotein biosynthesis defect causes progressive encephalopathy with elevated lactate. *Neurology*. 2015;**85**(4):306-315.
75. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev*. 2002;**23**(1):38-89.
76. Dumitrescu AM, Liao XH, Abdullah MS, et al. Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. *Nat Genet*. 2005;**37**(11):1247-1252.
77. Guo Q, Wu Y, Hou Y, et al. Cytokine secretion and pyroptosis of thyroid follicular cells mediated by enhanced NLRP3, NLRP1, NLRC4, and AIM2 inflammasomes are associated with autoimmune thyroiditis. *Front Immunol*. 2018;**9**:1197.
78. Vargas R, Videla LA. Thyroid hormone suppresses ischemia-reperfusion-induced liver NLRP3 inflammasome activation: Role of AMP-activated protein kinase. *Immunol Lett*. 2017;**184**:92-97.
79. Agnihothri RV, Courville AB, Linderman JD, et al. Moderate weight loss is sufficient to affect thyroid hormone homeostasis and inhibit its peripheral conversion. *Thyroid*. 2014;**24**(1):19-26.
80. Economidou F, Douka E, Tzanela M, Nanas S, Kotanidou A. Thyroid function during critical illness. *Hormones (Athens)*. 2011;**10**(2):117-124.