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A Common variation in Deiodinase 1 gene *DIO1* is associated with the relative levels of free thyroxine and triidothyronine.

Short title: Common variation in *DIO1* is associated with free T3/T4 ratio

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Abstract

Introduction: Genetic factors influence circulating thyroid hormone levels but the common gene variants involved have not been conclusively identified. The genes encoding the iodothyronine deiodinases are good candidates as they alter the balance of thyroid hormones. We aimed to thoroughly examine the role of common variation across the three deiodinase genes in relation to thyroid hormones.

Methods: We used HapMap data to select single nucleotide polymorphisms that captured a large proportion of the common genetic variation across the three deiodinase genes. We analyzed these initially in a cohort of 552 people on thyroxine replacement. Suggestive findings were taken forward into three additional studies in people not on thyroxine (total n=2,513) and meta-analyzed for confirmation.

Results: A single nucleotide polymorphism (SNP) in the *DIO1* gene, rs2235544, was associated with the free T3 to free T4 ratio with genome wide levels of significance ($p=3.6x10^{-13}$). The C-allele of this SNP was associated with increased D1 function with resulting increase in free T3/T4 ratio and free T3, decrease in free T4 and reverse T3. There was no effect on serum TSH levels. None of the SNPs in the genes coding for D2 or D3 had any influence on hormone levels.

Conclusions: This study provides convincing evidence that common genetic variation in *DIO1* alters deiodinase function resulting in an alteration in the balance of circulating free T3 to free T4. This should prove a valuable tool to assess the relative effects of circulating free T3 versus free T4 on a wide range of biological parameters.

Introduction

There is known to be a genetic component to circulating thyroid hormone levels (1, 2), but the common gene variants involved have not been conclusively identified.

The three deiodinase enzymes (D1, D2 and D3) play a vital role in maintaining euthyroidism both at a serum and local tissue level (3). Their action is important in maintaining different levels of thyroid hormone activity in different tissues, in different disease states and at different stages of development. They achieve this by altering the balance of the thyroid hormones between the active hormone 3,5,3'-triiodothyronine (T3), the inactive pro-hormone thyroxine (T4) which can be activated by conversion to T3, and the inactivated metabolites 3,3',5'- triiodothyronine (reverse T3, rT3) and diiodothyronine (T2).

D1 and D2 are predominantly activating enzymes; both convert T4 to T3 (and rT3 to T2) by outer ring deiodination. D1 is found in liver, kidney, thyroid and pituitary in humans, and D2 in skeletal muscle, CNS, pituitary, thyroid, heart and brown adipose tissue (3). Predictions based on isolated cell deiodinase activity and reported tissue activities in humans suggest that both are responsible for maintaining serum levels of T3, although D2 predominates in hypothyroidism and D1 in hyperthyroidism (4). D3 inactivates thyroid hormones by inner ring deiodination, converting T3 to T2 and T4 to rT3. It has been found in the CNS and placenta in adults, and in many additional tissues in the fetal state (3). A more detailed description of deiodinase action can be found in recent reviews (3, 5).

Previous studies have suggested that genetic variation in the deiodinase enzyme encoding genes may influence thyroid hormone levels and ratios (6-8). The most comprehensive of these studies, of 4 Single Nucleotide Polymorphisms (SNPs) in 995 elderly Dutch individuals, found the strongest association between rs11206244 in the *DIO1* gene and T3/rT3 ratio ($p<0.001$), and weaker associations with T3 ($p=0.004$), free T4 ($p=0.04$) and rT3 ($p=0.03$) (6). Further evidence is required to assess more comprehensively the role of common variation in the *DIO1* genes. It is now widely accepted that genetic association studies need to cover a large proportion of variation across a gene and that levels of statistical confidence need to exceed $p<5x10^{-7}$. None of these variants have been shown to influence TSH levels, although TSH secretion is controlled by negative feedback of T4 and T3 on the hypothalamus and pituitary. It should also be noted that unlike thyroid hormone receptors, there are no known individuals with inactivating mutations of the deiodinase genes, and that deiodinase knockout mice display quite mild phenotypes (9, 10). This means our understanding of the importance of genetic variation in the deiodinase genes in humans is limited.

In this study we aimed to thoroughly assess the role of common variation in the deiodinase genes in relation to thyroid hormone levels and the relationship of circulating concentrations of free T3 (fT3) to free T4 (fT4). We selected SNPs that covered a large proportion of common variation across the three *DIO* genes. We initially examined the role of this variation in a study of individuals with little or no thyroidal production of fT3 on thyroxine replacement therapy as we would expect functional variants to have greatest effect in this population. We then examined associations in three additional studies of individuals without known thyroid disease. We show that a common variant in intron 3 of the *DIO1* gene, rs2235544 is a clear genetic marker associated with changes in the conversion of fT4 to fT3 but without altering TSH levels.

Subjects and Methods

Details of the studies used are given in **Table 1**. The initial analysis was performed on the WATTS (Weston Area T3/T4 Study) cohort of 552 individuals with thyroid disease being treated with thyroxine. We followed up SNPs reaching suggestive levels of significance ($p<0.05$) in three further studies consisting of 3,424 individuals (including 911 pregnant

females): two population studies EFSOCH (Exeter Family Study of Childhood Health) and InCHIANTI (Invecchiare in Chianti); and a study of people being tested for abnormal thyroid function on the basis of clinical indication by their GPs - DEPTH (DEPression and THyroid disease). In all cohorts except WATTS individuals with any history of thyroid disease or a TSH outside the range of 0.05 – 10.0 mU/l were excluded, as were individuals taking medications known to alter thyroid hormone levels (amiodarone, lithium, carbimazole, methimazole, propylthiouracil). All individuals were of white European ancestry.

Subjects/Cohorts

WATTS

The WATTS cohort consists of people on thyroxine replacement, recruited from GP practices in the Bristol and Weston-super-Mare areas in the West of England between March 2000 and June 2002. Entry criteria have been previously published (11). They were involved in a study on the benefits of combined T3/T4 therapy; however in this study only baseline data was used (on a stable dose of T4 only). The study was approved by the local research ethics committees in Bristol.

EFSOCH

The EFSOCH study is a prospective study of birth weight and early postnatal growth. Blood was taken from mothers, fathers and offspring who lived in the central part of Exeter, Devon, UK. All mothers in the study were pregnant at the time of blood sampling. Therefore the fathers and pregnant mothers were analyzed separately, as pregnancy has well known effects on thyroid hormone levels, and may also have effects on deiodinase function. Study design and protocol have been described in detail previously (12).

InCHIANTI

The InCHIANTI study is a representative, population based sample of residents from two small towns near Florence, Italy, initially recruited to investigate risk factors for the onset of disability in older persons. It included 298 subjects aged <65 years and 1155 aged ≥ 65 years and data collection took place between September 1998 and March 2000. The study design and protocol have been described in detail previously (13). The study was approved by the INRCA ethical committee.

DEPTH

The Depth study is a prospective study to investigate the relationship between thyroid function and mood in a population with no known thyroid disease, presenting to primary care. Participants comprised patients not on thyroxine and with no previously documented thyroid abnormality in whom the general practitioner considered that thyroid function testing was clinically indicated. It included five GP practices in Bristol, UK and data collection took place between September 2005 and July 2007. Participants had a venous blood sample taken for thyroid function analysis, DNA extraction and filled out two questionnaires with mood assessments, the 12 question version of the General Health Questionnaire (GHQ-12) and the Patient Health Questionnaire (PHQ). The study was approved by the local research ethics committees in Bristol.

Biochemical measurements

WATTS

Serum TSH and fT4 were measured from a serum sample by RIA (Diagnostic product Corp. Los Angeles, CA). Free T3 was measured by chemiluminescence assay (Elecsys system 1010; Roche diagnostics, Mannheim, Germany). The laboratory reference ranges were TSH 0.3-4.0 mU/l, fT4 10-24 pmol/l and fT3 2.8-7.1 pmol/l. Coefficients of variation (CVs) were: TSH 5.5-8.0%, fT4 7.7-10.0% and fT3 11.7-12.6%. Reverse T3 was measured by an in-house RIA (Erasmus University Medical Centre) (14).

EFSOCH

Serum TSH, fT4, and fT3 were analysed using the electrochemiluminescent immunoassay, run on the Modular E 170 Analyzer (Roche, Burgess Hill, UK). The manufacturers' population

reference ranges were: TSH 0.35-4.5 mU/l, fT4 11-24 pmol/l and fT3 3.9-6.8 pmol/l. The between batch coefficient of variations (CVs) were: TSH 5.4%, fT4 7% and fT3 4.2%.

InCHIANTI

TSH, fT4 and fT3 were measured in EDTA plasma using chemiluminescent assays (Vitros TSH Reagent, Ortho-Clinical Diagnostics, Johnson & Johnson Medical S.p.A Section). Reference normal ranges were: TSH 0.46-4.68 mIU/l, fT4 10-28 pmol/l, and fT3 4.3 to 8.1 pmol/l. Intra-assay CVs were: TSH < 5.3%, fT4 <5.3% and fT3 <5.1%.

DEPTH

Serum TSH and free T4 were measured from serum samples by chemiluminescent immunometric assay on the Immulite 2000 (Siemens). Free T3 was measured by electrochemiluminescence immunoassay on the Elecsys 1010 (Roche diagnostics, Mannheim, Germany). The laboratory reference ranges were TSH: 0.3 – 4.0 mU/l, fT4: 10-24 pmol/l, fT3: 2.8-7.1 pmol/l. Intra-assay CVs were: TSH <6.3%, fT4 <7.5%, and fT3 <2.2%.

Tag SNP selection and genotyping

We used genotype data from the European individuals in the International Haplotype Mapping Project (http://www.hapmap.org), to select a set of Single Nucleotide Polymorphisms that capture the majority of common variation across the three deiodinase genes (*DIO1*, *DIO2* and *DIO3*) including 50kb either side of the genes. We used a minor allele frequency (MAF) of at least 10%. The 21, 7 and 7 SNPs in the *DIO1*, *DIO*2 and *DIO3* genes required 9, 4 and 6 SNPs respectively to capture all common variants with an r^2 >0.8. These were, for D1: rs11206237, rs11206244, rs2235544, rs2268181, rs2294511, rs2294512, rs4926616, rs731828 and rs7527713, for D2: rs12885300, rs225011, rs225014 and rs225015 and for D3: rs1190716, rs17716499, rs7150269, rs8011440, rs945006 and rs1190715. In previous studies, rs11206244 has also been referred to as D1a-C/T and rs225014 has been referred to as D2-Thr92Ala (6, 8).

Genotyping for the WATTS and Depth cohorts was performed by KBiosciences (http://www.kbioscience.co.uk) using their own novel fluorescence-based competitive allele-specific PCR (KASPar). Assays were designed for each of the 19 SNPs by KBiosciences. Design of an assay for the SNP rs1190715 was not possible; hence genotyping was performed on 18 SNPs only. In WATTs, two further SNPs, rs1190716 and rs12885300, failed QC because of a high number of duplicate errors (>1%) and deviation from Hardy Weinberg equilibrium ($p<0.05$) respectively. The percentage of duplicate samples included was 20%. Concordance between duplicate samples for the 16 SNPs used was ≥ 99%. Use of these 16 SNPs resulted in coverage of 100%, 85% and 71% of the common (MAF>10%), HapMap based, variation in *DIO1, DIO2* and *DIO3* respectively at r^2>0.8.

Genotyping on the EFSOCH cohort was performed in-house (Peninsula Medical School) using TaqMan SNP genotyping assay (Applied Biosystems) according to the manufacturer's protocol. The percentage of duplicate samples included was 12%. Concordance between duplicate samples was ≥ 99% for rs2235544.

Genotyping for the InCHIANTI cohort was performed as part of a genome wide association study using the Illumina Infinium HumanHap550 genotyping chip. Samples were initially assessed for genotype success rate (>98%) and concordance of reported and genotype-based gender.

Statistical analysis

We used a log transformation for serum TSH but fT3, rT3 and fT4 did not require normalisation. To detect an association between thyroid parameters and genotypes linear regression was used, with log TSH, fT4, fT3 or rT3 as the dependent variables and genotype (as 0, 1 or 2 alleles) as the independent. The analysis was performed both unadjusted, and using age and

sex as covariates. Analyses were performed using STATA version 9.0 (StataCorp).

Meta-analysis was performed using an inverse variance weighting method as implemented using the metan command of STATA v9.0. We tested for heterogeneity using the Q statistics and I^2 metric as implemented in STATA v9.0.

Results

Association of deiodinase gene variation in patients on thyroxine replacement therapy

We first tested deiodinase gene variants in 552 individuals from the WATTS study. These individuals are hypothyroid with little or no endogenous thyroid hormone production and are treated with thyroxine. They produce little or no thyroidal T3 and are therefore an ideal group to assess function of enzymes that convert fT4 to fT3. Sixteen of the 18 SNPs were successfully genotyped and passed quality control criteria in the WATTs study. Two SNPs in the *DI01* gene, rs2235544 and rs11206244, were associated with fT3/fT4 ratio at p<0.01 (**Table 2**). The same SNPs were also associated with individual fT3, rT3 and fT4 levels but not TSH levels (see **Table 3 and supplementary Tables 1a to 1d**).

There were no associations at p<0.01 in the *DIO2* or *DIO3* genes with any of the ratio or individual thyroid hormone measures. There was evidence of linkage disequilibrium (LD) between these SNPs within our data, strongest between rs2294511 and rs4926616 (r^2=0.49), and rs2235544 and rs11206244 (r^2 =0.41). All other pairwise LD correlations within the DIO1 gene were r^2<0.16. The LD structure of the *DIO1* gene and associated statistics are shown in **Figure 1**.

Associations in population based samples

To provide more robust evidence, and to assess whether associations were specific to patients with thyroid disease or were present in the general population, we followed up SNPs associated at p<0.01 in males and pregnant females from a second study. The associations between rs2235544 and rs11206244 and fT3/fT4 ratio were confirmed in the EFSOCH males ($p=1.3x10^{-6}$) with the same allele A, being associated with decreased ratio (**supplementary Table 2**). There was no evidence of association in the pregnant females (p=0.07), in whom thyroid hormone levels were measured at 28 weeks gestation.

Given the LD (r^2=0.41) between rs2235544 and rs11206244, we performed conditional analysis including both SNPs in the regression model for fT3/fT4 ratio in both WATTs and EFSOCH males and meta-analyzed the two results. This revealed that rs2235544 was driving the association ($p=6.8x10^{-4}$ conditioning on rs11206244) rather than rs11206244 (p=0.46 conditioning on rs2235544).

The association between the A allele of rs2235544 and reduced fT3/fT4 ratio was further confirmed in the InCHIANTI study of healthy older males and females (n= 1200, $p=1.5x10^{-7}$) and was consistent in the smaller Depth study of predominantly females (n= 436, p=0.24). The association of rs2235544 with fT3/fT4 ratio and individual hormone levels in all studies is summarized in **Table 3**. The fT3/fT4 ratio in the WATTS population is significantly lower than in the populations with normal thyroid function, this is consistent with previous findings in patients on thyroxine therapy who have a higher fT4 and lower fT3 for the same TSH due to lack of thyroid T3 production (15-17). A meta-analysis of the population based studies, excluding pregnant females, showed strong evidence for an association in the general population ($p=3.6x10^{-13}$), with no evidence of heterogeneity (p = 0.13, I^2=50%). Each copy of the C-allele was associated with an increase in the fT3/fT4 ratio of approximately 0.2 standard deviations. FT3/fT4 ratios by genotype are shown in **Figure 2**. By contrast, there was no association of this allele with TSH either in any individual study or in a meta-analysis of all studies (p=0.90 – **Table 3**). There was no difference in the effect size between non-pregnant females and males (p>0.05).

Discussion

Our results show that common variation, in the *DIO1* gene, best tagged by rs2235544, is associated with circulating fT3/fT4 ratio both in a population of people on thyroid hormone replacement and in the general population. In contrast to previous studies we have assessed a large proportion of the common variation across the *DIO* genes and, importantly, the statistical confidence of our finding ($p=3.6x10^{-13}$) exceeds that necessary for multimarker genetic association studies (18). Our study therefore provides robust evidence that a common variant can affect serum thyroid hormone levels.

The effect of rs2235544 on thyroid hormone levels is consistent with the C-allele being associated with higher deiodinase enzymatic activity (increased fT3/fT4 ratio and fT3, decreased fT4 and rT3). This association is mainly driven by the strong association with decreased fT4 (see Table 3). This association was present both in subjects on thyroxine replacement, with no endogenous T3 production, and those with normal thyroid function, suggesting that a significant amount of serum T3 is derived from peripheral deiodination of T4 in both populations. These findings are consistent with those from the D1 knockout mouse, in which absent D1 activity results in raised fT4 and rT3 serum levels with a decreased fT3/fT4 ratio and no effect on fT3 or TSH (10) (resembling the effect of the A-allele if this is associated with decreased D1 activity). Previous studies identified putative associations between rs11206244 and fT4, fT3 and rT3 levels but at much lower levels of statistical confidence (6, 8). This SNP is partially correlated with rs2235544 but conditional analyses clearly showed rs2235544 is driving the association. Rs2235544 occurs in intron 3 of the *DIO1* gene. There are no SNPs in HapMap in the coding region of *DIO1* that are strongly correlated with rs2235544. There are also no SNPs within 100kb of the *DIO1* gene that are associated with altered *DIO1* expression levels in the recently published genome wide study of expression levels in lymphocytes (19). This means the mechanism by which this SNP or one in LD with it affects deiodinase function remains unknown. Taking into account the heritability estimates of twin studies for serum fT4 (39-65%) and fT3 (23-64%) (1, 2) we estimate, using the regression model, that rs2235544 is responsible for approximately 2% of genetic variance of fT4 and 1.5% of fT3.

No association was observed between any of the SNPs in *DIO2* or *DIO3* and circulating concentrations of thyroid hormone levels in our cohort of patients on thyroxine and this is consistent with several previous studies in patients not on thyroxine (6, 20, 21). This implies either that none of the common SNPs we have studied in these genes are linked to changes in enzyme function, or that these enzymes (D2 in particular) have a smaller role in determining serum (as opposed to intracellular) thyroid hormone levels than was previously thought (3, 4, 22). One of the three *DIO2* SNPs we studied, rs225014, has previously been reported to be associated with reduced enzyme activity in thyroid and skeletal muscle samples (23) although the effect was not apparent in transfected cell lines (24) and phenotype associations with *DIO2* SNPs have been inconsistent in other reports (6, 23, 25, 26). Hence further evidence is required of the functional association of common variation in the *DIO2* and *DIO3* genes before we can interpret the lack of association with serum thyroid hormone levels. Studies of the effects of a common variation in *DIO3* in pregnant women (not studied here) would also be of interest in view of the high levels of D3 expression in placental tissue.

Discovery of a marker which alters the ratio of circulating T3:T4 may help us answer important questions about thyroid hormone action in humans. T3 is the active hormone that binds to thyroid hormone receptors. However the local deiodination of T4 to intracellular T3 by the D2 enzyme means that circulating concentrations of both T3 and T4 levels contribute to intracellular T3 levels. Importantly, wide variation in the levels of deiodinase expression (and possibly also of thyroid hormone transporters (27)) between tissues results in wide variation in the relative contribution of the circulating concentrations of these two hormones to thyroid

hormone action. In rodent studies for example, it is estimated that serum T3 contributes 87% of intracellular T3 in the kidney but only 50% in the pituitary and just 20% in the cerebral cortex, the remainder coming from local deiodination of serum T4 (3). Hence, variation in the ratio of T3 to T4 in the circulation is likely to have different effects in different tissues, with potential impact on a wide range of important biological parameters such as body weight, serum lipids, heart rate, bone metabolism, central nervous systems development and psychological well-being. However, very large samples are likely to be required for robust exploration of these effects. To assess the effects of a SNP that alters fT3/fT4 ratio on secondary traits, power calculations need to consider both the correlation between fT3/fT4 ratio and the correlation between fT3/fT4 ratio and the secondary trait (28). For example, rs2235544 is associated with a ~0.2 standard deviation difference in fT3/fT4 ratio per allele. FT3/fT4 ratio is correlated with BMI in published studies with an r of approximately 0.28 (29). This means studies of the effects of rs2235544 on BMI need to include approximately 15,000 individuals to be powered to detect the expected effect of 0.056 (0.2 x 0.28) standard deviations BMI change per allele (at p=0.01 and 80% power).

The very clear lack of an association between rs2235544 and circulating concentrations of TSH across all our cohorts, despite its effects on the fT3/fT4 ratio, is interesting. It implies that rs2235544 can be used as a tool to explore the effects of changes in the balance of circulating T3 and T4 on different tissues in the absence of an increase in overall thyroid hormone production from the thyroid. It is also consistent with the observation that TSH levels correlate as well if not better with serum fT4 than fT3 in patients on thyroxine (11) and implies that for the pituitary the rise in circulating concentrations of fT3 compensates for the fall in fT4 associated with the C-allele of rs2235544. The pituitary itself does express D1 (3), although D2 may predominate in the feedback pathway

In conclusion our study provides the most convincing example of common DNA variation that alters circulating thyroid hormone ratios in the general population. This variant may be useful in dissecting causal relationships between thyroid hormone levels and related traits in different tissues.

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Figure legends.

Figure 1. Linkage Disequilibrium structure and associations in WATTS diagram of the *DIO1* gene.

Figure 2. FT3/fT4 ratio by genotype of rs2235544. Plot of the mean and 95% confidence intervals of fT3/fT4 ratio in the four studies.

Tables and Figures

Table 1 – Baseline characteristics

Characteristic	WATTS	EFS males	EFS pregnant females*	InCHIANTI	Depth
Number	552	877	911	1200	436
No female (%)	460 (83.3)	-	-	662 (55.2)	335 (76.8)
age (years)	57.2 ± 10.8	32.7 ± 5.9	30.4 ± 5.2	68.4 ± 15.5	43.7 ± 15.0
BMI (kg/m2)	29.2 ± 61	26.6 ± 3.8	24.0 ± 4.6	27.1 ± 4.1	-
free T4 (pmol/l)	21.1 ± 3.7	16.5 ± 2.1	12.2 ± 1.6	18.7 ± 4.6	16.5 ± 7.0
free T3 (pmol/l)	3.84 ± 0.72	5.51 ± 0.6	4.2 ± 0.5	6.63 ± 1.0	4.67 ± 0.72
TSH (mU/l) median (IQR)	0.91 (0.29, 2.08)	1.82 (1.33, 2.53)	1.85 (1.35, 2.50)	1.3 (0.84, 2.00)	1.5 (1.0, 2.4)

Mean ± SD unless otherwise specified
*BMI in pregnant females is based on self reported pre-pregnancy weight

Table 2 – Free T3 /free T4 ratio by genotype in WATTs cohort

SNP	common homozygous		heterozygous		minor homozygous		p for trend	
DIO1	mean free T3/free T4 (95%CI)	n	mean freeT3/free T4 (95%CI)	n	mean freeT3/free T4 (95%CI)	n	unadj.	adj.[a]
rs11206237	0.187 (0.183, 0.192)	399	0.187 (0.180, 0.195)	130	0.192 (0.177, 0.207)	16	0.82	0.96
rs11206244	**0.193 (0.187, 0.199)**	**239**	**0.186 (0.181, 0.192)**	**238**	**0.175 (0.166, 0.185)**	**69**	**0.005**	**0.004**
rs2235544	**0.177 (0.171, 0.184)**	**143**	**0.189 (0.184, 0.195)**	**288**	**0.196 (0.187, 0.205)**	**111**	**0.001**	**0.001**
rs2268181	0.187 (0.182, 0.192)	387	0.189 (0.182, 0.196)	140	0.192 (0.178, 0.205)	19	0.63	0.88
rs2294511	0.192 (0.186, 0.197)	240	0.185 (0.179, 0.190)	248	0.183 (0.171, 0.195)	56	0.08	0.08
rs2294512	0.186 (0.180, 0.191)	252	0.186 (0.180, 0.191)	245	0.205 (0.190, 0.220)	50	0.06	0.02
rs4926616	0.189 (0.183, 0.194)	245	0.188 (0.182, 0.194)	236	0.186 (0.174, 0.197)	56	0.67	0.68
rs731828	0.185 (0.179, 0.191)	183	0.189 (0.183, 0.194)	278	0.190 (0.179, 0.201)	84	0.32	0.19
rs7527713	0.187 (0.182, 0.192)	362	0.189 (0.182, 0.196)	157	0.184 (0.170, 0.198)	26	0.96	0.75
DIO2								
rs225011	0.184 (0.177, 0.190)	172	0.189 (0.183, 0.195)	264	0.190 (0.182, 0.198)	107	0.22	0.24
rs225014	0.186 (0.180, 0.192)	223	0.187 (0.181, 0.193)	236	0.193 (0.184, 0.203)	87	0.25	0.28
rs225015	0.187 (0.181, 0.193)	237	0.187 (0.181, 0.193)	236	0.193 (0.183, 0.204)	71	0.42	0.46
DIO3								
rs17716499	0.191 (0.185, 0.196)	202	0.184 (0.178, 0.189)	248	0.193 (0.183, 0.202)	95	0.89	0.97
rs7150269	0.191 (0.184, 0.197)	169	0.186 (0.180, 0.192)	254	0.187 (0.179, 0.196)	121	0.48	0.50
rs8011440	0.190 (0.184, 0.195)	215	0.186 (0.180, 0.192)	246	0.188 (0.178, 0.199)	85	0.65	0.54
rs945006	0.187 (0.183, 0.192)	438	0.187 (0.178, 0.196)	92	0.200 (0.156, 0.243)	10	0.69	0.55

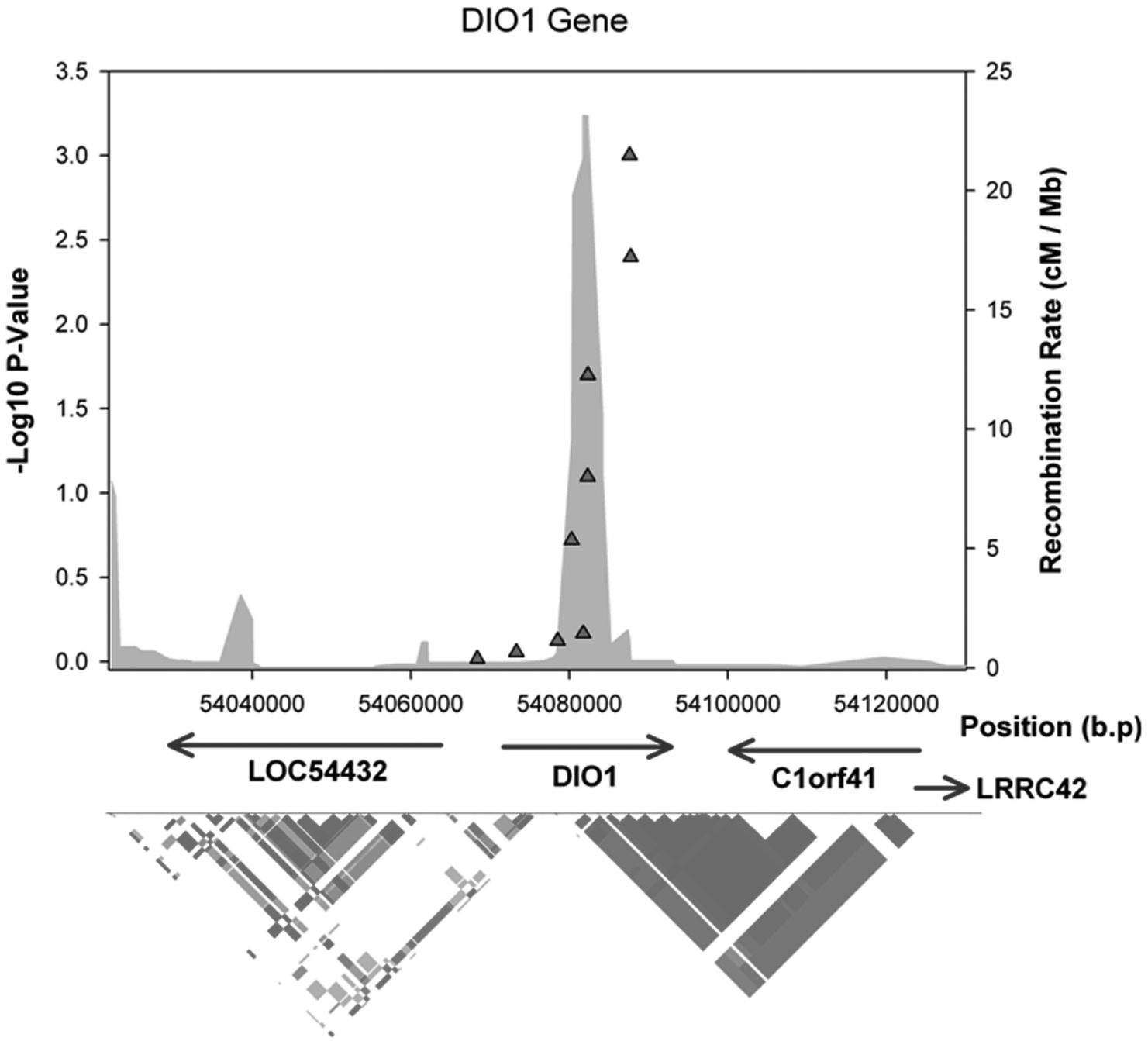
[a] – adjusted for age and sex

Table 3 - Relationships between rs2235544 and thyroid function in all studies, EFS_M = EFSOCH males, EFS_F = EFSOCH females.

Measure	Study	AA		AC		CC					
		mean (95%CI)	n	mean (95%CI)	n	mean (95%CI)	n	B coeff	SE	p for trend*	Meta-analysis p value**
fT3/fT4	Watts	0.18 (0.17, 0.18)	143	0.19 (0.18, 0.19)	288	0.20 (0.19, 0.21)	111	0.009	0.003	0.001	
	EFS_M	0.32 (0.32, 0.33)	224	0.34 (0.33, 0.34)	430	0.35 (0.34, 0.36)	201	0.011	0.002	1.3*10-6	
	InChianti	0.35 (0.34, 0.36)	244	0.37 (0.36, 0.37)	533	0.38 (0.37, 0.39)	320	0.015	0.003	1.5*10-7	
	Depth	0.29 (0.28, 0.30)	116	0.30 (0.29, 0.31)	192	0.30 (0.29, 0.31)	115	0.005	0.004	0.244	
											3.6x10-13
	EFS_F	0.34 (0.34, 0.35)	236	0.35 (0.35, 0.36)	450	0.35 (0.34, 0.36)	210	0.005	0.003	0.072	
free T3	Watts	3.75 (3.65, 3.85)	143	3.88 (3.80, 3.96)	288	3.91 (3.76, 4.07)	111	0.076	0.045	0.092	
	EFS_M	5.43 (5.36, 5.49)	225	5.53 (5.47, 5.58)	431	5.55 (5.46, 5.64)	201	0.053	0.027	0.05	
	InChianti	6.51 (6.42, 6.60)	244	6.58 (6.52, 6.64)	533	6.57 (6.49, 6.65)	320	0.034	0.030	0.261	
	Depth	4.66 (4.52, 4.79)	116	4.71 (4.61, 4.81)	193	4.65 (4.53, 4.78)	115	-0.015	0.045	0.750	
											0.058
	EFS_F	4.15 (4.08, 4.22)	237	4.20 (4.15, 4.24)	449	4.20 (4.13, 4.27)	209	0.029	0.024	0.226	
free T4	Watts	21.7 (21.1, 22.3)	143	21.1 (20.6, 21.5)	288	20.4 (19.7, 21.0)	111	-0.631	0.231	0.007	
	EFS_M	16.9 (16.6, 17.2)	224	16.5 (16.4, 16.7)	431	16.1 (15.9, 16.4)	201	-0.408	0.101	$5.8x10^{-5}$	
	InChianti	19.4 (18.8, 20.0)	244	18.5 (18.2, 18.9)	533	17.9 (17.5, 18.3)	324	-0.772	0.162	$2.1x10^{-6}$	
	Depth	16.5 (15.9, 17.1)	116	16.8 (15.4, 18.2)	193	15.9 (15.4, 16.4)	115	-0.361	0.464	0.440	
											2.1x10-9
	EFS_F	12.2 (12.0, 12.5)	236	12.1 (12.0, 12.3)	450	12.1 (11.9, 12.4)	209	-0.044	0.076	0.561	
TSH	Watts	0.79 (0.61, 1.03)	143	0.65 (0.55, 0.78)	288	0.67 (0.53, 0.85)	111	0.032	0.040	0.510	
	EFS_M	1.31 (1.27, 1.36)	220	1.29 (1.26, 1.31)	427	1.31 (1.27, 1.35)	200	-0.0008	0.011	0.938	
	InChianti	1.24 (1.13, 1.35)	234	1.25 (1.18, 1.33)	516	1.35 (1.25, 1.46)	311	0.046	0.030	0.127	
	Depth	2.81 (2.10, 3.70)	116	2.77 (2.25, 3.42)	193	2.15 (1.67, 2.77)	115	-0.056	0.042	0.180	
											0.90
	EFS_F	1.28 (1.24, 1.33)	236	1.29 (1.26, 1.32)	448	1.31 (1.28, 1.35)	208	0.010	0.011	0.357	

*Adjusted for sex and age.

**Excludes EFS females and WATTs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Recombination Rate (cM / Mb)
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2.5
2.0
1.5
1.0
0.5
0.0
25
20
15
10
5
0
54040000
54060000
54080000
54100000
54120000
Position (b.p)
LOC54432
DIO1
C1orf41
LRRC42

Figure 2 – fT3/fT4 ratio (and 95% Confidence intervals) by genotype rs2235544 in the 4 studies