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CLINICAL STUDY

A meta-analysis of the associations between common variation in the *PDE8B* gene and thyroid hormone parameters, including assessment of longitudinal stability of associations over time and effect of thyroid hormone replacement

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Abstract

Objective: Common variants in *PDE8B* are associated with TSH but apparently without any effect on thyroid hormone levels that is difficult to explain. Furthermore, the stability of the association has not been examined in longitudinal studies or in patients on levothyroxine (L-T_4).

Design: Totally, four cohorts were used ($n=2557$): the Busselton Health Study (thyroid function measured on two occasions), DEPTH, EFSOCH (selective cohorts), and WATTS (individuals on L-T_4).

Methods: Meta-analysis to clarify associations between the rs4704397 single nucleotide polymorphism in *PDE8B* on TSH, tri-iodothyronine (T_3), and T_4 levels.

Results: Meta-analysis confirmed that genetic variation in *PDE8B* was associated with TSH ($P=1.64\times10^{-10}$ 0.20 S.D./allele, 95% confidence interval (CI) 0.142, 0.267) and identified a possible new association with free T_4 ($P=0.023$, −0.07 S.D./allele, 95% CI −0.137, −0.01), no association was seen with free T_3 ($P=0.218$). The association between *PDE8B* and TSH was similar in 1981 (0.14 S.D./allele, 95% CI 0.04, 0.238) and 1994 (0.20 S.D./allele, 95% CI 0.102, 0.300) and even more consistent between PDE8B and free T_4 in 1981 (−0.068 S.D./allele, 95% CI −0.167, 0.031) and 1994 (−0.07 S.D./allele, 95% CI −0.170, 0.030). No associations were seen between *PDE8B* and thyroid hormone parameters in individuals on L-T_4.

Conclusion: Common genetic variation in *PDE8B* is associated with reciprocal changes in TSH and free T_4 levels that are consistent over time and lost in individuals on L-T_4. These findings identify a possible genetic marker reflecting variation in thyroid hormone output that will be of value in epidemiological studies and provides additional evidence that PDE8B is involved in TSH signaling in the thyroid.

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Introduction

Variation in thyroid function within the reference range is associated with important differences in key biological outcomes in the general population. These include body mass index, lipid levels, blood pressure, childhood development, and risk of fatal ischemic heart disease events (1–5). Although nominally informative, these studies have all relied upon measurements of serum thyroid hormone levels taken on a single occasion. This approach may not represent an individual's lifetime exposure and is also susceptible to confounding factors (6, 7). For example, cigarette smoking is associated with reduced TSH concentrations, (8) making it difficult to accurately assess the effect of thyroid dysfunction on cardiovascular disease (5).

Genetic association studies represent an alternative method to study life course differences in the thyroid

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hormone pathway and phenotypic outcomes. The genetic approach avoids confounding and reverse causation effects present in traditional epidemiological studies. It may also better reflect lifetime exposure to thyroid hormones than a single serum measurement. For the genetic approach to be practical, robust genetic associations with thyroid hormone levels need to be established.

At present, the genetic and temporal architecture of this important trait remains incompletely understood. Genomewide association studies (GWAS) and candidate gene studies have identified single nucleotide polymorphisms (SNPs) in a number of genes that have been associated with variance in TSH levels or the free thyroxine (T_4)/free tri-iodothyronine (T_3) ratio in the normal population (9–11). However, to date, no genetic variant has been confirmed at a high level of statistical significance to be associated with the overall thyroid function set point. Furthermore, no data exist on the temporal stability of genetic associations with thyroid function over time. Both these elements are key in determining whether a genetic marker can be found that is a valuable surrogate for lifetime thyroid hormone exposure.

Arnaud-Lopez *et al.* (10) identified that variation in rs4704397 SNP in *PDE8B* was associated with altered TSH levels in the reference range. This initial study, however, did not include free T_3 and free T_4 levels. As *PDE8B* encodes a cAMP phosphodiesterase enzyme (12), strongly expressed in the thyroid gland (13–15) but undetectable in the pituitary gland (16), it was postulated that its influence on thyroid hormone parameters was via the hydrolysis and inactivation of cAMP in the thyroid in response to TSH signaling. This work raises the possibility that variation in *PDE8B* influences the thyroid hormone set point by making the thyroid less responsive to TSH, equivalent to a mild degree of thyroid failure resulting in higher TSH levels. Such a mode of action would be expected to also result in changes in thyroid hormone levels, in a reciprocal direction to the change in TSH.

Intriguingly, a recent study of 1017 pregnant women (17) replicated the association of this SNP with TSH levels but failed to find any evidence of association with free T_3 and free T_4 levels. However, due to the log-linear relationship between TSH and thyroid hormone levels, small changes in thyroid hormone levels result in relatively large changes in TSH; this study might, therefore, have been underpowered to detect associations between *PDE8B* and free T_3 and free T_4 levels.

In this study, we undertook a meta-analysis to investigate the association between variation at rs4704397 SNP in *PDE8B* on free T_3 and free T_4 levels in the general population and in individuals on thyroid hormone replacement and assessed the temporal stability of its effects in the Busselton cohort (18). As a control, we also studied the temporal stability of the rs2235544 SNP in the deiodinase 1 (*DIO1*) gene which our group previously identified was associated with altered free T_3/free T_4 ratio ($P=3.6\times10^{-13}$ effect size 0.2 s.d./allele s.e.m. 0.003) and altered free T_4 levels ($P=2.1\times10^{-9}$) but not serum TSH levels (11). Furthermore, we aimed to test whether variation in *PDE8B* was still associated with TSH in individuals on L-T_4.

Finally, we also explored two new candidate SNPs, rs10499559 in *RAPGEF5* and rs9322817 in *HACE1*. These two SNPs had the strongest associations with TSH ($P=8.3\times10^{-6}$ and 6.5×10^{-6} respectively) where the identity of the gene tagged by the SNP was defined (19) using generalized estimating equations (GEE), from a GWAS of the Framingham Heart Study. The GEE analysis from this study was utilized as it estimates the average response over a population, rather than familial aggregation and hereditability. Therefore, SNPs identified with the GEE approach would be potentially very applicable for use in Mendelian Randomization studies in epidemiological cohorts.

Although these SNPs could have been identified due to a type 1 error, *RAPGEF5* is a member of the RAS subfamily of GTPases and is the key link between cell surface receptors and RAS activation. It is expressed in a wide variety of tissues including the thyroid, and we postulated that it may also be involved in TSH signaling. *HACE1* is expressed in multiple tissues including the heart, brain, and kidney but is not thought to be present in the thyroid gland. We postulated that its association with TSH may be due to differences in detection of thyroid hormone in the brain.

Subjects and methods

In this study, four cohorts were used: the Busselton Health Study (Busselton), the Depression and Thyroid Disease study (DEPTH), The Exeter Family Study of Childhood Health (EFSOCH), and The Weston Area T_3 T_4 Study (WATTS). These studies have all been described previously (11, 20–22). Busselton is a community-based cohort in which individuals were tested for thyroid function on two occasions 13 years apart (1981 and 1994), DEPTH is a primary care cohort of individuals referred for thyroid function testing, EFSOCH is a cohort of male partners of pregnant women recruited for a family study, and WATTS is a cohort of patients on thyroid hormone replacement recruited for an intervention study. Baseline characteristics of all cohorts are presented in Table 1. Further details of the cohorts/collections can be found in the Supplementary data, see section on supplementary data given at the end of this article. All individuals in all cohorts were of white European ancestry.

To examine the association of genetic variation with endogenous thyroid hormone levels separately from the effects of disease processes or medication that affects thyroid function, individuals with any TSH, free T_3, or

Table 1 Baseline characteristics of the cohorts (S.D. in brackets).

Characteristic	Busselton	DEPTH	WATTS	WATTS abnormal TSH removed	EFSOCH
n	825	387	544	387	801
% Male	50.1	22.7	16.5	14.5	100
Age (years)	45.1 (±14.2)	43.6 (±14.7)	57.0 (±11.1)	55.6	32.7 (±5.9)
TSH (mU/l)	1.47	1.72	1.47	1.61	1.99
Free T_3 (pmol/l)	5.53 (±0.53)	4.67 (±0.63)	3.84 (±0.72)	3.78 (±0.69)	5.51 (±0.53)
Free T_4 (pmol/l)	16.6 (±2.64)	16.3 (±2.65)	21.1 (±3.68)	20.3 (±3.23)	16.5 (±2.02)

free T_4 levels outside the reference ranges for the assays used (see Supplementary data) were excluded from the Busselton, DEPTH, and EFSOCH cohorts. These exclusions were not applied to the WATTS cohort in which subjects were on thyroid hormone replacement and WATTS was, therefore, excluded from the meta-analysis of the cohorts. Subgroup analysis was subsequently performed for the WATTS-cohort individuals with TSH, free T_3, or free T_4 levels outside the reference range excluded.

Statistical analyses

Descriptive statistics of the three cohorts are presented as means and S.D. TSH results across the cohorts were not normally distributed; a $\log_e$ transformation was, therefore, used to normalize the data.

Within each cohort, the association per minor allele of the different genotypes and serological markers was assessed using ordinary least squares (OLS) linear regression. Results are presented in both 'natural' and 'standardized' (per S.D.) units; all models were adjusted for age and sex. Simple inverse variance weighted (fixed effects) meta-analysis was performed to pool study specific results and combined estimates and *P* values presented along with metrics of heterogeneity. Additional individual patient data (IPD) meta-analysis was performed on genetic markers shown to be associated with serological markers (i.e. $P<0.05$). The IPD meta-analysis was performed with a multilevel regression model using restricted maximum likelihood estimation; cohort was entered as a random effect to preserve the hierarchical nature of the data. IPD meta-analysis models were successively adjusted for sex, age, free T_3, and free T_4. We tested for heterogeneity using the Q statistics.

To assess the temporal stability of the genetic effect, the Busselton cohort was studied using a series of multivariate seemingly unrelated regression (SUR) models. SUR is a generalization of OLS linear regression, which allows simultaneous model fitting between two different outcomes (serological levels in 1981 and 1994), and a consistent genetic exposure. The SUR enables the genetic effect between 1981 and 1994 to be tested and the residual (error) correlation between 1981 and 1994 to be explored.

All data analysis was performed using STATA version 11.0 (STATACORP, College Station, TX, USA).

Results

Study populations

Circulating levels of free T_3 and free T_4 were approximately normally distributed in the Busselton, DEPTH, EFSOCH, and WATTS cohorts. Log transformation of TSH led to a near-normal distribution. Table 2

Table 2 Genotype frequencies and Hardy–Weinburg equilibrium (χ^2) by cohort.

		Genotype and frequency (%)								
Gene SNP	Cohort	A:A	A:G	G:G	A:C	C:C	C:T	T:T	Total	χ^2
PDE8B (rs4704397)	Busselton	116 (14.1)	397 (45.2)	317 (38.4)					825	0.07
	DEPTH	66 (17.1)	201 (51.9)	120 (31.1)					387	1.36
	WATTS	87 (16.9)	243 (41.2)	185 (35.9)					515	0.22
	EFSOCH	131 (16.35)	374 (46.7)	296 (37.0)					801	0.05
DIO1 (rs223554)[a]	Busselton	213 (25.9)			393 (47.8)	216 (26.2)			822	1.58
	DEPTH	103 (27.2)			176 (46.8)	98 (25.9)			377	1.65
RAPGEF5 (rs10499559)	Busselton					623 (75.3)	187 (23.0)	20 (2.4)	827	1.73
	DEPTH					284 (77.7)	74 (20.1)	10 (2.72)	368	3.5
	WATTS					400 (82.1)	72 (14.7)	15 (3.1)	487	21.8
HACE1 (rs9322817)	Busselton	312 (37.8)			390 (47.2)	123 (14.9)			825	0
	DEPTH	145 (41.2)			157 (44.6)	50 (14.2)			352	1.6
	WATTS	171 (38.7)			195 (44.2)	75 (17.1)			441	2.26

[a]Incidences of the rs2235544 (*DIO1)* genotypes in the WATTS cohort has been published previously (11).

demonstrates that all SNPs were in Hardy–Weinberg equilibrium (HWE) in all cohorts except for rs10499559 in *RAPGEF5* in the WATTS cohort. There were difficulties in genotyping this SNP from the DNA stores available, and this led to an unusually high error rate; furthermore, there were difficulties in distinguishing between the CC and the CT genotype. We have presented the data for this SNP in WATTS, but it should be interpreted with caution.

Effects on TSH and serum thyroid hormone levels in individuals with intact thyroid function

The meta-analysis of three cohorts of individuals not on thyroid hormone replacement reconfirmed the association between the rs4704397 SNP in the *PDE8B* on TSH levels ($P=1.64\times10^{-10}$). Table 3 shows results for the individual cohorts, and the results of the meta-analysis are shown in Table 4. Minimal attenuation of the effect was observed (see Fig. 1) after adjusting for multiple covariates including free T_4, and only a small increase in the S.E.M. was observed, resulting in a small increase in the P value from $P=2.95\times10^{-10}$ (unadjusted) to $P=1.64\times10^{-10}$ (fully adjusted). Meta-analysis also revealed an association with free T_4 levels ($P=0.023$), in the opposite direction to the association seen with TSH.

Our meta-analysis also validated the association with the rs223554 SNP in the *DIO1* gene on free T_4 ($P=0.001$) and free T_3/free T_4 ratio ($P=9.26\times10^{-4}$) and confirmed that there was no association with TSH ($P=0.32$).

The effect of variation in the PDE8B and DIO1 genes on serum thyroid hormone and TSH levels over time in the same individuals in the Busselton cohort

In the Busselton cohort, the effect size per minor A allele of the rs4704397 SNP of the *PDE8B* gene on TSH levels in 1981 was 0.141 S.D./allele ($P=0.005$ (95% confidence interval (CI) 0.04, 0.24)), and on repeat testing in 1994, there was no evidence for difference in effect estimate at 0.189 S.D./allele ($P=0.0001$ (95% CI 0.09, 0.28)). There was also a similar effect size per minor A allele on free T_4 in 1981 of −0.068 S.D./allele (95% CI −0.167, 0.031)) and in 1994 −0.07 S.D./allele (95% CI −0.170, 0.030)). Testing the difference in genetic effects for *PDE8B* on TSH between 1981 and 1994 showed that there was no evidence of any difference ($P=0.32$). The residual error correlation between 1981 and 1994 was also very high ($r=0.5467$, $P=0.0001$), again indicating commonality of effect.

Very similar results were obtained for the effect size per minor C allele of the rs2235544 SNP of the *DIO1* gene on T_4 levels in 1981: −0.136 S.D./allele ($P=0.0054$ (95% CI −0.23, −0.042)) versus 0.131 S.D./allele ($P=0.006$ 95% CI −0.225, −0.037) in 1994. Testing the difference in genetic effects between 1981 and 1994 showed that there was no evidence of any difference ($P=0.93$) and the residual error correlation between 1981 and 1994 was also high ($r=0.368$), again indicating commonality of effect.

The associations between PDE8B and thyroid hormone parameters on TSH in individuals on thyroid hormone replacement

The WATTS cohort (individuals on thyroid hormone replacement) was analyzed separately. In this cohort, unlike the general population, we observed no evidence of association with the rs4704397 SNP in the *PDE8B* gene on TSH levels ($P=0.99$). Similarly, no effect of this *PDE8B* SNP was seen on free T_4 ($P=0.59$) or free T_3 levels ($P=0.90$). By contrast, an effect was seen on free T_4/free T_3 ratio with the *DIO1* SNP in this cohort as previously reported (11).

Our meta-analysis failed to confirm any association between genetic variation in rs10499559 in *RAPGEF5* on TSH ($P=0.67$), free T_3 ($P=0.21$), or free T_4 ($P=0.49$). Our meta-analysis also found no evidence of association between genetic variation in rs9322817 in *HACE1* on TSH ($P=0.93$), free T_3 ($P=0.94$), or free T_4 ($P=0.09$).

Discussion

Our meta-analysis has confirmed that the rs4704397 SNP in the *PDE8B* gene is associated with variation in TSH levels, in keeping with the initial report of Arnaud-Lopez *et al.* (10) and a subsequent study in pregnant women (20). However, the additional power available to us in this meta-analysis enabled us to detect that this SNP is also reciprocally associated with free T_4 levels. For each additional minor A allele at this SNP, there was an increase in TSH levels and a reduction in free T_4 levels, indicating relative hypothyroidism (Fig. 1). Although the level of significance for the association with free T_4 remains modest, and further larger confirmatory studies are required to validate this, our finding is supported by its replication in the same individuals 13 years later in the Busselton cohort. Associations with the rs2235544 SNP in *DIO1* were also consistent over time, but by contrast, this SNP was associated with free T_4/free T_3 ratio and not TSH (11). We found no evidence of association between SNPs, rs10499559 in *RAPGEF5* gene and rs9322817 in *HACE1* gene, on thyroid hormone parameters.

These findings are important for two reasons. First, they potentially resolve the paradox raised by Shields *et al.* (17), which demonstrated an association with the rs4704397 SNP of *PDE8B* on TSH, but no evidence of association with free T_3 or T_4. Therefore, we have provided evidence that this SNP is a valuable marker for

Table 3 The effect of variation in rs4704397 in *PDE8B*, rs223554 in *DIO1,* rs10499559 in *RAPGEF5*, and rs9322817 in *HACE1* on TSH and serum thyroid hormone levels by cohort.

		β estimate				
	n	Natural	Std.	**Std. 95% CI**	***P***	r^2
TSH						
PDE8B						
Busselton 1981	825	0.066	0.139	0.040 to 0.238	0.0062	0.011
Busselton 1994	825	0.095	0.201	0.102 to 0.300	0.0001	0.031
DEPTH	387	0.122	0.225	0.079 to 0.371	0.0026	0.025
WATTS	515	0	0	−0.123 to 0.122	0.9970	0.001
EFSOCH	801	0.096	0.199	0.101 to 0.297	0.0001	0.02
DIO1						
Busselton 1981	822	0.029	0.06	−0.035 to 0.155	0.2160	0.004
Busselton 1994	818	0.001	0.001	−0.093 to 0.096	0.9770	0.012
DEPTH	377	−0.068	−0.125	−0.262 to 0.013	0.0760	0.012
RAPGEF5						
Busselton 1981	827	0.017	0.037	−0.104 to 0.177	0.6098	0.002
Busselton 1994	822	−0.016	−0.033	−0.173 to 0.107	0.6453	0.012
DEPTH	368	−0.005	−0.009	−0.217 to 0.199	0.9302	0.002
WATTS	487	−0.165	−0.104	−0.291 to 0.083	0.2779	0.006
HACE1						
Busselton 1981	825	0.034	0.072	−0.027 to 0.171	0.1527	0.005
Busselton 1994	821	0.008	0.017	−0.082 to 0.116	0.7333	0.012
DEPTH	352	−0.028	−0.052	−0.203 to 0.098	0.4950	0.002
WATTS	441	−0.022	−0.014	−0.147 to 0.12	0.8405	0.002
Free T_3[a]						
PDE8B						
Busselton 1994	825	−0.034	−0.048	−0.147 to 0.050	0.3343	0.04
DEPTH	387	−0.03	−0.048	−0.195 to 0.100	0.5271	0.001
WATTS	515	0.006	0.008	−0.116 to 0.133	0.8955	0.004
EFSOCH	801	−0.014	−0.027	−0.123 to 0.070	0.5859	0.053
DIO1						
Busselton 1994	818	0.066	0.094	0.000 to 0.187	0.0492	0.043
DEPTH	377	0.003	0.005	−0.133 to 0.144	0.9399	0
RAPGEF5						
Busselton 1994	822	−0.063	−0.089	−0.227 to 0.049	0.2054	0.04
DEPTH	368	−0.024	−0.038	−0.247 to 0.171	0.7242	0.001
WATTS	487	0.11	0.152	−0.038 to 0.343	0.1181	0.007
HACE1						
Busselton 1994	821	−0.012	−0.017	−0.115 to 0.081	0.7352	0.039
DEPTH	352	0.018	0.029	−0.122 to 0.179	0.7111	0.001
WATTS	441	0.08	0.11	−0.023 to 0.243	0.1058	0.007
Free T_4						
PDE8B						
Busselton 1981	825	−0.179	−0.068	−0.167 to 0.031	0.1791	0.029
Busselton 1994	825	−0.186	−0.07	−0.170 to 0.030	0.1696	0.003
DEPTH	387	−0.276	−0.104	−0.251 to 0.043	0.1665	0.006
WATTS	515	0.126	0.034	−0.090 to 0.158	0.5897	0.001
EFSOCH	801	−0.13	−0.064	−0.163 to 0.035	0.2032	0.002
DIO1						
Busselton 1981	822	−0.35	−0.133	−0.226 to −0.040	0.0052	0.035
Busselton 1994	818	−0.341	−0.129	−0.224 to −0.034	0.0079	0.01
RAPGEF5						
Busselton 1981	827	0.119	0.045	−0.093 to 0.184	0.5219	0.026
Busselton 1994	822	−0.05	−0.019	−0.160 to 0.122	0.7924	0.001
DEPTH	368	−0.239	−0.09	−0.300 to 0.119	0.3989	0.002
WATTS	487	0.055	0.015	−0.171 to 0.201	0.8751	0.001
HACE1						
Busselton 1981	825	−0.048	−0.018	−0.116 to 0.079	0.7160	0.027
Busselton 1994	821	−0.102	−0.039	−0.138 to 0.061	0.4471	0.002
DEPTH	352	−0.387	−0.146	−0.293 to 0.001	0.0524	0.016
WATTS	441	−0.045	−0.012	−0.142 to 0.118	0.8544	0

Std., standardized.
[a]Free T_3 levels not available in the Busselton 1981 data set.

Table 4 Meta-analysis to show associations between variation in rs4704397 in *PDE8B*, rs223554 in *DIO1*, rs10499559 in *RAPGEF5*, and rs9322817 in *HACE1* on TSH and thyroid hormone levels. Cohort 1= Busselton, Cohort 2= Depth, and Cohort 3= EFSOCH.

Cohorts	Gene	β	95% CI	*P* value	Q	p(Q)≥
TSH						
1, 2, 3	*PDE8B*	0.205	(0.142, 0.267)	1.64×10^{-10}	0.093	0.955
1, 2	*DIO1*	−0.04	(−0.118, 0.038)	0.324	2.188	0.139
1, 2	*RAPGEF5*	−0.026	(−0.142, 0.091)	0.667	0.035	0.861
1, 2	*HACE1*	−0.004	(−0.087, 0.079)	0.928	0.562	0.453
T_3						
1, 2, 3	*PDE8B*	−0.039	(−0.102, 0.023)	0.218	0.106	0.948
1, 2	*DIO1*	0.066	(−0.011, 0.144)	0.093	7.501	0.006
1, 2	*RAPGEF5*	−0.073	(−0.189, 0.042)	0.211	0.159	0.69
1, 2	*HACE1*	−0.003	(−0.086, 0.079)	0.936	0.251	0.616
T_4						
1, 2, 3	*PDE8B*	−0.074	(−0.137, −0.010)	0.023	0.205	0.902
1, 2	*DIO1*	−0.132	(−0.210, −0.054)	0.001	0.014	0.907
1, 2	*RAPGEF5*	−0.041	(−0.158, 0.076)	0.491	0.304	0.582
1, 2	*HACE1*	−0.072	(−0.155, 0.010)	0.086	1.365	0.243
T_3:T_4						
1, 2, 3	*PDE8B*	0.027	(−0.036, 0.089)	0.407	0.469	0.791
1, 2	*DIO1*	0.148	(0.071, 0.226)	9.2×10^{-4}	0.158	0.691
1, 2	*RAPGEF5*	−0.018	(−0.133, 0.098)	0.737	0.749	0.387
1, 2	*HACE1*	0.055	(−0.027, 0.138)	0.189	2.617	0.106

use in large epidemiological studies to examine the impact of small reductions in thyroid gland function on biological phenotypes and common diseases in the population. Such an approach can also then be used to explore the potential benefits of thyroid hormone supplementation in subjects with thyroid function in the lower parts of the reference range.

Second, our finding of reciprocal changes in TSH and free T_4 with common genetic variation in *PDE8B* provides further support for the suggestion, arising from the data of Arnoud-Lopez *et al.* (10) that *PDE8B* plays a role in TSH signaling in the thyroid gland rather than operating at the level of central control of TSH secretion. This is entirely consistent with the predominant expression of the *PDE8B* gene in the thyroid gland (14), with expression to a lesser extent in the brain (13).

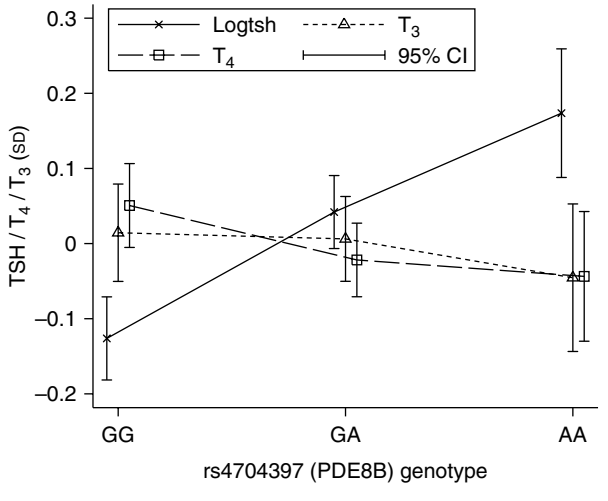

Figure 1 Standardized TSH, free T_3, and free T_4 levels by rs4704397 (*PDE8B*) genotype.

The observation that the association with TSH is lost in subjects on thyroid hormone replacement is unlikely to be an issue of statistical power, as an association with TSH was seen in the DEPTH cohort (not on T_4) that is smaller in number than WATTS (Table 3). This finding is also consistent with *PDE8B* having an effect at the level of the thyroid, as in these individuals thyroid responsiveness to TSH will play little or no role in determining thyroid hormone levels. In this context, it is interesting to note that individuals in the WATTS cohort have a higher free T_4 ($P\leq0.001$) and a lower free T_3 ($P=0.04$), despite having a similar TSH ($P=0.74$) level to subjects in the Busselton cohort (general population) in keeping with previous reports (23). This trend persisted even when individuals with abnormal and suppressed TSH levels were removed from the WATTS cohorts; individuals in WATTS had a higher T_4 ($P<0.001$) and a lower free T_3 ($P=0.03$) despite having similar TSH levels ($P=0.24$). This observation emphasizes that although thyroid hormone replacement may restore an individual's TSH levels to within the 'normal population range', this may be outside an individual's genetically determined set point.

The rs4704397 SNP is present in intron 1 of the *PDE8B* gene. However, it is possible that this SNP is not itself functional but linked to functional locus outside intron 1. Arnaud-Lopez *et al.* (10) have previously demonstrated that the rs4704397 SNP shows linkage disequilibrium with SNPs in neighboring regions. It is, however, unlikely to be involved in a coding region because sequence analysis of 20 subjects homozygous for the rare and 20 subjects for the common allele of rs4704397 did not demonstrate any coding variants

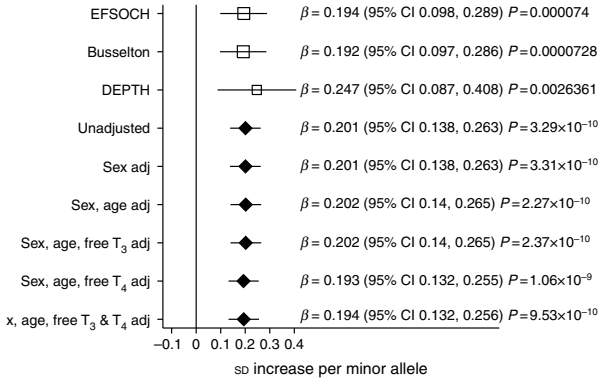

Figure 2 Association between *PDE8B* and TSH levels by cohort and adjustment.

(10). We propose that the presence of additional minor A alleles results in increased phosphodiesterase activity of PDE8B and hence a reduced ability of the thyroid gland to generate free T_4 (and free T_3) when stimulated by TSH. This ultimately results in a reduction in free T_4 and free T_3 levels with a subsequently higher steady TSH level. As its mechanism of effect had been in the brain in altering the detection and subsequent set point of TSH, one would have expected higher TSH levels to be associated with higher thyroid hormone levels rather than a reciprocal relationship. Consistent with this, a recent report shows that variants in *PDE8B* associated with increased TSH levels segregate separately from a rare inactivating variant (H305P) in PDE8B (24).

The lack of association with free T_3 levels in this study is likely to relate to the observation that TSH levels are more sensitive to free T_4 than free T_3 levels, both outside (25) and within (20) the normal range. Therefore, we suggest that the rs47044397 SNP probably influences free T_3 levels in addition to free T_4 (and indeed the trends in changes in free T_3 were consistently in the same direction as free T_4 and reciprocal to TSH – Tables 3 and 4), but the current meta-analysis was still underpowered to detect this. Adjusting for free T_4 and free T_3 levels only partially attenuates the effect of the *PDE8B* SNP on TSH levels (Fig. 2). This implies that even given the log-linear relationship between TSH and free T_4, the fall in T_4 is insufficient to account for the whole rise in TSH. This discrepancy currently remains unexplained.

We were unable to confirm the association between the rs10499559 SNP in *RAPGEF5* and the rs9322817 SNP in *HACE1* with serum TSH levels. Genetic variation in rs932281 in *HACE1* was evenly distributed in our cohorts, and our study was sufficiently powered to detect an effect of 0.10 s.d./allele. The initial identification of an association between this SNP and TSH may be due to a type 1 error or else its effect is only modest. By contrast, the mean allele frequency of rs10499559 in *RAPGEF5* is low, at 0.15, in our cohorts and we were, therefore, only sufficiently powered to detect an association of 0.15 s.d./allele or higher. This SNP will require further replication studies in several large epidemiological cohorts with thyroid function available to fully assess its effect.

We were able to demonstrate the continuing association between genetic variation due to SNPs on TSH and thyroid hormone levels, and to our knowledge, this report is the first to provide evidence for this. The marked similarity in effect size per additional minor allele for both the rs4704397 SNP in *PDE8B* on TSH and free T_4 and the rs2235544 SNP in *DIO1* on free T_4 over a 13-year period provides compelling evidence for the consistent associations of these SNPs on thyroid hormone parameters over time. Indeed, over this time interval, there was a mean increase in serum TSH of 0.31 μIU/ml, and yet the relative genetic effect of these SNPs remained constant. This suggests that the genetic approach better reflects lifelong trends in TSH and thyroid hormone levels compared with individual serum values.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-10-0938.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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