Common Variation in the *DIO2* Gene Predicts Baseline Psychological Well-Being and Response to Combination Thyroxine Plus Triiodothyronine Therapy in Hypothyroid Patients

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Introduction: Animal studies suggest that up to 80% of intracellular T_3 in the brain is derived from circulating T_4 by local deiodination. We hypothesized that in patients on T_4 common variants in the deiodinase genes might influence baseline psychological well-being and any improvement on combined T_4/T_3 without necessarily affecting serum thyroid hormone levels.

Methods: We analyzed common variants in the three deiodinase genes *vs.* baseline psychological morbidity and response to T_4/T_3 in 552 subjects on T_4 from the Weston Area T_4 T_3 Study (WATTS). Primary outcome was improvement in psychological well-being assessed by the General Health Questionnaire 12 (GHQ-12).

Results: The rarer CC genotype of the rs225014 polymorphism in the deiodinase 2 gene (*DIO2*) was present in 16% of the study population and was associated with worse baseline GHQ scores in patients on T_4 (CC *vs.* TT genotype: 14.1 *vs.* 12.8, $P = 0.03$). In addition, this genotype showed greater improvement on T_4/T_3 therapy compared with T_4 only by 2.3 GHQ points at 3 months and 1.4 at 12 months ($P = 0.03$ for repeated measures ANOVA). This polymorphism had no impact on circulating thyroid hormone levels.

Conclusions: Our results require replication but suggest that commonly inherited variation in the *DIO2* gene is associated both with impaired baseline psychological well-being on T_4 and enhanced response to combination T_4/T_3 therapy, but did not affect serum thyroid hormone levels. (***J Clin Endocrinol Metab*** **94: 1623–1629, 2009)**

Up to 3% of the population in Western countries is on thyroid hormone replacement (1), the majority on T_4 alone. However, the adequacy of this to replace physiological requirements and reverse patients' symptoms remains controversial due to several observations. In thyroidectomized rats, Escobar-Morreale *et al.* reported that it was not possible to normalize tissue levels of thyroid hormone (T_4 and T_3) by replacement with T_4 alone (2, 3) or T_3 alone (4). In humans, patients on T_4 monotherapy have a significantly higher serum T_4 to T_3 ratio for a similar TSH than people with normal thyroid function (5, 6). Some markers of thyroid hormone action, such as IGF-1 may not normalize on T_4 monotherapy (7). Finally, a significant number

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Abbreviations: C, Cytosine; fT3, free T_3; fT4, free T_4; GHQ-12, General Health Questionnaire, 12-question version; HAD, Hospital Anxiety and Depression Scale questionnaire; SNP, single-nucleotide polymorphism; T, thymine; TSQ, thyroid symptom questionnaire; WATTS, Weston Area T^4/T^3 Study.

of patients report persistent symptoms despite titration of T_4 replacement to adequate serum levels of thyroid hormone and normalization of TSH levels (8, 9). These observations led to the proposition that combination therapy with both T_4 and the T_3 might be more effective. However, although in the initial study of combination therapy (T_4 and T_3), patients treated with the combination appeared to have improved well-being (10), 10 subsequent larger studies failed to confirm this benefit (11–20) and in at least one case demonstrated a large and sustained placebo effect (18). Metaanalysis of these trials (21) showed no benefit from combination therapy and a carefully controlled study of overreplacement with T_4 also failed to show benefit (22). Doubt remains though because an excess psychological morbidity among patients on T_4 has been documented in three separate large community-based studies (9, 23, 24), and also anecdotally there remain patients who feel better on combination therapy.

One possibility that might resolve these issues is the existence of a subgroup of patients who require combination therapy due to an inherited abnormality. If this group represented less than 20% of the population, such patients may be too infrequent for their presence to be detected in the intervention trials but could still account for significant morbidity in patients on T_4. The three iodothyronine deiodinases represent possible candidate loci for such genetic variation, because these are responsible for the interconversion of T_4 and T_3 (25). The Weston Area T_4/T_3 Study is the largest study of thyroid hormone replacement yet conducted (n = 697) (18). We have taken advantage of the greater statistical power of this study to explore the role of common polymorphisms in the three deiodinase genes in determining psychological well-being and the response to partial T_3 replacement.

Subjects and Methods

Sample population

Subjects were 552 people in the Weston Area T^4/T^3 Study (WATTS) who had DNA available for genotyping (total study participants = 697). The study design has been previously described (18), but briefly, subjects on a stable dose of T_4 therapy 100 μg or more per day were recruited from 28 primary care practices in the Weston-superMare and Bristol areas of the United Kingdom and randomized to either combination T_4/T_3 therapy (original dose minus 50 μg of T_4 and added 10 μg T_3) or original dose of T_4 alone. Biochemical, physical, and psychological assessments were made at baseline and 3 and 12 months. The trial was double blinded and results analyzed on an intention-to-treat basis. The study protocol was approved by the local research ethics committee.

Biochemical methods

Serum TSH and free T_4 (fT4) were measured from a serum sample by RIA (Diagnostic Product Corp., Los Angeles, CA). Free T_3 (fT3) was measured by chemiluminescence assay (Elecsys system 1010; Roche Diagnostics, Mannheim, Germany). The laboratory reference ranges were: TSH, 0.3–4.0 mU/liter; fT4, 10–24 pmol/liter; and fT3, 2.8–7.1 pmol/liter. Coefficients of variation were: TSH, 5.5–8.0%; fT4, 7.7–10.0%; and fT3, 11.7–12.6%.

Tag single-nucleotide polymorphism (SNP) selection and genotyping

We used genotype data from the Caucasian European individuals in the International Haplotype Mapping Project (http://www.hapmap.org) to select a set of SNPs that capture the majority of common variation across the three deiodinase genes (*DIO1*, *DIO2*, and *DIO3*) including 50 kb either side of the genes. We used a minor allele frequency of at least 10%. The 21, seven, and seven SNPs in the *DIO1*, *DIO2*, and *DIO3* genes required nine, four, and six SNPs, respectively, to capture all common variants with an $r^2 > 0.8$. These were: D1, rs11206237, rs11206244, rs2235544, rs2268181, rs2294511, rs2294512, rs4926616, rs731828, and rs7527713; D2, rs12885300, rs225011, rs225014, and rs225015; and D3, rs1190716, rs17716499, rs7150269, rs8011440, rs945006, and rs1190715. We used only SNPs that were in Hardy Weinberg equilibrium ($P > 0.05$) and were genotyped in at least 97.5% of the samples in the final analyses. We examined the association between these SNPs and baseline (before randomisation) psychological well-being.

Genotyping 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 and two further SNPs (rs1190716 and rs12885300) failed quality control. The percentage of duplicate samples included was 20% and concordance between duplicate samples was 99% or greater. Use of these 16 SNPs resulted in coverage of 100, 85, and 71% of the common (minor allele frequency > 10%), HapMap based, variation in *DIO1*, *DIO2*, and *DIO3*, respectively, at $r^2 > 0.8$.

Psychological assessments

At each visit, the patient's psychological well-being was assessed by the following self-report scales: the General Health Questionnaire, 12-question version (GHQ-12) (26, 27), a disease-specific thyroid symptom questionnaire (TSQ) (9), and the Hospital Anxiety and Depression Scale questionnaire (HAD) (28). In addition, patients completed a satisfaction question on a 5-point scale on the second and third visits. The GHQ-12 and TSQ were scored by both linear (Likert method) and categorical methods (score ≥3 by GHQ method) (26, 27). HAD is divided into seven questions for anxiety (HAD-A) and seven questions for depression (HAD-D) all scored 0–3. HAD anxiety or depression caseness was defined as a total score from the seven questions 8 or greater, which has been shown to provide the best sensitivity and specificity for case finding (28).

For response to T_4/T_3 or T_4-only therapy, improvement in GHQ-12 score was used as the primary end point as this was the endpoint that the original study was powered to measure (18). All other measures were analyzed as secondary end points.

Statistical analysis

To ensure there were no significant differences between the two study groups, baseline characteristic means were compared by ANOVA and proportions by χ^2 test. Initial analysis of the relationship between psychological well-being and genotype at baseline was performed by linear regression, with total GHQ Likert score as the dependent variable and genotype as the independent variable, with each allele considered additive. Logistic regression was also used with GHQ, HAD-D, or HAD-A caseness as the dependent variable. For response to therapy repeated-measures ANOVA was used with the scales, which were normally distributed (total GHQ Likert score, total TSQ Likert score, and satisfaction question score). The total number of missing values was low (<5%). To ensure this did not create error missing values were imputed using the missing values analysis function on SPSS (Chicago, IL), using regression methods to estimate values and adding a random regression residual. This did not significantly change the original estimates, which are displayed in the results section. For GHQ and TSQ, the scores at 3 and 12 months were the repeated measures, the study treatment arm, and genotype were the between-subject effects and baseline (before randomization) score was adjusted for as a covariate. The repeated-measures analysis includes two-way interactions between study treatment arm and genotype and genotype and baseline score. For rs225014, genotype was analyzed as both additive (each allele increases the association) and with the T-allele as dominant (TT and TC combined *vs.* CC) as a dominant

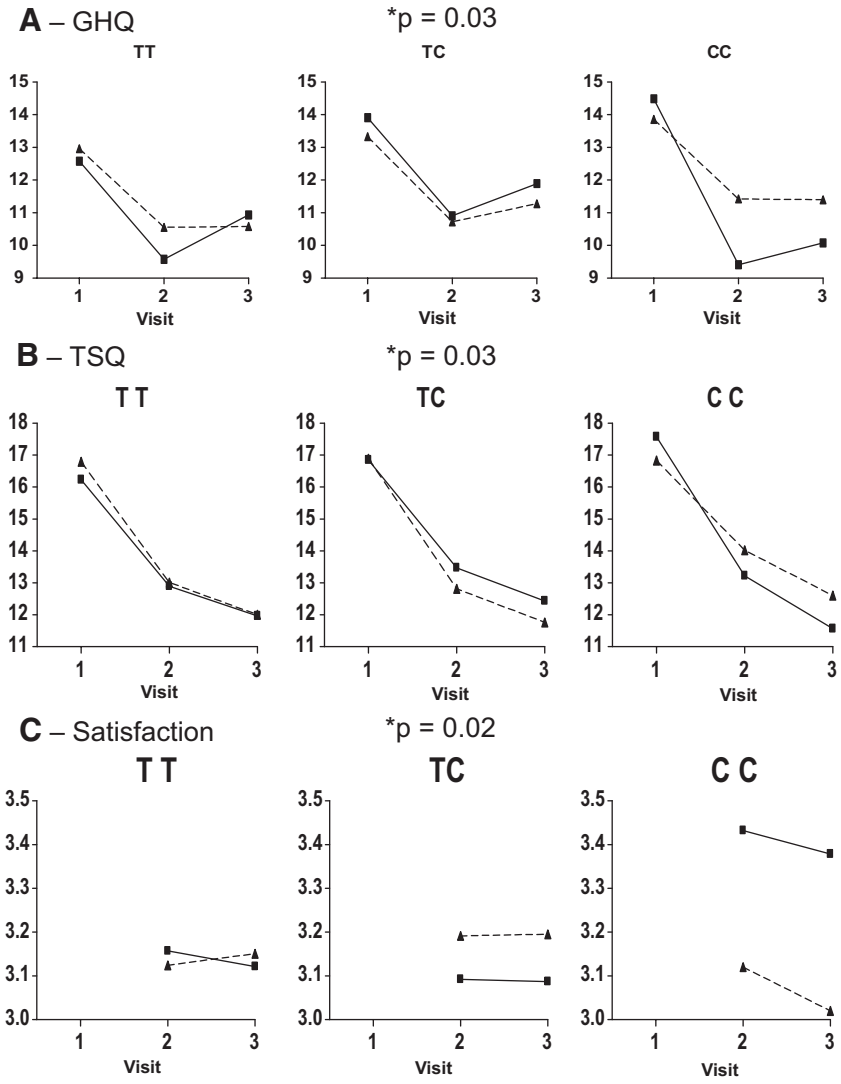

FIG. 1. Response to therapy by genotype rs225014 as measured by GHQ (A), TSQ (B), and satisfaction score (C). *Squares* and *continuous line*, T_4/T_3 group; *triangles* and *dashed line*, T_4-only group. *P* values reflect the significance of an effect of the CC genotype on difference in scores by treatment arm using repeated-measures ANOVA. *, $P < 0.05$. There was a significant effect of the interaction between the CC genotype and treatment arm at follow-up (visits 2 and 3) on GHQ scores, TSQ scores, and satisfaction. There are no baseline (visit 1) scores for satisfaction with therapy (C) because this was not assessed at baseline. For GHQ and TSQ scores, higher scores indicate worse well-being, whereas for satisfaction higher scores indicate more satisfied.

effect was suggested by the graphs (Fig. 1) and has been proposed previously (29). For satisfaction score, no baseline score was adjusted for because there was no baseline assessment. No correction was made for multiple testing because, despite being the largest study to date, it is still underpowered to detect all but very large differential gene-treatment effects. Instead, we have chosen to report the *P* values and associations, which should be considered suggestive, and have qualified our findings by stating clearly that the results need replicating as a risk of type I statistical error exists. Analyses were performed on Stata version 9.0 (www.stata.com) and SPSS version 14.0 (www.spss.com).

Results

Descriptive statistics of the two groups are displayed in Table 1. The treatment groups were not significantly different at baseline in any of the parameters studied.

Genotype and psychological well-being at baseline

The relationship between the 16 SNPs covering the three deiodinase genes and baseline psychological well-being is shown in Table 2. Two SNPs in the *DIO2* gene; rs225014 and rs225015 showed an association at the $P < 0.05$ level of significance. By contrast, the other SNPs analyzed, including all of those from *DIO1* and *DIO3* did not show any association. Because the two D2 SNPs that had showed an association, rs225014 and rs225015, are in strong linkage disequilibrium with an r^2 of 0.88 in this population, and rs225014 is also in linkage disequilibrium with the third SNP studied in this gene, rs225011 (r^2 of 0.59), further analysis is shown on rs225014 alone. In this SNP the possible base combinations of thymine (T) and cytosine (C) are TT, TC and CC. For GHQ-12, each C allele of rs225014 was associated with an average increase of 0.71 GHQ points (worse well-being, *P* for the trend = 0.02) with a difference between the CC and TT alleles of 1.3 points.

Table 3 shows the relationship between rs225014 genotype and baseline psychological well-being for other parameters measured in WATTS. The scores for GHQ-12 from Table 2 are included for comparison. An association with HAD-D (depression) caseness in the same direction as GHQ was seen ($P = 0.01$). Each C allele was associated with a 49% increase in odds of being a HAD-D case ($P = 0.01$) and as a result caseness was almost twice as great in subjects homozygous for the CC genotype as in subjects with the TT genotype (24 *vs.* 13%). No significant differences were seen in the other psychological scores, although all the scores appeared to increase in the same direction across the genotypes (Table 3), with the TT genotype having the lowest score and TC intermediate and CC the worst score. We published previously that rs225014 did not have any detectable effect on baseline thyroid function in this cohort, and hence, this effect appears to be independent of serum thyroid hormone levels (30).

Genotype and response to therapy

Results of repeated-measures ANOVA for response to treatment by genotype and treatment arm for rs225014 are shown in Fig. 1. *P* values indicate an effect of an interaction between treatment arm (T_4/T_3 *vs.* T_4) and the CC genotype on mean scores at both follow-up visits. Note the higher baseline scores for GHQ in the CC genotype as in Table 2. As described in the initial report (18), both treatments resulted in an improvement from baseline consistent with a strong placebo effect. However, when analyzed

TABLE 1. Baseline characteristics of sample population

	T_4/T_3 treatment	T_4 only	*P*[a]
n	270	282	
Sex (percent female)	82.2	84.4	0.49
Age (yr), mean	56.7 ± 11.1	57.6 ± 10.5	0.35
BMI (kg/m^2)	29.0 ± 5.8	29.5 ± 6.3	0.37
Serum TSH, median (interquartile range) (mU/liter)	1.00 (0.30, 2.15)	0.86 (0.27, 2.03)	0.92
fT4 (mean ± SD) (pmol/liter)	21.2 ± 3.6	21.0 ± 3.7	0.58
fT3 (mean ± SD) (pmol/liter)	3.85 ± 0.69	3.84 ± 0.75	0.81
Positive TPOAb	53.2%	57.1%	0.35
GHQ case	42.0%	42.9%	0.94
HAD-D case	19.6%	16.3%	0.31
HAD-A case	45.2%	42.9%	0.59
TSQ case	60.0%	65.3%	0.20

Characteristics compared between two study arms by ANOVA or χ^2 test as appropriate. TPOAb, Anti-thyroid peroxidase antibody.

[a] ANOVA or χ^2 test as appropriate.

by dominant effects (*P* values represent interaction between treatment arm and presence of CC genotype), there was a significant interaction between treatment arm and genotype on improvement in GHQ ($P = 0.03$), TSQ ($P = 0.03$), and satisfaction scores ($P = 0.02$) (Fig. 1). This suggests an improved response to combination therapy in this genotype, the same genotype that had the poorest psychological well-being at baseline (on T_4 only). In addition, when analyzed as an additive model, trends toward an interaction between treatment arm and genotype on improvement in GHQ ($P = 0.07$), TSQ ($P = 0.06$), and satisfaction scores ($P = 0.06$) were also seen. In the CC genotype, the mean fall in GHQ score was 2.33 (95% confidence interval 0.38–4.38) points greater with T_4/T_3 than with T_4 only at 3 months and 1.44 (−0.25 to 3.12) points greater at 12 months. No difference was seen in response to the two treatments for the subjects with the other genotypes.

By contrast with the above effects, rs225014 genotype did not predict decrease in numbers of HAD-D or HAD-A cases, or improvement in mean HAD-D or HAD-A scores in either of the study groups. HAD-D and HAD-A cases improved on treatment in both treatment arms, with no difference in improvement due to genotype (*P* for trend all >0.20).

The rs225014 genotype frequencies were not significantly different between the two study groups (frequency of TT, TC, and CC genotypes: 40.6, 45.5, and 13.9%, respectively, in T_4/T_3 group and 41.1, 41.1, and 17.9% in T_4 only group, $P = 0.38$).

Genotype and thyroid function in response to treatment

Although no significant differences were seen in baseline thyroid function when analyzed by genotype across the whole cohort (30), it remained possible that serum levels might alter differently by genotype in response to T_4/T_3 treatment. Table 4 shows thyroid function by genotype for each of the treatment groups at the three time points. No significant difference in thyroid function by genotype was seen after T_4/T_3 treatment. There was a statistically significant difference (at the $P < 0.05$ level) in serum TSH levels by

TABLE 2. Relationship between genotype and GHQ-12 scores at baseline in all studied SNPs

	Common homozygous		Heterozygous		Minor homozygous		
	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	*P*
DIO1							
rs11206237	399	13.4 (12.9, 13.9)	130	13.5 (12.6, 14.4)	16	11.7 (9.5, 13.9)	0.56
rs11206244	239	13.2 (12.5, 13.8)	238	13.6 (12.9, 14.3)	69	13.2 (12.1, 14.2)	0.70
rs2235544	143	13.6 (12.8, 14.4)	288	13.4 (12.8, 14.0)	111	13.1 (12.2, 14.1)	0.50
rs2268181	387	13.4 (12.9, 14.0)	140	13.3 (12.5, 14.2)	19	11.9 (10.0, 13.9)	0.37
rs2294511	240	13.2 (12.6, 13.8)	248	13.6 (12.9, 14.3)	56	13.2 (11.9, 14.5)	0.71
rs2294512	252	13.7 (13.1, 14.4)	245	13.0 (12.3, 13.7)	50	13.2 (11.8, 14.6)	0.18
rs4926616	245	13.2 (12.5, 13.8)	236	13.7 (13.0, 14.4)	56	13.1 (11.7, 14.5)	0.63
rs731828	183	13.4 (12.7, 14.1)	278	13.2 (12.6, 13.8)	84	13.8 (12.7, 14.9)	0.70
rs7527713	362	13.5 (12.9, 14.0)	157	13.1 (12.3, 13.9)	26	13.0 (11.1, 15.0)	0.46
DIO2							
rs225011	172	12.6 (11.9, 13.3)	264	13.7 (13.1, 14.3)	107	13.7 (12.5, 14.9)	0.06
rs225014	**223**	**12.8 (12.2, 13.4)**	**236**	**13.6 (13.0, 14.3)**	**87**	**14.1 (12.8, 15.5)**	**0.02**
rs225015	**237**	**12.9 (12.3, 13.5)**	**236**	**13.6 (12.9, 14.3)**	**71**	**14.3 (12.8, 15.8)**	**0.03**
DIO3							
rs17716499	202	12.9 (12.3, 13.6)	248	13.6 (12.9, 14.3)	95	13.7 (12.7, 14.6)	0.19
rs7150269	121	13.6 (12.6, 14.5)	254	13.4 (12.8, 14.1)	169	13.1 (12.4, 13.8)	0.44
rs8011440	215	13.1 (12.5, 13.8)	246	13.5 (12.8, 14.2)	85	13.4 (12.3, 14.5)	0.51
rs945006	438	13.4 (12.9, 13.8)	92	13.4 (12.3, 14.4)	10	13.4 (9.7, 17.1)	0.98

Bold values indicate $P < 0.05$. CI, Confidence interval.

TABLE 3. Genotype of rs225014 and all psychological parameters at baseline

	Common homozygous (TT)		Heterozygous (TC)		Minor homozygous (CC)		
	n	Mean (95% CI) or percent of cases	n	Mean (95% CI) or percent of cases	n	Mean (95% CI) or percent of cases	*P*
GHQ Likert score	223	12.8 (12.2, 13.4)	236	13.6 (13.0, 14.3)	87	14.1 (12.8, 15.5)	0.02
GHQ case	217	39.2%	235	43.8%	86	46.5%	0.20
HAD-D case	223	13.0%	236	20.8%	87	24.1%	0.01
HAD-A case	223	40.4%	236	46.6%	87	48.3%	0.14
TSQ Likert score	216	16.5 (15.9, 17.1)	231	16.9 (16.3, 17.4)	83	17.2 (16.2, 18.1)	0.23
TSQ case	216	62.0%	231	62.3%	83	66.3%	0.56

CI, Confidence interval.

genotype in the T_4-only group at baseline; however, this is likely to represent a chance finding because it is not present in the T_4/T_3 group at baseline and does not persist at the 3- and 12-month visits, despite the T_4 group remaining on the same treatment.

Discussion

Our results suggest that common variation in the *DIO2* gene as tagged by the SNP rs225014 may predict both poorer psychological well-being on T_4 monotherapy and improved response to combination T_4/T_3 therapy in patients on thyroid hormone replacement. This result needs replicating because we have tested 16 SNPs across three genes. Although our study is the largest available T_4/T_3 trial, it is still underpowered to reliably detect gene-treatment interactions. However, if replicated, this is likely to reflect an effect on local deiodination of T_4 by the D2 deiodinase in the brain because variation in rs225014 has no effect on circulating thyroid hormone levels (30). The CC genotype of rs225014 is present in a relatively small proportion of the population on T_4, approximately 16% in our study population, and hence, previous studies are likely to have been underpowered to see this effect.

At baseline on T_4, there is an association with genotype of all three *DIO2* SNPs studied and psychological well-being, as measured by GHQ score, at a $P < 0.1$ level; however, no association with any of the *DIO1* or *DIO3* SNPs (Table 2). In each case the rare genotype (CC with rs225014) was associated with poorer well-being. There was also a significant association at baseline with HAD-D caseness, and four other measures showed stepwise changes in the same direction but did not reach statistical significance (Table 3). After intervention there was evidence of a significant effect of the CC genotype on the response to T_4/T_3 compared with T_4 only as measured by GHQ, TSQ, and satisfaction score (Fig. 1). By contrast, there was a consistent failure to observe any effect of intervention by genotype on HAD scores, despite the associations with HAD-D seen at baseline (Table 3). This may relate to a lack of sensitivity to change in the HAD scoring system, which, unlike the other scales, asks subjects to score particular symptoms without reference to a time period or how they would normally feel.

Wide variation exists in deiodinase expression between tissues resulting in important variation in the relative contribution of the serum concentrations of T_4 and T_3 to thyroid hormone action. In rodent studies it is estimated that serum T_3 contributes 87% of intracellular T_3 in the kidney but only 50% in the pituitary and just 20% in the cerebral cortex, the remainder coming from local deiodination of serum T_4 by D2 (25). Our observation that common genetic variation in the *DIO2* gene but not the *DIO1* or *DIO3* genes could be relevant to psychological well-being is interesting because the D1 enzyme is not expressed in the brain (25) and D3 is a deactivating enzyme. Therefore, D2 is the only enzyme able to convert T_4 to T_3 in the brain and is likely to play a key role in determining the ability of the brain to respond

TABLE 4. Thyroid function by genotype rs225014 and intervention at three time points

	T_4/T_3 treatment				T_4 only			
Genotype	TT	TC	CC	*P*[a]	TT	TC	CC	*P*[a]
Baseline fT4	21.3 ± 3.7	21.1 ± 3.6	20.9 ± 3.9	0.54	21.4 ± 3.9	20.9 ± 3.6	20.5 ± 3.8	0.14
3-month fT4	13.7 ± 3.5	13.9 ± 3.4	14.2 ± 4.3	0.43	19.8 ± 3.5	19.7 ± 3.4	19.1 ± 3.4	0.32
12-month fT4	14.6 ± 3.5	14.4 ± 3.4	14.8 ± 3.7	0.99	20.7 ± 3.5	20.2 ± 3.4	19.5 ± 2.9	0.06
Baseline fT3	3.87 ± 0.67	3.78 ± 0.73	4.10 ± 0.59	0.38	3.86 ± 0.86	3.87 ± 0.64	3.74 ± 0.70	0.34
3-month fT3	3.84 ± 0.84	3.81 ± 0.82	4.10 ± 0.72	0.19	3.83 ± 0.62	3.84 ± 0.70	3.89 ± 0.65	0.63
12-month fT3	3.74 ± 0.58	3.70 ± 0.90	3.88 ± 0.77	0.56	3.64 ± 0.68	3.63 ± 0.65	3.52 ± 0.56	0.31
Baseline TSH	1.00 (0.30, 2.00)	1.08 (0.34, 2.18)	0.87 (0.10, 2.67)	0.39	0.69 (0.24, 1.58)	0.92 (0.26, 2.33)	1.10 (0.47, 2.14)	0.04
3-month TSH	2.83 (0.88, 4.97)	2.14 (0.88, 4.16)	1.56 (0.55, 3.81)	0.07	0.71 (0.26, 1.36)	0.64 (0.16, 1.69)	0.69 (0.26, 2.38)	0.68
12-month TSH	2.36 (0.88, 5.37)	2.35 (0.87, 4.40)	1.52 (0.71, 4.71)	0.48	0.55 (0.20, 1.60)	0.71 (0.15, 1.94)	0.84 (0.24, 2.30)	0.36

Values are mean ± SD for fT4 and fT3 and median (interquartile range) for TSH.

[a] Adjusted for age and sex.

to circulating T_4 levels. Indeed, common variation in D2 activity may represent the best available marker of intracellular T_3 levels in the brain. The lack of effect of the *DIO2* polymorphisms on serum thyroid hormone levels (30–32) means that without performing genetic testing, it is impossible to select out the group likely to respond to combination therapy for subgroup analysis in intervention trials. One previous study has shown an association between a *DIO2* polymorphism, rs12885300, and serum thyroid hormone levels in young blood donors but not an elderly population (33) or in a subsequent study (31). This SNP was excluded from analysis in our sample due to failed quality control; however, it is unlikely to have influenced the results because we have shown no difference in serum thyroid hormone levels by genotype during the trial (Table 4), supporting the view that differences in serum hormone levels are not responsible for the differences in well-being seen.

D2 activity is regulated according to local thyroid hormone levels in the brain and other tissues and hence can protect against hypothyroidism at a local level by increasing its activity (34, 35). This regulation is mostly brought about by substrate (T_4)-induced ubiquitination (36). The *DIO2* SNP of interest in the current study is located in exon 3 of the D2 gene resulting in a Thr92Ala substitution in the instability loop in D2, which is closely linked to ubiquitination and a key determinant of turnover rate (37). *In vitro* studies have not shown any difference in the enzymatic function of D2 compared with the wild-type when this polymorphism is transfected into cells (29, 32); however, an *in vivo* study did show decreased D2 velocity in skeletal muscle and thyroid biopsy samples in type 2 diabetes with the rare genotype (29). In addition, a recent report has shown an association between the CC genotype and osteoarthritis, which given the action of D2 at the growth plate is again consistent with decreased function (38). Potentially the Thr92Ala substitution may effect ubiquitination impairing its ability to increase its activity with low T_4 levels, reducing the ability to maintain homeostasis and increasing dependence on serum T_3 as a source of T_3 in the brain. While in the rat model brain, T_3 shows remarkable stability to different concentrations of infused T_4 (2), it is likely that a significant amount of this homeostasis is derived from the ability of D2 within the brain to up-regulate in conditions of low local thyroid hormone concentrations.

Previous studies on the benefits of combination therapy on psychological well-being in patients on thyroid hormone replacement, including the WATTS study itself and a metaanalysis of the major studies (21), have not shown any significant advantage over T_4 monotherapy. The key issue here is likely to be one of statistical power. Our results suggest that the relevant *DIO2* alleles that might confer responsiveness to combination therapy are only present in 16% of the population on T_4. Other than WATTS, the studies of combination therapy have all had study populations of less than 141 subjects and more commonly around 40 subjects. Hence, only between three and 10 subjects in each study arm would have the potentially responsive genotype, giving little power to detect effects on baseline scores and even less power to detect differences in response to therapy in which subjects are divided into intervention groups. The largest of these studies (n = 141) did examine *DIO2* polymorphisms and showed a nonsignificant trend toward the CC genotype of rs225014, giving poorer scores in baseline assessments of general well-being such as the Rand and Symptom Checklist, a 90-item self-report scale, which would be consistent with our findings, (39). In the WATTS study itself (total n = 697), we would estimate that approximately 50 subjects in the intervention group (of 344) would have had the genotype, which would respond to combination therapy, but given the large overall placebo effect, the differential change in these subjects was not detected in the initial analysis of the whole cohort.

The observation that genetic variation in *DIO2* may influence an individual's ability to respond to T_3 (and T_4) is important for several reasons. First, it may help to explain the excess of patients who do not feel back to normal on T_4 replacement therapy alone (9, 23, 24). In our initial cross-sectional study on well-being on T_4 replacement, subjects on T_4 had an average score 0.7 GHQ points higher compared with age- and sex-matched controls (9); hence, our finding of an extra 0.71 GHQ score points at baseline for each C allele suggests that subjects with one or two copies of this allele might explain a significant part of this difference. Subjects on T_4 replacement are likely to be particularly susceptible to impaired D2 function because they have a lower circulating T_3 to T_4 ratio compared with subjects with an intact thyroid axis (5, 6). Second, this effect provides evidence that thyroid hormone activity, presumably in the brain, plays a role in psychological well-being and mood, something that has previously been shown only in animal studies (40). Finally, there are other candidate genes in addition to *DIO2*, notably thyroid hormone transporters that may also influence psychological well-being, and our findings suggest that common genetic variation in these other loci should also be explored.

Conclusion

Genetic polymorphisms in the *DIO2* gene may affect psychological well-being in patients on T_4 replacement and predict those who will have improved well-being in response to combination therapy with T_3. Replication of this result, including prospective studies with genotype-selected populations, are required before changes in treatment approach can be recommended in routine practice.

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