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No Effect of the Thr92Ala Polymorphism of Deiodinase-2 on Thyroid Hormone Parameters, Health-Related Quality of Life, and Cognitive Functioning in a Large Population-Based Cohort Study

Hanneke J.C.M. Wouters,¹ Hannah C.M. van Loon,¹ Melanie M. van der Klauw,¹ Martin F. Elderson,¹ Sandra N. Slagter,¹ Anneke Muller Kobold,² Ido P. Kema,² Thera P. Links,¹ Jana V. van Vliet-Ostaptchouk,¹ and Bruce H.R. Wolffenbuttel¹

Introduction: The presence of the Thr92Ala polymorphism of deiodinase-2 (D2) has been thought to have several effects. It may influence its enzymatic function, is associated with increased expression of genes involved in oxidative stress in brain tissue, and may predict favorable response to combination levothyroxine (LT4) plus triiodothyronine (T3) therapy. It was hypothesized that homozygous carriers of the D2-92Ala allele have different thyroid hormone parameters, and reduced health-related quality of life (HRQoL) and cognitive functioning.

Methods: In 12,625 participants from the LifeLines cohort study with genome-wide genetic data available, the effects of the Thr92Ala polymorphism (rs225014) were evaluated in the general population and in 364 people treated with thyroid hormone replacement therapy, the latter mainly because of primary hypothyroidism. In addition to evaluating anthropometric data, medication use, and existence of metabolic syndrome, HRQoL was assessed with the RAND 36-Item Health Survey, and the Ruff Figural Fluency Test was used as a sensitive test for executive functioning. Data on thyrotropin, free thyroxine (fT4), and free T3 (fT3) levels were available in a subset of 4479 participants.

Results: The mean age ($\pm$ standard deviation) was 53 ± 12 years and the body mass index was 27.0 ± 4.5 kg/m² in the LT4 users compared with 48 ± 11 years and 26.2 ± 4.1 kg/m² in participants from the general population. The Ala/Ala genotype of the D2-Thr92Ala polymorphism was present in 11.3% of LT4 users and in 10.7% of the general population. In total, 3742/4479 subjects with thyroid hormone data available had normal TSH (0.4–4.0 mIU/L), and 88% of LT4 users were females. LT4 users had higher fT4, lower fT3, and a lower fT3/fT4 ratio, and female patients had lower scores on the HRQoL domains of physical functioning, vitality, mental health, social functioning, bodily pain, and general health compared with those not using LT4 ($p < 0.005$). Executive functioning scores, as part of cognitive functioning, were comparable between female LT4 users and the general population. In both groups, the D2-Thr92Ala polymorphism was not associated with differences in TSH, fT4, fT3, the fT3/fT4 ratio, presence of metabolic syndrome or other comorbidities, use of medication, HRQoL, and cognitive functioning.

Conclusion: The Thr92Ala polymorphism of D2 was not associated with thyroid parameters, HRQoL, and cognitive functioning in the general population and in participants on thyroid hormone replacement therapy.

Keywords: TSH, free T4, general population, quality of life, deiodinase-2, polymorphism, executive functioning

Introduction

PRIMARY HYPOTHYROIDISM is a common endocrine disorder. Its prevalence in Europe is approximately 3% (1). Thyroid hormone replacement with synthetic thyroxine (T4)—levothyroxine (LT4)—is the standard treatment according to current guidelines. The goals of this treatment are to

reduce or abolish symptoms and to normalize plasma levels of thyrotropin (TSH) and thyroid hormones (2). Unfortunately, some patients continue to experience symptoms and disturbed well-being, despite the fact that they are biochemically euthyroid (3–5). Gullo *et al.* (6) showed that free T4 (fT4) levels were significantly higher and that free triiodothyronine (fT3) levels and the fT3/fT4 ratio were significantly lower during

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thyroid hormone replacement therapy, despite normal TSH levels. Thus, TSH, the main treatment target, may not be a good marker for adequate thyroid hormone replacement (7). It has been suggested that combination therapy of LT4 with synthetic T3—liothyronine—may be more effective than LT4 alone. Results from clinical trials have reported that some patients experience improved well-being using combination therapy, but this issue remains controversial (8,9).

In addition to health-related quality of life (HRQoL), impairments of thyroid function may interfere with cognitive functioning. Earlier studies have indicated that decreased thyroid function is especially associated with decreased information processing speed, poor learning, and reduced efficiency in executive functions (10). Whether these functions improve with thyroid hormone replacement therapy is still unclear (11).

The thyroid gland produces both T3 and T4. Only 20% of T3 is directly secreted from the thyroid gland, and 80% of T3 is formed by deiodination of T4 in extrathyroidal tissue. This reaction is catalyzed by two types of deiodinase enzymes: deiodinase-1 (D1) and deiodinase-2 (D2) (12). D1 is the predominant deiodinating enzyme in the liver and kidney, whereas D2 is predominant in the thyroid gland, brain, pituitary gland, skeletal muscle, heart, and skin. Deiodinase-3 (D3) is the main inactivating enzyme, which can inactivate both T4 (by conversion to reverse T3) and T3 (by conversion to T2) (13). Together, these three enzymes regulate the normal euthyroid state in the blood and tissues.

During recent years, it has been demonstrated that certain genetic polymorphisms of deiodinases may be associated with variation in thyroid hormone levels (14–17). Even a modest variation in thyroid hormone levels can be associated with substantial phenotypic effects (18). The common single nucleotide polymorphism (SNP) rs225014 in the *DIO2* gene results in a single amino acid substitution at position 92 (19), in which threonine (Thr) is replaced by alanine (Ala). The functional effect of this replacement has not yet been fully elucidated. Torlontano *et al.* (20) showed that athyreotic differentiated thyroid cancer patients who are homozygous carriers of the D2-92Ala allele needed higher doses of LT4 to achieve target TSH levels. Several other studies failed to detect an association between the polymorphism and thyroid function tests (14–16,21,22), although recently it was suggested that the presence of this polymorphism could be associated with a higher than normal fT4/fT3 ratio (23). Panicker *et al.* (24) reported that patients with the Ala/Ala genotype of the D2-Thr92Ala polymorphism had a significantly lower psychological well-being and greater improvement of general health when treated with combined LT4/liothyronine therapy compared with LT4 alone. A recent study renewed interest in the genetic variation of D2, as it showed that homozygous carriers of the D2-92Ala allele exhibited increased expression of genes associated with oxidative stress and inflammation in brain tissue (25), and concluded that this could contribute to neurocognitive symptoms of affected carriers. Other studies have suggested an association between the D2-Thr92Ala polymorphism and insulin resistance, bipolar disorder, mental retardation, hypertension, and osteoarthritis (22).

It was hypothesized that homozygous carriers of the D2-92Ala allele can be recognized by having a low fT3/fT4 ratio, more frequently have high blood pressure and metabolic

syndrome, experience lower HRQoL, and have impaired cognitive functioning in comparison with subjects who are heterozygous Thr/Ala or homozygous Thr/Thr carriers. This hypothesis was tested in healthy individuals from the general population and in patients using thyroid replacement therapy, who participated in a large population-based study.

Materials and Methods

Subjects

This cross-sectional study used data from subjects participating in the LifeLines cohort study. LifeLines is a multidisciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of persons living in the north of Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics (26). The methodology has been described previously (27). All participants were between 18 and 90 years old at the time of enrollment. They provided written informed consent before participating in the study. The study protocol was approved by the medical ethical review committee of the University Medical Center Groningen.

The present study intended to include the 13,194 subjects in whom whole-genome SNP data were available. Subjects who were not of Western European descent and those who were current users of drugs known to influence thyroid hormone parameters such as liothyronine, lithium, amiodarone, methimazole, propylthiouracil, and corticosteroids were excluded. Participants with a CRP level >10 mg/L were also excluded because inflammation may disturb the activity of the deiodinase enzymes (28). Therefore, data for 12,625 participants could be analyzed, and of these, thyroid function parameters were available for 4479 participant. A total of 364 (3%) participants were using thyroid hormone replacement therapy with LT4. The majority of them used LT4 for primary hypothyroidism, and others after treatment for thyrotoxicosis or after thyroid surgery. The level of detail of the LifeLines data set does not allow further refinement of causes for LT4 use.

Clinical examination

Subjects completed a self-administered questionnaire on medical history, past and current diseases, use of medication, and health behavior at home. Medication was verified by a certified research assistant. Body weight was measured without shoes to the nearest 0.1 kg. Height and waist and hip circumference were measured to the nearest 0.5 cm. Waist circumference was measured in a standing position with a tape measure all around the body, at the level midway between the lower rib margin and the iliac crest. Body mass index (BMI) was calculated by dividing weight in kilograms by the squared height in meters (kg/m^2). Systolic and diastolic blood pressure were measured every minute for a period of 10 minutes using a DINAMAP Monitor. The size of the cuff was chosen according to the arm circumference. The average of the last three measurements was reported for each of the blood pressure levels.

HRQoL

HRQoL was measured using the RAND 36-Item Health Survey (27). The RAND-36 includes exactly the same items as the 36-Item Short Form Health Survey v1.0. However, the scoring for the two domains of bodily pain and general health are slightly different. This generic questionnaire consists of 36 items in eight subscales: physical functioning (10 items), role limitations due to physical health problems (four items), bodily pain (two items), general health (five items), vitality (four items), social functioning (two items), role limitations due to emotional problems (three items), and general mental health (five items) (29). Scores on the eight domains are generated as described elsewhere (27,29,30). A higher score reflects a better health status and well-being. Since there is a difference in HRQoL scores between men and women, data were analyzed separately for each sex. As HRQoL scores are not normally distributed, for each domain a sex-specific cutoff point was used at the 75th percentile of the healthy non-obese general population, as described by Slagter *et al.* (30). Participants with a score lower than this cutoff were considered to have an abnormally low score for this specific domain.

Cognitive functioning

Executive functioning, as part of cognitive functioning, was assessed in LifeLines with the Ruff Figural Fluency Test (RFFT) (31). Participants received five sheets of paper with 35 squares with five-dot patterns (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/thy). In parts 1–3, the same dot pattern was used, but in parts 2 and 3 different distractions were added. In parts 4 and 5, the dots were placed differently in the square (Supplementary Fig. 1). The participants were asked to draw as many designs as possible by connecting two or more dots with a straight line, and one minute was allowed for each part to be completed. The outcome of the RFFT is the sum of the number of unique designs in all five parts and the sum of the number of repetitions in all five parts. The total sum of designs had a range from 0 to 175. Repetitions of designs were scored as perseverative errors. Since sex, age, and educational level (according to the International Standard Classification of Education) influence the RFFT scores, z-scores of unique designs were calculated in order to correct for these parameters. Perseverative errors are given as median and range. No other cognitive function tests have been performed in LifeLines.

Genomics

The genotype data for the Thr92Ala (rs225014) SNP were derived from genome-wide genotyping performed with the Illumina HumanCytoSNP12 v2 beadchip assay (Illumina, Inc., San Diego, CA) as described previously (32). Frequency of the allele distribution of the D2-Thr92Ala polymorphism (common D2-Thr92 or T-allele, minor D2-Ala92 or C-allele) was calculated with PLINK (33).

Biochemical measurements

Blood samples were collected in the morning after an overnight fast, directly into tubes containing heparin, and centrifuged. Measurements were performed in the clinical

chemistry laboratory of the University Medical Center Groningen. Serum levels of total and high-density lipoprotein cholesterol were measured using an enzymatic colorimetric method, triglycerides using a colorimetric UV method, and low-density lipoprotein cholesterol using an enzymatic method, all on a Roche Modular P chemistry analyzer (Roche, Basel, Switzerland). HbA1c was measured using a turbidimetric inhibition immunoassay on a Cobas Integra 800 CTS analyzer (Roche Diagnostics Nederland BV, Almere, Netherlands). Fasting blood glucose was measured using a hexokinase method, and hs-CRP was analyzed using nephelometry (BN II system; Siemens, Marburg, Germany). Results of the thyroid hormone status were available in a subset of 4479 participants. TSH, fT4, and fT3 were assayed by electrochemiluminescent immunoassay on the Roche Modular E170 Analyzer using kits provided by the manufacturer (Roche, Basel, Switzerland) (27). Normal values are 11–20 pmol/L for fT4 and 4.4–6.7 pmol/L for fT3. The general Dutch population is iodine sufficient. Tests to measure antithyroid peroxidase antibody levels were not performed.

Data description and statistical analysis

The number of different medications used by a participant was considered as a proxy for multi-morbidity (34). The presence of the metabolic syndrome was calculated according to the revised criteria of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP ATP III) (35).

The initial analyses showed that there were differences in sex distribution, age, and BMI between participants using LT4 and the general population, and 88% of the LT4 users were female. This would have influenced the interpretation of the thyroid hormone parameters, as well as the scores of HRQoL and executive functioning. Therefore, the data were reevaluated in the female participants only after matching the LT4 users 1:5 with control participants for age and BMI. Matching was adequate, with mean age being 52.7 ± 11.4 versus 52.1 ± 11.1 years, and BMI 27.0 ± 4.6 versus 26.9 ± 4.4 kg/m² in LT4 users versus the matched control group, respectively.

SNPs were coded by genotype as 0, 1, and 2, with individuals homozygous for the non-risk allele as the reference group. To examine the main effect of the D2-Thr92Ala SNP, the three genotypes (Thr/Thr, Thr/Ala, and Ala/Ala) were considered separately under the additive model.

All analyses were conducted using IBM SPSS Statistics for Windows v22 (IBM, Armonk, NY). Data are presented as mean $\pm$ standard deviation (SD), or median and interquartile range when not normally distributed. Means were compared between groups with analysis of variance. When variables were not normally distributed, medians were compared with the nonparametric Kruskal–Wallis test. The chi-square test was used to analyze categorical variables. To adjust for multiple comparisons, a *p*-value of <0.01 was considered statistically significant.

Results

Relevant baseline characteristics are shown in Table 1 for participants using LT4 and those from the general population. LT4 users more often were women, and they were older than participants from the general population. Both groups had similar TSH levels. There were, however, significant

TABLE 1. CHARACTERISTICS OF THE STUDY PARTICIPANTS

	General population, N = 12,261	LT4 users, N = 364	p-Value
Sex, M/F (%)	5278/6983 (43/57)	43/321 (11/88)	<0.001
Age (years)	48.4 ± 11.2	53.1 ± 11.6	<0.001
BMI (kg/m ²)	26.2 ± 4.1	27.0 ± 4.5	<0.001
TSH (mIU/L) ^a	2.15 (1.54–3.01)	2.68 (0.71–4.51)	0.245
fT3 (pmol/L) ^a	5.2 ± 0.8	4.5 ± 0.7	<0.001
fT4 (pmol/L) ^a	15.7 ± 2.3	18.4 ± 3.3	<0.001
Systolic BP (mmHg)	128 ± 16	128 ± 17	0.888
Diastolic BP (mmHg)	75 ± 9	75 ± 9	0.198
Heart rate (beats/min)	72 ± 11	71 ± 10	0.033
Creatinin (μmol/L)	74 ± 15	72 ± 13	0.001
Glucose (mmol/L)	5.1 ± 0.9	5.2 ± 1.1	0.001
HbA1c (%)	5.6 ± 0.5	5.7 ± 0.5	<0.001
Total cholesterol (mmol/L)	5.1 ± 1.0	5.2 ± 1.0	0.177
HDL cholesterol (mmol/L)			
Males	1.27 ± 0.31	1.31 ± 0.33	0.412
Females	1.58 ± 0.38	1.54 ± 0.37	0.059
LDL cholesterol (mmol/L)	3.29 ± 0.89	3.30 ± 0.89	0.816
Triglycerides (mmol/L)	1.27 ± 0.85	1.20 ± 0.65	0.158
% with metabolic syndrome	20	27	0.001
% of participants using:			
Blood pressure lowering drugs	15	29	<0.001
Cholesterol-lowering drugs	8	17	<0.001
Number of medications other than thyroid hormone	1.1	1.9	<0.001

Data are given as mean ± SD, or median (IQR) when not normally distributed.

^aAvailable in 4336 participants from the general population, and 143 LT4 users.

LT4, levothyroxine; M, males; F, females; BMI, body mass index; TSH, thyrotropin; fT4, free thyroxine; fT3, free triiodothyronine; BP, blood pressure; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SD, standard deviation; IQR, interquartile range.

differences of fT3 and fT4 between those with and without LT4 therapy. fT3 was lower and fT4 was higher in the individuals using LT4. Patients treated with LT4 reported significantly more comorbidities such as atherosclerosis, gallstones, Parkinson's disease, anemia, osteoporosis, and depression compared with the general population. Also, the LT4 group used a higher number of other medications (median 1.9 vs. 1.1 other drugs). In particular, lipid-lowering medication was used more often (Table 1).

The Ala/Ala genotype of the D2-Thr92Ala polymorphism was present in 11.3% of the LT4 users and 10.7% in the general population, whereas the Thr/Thr genotype frequency was 44.8% and 44.2%, respectively. Only 52% of LT4 users had a TSH level within the normal range (0.4–4.0 mIU/L), while 17% had a TSH level <0.4 mIU/L, and 31% had a TSH level >4.0 mIU/L. In the general population, 88% had normal TSH values. There were no significant associations between the number of D2-Ala92 alleles and the TSH classes in the LT4 users (data not shown) as well as in the general population (Supplementary Table S1).

Table 2 depicts the characteristics and thyroid hormone parameters between the different genotype groups of the D2-Thr92Ala SNP for the total study population of 12,625 participants. No statistically significant differences were observed in these parameters. Also, lipid levels and percentage of participants fulfilling the criteria for the metabolic syndrome were not different in individuals carrying the Ala/Ala genotype compared to the other two genotype groups, and neither was the percentage of participants using blood pressure or cholesterol-lowering drugs. Thyroid hormone

parameters were also similar when these parameters were evaluated in the different genotype groups separately for female LT4 users and their matched control group (Table 3). A lower fT3/fT4 ratio in the Ala/Ala carriers was not observed in participants from the general population, LT4 users, or matched non-LT4 users.

HRQoL and cognitive functioning

As the number of males using LT4 was limited (43/364), their HRQoL was not evaluated in comparison to males from the general population, and only data from the female population are reported. Compared with matched controls, a significantly larger proportion of female LT4 users had a score lower than the cutoff value, indicating a poor score, in six of the eight subscales (i.e., physical functioning, vitality, mental health, social functioning, bodily pain, and general health; Table 4). There was no difference in cognitive functioning between females using LT4 and the matched control group in both the creation of unique designs ($p = 0.420$), their z-scores ($p = 0.949$), and perseverative errors ($p = 0.701$; Table 5).

An association could not be demonstrated between the number of Ala alleles and HRQoL in the general population (Fig. 1), in LT4 users, and in their matched controls (Supplementary Table S2). Also, an association was not observed between the number of the D2-Ala92 alleles and cognitive functioning (unique patterns of z-score) in the LT4 users ($p = 0.912$), the matched control group ($p = 0.083$), and the general population ($p = 0.372$; Table 5).

TABLE 2. D2-THR92ALA POLYMORPHISM AND CLINICAL CHARACTERISTICS IN THE TOTAL STUDY POPULATION

D2-Thr92Ala	Thr/Thr	Thr/Ala	Ala/Ala	p-Value
N	5581	5690	1354	
Sex (% M/F)	42/58	42/58	44/56	0.460
Age (years)	48±11	49±11	48±11	0.100
BMI (kg/m ²)	26.2±4.1	26.2±4.1	26.3±4.2	0.609
TSH (mIU/L) ^a	2.16 (1.55–3.05)	2.17 (1.51–3.03)	2.09 (1.53–2.92)	0.185
fT4 (pmol/L) ^a	15.9±2.4	15.7±2.3	15.7±2.6	0.022
fT3 (pmol/L) ^a	5.2±0.8	5.2±0.7	5.2±0.9	0.789
fT3/fT4 ratio ^a	0.33±0.05	0.34±0.05	0.34±0.05	0.012
Systolic BP (mmHg)	128±16	128±16	128±15	0.757
Diastolic BP (mmHg)	75±9	75±9	76±9	0.509
Heart rate (beats/min)	72±11	72±11	72±11	0.174
Creatinin (μmol/L)	75±14	74±13	75±21	0.060
Glucose (mmol/L)	5.1±0.9	5.1±0.8	5.2±0.9	0.114
HbA1c (%)	5.6±0.5	5.6±0.4	5.6±0.5	0.816
Total cholesterol (mmol/L)	5.1±1.0	5.1±1.0	5.1±1.0	0.723
HDL cholesterol (mmol/L)				
Males	1.28±0.32	1.27±0.30	1.28±0.31	0.106
Females	1.58±0.39	1.58±0.38	1.56±0.38	0.640
LDL cholesterol (mmol/L)	3.29±0.90	3.29±0.89	3.30±0.89	0.891
Triglycerides (mmol/L)	1.04 (0.76–1.50)	1.06 (0.77–1.49)	1.04 (0.78–1.48)	0.487
% with metabolic syndrome	19.9	20.2	20.0	0.920
% of participants using:				
Blood pressure lowering drugs	14.9	15.9	15.1	0.338
Cholesterol-lowering drugs	8.2	7.8	6.6	0.140
Number of medications other than thyroid hormone	1.2	1.2	1.2	0.390

Data are given as mean ± SD, median (IQR) when not normally distributed, or percentage.

^aAvailable for 4336 participants from the general population, and 143 LT4 users.

Discussion

In this large cross-sectional study, participants using LT4 had a lower fT3 and a higher fT4, while their TSH was comparable to the general population. Also, female LT4 users had lower HRQoL in six of the eight domains of the RAND-36 questionnaire (physical functioning, vitality, mental health,

social functioning, bodily pain, and general health), but a difference in cognitive functioning was not observed. In contrast to the hypothesis, the D2-Thr92Ala polymorphism was not associated with any of the thyroid parameters, percentage of participants with the metabolic syndrome, percentage of participants using blood pressure or cholesterol-lowering drugs, HRQoL, and cognitive functioning either in the general

TABLE 3. D2-THR92ALA POLYMORPHISM AND THYROID FUNCTION PARAMETERS IN FEMALE SUBJECTS USING LT4 AND THE GENERAL POPULATION MATCHED FOR AGE AND BODY MASS INDEX

D2-Thr92Ala	Thr/Thr	Thr/Ala	Ala/Ala	p-Value
<i>Matched non-LT4 users</i>				
N	733	694	154	
Age (years)	52±9	52±9	52±10	0.913
BMI (kg/m ²)	26.7±4.2	26.9±4.6	27.4±4.6	0.181
TSH (mIU/L) ^a	2.38 (1.69–3.41)	2.33 (1.56–3.24)	2.50 (1.77–3.21)	0.439
fT4 (pmol/L) ^a	15.6±2.0	15.4±2.2	15.8±4.8	0.383
fT3 (pmol/L) ^a	5.1±0.7	5.1±0.7	5.3±2.0	0.350
fT3/fT4 ratio ^a	0.33±0.05	0.33±0.05	0.33±0.05	0.548
<i>Subjects using LT4</i>				
N	146	140	35	
Age (years)	52±11	54±12	51±11	0.325
BMI (kg/m ²)	27.1±4.7	26.7±4.6	27.0±4.1	0.802
TSH (mIU/L) ^b	2.69 (0.72–4.43)	2.71 (0.65–4.50)	2.60 (1.29–4.00)	0.997
fT4 (pmol/L) ^b	18.3±3.4	18.4±3.3	18.5±3.2	0.975
fT3 (pmol/L) ^b	4.5±0.8	4.5±0.7	4.8±0.5	0.399
fT3/fT4 ratio ^b	0.25±0.05	0.25±0.06	0.26±0.03	0.706

^aAvailable in 628 participants.

^bAvailable in 131 participants.

Data are given as mean ± SD, or median (IQR).

TABLE 4. HRQoL AND COGNITIVE FUNCTIONING IN FEMALE LT4 USERS AND MATCHED PARTICIPANTS FROM THE GENERAL POPULATION

HRQoL domain + cutoff value indicating a poor score	Non LT4 users, N = 1581	LT4 users, N = 321	p-Value
<i>HRQoL</i>			
Physical functioning <85 (%)	27.4	35.6	0.003
Role limitations due to physical health problems <100 (%)	23.7	29.8	0.014
Role limitations due to emotional problems <100 (%)	13.9	15.2	0.292
Vitality <55 (%)	18.1	30.8	<0.001
Mental health <72 (%)	20.5	30.5	<0.001
Social functioning <75 (%)	17.4	25.1	0.001
Bodily pain <67.4 (%)	15.4	23.8	<0.001
General health <65 (%)	25.8	37.5	<0.001
<i>RFFT scores</i>			
Unique designs	N = 1384 75 ± 23	N = 276 74 ± 23	0.420
z-Scores for unique designs	-0.09 ± 0.97	-0.07 ± 0.95	0.949
Perseverative errors	10 (5–19)	9 (5–19)	0.701

Data are given as percentage for the different HR-QoL domains, and as mean ± SD and median (IQR) for RFFT scores. For each HR-QoL domain a sex-specific cutoff point at the 75th percentile of the healthy non-obese general population was used, as described by Slagter *et al.* (30). HR-QoL, health-related quality of life; RFFT, Ruff Figural Fluency Test; NS, not significant.

population or subjects on thyroid hormone replacement therapy or in matched controls.

Renewed interest in the effects of polymorphisms of deiodinases came after the elegant recent study by McAninch *et al.*, who showed that the D2-Ala92 polymorphism was associated with increased expression of genes associated with oxidative stress and inflammation in brain tissue (36). These are relevant findings for the clinician, considering that 10–12% of patients treated for primary hypothyroidism experience complaints such as fatigue, lack of energy, reduced exercise capacity, and disturbances in concentration and mental functioning, despite optimal thyroid hormone replacement therapy. It has been suggested that some of these patients can be identified by a lower fT3/fT4 ratio (23), and may favorably respond to combination therapy of LT4 and liothyronine (24). Thus, it may be warranted to identify those patients in clinical care, preferentially based on simple parameters such as thyroid hormone measurements, who may show the best response to this treatment. In this study, there was no association between the plasma level of fT4, fT3, and

the fT3/fT4 ratio and the number of D2-Ala92 alleles. There are two studies that indirectly showed that the T4 to T3 conversion could be diminished in subjects carrying the D2-Thr92Ala polymorphism. Butler *et al.* showed that the Thr92Ala polymorphism is associated with a delayed T3 secretion in response to TRH stimulation (37). Torlantano *et al.* showed that thyroidectomized patients carrying the Thr92Ala polymorphism needed higher doses of LT4 to achieve target TSH levels (20). Other studies failed to confirm these findings (38,39).

The current study evaluated HRQoL with the RAND-36 questionnaire, a generic questionnaire on well-being, of which physical functioning, vitality, and general health are prominent domains. The RAND-36 instrument proved able to discriminate differences in HRQoL, as it was found that more LT4 users reported impaired HRQoL compared with the matched euthyroid participants. Women using LT4 experienced a significant reduction in HRQoL in several RAND-36 domains. However, no differences in any of the RAND-36 domain scores were observed between the Thr/Thr and the

TABLE 5. SCORES ON THE RFFT ACCORDING TO THE D2-THR92ALA POLYMORPHISMS IN WOMEN FROM THE GENERAL POPULATION, IN FEMALE LEVOTHYROXINE USERS AND MATCHED PARTICIPANTS

		D2-Thr92Ala polymorphism			p-Value
		Thr/Thr	Thr/Ala	Ala/Ala	
Non-LT4 users	N	2707	2799	637	
	Unique designs	79 ± 24	78 ± 24	81 ± 24	0.048
	z-Scores for unique designs	-0.03 ± 1.00	-0.07 ± 1.02	-0.02 ± 1.04	0.372
	Perseverative errors	9 (5–17)	9 (5–17)	9 (5–17)	0.811
Matched non-LT4 users	N	646	610	128	
	Unique designs	77 ± 23	73 ± 23	75 ± 24	0.026
	z-Scores for unique designs	-0.01 ± 0.96	0.13 ± 0.98	-0.12 ± 0.97	0.083
	Perseverative errors	10 (5–17)	10 (5–21)	11 (6–22)	0.459
LT4 users	N	126	118	32	
	Unique designs	74 ± 23	73 ± 25	76 ± 18	0.780
	z-Scores for unique designs	-0.09 ± 0.96	-0.05 ± 1.00	-0.11 ± 0.13	0.912
	Perseverative errors	10 (5–22)	9 (4–18)	7 (5–16)	0.750

Data are given as mean ± SD, or median (IQR).

FIG. 1. Percentages of female participants in the general population with a poor health-related quality of life domain score, according to Thr or Ala carriership. The corresponding cutoff value derived from the scores in the normal weight population for the individual domains (25th percentile): PF, physical functioning (85.0); RP, role limitations due to physical health problems (100.0); BP, bodily pain (67.4); GH, general health (65.0); VT, vitality (55.0); SF, social functioning (75.0); RE, role limitations due to emotional problems (100.0); MH, mental health (72.0). There are no statistically significant differences between Thr/Thr, Thr/Ala, and Ala/Ala.

Ala/Ala carriers in all three groups evaluated (the general population, the LT4 users, and their matched controls), which confirms an earlier report by Appelhof *et al.* (40). It has been known from several studies that comorbidities may strongly influence HRQoL. In the present population, participants treated with LT4 used more other medications (median 1.9 vs. 1.1) than those not using LT4. The number of medications used is considered as a proxy for the presence of other comorbid disorders (34). In particular, lipid-lowering drugs were more often used by LT4 users. Also, more LT4 users reported complaints related to gallbladder disease, anemia, atherosclerosis, and depression.

In addition to the more traditional HRQoL assessment, it was possible to assess potential differences in concentration and mental functioning in the present population with the RFFT. This test measures the ability to draw as many unique designs as possible within a set time period, and is a sensitive cognitive test for changes in nonverbal fluency in young as well as old persons (31,41–43). The test provides insight in many different cognitive abilities that range from initiation and planning to divergent reasoning and mental flexibility. These characteristics and the limited time that is allowed to administer the test make the RFFT a useful outcome measure for cognitive functioning and early cognitive decline (42,43). No differences were observed in RFFT scores between participants who used LT4 and those who did not. This is in accordance with the study by Samuels *et al.*, in which executive functioning was assessed with the Letter Cancellation Test, Train Making Test, and the Iowa Gambling Test (44). Another study by Samuels *et al.* did show a significantly lower cognitive functioning and HRQoL in patients treated with LT4 compared to healthy controls, which it was suggested was related to suboptimal treatment (45).

The findings by McAninch *et al.* (36) suggest that the D2-Thr92Ala polymorphism could be a risk factor for neurodegenerative diseases and neuropsychological impairment. In the current study, carriers of the two D2-Ala92 alleles did not show a worse score compared to the other two groups. To the authors' knowledge, no other studies have been performed that investigated the association between the D2-Thr92Ala polymorphism and executive functioning. Replication of the present findings in other cohorts and perhaps with other techniques assessing cognitive functioning may be warranted. The single amino acid substitution of Thr for Ala lies within the 18 amino acid loop that controls susceptibility to T4-induced ubiquitination of D2 (46). This mutated enzyme is ectopically located in the Golgi apparatus and has a longer half-life. Also the Golgi apparatus has a different structure. The cellular abnormalities result in alterations at the transcriptional level and are independent of D2-mediated T3 production. These transcriptional changes are associated with mitochondrial dysfunction, inflammation, apoptosis, changes in DNA repair, and growth factor signaling (36). This may explain why it has been suggested that the D2-Thr92Ala polymorphism may result in such diverse phenotypic outcomes, such as insulin resistance, bipolar disorder, mental retardation, hypertension, and osteoarthritis (22). However, the current study did not find any association between the D2-Ala92 polymorphism and metabolic syndrome or hypertension, or their treatment.

There are some strengths and limitations to this study. More than 12,600 individuals from the general population were evaluated, which is one of the largest cohorts to date of people in whom both information on the D2-Thr92Ala polymorphism, thyroid hormone parameters, and HRQoL was available. A limitation of this study is that the analyses were mainly limited to female users of LT4, as <12% of subjects using thyroid replacement therapy were male. Considering the 3% prevalence of primary hypothyroidism in the general population, only a small number of LT4 users could be included in this cross-sectional study. Nevertheless, this study was specifically chosen to be performed in the general population, as evaluating LT4 users treated in other settings, for instance an endocrinology outpatient clinic, may overestimate the prevalence of residual complaints and reduced HRQoL. Typically, patients with more severe complaints are more likely to be referred to an endocrinologist.

In conclusion, this study demonstrates that women using thyroid hormone replacement therapy experience a lower HRQoL in six domains of the RAND-36 questionnaire (physical functioning, vitality, mental health, social functioning, bodily pain, and general health) compared with matched participants from the general population. The Thr92Ala polymorphism of D2 was not associated with thyroid parameters, elevated blood pressure, metabolic syndrome, HRQoL, or executive functioning in the general population and in subjects using thyroid replacement therapy.

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Author Disclosure Statement

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References

- Garmendia Madariaga A, Santos Palacios S, Guillen-Grima F, Galofre JC 2014 The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. *J Clin Endocrinol Metab* **99**:923–931.
- Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM; American Thyroid Association Task Force on Thyroid Hormone Replacement 2014 Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association Task Force on Thyroid Hormone Replacement. *Thyroid* **24**:1670–1751.
- Canaris GJ, Manowitz NR, Mayor G, Ridgway EC 2000 The Colorado thyroid disease prevalence study. *Arch Intern Med* **160**:526–534.
- Saravanan P, Chau WF, Roberts N, Vedhara K, Greenwood R, Dayan CM 2002 Psychological well-being in patients on “adequate” doses of L-thyroxine: results of a large, controlled community-based questionnaire study. *Clin Endocrinol (Oxf)* **57**:577–585.
- Wiersinga WM, Duntas L, Faddeyev V, Nygaard B, Vanderpump MP 2012 2012 ETA Guidelines: the use of L-T4 + L-T3 in the treatment of hypothyroidism. *Eur Thyroid J* **1**:55–71.
- Gullo D, Latina A, Frasca F, Le Moli R, Pellegriti G, Vigneri R 2011 Levothyroxine monotherapy cannot guarantee euthyroidism in all athyreotic patients. *PLoS One* **6**:e22552.
- Alevizaki M, Mantzou E, Cimponeriu AT, Alevizaki CC, Koutras DA 2005 TSH may not be a good marker for adequate thyroid hormone replacement therapy. *Wien Klin Wochenschr* **117**:636–640.
- Bunevicius R, Kazanavicius G, Zalinkevicius R, Prange AJ Jr 1999 Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. *N Engl J Med* **340**:424–429.
- Escobar-Morreale HF, Botella-Carretero JJ, Morreale de Escobar G 2015 Treatment of hypothyroidism with levothyroxine or a combination of levothyroxine plus L-triiodothyronine. *Best Pract Res Clin Endocrinol Metab* **29**:57–75.
- Davis JD, Tremont G 2007 Neuropsychiatric aspects of hypothyroidism and treatment reversibility. *Minerva Endocrinol* **32**:49–65.
- Wekking EM, Appelhof BC, Fliers E, Schene AH, Huyser J, Tijssen JG, Wiersinga WM 2005 Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. *Eur J Endocrinol* **153**:747–753.
- Pilo A, Iervasi G, Vitek F, Ferdeghini M, Cazzuola F, Bianchi R 1990 Thyroidal and peripheral production of 3,5,3'-triiodothyronine in humans by multicompartmental analysis. *Am J Physiol* **258**:E715–E726.
- Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR 2002 Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* **23**:38–89.
- Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, Visser TJ 2003 Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. *J Clin Endocrinol Metab* **88**:2880–2888.
- Peeters RP, van den Beld AW, Attalki H, Toor H, de Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG, Visser TJ 2005 A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. *Am J Physiol Endocrinol Metab* **289**:E75–E81.
- de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ, Breteler MM 2007 The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. *J Clin Endocrinol Metab* **92**:636–640.
- Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JR, Weedon MN, Singleton A, Hernandez D, Evans J, Durant C, Ferrucci L, Melzer D, Saravanan P, Visser TJ, Ceresini G, Hattersley AT, Vaidya B, Dayan CM, Frayling TM 2008 A common variation in deiodinase 1 gene *DIO1* is associated with the relative levels of free thyroxine and triiodothyronine. *J Clin Endocrinol Metab* **93**:3075–3081.
- Taylor PN, Razvi S, Pearce SH, Dayan CM 2013 Clinical review: a review of the clinical consequences of variation in thyroid function within the reference range. *J Clin Endocrinol Metab* **98**:3562–3571.
- Celi FS, Canettieri G, Mentuccia D, Proietti-Pannunzi L, Fumarola A, Sibilla R, Predazzi V, Ferraro M, Andreoli M, Centanni M 2000 Structural organization and chromosomal localization of the human type II deiodinase gene. *Eur J Endocrinol* **143**:267–271.
- Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S, Trischitta V 2008 Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. *J Clin Endocrinol Metab* **93**:910–913.
- Grarup N, Andersen MK, Andreasen CH, Albrechtsen A, Borch-Johnsen K, Jorgensen T, Auwerx J, Schmitz O, Hansen T, Pedersen O 2007 Studies of the common *DIO2* Thr92Ala polymorphism and metabolic phenotypes in 7342 Danish white subjects. *J Clin Endocrinol Metab* **92**:363–366.
- Verloop H, Dekkers OM, Peeters RP, Schoones JW, Smit JW 2014 Genetics in endocrinology: genetic variation in deiodinases: a systematic review of potential clinical effects in humans. *Eur J Endocrinol* **171**:R123–R135.
- Wartofsky L 2013 Combination L-T3 and L-T4 therapy for hypothyroidism. *Curr Opin Endocrinol Diabetes Obes* **20**:460–466.
- Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, Dayan CM 2009 Common variation in the *DIO2* gene predicts baseline psychological well-being and response

- to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. *J Clin Endocrinol Metab* **94**:1623–1629.
25. McAninch EA, Bianco AC 2015 New insights into the variable effectiveness of levothyroxine monotherapy for hypothyroidism. *Lancet Diabetes Endocrinol* **3**:756–758.
 26. Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, van Dijk F, van Zon SK, Wijmenga C, Wolffenbuttel BH, Stolk RP 2015 Cohort profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol* **44**:1172–1180.
 27. Klaver EI, van Loon HC, Stienstra R, Links TP, Keers JC, Kema IP, Muller Kobold AC, van der Klauw MM, Wolffenbuttel BH 2013 Thyroid hormone status and health-related quality of life in the LifeLines cohort study. *Thyroid* **23**:1066–1073.
 28. Boelen A, Kwakkel J, Alkemade A, Renckens R, Kaptein E, Kuiper G, Wiersinga WM, Visser TJ 2005 Induction of type 3 deiodinase activity in inflammatory cells of mice with chronic local inflammation. *Endocrinology* **146**:5128–5134.
 29. VanderZee KI, Sanderma R, Heyink JW, de HH 1996 Psychometric qualities of the RAND 36-Item Health Survey 1.0: a multidimensional measure of general health status. *Int J Behav Med* **3**:104–122.
 30. Slagter SN, van Vliet-Ostaptchouk JV, van Beek AP, Keers JC, Lutgers HL, van der Klauw MM, Wolffenbuttel BH 2015 Health-related quality of life in relation to obesity grade, type 2 diabetes, metabolic syndrome and inflammation. *PloS One* **10**:e0140599.
 31. van Eersel ME, Joosten H, Koerts J, Gansevoort RT, Slaets JP, Izaks GJ 2015 Longitudinal study of performance on the Ruff Figural Fluency Test in persons aged 35 years or older. *PLOS ONE* **10**:e0121411.
 32. Jansen H, Stolk RP, Nolte IM, Kema IP, Wolffenbuttel BH, Snieder H 2013 Determinants of HbA1c in nondiabetic Dutch adults: genetic loci and clinical and lifestyle parameters, and their interactions in the LifeLines cohort study. *J Intern Med* **273**:283–293.
 33. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC 2007 PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**:559–575.
 34. Schubert CC, Boustani M, Callahan CM, Perkins AJ, Carney CP, Fox C, Unverzagt F, Hui S, Hendrie HC 2006 Comorbidity profile of dementia patients in primary care: are they sicker? *J Am Geriatr Soc* **54**:104–109.
 35. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JJ, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr 2009 Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* **120**:1640–1645.
 36. McAninch EA, Jo S, Preite NZ, Farkas E, Mohacsik P, Fekete C, Egri P, Gereben B, Li Y, Deng Y, Patti ME, Zevenbergen C, Peeters RP, Mash DC, Bianco AC 2015 Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated syndromes. *J Clin Endocrinol Metab* **100**:920–933.
 37. Butler PW, Smith SM, Linderman JD, Brychta RJ, Alberobello AT, Dubaz OM, Luzon JA, Skarulis MC, Cochran CS, Wesley RA, Pucino F, Celi FS 2010 The Thr92Ala 5' type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: a pharmacogenomic study. *Thyroid* **20**:1407–1412.
 38. Heemstra KA, Hoftijzer HC, van der Deure WM, Peeters RP, Fliers E, Appelhof BC, Wiersinga WM, Corssmit EP, Visser TJ, Smit JW 2009 Thr92Ala polymorphism in the type 2 deiodinase is not associated with T4 dose in athyroid patients or patients with Hashimoto thyroiditis. *Clin Endocrinol (Oxf)* **71**:279–283.
 39. Santoro AB, Vargens DD, Barros Filho Mde C, Bulzico DA, Kowalski LP, Meirelles RM, Paula DP, Neves RR, Pessoa CN, Struchine CJ, Suarez-Kurtz G 2014 Effect of UGT1A1, UGT1A3, DIO1 and DIO2 polymorphisms on L-thyroxine doses required for TSH suppression in patients with differentiated thyroid cancer. *Br J Clin Pharmacol* **78**:1067–1075.
 40. Appelhof BC, Peeters RP, Wiersinga WM, Visser TJ, Wekking EM, Huyser J, Schene AH, Tijssen JG, Hoogendijk WJ, Fliers E 2005 Polymorphisms in type 2 deiodinase are not associated with well-being, neurocognitive functioning, and preference for combined thyroxine/3,5,3'-triiodothyronine therapy. *J Clin Endocrinol Metab* **90**:6296–6299.
 41. Ruff RM, Light RH, Evans RW 1987 The Ruff Figural Fluency Test: a normative study with adults. *Devel Neuropsychol* **3**:37–51.
 42. Brummelman P, Elderson MF, Dullaart RP, van den Bergh AC, Timmer CA, van den Berg G, Koerts J, Tucha O, Wolffenbuttel BH, van Beek AP 2011 Cognitive functioning in patients treated for nonfunctioning pituitary macroadenoma and the effects of pituitary radiotherapy. *Clin Endocrinol (Oxf)* **74**:481–487.
 43. Izaks GJ, Joosten H, Koerts J, Gansevoort RT, Slaets JP 2011 Reference data for the Ruff Figural Fluency Test stratified by age and educational level. *PloS One* **6**:e17045.
 44. Samuels MH, Kolobova I, Smeraglio A, Peters D, Janowsky JS, Schuff KG 2014 The effects of levothyroxine replacement or suppressive therapy on health status, mood, and cognition. *J Clin Endocrinol Metab* **99**:843–851.
 45. Samuels MH, Schuff KG, Carlson NE, Carello P, Janowsky JS 2007 Health status, psychological symptoms, mood, and cognition in L-thyroxine-treated hypothyroid subjects. *Thyroid* **17**:249–258.
 46. Callebaut I, Curcio-Morelli C, Mornon JP, Gereben B, Buettner C, Huang S, Castro B, Fonseca TL, Harney JW, Larsen PR, Bianco AC 2003 The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. *J Biol Chem* **278**:36887–36896.

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