Differentiated thyroid carcinoma : studies on diagnosis, treatment-related side effects and well-being

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CHAPTER 6

Genetic variation in deiodinases: a systematic review of potential clinical effects in humans

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Abstract

Iodothyronine deiodinases represent a family of selenoproteins involved in peripheral and local homeostasis of thyroid hormone action. Deiodinases are expressed in multiple organs and thyroid hormone affects numerous biological systems, thus genetic variation in deiodinases may affect multiple clinical endpoints. Interest in clinical effects of genetic variation in deiodinases has clearly increased. We aimed to provide an overview for the role of deiodinase polymorphisms in human physiology and morbidity. In this systematic review, studies evaluating the relationship between deiodinase polymorphisms and clinical parameters in humans were eligible. No restrictions on publication date were imposed. The following databases were searched up to August 2013: Pubmed, EMBASE (OVID-version), Web of Science, COCHRANE Library, CINAHL (EbscoHOST-version), Academic Search Premier (EbscoHOST-version), and ScienceDirect. Deiodinase physiology at molecular and tissue level is described, and finally the role of these polymorphisms in pathophysiological conditions is reviewed. Deiodinase type 1 (D1) polymorphisms particularly show moderate-to-strong relationships with thyroid hormone parameters, IGF1 production, and risk for depression. D2 variants correlate with thyroid hormone levels, insulin resistance, bipolar mood disorder, psychological well-being, mental retardation, hypertension, and risk for osteoarthritis.
D3 polymorphisms showed no relationship with inter-individual variation in serum thyroid hormone parameters. One D3 polymorphism was associated with risk for osteoarthritis. Genetic deiodinase profiles only explain a small proportion of inter-individual variations in serum thyroid hormone levels. Evidence suggests a role of genetic deiodinase variants in certain pathophysiological conditions. The value for determination of deiodinase polymorphism in clinical practice needs further investigation.

Introduction

The relationship between variation in thyroid hormone pathway genes and their effects on clinical phenotypes represents a relatively new field of research. In the last few years, the influence of deiodinase polymorphisms on various thyroid hormone-related endpoints is considered a topic of particular interest. Iodothyronine deiodinases represent a family of selenoproteins involved in local and periphe-

ral homeostasis of thyroid hormones. Three deiodinases have been described. Deiodinase type 1 (D1) and D2 play a major role in conversion of thyroxine (T4) to biologically active tri-iodothyronine (T3) and in clearance of reverse T3 (rT3) (1). Although both deiodinases are determinants of peripheral thyroid hormone levels, D2 is particularly renowned for its role in local T3 production (2, 3). D3 is the main T3-inactivating enzyme, regulating the conversion of T3 to T2 and T4 to rT3 (1). Deiodinases have a tissue-specific expression pattern (1, 2). D1 protein is mainly expressed in liver, kidney, thyroid, and euthyroid pituitary (1). D2 protein is detected in the CNS, pituitary, skeletal muscle, thyroid, heart, bone, and brown adipose tissue (2). D3 is located in the CNS and placenta in adult life and is predominantly expressed in many tissues during fetal life (1, 2).

Bearing in mind that deiodinases affect thyroid hormone regulation in a wide range of tissues, genetic variation in these seleno-enzymes might affect various organ systems. In the last few years, several deiodinase polymorphisms with putative impact on functional enzyme activity have been detected (4, 5, 6, 7, 8). In this systematic review, available evidence for the impact of deiodinase polymorphisms on various clinical domains in humans is summarized. Furthermore, we aimed to evaluate whether determination of a genetic deiodinase profile may be a valuable new endocrinological tool in future clinical practice.

Methods

Eligibility criteria and search strategy

Studies evaluating the relationship between deiodinase polymorphisms and clinical parameters in human populations were eligible. No restrictions on publication date were imposed. The search strategy was composed in cooperation with a trained librarian. The following databases were searched up to 13 August 2013: Pubmed, EMBASE (OVID-version), Web of Science, COCHRANE Library, CINAHL (EbscoHOST-version), Academic Search Premier (EbscoHOST-version), and ScienceDirect. For two concepts (deiodinase and polymorphisms), relevant keyword variations were used, not only variations in the controlled vocabularies of the various databases, but also free text word variations. This search strategy was optimized for all consulted databases. Reference lists of studies eligible for inclusion were hand-searched and checked for additional relevant articles.

Table 1 Overview detailing the prevalence of frequently investigated D1 and D2 polymorphisms in different populations

Study	D1-C785T polymorphism (T-allele frequency)	N (number of patients	Origin of population
Cooper-Kazaz et al, 2009 (44)	0.42	64	Patients diagnosed with major depression
De Jong et al, 2007 (20)	0.36 [k]	995	Elderly Caucasians
Hoftijzer et al, 2011 (15)	0.36 [k]	148	Patients treated for DTC
Panicker et al, 2008 (10)	0.34 [k]	546	Patients on thyroid hormone replacement therapy
Peeters et al, 2003 (17)	0.34	156	Healthy European blood donors
Peeters et al, 2005 (24)	0.34	349	Healthy elderly men
Procopciuc et al, 2012 (23)	0.24 (C) /0.37 (PW)	100	Pregnant women: healthy controls (N=50) and subjects with preeclampsia (N=50)
Van der Deure et al, 2009 (11)	0.34	1192	Healthy Danish twins
Cooper-Kazaz et al, 2009 (44)	0.09	64	Patients diagnosed with major depression
De Jong et al, 2007 (20)	0.10 [a]	995	Elderly Caucasians
Hoftijzer et al, 2011 (15)	0.09 [a]	147	Patients treated for DTC
Peeters et al, 2003 (17)	0.10	156	Healthy European blood donors
Peeters et al, 2005 (24)	0.11	349	Healthy elderly men
Van der Deure et al, 2009 (11)	0.11	1076	Healthy Danish twins
Appelhof et al, 2005 (27)	0.40	141	Patients with PAH
Babenko et al, 2012 (37)	0.24 (C) /0.32 (Graves)	315	Russian population: healthy blood donors (N=135) and patients with Graves' disease (N=180)
Brouwer et al, 2006 (45)	0.31 [k]	93	Patients diagnosed with major depression
Butler et al, 2010 (34)	0.41	83	Healthy volunteers
Canani et al, 2005 (5)	0.41	183	Patients with DM-II
Canani et al, 2007 (67)	0.41	315	Patients with DM-II
Cooper-Kazaz et al, 2009 (44)	0.41	64	Patients diagnosed with major depression
De Jong et al, 2007 (20)	0.37 [k]	995	Elderly Caucasians
Dora et al, 2010 (58)	0.38 (C)/0.38 (DM-II)	1573	Patients with DM-II (N=1057) and controls without DM-II (N=516)
Estivalet et al, 2011 (59)	0.390	721	Patients with DM-II
Fiorito et al, 2007 (60)	0.36	590	Non-diabetic Caucasians
Grarup et al, 2007 (61)	0.36	5843	Danish white subjects
Grineva et al, 2009 (33)	0.25 [k]	145	Patients with Graves' disease
Guerra et al, 2012 (30)	0.36 (C)/0.38 (TA)	216	Healthy controls (N=106) and subjects with thyroid autoimmunity (N=110)
Gumieniak et al, 2007 (31)	0.320 (NTP)/0.390 (HTP)	372	Normotensive (N=68) and hypertensive (N=304) euthyroid subjects
Guo et al, 2004 (76)	0.38 (C)/0.35 (MR)	543 [c]	Controls (N=331) and mentally retarded (N=96) derived from iodine-deficient areas in China
He et al, 2009 (46)	0.41 (C)/0.53 (BPAD)	563	Chinese Han population: C (N=284) and patients with BPAD (N=279)
Heemstra et al, 2009 (28)	0.40 (DTC)/0.40 (HT)	295	Patients treated for DTC (N=154) and patients with HT (N=141)
Hoftijzer et al, 2011 (15)	0.40 [k]	148	Patients treated for DTC
Study	**D1-C785T polymorphism (T-allele frequency)**	**N (number of patients**	**Origin of population**

Ma et al, 2011 (75)	0.40 (C)/0.31 (SS)/0.26 (ALI) [c] and 0.44 (C)/ 0.44 (SS)/ 0.46 (ALI) [d]	405 [c]/302 [d]	European Americans: C(N=188); SS (N=139); ALI(N=78) African Americans: C(N=187); SS (N=74); ALI(N=41)
Maia et al, 2007 (63)	0.37	1633	White subjects of mixed European ancestry
Mentuccia et al, 2002 (4)	0.35	972	Non-diabetic Caucasians
Mentuccia et al, 2005 (32)	0.30	1268	Old order Amish population
Meulenbelt et al, 2008 (39)	0.35	360	Caucasian subjects with symptomatic osteoarthritis
Nair et al, 2012 (64)	0.82	300 [f]	Pima Indians
Panicker et al, 2009 (43)	0.36 (T4) [k g]/0.38 (T4/T3) [k h]	552	Patients on thyroid hormone replacement therapy (T4 only (N=282) and T4/T3 combined (N=270))
Peeters et al, 2003 (17)	0.39	156	Healthy European blood donors
Torlantano et al, 2008 (29)	0.39	191	Patients treated for DTC
Van der Deure et al, 2009 (69)	0.37 [i]/0.38 [j]	2441	Two cohorts of elderly Caucasians: N=1444/N=997
Xiong et al, 2010 (82)	0.44 (C) /0.46 (KBD)	473	Chinese Han population: Controls (N=161) and patients with KBD (N=312)
Zhang et al, 2012 (77)	0.561	1461	Chinese Han population
Appelhof et al, 2005 (27)	0.32	141	Patients with PAH
Cooper-Kazaz et al, 2009 (44)	0.30	64	Patients diagnosed with major depression
De Jong et al, 2007 (20)	0.35 [k]	995	Elderly Caucasians
He et al, 2009 (46)	0.26 (C)/0.19 (BPAD)	563	Chinese Han population: PHC (N=284) and patients with BPAD (N=279)
Hoftijzer et al, 2011 (15)	0.31 [k]	147	Patients treated for DTC
Ma et al, 2011 (75)	0.36 (C)/0.37 (SS)/0.41 (ALI) [c] and 0.06 (C)/ 0.10 (SS)/ 0.12 (ALI) [d]	405 [c]/302 [d]	European Americans: C(N=188); SS (N=139); ALI(N=78) African Americans: C(N=187); SS (N=74); ALI(N=41)
Meulenbelt et al, 2008 (39)	0.34[e]	360	Caucasian subjects with symptomatic osteoarthritis
Peeters et al, 2005 (6)	0.34 (HBD)/0.34 (HEM)	505	Healthy European blood donors (N=156) and healthy elderly men (N=349)
Van der Deure et al, 2009 (69)	0.36 [i]/0.35 [j]	2441	Two cohorts of elderly Caucasians: N=1444/N=997

a = including studies in which allele frequencies of the polymorphism were reported or could be extracted
b Including borderline mentally retarded patients N=116; for genotype frequency see article)
c=determined in European Americans
d=determined in African Americans
e= in siblings affected by osteoarthritis sharing 0 alleles identical by descent (see article for more details)
f=including diabetic subjects (N=150) and non-diabetic subjects (N=150)
g= allele frequency determined in a study including patients on thyroid hormone replacement, randomized to T4 therapy only group
h= allele frequency determined in a study including patients on thyroid hormone replacement, randomized to combined T4/T3 therapy
i= The Rotterdam Study is a population-based, prospective cohort study on chronic and disabling diseases in the elderly.
j= The Rotterdam Scan Study is an ongoing prospective population based cohort study designed to investigate causes and consequences
of age-related brain changes on MRI

k= extracted from reported genotype frequencies
C=controls
PAH= primary autoimmune hypothyroidism
HTP=hypertensive patients
NTP=normotensive patients
BPAD=Bipolar Affective disorder
PHC=psychiatrically healthy controls
DM-II=Diabetes Mellitus type 2
SS=patients with severe sepsis
ALI=patients with severe sepsis and acute lung injury
HBD=healthy blood donors
HEM= healthy elderly men
KBD= Kashin-Beck disease
DTC= differentiated thyroid carcinoma
PW=preeclamptic women

Effect of deiodinase polymorphisms on mRNA and protein synthesis

To provide a better understanding of the putative effects of deiodinase polymorphisms on clinical parameters in our systematic review of literature, we first aimed to describe available evidence on the impact of deiodinase variants on the mRNA and deiodinase protein level.

D1 polymorphisms ➤ The human D1 gene (2–2.1 kb) is located on chromosome 1p32–p33 and comprises four exons (9). Three polymorphisms, D1–rs2235544; D1–rs11206244 (DI-C785T); and D1–rs12095080 (DI-A1814G), are highlighted as potential candidates associated with physiological and pathological processes in humans. The D1–rs2235544 polymorphism is located in intron 3 of the D1 gene (10); D1–rs11206244 and D1–rs12095080 are located in the 3'-UTR of the mRNA (7). An in vitro study revealed no clear effect of the C785T or A1814G variant on mRNA level, mRNA decay rate, or D1 activity (11). A large genome-wide study revealed that within 100 kb of the D1 gene no association between genetic variants and altered D1 expression levels in lymphocytes could be found, thereby questioning the effect of genetic variants in the D1 gene (10, 12). As functionality of D1 polymorphisms has not yet been shown, more research is warranted to determine its exact influence on D1 activity at gene and protein level.

D2 polymorphisms ➤ The human D2 gene (15 kb) maps to chromosome 14q24.2–q24.3 and consists of three exons (13). The D2–rs12885300 (D2-ORFa-Glu3Asp) and D2–rs225014 (D2-Thr92Ala) polymorphisms in exons 1 and 2, respectively, have been frequently evaluated in clinical research.

The D2-ORFa-Glu3Asp polymorphism is located in the short open reading frame within the 5'-UTR of the D2 gene (6). An in vitro study by Coppotelli et al. (8) showed that the minor allele variant of D2-ORFa-Glu3Asp polymorphism was associated with an increased gene transcription and ultimately increased D2 activity. In a second in vitro study by Gereben et al. (14), however, the D2-ORFa-Glu3Asp polymorphism did not affect the expression of a D2 sequence reporter mRNA but did decrease D2 activity by about fivefold. Hoftijzer et al. (15) proposed that the discrepancies between the two studies may be related to the reporter genes used (e.g. a selenoprotein and a non-selenoprotein). From the study of Gereben et al., it may be concluded that the D2 activity/D2 mRNA ratio is low, suggesting a post-translational regulation (15).

The Thr92Ala polymorphism is characterized by the first amino acid substitution located in the instability loop of D2, a site of importance for D2 turnover rate (16). In vitro studies were inconsistent on the functionality of the Thr92Ala variant. D2 enzyme velocity was decreased in skeletal muscle and thyroid tissue samples of type 2 diabetes mellitus (DM2) patients who were homozygous for the Thr92Ala variant (5). In contrast, in vitro analysis in cells transfected with WT or variant D2 revealed no significant differences in D2 activity between the genotypes (17). This discrepancy might be explained by linkage of D2-Thr92Ala to a functional variant elsewhere in the genome. Table 1 provides an overview detailing the prevalence of frequently investigated D1 and D2 polymorphisms in different populations. See supplementary table 1 (see section on supplementary data given at the end of this article) for an overview of studies and their primary clinical endpoints

D3 polymorphisms ➤ The human D3 gene maps to chromosome 14q32.2, consists of one exon, and is located in a human imprinted domain (18). To our knowledge, the effect of DIO3 variants on functional characterization of the DIO3 protein has not been studied. The effect of D3 variants on D3 activity might be hampered by the epigenetic process of genetic imprinting. Effects of D3 polymorphisms on thyroid hormone homeostasis depend on the parental origin of the variant allele (7).

Organ and tissue-specific effects of deiodinase polymorphisms

Pituitary–thyroid axis

D1 polymorphisms ➤ The impact of the D1-C785T polymorphism on basal serum thyroid hormone parameters has been evaluated in several studies. The D1-785T variant was related to higher serum concentrations of fT4 (10, 11, 19, 20, 21, 22, 23) and rT3 (11, 17, 19, 20), lower serum concentrations of T3 or fT3 (10, 20, 23, 24), and lower T3/rT3 or fT3/fT4 ratios (10, 11, 17, 19, 20), overall pointing toward a decreased conversion of T4 to T3 by diminished D1 protein expression or activity. More specifically, the D1-C785T polymorphism was linked to higher rT3 serum levels and a lower T3/rT3 ratio in healthy young mixed cohorts (mean age, 36.7 and 46.2 years) (11, 17), healthy young men (mean age, 25–45 years) (19), and a cohort of elderly subjects (mean age, 72.2 years) (20). Although in elderly populations (mean age, >70 years) the D1-785T variant was associated with lower T3 serum levels (20, 24), this could not be replicated in healthy young mixed cohorts

(mean age, 36.7 and 46.2 years) (11, 17). D1 may have larger contribution to serum T3 production in older age. In young subjects, a decreased T3 production may be masked by the production of serum T3 by D2 in skeletal muscle. Throughout adult life, skeletal muscle size and strength gradually decline, resulting in a decrease in D2-expressing skeletal muscle (7). Therefore, potential effects of D1 polymorphisms on serum T3 production might only result in changes in serum T3 levels in elderly subjects. Research by Panicker et al. (10) showed higher fT4 levels, lower fT3 levels, and a lower fT4/fT3 ratio in carriers of the D1-785T variant in a large population (n=552) of patients on thyroid hormone replacement and this tendency was confirmed in patients not on thyroid hormone replacement therapy. Concordantly, Philibert et al. (22) reported an association between higher fT4 levels and the D1-785T allele in large cohorts including White and Afro-American subjects. Medici et al. (21) performed a large-scale association analysis in four large independent cohorts (n=3777) and detected a robust association between the D1-C785T polymorphism and serum fT4, with higher fT4 in carriers of the D1-785T variant allele. In a small study including preeclamptic women, the variant was associated with higher T4 and lower fT3 levels (23). Collectively, these findings support the hypothesis of a decreased D1 activity in carriers of the D1-785T variant, inducing a lower conversion of T4 to T3 and lower rT3 clearance.

The minor allele variant (T-allele) of the D1–rs2235544 polymorphism was associated with variation in serum iodothyronine levels, evidenced by significantly lower serum fT4 levels and higher fT3/fT4 ratio in large euthyroid as well as non-euthyroid populations (10, 19, 21, 22, 25). Recent evidence supports a strong correlation between fT4 levels and D1–rs2235544 polymorphism in a large meta-analysis (n>40 000 subjects; P=7.87!10K32) (26). These findings might point toward an increased D1 activity of the T-allele.

The D1-A1814G polymorphism was associated with a higher T3/rT3 ratio in two independent cohorts of elderly and young subjects (17, 20), suggesting that this genetic variant may confer a decreased functional activity to the
D1 protein. On the contrary, the genetic variant had no significant impact on thyroid hormone levels or thyroid hormone ratios in another large healthy population of relatively young age (mean age, 36 years) (11).

Although the impact of genetic profile on inter-individual variation in serum thyroid

hormone levels is assumed to be large, it is important to consider that the solitary effect of D1 polymorphisms on thyroid function is assumed to be relatively small (variation explained by D1–rs2235544 and DI-C785T for serum fT4, 2 and 0.87% respectively) (10, 11).

D2 polymorphisms ➤ The lack of association between the D2-Thr92Ala polymorphism and serum iodothyronine or thyroid-stimulating hormone (TSH) levels has been consistently reported in the following studies including healthy as well as diseased populations: patients with primary autoimmune hypothyroidism (10, 27); cohorts of athyreotic differentiated thyroid carcinoma (DTC) patients on TSH suppression therapy (28, 29); a cohort of females only (30); middle-aged euthyroid subjects (31); non-diabetic Amish order subjects (32); young healthy blood donors (17); elderly subjects (mean age, >70 years) of Caucasian origin (6, 20); and young healthy euthyroid men (19). In one report, including patients with Graves's disease, carrier status of the 92Ala variant was associated with a decreased T3/T4 ratio and higher serum auto-antibody levels (anti-TPO and anti-Tg) (33).

Butler et al. (34) reported that the homozygous status for the 92Ala allele was associated with a decreased TSH-stimulated release of T3 from the thyroid in healthy subjects, indicative of a lower intrathyroidal conversion of T4 to T3. Supportive of this hypothesis are the observations of Torlontano et al. reporting a need for higher levothyroxine doses to suppress TSH levels in athyreotic DTC patients carrying the 92Ala allele. However, there was no difference in T4 levels between the genotype groups and this would have been expected if higher levels of T4 were needed to suppress the TSH. In addition, an impact of D2 variants on T4 dose could not be replicated in other cohorts on T4 replacement for primary hypothyroidism (28, 35). Interestingly, the D2-92Ala allele was associated with an increased risk for Graves' disease in a Russian population (36). On the other hand, in a Russian population the 92Ala variant was suggested to be protective regarding risk for Graves' disease development, severity of disease, and rate of remissions in Graves' patients (37). Guerra et al. (30), however, reported that the 92Ala variant was not associated with an increased risk of thyroid autoimmunity (defined as TPO-antibody level >100 U/ml).

The D2-ORFa-Gly3Asp polymorphism was associated with lower fT4, T4, and rT3 serum concentrations and higher T3/rT3 and T3/T4 ratio in healthy blood donors

(6), but not in two populations of healthy elderly men (6, 20). It was hypothesized that these conflicting results could be explained by an aging-related decrease in muscle mass, a major depot of D2 expression, which could lead to a decreased impact of potential functional activity of D2 on serum thyroid hormone parameters. Hoftijzer et al. (15) reported that the carriers of the D2-ORFa-3Asp variant had a lower feedback of fT4 on pituitary TSH secretion, suggesting that this polymorphism was associated with an altered setpoint of the pituitary– thyroid axis. Peltsverger et al. (38) reported that the D2-ORFa-3Asp variant might confer an altered pattern of thyroid hormone secretion by showing that acute TRH-induced TSH stimulation led to a blunted fT4 secretion in carriers of the 3Asp allele, consistent with an increased activity of the D2 enzyme.

D3 polymorphisms ➤ D3 polymorphisms have not been associated with TSH or iodothyronine levels in any study evaluating the impact of D3 variants on serum thyroid hormone parameters (10, 17, 21).

GH–IGF1 axis and body composition

Thyroid function is considered to affect the growth hormone (GH)–insulin-like growth factor (IGF1) axis, with hypothyroidism and hyperthyroidism associated with lower and normal or higher IGF1 levels respectively (24).

A haplotype containing the minor allele of the D1-C785T variant and the major allele of the D1-A1814G variant (both alleles with potential decreased D1 activity) was associated with higher free IGF1 levels and higher IGF1-related endpoints (such as muscle mass and muscle strength) in two populations comprising younger (mean age, 46 years) and older (mean age, 71 years) patients respectively (24). Roef et al. (19) investigated the contribution of D1 (D1-C785T and D1-rs2235544) and D2 polymorphisms (D2-Thr92Ala) on variation in body composition in a group of healthy young euthyroid men (n=677) and found that minor allele carriers of the D1-C785T polymorphism had significantly higher body height and higher values of other anthropometric variables (armspan, calf height, sitting, and sternum height). Another study reported that homozygous female carriers of the Thr92Ala minor allele had significantly shorter stature (mean, 162 cm) compared with subjects with other D2-Thr92Ala genotype status (mean, 166 cm, P=0.001) (39). These findings might point to a potential role of deiodinase genes in determination of body height.

Cognitive function and affective disorders

Evidence suggests that thyroid hormones affect neurotransmitter activity, such as the serotonergic neurotransmission (40). The brain serotonin system is considered to be frequently involved in the pathogenesis of affective disorders and psychotropics are believed to exert their effects upon this system. Some studies in humans showed that thyroid hormone abnormalities, i.e. hypothyroidism, may be linked to reduced 5-HT (serotonin) responsiveness (40). As deiodinase activity is important for local T3 availability in the brain and thyroid hormone status has been associated with cognitive functioning and emotional well-being (2, 41), it has been speculated that deiodinase polymorphisms could affect neuropsychological parameters. Based on the notion that thyroid hormone abnormalities are associated with cognitive impairment and dementia (42), de Jong et al. (20) evaluated whether D1 and D2 polymorphisms were related to early magnetic resonance markers of Alzheimer's disease in elderly patients, for which no relationship was found.

Panicker et al. (43) demonstrated in a large cohort of patients on thyroid hormone replacement therapy that the D2-Thr92Ala variant was associated with worse baseline psychological well-being in patients on T4 replacement therapy and improved response to combined T4–T3 therapy. No impact of D1 or D3 polymorphisms on study outcomes was found. Confirmation of these findings in other cohorts is warranted. In a relatively small population of patients with primary autoimmune hypothyroidism (n=141), D2 variants (D2-ORFa-Gly3Asp and D2-Thr92Ala) have shown no association with well-being, neurocognitive function, or preference for combined T4–T3 therapy (27).

The influence of deiodinase polymorphisms on the development, severity, and treatment response of psychiatric disorders has not been investigated extensively. The D1-C785T polymorphism was associated with lifetime major depression in white female subjects from high-risk cohorts (22).

The hypothesis was postulated that patients with a genetically determined decreased conversion of T4 to T3 might benefit from the additive effect of T3 to antidepressants. Cooper-Kazaz et al. (44) reported that in a population of patients diagnosed with major depression, DI-785T carriers showed better treatment response to combined antidepressant sertraline/T3 therapy compared with non-carriers on combined treatment. Other D1 and D2 polymorphisms (D1-A1814G, D2-ORFa-

Gly3Asp, and D2-Thr92Ala) did not show a relationship with therapeutic response in this study. Brouwer et al. (45) reported that the Thr92Ala polymorphism was not associated with response rate to antidepressant therapy with paroxetine in 96 patients treated for major depression.

In the Chinese Han population, He et al. (46) found that the D2-ORFa-Gly3Asp variant and D2-92Ala variant were linked to higher risk for bipolar mood disorder. Recently, Colak et al. (47) have reported an association between susceptibility for schizophrenia and the D2-Thr92Ala polymorphism.

Bone metabolism

Thyroid hormones are considered to be key elements in the regulation of bone matrix synthesis (48). T3 regulates cell differentiation, bone matrix synthesis, and degradation in osteoblasts. D2 activity in these cells fulfills a vital role in the maintenance of optimal bone mineralization and strength. Bone derived from D2-knockout mice showed reduced toughness, was brittle, and had increased vulnerability to fracture, emphasizing that D2 expression is an important prerequisite for optimal bone remodeling (49). In human adults, hypothyroidism as well as hyperthyroidism is associated with an increased risk of bone fractures (50). Variation in deiodinases has been described as a potential genetic determinant of bone pathology. Two studies investigated the effect of deiodinase polymorphisms on bone-related parameters. The D2-Thr92Ala polymorphism was associated with a decreased femoral neck and total hip bone mineral density and several markers of bone turnover in 154 athyreotic patients treated for DTC (51), indicating a role for D2 in regulation of bone formation. However, in a study of 641 young (25–45 years) healthy men, no effect of D1 (D1–rs22335544; D1-C785T) or D2 variants (D2-Thr92Ala) on bone mass could be found (52).

Risk for osteoarthritis

T3 is considered an important regulator of chondrocyte cell growth and differentiation in the endochondral growth plate (53). Variation in local T3 bioavailability, for example, caused by certain genetic deiodinase profiles might affect cartilage homeostasis in health and disease. The D2-Thr92Ala polymorphism was related to higher risk for generalized osteoarthritis and further study in three large cohorts

showed that a haplotype including the minor allele of D2-Thr92Ala and the major allele of the D2-ORFa-Gly3Asp polymorphism was similarly associated with osteoarthritis (39). In contrast, a D3 polymorphism (D3–rs945006) was associated with a decreased risk for osteoarthritis, suggestive of a protective effect of this genetic variant (18). These findings generated the hypothesis that local T3 availability, regulated by the opposite functions of D2 and D3, may be an important determinant of susceptibility to symptomatic osteoarthritis development. Bos et al. (54) demonstrated that D2 was upregulated in osteoarthritis-affected joints and that an allelic imbalance (difference in expression of alleles) inpatients might explain the positive association between the D2-92Ala variant and osteoarthritis. Waarsing et al. (55) postulated that deiodinase variants might increase vulnerability of cartilage in patients with a predispository non-optimal bone shape and showed a higher risk for osteoarthritis in carriers of the D2-ORFa-Gly3Asp polymorphism. The exact role of deio-dinase polymorphism in the genesis or progression of osteoarthritis has yet to be disentangled.

Lipoprotein metabolism

T3 regulates the expression of genes coding for proteins involved in energy utilization, lipolysis, and lipogenesis (56). Furthermore, T3 has a positive impact on upregulation of the LDL-receptor and thereby on cholesterol uptake and serum levels of cholesterol (57). Deiodinase polymorphisms may affect intracellular thyroid hormone concentration and thereby lipoprotein metabolism. The effect of the Thr92Ala polymorphism on serum lipid parameters was evaluated in various diabetic and non-diabetic cohorts (5, 32, 38, 58, 59,
60,61). No associations between HDL, LDL, total choles-terol, or triglyceride levels and genotype status were found in any of the reports studying this relationship.

BMI and obesity

T3 is considered to be a key modulator of energy expenditure and metabolic rate (62). Under certain circumstances, D2 knockout mice show an increased risk of obesity (62). In humans, subtle changes in deiodinase enzyme activity might affect energy metabolism and as such BMI or risk for obesity.

No effect of D1 polymorphisms on BMI was found in a cohort of healthy elderly men (n=350) (24). The isolated effect of the D2-Thr92Ala polymorphism demon-

strated no significant effect on BMI in a variety of studies in which this potential association was investigated (5, 28, 29, 32, 34, 60, 61,63). In 960 non-diabetic Caucasians, carriers of both a variant (Trp64Arg) located in the b3-adrenergic receptor (ADRB3) and the minor allele of the D2-Thr92Ala variant displayed an increased BMI (4). Albeit, these findings were not replicated in another two cohorts including healthy Danish subjects and Pima Indians respectively (61, 64). In 139 patients with primary autoimmune hypothyroidism, carriers of the D2-92Ala allele had significantly higher BMI values (27). The D2–rs7140952 polymorphism was significantly associated with central obesity in a population with primary hypothyroidism (35).

Blood pressure and hypertension

The vasodilatory effect of T3 and the expression and regulatory function of D2 in vascular smooth muscle cells hint toward a potential effect of variants in deiodinases on blood pressure (65, 66). The impact of deiodinase polymorphisms on cardiovascular parameters has primarily been restricted to the outcome blood pressure and demonstrates equivocal results. The D2-Thr92Ala polymorphism was associated with higher risk for arterial hypertension in 372 euthyroid subjects not on thyroid hormone replacement therapy (31). The only other positive association between this variant and blood pressure was reported by Fiorito et al. (60). Patients who are carriers of both the minor variant of D2-Thr92Ala and PPARg2–Pro12Ala polymorphism had significantly higher diastolic and systolic blood pressures. Other studies detected opposite effects. The D2-Thr92Ala polymorphism had no impact on blood pressure or risk for arterial hypertension in the two studies including only DM2 patients as well as a large unselected community-based cohort (58, 67, 68). Similarly, van der Deure et al. (69) assessed the relationship between the Thr92Ala polymorphism and blood pressure or hypertension in two large cohorts of elderly subjects and found no consistent significant associations. The 92Ala allele may confer a decreased risk for thyrotoxicosis-related cardiomyopathy (i.e. left ventricle hypertrophy) inpatients with Graves' disease, of which the authors proposed a potential protective effect of this genetic variant against hyperthyroidism-induced changes in heart tissue (33). Recently, Al-azzam et al. (35) has detected an association between the D2–rs7140952 polymorphism with

diastolic and systolic blood pressure in patients on thyroid hormone replacement therapy.

Insulin resistance and DM2

Thyroid hormones have been associated with glucose homeostasis and insulin sensitivity in numerous experimental and epidemiological studies (70). Therefore, certain deiodinase profiles affecting peripheral or local thyroid hormone concentrations maybe associated with the risk of insulin resistance or contribute to the development of DM2.

Research particularly focused on the role of the D2-Thr92Ala polymorphism and was done in mixed-cohorts including both diabetic and non-diabetes cohorts (n=5): studies including only DM2 patients (n=2) and studies including only non-diabetic subjects (n=3). In the genetic association studies (n=5), the relationship between D2-Thr92Ala genotype status and risk for DM2 was examined. Three independent case–control studies revealed no significant association between the D2-Thr92Ala variant and risk for DM2 (32, 61, 63). However, Dora et al. (58) performed a case–control study and a meta-analysis of the previously published studies and concluded that homozygous carriers had a higher risk for DM2 and the meta-analysis supported these findings by showing that carriers of the 92Ala allele had increased risk for DM2. The latest study on this subject from Nair et al. (64) showed that in a large cohort of Pima Indians no clear impact of D2 variants (including Thr92Ala) on insulin resistance or DM2 risk could be detected.

In two studies including only DM2 patients, a greater insulin resistance (reflected by higher fasting insulin and HOMA-IR) was reported in homozygous carriers of the D2-92Ala allele (5, 59). Dora et al. (58) replicated these results in a subset of their case–control study, including DM2 patients of European descent.

In non-diabetic cohorts, research on the relationship between deiodinase polymorphisms and measures of insulin resistance (i.e. HOMA-IR) yielded several non¬significant reports (31, 32, 60, 61, 64). Of note, a small number of DM2 patients (4.2%) were included in the study of Grarup et al. (61). In one study, a subanalysis including a population of non-diabetic women who underwent euglycemic–hyperinsulinemic clamps to diagnose insulin resistance, a consistent strong relationship between the Thr92Ala polymorphism and lower glucose disposal rate

was found, supportive of an increased risk for insulin resistance (4). Al-azzam et al. (35) found no association between diabetes and various D2 polymorphisms in a population of patients with primary hypothyroidism.

Interaction with other polymorphisms

Estivalet et al. (59) reported that carriers of both the PPARg2–Pro12Ala and D2-Thr92Ala polymorphisms had a significantly higher insulin resistance (as measured by HOMA-IR) in DM2 patients. These findings could not be replicated by Fiorito et al. (60), reflected by a non-significant impact of combined carrier status for these polymorphisms on insulin resistance (HOMA-IR) in a cohort of non-diabetic subjects.

Gynecological parameters

Preeclampsia tends to be accompanied by lower T3 serum levels and evidence suggests hypothyroidism could be a risk factor for this clinical entity (71, 72, 73, 74). In a preliminary report by Procopciuc et al. (23), the D1-C785T polymorphism (previously linked to decreased conversion of T4 to T3) was associated with degree of severity of preeclampsia, and severe preeclamptic women carrying the D1-785T variant delivered neonates with significantly lower birth weight at a significantly lower gestational age. The exact relationship is far from clear and research in larger cohorts is warranted.

Sepsis and sepsis-related study endpoints (acute lung injury)

In two preclinical mouse models, induction of acute lung injury led to upregulation of D2 gene and D2 protein. Furthermore, a significant positive relationship between D2 expression and extent of lung injury was found (75). The interaction between thyroid hormone metabolism and inflammatory response is complex and far from elucidated. Genetic D2 variants might affect inflammatory response, by affecting D2 gene and protein levels. This hypothesis was strengthened by the observed increased magnitude of lung injury in a state of reduced D2 gene and protein in mice (75). In the same study, the D2-92Ala variant was associated with a reduced susceptibility for severe sepsis and sepsis-related acute lung injury in critically ill patients of European descent, but not in patients of African origin (75).

These findings may support the role of D2 in prevention of local thyroid hormone disruption in lung and other bodily tissues under inflammatory and mechanical stressors.

Iodine and selenium deficiency and effect of polymorphisms on disease

Vulnerability to the potential clinical effects of poly-morphisms may partly be dependent on deficiencies in chemical elements involved in proper functioning of pituitary–thyroid axis. Based on the notion that sufficient iodine availability is of vital importance in local T3 regulation for normal brain development during the fetal and early postnatal period, researchers sought to evaluate whether risk for mental retardation in iodine-deficient areas could be dependent on variation in deiodinases. Guo et al. (76) found a significant positive association between two D2 polymorphisms (rs225010 and r2225012) and risk for mental retardation in children in an iodine-deficient area of China. Recently, Zhang et al. (77) have confirmed that children's susceptibility to mental retardation might be related to certain D2 polymorphisms (rs225015, rs2267872, rs1388378). Of note, the D2-Thr92Ala polymorphism (rs225014) was not associated with mental retardation in either study. Deiodinases are selenoproteins and selenium is an important building block for their proper activity (78). Selenium deficiency has the potential to impair expression of D2 activity (79, 80). Vulnerability to the clinical effects of selenium deficiency might be dependent on genetic thyroid hormone profile. Gentschew et al. (81) reported that the significantly lower selenium levels detected inpatients with Crohn's disease may contribute to its development and found that variation in D1 and D2 was significantly associated with modified selenium serum levels. Albeit, after multiple testing, no significant associations remained. Xiong et al. (82) evaluated whether the D2-Thr92Ala variant could influence the risk of Kashin–Beck disease, a chronic endemic osteochondropathy found in selenium-deficient areas of China, south-east Siberia, and North-Korea, and found no significant association of this genetic variant with the disease. The interaction between selenium or iodine deficiency, genetic variation in deiodinases and health effects is a scarcely investigated domain and may be a worthwhile field of future research.

Points to consider in the interpretation of studies on genetic polymorphisms

Thyroid hormones exert a myriad of functions in a variety of tissues. Since deiodinases are key regulators of peripheral and local thyroid hormone metabolism, research on the effect of deiodinase gene variation has grown over the last few years and could be considered a valuable tool to investigate the effect of thyroid hormone regulation on the end-organ level and thus on the expression of clinical phenotypes. Although many studies showed evidence of deiodinase polymorphisms on thyroid hormone-related endpoints, clinical implications of deiodinase variants are far from clarified.

One of the major impediments to provide general statements about the impact of deiodinase polymorphisms on clinical outcomes is the diversity in study cohorts (83). The action of deiodinase variations on clinical endpoints may simply be non-uniform in different populations for several reasons:

I) for instance, aging has been suggested as a factor that could explain the different impact of D1 and D2 deiodinase variants found in younger and older European cohorts (7). The contribution of D2 to serum T3 production may be lower in elderly compared with younger cohorts, caused by a decrease in skeletal muscle mass, an important source of D2, during aging (3, 7).

II) In the CNS, upregulation of D2 and downregulation of D3 are physiological components of the response to iodine deficiency (84). Iodine deficiency might expose otherwise non-identified effects of genetic deiodinase variants. Studies were performed in different geographical areas in which dietary factors such as iodine status may vary.

III) Study populations were from diverse geographical background (i.e. the old Amish order, South-American, Danish, and Mixed European) with different genetic make-up and as such study results on the same subject matter may not be plainly comparable.

IV) Deiodinase levels may vary dependent on intactness of the pituitary–thyroid axis. Synthetic thyroid hormone analogs such as T4 could be potent inhibitors of deiodinases (1). Long-term TSH suppression therapy could lead to significant changes in thyroid hormone metabolism, best explained by downregulation of D1 and D2 activity and upregulation of D3 (85). Thyroid function

abnormalities (hypothyroidism and hyperthyroidism) are associated with changes in deiodinase levels (1).

V) It cannot be ignored that the functional characterization of deiodinase variants is far from complete (8, 11, 14, 17, 67). Although significant associations are rather robust it could not be excluded that genetic markers considered significant may be associated with other deiodinase polymorphisms which are in linkage disequilibrium with these variants.

Besides these true underlying differences, variation in sample size (decreased power to detect significant difference) might contribute to different effects of the polymorphisms in different studies. Subtle effects of deiodinase variants on thyroid hormone-related parameters might be difficult to detect in relatively small sample sizes (decreased power). Significant relationships detected in small studies should be replicated in larger patient cohorts to decrease the risk of adopting potential false-positive assumptions. The risk of publication bias in genetic association studies could also not be excluded.

Conclusions and role of deiodinase polymorphisms in the clinical setting

Although the effect of genetic variation on inter-individual TSH and thyroid hormone levels is relatively large (65%), evidence suggests that genetic deiodinase profiles only explain a small proportion of inter-individual variation in serum thyroid hormone levels. These observations are in line with the general notion that circulating thyroid hormone levels and related phenotypes are under control of a complex interplay between multiple genes and environmental factors. A multitude of studies determined a promising role of deiodinase variants on clinical outcomes. D1 polymorphisms particularly showed a strong relationship with serum thyroid hormone parameters (D1-C785T and D1– rs2234455), IGF1 production (D1-C785T) and risk for major depression (D1-C785T) and D2 variants with serum thyroid hormone parameters (D2-ORFa-Gly3Asp), insulin resistance parameters (D2Thr92Ala), bipolar mood disorder (D2-Thr92Ala), psychological well-being (D2Thr92Ala), mental retardation, hypertension (D2-Thr92Ala), and risk for osteoarthritis (D2-Thr92Ala). D3 polymorphisms showed no relationship with inter-individual variation in serum thyroid hormone parameters. A D3 polymorphism (D3–rs945006) was associated with risk for osteoarthritis.

Evidence suggests no major impact of deiodinase variants on risk for obesity or lipoprotein metabolism. Potential associations between deiodinase polymorphism status and risk for thyroid disease, sepsis, and pregnancy outcomes are noteworthy, but need further confirmation. In summary, this systematic review showed the variety in available evidence on the effect of deiodinase variants on clinical endpoints. The significance of deiodinase polymorphisms for clinical practice seems to be limited from the studies conducted so far, but future studies are needed to unravel the exact role of deiodinase polymorphisms as contributors or therapeutic targets in the clinical setting.

Supplementary table 1 General overview of studies and their primary clinical endpoints				
Study	**Deiodinase polymorphism**	**Number of patients**	**Clinical Endpoint**	**Clinical outcome**
			Pituitary thyroid axis	
Al-azzam et al, 2013 (35)	D2-225011, D2-225012 and other D2 variants	228	The effect on clinical and biochemical response to T4 replacement therapy in hypothyroid patients on T4 replacement therapy	No significant association
Babenko et al, 2012 (37)	D2-Thr92Ala	315	The effect on development of Graves' disease, treatment efficiency and rate of remission in a Russian population	The D2-92Ala variant was suggested to be protective regarding risk for Graves' disease development (lower frequency of the variant in Graves patients; P<0.0001), severity of disease and rate of remissions
Butler et al, 2010 (34)	D2-Thr92Ala	83	The impact on acute-TSH stimulated release of T3 from the thyroid in healthy volunteers	The homozygous status for D2-92Ala allele was associated with a decreased rate of acute-TSH stimulated release of T3 from the thyroid (P=0.029)
Chistakov et al, 2004 (36)	D2-Thr92Ala	252	The impact on risk for Graves' disease in a Russian population	The D2-92Ala allele was associated with increased risk for development of Graves' disease (P=0.031)
De Jong et al, 2007 (20)	D1-C785T ,D1-A1814G, D2-ORF-Gly_a-3Asp and D2-Thr92Ala	995	The effect on various serum thyroid hormone parameters in elderly subjects	The D1-785T variant was significantly associated with higher fT4 (P=0.04) and rT3 levels (P=0.03) and lower T3 (P=0.004) and T3/rT3 (P=<0.001). The D1-A1814G variant was associated with higher T3 (p=0.02) and T3/rT3 (P=0.06).
Guerra et al, 2012 (30)	D2-Thr92Ala	216	The effect on serum thyroid hormone parameters or thyroid autoimmunity in female subjects	No significant association
Grineva et al, 2009 (33)	D2-Thr92Ala	145	The effect on serum thyroid hormone parameters and thyreotoxic cardiomyopathy and echocardiography parameters in patients with Graves' disease	The D2-92Ala allele was associated with decreased T3/T4 ratio and higher levels of auto-antibodies (anti-TSH, anti-TPO and anti-Tg) (P<0.001 and P<0.01, respectively)
Heemstra et al, 2009 (28)	D2-Thr92Ala	295	The effect on thyroid hormone levels and T4 dosage in patients treated for DTC and a group of AIH patients	No significant association
Hoftijzer et al, 2011 (15)	D1-C785T ,D1-A1814G, D2-ORF-Gly_a-3Asp and D2-Thr92Ala	148	The effect on the set-point of the hypothalamus-pituitary-thyroid axis in patients treated for DTC	The homozygous status for the D2-ORF-3Asp variant was associated with a weaker negative feedback of fT4 on TSH in DTC patients on TSH suppression therapy (P=0.036)
Medici et al, 2011 (21)	D1-C785T, D1-rs2235544 and other D1/2/3 polymorphisms	3777 [b]	The association with serum TSH and fT4 levels in a combined meta-analysis of 4 studies including Caucasian subjects	The minor allele of the D1-C785T and rs2235544 variant was strongly positively and negatively associated with fT4 levels ($P=5*10^{-10}$ and $P=8*10^{-12}$, respectively)
Panicker et al, 2008 (10)	D1-C785T, D1-rs2235544 ,D2-Thr92Ala and other D1/D2 polymorphisms	552	The effect on serum thyroid hormone parameters in patients on thyroid hormone replacement therapy and patients not on thyroid hormone replacement therapy	The D1-C785T and D1-rs2235544 variants were significantly associated with lower and higher fT3/fT4 ratio, respectively (P=0.004 and $P=3.6x10^{-13}$, respectively)
Peeters et al, 2003 (17)	D1-C785T, D1-A1814G and D2-Thr92Ala	155	The effect on various serum thyroid hormone parameters in healthy European blood donors	The D1-785T variant was associated with increased rT3 (P=0.017) and rT3/T4 (P=0.01) and decreased T3/rT3 (P=0.003). D1-1814G variant with decreased rT3/T4 (P=0.024) and increased T3/rT3 (P=0.08).
Peeters et al, 2005 (6)	D2-ORF-Gly_a-3Asp and D2-Thr92Ala	156 (EBD) 349 (HEM)	The effect on various serum thyroid hormone parameters in healthy European blood donors and healthy elderly men	D2-ORFa-3Asp variant was associated with lower fT4 (P=0.001), T4 (P=0.01) and rT3 (P=0.01) and higher T3/rT3 (P=0.03) and T3/T4 (P=0.002) in healthy blood donors, but not in healthy elderly men.
Peltsverger et al, 2012 (38)	D2-ORF-Gly_a-3Asp	45	The thyroid hormone secretion in response to 500 mg intravenous TRH injection was studied in healthy volunteers	The D2-ORF-3Asp variant was associated with a blunted fT4 secretion after TRH-induced acute TSH stimulation.(P<0.01)
Porcu et al,2013 (26)	D1-rs2235544	N>17000	The effect on serum thyroid hormone parameters (TSH and FT4) in a meta-analysis including euthyroid individuals	The variant was positively associated with serum fT4 levels (effect size 0.138; $P=7.87x10^{-32}$)

Supplementary table 1 General overview of studies and their primary clinical endpoints

Study	Deiodinase polymorphism	Number of patients	Clinical Endpoint	Clinical outcome
Roef et al, 2003 (19)	D1-C785T; D1-rs2235544 and D2-Thr92Ala	677	The effect on serum thyroid hormone parameters in young healthy euthyroid men	The D1-785T variant was associated with higher fT4, rT3 and lower fT3/fT4, T3/rT3 and the D1-rs2235544 C variant with higher fT3/fT4, T3/rT3, fT3 and lower rT3.
Taylor et al, 2010 (25)	D1-rs2235544	917	The effect on serum thyroid hormone parameters in euthyroid patients	The variant was associated with T3/T4 ratio ($P=5.93x10^{-4}$)
Torlantano et al, 2008 (29)	D2-Thr92Ala	191	The effect on T4 doses needed to maintain target TSH levels in patients treated for DTC	The D2-92Ala variant was associated with a higher T4 dosage needed to suppress TSH levels ($P<0.05$)
Van der Deure et al, 2009 (11)	D1-C785T and D1-A1814G	1192	The effect on serum thyroid hormone parameters in a population of healthy Danish twins	The D1-785T variant was associated with higher fT4 ($P=0.004$), rT3 ($P<0.001$) and rT3/T4 ($P<0.001$)
			GH-IGF1 axis and body composition	
Peeters et al, 2005 (24)	D1-C785T and D1-A1814G	156 (EBD) 350 (HEM)	The impact on GH-IGF1 system in healthy blood donors and elderly men	A haplotype of both D1 variants was associated with increased IGF-I levels in both cohorts ($P=0.02$ and $P=0.01$). In elderly men the haplotype was linked to increased lean body mass ($P=0.03$) and improved muscle strength ($P=0.047$)
Roef et al, 2003 (19)	D1-C785T, D1-rs2235544 and D2-Thr92Ala	677	The effect on body composition in healthy euthyroid men	D1-785T variant was associated with significantly higher body height ($P=0.009$) and higher values of other anthropometric variables (armspan, calf height, sitting and sternum height). A negative association was found between the C-allele of rs 2235544 and body height ($P=0.02$)
			Cognitive function and affective disorders	
Appelhof et al, 2005 (27)	D2-ORF-Gly_a-3Asp and D2-Thr92Ala	141	Effect on well-being, neurocognitive function or preference for combined T4/T3 therapy in patients with PAH	No significant association
Brouwer et al, 2006 (45)	D2-Thr92Ala	93	The association with response rate to the antidepressant paroxetine in patients treated for major depression	No significant association
Cooper-Kazaz et al, 2009 (44)	D1-C785T ,D1-A1814G, D2-ORF-Gly_a-3Asp and D2-Thr92Ala	64	The association with response rate to combined antidepressant sertraline/T3 therapy in patients treated for major depression.	The DI-785T variant was associated with higher response rate to combined antidepressant sertraline/T3 therapy in patients treated for major depression ($P=0.01$)
Colak et al, 2013 (47)	D2-ORF-Gly_a-3Asp and D2-Thr92Ala	290 (SCH) 198 (C)	The effect on risk of schizophrenia	The D2-92Ala variant was significantly associated with increased risk of schizophrenia ($P=0.045$)
De Jong et al, 2007 (20)	D1-C785T ,D1-A1814G, D2-ORF-Gly_a-3Asp and D2-Thr92Ala	995	The association with early magnetic resonance markers of Alzheimer disease in elderly Caucasians	No significant association
Guo et al, 2004 (76)	D2-Thr92Ala, D2225012 and rs225010	543	The association of D2 polymorphisms with mental retardation in a population from iodine-deficient areas in China	Positive association of the D2-rs225012 ($P=0.00246$) and D2-rs225010 ($P=0.044$) polymorphism with mental retardation
He et al, 2009 (46)	D2-ORF-Gly_a-3Asp and D2-Thr92Ala	279 (BPAD) 284 (C)	The association between D2 polymorphisms and BPAD in Chinese Han population	The D2-ORF-3Gly_a variant and D2-92Ala variant were significantly associated with increased BPAD risk ($P=0.005$ and $P=7.0e-0.005$)
Panicker et al, 2009 (43)	A variety of D1/D2/D3 polymorphisms	552	The association with psychological well-being in patients on thyroid hormone replacement therapy	The D2-92Ala variant was associated with worse baseline psychological well-being on T4 ($P=0.02$) and improved response to combined T4/T3 therapy ($P<0.05$) No impact of the D1 or D3 polymorphisms on study outcomes.
Philibert et al, 2011 (22)	D1-C785T and other D1 polymorphisms	1555	The impact on risk for major depression in three independent populations	The DI-C785T polymorphism was associated with lifetime major depression in white female subjects from high-risk cohorts. ($P<0.004$)

Supplementary table 1 General overview of studies and their primary clinical endpoints

Study	Deiodinase polymorphism	Number of patients	Clinical Endpoint	Clinical outcome
Zhang et al, 2012 (77)	D2-Thr92Ala and other D2 polymorphisms	1461	The association with mental retardation in Chinese Han population derived from iodine deficient areas	The major allele of D2-rs225015 polymorphism was associated with mental retardation (P=0.020)
			Bone metabolism	
Heemstra et al, 2010 (51)	D2-Thr92Ala	154	The effect on bone mineral density and bone turnover markers were assessed in patients treated for DTC	The D2-92Ala variant was associated with a decreased femoral neck (P=0.022) and total hip bone mineral density (P=0.028) and several markers of bone turnover
Roef et al, 2011 (52)	D1-C785T, D1-rs2235544 and D2-Thr92Ala	677	The effect on bone mass parameters in healthy male siblings	No significant association
			Risk for osteoarthritis	
Bos et al, 2012 (86)	D2-Thr92Ala	31	To investigate allelic balance of the D2 variant in human osteoarthritis joints (see article for more details)	An allelic imbalance (difference in expression of alleles) in patients might explain the positive association between D2-Thr92Ala variant and osteoarthritis
Meulenbelt et al, 2008 (39)	D2-ORF-Gly$_a$-3Asp, D2-Thr92Ala and other D2 variants	>4500	The association with generalized osteoarthritis in 4 large cohorts including Caucasian subjects	The D2-Thr92Ala variant was related to higher risk for generalized osteoarthritis and further study in three large cohorts showed that a haplotype including the D2-92Ala variant and the major allele of the D2-ORFa-Gly3Asp variant was also associated with osteoarthritis (P=2.02X10^{-5})
Meulenbelt et al, 2011 (18)	Various D3 polymorphisms	3252 (OA) 2132 (C)	The association with generalized osteoarthritis in 4 large European populations	The minor allele variant of D3-rs945006 was associated with decreased risk of osteoarthritis (P=0.004)
Waarsing et al, 2011 (55)	D2-ORF-Gly$_a$-3As, D2-Thr92Ala	341	The effect on nonoptimal joint geometry and risk for osteoarthritis in Caucasian subjects	A higher risk for osteoarthritis and certain hip shapes in carriers of the D2-ORFa-Gly3Asp variant (P=0.005)
			Lipoprotein metabolism [a]	
			Body mass index and obesity [a]	
			Insulin resistance and DM-II	
Canani et al, 2005 (5)	D2-Thr92Ala	183	The effect on insulin resistance parameters in patients with DM-II and D2 enzyme velocity in thyroid and skeletal muscle samples of non-diabetic subjects	The homozygous state for the D2-92Ala variant was significantly associated with higher fasting plasma insulin (P=0.004) and decreased D2 enzyme velocity in thyroid (P=0.05) and skeletal muscle (p=0.04) samples
Dora et al, 2010 (58)	D2-Thr92Ala	1573	The association with risk of DM-II in a case-control study including diabetic and non-diabetic subjects	The homozygous state for D2-92Ala variant was significantly associated DM-II (P=0.03). The pooled effect of DM-II in a meta-analysis yielded a risk for DM-II (P=0.02)
Estivalet et al, 2011 (59)	D2-Thr92Ala	721	The effect on insulin resistance parameters in patients with DM-II	The homozygous state for D2-92Ala variant was significantly associated with more severe insulin resistance compared to patients heterozygous (P=0.022) or wild type (P=0.001) for the D2 variant
Fiorito et al, 2007 (60)	D2-Thr92Ala	590	The effect on insulin resistance parameters in non-diabetic whites	Gene-gene interaction between D2-Thr92Ala and Pro12Ala polymorphism and risk for metabolic syndrome (P=0.02)
Grarup et al, 2007 (61)	D2-Thr92Ala	5843	The association with risk of DM-II in a case-control study including diabetic and non-diabetic Danish white subjects	No significant association with risk for DM-II
Maia et al, 2007 (63)	D2-Thr92Ala	1633	The association with risk of DM-II in a case-control study including White subjects of mixed European ancestry	No significant association with diabetes intermediate trait levels or risk for DM-II
Mentuccia et al, 2002 (4)	D2-Thr92Ala	972	The effect on insulin resistance parameters in non-diabetic patients	The D2-92Ala variant was associated with insulin resistance parameters (P=0.0088)

Supplementary table 1 General overview of studies and their primary clinical endpoints

Study	Deiodinase polymorphism	Number of patients	Clinical Endpoint	Clinical outcome
Mentuccia et al, 2005 (32)	D2-Thr92Ala	1268	The effect on insulin resistance parameters in the old order Amish population	No clear association between D2-Thr92Ala and DM-II or impaired glucose intolerance
Nair et al, 2012 (64)	D2-Thr92Ala and other D2 variants	300	The association with development of DM-II in a native American population of Pima indians	A minor association between D2-Thr92Ala variant with early-onset DM-II (P=0.01) [e]
			Blood pressure and hypertension	
Canani et al, 2005 (5)	D2-Thr92Ala	183	The effect on blood pressure or risk for arterial hypertension in DM-II patients	No significant association
Fiorito et al, 2007 (60)	D2-Thr92Ala	590	The effect on blood pressure in non-diabetic whites	Significantly higher diastolic (P=0.02) and systolic blood pressure (P=0.01) in patients with D2-92Ala variant combined with the Pro12Ala polymorphism in the PPARγ2 gene
Grineva et al, 2009 (33)	D2-Thr92Ala	145	The effect on serum thyroid hormone parameters and thyreotoxic cardiomyopathy and echocardiography parameters in patients with Graves' disease	An association between the D2-92Ala variant and parameters of thyrotoxic cardiomyopathy in patients with Graves' disease (P<0.05)
Gumieniak et al, 2007 (31)	D2-Thr92Ala	372	The association with hypertension and hypertension related intermediate phenotypes in euthyroid subjects	The D2-92Ala variant was significantly associated with hypertension (odds ratio 2.11, P=0.01)
Maia et al, 2007 (63)	D2-Thr92Ala	1633	The effect on blood pressure or risk for arterial hypertension in a large community-based cohort of mixed European ancestry	No significant association
Van der Deure et al, 2009 (69)	D2-ORF-Gly$_9$-3Asp and D2-Thr92Ala	2441	The effect on blood pressure or risk for arterial hypertension in two large cohorts of elderly subjects	No significant association
			Gynaecological parameters	
Procopciuc et al, 2012 (23)	D1-C785T	100	The effect on degree of severity of preeclampsia and pregnancy outcome in pregnant women	The D1-785T variant was associated with significantly lower birth weight (P=0.023) of neonates at a significantly lower gestational age(P=0.035)
			Sepsis and sepsis-related study endpoints	
Ma et al, 2011 (75)	D2-Thr92Ala	405 (EA) 302 (AA)	Risk for sepsis and sepsis-related acute lung injury in European and African Americans	The D2-92Ala variant was associated with a reduced susceptibility for severe sepsis (P=0.009) and sepsis-related acute lung injury (P=0.004) in critically ill patients of European descent
			Iodine and selenium deficiency	
Gentschew et al, 2012 (81)	D1-A1814G , D2-ORF-Gly$_9$-3Asp, D2-Thr92Ala and other D1/D2 variants	798	The association with serum selenium levels in a Caucasian population	No significant association after correction for multiple testing
Guo et al, 2004 (76)	D2-Thr92Ala, D2225012 and rs225010	543	The association with mental retardation in Chinese Han population derived from iodine deficient areas	Positive association of the D2-rs225012 (P=0.00246) and D2-rs225010 (P=0.044) polymorphism with mental retardation in a patient population from an iodine-deficient area. No significant relationship with the Thr92Ala polymorphism
Xiong et al, 2010 (82)	D2-Thr92Ala	473	The effect on risk of Kashin-Beck disease in the Chinese Han population living in selenium deficient areas	No significant association
Zhang et al, 2012 (77)	D2-Thr92Ala and other D2 polymorphisms	1461	The association with mental retardation in Chinese Han population derived from iodine deficient areas	The major allele of D2-rs225015 polymorphism was associated with mental retardation (P=0.020)

List of abbreviations

AA = African Americans
AIH = autoimmune hypothyroidism
C = controls
DM-II= diabetes mellitus type 2
DTC = differentiated thyroid carcinoma
EA = European Americans
EBD = European blood donors
HEM = healthy elderly men
OA = osteoarthritis
PAH = primary autoimmune hypothyroidism
SCH = schizophrenia
a = *these (secondary) clinical endpoints were studied in several studies investigating the effect of deiodinase variants on other (primary) endpoints. Further details on the clinical outcomes are to be found in the text.*
b = *consisted of 4 independent cohorts (Rotterdam Study; Scan Study; Healthy Twin; Nijmegen Biochemical study)*
e = *not significant after correction for multiple testing*

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