For Some, L-Thyroxine Replacement Might Not Be Enough: A Genetic Rationale

Brian W. Kim and Antonio C. Bianco

Division of Endocrinology, Diabetes, and Metabolism, Miller School of Medicine, University of Miami, Miami, Florida 33136

Although the thyroid gland secretes a very small amount of active thyroid hormone (triiodothyronine or T_3), the major circulating form is thyroxine (T_4), and it is widely accepted that thyroid hormone replacement in patients with hypothyroidism can be fully accomplished with L-T_4 monotherapy [see 1995 American Thyroid Association guidelines (Ref. 1)]. The mechanistic basis for this belief stems from the actions of the iodothyronine deiodinases, enzymes that activate thyroid hormone by conversion of T_4 to T_3 (or inactivate both forms via further deiodination) (2). Thanks to the actions of the type 2 deiodinase (D2) and to some extent the type 1 deiodinase (D1), both endogenous T_4 and L-T_4 are converted to T_3 at a rate sufficient to maintain normal serum T_3 concentration. However, despite the extensive literature demonstrating that patients do well clinically on L-T_4 monotherapy, most clinicians have at least anecdotally seen benefits from combined T_3/T_4 therapy for some patients, prompting a search for the biological mechanism of the T_3 requirement. In the current issue of *JCEM*, Panicker *et al.* (3) examine this question from a novel perspective, asking whether a polymorphism in the *Dio*2 gene could explain a differential psychological response to the type of thyroid hormone replacement seen in a subpopulation of the Weston Area T_4/T_3 Study (WATTS) from the United Kingdom (3, 4).

D2 was initially characterized in the pituitary gland and hypothalamus, where it plays an important role in the TSH/TRH feedback mechanism (2). Subsequent studies found that D2 is also an important source of plasma T_3, *i.e.* plasma T_3 largely originates from the thyroid gland as T_4, to be converted to T_3 by D2 in peripheral tissues (5, 6). To understand how a *Dio*2 gene polymorphism could explain a differential neurological response to T_4 as opposed to T_3/T_4 therapy, it is critical to understand two properties of D2 physiology. First, when thyroid hormone levels fall, D2 activity increases thanks to decreased ubiquitination of the enzyme (7). As D2 activity increases in the face of falling T_4, the net effect is homeostatic, promoting maintenance of normal T_3 levels. Second, D2 is expressed in the endoplasmic reticulum, and D2-generated T_3 can accumulate in the nucleus of specific tissues while plasma thyroid hormone levels remain unchanged; *i.e.* D2 allows for local control of thyroid hormone signaling. For example, sympathetic stimulation of brown adipose tissue leads to cAMP generation, which in turn induces D2 and fills up the tissue with T_3, a crucial mechanism for adaptive thermogenesis (heat generation after exposure to cold or high caloric diet) (8). The example of D2 in brown adipose tissue also illustrates that dynamic changes in D2 activity can occur in settings beyond the realm of thyroid hormone homeostasis. Indeed novel "proactive" roles for D2 have been found in settings ranging from development to metabolism; for example, D2 ubiquitination is accelerated by the Hedgehog signaling pathway, linking deiodination to the development of the tibial growth plate (9), while at the same time D2 activity is increased via the G protein-coupled bile acid receptor 1-mediated (also known as TGR5) signaling cascade, which mediates some of the metabolic effects of bile salts (10). D2 activity is also induced by some dietary flavonols, suggesting the potential for nutritional or pharmacological manipulation of metabolism via control of D2 (11).

The homeostatic and proactive functions of D2 are both at work in the brain, where D2 is widely expressed (12, 13). Indirect evidence for a physiological role for D2 in the brain includes studies of thyroidectomized rats in which T_4 monoreplacement fails to normalize T_3 levels in some tissues but the cerebral cortex is spared (14). At the same time, D2 knockout mice exhibit a substantial reduction in brain T_3 levels but, to the extent that they have been tested, do not demonstrate any overt neurological phenotype (15). Thus, whereas the effects of T_3 on neurological function are well established, and an extensive literature regarding T_3 therapy and mood exists (16), except for the TSH feedback mechanism, it remains to be proven that D2-generated T_3 is important for neurological function in adult humans.

The observation of an unexpected spatial dichotomy currently prevents a full mechanistic accounting of the role of D2 in the brain: D2 is found in glial cells, whereas thyroid hormone receptors are predominantly expressed in neurons (17, 18). Thus, for D2-generated T_3 to have its proposed effects, it must

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Abbreviations: D1, Type 1 deiodinase; D2, type 2 deiodinase; SNP, single nucleotide polymorphism.

somehow transit from the glial cytoplasm to the nuclei of target neurons; it has been proposed that glial D2-generated T_3 acts in a paracrine fashion in the brain (19). Indirect support for this hypothesis could be taken from the observation that the T_3 transporter monocarboxylate transporter 8 plays a critical role in brain development (20, 21), but the transit of iodothyronines in the brain remains to be elucidated. The supply-side situation is complex as well; in the mediobasal hypothalamus, D2 expression is concentrated in specialized glial cells known as tanycytes, located in the floor and infralateral walls of the third ventricles. This location suggests that the cerebrospinal fluid may be a source of T_4 for hypothalamic D2 (22).

To date, there are no identified genetic syndromes involving mutations in the *Dio*2 gene. However, a number of single nucleotide polymorphisms (SNPs) have been identified, the best characterized being Thr92Ala, a common A-G polymorphism (T-C on the complementary forward strand) at codon 92 that predicts an Ala for Thr substitution (274 A-G, D2-G/A; GI 13654872; rs225014) (23). This SNP is common in various ethnic groups: its allele frequency is 0.35 in Caucasians and is particularly high at 0.75 in Pima Indians. A growing number of studies have examined correlations between the Thr92Ala polymorphism and disease or dysfunction in tissues where D2 is expressed, but the results have been mixed for metabolic endpoints (23–27) and for hypertension (28–30). Recently, the Thr92Ala D2 polymorphism was also linked to susceptibility for generalized osteoarthritis, a common late-onset articular joint disease (31); this is an interesting result, given the role of D2 in the developing growth plate (9). As for the brain, associations have been reported between the Thr92Ala polymorphism and TSH level as well as the dose of L-T_4 needed to normalize TSH (32, 33). However, investigators failed to find an association with response to paroxetine therapy in depressed patients (34), and a study from China found associations between other *Dio*2 SNPs and mental retardation but not Thr92Ala (35).

The publication by Panicker *et al.* (3) adds importantly to the literature because the WATTS data set represents one of the largest randomized studies of thyroid hormone replacement (4). The key findings are an association between the Thr92Ala polymorphism with lower baseline psychological well-being on L-T_4 alone, as well as with improvement on combination T_3/T_4 therapy compared with L-T_4 monotherapy. These results stand in contrast to a number of smaller trials that did not find associations with Thr92Ala and cognitive endpoints [see references in Panicker *et al.* (3)]. A complex clinical question arises from these findings: is the subset of patients with the Thr92Ala abnormal with respect to brain T_3 levels, and thus susceptible to cognitive or mood defects?

A key point underlying this and other Thr92Ala studies is whether or not the polymorphism has functional implications for D2 protein. On this score, the jury is still out. Although the substitution is nonconservative (aliphatic for polar), codon 92 is not in the catalytic active site of the enzyme, and it is not phylogenetically conserved: the homologous position is proline in rodents and glycine in chicken D2 (23). *In vitro* analysis has shown that the Thr92Ala substitution does not change the kinetics of the transiently expressed D2 enzyme (32). One study suggested that Thr92Ala decreases the activity of the endogenously expressed enzyme in human thyroid and skeletal muscle tissue (24). However, two subsequent studies found much lower levels of D2 activity (two orders of magnitude) in human skeletal muscle (36, 37), calling these findings into question. Furthermore, whereas codon 92 is located in the 18-amino acid region of D2 known to be important for regulation of the enzyme's ubiquitination, an *in vitro* mutational analysis failed to confirm any impact of Thr92Ala on protein stability (38). If the Thr92Ala substitution is relevant for D2 ubiquitination, the conditions or endogenous factors that bring out the phenotype must be identified and confirmed experimentally. Otherwise, one must consider the possibility that the positive findings of these genetic studies are based on linkage with another locus. What is also needed is a prospective trial studying the effect of the Thr92Ala substitution on the neuropsychiatric response to combined T_3/T_4 *vs.* monotherapy with L-T_4; this will be the true test of the fascinating implications raised in the current work.

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Address all correspondence and requests for reprints to: Brian W. Kim, Division of Endocrinology, Diabetes, and Metabolism, Dominion Towers, Suite 816, 1400 NW 10th Avenue, Miami, Florida 33136. E-mail: bkim@med.miami.edu.

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References

1. **Singer PA, Cooper DS, Levy EG, Ladenson PW, Braverman LE, Daniels G, Greenspan FS, McDougall IR, Nikolai TF** 1995 Treatment guidelines for patients with hyperthyroidism and hypothyroidism. JAMA 273:808–812
2. **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
3. **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
4. **Saravanan P, Simmons DJ, Greenwood R, Peters TJ, Dayan CM** 2005 Partial substitution of thyroxine (T4) with tri-iodothyronine in patients on T4 replacement therapy: results of a large community-based randomized controlled trial. J Clin Endocrinol Metab 90:805–812
5. **Geffner DL, Azukizawa M, Hershman JM** 1975 Propylthiouracil blocks extrathyroidal conversion of thyroxine to triiodothyronine and augments thyrotropin secretion in man. J Clin Invest 55:224–229
6. **Saberi M, Sterling FH, Utiger RD** 1975 Reduction in extrathyroidal triiodothyronine production by propylthiouracil in man. J Clin Invest 55:218–223
7. **Gereben B, Goncalves C, Harney JW, Larsen PR, Bianco AC** 2000 Selective proteolysis of human type 2 deiodinase: a novel ubiquitin- proteasomal mediated mechanism for regulation of hormone activation. Mol Endocrinol 14:1697–1708
8. **Bianco AC, Silva JE** 1987 Intracellular conversion of thyroxine to triiodothyronine is required for the optimal thermogenic function of brown adipose tissue. J Clin Invest 79:295–300
9. **Dentice M, Bandyopadhyay A, Gereben B, Callebaut I, Christoffolete MA, Kim BW, Nissim S, Mornon JP, Zavacki AM, Zeöld A, Capelo LP, Curcio-Morelli C, Ribeiro R, Harney JW, Tabin CJ, Bianco AC** 2005 The Hedgehog-inducible ubiquitin ligase subunit WSB-1 modulates thyroid hormone activation and PTHrP secretion in the developing growth plate. Nat Cell Biol 7:698–705
10. **Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, Schoonjans K, Bianco AC,**

Auwerx J 2006 Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 439:484–489
11. **Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeöld A, Bianco AC** 2008 Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev 29:898–938
12. **Campos-Barros A, Hoell T, Musa A, Sampaolo S, Stoltenburg G, Pinna G, Eravci M, Meinhold H, Baumgartner A** 1996 Phenolic and tyrosyl ring iodothyronine deiodination and thyroid hormone concentrations in the human central nervous system. J Clin Endocrinol Metab 81:2179–2185
13. **Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, Hume R, Morreale de Escobar G** 2004 Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab 89:3117–3128
14. **Escobar-Morreale HF, Obregón MJ, Escobar del Rey F, Morreale de Escobar G** 1995 Replacement therapy for hypothyroidism with thyroxine alone does not ensure euthyroidism in all tissues, as studied in thyroidectomized rats. J Clin Invest 96:2828–2838
15. **Galton VA, Wood ET, St Germain EA, Withrow CA, Aldrich G, St Germain GM, Clark AS, St Germain DL** 2007 Thyroid hormone homeostasis and action in the type 2 deiodinase-deficient rodent brain during development. Endocrinology 148:3080–3088
16. **Jackson IM** 1998 The thyroid axis and depression. Thyroid 8:951–956
17. **Guadaño-Ferraz A, Obregón MJ, St Germain DL, Bernal J** 1997 The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. Proc Natl Acad Sci USA 94:10391–10396
18. **Tu HM, Kim SW, Salvatore D, Bartha T, Legradi G, Larsen PR, Lechan RM** 1997 Regional distribution of type 2 thyroxine deiodinase messenger ribonucleic acid in rat hypothalamus and pituitary and its regulation by thyroid hormone. Endocrinology 138:3359–3368
19. **Bernal J, Guadaño-Ferraz A, Morte B** 2003 Perspectives in the study of thyroid hormone action on brain development and function. Thyroid 13:1005–1012
20. **Dumitrescu AM, Liao XH, Abdullah MS, Lado-Abeal J, Majed FA, Moeller LC, Boran G, Schomburg L, Weiss RE, Refetoff S** 2005 Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. Nat Genet 37:1247–1252
21. **Friesema EC, Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ** 2004 Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. Lancet 364:1435–1437
22. **Lechan RM, Fekete C** 2005 Role of thyroid hormone deiodination in the hypothalamus. Thyroid 15:883–897
23. **Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi FS** 2002 Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the β-3-adrenergic receptor. Diabetes 51:880–883
24. **Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, Maia AL** 2005 The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 90:3472–3478
25. **Mentuccia D, Thomas MJ, Coppotelli G, Reinhart LJ, Mitchell BD, Shuldiner AR, Cell FS** 2005 The Thr92Ala deiodinase type 2 (DIO2) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. Thyroid 15:1223–1227
26. **Grarup N, Andersen MK, Andreasen CH, Albrechtsen A, Borch-Johnsen K, Jørgensen 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
27. **Mentuccia D, Thomas MJ, Coppotelli G, Reinhart LJ, Mitchell BD, Shuldiner AR, Celi FS** 2005 The Thr92Ala deiodinase type 2 (DIO2) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. Thyroid 15:1223–1227
28. **van der Deure WM, Peeters RP, Uitterlinden AG, Hofman A, Breteler MM, Witteman J, Visser TJ** 16 October 2008 Impact of thyroid function and polymorphisms in the type 2 deiodinase on blood pressure: the Rotterdam Study and the Rotterdam Scan Study. Clin Endocrinol (Oxf) 10.1111/j.1365-2265.2008.03447.x
29. **Gumieniak O, Perlstein TS, Williams JS, Hopkins PN, Brown NJ, Raby BA, Williams GH** 2007 Ala92 type 2 deiodinase allele increases risk for the development of hypertension. Hypertension 49:461–466
30. **Maia AL, Hwang SJ, Levy D, Larson MG, Larsen PR, Fox CS** 2008 Lack of association between the type 2 deiodinase A/G polymorphism and hypertensive traits: the Framingham Heart Study. Hypertension 51:e22–e23
31. **Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk HJ, Kroon HM, Nakajima M, Ikegawa S, Uitterlinden AG, van Meurs JB, van der Deure WM, Visser TJ, Seymour AB, Lakenberg N, van der Breggen R, Kremer D, van Duijn CM, Kloppenburg M, Loughlin J, Slagboom PE** 2008 Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. Hum Mol Genet 17:1867–1875
32. **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
33. **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
34. **Brouwer JP, Appelhof BC, Peeters RP, Hoogendijk WJ, Huyser J, Schene AH, Tijssen JG, Van Dyck R, Visser TJ, Wiersinga WM, Fliers E** 2006 Thyrotropin, but not a polymorphism in type II deiodinase, predicts response to paroxetine in major depression. Eur J Endocrinol 154:819–825
35. **Guo TW, Zhang FC, Yang MS, Gao XC, Bian L, Duan SW, Zheng ZJ, Gao JJ, Wang H, Li RL, Feng GY, St Clair D, He L** 2004 Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. J Med Genet 41:585–590
36. **Mebis L, Langouche L, Visser TJ, Van den Berghe G** 2007 The type II iodothyronine deiodinase is up-regulated in skeletal muscle during prolonged critical illness. J Clin Endocrinol Metab 92:3330–3333
37. **Grozovsky R, Ribich S, Rosene ML, Mulcahey MA, Huang SA, Patti ME, Bianco AC, Kim BW** 26 November 2008 Type 2 deiodinase expression is induced by PPAR-γ agonists in skeletal myocytes. Endocrinology 10.1210/en.2008-0938
38. **Zeöld A, Pormüller L, Dentice M, Harney JW, Curcio-Morelli C, Tente SM, Bianco AC, Gereben B** 2006 Metabolic instability of type 2 deiodinase is transferable to stable proteins independently of subcellular localization. J Biol Chem 281:31538–31543