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VIEWPOINT

Targeting the right population for T3 + T4 combined therapy: where are we now and where to next?

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Abstract

The universal applicability of levothyroxine (LT4) monotherapy for the treatment of hypothyroidism has been questioned in recent years. Indeed, it is now clear that about 10–15% of LT4-treated hypothyroid patients are dissatisfied with their treatment. It is plausible that this subset of hypothyroid patients may need T3 + T4 combined therapy to restore peripheral euthyroidism. To address this issue, many clinical trials have investigated the effect of T3 + T4 combinations versus standard LT4-based therapy. However, to date, results have been inconclusive, mainly due to the lack of markers that identify candidates for combination therapy. A breakthrough in this field came with the recent finding that several single-nucleotide polymorphisms in the deiodinase genes are associated with the persistence of hypothyroid symptoms in biochemically euthyroid LT4-treated patients, and are thus markers of candidates for combination therapy. In addition, whole-genome association studies are expanding our knowledge of other genes of the thyroid hormone (TH) pathway that affect serum TH levels. To target the right population for the T3 + T4 combined therapy, the next step is to translate these new findings into prospective trials. Hopefully, this will pave the way to personalized therapy for each hypothyroid patient.

Keywords Hypothyroidism · Personalized therapy · SNPs · Deiodinases · TH pathway

Viewpoint

The standard of care for hypothyroidism is based on monotherapy with levothyroxine (LT4), which is a synthetic levo isomer of the prohormone T4 [1]. Consequent to the peripheral action of the deiodinase enzymes, LT4 restores circulating T3 levels and results in clinical euthyroidism thereby obviating the need for levo-triiodothyronine (LT3) replacement. In fact, the production of the active hormone T3 largely depends on the T4-to-T3 conversion by deiodinases type 1 (D1) and type 2 (D2) that provide almost 80% of the total daily T3 requirement [2]. In routine practice, LT4 replacement therapy is adjusted to obtain normal thyroid stimulating hormone (TSH) levels that are a marker of both clinical and biochemical euthyroidism [1]. However, normal TSH levels do not invariably equate with therapeutic success. Indeed, about 10–15% of LT4-treated hypothyroid patients complain of unresolved hypothyroid symptoms notwithstanding TSH levels within the normal range [3]. Given that the prevalence of hypothyroidism in iodine-sufficient countries is 1–2% [4], millions of hypothyroid patients are dissatisfied with their treatment.

Clinical studies that explored T3 levels in LT4-treated athyreotic patients indicate that a consistent percentage of patients undergo a drop in serum T3 concentration and a shift in the T4/T3 ratio toward higher values (about 15% and 30%, respectively) compared with the presurgical state [5, 6]. The clinical relevance of the lower T3 concentrations in hypothyroid patients clearly emerges from two recent retrospective studies [7, 8]. In the first study, Peterson et al. [7] analyzed 52 clinical parameters of thyroid hormone (TH) action in 469 LT4-treated subjects matched for age, gender, and TSH levels to healthy controls. Serum total and free T3 levels were 10% and 5%, respectively, lower in LT4-treated subjects than in healthy controls, and the total T4/T3 ratio in LT4-treated subjects was 20% higher than in controls. Strikingly, 12 of the 52 clinical parameters, including increased body mass index and greater use of statins and antidepressants, differed significantly between hypothyroid patients and healthy controls. In the second study of 133 LT4-treated thyroidectomized patients, Ito

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Table 1 Effect on TH levels associated to the expression of SNPs in *DIO1* and *DIO2*

Gene	SNP	Effect	Participants	Ref.
D1	rs2235544	Lower FT4/FT3 ratio Higher T3 levels	552 hypothyroid individuals from WATTS study	[12]
		Lower FT4/FT3 ratio	227 patients affected by cerebral tumors	[13]
	rs11206244 (C785T)	Lower T3/rT3 ratio Higher rT3 levels	156 healthy blood donors	[14]
		Lower T3/rT3 ratio Higher rT3 levels Lower T3 levels Higher FT4 levels	995 participants in the Rotterdam Scan Study	[15]
		Higher FT4 levels	GWAS in 3777 subjects	[16]
	rs12095080 (A1814G)	Higher T3/rT3 ratio	156 healthy blood donors	[14]
		Higher T3/rT3 ratio Higher T3 levels	995 participants in the Rotterdam Scan Study	[15]
D2	rs225014 (Thr92Ala)	Unchanged	552 hypothyroid individuals from WATTS study	[12]
		Unchanged	995 participants in the Rotterdam Scan Study	[15]
		Decreased FT3 levels	102 athyreotic patients	[20]
	rs12885300 (ORFa-Gly3Asp)	Lower FT4 & rT3 Unaltered T3 & TSH	156 healthy blood donors	[17]
		Unchanged	995 participants in the Rotterdam Scan Study	[15]
		Altered HPT set point Weaker T4-feedback	151 athyreotic patients	[18]
		Blunted rise in FT4 after TRH test	45 healthy volunteers	[19]
		Unchanged	102 athyreotic patients	[20]

et al. [8] found that patients with TSH within normal levels (i.e., 0.3–5 μIU/mL) had lower T3 levels than before surgery as well as variations in lipid and bone metabolism markers that tended toward a hypothyroid state. Lastly, in a recent meta-analysis of objective markers of TH signaling in LT4-treated hypothyroid patients, McAninch et al. [9] found that LT4-treated patients had higher serum levels of LDL-cholesterol and triglycerides, notwithstanding normal TSH values, than healthy controls.

The above reports of a significant association between low T3 and impaired TH action in LT4-treated hypothyroid patients raise the critical question as to whether decreased serum T3 levels correspond to insufficient T3 at tissue level. Obviously, there is not a unique answer to this question because different cells in the body use different intracellular tools to contrast T3 deficiency [2]. However, it is now clear that deiodinases play an essential role in the homeostasis of tissue TH levels and that polymorphisms in the D1 and D2 genes (*DIO1* and *DIO2*) can affect serum T3 levels in LT4-treated hypothyroid patients [10]. In fact, all T3 production in athyreotic patients is dependent on deiodinase activity and the efficiency in T4-to-T3 conversion may affect serum T3 levels [10].

Both D1 and D2 play a role in the homeostatic control of serum T3, although with different properties. D1 is believed to provide one-fifth of extrathyroidal T3 whereas the rest derives from D2 activity, although, at least in humans, the exact relative contribution of these two enzymes remains to be defined [2]. D1 is located in the plasma membrane and is abundantly present in *DIO1*-expressing tissues such as liver and kidney. Most of the T3 produced by D1 rapidly exits cells and contributes to the extracellular pool of T3, while only a minor amount enters the nucleus to bind to the TH nuclear receptor [11]. Conversely, the D2-produced T3 slowly (in about 8 h) equilibrates with serum T3 level, and exerts its action by finely regulating the nuclear pool of T3. In fact, D2 is retained in the endoplasmic reticulum, close to the nucleus, and provides a rapidly available source of T3 to meet the local needs of *DIO2*-expressing cells [11].

Dozens of single-nucleotide polymorphisms (SNPs) in *DIO1* and *DIO2* have been examined to evaluate whether they affect serum TH levels, and at least five SNPs were found to influence serum TH homeostasis (see Table 1) [12–20]. In *DIO1*, rs223554 and rs12095080 have been related to higher T3 levels, higher T3/rT3 ratio and lower FT4/FT3 ratio, which suggests increased T4-to-T3

conversion [12–15]. Low T3 levels and high rT3 levels have been found in association with rs11206244, even though this SNP is in linkage disequilibrium with rs223554 [12, 16] that is the driver association. Two polymorphisms in *DIO2*, namely, rs225014 (Thr92Ala) and rs12885300 (ORFa-Gly3Asp), affect TH parameters [12, 15, 17–20]. The Thr92Ala polymorphism is the most well studied because of its high frequency in the Caucasian population (12–16%) [21, 22]. Moreover, it has been found in various clinical conditions [21, 23]. The expression of $DIO2^{Ala/Ala}$ does not affect serum TH levels in healthy subjects [15] but it is associated with lower FT3 levels than those induced by $DIO2^{Thr/Thr}$ in athyreotic patients [20] and requires higher LT4 doses to normalize TSH levels [24]. Furthermore, the $D2^{Ala/Ala}$ protein reduces the T4-to-T3 conversion in myoblasts and in thyrotrophic cell models [20], and causes localized hypothyroidism in distinct brain areas of Ala92-DIO2 mice despite normal TH serum levels [25].

Notwithstanding this large body of data, the association between deiodinase SNPs and serum T3 levels and other clinical parameters in LT4-treated hypothyroid patients remains largely obscure due mainly to study limitations. First, most available studies are retrospective and included highly heterogeneous populations ranging from healthy blood donors to athyreotic subjects. Next, the expression of single SNPs results in narrow TH variations (in particular for free T3) that are difficult to replicate. Lastly, the deiodinases are only part of the complex cellular signaling that drives LT4 responsiveness. In fact, deiodinases act along the TH pathway together with transporters, receptors, and intracellular cofactors [11]. Therefore, the largest possible number of peripheral TH metabolism players must be investigated to exclude genetic-based defects of LT4 responsiveness in individual patients.

In support of the above concept, genome-wide association studies (GWAS) are enlarging the number of genes known to be involved in TH peripheral metabolism [26, 27]. A recent meta-analysis of GWAS that tested 72,167 subjects with normal TSH found 63 independent genetic associations (including 35 novel loci) with circulating TSH and FT4 levels [27]. Among these, two SNPs in the genes of a metabolizing enzyme and a novel TH transporter, *AADAT*-rs6854291 and *SLC17A4*-rs9356988, respectively, were found to significantly affect the T4/T3 ratio and T3 levels. Other studies showed that SNPs in TH transporter and receptor genes can affect TH serum levels, alone or in combination with SNPs in *DIO2* [28, 29]. In a study of 100 athyreotic patients, Cantara et al. [30] found no impact on T3 levels with the expression of rs17606253 in the TH transporter *MCT10* gene. However, a trial conducted in 45 hypothyroid patients demonstrated that the double expression of rs225014 in *DIO2* and rs17606253 in *MCT10* was associated with a stronger preference for the T3 + T4 combination versus LT4 monotherapy [31].

In conclusion, numerous SNPs in the TH pathway genes have been related to low T3 levels and to the persistence of symptoms in hypothyroid patients. However, to date there are no evidences that T3 supplementation can overcome persistent hypothyroid symptoms. One explanation for the lack of conclusive data is that trials may have "missed" the relatively small percentage of patients that fail to respond to LT4 properly. To obviate this issue, clinical trials that aim to evaluate the effect of the T3 + T4 combination should recruit patients based on their genetic profile as discussed herein. Studies conducted in such selected populations will probably be able to target the most promising candidates for T3 + T4 combination therapy. Hopefully, future studies will reveal a gene profile or a personal signature by which to predict whether a given patient requires T3 supplementation.

In summary, despite many open questions, we are now on the right path to identifying the best personalized treatment for each patient affected by hypothyroidism.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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