Pathophysiological relevance of deiodinase polymorphism

Antonio C. Bianco[a] *and Brian S. Kim*[b]

Purpose of review
To assess new findings and clinical implications of deiodinase gene polymorphism. Deiodinases are enzymes that can activate or inactivate thyroid hormone molecules. Whereas the types 1 and 2 deiodinase (D1 and D2) activate thyroxine (T4) to 3,5,3′-triiodothyronine (T3) via deiodination of T4's outer ring, D1 and D3 inactivate both T4 and T3 and terminate thyroid hormone action via deiodination of T4's inner molecular ring. A number of polymorphisms have been identified in the three deiodinase genes; the most investigated and likely to have clinical relevance is the Thr92 substitution for Ala substitution in DIO2 (Thr92Ala-DIO2). There are a number of reports describing the association between the Thr92Ala-DIO2 polymorphism and clinical syndromes that include hypertension, type 2 diabetes, mental disorders, lung injury, bone turnover, and autoimmune thyroid disease; but these associations have not been reproduced in all population studies.

Recent findings
A new report indicates that carriers of the Thr92Ala-DIO2 polymorphism exhibit lower D2 catalytic activity and localized/systemic hypothyroidism. This could explain why certain groups of levothyroxine-treated hypothyroid patients have improved quality of life when also treated with liothyronine (LT3). Furthermore, Ala92-D2 was abnormally found in the Golgi apparatus, what could constitute a disease mechanism independent of T3 signaling. Indeed, brain samples of Thr92Ala-DIO2 carriers exhibit gene profiles suggestive of brain degenerative disease. In addition, African American carriers of Thr92Ala-DIO2 exhibit an about 30% higher risk of developing Alzheimer's disease.

Summary
The finding of deiodinase polymorphisms that can diminish thyroid hormone signaling and/or disrupt normal cellular function opens the door to customized treatment of hypothyroidism. Future studies should explore how the racial background modulates the clinical relevance of the *Thr92Ala-DIO2* gene polymorphism.

Keywords
deiodinase polymorphism, hypothyroidism, thyroid, thyroid hormone., thyroxine

INTRODUCTION

Thyroid hormone regulates a wide array of developmental and physiologic processes, affecting virtually every tissue in the human body throughout all phases of life [1]. Both thyroxine (T4) and the biologically active 3,5,3′-triiodothyronine (T3) have stable levels in the circulation, a finding that at face value may seem to oppose the idea that these molecules initiate or terminate important biological processes. However, the modern paradigm of thyroid hormone action posits that thyroid hormone does have dynamic regulatory functions made possible by 'local' molecular mechanisms by which thyroid signaling can be altered at the level of the target cell – both in the short and long term – even as circulating levels remain stable [2]. This 'local' control model is based on three key factors. First, the lipid bilayer that constitutes the plasma membrane is not significantly permeable to T3 or T4, such that both molecules only enter cells through specific transporters that are embedded in the plasma membrane [3▪▪]. Second, once inside the target cells, thyroid hormones are

[a]Division of Endocrinology, University of Chicago and [b]Division of Endocrinology and Metabolism, Rush University Medical Center, Chicago, Illinois, USA

Correspondence to Antonio C. Bianco, MD, PhD, Section of Endocrinology, Diabetes & Metabolism, University of Chicago Medical Center, 5841 S. Maryland Avenue, MC1027, Room M267, Chicago, IL 60637, USA. Tel: +1 312 775 4493; E-mail: abianco@deiodinase.org

Curr Opin Endocrinol Diabetes Obes 2018, 25:000–000
DOI:10.1097/MED.0000000000000428

KEY POINTS

- The Thr92Ala-DIO2 polymorphism might reduce thyroid hormone signaling and cause systemic and/or localized hypothyroidism.
- The central nervous system of carriers of the Thr92Ala-DIO2 polymorphism exhibit changes in gene profile suggestive of brain degenerative disease.
- African American carriers of the Thr92Ala-DIO2 polymorphism have are at a 30% higher risk of developing Alzheimer's disease.

activated or inactivated by deiodinases, enzymes that thereby control the intracellular level of T3. The type 2 deiodinase (D2) converts T4 to T3 and is the main activating deiodinase in humans, whereas the type 3 deiodinase (D3) inactivates both T4 and T3 [4]. The third factor is that intracellular T3 binds to and activates thyroid hormone receptors that modulate transcription of target genes. Additional local control at the receptor level is achieved via the existence of two different T3 receptors, TRα and TRβ, which have distinct patterns of tissue-specific distribution; these receptors also control different sets of genes [5]. Ultimately, the thyroid-hormone dependent behavior of a cell or tissue depends not just on the supply of T3 and T4 from the plasma, but also on the unique blend of transporters, deiodinases, and receptor subtypes expressed in that cell.

DEIODINASE GENE POLYMORPHISM AND THYROID HORMONE SIGNALING

The purpose of this article is to review recent progress in the deiodinase field, focusing on single nucleotide polymorphisms (SNPs) of the deiodinase genes and their associated clinical syndromes. A gene polymorphism is a common variation in a DNA sequence in which the least common allele has a frequency of at least 1%. This is in contrast to a mutation, which is any change in a DNA sequence away from normal that is present in less than 1% of the population. The original study that shed light on the importance of deiodinase SNPs came as a result of molecular scanning of DIO2 in 50 obese Caucasians, which identified a Thr92Ala variant associated with lower glucose disposal rates [6]. Carriers of the Thr92Ala-DIO2 polymorphism exhibited strong association with insulin resistance and, in individuals who also carried the Trp64Arg ADRB3 variant, an increased BMI. Subsequent population-based studies have suggested associations between Thr92Ala-DIO2 with hypertension [7], insulin resistance [6,8], type 2 diabetes [9], bipolar disorder [10], mental retardation [11], low intelligence quotient (IQ) [12], recovery from lung injury [13], osteoarthritis [14], and increased bone turnover [15]. A recent study of autoimmune thyroid disease such as Graves' and Hashimoto's diseases revealed that Thr92-DIO2 genotype is less frequent in these patients, especially in Hashimoto's disease, when compared with controls [16]. Clinical phenotypes related to other deiodinase gene (*DIO1, DIO3*) polymorphisms have been reported but have been less well studied to date (see [17] for review).

The Thr92Ala-DIO2 polymorphism is relatively common, present in 12–36% of the population [18]. Given the role of DIO2 in the tissue-specific determination of local thyroid hormone levels, it is particularly intriguing that the Thr92Ala-DIO2 polymorphism has been linked to altered responsiveness of hypothyroid patients to thyroid hormone replacement therapy [19,20]. Although most patients do well on replacement therapy with levothyroxine (LT4), a minority (about 15%) remain symptomatic despite being achieving normal thyroid-stimulating hormone (TSH) levels [21,22]. Residual clinical manifestations of hypothyroidism include objective parameters, that is, higher BMI, greater use of beta-blockers, statins or antidepressant medication [22], and lower energy expenditure [23], as well as subjective aspects, that is, difficulty in weight management, fatigue or low-energy levels, and problems with mood and memory [24]. In a study performed in the United Kingdom, carriers of the Thr92Ala-DIO2 polymorphism exhibited better quality of life outcomes in response to combination therapy with LT4 and LT3 as compared with LT4 alone [21]. One hypothesis to explain this association could be that carriers of the Thr92Ala-DIO2 polymorphism suffer from localized hypothyroidism, presumably related to defective local LT4 activation that can be overcome using LT3. Further evidence for a clinically relevant phenotype of this polymorphism comes from a Danish study of 45 overtly autoimmune, hypothyroid patients who participated in a prospective, double-blind, crossover study regarding combination therapy [25▪]. The Danish patients were randomized to receive either 3 months of LT4 therapy followed by 3 months of combination therapy or vice versa, adjusted to obtain normal serum TSH values. Clinical outcomes were evaluated considering polymorphisms in DIO2 (Thr92Ala-DIO2) and on the cellular membrane transport-facilitating monocarboxylate transporter (MCT10) gene (*rs17606253*). The major finding was that the combination of polymorphisms in Thr92Ala-DIO2 and MCT10 on the same patient enhanced patients' preference for

LT4 combined with LT3 replacement therapy [25▪]. These findings suggest that LT4-treated thyroidectomized patients carrying the Thr92Ala-DIO2 polymorphism might be at a greater risk of systemic and/or localized hypothyroidism.

Perhaps unsurprisingly, the clinical syndrome associations with *DIO2* gene polymorphisms have not been reproduced in all population studies [4,18,26]. This type of study design is prone to false-positive findings, and of course racial and other background factors may play important roles in such associations. Studies with negative findings include a Dutch study in which the effects of the Thr92Ala-DIO2 polymorphism were evaluated in 12 625 individuals, including 364 patients on thyroid hormone replacement therapy. The analyses involved anthropometric data, medication use and existence of metabolic syndrome, quality of life assessed with the RAND 36-Item Health Survey, and executive function with the Ruff Figural Fluency Test. In this case, there was no association between the Thr92Ala-DIO2 polymorphism and thyroid parameters, quality of life, or cognitive functioning in the general population or in participants on either form of thyroid hormone replacement therapy [26]. In a similar study conducted in Korean patients, no associations were observed with SNPs in DIO2 or DIO3 and wellbeing in hypothyroid patients [27]. These negative findings remind one that SNP associations seen in one sub-population may not apply to another.

ABNORMAL CELLULAR PROPERTIES OF Thr92Ala-D2

How could the Thr92Ala-DIO2 polymorphism participate as a disease mechanism and/or affect clinical outcomes? The most obvious possibility is that Thr92Ala substitution affects D2 catalytic activity. Impaired T3 production would be predicted to cause either or both systemic/localized hypothyroidism. In this regard, the Thr92Ala substitution occurs at a residue that is relatively distant from the catalytic active site of the enzyme [28]. In-vitro studies show that Thr92Ala-D2 converts T4 to T3 with normal kinetics when transiently expressed in HEK-293 cells [29]. In fact, different laboratories have studied the Ala92-D2 enzyme extensively *in vitro* and no evidence of reduced catalytic activity have been reported [30]. In contrast, studies *in vivo* suggest that Thr92Ala-D2 might affect TSH secretion [31] and/or LT4 bioavailability [32]. More recently, a comparison of presurgical hormonal status of LT4-treated thyroidectomized individuals with their postsurgery status revealed an association between low FT3 values and Thr92Ala-DIO2 polymorphism [33]. In the same study, evidence of reduced Thr92Ala-D2 in-vivo activity was obtained in Ala92-D2-expressing murine myoblasts and in Ala92-D2-expressing primary cultures of pituitary cells from Dio2-null mice [33]. Thus, whether the Thr92Ala polymorphism affects the catalytic activity of the enzyme remains a subject of debate.

If altered catalysis is not the basis for clinical syndromes, another possibility is abnormal cell handling of the Thr92Ala-D2. D2 is a selenoprotein that resides in the endoplasmic reticulum (ER) [34,35]. Thanks to the physical proximity and functional relationship between ER and nuclear membrane, D2-generated T3 feeds the nuclear compartment with intracellularly generated T3. The D2 protein has not been crystalized but three-dimensional (3D) modeling based on hydrophobic cluster analyzes [28] identified a unique 18-residue 'instability' loop in the D2 molecule that mediates binding to ubiquitin ligases [36,37], hence D2 ubiquitination [38–40]. Subsequently, ubiquitinated D2 is retro-translocated to the cytoplasm where it is degraded by the proteasomes [41]. This model has been substantiated via experiments in which D2's instability is transferrable to other proteins as seen when the otherwise stable protein Sec62 becomes unstable after fusion to D2's 18-residue instability loop [42]. Site-directed mutagenesis of this loop identified a conserved stretch of six amino acids critical for binding to ubiquitin ligases. Remarkably, the Thr92 to Ala substitution is contained within this six amino-acid stretch, and thus slows down the rate of D2 turnover via interference with ubiquitination [43]. Furthermore, electron microscopy studies of Ala92-D2-expressing cells have identified the ectopic presence of Ala92-D2 in the Golgi apparatus giving it a perturbed morphology, in contrast to Thr92-D2 that could not be found in the Golgi [43]. Cells expressing Ala92-D2 also exhibited alteration in expression of Golgi markers, a finding that was normalized with antioxidant treatment. Thus, although the obvious first pass hypothesis would be that DIO2 SNPs have pathophysiological effects mediated by alterations in local thyroid hormone signaling, it may also affect the cell via altering the Golgi apparatus.

One recent microarray-based study provides support for this 'non-T3 mediated' Golgi mechanism. Transcriptome profiling of cells that were engineered to express Thr92-D2 or Ala92-D2 and also brain samples of young adults carrying the Thr92Ala-DIO2 polymorphism who had died of trauma injuries identified unique modifications of gene expression common to both the Ala92-D2-expressing cells and the temporal poles of carriers of the Thr92Ala-DIO2 polymorphism; these genes

were not obviously related to T3 signaling and included evidence of upregulation of pathways related to the mitochondria, Golgi apparatus/ER transport, oxidative stress, and apoptosis [43]. Notably, in both models, there was molecular and physiological evidence of dysregulation in EGF receptor signaling, a pathway known to be altered in oxidative stress and play an important role in cognitive development and function [44]. Further studies are needed to understand the molecular consequences of ThrAla92-DIO2 accumulation in the Golgi apparatus.

DIO POLYMORPHISMS IN NEURODEGENERATIVE DISEASE

One of the more intriguing observations from the transcriptome-focused recent studies in young adults who died of trauma injuries is that Thr92AlaDIO2 carriers exhibit transcriptional alterations in processes typically associated with neurodegenerative diseases, such as amyloid-beta (Aβ) peptide processing [43]. The hypothesis that carriers of the Thr92Ala-DIO2 polymorphism have an increased risk for incident Alzheimer's disease has been investigated in a subsequent study, with a number of circumstantial findings supporting an association. For example, the epidemiology and tissue pathology of Alzheimer's disease vary by ethnicity in parallel with Thr92Ala-DIO2 [45]. There is higher incidence and prevalence of Alzheimer's disease in African Americans compared with European Americans [46,47]; in studies that involved 3054 African Americans and 9304 European American subjects, the Thr92Ala-DIO2 polymorphism was found to be more prevalent in African Americans than European Americans, and to be associated with the development of Alzheimer's disease in African Americans but not European Americans [48▪]. These findings were based on large, well characterized primary and replication population studies containing both African American and European American participants in addition to a complimentary molecular analysis of both African American and European American brain samples. One negative study showing that Thr92Ala-DIO2 was not identified in a genome-wide association study (GWAS) with African American participants [49] may have been underpowered to detect the link because of its moderate effect size. Future studies will be needed to examine whether Thr92AlaD2 is a risk factor for neurodegenerative disease in African Americans and that Thr92AlaD2 may represent one factor contributing to racial discrepancies in incident Alzheimer's disease.

Altered Golgi trafficking of amyloid precursor protein (APP) is implicated in development of Alzheimer's disease as Aβ peptide accumulation causes Golgi structural defects that further affect APP trafficking and processing [50]. Thus, it is conceivable that the cellular and Golgi perturbation associated with Thr92AlaD2 expression could promote dysfunction in cellular pathways involved in Aβ peptide processing and contribute to development of Alzheimer's disease. Another possibility may be that the polymorphism promotes mitochondrial dysfunction: mitochondrial dysfunction and Aβ accumulation likely contribute to oxidative stress in Alzheimer's disease [51]. Indeed, mitochondrial dysfunction and oxidative stress markers were present in the transcriptomes of human temporal pole samples from Thr92AlaD2 carriers and in the cell model of Thr92AlaD2 expression; some of these alterations in Thr92AlaD2-expressing cells were reversed upon antioxidant treatment [43]. Of course, it must be remembered that there could be yet unidentified causal markers that could be contributing to the racially dependent phenotype. Ultimately, understanding the mechanisms underlying the Thr92Ala-DIO2 association with neurodegenerative disease may prove to be important for disease prevention.

CONCLUSION

A number of polymorphisms have been identified in the three deiodinase genes. The most investigated and likely to have clinical relevance is *Thr92Ala-DIO2*. It is questionable whether the Thr92 substitution for Ala affects the catalytic activity of the enzyme and causes localized/systemic hypothyroidism. If confirmed, this could explain why certain populations of hypothyroid patients exhibit better clinical outcomes when treated with combination therapy containing LT3. On the other hand, the in-vitro data clearly indicates that this polymorphism is associated with ectopic localization in the Golgi apparatus, what could constitute a disease mechanism *per se*. Indeed, temporal pole samples of carriers of the Thr92Ala-DIO2 polymorphism exhibit a transcriptome that is suggestive of brain degenerative disease. Future studies should confirm these results and further explore how the racial background modulates the clinical relevance of the *Thr92Ala-DIO2* gene polymorphism. This avenue of research may someday allow us to understand why some patients seem to fail T4 monotherapy, and help guide prevention strategies for neurodegenerative disease.

Acknowledgements

None.

Financial support and sponsorship

The present work was supported by grants from the NIDDK, DK58538, and DK65055, to A.C.B.

Conflicts of interest

A.C.B. is a consultant for Sentier Therapeutics LLC and Synthonics Inc. The remaining authors have no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism Physiol Rev 2014; 94:355–382.
2. Gereben B, Zavacki AM, Ribich S, *et al.* Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev 2008; 29:898–938.
3. ■■ Groeneweg S, Visser WE, Visser TJ. Disorder of thyroid hormone transport into the tissues. Best Pract Res Clin Endocrinol Metab 2017; 31:241–253.

It provides exceptional summary and insights of how thyroid hormone trtansporters play a role in health and disease.

4. Gereben B, McAninch EA, Ribeiro MO, Bianco AC. Scope and limitations of iodothyronine deiodinases in hypothyroidism. Nat Rev Endocrinol 2015; 11:642–652.
5. Brent GA. Mechanisms of thyroid hormone action. J Clin Invest 2012; 122:3035–3043.
6. Mentuccia D, Proietti-Pannunzi L, Tanner K, *et al.* 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 beta-3-adrenergic receptor. Diabetes 2002; 51:880–883.
7. Gumieniak O, Perlstein TS, Williams JS, *et al.* Ala92 type 2 deiodinase allele increases risk for the development of hypertension. Hypertension 2007; 49:461–466.
8. Estivalet AA, Leiria LB, Dora JM, *et al.* D2 Thr92Ala and PPARgamma2 Pro12Ala polymorphisms interact in the modulation of insulin resistance in type 2 diabetic patients. Obesity (Silver Spring) 2010; 19:825–832.
9. Nair S, Muller YL, Ortega E, *et al.* Association analyses of variants in the DIO2 gene with early-onset type 2 diabetes mellitus in Pima Indians. Thyroid 2012; 22:80–87.
10. He B, Li J, Wang G, *et al.* Association of genetic polymorphisms in the type II deiodinase gene with bipolar disorder in a subset of Chinese population. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33:986–990.
11. Guo TW, Zhang FC, Yang MS, *et al.* Positive association of the DIO2 (deiodinase type 2) gene with mental retardation in the iodine-deficient areas of China. J Med Genet 2004; 41:585–590.
12. Taylor P, Sayers OO, Pearce A, *et al.* Effect of low thyroid hormone bioavailability on childhood cognitive development: data from the Avon Longitudinal Study of Parents and Children birth cohort. Lancet 2014; 383:S100.
13. Ma SF, Xie L, Pino-Yanes M, *et al.* Type 2 deiodinase and host responses of sepsis and acute lung injury. Am J Respir Cell Mol Biol 2011; 45:1203–1211.
14. Meulenbelt I, Min JL, Bos S, *et al.* Identification of DIO2 as new susceptibility locus for symptomatic osteoarthritis. Hum Mol Genet 2008; 17:1867–1875.
15. Heemstra KA, Hoftijzer H, van der Deure WM, *et al.* The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density. J Bone Miner Res 2010; 25:1385–1391.
16. Inoue N, Watanabe M, Katsumata Y, *et al.* Functional polymorphisms of the type 1 and type 2 iodothyronine deiodinase genes in autoimmune thyroid diseases. Immunol Invest 2018; 47:534–542.
17. Verloop H, Dekkers OM, Peeters RP, *et al.* Genetics in endocrinology: genetic variation in deiodinases: a systematic review of potential clinical effects in humans. Eur J Endocrinol 2014; 171:R123–R135.
18. Dora JM, Machado WE, Rheinheimer J, *et al.* Association of the type 2 deiodinase Thr92Ala polymorphism with type 2 diabetes: case-control study and meta-analysis. Eur J Endocrinol 2010; 163:427–434.
19. Panicker V, Saravanan P, Vaidya B, *et al.* 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 2009; 94:1623–1629.
20. Kim BW, Bianco AC. For some, L-thyroxine replacement might not be enough: a genetic rationale. J Clin Endocrinol Metab 2009; 94:1521–1523.
21. Saravanan P, Chau WF, Roberts N, *et al.* Psychological well being in patients on 'adequate' doses of l-thyroxine: results of a large, controlled community-based questionnaire study. Clin Endocrinol (Oxf) 2002; 57:577–585.
22. Peterson SJ, McAninch EA, Bianco AC. Is a normal TSH synonymous with 'euthyroidism' in levothyroxine monotherapy? J Clin Endocrinol Metab 2016; 101:4964–4973.
23. Samuels MH, Kolobova I, Smeraglio A, *et al.* Effects of levothyroxine replacement or suppressive therapy on energy expenditure and body composition. Thyroid 2016; 26:347–355.
24. Peterson SJ, Cappola AR, Castro MR, *et al.* An online survey of hypothyroid patients captured predominantly dissatisfied individuals. Thyroid 2018; 28:707–721.
25. ■ Carle A, Faber J, Steffensen R, *et al.* Hypothyroid patients encoding combined MCT10 and DIO2 gene polymorphisms may prefer L-T3 + L-T4 combination treatment - data using a blind, randomized, clinical study. Eur Thyroid J 2017; 6:143–151.

First description of the clinical impact of the combined polymorphism involving Dio2 and MCT10.

26. Wouters HJ, van Loon HC, van der Klauw MM, *et al.* No effect of the Thr92Ala polymorphism of deiodinase-2 on thyroid hormone parameters, health-related quality of life, and cognitive functioning in a large population-based cohort study. Thyroid 2017; 27:147–155.
27. Young Cho Y, Jeong Kim H, Won Jang H, *et al.* The relationship of 19 functional polymorphisms in iodothyronine deiodinase and psychological well being in hypothyroid patients. Endocrine 2017; 57:115–124.
28. Callebaut I, Curcio-Morelli C, Mornon JP, *et al.* The iodothyronine selenodeiodinases are thioredoxin-fold family proteins containing a glycoside hydrolase clan GH-A-like structure. J Biol Chem 2003; 278:36887–36896.
29. Canani LH, Capp C, Dora JM, *et al.* 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 2005; 90:3472–3478.
30. Peeters RP, van Toor H, Klootwijk W, *et al.* Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. J Clin Endocrinol Metab 2003; 88:2880–2888.
31. Butler PW, Smith SM, Linderman JD, *et al.* The Thr92Ala 5′ type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: a pharmacogenomic study. Thyroid 2010; 20:1407–1412.
32. Arici M, Oztas E, Yanar F, *et al.* Association between genetic polymorphism and levothyroxine bioavailability in hypothyroid patients. Endocr J 2018; 65:317–323.
33. Castagna MG, Dentice M, Cantara S, *et al.* DIO2 Thr92Ala reduces deiodinase-2 activity and serum-T3 levels in thyroid-deficient patients. J Clin Endocrinol Metab 2017; 102:1623–1630.
34. Curcio C, Baqui MM, Salvatore D, *et al.* The human type 2 iodothyronine deiodinase is a selenoprotein highly expressed in a mesothelioma cell line. J Biol Chem 2001; 276:30183–30187.
35. Baqui MM, Gereben B, Harney JW, *et al.* Distinct subcellular localization of transiently expressed types 1 and 2 iodothyronine deiodinases as determined by immunofluorescence confocal microscopy. Endocrinology 2000; 141:4309–4312.
36. Dentice M, Bandyopadhyay A, Gereben B, *et al.* The Hedgehog-inducible ubiquitin ligase subunit WSB-1 modulates thyroid hormone activation and PTHrP secretion in the developing growth plate. Nat Cell Biol 2005; 7:698–705.
37. Zavacki AM, Arrojo e Drigo R, Freitas BC, *et al.* The E3 ubiquitin ligase TEB4 mediates degradation of type 2 iodothyronine deiodinase. Mol Cell Biol 2009; 29:5339–5347.
38. Botero D, Gereben B, Goncalves C, *et al.* Ubc6p and ubc7p are required for normal and substrate-induced endoplasmic reticulum-associated degradation of the human selenoprotein type 2 iodothyronine monodeiodinase. Mol Endocrinol 2002; 16:1999–2007.
39. Gereben B, Goncalves C, Harney JW, *et al.* Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. Mol Endocrinol 2000; 14:1697–1708.
40. Kim BW, Zavacki AM, Curcio-Morelli C, *et al.* Endoplasmic reticulum-associated degradation of the human type 2 iodothyronine deiodinase (D2) is mediated via an association between mammalian UBC7 and the carboxyl region of D2. Mol Endocrinol 2003; 17:2603–2612.
41. Arrojo EDR, Egri P, Jo S, *et al.* The type II deiodinase is retrotranslocated to the cytoplasm and proteasomes via p97/Atx3 complex. Mol Endocrinol 2013; 27:2105–2115.
42. Zeold A, Pormuller L, Dentice M, *et al.* Metabolic instability of type 2 deiodinase is transferable to stable proteins independently of subcellular localization. J Biol Chem 2006; 281:31538–31543.
43. McAninch EA, Jo S, Preite NZ, *et al.* Prevalent polymorphism in thyroid hormone-activating enzyme leaves a genetic fingerprint that underlies associated clinical syndromes. J Cin Endocrinol Metab 2015; 100:920–933.

44. Futamura T, Kakita A, Tohmi M, *et al.* Neonatal perturbation of neurotrophic signaling results in abnormal sensorimotor gating and social interaction in adults: implication for epidermal growth factor in cognitive development. Mol Psychiatry 2003; 8:19–29.

45. Barnes LL, Leurgans S, Aggarwal NT, *et al.* Mixed pathology is more likely in black than white decedents with Alzheimer dementia. Neurology 2015; 85:528–534.

46. Steenland K, Goldstein FC, Levey A, Wharton W. A meta-analysis of Alzheimer's disease incidence and prevalence comparing African-Americans and Caucasians. J Alzheimer's Dis 2016; 50:71–76.

47. Barnes LL, Bennett DA. Alzheimer's disease in African Americans: risk factors and challenges for the future. Health Aff (Millwood) 2014; 33:580–586.

48. McAninch EA, Rajan KB, Evans DA, *et al.* A common DIO2 polymorphism and ■ alzheimer's disease dementia in African And European Americans. J Clin Endocrinol Metab 2018; 103:1818–1826.
First description that Dio2 polymorphisms can have an impact on cognition and degeneration of the central nervous system.

49. Reitz C, Jun G, Naj A, *et al.,* Alzheimer Disease Genetics Collaboration. Variants in the ATP-binding cassette transporter (ABCA7), apolipoprotein E 4, and the risk of late-onset Alzheimer disease in African Americans. JAMA 2013; 309:1483–1492.

50. Joshi G, Wang Y. Golgi defects enhance APP amyloidogenic processing in Alzheimer's disease. Bioessays 2015; 37:240–247.

51. Querfurth HW, LaFerla FM. Alzheimer's disease. New Engl J Med 2010; 362:329–344.