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From DIO2 Genotype to Personalized Medication

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Abstract

Hypothyroidism is a syndrome in which the metabolic process of the entire body is degraded due to a lack of thyroid hormones. The single nucleotide polymorphism (SNP) information from the DIO2 gene found in the patient's genome is currently used in combination therapy: a treatment for hypothyroidism by providing patients with levothyroxine and liothyronine together. In patients with rs225014 (T→C) transition in DIO2, the conversion from prohormone thyroxine (T4) to bioactive thyroid hormone (T3) proceeds inefficiently even if the level of T4 is increased through Levothyroxine (LT4). However, there is a lack of information to explain how the SNP of DIO2 is linked with the effectiveness of the treatment, and some findings are incomplete and conflicting. In this study, we analyzed the cellular localization of the DIO2 gene, and investigated the tissue-specific gene expression of DIO2 in our body organs. We also conducted a gene network analysis of DIO2 to find the closely related genes to DIO2. Our result shows that the DIO2 gene is closely associated with eight proteins that function as Ubiquitin-dependent proteins. Thus, this study investigated for the first time that DIO2 functions as a homeostasis regulator for thyroid hormones and is closely involved in the proteolytic regulatory system by ubiquitin. The findings provide more information on DIO2 gene function, which can be applied to the development of personalized treatments for hypothyroidism, suggesting that the drugs can be prescribed more precisely and accurately.

Keywords: Hypothyroidism, Personalized Medication, DIO2, Ubiquitination, Gene Network Analysis

Introduction

Hypothyroidism is a condition in which the thyroid gland cannot create enough thyroid hormone that humans need. This negatively affects general human metabolism such as gaining or losing weight, disrupting heart rate and body temperature, and weakening muscles. (1)

Out of thousands of medicines, the most frequently used medicine in the world is levothyroxine (LT4). (2) LT4 dosing normalizes T4, a hormone type of medicine that replaces malfunction hormones in the thyroid gland and helps our body remain at the thyroid hormone level. (3) T3, the metabolically active form, derives from extrathyroidal conversion of T4 by deiodinase 2 (D2) enzyme encoded by DIO2 gene. In thyroid-deficient patients, decreased levels of free T3 have been associated with the polymorphism rs225014 T/C in DIO2 (4)

There are two types of substitutions of nucleotide sequences of genes: mutation and single nucleotide polymorphism. Mutation occurs when there is an error during DNA replications which rarely occurs. It can affect the physical appearance of an organism. In contrast to that, single nucleotide polymorphism is more likely to occur in organisms and not all of them change the physical appearance of an organism. Still, it may

create unique characteristics of the organism. (5) Many scientists hypothesize that the unique characteristics created due to SNP would be why the patients with the rs225014 T/C on DIO2 suggested the combination therapy. (6)

Various SNPs exist in the DIO2 gene. However, one of the most significant SNP is rs225014. It is a type of SNP that occurs in DIO2, which encodes the (T) allele to the Thr (threonine) and (C) allele to the Ala (alanine). Encoding (T) allele to the Thr is more common than the (C) allele to Ala because more people tend to have (T) allele in their DIO2 sequences. It can cause various conditions such as osteoarthritis and psychological disorder. However, this SNP is significant to the study because it can cause thyroid hormone metabolism. The previous research indicated that hypothyroid patients who did not show much improvement using LT4 therapy tend to have lower brain thyroid levels which correlate to rs225014. (7) On the other hand, people with precedence of the rs225014 showed more improvement on the combination therapy. (8) In short, the presence of rs225014 seems to impact hyperthyroid patients' preference of the combination therapy.

The guidelines on T4 + T3 combination therapy were published in 2012. However, dissatisfaction with the outcome of T4 monotherapy remains high. The purpose of this research investigates the general information on DIO2 gene and protein networks. In addition, this study investigated that DIO2 functions as a homeostasis regulator for thyroid hormones and is closely involved in the proteolytic regulatory system by ubiquitin.

Materials and Methods

DIO2 protein localization and tissue-specific expression

The basic gene information was obtained from GeneCard. (9) The information on gene position, summary, protein information, and protein localization was provided. It provides a database of human genes that provides concise genomic-related information with all known and predicted human genes. It also provides genomic, proteomic, transcriptomic, genetic, and functional information on all known and predicted human genes.

DIO2 functional protein association network analysis by STRING

DIO2 functional protein association network was analyzed by STRING. (10) It is a database of known and predicted protein-protein interactions. The physical interactions and functional associations were analyzed from computational prediction. The interaction database was constructed based on the five main sources: genomic context prediction, high-throughput lab experiments, co-expression, automated text mining, previous knowledge in the database.

DIO2 associated proteins biological pathway analysis by GeneMANIA

GeneMANIA predicts gene function by integrating multiple functional association networks. It predicts gene function from a single process-specific network using label propagation. It provides genome-wide predictions that achieve an accurate seed gene list without relying on a pre-specified association network. (11)

Results and Discussions

Compartments program, a subcellular localization web database, was used to analyze the DIO2 protein subcellular localization. (12) This program predicts the protein subcellular localization based on the literature manual curation, microscopy-based screens, and predictions from primary sequence. The confidence scale ranges from one for low confidence to five for high confidence. DIO2 is predicted to be localized on the plasma membrane with a confidence level of four. It is also predicted to be localized on extracellular, mitochondria, and endoplasmic reticulum with a confidence level of three (Figure 1). Other places in a cell where DIO2 protein is being localized are cytoskeleton, peroxisome, nucleus, cytosol, endosome, lysosome, and Golgi apparatus. This

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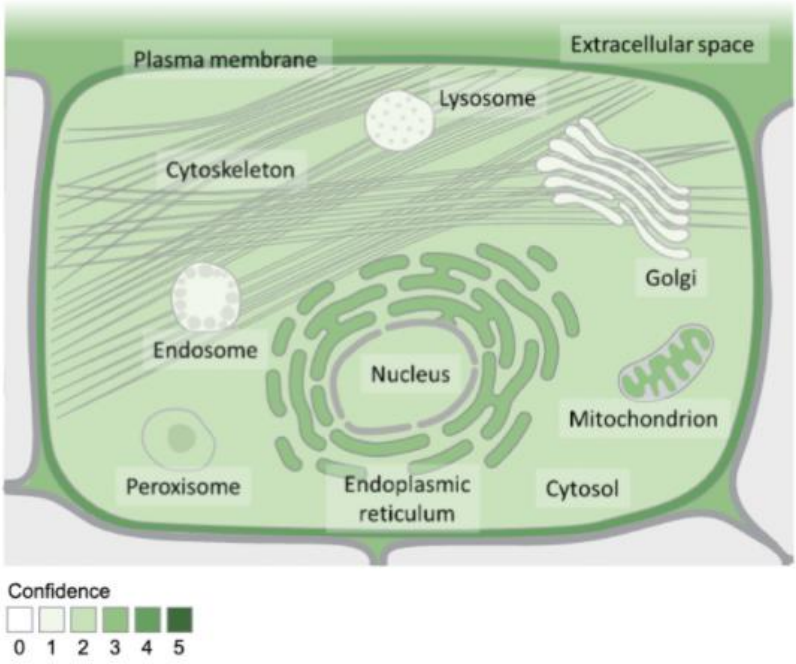

Compartment	Confidence
plasma membrane	4
extracellular	3
mitochondrion	3
endoplasmic reticulum	3
cytoskeleton	2
peroxisome	2
nucleus	2
cytosol	2
endosome	1
lysosome	1
golgi apparatus	1

FIGURE 1. DIO2 protein subcellular localization analysis. DIO2 protein mainly localizes to the plasma membrane, extracellular, mitochondria, and endoplasmic reticulum.

shows how DIO2 protein is localized mostly everywhere in a cell, both inside and outside of it. However, the further cell experiment is needed to verify DIO2 subcellular localization in various types of human cells.

DIO2 tissue-specific mRNA expression was analyzed by RNA expression overview from the protein atlas database. It provides RNA-data from three different sources, respectively: Internally generated Human Protein Atlas (HPA) RNA-seq data, RNA-seq data from the Genotype-Tissue Expression (GTEx) project and CAGE data from FANTOM5 project, as well as the consensus dataset which is based on a combination of all three sources. (13) Color-coding is based on tissue groups, each consisting of tissues with functional features in common. Figure 2 shows that DIO2 is mostly expressed in the thyroid gland and cervix uterine. The thyroid gland releases T4 thyroid hormones, which are necessary for our body cells to work properly. Since the main function of DIO2 is transitioning T4 to T3, high expression of DIO2 in thyroid gland may play an important role in producing T3 in human body. In the case of the cervix uterine, thyroid hormones are also necessary for its proper functioning. It regulates the metabolism and the development of ovarian, uterine, and placental tissues (14). As shown in both the thyroid gland and cervix, DIO2 is most likely to be expressed in organs that function within thyroid hormones.

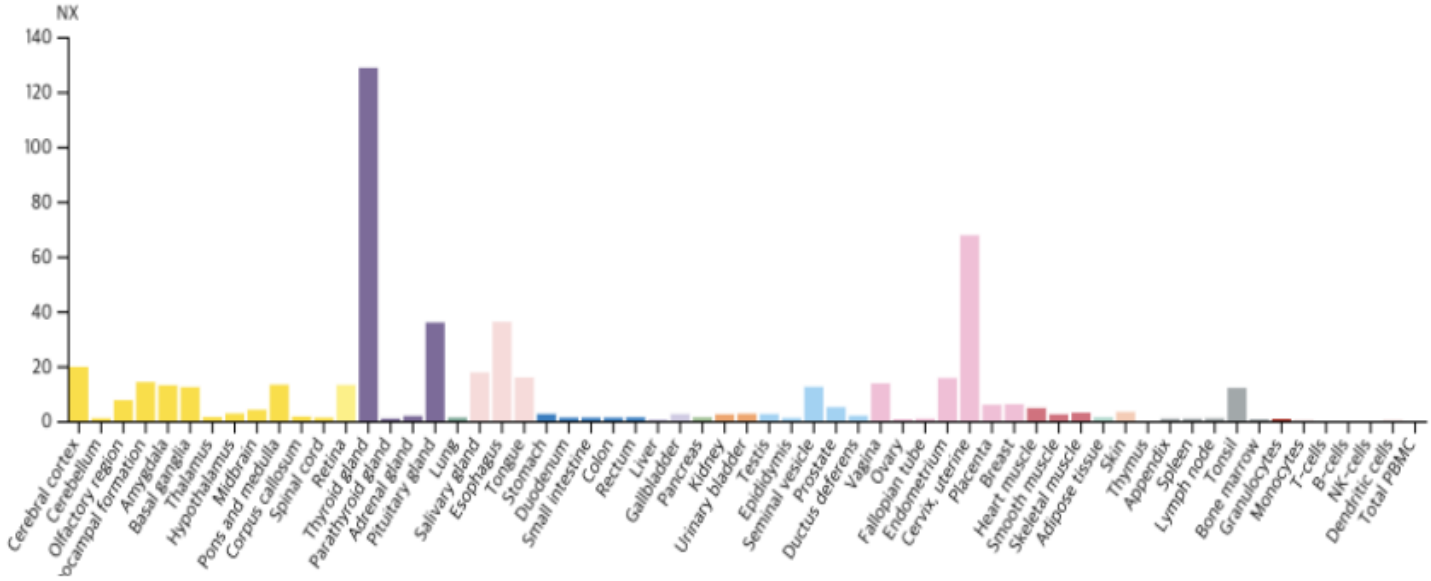

FIGURE 2. DIO2 gene is mainly expressed in the thyroid gland and cervix, uterine in human tissue. NX on the y-axis indicates the expression level.

To investigate the functional role of close relative genes of DIO2, we performed protein network analysis. Figure 3 shows 17 genes that are closely related to DIO2: CUL5, UBE2G1, TCEB1, RBX1, PSMD4, VCP, TCEB2, MARCH5, USP33, UBE2J1, WSB1, USP20, SULT1A1, SULT1A2, DIO3, UGT2B11, and UGT2B10. Identifying each one of their unique abilities and how it relates to DIO2 can bring new insights which can extend to existing studies. However, further analysis was required to find the novel relationship between DIO2 relative genes. To further investigate the biological pathway associated with these genes, 17 genes were used to analyze the protein physical interaction network and biological pathway in figure 4.

As shown in figure 3, more in-depth observation of those 17 genes is necessary. This figure provides closer observation on the relationship of those genes along with their protein. Figure 4 shows the graphical illustration of all 17 genes on the left column with the direct protein physical interaction and biological pathway. The red mark indicates the proteins associated with ubiquitin ligase complex pathways. The blue mark indicates the proteins associated with the ubiquitin-protein transferase activity. The yellow mark indicates the proteins associated with proteasome-mediated ubiquitin-dependent protein catabolic processes. Interestingly, protein ubiquitin is involved in the relationships between those 17 genes. This is significant because it portrays a common protein that all those genes contain, thus closely related.

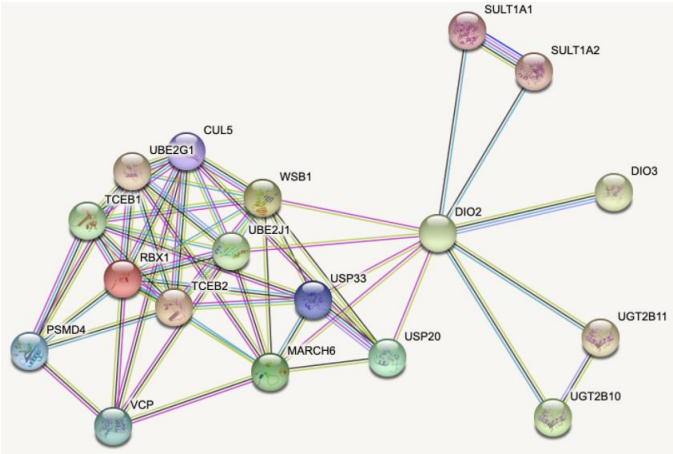

FIGURE 3. Protein network analysis of DIO2 and associated network proteins.

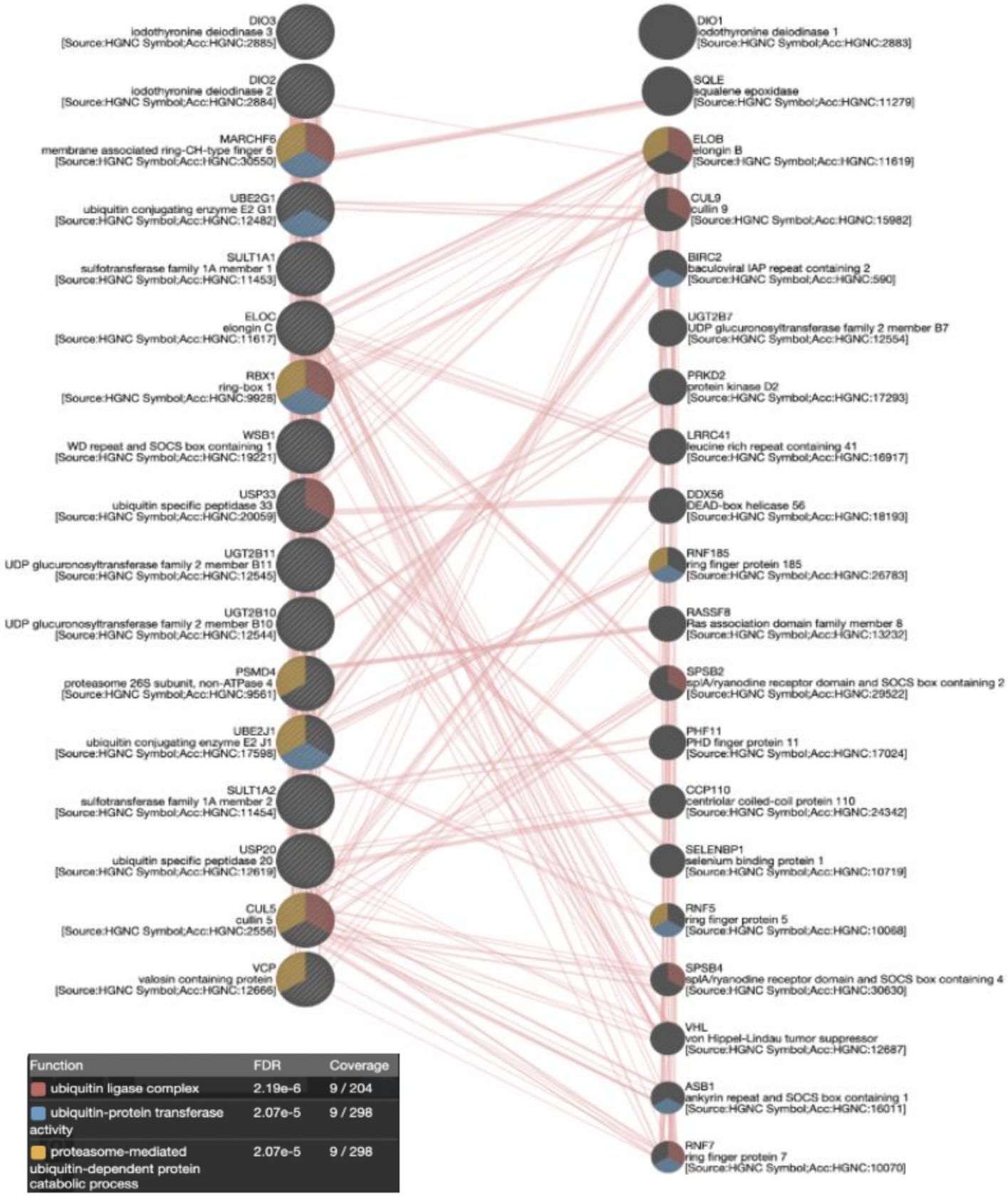

Figure 4. DIO2 protein physical interaction and biological pathway analysis. The red line shows the physical interaction of proteins within the selected proteins.

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Ubiquitin plays a vital role in protein quality control and cellular regulation. Many proteins might be damaged by heat, oxidation, inherited mutation, heavy metals/metalloids, abnormal amino acids, or translation inhibition during and after protein synthesis. Ubiquitin helps these damaged proteins to be degraded through protein degradation, which is called protein quality control. Furthermore, ubiquitin can also degrade proteins that can impair cell cycle, transcription, DNA repair, stress responses, protein sorting, and apoptosis: this is called cellular regulation. By helping these proteins to go through protein degradation, ubiquitin is an essential factor in our body functions.

Next, the relationship between ubiquitin pathway and rs225014 polymorphism in DIO2 was investigated. The previous study indicated that D2, DIO2 encoded protein, is regulated to local thyroid hormone levels in the brain and other tissues. Therefore, increased activity of D2 is known to protect against hypothyroidism. This regulation is mostly brought about by substrate (T4)-induced ubiquitination (15). The DIO2 rs225014 polymorphism is positioned in exon 3 of the DIO2 gene resulting in a Thr92Ala amino acid substitution, which is closely linked to ubiquitination and a key determinant of turnover rate. (16) A recent report, which showed an association between CC genotype and osteoarthritis, showed that CC genotype decreased the activity of D2 in humans (17). Therefore, it is possible that Thr92Ala substitution may cause ubiquitination impairing that reduce the ability to maintain homeostasis of serum T3 level. In conclusion, these results are consistent with the previous findings that patients with CC genotype should be prescribed the T4+T3 combination therapy.

CONCLUSION

This study prompts the new potential reasoning for why the presence of rs225014 in DIO2 in patients affects the effectiveness of the combination therapy. Through the close investigations on DIO2, we were able to find how rs225014 in DIO2 might have affected the ubiquitination and lead to impair T3 hormones level. CC genotype in rs225014 may cause ubiquitination impairment of D2, which leads to reducing the ability to maintain homeostasis of T3. In conclusion, this study provides a novel protein interaction of DIO2, which may provide vital information for a more precise prescription for hypothyroidism patients.

References

Chiovato L, Magri F, Carlé A. Hypothyroidism in context: where we've been and where we're going. Adv Ther. 2019 Sep 4;36(Suppl 2):47–58.

Hughes M, Inglese J, Kurtz A, Andalibi A, Patton L, Austin C, et al. Early Drug Discovery and Development Guidelines: For Academic Researchers, Collaborators, and Start-up Companies. In: Sittampalam GS, Coussens NP, Nelson H, Arkin M, Auld D, Austin C, et al., editors. Assay Guidance Manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004.

Chen Y, Tai H-Y. Levothyroxine in the treatment of overt or subclinical hypothyroidism: a systematic review and meta-analysis. Endocr J. 2020 Jul 28;67(7):719–32.

Wiersinga WM. T4 + T3 combination therapy: any progress? Endocrine. 2019 Oct 15;66(1):70–8.

Vignal A, Milan D, SanCristobal M, Eggen A. A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol. 2002 Jun;34(3):275–305.

Park E, Jung J, Araki O, Tsunekawa K, Park SY, Kim J, et al. Concurrent TSHR mutations and DIO2 T92A polymorphism result in abnormal thyroid hormone metabolism. Sci Rep. 2018 Jul 4;8(1):10090.

Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, 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 May;94(5):1623–9.

Wouters HJCM, van Loon HCM, van der Klauw MM, Elderson MF, Slagter SN, Kobold AM, 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 Feb;27(2):147–55.

Safran M, Dalah I, Alexander J, Rosen N, Iny Stein T, Shmoish M, et al. GeneCards Version 3: the human gene integrator. Database (Oxford). 2010 Aug 5;2010:baq020.

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res. 2019 Jan 8;47(D1):D607–13.

Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 2010 Jul;38(Web Server issue):W214-20.

Binder JX, Pletscher-Frankild S, Tsafou K, Stolte C, O'Donoghue SI, Schneider R, et al. COMPARTMENTS: unification and visualization of

protein subcellular localization evidence. Database (Oxford). 2014 Feb 25;2014(2014):bau012.

Pontén F, Jirström K, Uhlen M. The Human Protein Atlas--a tool for pathology. J Pathol. 2008 Dec;216(4):387–93.

Silva JF, Ocarino NM, Serakides R. Thyroid hormones and female reproduction. Biol Reprod. 2018 Nov 1;99(5):907–21.

Gereben B, Goncalves C, Harney JW, Larsen PR, Bianco AC. Selective proteolysis of human type 2 deiodinase: a novel ubiquitin-proteasomal mediated mechanism for regulation of hormone activation. Mol Endocrinol. 2000 Nov;14(11):1697–708.
Dentice M, Bandyopadhyay A, Gereben B, Callebaut I, Christoffolete MA, Kim BW, 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 Jul;7(7):698–705.

Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk H-J, et al. Identification of DIO2 as a new susceptibility locus for symptomatic osteoarthritis. Hum Mol Genet. 2008 Jun 15;17(12):1867–75.