

# Gene Network Analysis of DIO2 and Thyroid Hormone Replacement Therapy

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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

## 1. 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 mal-function 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.

## 2. Research Method

### 2.1 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.

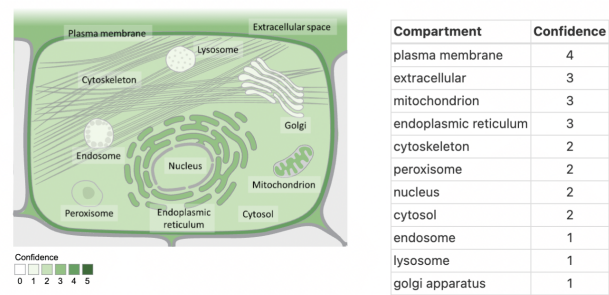
### 2.2 DIO2 functional protein association network analysis by STRING

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.

### 2.3 DIO2 associated proteins biological pathway analysis by GeneMANIA

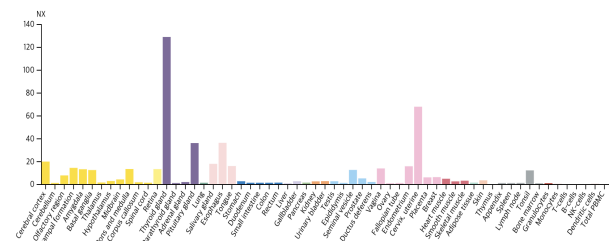
GeneMANIA predicts gene function by integrating multiple functional association networks. It predicts gene function from a single process-specific network using label propagation. It provides genome-wide predictions that achieve an accurate seed gene list without relying on a pre-specified association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

## 3. Result



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

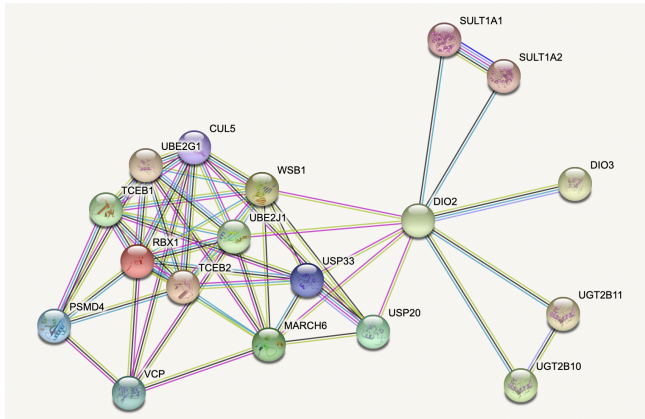
Compartments program, a subcellular localization web database, was used to analyze the DIO2 protein subcellular localization. [12] This program predicts the protein subcellular localization based on the literature manual curation, microscopy-based screens, and predictions from primary sequence. The confidence 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 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.



**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.

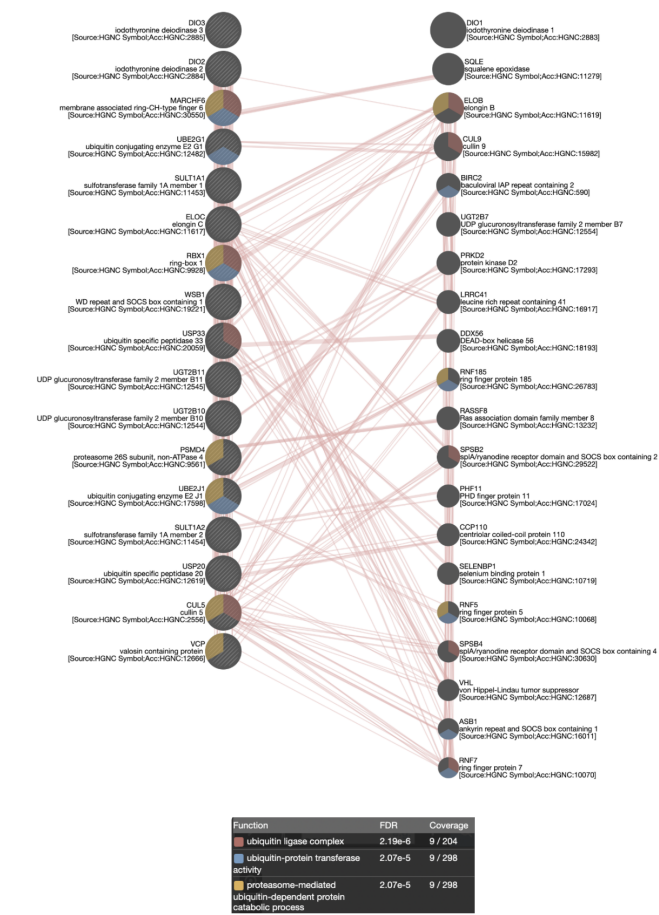
DIO2 tissue-specific mRNA expression was analyzed by RNA expression overview from the protein atlas database. It provides RNA-data from three different sources, respectively: Internally generated Human Protein Atlas (HPA) RNA-seq data, RNA-seq data from the

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

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.



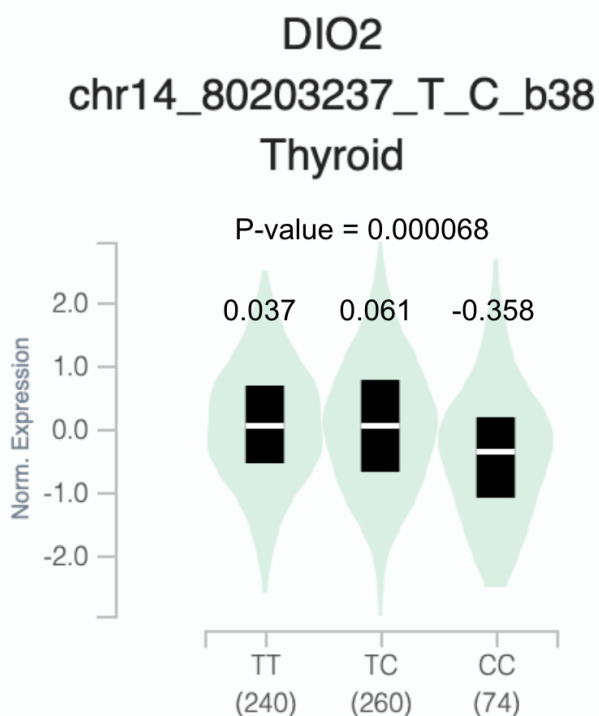
**Figure 4.** DIO2 protein physical interaction and biological pathway analysis. The red line shows the physical interaction of proteins within the selected proteins.

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.

Ubiquitin plays a vital role in protein quality control and cellular regulation. Many proteins might be damaged by heat, oxidation, inherited mutation, heavy

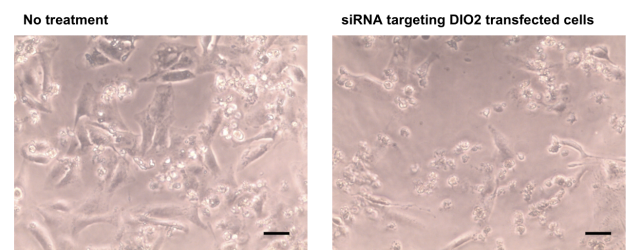
metals/metalloids, abnormal amino acids, or translation inhibition during and after protein synthesis. Ubiquitin helps these damaged proteins to be degraded through protein degradation, which is called protein quality control. Furthermore, ubiquitin can also degrade proteins that can impair cell cycle, 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 reduces 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.



**Figure 5.** Violin plot of DIO2 gene expression based on the sequence of rs225014. The mRNA expression level of DIO2 based on the patient's genotype TT (n=240), TC (n=260), CC (n=74) was analyzed in thyroid tissue. The median value is indicated on the graph.

We analyzed the mRNA expression level of DIO2 based on the patient's genotype of rs225014 polymorphism. Three types of rs225014 polymorphism were analyzed: TT, TC, and CC. The number of patients analyzed in this data was 240 for TT, 260 for TC, and 74 for CC. The median value of DIO2 expression level is 0.037 for TT, 0.061 for TC, and -0.358 for CC. This result indicates that CC genotype decreased the expression level of DIO2 in thyroid tissue. This data was consistent with the previous finding that CC genotype decreased the activity of D2 in humans [17].



**Figure 6.** siRNA targeting DIO2 transfection decreased the thyroid cell number. The image of the cells was represented. scale bar = 20  $\mu$ m

To mimic the thyroid cells with CC genotype of rs225014 polymorphism, we transfect the siRNA targeting DIO2 in thyroid cells. Since siRNA targeting DIO2 specifically degrades the DIO2 mRNA, the expression level of DIO2 would be decreased compared to the no treatment cells. Figure 6 shows the image of two conditions on thyroid cells: 1) no treatment (negative control) and 2) siRNA targeting DIO2 transfected cells. The cell number in the image indicates the live thyroid cells attached to the cell culture plate. The number of cells was decreased in siRNA targeting DIO2 transfected cells. This result demonstrates that decreased DIO2 expression level may reduce the live thyroid cells, which mediates the abnormal function of the thyroid and decreased activity of D2.

#### 4. 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.

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