



Type 2 Deiodinase A/G (Thr92Ala) Polymorphism and Circulating Thyroid Hormone Level of Childbearing Age Women in Area Replete with Iodine Deficiency Disorders

Agus Wibowo^{1*}, Taufiq Hidayat², Sri Nuryani Wahyuningrum¹

¹National Institute Health Research and Development Station of Magelang, Indonesia Ministry of Health, Magelang, Indonesia;

²National Research and Innovation Agency, Cibinong Science Center, Bogor, Indonesia

Abstract

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***Correspondence:** Agus Wibowo, National Health Research and Development Station of Magelang, National Institute Health and Research Development, Indonesian Ministry of Health, Kapling jayan Borobudur, Magelang Central Java, Magelang 56553, Indonesia. E-mail: bowo6996@gmail.com

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BACKGROUND: Iodothyronine deiodinase (DIO) is an enzyme that regulates thyroid hormone activity. DIO consists of three types: deiodinase 1 (D1), 2 (D2), and 3 (D3). D2 is a gene that plays an important role in regulation of the biochemistry of the thyroid hormone in several tissues. D2 also plays a role in the production of triiodothyronine and controlling thyroid hormone signals. This study measured the observation that about 15% of the normal population show that D2 gene polymorphism (Thr92Ala) potentially affects the activity of D2.

AIM: This study aimed to determine D2 polymorphisms and their association with thyroid hormone levels in women of childbearing age in replete iodine deficiency disorder areas.

METHODS: Total number of subjects was 131. Analysis of serum TSH, T_3 , fT_3 , T_4 , and fT_4 levels was done using ELISA. Polymorphism of Thr92Ala was analyzed by PCR-RFLP method.

RESULTS: The results showed that the frequencies of the genotypes Thr92Ala were AA 16.79%, AG 41.22%, and GG 41.99%, whereas the allele frequency A 37.5% and G 62.5% (p HWE = 0.171). In this study, we found no differences of TSH and thyroid hormone level between group of each allele. Mean of TSH and thyroid hormone level was on normal range.

CONCLUSION: This D2 polymorphism is associated with fT_4 levels rather than fT_3 but not statistically significant. Heterozygous alleles at D2 AG have higher TSH levels compared with homozygous alleles.

Introduction

Iodine is the essential micronutrient for thyroid hormone biosynthesis in humans. The impact of this biosynthesis of thyroid hormones is to ensure the availability of iodine in the thyroid gland homeostasis. Biosynthesis of thyroid hormones consists of two steps. The first step is the activation of membrane transporters by the Sodium/Natrium Iodine Symporter (*NIS*) gene to accumulate iodine in the thyroid cells. The second is the recycling of iodine through monoiodotyrosine (T_1) and diiodotyrosine (T_2) deiodination by iodothyronine deiodinase. This second step is the main product of thyroid hormone synthesis [1], [2]. Iodothyronine deiodinase is an enzyme that regulates thyroid hormone activity and plays a role in activating thyroid hormone precursor in the form of thyroxine (T_4) into the active form of triiodothyronine (T_3), by reducing the iodine-specific group of T_4 precursor molecules. It also plays a role in thyroid hormone inactivation by converting T_3 hormone into a reverse form of T_3 (rT_3),

an inactive form, when an excess level of T_3 occurs. The availability of T_3 is primarily regulated by three different selenodeiodinases, which are deiodinase 1 (D1), 2 (D2) and 3 (D3), and each of them plays a different physiological role [3], [4], [5], [6].

Deiodinase 2 (D2) is an enzyme that plays an important role in the regulation of thyroid hormones in some tissues. It deiodinates the phenol group of the T_4 outer ring to convert T_4 to T_3 and influences thyroid hormone signaling in T_3 production. A large number of clinical studies also indicated that D2 is essential in influencing thyroid hormone signaling. This study comes from the observation that about 15% of the normal population shows the Dio2 (Thr92AlaD2) gene polymorphism, which potentially affects the activity of D2 and the production of localized T_3 in tissue [7].

A single nucleotide polymorphism (SNP), Thr92Ala, has been reported in the general population. Thr92Ala single nucleotide polymorphism (SNP), a threonine (Thr) changes to alanine (Ala) at codon 92 (14q24.2-q24.3), has been reported in the general

population [8]. Thr92Ala polymorphism has been linked to increased risk for hypothyroidism, Graves' disease, osteoarthritis, hypertension, response to T3 or T4 treatment, a decreased bone mass, and higher bone turnover, the Ala allele in homozygosis was associated with greater insulin resistance in type 2 diabetes (DM2) patients [8], [9], [10].

T₃ production, mediated by D2, serves as an additional source of T₃ for thyroid receptors' (TRs) increased thyroid hormone signals on discrete or tissue cells. This pathway has been shown to play an important role in a several of biological systems such as the pituitary gland and feedback mechanisms of T₄ hypothalamus mediators (cochlear and retinal development, brown adipose tissue development, metabolic control, bone maturation, myogenesis, and muscle regeneration). Furthermore, in the central nervous system, D2 is expressed in glial cells, whereas most TRs are expressed in neurons. Thus, glial-cell-derived T₃ enters the nearest neuron through the hormone-transporting thyroid and creates a transcription site, which involves a paracrine signal that activates neuronal gene expression in the brains of mice and human cells [11], [12], [13], [14], [15], [16], [17].

Polymorphism is a frequent variation in the nucleotide sequence in the genome and happens in at least 1% of the population [18]. This genetic variation plays an important role in the serum thyroid variation and affects levels of thyroid hormones biochemically between individuals. Parameters of serum thyroid hormones in healthy people indicate the variation between individuals, although still within the adjacent normal range. Environmental and genetic factors contribute significantly to the variation of thyroid function tests performed among individuals [5].

Indonesia is one of the developing countries that still have problems with iodine deficiency disorders (IDD). Research about the genetic variation of iodothyronine deiodinase genes in Indonesia is still rare, especially in populations who live in IDD endemic areas. This study aimed to determine the polymorphism of the D2 gene, Dio Thr92Ala (D2 A/G), in women in a childbearing age population of an IDD endemic area, and associate it with thyroid-stimulating hormone (TSH) and thyroid hormone levels.

Materials and Methods

Study population

This is a cross-sectional study. Blood was collected from 131 women of childbearing age in a replete IDD area, Samigaluh Regency of Yogyakarta, Indonesia. Based on the World Health Organization (WHO) criteria, women of childbearing age were included in the IDD risk group. Respondents are

women of childbearing age who live in the study area for approximately 17 years, are not pregnant and do not suffer from serious diseases, and do not receive long-term treatment. The whole process strictly followed the guidelines and regulations set by the Helsinki Declaration of 1975. Informed consent was given to all participant. This study was agreed by the National Commission for Health Research Ethics at the Health Research and Development Agency.

Serum analysis

Thyroid hormone levels

Serum, TSH (μ U/mL), fT₃ (pg/mL), fT₄ (ng/dL), T₃ (ng/dL), and T₄ (ng/dL) were measured by Enzyme-linked Immunosorbent Assay (ELISA) (Human.de, Germany).

DNA isolation

DNA was extracted from 2 ml of EDTA blood, using the GeneJet DNA extraction (Thermo Fisher Scientific, EU Lithuania) with a slightly modified procedure in the lysis solution. Modifications were made by adding 50 μ L of lysis buffer than required to make the process better. After isolation, DNA samples were diluted to concentrations of 50 ng/l (stock) and 10 ng/ μ l (work solution). A quantitative spectrophotometric assay of DNA was done. The absorbance quotient (OD260/OD280) signifies the purity of DNA. Variation of D2 was analyzed with thermocycler (Polymerase Chain Reaction/PCR; Veriti Applied Biosystem) and products were verified by 2% agarose gel electrophoresis with TAE buffer.

Restriction fragment length polymorphism (RFLP)

Polymorphism of D2A/G was analyzed using the RFLP method and DNA fragment was cut by restriction enzyme *Rsa*I. Incubation is carried out at 37°C in 3 h and deactivation at 80°C at 20 min. Restriction product was AA: 405 bp and GG: 380 bp. The primers were as follows: 5'-GATAGTAAAGAATAACAGCCTTGGCT-3' (forward) and 5'-CAGCTATCTTCTCCTGG-ATACCA-3' (reverse). The PCR conditions were: 30 sec at 96°C, 30 cycles of 90 s at 94°C, 1 min at 58°C annealing temperature, and 1 min at 72°C; finally, 5 min at 72°C.

Statistical analysis

Data were analyzed using SPSS 16.0 for Windows. Mean differences of TSH and thyroid hormone levels based on genotype alleles groups were tested by ANOVA. Association between TSH, thyroid hormone, and alleles group was tested using a linear regression model. Deviation from the Hardy-Weinberg equilibrium

was analyzed using an X^2 test. p values were two-sided, and $p < 0.05$ was considered significant.

Results

Population descriptive

Population characteristics in Table 1 show that the mean of population age is 33.74 ± 7.95 years old and having normal BMI average. Although the subject population live in an IDD endemic area, they have TSH and thyroid hormone average levels within normal range. This finding could mean that there has been an improvement in the health status associated with IDD in the Samigaluh area, compared with the previous data [19]. IDD can trigger hypothyroidism with high levels of TSH followed by decreasing of ft_4 level. High levels of TSH may stimulate excessive thyroid gland activation, causing epithelium hyperplasia of thyroid gland [20].

Table 1: Descriptive statistics of the population

Variables	Mean \pm SD	Reference value
Age (year)	33.74 ± 7.95	
BMI (kg/m^2)	22.76 ± 4.18	
TSH ($\mu IU/mL$)	3.31 ± 4.11	0.6–4
T_4 of this study population ($\mu g/dl$)	9.51 ± 1.89	4.8–11.6
ft_4 of this study population (ng/dl)	1.24 ± 0.26	0.80–2.00
T_3 (ng/dl)	1.31 ± 0.29	0.69–2.02
ft_3 level (pg/mL)	2.11 ± 0.43	1.40–4.20

Reference value used derived from reagent Kit. $n = 131$. TSH: Thyroid stimulating hormone, T_4 : Thyroxine, ft_4 : Free T_4 , T_3 : Triiodothyronine, ft_3 : Free T_3 , SD: Standard deviation, BMI: Body mass index.

Figure 1 shows D2 A/G gene cutting that used *RasI* restriction enzyme, consisting of three genotypes alleles: homozygote AA size 405 bp (no: 12, 15, 16, and 17) and GG: 385 bp (no: 10 and 14), and heterozygous AG: 405, 385 bp (No:11).

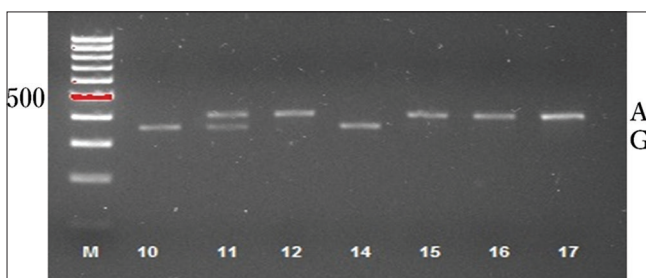


Figure 1: PCR-RFLP gel electrophoresis of D2 Thr92Ala (A/G)

Identification of variant genotype

The homozygous GG gene was commonly found in the study population (42%). Subjects in our population were in Hardy–Weinberg's assumptions (Table 2).

Table 2: Thr92Ala variant in studied subjects

Polymorphism	Genotype frequency (%)			Allele frequency (%)	p HWE
	AA	AG	GG		
D2 A/G	22 (16.79)	54 (41.22)	55 (41.99)	A = 98 (37.5) G = 164 (62.5)	0.171

HWE: Hardy–Weinberg Equilibrium.

Association of D2 Thr92Ala with circulating TSH and thyroid hormone level

The relationship between polymorphism of D2 A/G with TSH and thyroid hormone levels is described in Figure 2. In this study, we found no differences in TSH and thyroid hormones mean values among different genotypes of the Thr92Ala variant using ANOVA. Mean of TSH and thyroid hormone level was on normal range.

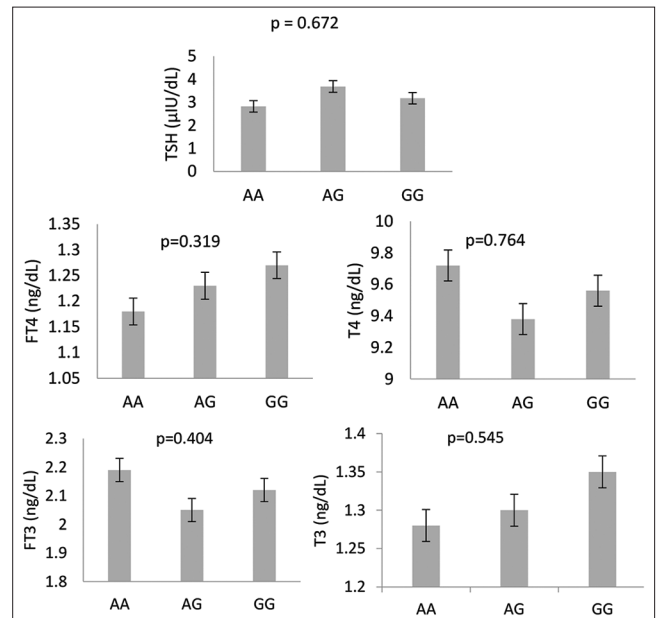


Figure 2: Difference in TSH, FT4, T4, FT3 and T3 level based on D2 genotypes : AA, AG and GG

Discussion

Thyroid hormones are essential hormone which regulates development of the human body, physiologic processes and energy metabolism [6], [21]. Abnormalities in the production of thyroid hormones can cause hypothyroid or hyperthyroid disease. In IDD area the relationships between dietary iodine intake, endemic goiter and prevalence of clinical or subclinical thyroid are controversial [22]. Deiodinases (Dio) are enzymes that can activate or inactivate thyroid hormone molecules. D2 activates thyroxine (T_4) to 3,5,3'- triiodothyronine (T_3) [21].

Single nucleotide polymorphism (SNP) in deiodinase genes has been widely identified, although their functional relevance remains to be assessed. The most characteristic SNP in deiodinase genes is Thr92Ala in gene D2 (D2-G/A; Thr92Ala; GI 13654872; and rs225014) [21]. The SNP is common in various ethnic groups; the frequency of alleles is 0.35 in Caucasians and very high (0.75) in Pima Indians. The Thr92Ala alteration in the coding region D2 does not interfere with the kinetics of temporarily expressed D2

enzymes but are associated with decreasing enzyme velocity in thyroid and skeletal muscles [2], [21].

Hardy–Weinberg's equilibrium law describes the relationship between allele frequencies and genetic frequencies in ideal populations [20], [23]. In this study, several factors may influence allele frequency balance in a population, such as individual migration into or out of the population, the presence of allele mutations, and the absence of random marriage. The population of a society is highly dynamic, and the occurrence of migration and contact with other populations is inevitable. For example, an individual in the population can have more offspring than another individual, thus contributing to the disproportion of the number of alleles in later generations. In a real population, it is rarely encountered that the distribution suits the Hardy–Weinberg model [20]. In the two studies conducted by Peeters *et al.* [2], [6], [24], polymorphisms of D2 A/G in an adult Rotterdam population also showed P HWE > 0.05 of the Hardy–Weinberg model. GG heterozygote genotype frequency was highest in the subject population compared to homozygous AA and AG, with G allele carrier subjects higher than A allele. In this study, frequencies in G allele were higher than the A allele, the GG genotype was higher than the AA and AG genotype, while, in the Peeters *et al.* study, the AA genotype had a higher frequency compared to the GG genotype [6].

The Thr92Ala-DIO2 polymorphism participates as a thyroid disease mechanism and/or affects clinical outcomes by substitution affecting D2 catalytic activity [21]. Effects of D2 A/G polymorphism (Thr92Ala) on thyroid hormone homeostasis and thyroid function have been investigated. A correlation has been observed between D2 Thr92Ala and serum TSH polymorphisms but not serum thyroid hormone levels in healthy individuals [2], [6], [24]. One study on the Dutch population also confirmed that heterozygous individuals for Thr92Ala D2 polymorphism had lower serum TSH compared with individuals with wild-type D2, and remarkably, also for homozygous individuals for Thr92Ala D2 polymorphism [2], [6]. It is in different with this study that TSH levels in heterozygous individuals are higher than homozygotes (Figure 2). Other studies have shown that thyroidectomy patients with Thr92Ala variant require higher T4 doses to achieve target TSH levels. However, Thr92Ala variant do not correlate with differences in general conditions, neurocognitive function, or response to combination T4/T3 therapy in patients treated for hypothyroidism. Further, research is needed with a larger number of samples so that it can strengthen the conclusions obtained.

Conclusion

The Thr92Ala gene in IDD endemic replete area population in Samigaluh Yogyakarta had

genotype frequencies of AA 16.79%, AG 41.22%, and GG 41.99%, with allele frequency A 37.5% and G 62.5%. Heterozygous genotypes AG carriers had higher TSH levels compared with homozygous alleles, but no significant relationship was found between polymorphism D2 A/G with TSH and thyroid hormones level.

Declarations

Ethics approval and consent for participate

Ethics approval and informed consent were required in our study. The research protocol was approved by the Research Ethical Committee of the National Institute Research and Development, Indonesia Ministry of Health. The research also conformed to the ethical guidelines of the Declaration of Helsinki, as revised in 2013. A written informed consent was obtained from all participants before enrollment in the study.

Availability of Data and Material

The data and material will be available with the corresponding author, on reasonable request.

Authors' Contributions

RAW and SNW: Idea/concept by RAW, SNW. Design was contributed by RAW TH, SNW, Supervision and controlling data. Data collection/processing by RAW, TH, and SNW. Analysis/interpretation was done by RAW, SNW, and TH. Revision of the article was done by RAW, TH, and SNW. All authors have read and approved the final manuscript.

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