Journal of the Endocrine Society, 2021, Vol. 5, No. 4, 1–6
doi:10.1210/jendso/bvab012
Case Report

ENDOCRINE SOCIETY

OXFORD

Case Report

Familial Dysalbuminemic Hyperthyroxinemia as a Cause for Discordant Thyroid Function Tests

Matthew J. M. Ting,[1] Rui Zhang,[2] Ee Mun Lim,[1,2] Bryan K. Ward,[1,3] Scott G. Wilson,[1,4,5] and John P. Walsh[1,6]

[1]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Australia; [2]PathWest Laboratory Medicine, Nedlands, Australia; [3]Harry Perkins Institute of Medical Research, Centre for Medical Research, QEII Medical Centre, University of Western Australia, Nedlands, Australia; [4]School of Biomedical Sciences, University of Western Australia, Crawley, Australia; [5]Department of Twin Research and Genetic Epidemiology, King's College London, London, UK; and [6]Medical School, University of Western Australia, Crawley, Australia

ORCiD numbers: 0000-0003-2505-9423 (M. J. M. Ting); 0000-0001-9251-2107 (B. K. Ward); 0000-0002-1766-2612 (J. P. Walsh).

Received: 10 November 2020; Editorial Decision: 26 January 2021; First Published Online: 1 February 2021; Corrected and Typeset: 4 March 2021.

Abstract

Introduction: Discordant thyroid function tests are routinely encountered in clinical practice. Differential diagnoses include acute thyroxine (T4) ingestion, laboratory interference from heterophilic antibodies, thyroid hormone resistance, thyroid-stimulating hormone (TSH)-secreting pituitary adenomas, and T4 protein binding abnormalities. The impact of abnormal binding proteins may be less recognized since widespread use of free T4 (FT4) assays compared to older total T4 assays.

Case report: A 69-year-old female was referred for assessment of discordant thyroid function tests. Biochemistry since July 2015 showed persistently elevated FT4 levels by immunoassay ranging between 25 to 34 pmol/L with normal or slightly decreased TSH ranging between 0.05 to 2.74 mU/L. The patient was clinically euthyroid on 100 mcg daily of levothyroxine for Hashimoto's thyroiditis. FT4 measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was 19.5 pmol/L. Exome sequencing (confirmed by Sanger sequencing) detected a guanine to adenine substitution at residue 725 of the *ALB* gene previously associated with dysalbuminemic hyperthyroxinemia. The patient's daughter had similar thyroid function tests and the same genetic variant. FT4 results from 3 different automated immunoassays showed the Roche Cobas and Siemens Centaur platforms to be most affected by the variant, and Abbott Architect had the best agreement with LC-MS/MS.

Conclusion: Familial dysalbuminemic hyperthyroxinemia is a potential cause of discordant thyroid function tests. Clinicians suspecting protein-binding abnormalities may further investigate using reference methods such as LC-MS/MS and equilibrium dialysis

ISSN 2472-1972

© The Author(s) 2021. Published by Oxford University Press on behalf of the Endocrine Society.
This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (http://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

if available. The increasing accessibility of exome sequencing offers a cost-effective method of diagnosing genetic variants that cause discordant thyroid function tests.

Key Words: euthyroid hyperthyroxinemia, thyroxine, albumin

Thyroxine (T4) is the predominant circulating thyroid hormone and is solely released by the thyroid gland. A majority of T4 circulates in the periphery bound by 3 carrier proteins: T4-binding globulin (TBG), transthyretin (TTR), and albumin (ALB). TBG is responsible for transporting approximately 70% of circulating T4 whereas TTR and ALB bind 20% and 10% of T4, respectively [1]. However, it is the free fraction of T4 that is available for peripheral tissue uptake and regulation of negative feedback of the hypothalamic-pituitary-thyroid axis. Genetic variations in TBG, TTR, and ALB can change their binding affinity for T4 and therefore affect total T4 levels, causing euthyroid hyperthyroxinemia. This typically results in discordant patterns of thyroid function tests where T4 levels are raised and thyroid-stimulating hormone (TSH) is unsuppressed.

Early tests used to quantify circulating thyroid hormone employed total T4 assays, which were heavily influenced by changes in T4-binding proteins. When these methods were in routine use, euthyroid hyperthyroxinemia was commonly encountered such as in pregnancy and use of exogenous estrogens in the oral contraceptive pill [2]. Although these problems are encountered less frequently since the widespread use of free T4 (FT4) assays, protein binding abnormalities should not be overlooked as a cause of discordant thyroid function tests. Variations in T4 protein binding can produce spuriously elevated FT4 levels on immunoassay, and it is important to recognize them as differential diagnoses when faced with euthyroid hyperthyroxinemia. Familial dysalbuminemic hyperthyroxinemia (FDH) is an autosomal dominantly inherited condition resulting in increased binding affinity of T4 to ALB and raised total T4 levels first described in 1979 [3,4]. We report a mother and daughter with FDH due to a genetic mutation in the ALB gene causing raised FT4 readings.

Case Report

A 69-year-old woman was referred to the Thyroid Clinic with discordant thyroid function tests. FT4 levels dating back four years showed high levels between 25 to 34 pmol/L with unsuppressed TSH levels either within, or slightly below, the reference range (Table 1). The patient described some tiredness and anxiety due to social stressors but denied any other symptoms of thyrotoxicosis. Pertinent past medical history consisted of primary hypothyroidism secondary to Hashimoto's thyroiditis, which was diagnosed in her 30s on the basis of raised TSH levels of 9.5 mU/L with positive thyroperoxidase antibodies. In addition, she had dyslipidemia, premature atrial contractions, and previous breast cancer, which is in remission. The patient reported to take 100 mcg of levothyroxine a day with good adherence, magnesium supplementation, and glucosamine. Previously, attempts at reducing levothyroxine dosage resulted in symptoms of hypothyroidism. There was a strong family history of hypothyroidism affecting her mother, both sisters, brother, and one daughter. Interestingly, her daughter was also reported to have similar abnormalities in her thyroid function tests, showing high FT4 levels with unsuppressed TSH. On examination, the patient was clinically euthyroid and weighed 58.4 kg at a height of 148.8 cm (body mass index = 26.4 kg/m^2). There was a mild, long-standing fine tremor in both hands, but no goiter or signs of orbitopathy were present.

Incubation of the patient's serum with heterophilic blocking tubes was performed to exclude heterophile antibodies as a cause of assay interference. For genetic testing, DNA was extracted from whole blood using the QIAamp DNA blood midi kit (Qiagen, Venlo, Limburg, Netherlands) for exome sequencing or the High Pure PCR Template Preparation kit, version: 2.3 for Sanger sequencing. Exome capture was performed using Agilent SureSelect XT Human All Exon v6 with subsequent sequencing (100 bp pair-end) on Illumina HiSeq2500; further processing of genetic data was as described previously [5] except that only genes relevant to thyroid function were considered in the analysis. Exome sequencing failed to detect any variants in the thyroid hormone receptor gene making thyroid hormone resistance less likely. Similarly, no variants were found in *TBG* and *TTR* genes, but an *ALB* gene variant (rs75002628) associated with FDH was detected (NM_000477; exon7; hg38: c.G725A:p.R242H). Bioinformatics analysis predicted the variant to be disease causing and further analysis using the Genetic Variant Interpretation Tool based on American College of Medical Genetics standards and guidelines [6] classified the variant as Pathogenic (II). Confirmatory Sanger sequencing was performed on the polymerase chain reaction (PCR) product generated from the patient's DNA using previously described intronic primers that amplify the entire exon 7 of the *ALB* gene containing the G725A variant [7]. PCR amplification was performed using Go

Table 1. Thyroid function tests for patient from 2015 to 2019

Test	1/23/15	7/8/15	12/14/15	4/20/16	10/21/16	11/18/17[a]	2/21/17[a]	8/29/17[a]	2/21/18	5/1/18	10/12/18	9/23/19
TSH (mU/L)	2.32	2.74	0.10	0.30	0.69	0.17	0.05	0.20	0.48	1.63	0.68	0.25
FT4 (pmol/L)	28	34	25	26	28	33	33	26	22	27	24	18
FT3 (pmol/L)	3.8	4.1	6.0	5.5		4.7			4.3			4.5
TPO Ab (IU/ml)	85.5		49.3		50.2	53					58.9	
TG Ab (IU/ml)	56.1		38.4		27.8						18.4	
TRAB (U/L)						<0.3						

Roche Cobas reference ranges: TSH, 0.5-5.5 mU/L; FT4, 11.0-21.0 pmol/L; FT3, 3.1-6.0 pmol/L; TPO Ab, <34 IU/mL; TG Ab: <115 IU/mL. Abbot Architect reference ranges: TSH, 0.40-4.00 mU/L; FT4, 9-19 pmol/L; FT3, 3.0-5.5 pmol/L; TPO Ab, <6 kU/L; TRAB: <1.8 U/L. Tests performed on the Roche Cobas Immunoassay unless otherwise noted.

Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; TG Ab, thyroglobulin antibodies; TPO Ab, thyroperoxidase antibodies; TRAB, TSH receptor antibodies; TSH, thyroid stimulating hormone.

[a]Performed on Abbot Architect.

Taq Flexi DNA polymerase (Promega Corp, Madison, WI, USA) in a reaction volume of 25 µL containing 1x Go Taq Flexi buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each primer, 1.0 U polymerase and 250 to 450 ng template DNA with cycling conditions as follows: 95°C/5 min, 35 cycles of 94°C/20 sec, 58°C/20 sec, 72°C/90 sec, and then 72°C/5 min. Bidirectional sequencing was performed at the Australian Genome Research Facility as described previously [5]. Examination of the sequencing chromatogram (Fig. 1A) confirmed the heterozygous presence of the variant. The patient's daughter subsequently underwent genetic testing by Sanger sequencing with the same variant being detected (Fig. 1B).

Both the patient and her daughter then had blood samples drawn for TSH, FT4, and free triiodothyronine (FT3) analysis using 3 different automated immunoassay platforms as well as using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The immunoassays were of chemiluminescence subtype on the Architect analyzer (Abbott Diagnostics, Australia) and Centaur (Siemens, Australia), and electrochemiluminescence on Cobas e602 (Roche Diagnostics, Australia). Information on the antibodies utilized in the immunoassays can be obtained from the Antibody Registry: Abbott Architect—TSH [8], FT4 [9], and FT3 [10]; Siemens Centaur—TSH [11], FT4 [12], and FT3 [13]; Roche Cobas—TSH [14], FT4 [15], FT3 [16]. The LC-MS/MS FT4 and FT3 assay was a custom developed assay using the ThermoFisher Scientific rapid equilibrium dialysis) plate (product no. 15036) to extract FT4 and FT3 from plasma serum samples. The rapid equilibrium dialysis plate is composed of disposable high-density polypropylene membrane inserts to which plasma serum samples were added. Buffer solution containing sodium phosphate and sodium chloride was added to the chamber surrounding the membrane inserts. The equilibrium dialysis was carried out in a temperature controlled mixer set at 100 rpm. The mixer was operated at 37°C with the process lasting 4 h. At the end of equilibrium dialysis, buffer solution was collected and analyzed by LC-MS/MS. The LC-MS/MS analysis of FT4 and FT3 was carried out on a Waters Xevo TQ-S tandem mass spectrometer coupled with a Waters Acquity® H-class UPLC system with quaternary pumping capability. An Acquity® analytical column (C18 100 × 2.1 mm, 1.7 µ) was used for the separation of the analytes with a 6-min UPLC gradient at a flowrate of 0.3 mL/min. Mobile phase A and B consisted of HPLC grade water containing 0.1% formic acid and 100% methanol, respectively. Multiple reaction monitoring mode was used for the analysis.

All investigations were performed as part of routine clinical care with the patients' consent. As such, institutional ethics committee approval was not required.

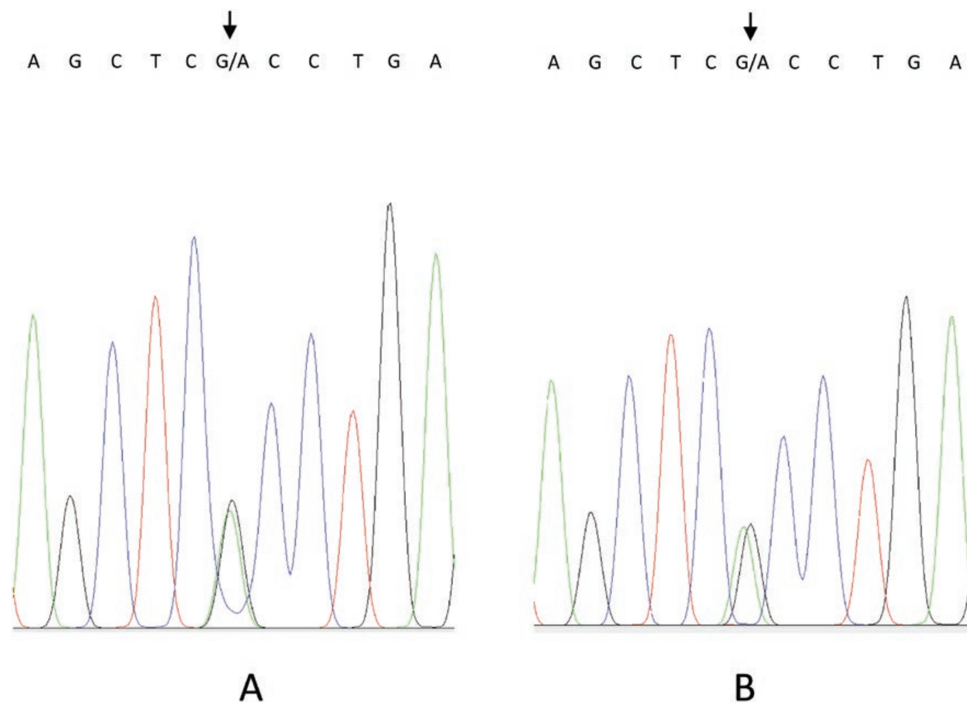

Figure 1. Sanger sequencing chromatograms showing the heterozygous G725A variant (arrowed) in exon 7 of the *ALB* gene of the mother (A) and her daughter (B) presenting with features consistent with dysalbuminemic hyperthyroxinemia. The sequence variant was confirmed in both cases with the reverse primer; only the forward primer sequences are presented.

The results of the previously described thyroid function tests are detailed in Table 2. FT4 levels measured using LC-MS/MS showed more concordant, high-normal values. Comparison of different immunoassay platforms revealed the Abbot Architect to most approximate LC-MS/MS results as opposed to Roche Cobas and Siemens Centaur platforms.

Discussion

This case report describes a mother and daughter with FDH caused by a variant in the *ALB* gene (NM_000477; exon7; hg38: c.G725A:p.R242H), which has been previously reported (as R218H; amino acid numbering excluding the signal peptides) [7]. In this condition, the ALB variant has greater affinity for T4 compared with wild-type ALB thereby increasing total T4 levels, but free hormone levels as measured by equilibrium dialysis remain unchanged and patients are clinically euthyroid [3]. Affected individuals have normal serum levels of ALB [17], which binds T4 more avidly as a result of reduced steric occlusion of T4 binding sites. This is due to the amino acid substitution causing a smaller side chain of the domain 2 ligand binding site [18]. FT4 levels in these individuals measured by immunoassay are artefactually

Table 2. Comparison of different thyroid function test platforms for patient and daughter

Laboratory platform	Patient	Daughter
Immunoassay: Abbot Architect		
TSH (mU/L)	0.74	3.60
FT4 (pmol/L)	22	19
FT3 (pmol/L)	3.4	3.8
Immunoassay: Roche Cobas		
TSH (mU/L)	1.41	4.40
FT4 (pmol/L)	34	30
FT3 (pmol/L)	3.89	3.73
Immunoassay: Siemens Centaur		
TSH (mU/L)	1.13	4.26
FT4 (pmol/L)	30	25
FT3 (pmol/L)	4.8	4.4
LC-MS/MS		
TSH (mU/L)	—	—
FT4 (pmol/L)	19.5	19.0
FT3 (pmol/L)	2.5	1.6

Abbot Architect reference ranges: TSH, 0.40-4.00 mU/L; FT4, 9-19 pmol/L; FT3, 3.0-5.5 pmol/L. Roche reference ranges: TSH, 0.5-5.5 mU/L; FT4, 11.0-21.0 pmol/L; FT3, 3.1-6.0 pmol/L. Siemens Centaur reference ranges: TSH, 0.4-4.0 mU/L; FT4, 10-20 pmol/L; FT3, 3.5-6.5 pmol/L. Reference ranges for the LC-MS/MS assay have not been developed locally.

Abbreviations: FT3 = free triiodothyronine; FT4 = free thyroxine; LC-MS/MS = liquid chromatography-tandem mass spectrometry; TSH = thyroid-stimulating hormone.

elevated, but they have normal TSH and FT3 levels and responses to thyrotropin-releasing hormone are unchanged.

Although current FT4 immunoassays theoretically measure free rather than bound T4, some are still affected by FDH to varying degrees. T4 analogues used in current immunoassays also bind more avidly to variant ALB found in FDH, causing falsely raised FT4 readings [19]. The addition of a wash step to remove binding proteins prior to incubating the T4 analogue used in 2-step immunoassays reduces the effect of protein binding abnormalities [20]. These differences are highlighted in our report showing measurements using the Abbot Architect (2-step) platform were more accurate compared to the Roche Cobas and Siemens Centaur (1-step) platforms. These results are consistent with those of a recent report that FDH caused by the R242H variant (R218H if the signal peptides are excluded) also interferes with FT4 measurement by immunoassay, although the use of chloride rich incubation buffers can result in higher FT4 readings in 2-step assays as well [21].

The prevalence of FDH has been reported to be between 0.01% to 1.8% depending on the population [20]. Because screening for thyroid dysfunction is usually done by measuring TSH levels alone, cases of FDH may remain undetected if FT4 levels are never measured, and reported prevalence may be underestimated. Once identified, individuals with FDH should be counseled on the condition to avoid unnecessary treatment for hyperthyroidism as the condition is benign and does not require intervention. Similarly, screening of family members should be undertaken for the same purposes. Additionally, patients with concurrent primary hypothyroidism should have levothyroxine dosage titrated based on TSH levels rather than FT4 to avoid undertreatment.

Discordant thyroid function tests are not uncommon in routine clinical practice. In addition to protein binding abnormalities, other causes of raised FT4 with unsuppressed TSH include acute T4 ingestion, medications that inhibit T4 to T3 conversion such as amiodarone, glucocorticoids, and beta-blockers; heterophilic antibody interference; thyroid hormone resistance; and TSH-secreting pituitary adenomas [1]. Measurement of FT3 levels can help differentiate between some potential causes—FT3 levels are usually low when medications reduce T4 to T3 conversion. It is important to consider protein binding abnormalities as a potential cause that can allow the avoidance of unnecessary testing such as thyrotropin-releasing hormone stimulation tests or pituitary magnetic resonance imaging. When suspected, further investigation with reference methods such as equilibrium dialysis or LC-MS/MS can be helpful in obtaining accurate FT4 levels.

Our report demonstrates the utility of exome sequencing in identifying individuals with suspected variants in protein binding. This method allows for concurrent exclusion of thyroid hormone resistance by testing for variants in the thyroid hormone receptor beta gene. As exome sequencing becomes more readily available and the cost continues to fall, it should be considered as a cost-effective, noninvasive method of investigating discordant thyroid function tests when protein binding abnormalities or thyroid hormone resistance are considered.

Summary

Discordant thyroid function tests are commonly encountered in routine practice. Although modern FT4 assays have reduced the impact of fluctuations in T4 binding proteins on thyroid function tests, protein binding abnormalities such as FDH can affect FT4 assays and should not be overlooked. Identifying patients with this condition is important to avoid unnecessary investigations and treatments for TSH-dependent hyperthyroidism. Two-step FT4 immunoassays are less affected by protein binding abnormalities compared to their 1-step counterparts. As exome sequencing becomes more widely available, it may be possible to use it as an effective method of evaluating genetic variations that cause discordant thyroid function tests.

Additional Information

Correspondence: Matthew J. M. Ting, Department of Endocrinology and Diabetes, Level 1, C block, Queen Elizabeth II Medical Centre, 6 Verdun Street, Nedlands, Western Australia 6009, Australia. Email: matthew.ting@health.wa.gov.au.

Disclosure Summary: The authors have nothing to disclose.

Data Availability: Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in references.

References

1. Salvatore D, Cohen R, Kopp PA, Larsen PR. Thyroid pathophysiology and diagnostic evaluation. In: Melmed S, Auchus RJ, Goldfine AB, Koenig RJ, Rosen CJ, eds. *Williams Textbook of Endocrinology*. 14th ed. Elsevier; 2020:332-363.
2. Ain KB, Mori Y, Refetoff S. Reduced clearance rate of thyroxine-binding globulin (TBG) with increased sialylation: a mechanism for estrogen-induced elevation of serum TBG concentration. *J Clin Endocrinol Metab*. 1987;**65**(4):689-696.
3. Lee WN, Golden MP, Van Herle AJ, Lippe BM, Kaplan SA. Inherited abnormal thyroid hormone-binding protein causing selective increase of total serum thyroxine. *J Clin Endocrinol Metab*. 1979;**49**(2):292-299.
4. Hennemann G, Krenning EP, Otten M, Docter R, Bos G, Visser TJ. Raised total thyroxine and free thyroxine index but normal free thyroxine. *Lancet*. 1979;**313**(8117):639-642.

5. Magno AL, Leatherbarrow KM, Brown SJ, Wilson SG, Walsh JP, Ward BK. Functional analysis of calcium-sensing receptor variants identified in families provisionally diagnosed with familial hypocalciuric hypercalcaemia. *Calcif Tissue Int*. 2020;**107**(3):230-239.
6. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;**17**(5):405-424.
7. Petersen CE, Scottolini AG, Cody LR, Mandel M, Reimer N, Bhagavan NV. A point mutation in the human serum albumin gene results in familial dysalbuminaemic hyperthyroxinaemia. *J Med Genet*. 1994;**31**(5):355-359.
8. RRID:AB_2883972.
9. RRID:AB_2801665.
10. RRID:AB_2885163.
11. RRID:AB_2827386.
12. RRID:AB_2827385.
13. RRID:AB_2783802.
14. RRID:AB_2756377.
15. RRID:AB_2756378.
16. RRID:AB_2827368.
17. Stockigt JR, Topliss DJ, Barlow JW, White EL, Hurley DM, Taft P. Familial euthyroid thyroxine excess: an appropriate response to abnormal thyroxine binding associated with albumin. *J Clin Endocrinol Metab*. 1981;**53**(2):353-359.
18. Ryan JB, Brennan SO, Potter H, Wolmarans L, Florkowski CM, George PM. Familial dysalbuminaemic hyperthyroxinaemia: a rapid and novel mass spectrometry approach to diagnosis. *Ann Clin Biochem*. 2016;**53**(Pt 4):504-507.
19. Stockigt JR, de Garis M, Csicsmann J, Barlow JW, White EL, Hurley DM. Limitations of a new free thyroxine assay. *Clin Endocrinol*. 1981;**15**(3):313-318.
20. Favresse J, Burlacu MC, Maiter D, Gruson D. Interferences with thyroid function immunoassays: clinical implications and detection algorithm. *Endocr Rev*. 2018;**39**(5):830-850.
21. Khoo S, Lyons G, McGowan A, et al. Familial dysalbuminaemic hyperthyroxinaemia interferes with current free thyroid hormone immunoassay methods. *Eur J Endocrinol*. 2020;**182**(6):533-538.