Utility of systematic TSHR gene testing in adults with hyperthyroidism lacking overt autoimmunity and diffuse uptake on thyroid scintigraphy

Kashyap A. Patel1,2 | Bridget Knight1,3 | Aftab Aziz4 | Tarig Babiker4 | Avades Tamar2 | Joanna Findlay3 | Sue Cox | Ioannis Dimitropoulos4 | Carolyn Tysoe6 | Vijay Panicker7 | Bijay Vaidya1,2

1The Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK
2Department of Diabetes and Endocrinology, The Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
3NIHR Clinical Research Facility, R&D Department, The Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
4Department of Diabetes and Endocrinology, University Hospitals Plymouth NHS Trust, Plymouth, UK
5Department of Diabetes and Endocrinology, Torbay and South Devon, NHS Foundation Trust, Torbay, UK
6Department of Molecular Genetics, The Royal Devon and Exeter NHS Foundation Trust, Exeter, UK
7Department of Endocrinology, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia

Correspondence
Kashyap A. Patel, The Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter, UK.
Email: k.a.patel@exeter.ac.uk

Funding information
This study was funded by Royal Devon and Exeter Hospital Small Grant Scheme and Society for Endocrinology Early Career Grant awarded to KAP. KAP has a postdoctoral fellowship funded by the Wellcome Trust (Grant Number 110082/Z/15/Z).

Summary
Objective: Patients with hyperthyroidism lacking autoimmune features but showing diffuse uptake on thyroid scintigram can have either Graves’ disease or germline activating TSH receptor (TSHR) mutation. It is important to identify patients with activating TSHR mutation due to treatment implication, but the overlapping clinical features with Graves’ disease make it difficult to discriminate these two conditions without genetic testing. Our study aimed to assess the potential of systematic TSHR mutation screening in adults with hyperthyroidism, showing diffuse uptake on thyroid scintigraphy but absence of TSH receptor antibodies (TRAb) and clinical signs of autoimmunity.

Design: A cross-sectional study of Caucasian adults with hyperthyroidism, managed at three endocrine centres in the South West, UK, from January 2006 to April 2017.

Methods: We recruited 78 adult Caucasian patients with hyperthyroidism showing diffuse uptake on 99mTc-pertechnetate thyroid scintigraphy but without TRAb and other autoimmune clinical features of Graves’ disease (such as thyroid-associated ophthalmopathy or dermopathy). Genomic DNA of these patients was analysed for variants in the TSHR gene.

Results: Genetic analysis identified 11 patients with four variants in TSHR [p.(Glu34Lys), p.(Asp36His), p.(Pro52Thr) and p.(Ile334Thr)]. None of these variants were pathogenic according to the American College of Medical Genetics and Genomics guideline.

Conclusions: Activating TSHR mutations are a rare cause of nonautoimmune adult hyperthyroidism. Our study does not support the routine genetic testing in adult patients with hyperthyroidism showing diffuse uptake on scintigraphy but negative TRAb and lacking extrathyroidal manifestations of Graves’ disease.

KEYWORDS
Graves’ disease, hyperthyroidism, thyroid scintigraphy, TRAb, TSHR
Activating TSH receptor (TSHR) mutations are a rare cause of nonautoimmune hyperthyroidism, although the true prevalence in adults is unknown.1-3 The majority of these cases are familial, with autosomal dominant patterns of inheritance, but mutation can also arise de novo.1,2,4 Identification of these patients has important clinical implications. Hyperthyroidism due to activating TSHR mutations does not remit following antithyroid drug treatment, and patients usually require total thyroidectomy or radioiodine ablation as a definitive treatment.1,2 A genetic diagnosis at the time of first presentation could, potentially, prevent unnecessary and prolonged treatment with antithyroid drugs, as well as reducing the associated morbidity and cost. This would also enable screening immediate family members, leading to earlier identification and treatment of undiagnosed hyperthyroidism.

It is difficult to identify patients with hyperthyroidism due to activating TSHR mutation, as their clinical features show significant overlap with Graves’ disease. Similar to Graves’ disease, presentation is highly variable, from subclinical hyperthyroidism to severe hyperthyroidism,1,2,5 with ages at presentation ranging from the neonatal period up to 60 years,1,6 and has a diffuse uptake on thyroid scintigraphy.2 A family history of thyrotoxicosis is not always present in patients with activating TSHR mutation due to variable penetrance and seen in 30% of patients with Graves’ disease1; thus, it is not very useful in discriminating these two aetiologies. Contrary to Graves’ disease, patients with an activating TSHR mutation do not have TSHR antibodies (TRAb) or extrathyroidal autoimmune clinical manifestations such as thyroid-associated ophthalmopathy and dermopathy.1,2 However, around 3%-5% patients with Graves’ disease also do not have TRAb when analysed with the second- or third-generation assays,8,9 and only about 25% will have extrathyroidal manifestations, such as thyroid-associated ophthalmopathy.10 Altogether these data suggest that it is difficult to identify patients with activating TSHR mutation in routine clinical practice without systematic genetic testing. However, utility of this approach is not known. Therefore, we aim to assess the utility of systematic genetic testing for TSHR mutations in adults with hyperthyroidism showing diffuse uptake on thyroid scintigraphy but TRAb negative and lacking extrathyroidal manifestations of Graves’ disease.

2 | SUBJECTS AND METHODS

2.1 | Study participants

We recruited patients from three endocrine centres (Exeter, Plymouth and Torbay) in the South West, England (UK). The inclusion criteria were as follows: age at recruitment >18 years, biochemically confirmed overt or subclinical hyperthyroidism according to the American Thyroid Association guideline,11 absence of TRAb, diffuse uptake on the thyroid scintigraphy and Caucasian ethnicity. We excluded patients with any extrathyroidal manifestations of Graves’ disease, such as thyroid-associated ophthalmopathy or dermopathy, and patients with any other known cause of hyperthyroidism (eg, toxic nodular disease, thyroiditis and drug-induced thyrotoxicosis).

2.2 | Study recruitment

We identified 275 patients from the clinical records who had thyroid scintigraphy and had absence of TRAb from all three centres over 11 years (2006-2017). Local endocrinologists performed TRAb and thyroid scintigraphy at presentation as part of the routine clinical care. The research team reviewed all thyroid scintigraphy to identify patients with diffuse uptake. The clinical records were reviewed to assess the eligibility of these patients. Of the initial 275 potential participants identified, three had died, 41 had decreased or no uptake on scintigraphy, 98 had patchy/localized uptake, two had Graves’ ophthalmopathy, and four had drug-related thyrotoxicosis, and they were therefore excluded. All 127 patients who fulfilled the entry criteria were approached and invited to take part in the study, and 78/127 (61%) were recruited (49 refused or were unable to be contacted directly). We estimated the frequency of activating TSHR mutation in adults with hyperthyroidism lacking overt autoimmunity and diffuse uptake on thyroid scintigraphy to be ~4.5% based on two previous studies.12,13 This provided a sample size of 67 to find at least one individual with a mutation at P < 0.05.

2.3 | DNA and data collection

We collected one-off blood or saliva sample for DNA extraction. Demographics and other clinical features were collected from the clinical records. If an individual had multiple episodes of the thyrotoxicosis, the data for the episode during which thyroid scintigraphy and TRAb were performed were used in the study.

All three centres have been using a second-generation enzyme-linked immunoassay from EUROIMMUN AG, Germany, to measure TRAb throughout the study period. Patients with borderline or positive results were excluded from the study (>1.8 IU/L). Thyroid function tests were measured locally as part of routine care by each centre independently. Manufacturers’ reference ranges of TSH, free thyroxine (FT4) and free triiodothyronine (FT3) for the assays for each centre were used to confirm biochemical thyrotoxicosis. The nuclear medicine department of each centre performed thyroid scintigraphy according to their local protocol.

NRES Committee North East - Newcastle & North Tyneside, UK, gave ethical approval for the study (IRAS no 153183). All the study participants gave the informed written consent.

2.4 | Data analysis

Statistical analysis was conducted using Stata® 15.1 (StataCorp, College Station, TX, USA). The Mann-Whitney U test was used for comparing continuous variable, whereas Fisher’s exact test was used for comparing categorical variable.
2.5 DNA analysis

Genomic DNA was extracted from peripheral blood or salivary cells using standard procedures in the molecular genetics laboratory, Royal Devon and Exeter Hospital, Exeter, UK. The entire coding region and intron-exon boundaries of all 10 exons of the TSHR gene were amplified by polymerase chain reaction and Sanger-sequenced using BigDye Terminator v3.1 cycle sequencing. Sequences were compared to the reference sequence (NM_000369.2) using Mutation Surveyor software (SoftGenetics, State College, PA, USA). The changes in sequence were assessed and reported according to the American College of Medical Genetics and Genomics (ACMG) guideline.14

3 RESULTS

3.1 Study cohort characteristics

The clinical characteristics of the study cohort are shown in Table 1. The median age at diagnosis of hyperthyroidism was 52 years, 76% were female, and 42% had a palpable diffuse goitre. None had thyroid-associated ophthalmopathy, dermopathy or TRAb, all had diffuse uptake on thyroid scintigraphy, and 35% had a family history of thyrotoxicosis.

| Clinical characteristics | Whole cohort N = 78 | Patients without TSHR variant, n = 67 | Patients with benign TSHR variant, n = 11 | P       |
|--------------------------|---------------------|----------------------------------------|-----------------------------------------|---------|
| Age at diagnosis (y)     | 52.4 (43.8-69.5)     | 51.6 (42.4-67.9)                       | 60.6 (51.9-73.1)                        | 0.13    |
| Female (%)               | 59 (76)             | 51 (76)                                | 8 (73)                                 | 1       |
| Duration since the presenta- | 2.7 (1-4.8)         | 2.8 (1-4.7)                           | 2.6 (1-6.5)                            | 0.81    |
| tion with thyrotoxicosis at the time of recruitment (y)  |                     |                                       |                                         |         |
| Hyperthyroidism (%)      |                     |                                       |                                         |         |
| Overt hyperthyroidism    | 75 (95)             | 64 (96)                                | 11 (100)                               | 1       |
| Subclinical hyperthyroidism | 3 (5)               | 3 (4)                                  | 0                                      |         |
| Presence of goitre (%)   | 33 (42)             | 25 (37)                                | 8 (73)                                 | 0.05    |
| Family history of hyperthyroidism (%) | 27 (35) | 22 (33) | 5 (45) | 0.5    |
| Thyroid-associated ophthalmopathy or dermopathy (%) | 0 | 0 | 0 |         |
| Presence of TSH receptor antibodies (%) | 0 | 0 | 0 |         |
| Diffuse uptake on thyroid scintigraphy (%) | 78 (100) | 67 (100) | 11(100) |         |
| Treatment of hyperthyroidism |                     |                                       |                                         |         |
| Antithyroid drug          | 61 (78)             | 51 (76)                                | 10 (91)                                | 0.76    |
| Radioiodine ablationa     | 15 (19)             | 14 (21)                                | 1 (9)                                  |         |
| Total thyroidectomya      | 2 (3)               | 2 (3)                                  | 0                                      |         |

Median (IQR) for age at diagnosis and duration. N (%) for the others. P-value is for comparison between patients with or without TSH receptor (TSHR) variant.

3.2 Analysis of TSHR variants

Genetic analysis identified 11 patients with four variants in TSHR (Table 2). None of these variants were novel or pathogenic according to the ACMG guideline.14 p.(Glu34Lys), p.(Asp36His) and p.(Pro52Thr) were definitely benign according to the ACMG guideline. The p.(Glu34Lys) variant was seen in an individual with congenital hypothyroidism and the probands’ mother with Graves’ disease (Table 2). Both p.(Glu34Lys) and p.(Asp36His) had in vitro evidence of normal or low activity.16,17 These data together suggest that these are not activating pathogenic variants.

3.3 Analysis of TSHR variant p.(Ile334Thr)

p.(Ile334Thr) variant was not reported in the literature. It is classified as a likely benign variant according to the ACMG guideline.14 The variant is in the extracellular domain outside the mutation hot
Based on the frequency of this variant in gnomAD, for this variant to be pathogenic, monogenic disease would have to be seen in one in 5000 population, which is far more common than the estimated prevalence of hyperthyroidism due to activating TSHR mutations (~<1:15 000 children in Denmark). None of the previously published pathogenic activating TSHR mutations are seen in gnomAD. Furthermore, the proband with this variant in our study was diagnosed at 60 years of age and had no family history of thyrotoxicosis. She also remitted after 18 months of antithyroid drug treatment which is unlikely if she had activating TSHR gene mutation.

### TABLE 2

| TSHR variants | No. of patients | Exon | Location of variant | Phenotype previously reported in literature | No. and freq. of heterozygotes in gnomAD, all ethnicity | No. and freq. of heterozygotes in gnomAD, European (non-Finnish) | No. of homozygotes in gnomAD | In vitro activity of variant in literature | ACMG classification of variants |
|---------------|-----------------|------|---------------------|--------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------|-----------------------------|------------------------------------------|----------------------------------|
| c.100G>A, p.(Glu34Lys) (rs45499704) | 1 | 1 | Extracellular | Low [19] | 60/137 988 (0.043%) | 41/62 412 (0.065%) | 12 | - | Low (19) |
| c.106G>C, p.(Asp36His) (rs61747482) | 2 | 1 | Extracellular | No impact [18] | 1439/137 892 (1.04%) | 1146/62 646 (1.83%) | - | - | Benign |
| c.154C>A, p.(Pro52Thr) (rs2234919) | 7 | 1 | Extracellular | Graves’ disease with ophthalmopathy [16, 17] | 15 958/137 484 (11.6%) | 7092/62 256 (11.3%) | 1071 | - | Benign |
| c.1001T>C, p.(Ile334Thr) (rs553893026) | 10 | 10 | Extracellular | - | 5/123 129 (0.004%) | 2/55 855 (0.003%) | 0 | - | Benign |

**DISCUSSION**

Our study identified that the systematic assessment for activating TSHR mutations in adult patients with hyperthyroidism with diffuse uptake on scintigraphy, negative TRAb and absence of extrathyroidal manifestations of Graves’ disease (such as thyroid-associated ophthalmopathy or dermopathy) did not identify a single case. Our study, therefore, is unable to support the routine genetic testing for TSHR mutations in these patients.

This is the largest study in adult Caucasian patients with hyperthyroidism with diffuse goitre and lacking overt autoimmunity, and we did not find any individuals with a pathogenic variant in the TSHR gene. Our study results differ from a previous study in adult Japanese hyperthyroid patients with diffuse goitre and without TRAb (n = 89). This study found activating TSHR mutations in four patients, giving a prevalence of 4.5%. This discrepancy in the results may be due to different populations characteristics; for example, our study participants were older, median age 52 vs 40 years. The high frequency in the Japanese population may be due to founder effects as seen in other monogenic diseases in this population. The negative results in our study may also be due to a type II error (false negative), although we were powered to detect a 4.5% frequency with 95% confidence. This suggests that the accurate assessment of prevalence of activating TSHR mutation in adult Caucasian patients may need a larger sample size. For example, Nishihara et al found only 89 patients with diffuse goitre and lacking overt autoimmunity from 24 623 hyperthyroid patients referred to them over 9 years.

Our results suggest that routine screening for TSHR gene may not be a useful practice in adult Caucasian patients with hyperthyroidism lacking overt autoimmunity and diffuse goitre due to the rarity of the disease. The European Thyroid Association (ETA) have previously issued recommendations on when and whom to select for genetic testing for TSHR. One-third of our cohort had a family history of thyrotoxicosis, and fulfilled the ETA criteria for genetic testing for TSHR mutation. Our results do not support routine genetic testing in adult onset hyperthyroidism even in the presence of family history of thyrotoxicosis; however, it may be useful for families with multigenerational history of thyrotoxicosis.

Our results are not applicable to children, and these patients should
be evaluated for activating TSHR mutation as per the guideline.\textsuperscript{3} This is supported by the study in Danish children that showed 6% of children with nonautoimmune hyperthyroidism have activating TSHR mutation.\textsuperscript{12}

We acknowledge several study limitations: we only recruited 61% patients from the eligible cohort, and a larger sample size would have increased the power to identify a lower frequency of activating TSHR mutation. We did not have data on Doppler thyroid ultrasound or thyroid-stimulating immunoglobulin to exclude Graves’ disease. Although these two tests would have reduced the number of eligible patients, it would not have changed the results of our study due to the inclusive nature of our study eligibility criteria. We excluded patients with multinodular goitre in our study as it is seen in <15% of individual with activating TSHR mutation. This along with relatively common occurrence of non-genetic multinodular goitre in adult population would have provided very low positive predictive value of multinodular goitre to identify TSHR mutation.\textsuperscript{2} We also did not take the treatment history of patients (e.g., relapse after antithyroid drug treatment) into consideration when selecting potential participants as we wanted to establish the utility of genetic testing at the time of diagnosis during routine clinical care.

In conclusion, our systematic assessments of adult Caucasian patients with hyperthyroidism showed that activating TSHR gene mutations are a rare cause of nonautoimmune hyperthyroidism. Our results do not support the routine genetic testing for adult patients with hyperthyroidism showing diffuse uptake on scintigraphy but lacking autoimmune features of Graves’ disease.

ACKNOWLEDGEMENTS

We would like to acknowledge Bradley Harman and Rebecca Ward for undertaking Sanger sequencing.

CONFLICT OF INTEREST

The authors report no conflict of interests that could be perceived as prejudicing the impartiality of the research reported.

AUTHORS’ CONTRIBUTIONS

KAP and BV designed the study; wrote the first draft of the manuscript, which was modified by all authors; and are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. KAP, BK VP researched data. KAP, BK, AA, TB, AT, SC, ID helped in data collection and study recruitment. CT did the genetic analysis. All authors contributed to the discussion and reviewed or edited the manuscript.

REFERENCES

1. Gozu HI, Lublinghoff J, Bircan R, Paschke R. Genetics and phenomics of inherited and sporadic non-autoimmune hyperthyroidism. Mol Cell Endocrinol. 2010;322(1–2):125-134.
2. Hebrant A, van Staveren WC, Maenhaut C, Dumont JE, Leclere J. Genetic hyperthyroidism: hyperthyroidism due to activating TSHR mutations. Eur J Endocrinol. 2011;164(1):1-9.
3. Paschke R, Niedziela M, Vaidya B, Persani L, Rapoport B, Leclere J. 2012 European thyroid association guidelines for the management of familial and persistent sporadic non-autoimmune hyperthyroidism caused by thyroid-stimulating hormone receptor germline mutations. Eur Thyroid J. 2012;1(3):142-147.
4. Watkins MG, Dejkhamron P, Huo J, Vazquez DM, Menon RK. Persistent neonatal thyrotoxicosis in a neonate secondary to a rare thyroid-stimulating hormone receptor activating mutation: case report and literature review. Endocr Pract. 2008;14(4):479-483.
5. Duprez L, Parma J, Van Sande J, et al. Germline mutations in the thyrotopin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. Nat Genet. 1994;7(3):396-401.
6. Karges B, Krause G, Homoki J, Depati KM, de Roux N, Karges W. TSH receptor mutation V509A causes familial hyperthyroidism by release of interhelical constraints between transmembrane helices TMH3 and TMH5. J Endocrinol. 2005;186(2):377-385.
7. Manji N, Carr-Smith JD, Boelaert K, et al. Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype. J Clin Endocrinol Metab. 2006;91(12):4873-4880.
8. Tozzoli R, Bagnasco M, Giavarina D, Bizzaro N. TSH receptor autoantibody immunoassay in patients with Graves’ disease: improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis. Autoimmun Rev. 2012;12(2):107-113.
9. Vos XG, Smit N, Endert E, Tijssen JG, Wiersinga WM. Frequency and characteristics of TBII-seronegative patients in a population with untreated Graves’ hyperthyroidism: a prospective study. Clin Endocrinol (Oxf). 2008;69(2):311-317.
10. Tanda ML, Plantanida E, Liparulo L, et al. Prevalence and natural history of Graves’ orbitopathy in a large series of patients with newly diagnosed Graves’ hyperthyroidism seen at a single center. J Clin Endocrinol Metab. 2013;98(4):1443-1449.
11. Ross DS, Burch HB, Cooper DS, et al. 2016 American thyroid association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid. 2016;26(10):1343-1421.
12. Lavard L, Jacobsen BB, Perrild H, Vassart G, Parma J. Prevalence of germline mutations in the TSH4 receptor gene as a cause of juvenile thyrotoxicosis. Acta Paediatr. 2004;93(9):1192-1194.
13. Nishihara E, Fukata S, Hishinuma A, Amino N, Miyauchi A. Prevalence of thyrotropin receptor germline mutations and clinical courses in 89 hyperthyroid patients with diffuse goiter and negative anti-thyrotropin receptor antibodies. Thyroid. 2014;24(5):789-795.
14. Richards S, Aziz N, Bale S, et al. 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.
15. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-291.
16. Bahn RS, Dutton CM, Heufelder AE, Sarkar G. A genomic point mutation in the extracellular domain of the thyrotropin receptor in patients with Graves' ophthalmopathy. *J Clin Endocrinol Metab*. 1994;78(2):256-260.

17. Bohr UR, Behr M, Loos U. A heritable point mutation in an extracellular domain of the TSH receptor involved in the interaction with Graves’ immunoglobulins. *Biochim Biophys Acta*. 1993;1216(3):504-508.

18. Gustavsson B, Eklof C, Westermark K, Westermark B, Heldin NE. Functional analysis of a variant of the thyrotropin receptor gene in a family with Graves' disease. *Mol Cell Endocrinol*. 1995;111(2):167-173.

19. Lado-Abeal J, Castro-Piedras I, Palos-Paz F, Labarta-Aizpun JI, Albero-Gamboa R. A family with congenital hypothyroidism caused by a combination of loss-of-function mutations in the thyrotropin receptor and adenylate cyclase-stimulating G alpha-protein subunit genes. *Thyroid*. 2011;21(2):103-109.

20. Whiffin N, Minikel E, Walsh R, et al. Using high-resolution variant frequencies to empower clinical genome interpretation. *Genet Med*. 2017;19(10):1151-1158.

21. Saga M, Mashima Y, Kudoh J, Oguchi Y, Shimizu N. Gene analysis and evaluation of the single founder effect in Japanese patients with Oguchi disease. *Jpn J Ophthalmol*. 2004;48(4):350-352.

How to cite this article: Patel KA, Knight B, Aziz A, et al. Utility of systematic TSHR gene testing in adults with hyperthyroidism lacking overt autoimmunity and diffuse uptake on thyroid scintigraphy. *Clin Endocrinol (Oxf)*. 2019;90:328-333. [https://doi.org/10.1111/cen.13892](https://doi.org/10.1111/cen.13892)