# Cardiovascular morbidity and mortality in patients with Resistance to Thyroid Hormone β (RTHβ): a linked record study

Okosieme OE[1,2], Usman D[1], Taylor PN[1], Dayan CM[1], Lyons G[3], Moran C[4, 5, 6], Chatterjee K[3], Rees DA[7]

Prof Onyebuchi E Okosieme, MD

Dr Danyal Usman, MBBCh

Dr Peter N Taylor, PhD

Prof Colin M Dayan, PhD

Greta Lyons, RGN

Dr Carla Moran, FRCPI

Prof Krishna Chatterjee, FRCP

Prof Dafydd Aled Rees, PhD

[1]Thyroid Research Group, Systems Immunity Research Institute, Cardiff University School of Medicine, UK

[2]Diabetes and Endocrinology Department, Prince Charles Hospital, Cwm Taf Morgannwg Health Board, Merthyr Tydfil, UK

[3]Wellcome Trust-MRC Institute of Medical Science, University of Cambridge, UK

[4]Endocrine Section, Beacon Hospital, Dublin, Ireland

[5]Endocrine Department, St Vincent's University Hospital, Dublin, Ireland

[6]School of Medicine, University College Dublin

[7]Neuroscience and Mental Health Innovation Institute, Cardiff University, UK

**Short title:** Morbidity and mortality in Resistance to Thyroid Hormone β

**Corresponding author:** Onyebuchi Okosieme, Thyroid Research Group, Systems Immunity Research Institute, Cardiff University, Heath Park, Cardiff CF14 4XW, United Kingdom.

**Telephone:** 00441685728353

**Email:** OkosiemeOE@Cardiff.ac.uk

**Keywords:** Resistance to thyroid hormone; Hyperthyroidism, Hypothyroidism, Cardiovascular disease; Mortality; Atrial Fibrillation, Heart Failure

Word count: 3829
Abstract word count: 277
Number of tables: 3
Number of figures: 3
Number of supplementary tables: 6
Number of supplementary figures: 2

## ABSTRACT

**Background:** Individuals with Resistance to Thyroid Hormone due to mutations in the thyroid hormone receptor-β gene (RTHβ) exhibit impaired tissue sensitivity to thyroid hormones but retain sensitivity in cardiac tissue. Long-term health and survival outcomes in this rare disorder have not been evaluated. We investigated all-cause mortality and cardiovascular event risk in an RTHβ patient cohort, followed-up in Welsh endocrine clinics.

**Methods:** In a retrospective cohort design, genetically confirmed RTHβ patients (n=55) with age- and sex-matched population controls (n=2750), were linked to outcomes in datasets within the Welsh Secure Anonymised Information Linkage (SAIL) Databank. Kaplan-Meier and Cox regression models analysed associations of RTHβ with all-cause mortality and cardiovascular events.

**Findings:** Compared to controls, patients exhibited increased risks for all-cause mortality (Hazard ratio [HR] 2·84, 95% Confidence Interval [95%CI] 1·59–5·08), atrial fibrillation (HR 10·56, 95%CI 4·72–23·63), heart failure (HR 6·35, 95%CI 2·26–17·86), and major adverse cardiovascular events (MACE) comprising cardiovascular death, acute myocardial infarction, heart failure, or strokes (HR 3·49, 95%CI 2·04–5·99). The median age of first occurrence of any adverse event was 11 years earlier in patients (56 yrs, 95%CI 44, 65) compared to controls (67 yrs, 95%CI 65, 70). Cubic spline analyses showed positive associations between FT4 concentrations at diagnosis and mortality or MACE, with FT4>30 pmol/L conferring increased risk. Compared to no intervention, treatment with antithyroid drugs, surgical/radioiodine gland ablation or thyroxine did not control thyroid hormone excess.

**Interpretation:** We have documented reduced survival and increased cardiovascular morbidity in an RTHβ cohort for the first time. These outcomes may be driven by lifelong cardiac exposure to thyroid hormone excess, and effective therapies, targeting hormone resistant pathways, may potentially curtail this risk.

## RESEARCH IN CONTEXT

### Evidence before the study

Resistance to Thyroid Hormone-β (RTHβ) is a rare genetic disorder in which hormone resistance in the pituitary-thyroid axis results in elevated, circulating thyroid hormones. Although a subset of affected individuals exhibit features of hyperthyroidism, whether the condition increases long-term risks of cardiovascular disease or death is unknown. We searched PubMed, without date or language restrictions, for articles assessing cardiovascular morbidity or mortality outcomes in this disorder. We used the search terms ("Resistance to Thyroid hormone" OR "Thyroid Hormone Resistance") AND ("mortality" OR "survival" OR "Cardiovascular" OR "Atrial fibrillation" OR "Heart Failure" OR "Myocardial Infarction" OR "Stroke"). Most studies were case reports or small case series which reported cardiovascular manifestations in individual patients. We found no previous study evaluating cardiovascular risk or mortality in this disorder.

### Added value of this study

This study represents the first assessment of cardiovascular morbidity and mortality outcomes in an RTHβ patient cohort. Using a linked health record approach, we have documented reduced survival and increased cardiovascular risk in RTHβ patients in Wales over a 22-year period. Compared to the background population, RTHβ patients exhibited a three-fold increase in all-cause mortality or major cardiovascular events, including excess risks of atrial fibrillation and heart failure. In addition, we observed positive associations between thyroid hormone (FT4) levels at diagnosis and subsequent cardiovascular morbidity or death, suggesting that lifelong exposure to elevated thyroid hormones mediates the excess morbidity and mortality in RTHβ. Furthermore, we observed that conventional treatments for hyperthyroidism (i.e., antithyroid drugs, radioiodine ablation of the thyroid gland, or thyroidectomy) did not control thyroid hormone levels effectively.

### Implications of all the available evidence

Individuals with RTHβ exhibit a significant burden of excess cardiovascular disease that is not mitigated by current therapies. Our findings suggest that aggressive management of cardiovascular risk in RTHβ patients is indicated. Clinical trials, addressing an unmet need for effective therapies that target hormone resistant pathways, are warranted in this disorder.

## INTRODUCTION

Resistance to Thyroid Hormone β (RTHβ) is a dominantly-inherited disorder due to mutations in the thyroid hormone receptor β (TRβ) gene. Affected individuals exhibit impaired sensitivity of target tissues to thyroid hormones, with reduced intracellular action of the biologically active hormone triiodothyronine (T3) (1, 2). The mutant receptor also exerts dominant-negative effects in the hypothalamic-pituitary-thyroid axis, thus disrupting feedback regulation and giving rise to the biochemical hallmark of the condition, i.e., elevated circulating thyroid hormones, free thyroxine (FT4) and free triiodothyronine (FT3), with non-suppressed thyroid stimulating hormone (TSH) (2). RTHβ is estimated to occur in about 1-2 per 40,000 live births and affects both sexes equally (1). To date, more than 3000 cases, involving over 1000 families harbouring 240 different TRβ mutations, have been recorded (2-4). The clinical phenotype is variable, ranging from asymptomatic cases to patients exhibiting clinical and metabolic features of hyperthyroidism or hypothyroidism (5). Some patients also display cardiovascular manifestations of thyrotoxicosis, such as tachycardia, heart failure (5, 6), or myocardial dysfunction (7-9). In addition, rare patients with homozygous RTHβ can develop life-threatening and sometimes fatal cardiomyopathy (6, 10).

Despite these recognised cardiovascular manifestations, long-term outcome data is lacking, and it is unknown whether RTHβ is associated with excess cardiovascular morbidity and mortality risk. Patients with hyperthyroidism due to intrinsic thyroid gland disease such as Graves' disease or toxic nodular goitre have an increased risk of cardiovascular death (11), an outcome that is driven by thyroid hormone excess (12-14) and prevented by controlling biochemical hyperthyroidism (12). Current treatment strategies for RTHβ are inconsistent and range from no treatment at all, to controlling

autonomic symptoms with beta blockade, limiting circulating thyroid hormone excess with antithyroid drugs, or ablating the thyroid gland with radioiodine or surgery followed by Levothyroxine replacement (6). However, while these therapies have proven efficacy in conventional hyperthyroidism, their effectiveness in controlling thyroid hormone levels in individuals with RTHβ is unproven. Here, using linked health records in a genetically-confirmed cohort of RTHβ patients in Wales, we have compared cardiovascular morbidity and mortality in patients to that of a background population and examined the relationship between thyroid hormone levels and health outcomes.

## METHODS

*Patients and controls*

The study is a retrospective cohort study using linked datasets within the Secure Anonymised Information Linkage (SAIL) Databank (Swansea University, Swansea, UK) (15). We identified records of all RTHβ patients in Wales, without age exclusion, in whom this disorder was suspected and then diagnosed in research or clinical genetic laboratories at Addenbrookes Hospital, Cambridge, UK between January 1997 and December 2019. Patients in whom RTHβ was suspected clinically but not confirmed by genetic testing, were excluded. Demographic, clinical, and laboratory records of patients with a confirmed diagnosis of RTHβ were collected from routine hospital records using a questionnaire. Age and sex-matched population controls for RTHβ patients were obtained from the background population in the Welsh Demographic Service and were matched to patients on birth-year (+/- 1 year) and sex within SAIL at a ratio of 50 controls per patient using a greedy matching algorithm with random matching without replacement (16). A matching ratio of 50:1 was chosen to optimise statistical power based on our power calculation which showed benefits to study power of increasing control to patient ratios, as has previously been shown in

power calculations of data with limited number of cases (17). Power considerations in the study design are detailed in page 3 of the supplementary appendix and in supplementary figure 1.

*Data linkage*

SAIL is a privacy-protecting database of routinely collected health data run by the Population Data Science Group at Swansea University, Wales (15). The databank contains over two billion anonymised, person-based records, including approximately 3.1 million individuals resident in Wales and registered with a GP practice. SAIL is linked to other health and social care datasets including the Patient Episode Database for Wales (PEDW), the Office for National Statistics (ONS), and the Primary Care database (PCD) (18). In SAIL, records with a valid NHS number are linked deterministically following which unmatched records are subjected to probabilistic linkage using surname, forename, date of birth and postcode (15). Data anonymity is ensured through multi-party encryption together with a split-file data management procedure (15). For the study, clinical data (genetic, laboratory, and treatment) of the RTHβ cohort was imported into the SAIL databank, de-identified with an encrypted identification number, and then matched to controls within SAIL. Patients and controls were then linked to the study outcomes using the same datasets within SAIL. Mortality data was obtained from the ONS which contains individual records for all death registrations in England and Wales, including an underlying cause of death. Data on comorbidity and cardiovascular events were obtained from the PEDW and PCD datasets, comprising coverage of all attendance in participating general practices (80% of practices) as well as all inpatient admission episodes in Welsh NHS hospitals. These datasets include information on diagnoses, treatment, and surgical procedures.

*Outcomes*

All-cause mortality was the primary outcome, with secondary outcomes being hospital admissions with acute myocardial infarction, atrial fibrillation, heart failure, stroke or major adverse cardiovascular events (MACE) – a composite of cardiovascular death, acute myocardial infarction, heart failure and stroke. Cardiovascular death was any death attributed to acute myocardial infarction, heart failure, strokes, arrhythmias or sudden cardiac death. .

*Follow-up definitions*

For the main survival analysis we compared mortality and cardiovascular outcomes in patients versus controls using age as timescale to account for variability in disease detection times. Both patients and controls entered the study from 1st January 1997, or from their date of birth if this was after January 1997. The start date of January 1997 was chosen to correspond with the availability of complete hospital admission records in PEDW. Patients and controls were followed up till outcome event, emigration from Wales, or end of study period in April 2021. In a separate analysis we compared outcomes according to baseline characteristics including thyroid hormone levels at the time of RTHβ diagnosis. This analysis was confined to the RTHβ patient group alone and follow up time was from the date of genetic diagnosis of RTHβ until outcome event, emigration from Wales, or end of study period in April 2021.

*Data analysis*

Baseline data was summarised as means (standard deviation, SD) for normally distributed values or median (interquartile range, IQR) for data that is not normally distributed. Baseline differences between groups were analysed with chi-squared test, 2-tailed t-test, Mann-Whitney tests, or Kruskal Wallis tests as appropriate. We compared event rates in RTHβ patients versus age- and sex-matched controls using

Kaplan-Meier survival curves and the log rank test for significance. . Cox regression models, adjusted for sex and for baseline age, were also used to determine hazard ratios for mortality and cardiovascular outcomes. In sensitivity analyses, survival analyses was repeated using the Fine and Gray competing-risk sub-hazard model with non-cardiac death as a competing risk event (19).

In the second analysis confined to the RTHβ patient group alone, we used a Cox regression model to ascertain event risk in relation to baseline characteristics at the time of diagnosis, namely age, sex, comorbidity and circulating thyroid function measurements (FT4,FT3,TSH). Univariate and multivariate Cox-regression models were developed comprising age, comorbidity scores, and thyroid function tests as continuous variables, while sex was handled as a dichotomous variable (male vs. female). Comorbidity scores were derived using a modification of the Charlson Comorbidity Index (20) (supplementary table 1, appendix). FT4 and FT3 were analysed in separate models due to collinearity. Missing thyroid function data (FT4 ≤5%, TSH ≤5%, FT3 10·9%) was addressed using a complete case analysis and in sensitivity analysis we also analysed missing data using the method of multiple variable imputation by chained equations (details described in the appendix, page 4). The validity of the proportional hazard assumption was tested by Schoenfeld residuals test. To account for minor differences in laboratory reference ranges across laboratory sites and over time-periods of tests, the regression models for thyroid function were corrected for treatment centre and year of laboratory test, and additional analyses were conducted using multiples of the upper-reference limit for individual assays. Assay reference ranges are described in the appendix (supplementary table 2). The year of diagnosis was also evaluated to determine if changes in cardiovascular risk management strategies over time had any effect on outcomes.

Restricted cubic splines were used to model a possible non-linear relationship between baseline thyroid hormone concentrations and health outcomes. Four equally spaced knots were set at the 5th, 35th, 65th, and 95th percentiles according to Harrell's recommendation (21) (model described in the appendix, page 3-4). In addition, the relationship between thyroid hormone levels and age was examined using quantile mixed-effects models with baseline age and sex as fixed effects and participants as random effects (22). β-coefficients and 95% confidence intervals were derived from the mixed models to represent the change in thyroid hormone level per year of age. Lastly, we examined any potential impact of treatment on thyroid hormone levels using multiple linear regression models, with follow-up thyroid hormone levels (last available outpatient test) as the dependent variable and treatment modality, age, sex and baseline thyroid hormone levels as independent variables. Statistical analysis was performed via remote desktop with the SAIL Databank using Stata version 17·0 for Windows (Stata Corp., College Station, TXS, USA).

*Ethics and Governance*

Approval for access to anonymised linked data in the SAIL databank was granted by an independent Information Governance Review panel (SAIL-IGRP reference no: 0954). All proposals to use SAIL data are subject to review by the panel which comprises representatives from various public and health sectors including the National Research Ethics Service, Public Health Wales, and NHS Wales Informatics Service. Approval for clinical data collection for the RTH cohort was obtained from the Cardiff and Vale University Health Board Clinical Audit Department (reference no: 9392). Genetic investigations for the RTHβ mutation were undertaken with prior, informed written consent at the clinical genetics laboratory at Addenbrookes Hospital

in Cambridge, either for clinical indications or as part of ethically approved research protocols (Cambridgeshire LREC 98/154).

## RESULTS

*Cohort characteristics*

Sixty-one patients, referred from Welsh hospital clinics, in whom a genetic diagnosis of RTHβ had been made in research or clinical genetic laboratories at Addenbrookes Hospital, Cambridge were identified. Of these, 55 patients were registered in the SAIL databank, the remainder either lacking sufficient linkage details or not being registered in the databank (supplementary figure 2). Patients (n=55) and age and sex matched controls (n= 2750) were linked to outcomes using the same datasets in the databank and the characteristics of the cohort and controls (n=2750) are presented in Table 1. The majority of patients were female (60%), with a median age of 29 years at the beginning of study follow-up, median age of 43 years at the time of genetic diagnosis, and most patients being between age 40 and 60 years at the end of follow-up. Patients with RTHβ exhibited more significant comorbidities at the time of genetic diagnosis; as expected, median, circulating thyroid hormones (FT4, FT3) were elevated with non-suppressed TSH levels at baseline. A proportion of patients were treated with antithyroid drugs (13%), levothyroxine (24%) or radioiodine/thyroidectomy (11%), including patients who received multiple treatments concurrently or sequentially.

*Thyroid hormone receptor β mutations*

Sixteen different heterozygous TRβ mutations, localising to its hormone binding domain, were identified. Amino acid codons most frequently mutated were Arg243 (n=6), Arg338 (n=6), Arg383 (n=15), and Arg438 (n=8). Most mutations mapped to three, previously established, hotspots or clusters in the TRβ hormone binding domain, namely cluster 1 (A268D, A268G, R243W, R243Q), cluster 2 (A317T, D351G,

239 R333W, R320C, R338P, R338W), and cluster 3 (I431M, P453T, R438C, R438H). In
240 addition, several patients harboured mutations (n=7, R383C; n=8, R383H) involving a
241 hotspot residue outside the three clusters.

242 *Mortality and cardiovascular events in patients and controls*

243 All-cause mortality and cardiovascular event outcomes are presented in survival
244 curves (Figure 1). Overall, there were 320 deaths, with greater mortality in RTHβ
245 patients (n=12, 22%) than in controls (n=308, 11%). In addition, cardiovascular events
246 (MACE) were more frequent in RTHβ patients (n=14, 25%) than in controls (n=252,
247 9%). Kaplan Meier curves showed increased risk for all-cause mortality, MACE, atrial
248 fibrillation, and heart failure, but not for acute myocardial infarction or stroke, in RTHβ.
249 Median age of any first adverse event including death was 56 yrs (95%CI 44, 65) in
250 RTHβ patients compared to 67 yrs (95%CI 65, 70) in controls, reflecting an 11-year
251 difference between patients and controls. Median survival times could not be
252 calculated for individual outcomes since less than half of patients or controls
253 experienced outcomes by the end of follow-up. Increased mortality and cardiovascular
254 hazards remained significant in the adjusted Cox regression model (Figure 1) as well
255 as in the competing risk regression model that incorporated non-cardiac death
256 (supplementary table 3). Cox regression models stratified by sex showed no
257 interaction of outcomes with sex apart from a marginally increased hazard for atrial
258 fibrillation in men compared to women (P-value for interaction, 0·048) (supplementary
259 table 4).

260 *Baseline characteristics, mortality and cardiovascular events*

261 We examined associations between baseline characteristics (age at diagnosis, sex,
262 comorbidity score, TRβ mutation cluster, year of diagnosis, FT4, FT3, TSH) and all-
263 cause mortality and MACE in the RTHβ cohort, using Cox regression models. Survival

time denotes the period from diagnosis to event or censor. Older age at diagnosis and increased comorbidity score were associated with increased mortality and adverse cardiovascular events, whereas no association was seen with sex, TRβ mutation cluster type, or year of diagnosis (Table 2). FT4 concentration at baseline was associated with modest increases in mortality and MACE risk, both in an unadjusted model and in models adjusted for age, sex, baseline comorbidity, laboratory centre, and diagnosis year (Table 2). Hazard ratios for baseline FT3 concentration were also increased but did not reach statistical significance. No association between baseline TSH and outcomes was observed (Table 2). Due to small numbers in subgroups, other variables including treatment modality in subsets of the RTHβ cohort, were not examined further. The results remained unchanged when thyroid hormones were converted to multiples of the upper reference limit to account for assay differences (supplementary Table 5). In addition, hazard ratios for baseline thyroid hormones were also unchanged in the sensitivity analysis using multiple imputation (supplementary table 6 in the appendix).

Using a model with four, equally spaced, cubic splines, we explored a non-linear relationship between baseline FT4 concentration and mortality or MACE. We observed a positive, non-linear relationship between FT4 and both outcomes, with modest increases in risk with FT4 >30pmol/L (Figure 2). We also examined the relationship between age and thyroid hormones using mixed quantile mixed-effects models, with age and sex as fixed effects and subject as a random effect. This showed a reduction in median FT4 and FT3 but not TSH levels with age, with decline being most marked between childhood (0-19 years) and early adult life (20-39 years) and continuing into older (>60 yrs) age (Supplementary Figure 2).

Lastly, we examined the association between thyroid hormone and TSH concentrations and treatment modalities (antithyroid drugs, radioactive iodine, surgery, or levothyroxine) (Table 3). Patients commenced on Levothyroxine had higher TSH and lower FT4 concentrations at baseline than untreated patients. However, a multiple linear regression model showed that treatment modality did not influence subsequent thyroid hormone levels. This model, which included age, sex, baseline thyroid hormones and TSH, and treatment modality as independent variables, with thyroid hormones and TSH at follow-up as dependent variable, showed no difference in subsequent FT4, FT3, or TSH concentrations between treated and untreated patients (Table 3).

## DISCUSSION

Using a linked health record approach, we have shown an increased risk of cardiovascular morbidity and mortality in an RTHβ patient cohort in Wales. Compared to an age and sex-matched background population, RTHβ patients exhibited three-fold increased risk of all-cause mortality or major cardiovascular events, including excess risks for atrial fibrillation and heart failure. These risks were most marked in older individuals and in individuals with pre-existing comorbidities at the time of diagnosis. In addition, associations between FT4 concentrations at diagnosis and subsequent cardiovascular morbidity or death were observed, with a 7% increased risk of mortality per pmol/L rise in FT4. The relationship between FT4 and outcomes was non-linear, with FT4 concentration >30 pmol/L conferring increased risk. Our observations suggest that lifelong exposure to the high, circulating thyroid hormones characteristic of this disorder, mediates the excess mortality seen in our RTHβ cohort.

For the first time in an RTHβ patient cohort, we have documented survival and cardiovascular outcomes using a unique dataset captured over many years with a

313 robust record linkage approach. As would be expected for a rare disease study of
314 lifetime outcomes, our sample size was small, precluding robust subgroup analysis on
315 the effects of TRβ mutation type or treatment modality on outcomes. An estimated
316 prevalence of 1 in 40,000 predicts 75 individuals with RTHβ in Wales, indicating that
317 we captured about 80% (n=61) of our potential RTHβ population. A higher estimated
318 prevalence (1 in 19,000) reported in a different study might suggest that some affected
319 individuals in our population were not ascertained (4). Nevertheless, our patient
320 sample was adequately powered to show significant differences for our primary
321 mortality and cardiovascular outcomes. Future studies in a larger RTHβ cohort may
322 discern further relationships in terms of genetic sub-types, clinical phenotypes
323 including mode of presentation, and the impact of treatment modality on outcomes.

324 Cardiovascular manifestations of thyroid hormone excess are well recognised in both
325 heterozygous and homozygous RTHβ patients (5, 6, 23). Kahaly *et al*, have previously
326 documented elevated heart rate, cardiac output, stroke volume, and systolic aortic flow
327 velocity in RTHβ patients, with cardiac indices showing positive correlations with
328 elevated circulating thyroid hormone (8). Occurrence of tachycardia, rhythm
329 disturbance (in up to 20% of cases) and impaired cardiac function in other RTHβ
330 cohorts, has been reported (24, 25). On the other hand, elevated levels of circulating
331 thyroid hormone with non-suppressed TSH or raised serum cholesterol and
332 triglyceride in RTHβ patients, signify the presence of hormone resistance and a
333 relative hypothyroid state in some target tissues or organs (26). Differential expression
334 of TRβ isoforms likely accounts, in part, for such variable sensitivity to thyroid
335 hormones in target tissues. Thus, inhibition of wild-type receptor action by dominant-
336 negative TRβ mutants mediates hormone resistance in TRβ1 (liver, kidney) or TRβ2

337 (pituitary, hypothalamus) expressing tissues. Conversely, TRα-expressing tissues
338 (e.g. heart) retain sensitivity to elevated, circulating thyroid hormones (3).

339 Consistent with the above notion, previous studies in RTHβ patients have shown
340 positive correlations between the degree of mutant TRβ dysfunction and raised
341 circulating thyroid hormones and elevated low-density-lipoprotein (LDL) cholesterol
342 (reflecting hormone resistance within the pituitary-thyroid axis or liver) (27) or elevation
343 of resting metabolic rate (reflecting actions of elevated thyroid hormones on TRα-
344 expressing skeletal muscle and possibly myocardium) (28). Thus, it is conceivable that
345 the excess cardiovascular risk in RTHβ we have observed in this study is mediated by
346 direct effects of elevated thyroid hormones on cardiac rhythm and contractility or via
347 increased atherosclerosis risk secondary to known dyslipidaemia and systemic insulin
348 resistance (28). However, given the strong risks for disordered cardiac rhythm (atrial
349 fibrillation) and contractility (heart failure) observed in our RTHβ cohort in this study,
350 we favour deleterious cardiac effects of lifelong elevation in thyroid hormones as the
351 dominant mechanism.

352 Our observations are also biologically plausible in the wider context of well-established
353 associations between thyroid dysfunction and cardiovascular disease (29). Thus,
354 reduced survival in our RTHβ cohort is comparable to that of patients with conventional
355 hyperthyroidism (11). Using the same data-linkage approach as this study, we have
356 shown previously that cardiovascular events and mortality are increased in patients
357 with Graves' disease, with early, effective control of hyperthyroidism improving survival
358 (12). Furthermore, in hyperthyroidism, hazard ratios (HRs) for cardiovascular events
359 and mortality were particularly high in patients who responded poorly to treatment,
360 comparable to the magnitudes of the HRs observed in our RTHβ cohort (12). As seen
361 with RTHβ patients in this study, an association between the degree of exposure to

hyperthyroidism and reduced survival has been documented in several cohorts with hyperthyroidism (12-14), including positive associations between FT4 and adverse outcomes (12). Overall, our observations indicate that adverse cardiovascular sequelae and reduced survival in our RTHβ patient cohort is comparable to that of uncontrolled, conventional hyperthyroidism.

Our findings have implications for the management of RTHβ patients. Current treatment strategies are inconsistent and largely pragmatic in approach. Definitive treatment with surgery or radioiodine is ineffective, with frequent recurrence of thyrotoxicosis after radioiodine or thyroidectomy, due to growth of remnant thyroid tissue (6). Post ablation, levothyroxine replacement in markedly supraphysiological dosage is required to normalise TSH levels, with failure to achieve this predisposing to pituitary enlargement due to thyrotroph hyperplasia (30). Conversely, supraphysiological hormone replacement, restoring elevated pre-ablation thyroid hormone concentrations, carries attendant cardiac risks. Antithyroid drug treatment also evokes exaggerated increases in TSH production, stimulating goitre formation and ongoing, excess thyroid hormone synthesis (6). In our study, intervention with antithyroid drugs, radioiodine/surgical ablation or levothyroxine, either alone or in combination, had no sustained impact on elevated, thyroid hormone concentrations. It is interesting that thyroid hormone levels remained high even in patients that had some form of intervention, and it is likely that both exogenous and endogenous thyroid hormones may play a role as discussed above. However, our analysis was limited to evaluations of baseline and final thyroid hormone levels and further detailed analyses that accounts for repeated measures will be useful to confirm these findings. Overall, there was a gradual decline in circulating FT3 and FT4 concentrations at an older age

(supplementary figure 2), with baseline thyroid hormone levels best predicting thyroid status at long-term follow-up, and various treatments having no discernible effect.

Accordingly, in RTHβ, we suggest that there is an unmet need for treatments targeting thyroid hormone resistant pathways, including exploring the therapeutic potential of thyroid hormone analogues (3, 31). TRIAC (3,3',5-triiodothyroacetic acid), a thyroid hormone analogue which exhibits greater affinity for TRβ than TRα, with preferential activation of some TRβ mutants, acts centrally to inhibit TSH secretion thereby lowering thyroid hormone levels, while devoid of peripheral thyromimetic activity (32, 33) (3). In individual RTHβ patients, TRIAC treatment in varying dosage and duration lowers circulating FT4 and FT3, reduces goitre size and improves hyperthyroid features, but its effect on health outcomes remains unknown (3, 34, 35). However, patients in our cohort were not treated with TRIAC and so we could not assess its efficacy on thyroid hormone levels.

Pending future clinical trials, using standardised protocols, to evaluate effectiveness of TRIAC in controlling adverse cardiac sequelae or altering outcomes in RTHβ, we suggest that regular monitoring of cardiac health (e.g., electrocardiogram, cardiac telemetry, echocardiography), with aggressive modification of other risk factors in patients with cardiac dysfunction, may be warranted. Future studies will be needed to explore the impact of other factors relevant to cardiovascular outcomes such as socio-economic class and ethnicity which were not captured in our dataset or incorporated in our matching algorithms. Ultimately, effective therapies that target hormone resistance within the pituitary-thyroid axis, thereby lowering and curtailing adverse cardiac effects of thyroid hormone excess, are needed in this disorder.

**Acknowledgements and funding information**

This work was funded by grants from the Royal College of Physicians (grant number 515842, DAR), a Wellcome Trust Investigator Award (210755/Z/18/Z, KC) and the NIHR Cambridge Biomedical Research Centre (KC, CM).

**Author contributions**

Aled Rees, Peter Taylor, Onyebuchi Okosieme, Carla Moran, and Krishna Chatterjee, conceived and designed the study. Onyebuchi Okosieme undertook the statistical analysis with contributions from Peter Taylor and Aled Rees. Onyebuchi Okosieme wrote the first draft of the manuscript with revisions from Aled Rees, Krishna Chatterjee, Carla Moran, Pete Taylor, Greta Lyons, Danyal Usman, and Colin Dayan. All authors contributed to the final draft of the manuscript and approved the manuscript before submission for publication. Onyebuchi Okosieme and Aled Rees had full access to the study data and vouch for the integrity and accuracy of the data analysis.

**Data sharing statement**

The data that support the findings of this study are not publicly available due to privacy and data disclosure restrictions.

**Declaration of interests**

CM is an independent contractor to Egetis therapeutics, providing clinical perspectives on MCT8 deficiency and other potential rare diseases. She has received speaking fees from Bahrain Diabetes & Endocrine Review Conference, 2022, Qatar Diabetes, Endocrinology and Metabolic conference, 2022, Indiana University, USA, 2022, Indonesian society of Endocrinology meeting, 2022, 1st Annual Arab Thyroid Association Congress, 2022, European College of Veterinary Internal Medicine Annual Congress, 2021, Thyroid Disease, MSc Clinical Chemistry program, Trinity College Dublin 2016 to present. She is Chair, Study Review Committee on TRIAC withdrawal study (ReTRIACt), an international trial assessing effect of TRIAC withdrawal in patients with MCT8 deficiency. She is an expert endocrine advisor, Health products regulatory authority (HPRA), Ireland. She serves as Society for Endocrinology Program Committee Member (2019-2023). She was Secretary/Assistant Secretary, British Thyroid Association (2017 – 2022). She is Lead, European Thyroid Association Taskforce on Guidelines for Interference in Immunoassays of Hormones used in Thyroid Function Tests (2023 to present). She is Member of Committee, European Thyroid Association Guideline on Diagnosis and Management of Syndromes of Resistance to Thyroid Hormone. She is Member, expert working group on rare thyroid disorders for the European Reference Networks (2018 to present). She was National convenor, Society for Endocrinology Thyroid Network (2015 to 2020).

CMD has received travel and accommodation fees for speaking at the symposium of the European Thyroid Association (Sept 2022).

PNT is a member of the Clinical committee of the Society for Endocrinology and committee member of the British Thyroid Association

## REFERENCES


1. Refetoff S, Bassett JH, Beck-Peccoz P, Bernal J, Brent G, Chatterjee K, et al. Classification and proposed nomenclature for inherited defects of thyroid hormone action, cell transport, and metabolism. J Clin Endocrinol Metab. 2014;99(3):768-70.

2. Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochimica et biophysica acta. 2013;1830(7):3987-4003.

3. Groeneweg S, Peeters RP, Visser TJ, Visser WE. Therapeutic applications of thyroid hormone analogues in resistance to thyroid hormone (RTH) syndromes. Molecular and cellular endocrinology. 2017;458:82-90.

4. Vela A, Pérez-Nanclares G, Ríos I, Rica I, Portillo N, Castaño L. Thyroid hormone resistance from newborns to adults: a Spanish experience. J Endocrinol Invest. 2019;42(8):941-9.

5. Persani L, Campi I. Syndromes of Resistance to Thyroid Hormone Action. Experientia supplementum (2012). 2019;111:55-84.

6. Moran C, Habeb AM, Kahaly GJ, Kampmann C, Hughes M, Marek J, et al. Homozygous Resistance to Thyroid Hormone β: Can Combined Antithyroid Drug and Triiodothyroacetic Acid Treatment Prevent Cardiac Failure? J Endocr Soc. 2017;1(9):1203-12.

7. Owen PJ, Chatterjee VK, John R, Halsall D, Lazarus JH. Augmentation index in resistance to thyroid hormone (RTH). Clin Endocrinol (Oxf). 2009;70(4):650-4.

8. Kahaly GJ, Matthews CH, Mohr-Kahaly S, Richards CA, Chatterjee VK. Cardiac involvement in thyroid hormone resistance. J Clin Endocrinol Metab. 2002;87(1):204-12.

9. Pulcrano M, Palmieri EA, Mannavola D, Ciulla M, Campi I, Covelli D, et al. Impact of resistance to thyroid hormone on the cardiovascular system in adults. J Clin Endocrinol Metab. 2009;94(8):2812-6.

10. Ferrara AM, Onigata K, Ercan O, Woodhead H, Weiss RE, Refetoff S. Homozygous thyroid hormone receptor β-gene mutations in resistance to thyroid hormone: three new cases and review of the literature. J Clin Endocrinol Metab. 2012;97(4):1328-36.

11. Brandt F, Green A, Hegedüs L, Brix TH. A critical review and meta-analysis of the association between overt hyperthyroidism and mortality. Eur J Endocrinol. 2011;165(4):491-7.

12. Okosieme OE, Taylor PN, Evans C, Thayer D, Chai A, Khan I, et al. Primary therapy of Graves' disease and cardiovascular morbidity and mortality: a linked-record cohort study. Lancet Diabetes Endocrinol. 2019;7(4):278-87.

13. Lillevang-Johansen M, Abrahamsen B, Jorgensen HL, Brix TH, Hegedus L. Excess Mortality in Treated and Untreated Hyperthyroidism Is Related to Cumulative Periods of Low Serum TSH. J Clin Endocrinol Metab. 2017;102(7):2301-9.

14. Boelaert K, Maisonneuve P, Torlinska B, Franklyn JA. Comparison of mortality in hyperthyroidism during periods of treatment with thionamides and after radioiodine. J Clin Endocrinol Metab. 2013;98(5):1869-82.

15. Lyons RA, Jones KH, John G, Brooks CJ, Verplancke JP, Ford DV, et al. The SAIL databank: linking multiple health and social care datasets. BMC Med Inform Decis Mak. 2009;9:3.

16. Rosenbaum PR. Optimal Matching for Observational Studies. Journal of the American Statistical Association. 1989;84(408):1024-32.

17. Hennessy S, Bilker WB, Berlin JA, Strom BL. Factors influencing the optimal control-to-case ratio in matched case-control studies. Am J Epidemiol. 1999;149(2):195-7.

18. Ford DV, Jones KH, Verplancke JP, Lyons RA, John G, Brown G, et al. The SAIL Databank: building a national architecture for e-health research and evaluation. BMC Health Serv Res. 2009;9:157.

19. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. Journal of the American Statistical Association. 1999;94(446):496-509.

20. Sundararajan V, Henderson T, Perry C, Muggivan A, Quan H, Ghali WA. New ICD-10 version of the Charlson comorbidity index predicted in-hospital mortality. Journal of clinical epidemiology. 2004;57(12):1288-94.

21. Harrell FE, Jr., Lee KL, Pollock BG. Regression models in clinical studies: determining relationships between predictors and response. Journal of the National Cancer Institute. 1988;80(15):1198-202.

22. Liu Y, Bottai M. Mixed-effects models for conditional quantiles with longitudinal data. The international journal of biostatistics. 2009;5(1):Article 28.

23. Ono S, Schwartz ID, Mueller OT, Root AW, Usala SJ, Bercu BB. Homozygosity for a dominant negative thyroid hormone receptor gene responsible for generalized resistance to thyroid hormone. J Clin Endocrinol Metab. 1991;73(5):990-4.

24. Brucker-Davis F, Skarulis MC, Grace MB, Benichou J, Hauser P, Wiggs E, et al. Genetic and clinical features of 42 kindreds with resistance to thyroid hormone. The National Institutes of Health Prospective Study. Ann Intern Med. 1995;123(8):572-83.

25. Illouz F, Briet C, Mirebeau-Prunier D, Bouhours-Nouet N, Coutant R, Sibilia P, et al. Cardiac complications of thyroid hormone resistance syndromes. Annales d'endocrinologie. 2021;82(3-4):167-9.

26. Beck-Peccoz P, Chatterjee VK. The variable clinical phenotype in thyroid hormone resistance syndrome. Thyroid. 1994;4(2):225-32.

27. Hayashi Y, Weiss RE, Sarne DH, Yen PM, Sunthornthepvarakul T, Marcocci C, et al. Do clinical manifestations of resistance to thyroid hormone correlate with the

functional alteration of the corresponding mutant thyroid hormone-beta receptors? J Clin Endocrinol Metab. 1995;80(11):3246-56.

28. Mitchell CS, Savage DB, Dufour S, Schoenmakers N, Murgatroyd P, Befroy D, et al. Resistance to thyroid hormone is associated with raised energy expenditure, muscle mitochondrial uncoupling, and hyperphagia. J Clin Invest. 2010;120(4):1345-54.

29. Biondi B, Kahaly GJ. Cardiovascular involvement in patients with different causes of hyperthyroidism. Nat Rev Endocrinol. 2010;6(8):431-43.

30. Gurnell M, Rajanayagam O, Barbar I, Jones MK, Chatterjee VK. Reversible pituitary enlargement in the syndrome of resistance to thyroid hormone. Thyroid. 1998;8(8):679-82.

31. Groeneweg S, Peeters RP, Visser TJ, Visser WE. Triiodothyroacetic acid in health and disease. J Endocrinol. 2017;234(2):R99-r121.

32. Messier N, Laflamme L, Hamann G, Langlois MF. In vitro effect of Triac on resistance to thyroid hormone receptor mutants: potential basis for therapy. Molecular and cellular endocrinology. 2001;174(1-2):59-69.

33. Takeda T, Suzuki S, Liu RT, DeGroot LJ. Triiodothyroacetic acid has unique potential for therapy of resistance to thyroid hormone. J Clin Endocrinol Metab. 1995;80(7):2033-40.

34. Persani L, Borgato S, Romoli R, Asteria C, Pizzocaro A, Beck-Peccoz P. Changes in the degree of sialylation of carbohydrate chains modify the biological properties of circulating thyrotropin isoforms in various physiological and pathological states. J Clin Endocrinol Metab. 1998;83(7):2486-92.

35. Faglia G, Beck-Peccoz P, Piscitelli G, Medri G. Inappropriate secretion of thyrotropin by the pituitary. Horm Res. 1987;26(1-4):79-99.

## Figures and Tables Legend

**Table 1:** Cohort characteristics

Legend: a. Some patients received treatment with multiple modalities either concurrently (e.g., Levothyroxine plus antithyroid drug treatment) or sequentially (e.g., RAI or thyroidectomy after antithyroid drug treatment, or Levothyroxine after RAI or thyroidectomy), b. Antithyroid drugs for more than 3 months' duration, c. RAI, radioactive iodine

**Table 2:** Mortality and MACE outcomes according to baseline characteristics at the time of RTHβ diagnosis

Legend: a, CHR, crude hazard ratio, b, AHR, adjusted hazard ratio. Adjusted models were corrected for age, sex, comorbidity, and diagnosis year. In addition, thyroid hormones were adjusted for laboratory site. c, Thyroid hormone measurements were available for 53 (FT4, TSH) and 49 (FT3) patients respectively. Nomenclature of mutation clusters corresponds to previously defined hotspots in the TRβ hormone binding domain (clusters 1-3) and an additional amino acid position (cluster 4), outside the established hotspots. Age and thyroid hormones were handled as continuous variables and HR are as per year of age and per pmol/L (FT4, FT3) and mU/L (TSH), respectively. d, Diagnosis year was divided into tertiles from the earliest to the most recent diagnosis.

**Table 3:** Baseline and Follow-up thyroid hormone levels by treatment modality

Legend: Treatment groups comprised patients who had: (1) no treatment, (2) antithyroid drugs alone, (3) RAI (radioactive iodine)/surgery, or (4) Levothyroxine alone. Thyroid hormones (median, interquartile range) are presented at baseline and at follow-up (last available outpatient test). *β-coefficient, 95% confidence intervals (95%CI) are derived from multiple linear regression models examining the impact of treatment group (independent variable) on follow-up thyroid hormone levels (dependent variable), with models adjusted for age, sex, and baseline thyroid hormone level. P values for trend denote differences across groups while the regression P value is derived from the linear regression model. a, Levothyroxine vs. No treatment or vs. RAI/Surgery, b, Levothyroxine vs. No treatment or vs. antithyroid drugs, c, Levothyroxine vs. no treatment.

**Figure 1:** Selection of study population

**Figure 2:** Kaplan Meier curves for mortality and cardiovascular events in patients with RTHβ.

Legend: Survival curves for all-cause mortality and cardiovascular events are plotted for patients with Resistance to Thyroid Hormone β (RTHβ) compared to age and sex-matched controls from the background population. Log-rank p values are presented for the difference in survival between patients and controls while Hazard ratios (HR), 95% confidence intervals are derived from Cox regression models corrected for sex. MACE, major adverse cardiovascular events. Risk tables are not presented due to privacy restrictions.

**Figure 3:** Cubic splines for baseline FT4 and outcomes in the RTH cohort

Legend: Curves represent association between baseline FT4 and log hazard ratios (HR, line) with 95% confidence intervals (95% CI, shaded areas) for the risk of all cause mortality (panel A) or major adverse cardiovascular events (panel B). Curves were derived from restricted cubic splines with 4 equally spaced knots at the 5th, 35th, 65th, and 95th percentiles. Hazard ratios were based on a Cox regression model corrected for sex, baseline comorbidity, treatment with antithyroid drugs, Levothyroxine, and thyroidectomy.

**Supplementary Table 1:** Modified Charlson Comorbidity Scores

**Supplementary Table 2:** Thyroid hormone assay reference ranges

For all assays the coefficient of variation was <10%; CCIA, competitive chemiluminescent immunoassay, CMIA, chemiluminescent microparticle immunoassay; ECLIA, electrochemiluminescence immunoassay; MEIA, Microparticle Enzyme Immunoassay; 1. Bayer Diagnostics, Newbury, UK; 2. Abbott Diagnostics, Maidenhead, Berks, UK; 3. Diagnostics Product Corporation, Llanberis, Wales; 4. Roche Diagnostics GmbH, Mannheim, Germany, *from January to December 2013 the reference range for this assay was changed to 9·2—21·0 pmol/L, **from September 2009 to April 2011, reference range for this assay was 0·3-6·0 mU/L

**Supplementary Table 3:** Sub-hazard ratios for cardiovascular events stratified by sex incorporating non-cardiac death as competing risk

Sub hazard ratios (HR) and 95% Confidence intervals (95%CI) were derived from Competing risk regression models according to the Fine and Gray sub-distribution hazard model with non-cardiac death as a competing risk. MACE, major adverse cardiovascular events, P-int, P value for the interaction with sex. Numbers (%) are the number of events (%) in the group, S, small cells (count < 5) are suppressed due to privacy restrictions.

**Supplementary Table 4:** Supplementary Table 3: Hazard ratios for mortality and cardiovascular events stratified by sex

Hazard ratios (HR) and 95% Confidence intervals (95%CI) were derived from Cox regression models. MACE, major adverse cardiovascular events, P-int, P value for the interaction with sex. Numbers (%) are the number of events (%) in the group, S, small cells (count ≤ 5) are suppressed due to privacy restrictions.

**Supplementary Table 5:** Mortality and MACE outcomes in RTHβ by baseline characteristics with thyroid hormones analysed as multiples of the upper reference limits

a, CHR, crude hazard ratio, b, AHR, adjusted hazard ratio. Adjusted models were corrected for age, sex, comorbidity, and diagnosis year. In addition, thyroid hormones were adjusted for laboratory site. c, Thyroid hormones were available for 53 (FT4, TSH) and 49 (FT3) patients respectively. Nomenclature of mutation clusters corresponds to previously defined hotspots in the TRβ hormone binding domain (clusters 1-3) and an additional amino acid position (cluster 4), outside the established hotspots. Age and thyroid hormones were treated as continuous variables and HR are as per year of age and per pmol/L (FT4, FT3) and mU/L (TSH), respectively. d, Diagnosis year was divided into tertiles from the earliest to the most recent diagnosis.

**Supplementary Table 6:** Mortality and MACE outcomes in RTHβ depending on baseline thyroid function at the time of diagnosis by missing imputation method

**Supplementary Figure 1:** Power calculation estimates

Figure shows number of patients (black labels) and controls (blue labels) needed at various patient vs. control ratios to demonstrate a hazard ratio of 2·5 for mortality or MACE with a two-sided α of 0·05 and power (ß) of 0·8.

**Supplementary Figure 2:** Thyroid hormone levels by age group

Legend: Box plots illustrate median and inter-quartile range for thyroid hormone levels (FT4, FT3, TSH) by age-group using all available data. ß coefficients (95% confidence intervals) are estimates of the change in median hormone level per year and are derived from quantile mixed-effects models with age (years) as a fixed effect and subject as a random effect.

| | RTHβ | Controls | Total | P value |
|---|---|---|---|---|
| No | 55 | 2750 | 2805 | |
| Age at last follow-up, yrs | | | | |
| Mean (SD) | 48·8 (17·1) | 51·7 (18·6) | 51·6 (18·6) | 0·26 |
| Median (IQR) | 52 (36, 65) | 53 (36, 67) | 53 (36, 67) | 0·50 |
| <40 yrs | 15 (27·3) | 706 (25. 7) | 721 (25·7) | 0·71 |
| 40–60 yrs | 23 (41·8) | 1046 (38·0) | 1069 (38·1) | |
| >60 yrs | 17 (30·9) | 998 (36·3) | 1015 (36·2) | |
| Age at start of follow-up | | | | |
| Mean (SD) | 30.8 (13.8) | 33.1 (14.8) | 33.1 (14.8) | 0.24 |
| Median (IQR) | 26 (20, 39) | 29 (20, 40) | 29 (20, 40) | 0.23 |
| Sex, N (%) | | | | |
| Female | 33 (60) | 1650 (60) | 1683 (60) | 1·00 |
| Male | 22 (40) | 1100 (40) | 1122 (40) | |
| Baseline Comorbidity, N (%) | | | | |
| Charlson score 0 | 48 (87·3) | 2596 (94·4) | 2644 (94·3) | 0·02 |
| Charlson score ≥1 | 7 (12·7) | 154 (5·6) | 161 (5·7) | |
| Person years of follow-up | 2685 | 142056 | 144741 | |
| Age at diagnosis of RTHβ | 43·4 (33·0, 56·4) | — | — | — |
| Baseline thyroid hormones | | | | |
| FT4, pmol/L | 31·0 (26·8, 39·1) | — | — | — |
| FT3, pmol/L | 10·0 (7·7, 11·9) | — | — | — |
| TSH, mU/L | 2·47 (1·50, 4·89) | — | — | — |
| Treatment [a] | | | | |
| Beta-blockers | 14 (25.5%) | | | |
| Antithyroid drugs [b] | 7 (12·7%) | — | — | — |
| RAI [c] or thyroidectomy | 6 (10·9%) | — | — | — |
| Levothyroxine | 13 (23·6%) | — | — | — |

**Table 1: Cohort characteristics**

a. Some patients received treatment with multiple modalities either concurrently (i.e., Levothyroxine plus antithyroid drugs) or sequentially (i.e., RAI or thyroidectomy after antithyroid drugs or Levothyroxine after RAI or thyroidectomy), b. Antithyroid drugs for more than 3 months' duration, c. RAI, radioactive iodine

| | | Mortality | | | | MACE | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N (%) | CHR (95% CI)[a] | P-value | AHR (95% CI)[b] | P-value | CHR (95% CI) | P-value | AHR (95% CI) | P-value |
| Age | 55 | 1·07 (1·03–1·10) | <0·001 | 1·05 (0·99–1·11) | 0·067 | 1·05 (1·02–1·08) | <0·001 | 1·06 (1·02–1·10) | 0·004 |
| Sex | | | | | | | | | |
| Male | 22 (40) | Ref | | Ref | | Ref | | Ref | |
| Female | 33 (60) | 1·18 (0·36–3·93) | 0·786 | 0·73 (0·19–2·80) | 0·651 | 0·81 (0·33–2·01) | 0·653 | 0·55 (0·18–1·68) | 0.30 |
| Comorbidity | | | | | | | | | |
| Charlson 0 | 38 (87) | Ref | | Ref | | Ref | | Ref | |
| Charlson ≥1 | 7 (13) | 1·53 (1·11–2·12) | 0·010 | 1·52 (0·93–2·47) | 0·093 | 1·40 (1·08–1·82) | 0·012 | 1·40 (0·78–2·50) | 0.26 |
| Thyroid hormones[c] | | | | | | | | | |
| FT4 | 53 | 1·07 (1·02–1·11) | 0·004 | 1·07 (1·02–1·13) | 0·005 | 1·04 (1·00–1·07) | 0·024 | 1·04 (1·01–1·08) | 0.02 |
| FT3 | 49 | 1·12 (0·97–1·29) | 0·124 | 1·15 (0·98–1·35) | 0·252 | 1·05 (0·94–1·18) | 0·361 | 1·08 (0·95–1·22) | 0·26 |
| TSH | 53 | 1·05 (0·84–1·30) | 0·685 | 1·13 (0·91–1·40) | 0·080 | 0·96 (0·79–1·15) | 0·639 | 0·97 (0·81–1·16) | 0.71 |
| Mutation cluster | | | | | | | | | |
| Cluster 1 | 10 (15) | Ref | | Ref | | Ref | | Ref | |
| Cluster 2 | 18 (34) | 2·52 (0·28–22·84) | 0·413 | 1·72 (0·05–58·31) | 0·762 | 1·90 (0·39–9·25) | 0·428 | 2·12 (0·28–15·92) | 0.46 |
| Cluster 3 | 12 (23) | 0·73 (0·06–8·44) | 0·800 | 2·09 (0·10–43·28) | 0·632 | 0·90 (0·16–5·12) | 0·904 | 3·38 (0·46–24·69) | 0.23 |
| Cluster 4 | 15 (28) | 1·26 (0·13–11·80) | 0·842 | 2·52 (0·14–45·76) | 0·531 | 1·05 (0·20–5·47) | 0·958 | 1·01 (0·16–6·26) | 0·99 |
| Diagnosis year[d] | | | | | | | | | |
| 1st tertile | 19 (35) | Ref | | Ref | | Ref | | Ref | |
| 2nd tertile | 18 (33) | 0·69 (0·20–2·39) | 0·553 | 1·55 (0·07–36·00) | 0·783 | 0·90 (0·33–2·47) | 0·844 | 1·47 (0·13–17·12) | 0.76 |
| 3rd tertile | 18 (33) | 0·32 (0·36–2·85) | 0·307 | 0·72 (0·24–21·70) | 0·851 | 0·48 (0·12–1·85) | 0·283 | 1·16 (0·07–19·47) | 0·92 |

**Table 2: Mortality and MACE outcomes in RTHβ depending on baseline characteristics at the time of diagnosis**

a, CHR, crude hazard ratio, b, AHR, adjusted hazard ratio. Adjusted models were corrected for age, sex, comorbidity, and diagnosis year. In addition, thyroid hormones were adjusted for laboratory site. c, Thyroid hormones were available for 53 (FT4, TSH) and 49 (FT3) patients respectively. Nomenclature of mutation clusters corresponds to previously defined hotspots in

the TRβ hormone binding domain (clusters 1-3) and an additional amino acid position (cluster 4), outside the established hotspots. Age and thyroid hormones were treated as continuous variables and HR are as per year of age and per pmol/L (FT4, FT3) and mU/L (TSH), respectively. d, Diagnosis year was divided into tertiles from the earliest to the most recent diagnosis.

| | No treatment | Antithyroid Drugs | RAI/Surgery | Levothyroxine | P value for trend |
|---|---|---|---|---|---|
| **Number** | 34 | 6 | 6 | 9 | |
| **FT4, pmol/L** | | | | | |
| Baseline | 30·8 (26·8, 36·8) | 32·0 (28·2, 35·1) | 37·7 (31·1, 59·0) | 25·1 (20·2, 41·0) | 0·10 |
| Follow-up | 28·3 (24·1, 35·7) | 26·0 (20·2, 32·0) | 36·1 (34·0, 59·0) | 23·4 (20·0, 25·9) | 0.03[a] |
| β-coefficient (95%CI)* | Reference | -3·4 (-8·9, 2·1) | -1·2 (-7·3, 4·9) | -3·5 (-8·6, 1·5) | |
| Regression P value | Reference | 0·22 | 0·70 | 0·16 | |
| **FT3, pmol/L** | | | | | |
| Baseline | 9·0 (8·3, 11·8) | 11·3 (7·6, 11·7) | 11·3 (7·1, 16·6) | 10·0 (6·8, 12·0) | 0·94 |
| Follow-up | 8·8 (7·3, 10·4) | 11·3 (7·9, 11·6) | 9·0 (6·5, 11·9) | 7·5 (5·7, 11·9) | 0.68 |
| β-coefficient (95%CI)* | Reference | 0·4 (-1·8, 2·6) | -1·1 (-3·4, 1·3) | -0·9 (-2·8, 1·1) | |
| Regression P value | Reference | 0·76 | 0·36 | 0·36 | |
| **TSH, mU/L** | | | | | |
| Baseline | 2·3 (1·2, 3·5) | 1·2 (0·7, 3·5) | 5·5 (1·5, 10·4) | 5·3 (3·2, 7·0) | 0·01[b] |
| Follow-up | 2·1 (1·5, 3·5) | 3·2 (0·7, 4·5) | 6·2 (1·5, 12·5) | 4·3 (3·3, 6·4) | 0·06[c] |
| β-coefficient (95%CI)* | Reference | 1·4 (-1·1, 4·0) | 2·5 (-0·6, 5·5) | -0·1 (-2·5, 2,4) | |
| Regression P value | Reference | 0·26 | 0·11 | 0·96 | |

**Table 3: Thyroid hormone and TSH concentrations in RTHβ at baseline and follow-up following different treatment modalities**

Treatment groups comprised patients who had: (1) no treatment, (2) antithyroid drugs alone, (3) RAI (radioactive iodine) or surgery, or (4) Levothyroxine alone. Thyroid hormones (median, interquartile range) are presented at baseline and at follow-up (last available outpatient test). *β-coefficient, 95% confidence intervals (95%CI) are derived from multiple linear regression models examining the impact of treatment group (independent variable) on follow-up thyroid hormone levels (dependent variable), with models adjusted for age, sex, and baseline thyroid hormone level. P values for trend denote differences across groups while the regression P value is derived from the linear regression model. a, Levothyroxine vs. No treatment or vs. RAI/Surgery, b, Levothyroxine vs. No treatment or vs. antithyroid drugs, c, Levothyroxine vs. no treatment.

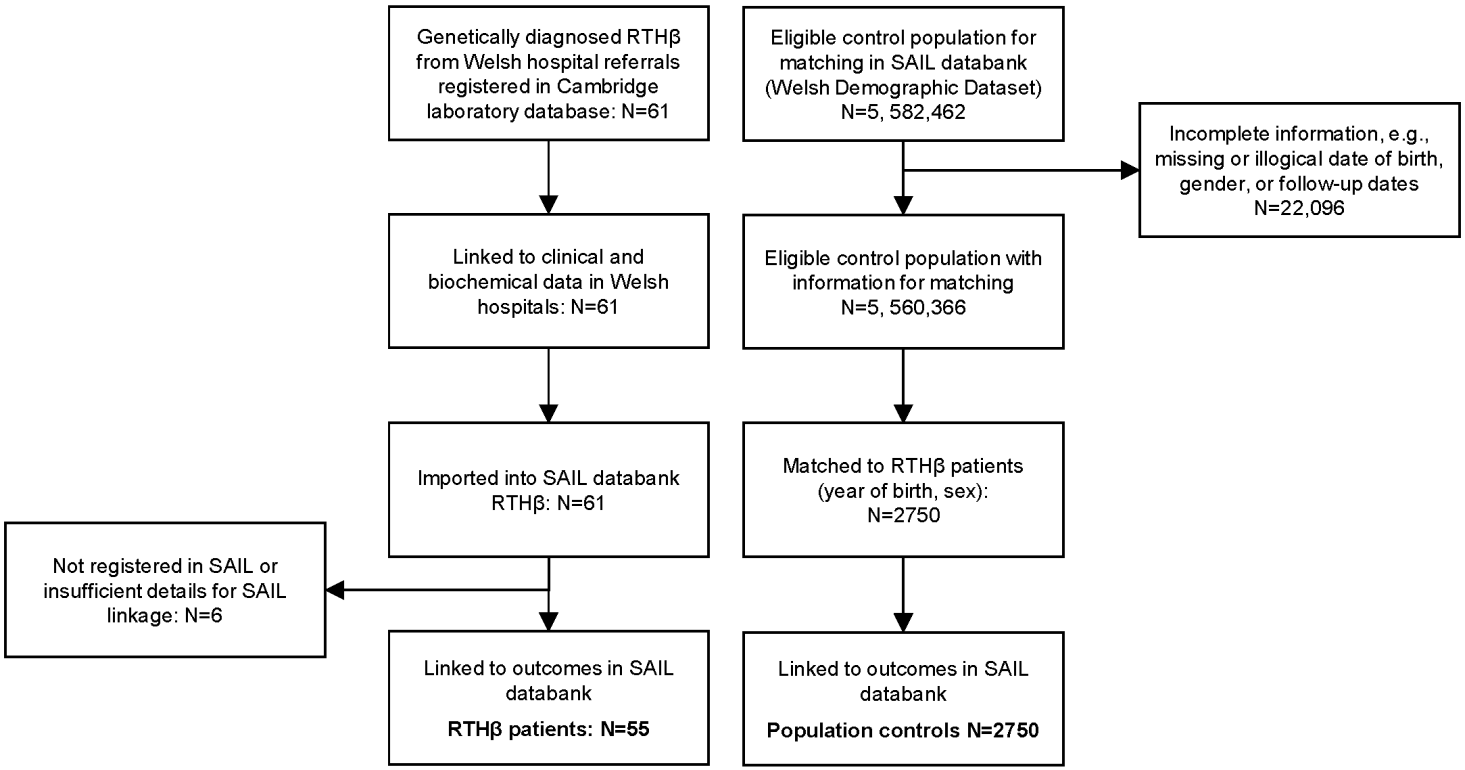


**Figure 1: Selection of study population**

**Figure 2: Kaplan Meier curves for mortality and cardiovascular events in patients with RTH**

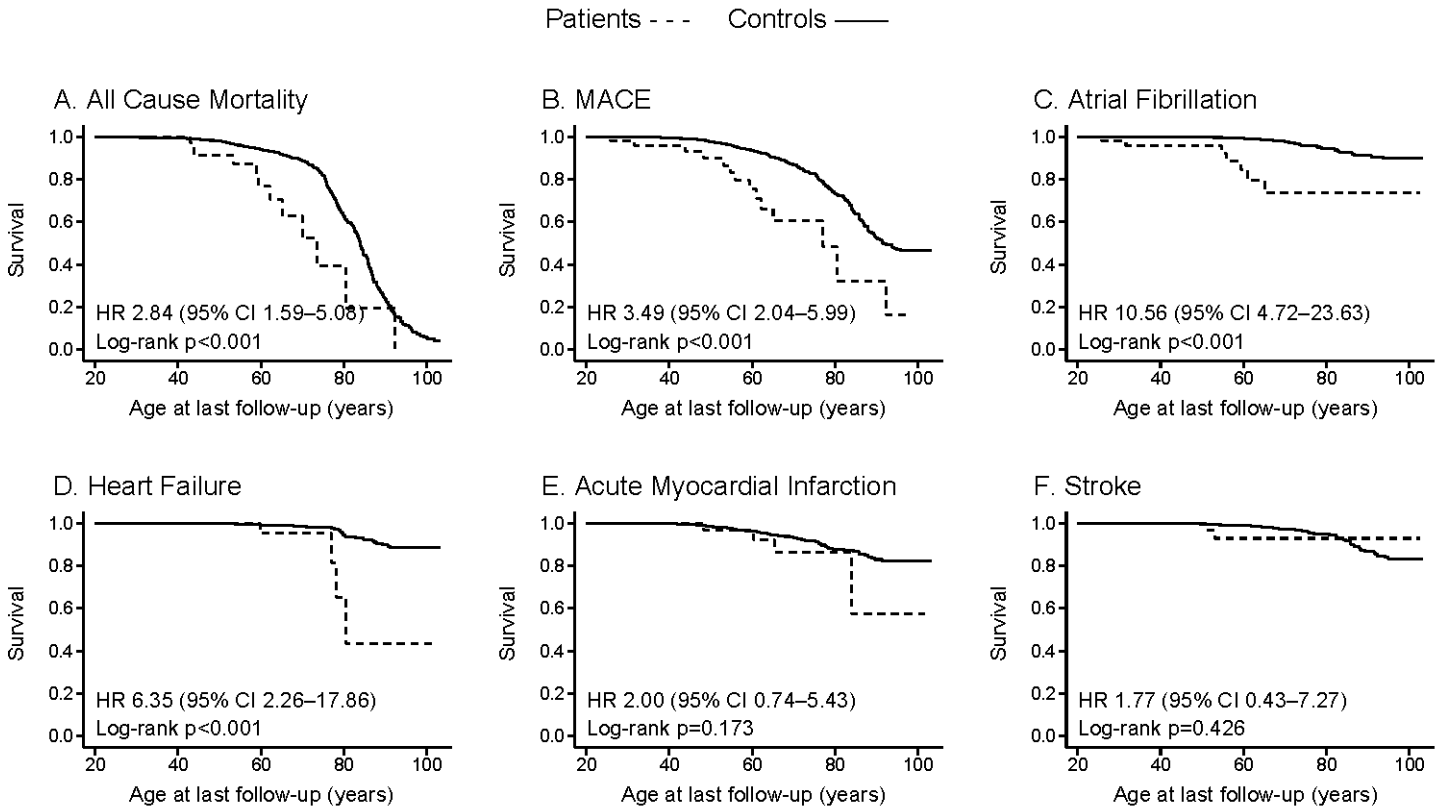

**Figure 3: Cubic splines for baseline FT4 and outcomes in the RTH cohort**