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Resistance to thyroid hormone caused by a mutation in thyroid hormone receptor (TR)α1 and TRα2: clinical, biochemical, and genetic analyses of three related patients

Carla Moran, Maura Agostini*, W Edward Visser, Erik Schoenmakers, Nadia Schoenmakers, Amaka C Offiah, Ken Poole, Odelia Rajanayagam, Greta Lyons, David Halsall, Mark Gurnell, Dionisios Chrysis, Alexandra Efthymiadou, Charles Buchanan, Simon Aylwin, Krishna K Chatterjee*

Summary

Background **The thyroid hormone receptor α gene (*THRA*) transcript is alternatively spliced to generate either thyroid hormone receptor (TR)α1 or a non-hormone-binding variant protein, TRα2, the function of which is unknown. Here, we describe the first patients identified with a mutation in *THRA* that affects both TRα1 and TRα2, and compare them with patients who have resistance to thyroid hormone owing to a mutation affecting only TRα1, to delineate the relative roles of TRα1 and TRα2.**

Methods **We did clinical, biochemical, and genetic analyses of an index case and her two sons. We assessed physical and radiological features, thyroid function, physiological and biochemical markers of thyroid hormone action, and *THRA* sequence.**

Findings **The patients presented in childhood with growth failure, developmental delay, and constipation, which improved after treatment with thyroxine, despite normal concentrations of circulating thyroid hormones. They had similar clinical (macrocephaly, broad faces, skin tags, motor dyspraxia, slow speech), biochemical (subnormal ratio of free thyroxine:free tri-iodothyronine [T_3], low concentration of total reverse T_3, high concentration of creatine kinase, mild anaemia), and radiological (thickened calvarium) features to patients with TRα1-mediated resistance to thyroid hormone, although our patients had a heterozygous mis-sense mutation (Ala263Val) in both TRα1 and TRα2 proteins. The Ala263Val mutant TRα1 inhibited the transcriptional function of normal receptor in a dominant-negative fashion. By contrast, function of Ala263Val mutant TRα2 matched its normal counterpart. In vitro, high concentrations of T_3 restored transcriptional activity of Ala263Val mutant TRα1, and reversed the dominant-negative inhibition of its normal counterpart. High concentrations of T_3 restored expression of thyroid hormone-responsive target genes in patient-derived blood cells.**

Interpretation **TRα1 seems to be the principal functional product of the *THRA* gene. Thyroxine treatment alleviates hormone resistance in patients with mutations affecting this gene, possibly ameliorating the phenotype. These findings will help the diagnosis and treatment of other patients with resistance to thyroid hormone resulting from mutations in *THRA*.**

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Introduction

The physiological actions of thyroid hormones are mediated by nuclear receptors (thyroid receptor [TR]α and TRβ) encoded by separate genes (*THRA* and *THRB*), which regulate gene expression in target tissues. TRs bind to DNA usually as a heterodimer with retinoid X receptor. When not bound to thyroid hormone, TR binds to the promoters of thyroid hormone-target genes—within gene promoter regions known as thyroid response elements—in complex with multiprotein corepressor complexes, inhibiting gene transcription. When thyroid hormone binds to a TR, a conformational change leads to dissociation of corepressor proteins and coactivator proteins can associate with the receptor, leading to transcriptional activation of target genes.[1]

Alternative splicing of the *THRA* transcript generates two subtypes of TRα with identical aminoterminal and DNA-binding domains, but different carboxyterminal regions. Because of this difference, TRα1 binds thyroid hormone. By contrast, TRα2 does not bind thyroid hormone but does bind DNA. TRα1 is most highly expressed in myocardium, skeletal muscle, the gastrointestinal tract, and the CNS; some forms of TRα1 that are truncated at the aminoterminus are expressed in mitochondria, affecting its function.[2,3] TRα2 is widely expressed, but its function is not understood.[3]

The incidence of resistance to thyroid hormone mediated by defective TRβ is roughly 1 in 40 000 people, and several hundred heterozygous mutations in *THRB* gene (most within three hotspots in the thyroid hormone-binding domain) have been identified in people with this disorder.[4] Consistent with the dominant inheritance pattern observed for this disorder, mutant TRβ proteins inhibit the function of their normal counterparts in a dominant-negative manner—probably caused by constitutive repression of target gene transcription

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*Contributed equally

University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science (C Moran MBBCh, M Agostini PhD, W E Visser MD, E Schoenmakers PhD, N Schoenmakers PhD, O Rajanayagam BSc, G Lyons RGN, M Gurnell PhD, Prof KK Chatterjee BMBCh), **Department of Clinical Biochemistry** (D Halsall PhD), **Department of Rheumatology** (K Poole PhD), **Addenbrooke's Hospital, Cambridge, UK; Academic Unit of Child Health, University of Sheffield, Sheffield, UK** (A C Offiah PhD); **Department of Paediatrics, Division of Endocrinology, Medical School University of Patras, Patras, Greece** (D Chrysis MD, A Efthymiadou MD); **and Department of Endocrinology** (S Aylwin FRCP) **and Department of Child Health** (C Buchanan MBChB), **King's College Hospital, London, UK**

Correspondence to: Prof Krishna K Chatterjee, University of Cambridge, Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK **kkc1@medschl.cam.ac.uk**

resulting from failure of the corepressor complex to dissociate from mutant TRβ.[5] Based on the homology (95%) of their thyroid hormone-binding domains, analogous mutations in human TRα had been predicted,[6] but only three different frameshift-stop or premature-stop mutations within a TRα1-specific, carboxyterminal-encoding exon have been described.[7–10] However, mouse models with TRα1 mutations in other regions of the gene[11–14] have been generated and have varying phenotypes, suggesting that molecular and clinical heterogeneity of the human disorder might be shown.

Here, we describe the first family with a *THRA* defect resulting in mutation of both TRα1 and TRα2.

Methods

The index patient (patient 1, a 60-year-old woman) had features suggesting hypothyroidism at age 2 years (increased bodyweight, poor linear growth, constipation, and a large, prominent tongue), but her thyroid hormone concentrations were within the normal ranges. Nevertheless, she was treated with thyroxine—which improved her growth and constipation—and has continued to take the drug since.

Her eldest son (patient 2, age 30 years) was delivered by caesarean section because of macrocephaly. At age 6 weeks, he had to switch from breastfeeding to bottle-feeding to correct poor nutritional intake. His subsequent growth and developmental milestones (using a ball, speech) were delayed and—because his mother had had similar symptoms—he started thyroxine treatment at age 3 years, despite his thyroid hormone concentrations being within the normal ranges. Although his growth and development improved, his motor coordination remained poor—causing imbalance, clumsiness, and poor handwriting—and he attended a specialist school for children with

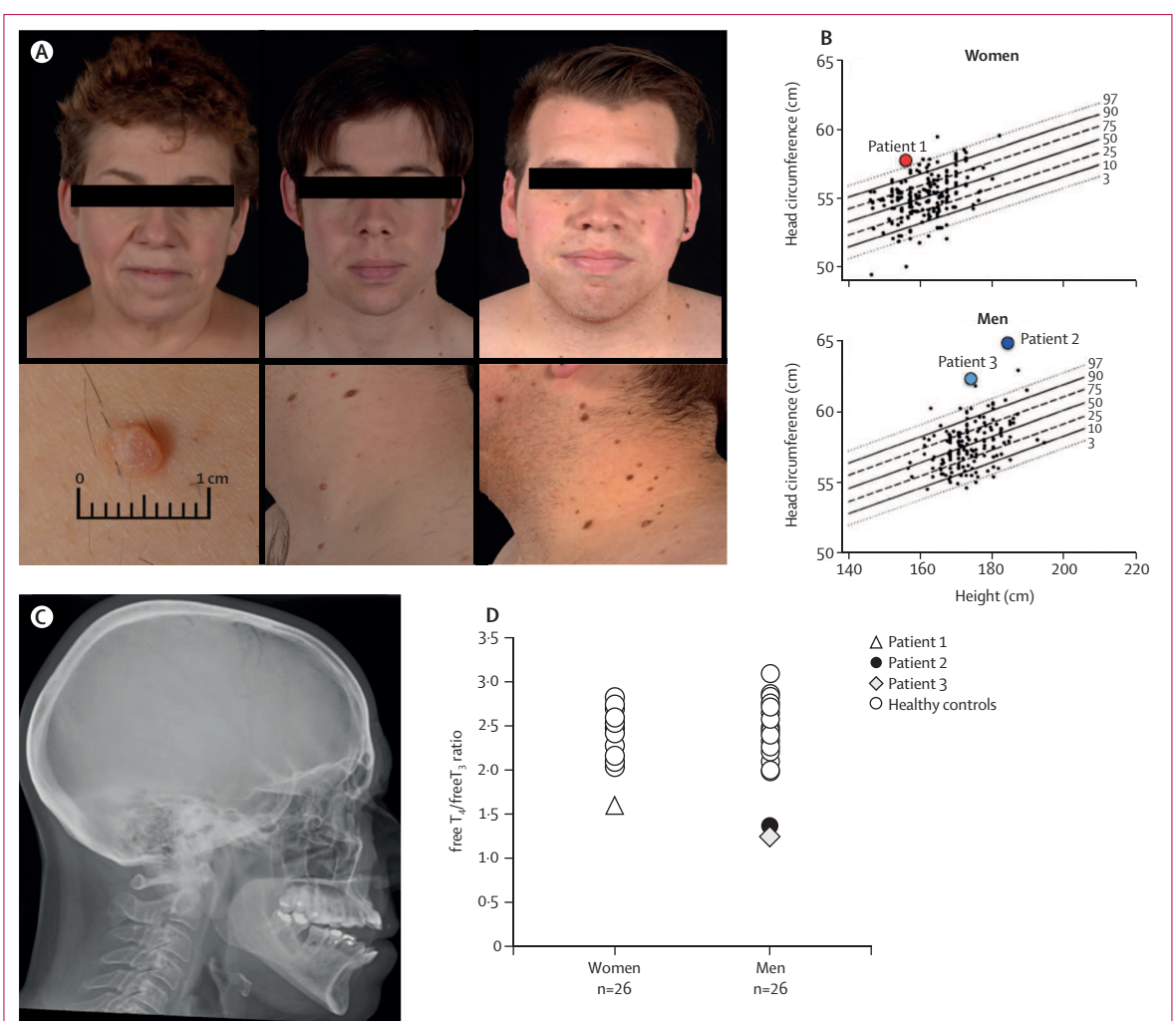

Figure 1: **Phenotypic features**
(A) Patients 1, 2, and 3. (B) Head circumferences by height and sex, divided into centiles (adapted with permission from Bushby and colleagues[15]). (C) Skull radiograph of patient 1. (D) Free thyroxine:free tri-iodothyronine ratios, compared with sex-matched healthy people of similar age (women 35–64 years, men 20–40 years).[15]

	Patient 1 (woman, age 61 years)		Patient 2 (man, age 30 years)		Patient 3 (man, age 26 years)		Reference values
Thyroxine dose (μg per day)	0	75	0	150	0	150	..
Weight (kg)	70·3	69·6	84·0	84·4	104·3	106·0	..
BMI (kg/m^2)	28·52	28·24	24·28	24·40	33·29	33·83	..
Sleeping heart rate (beats per min)	58	62	53	54	55	51	40–68*
Resting energy expenditure (Z score)	–1·93	–1·82	–3·06	–1·75	–3·35	–0·30	..
TSH (mU/L)	4·60	0·12	4·80	<0·03	3·2	0·54	0·35–5·50
Free thyroxine (pmol/L)	9·4	13·9	10·5	17·0	9·7	11·0	10·0–19·8
Total thyroxine (nmol/L)	60·0	96·1	76·6	130	66·3	83·6	69–141
Free T3 (pmol/L)	4·4	6·3	6·4	8·7	6·8	8	3·5–6·5
Total T3(nmol/L)	1·3	1·6	1·7	2·3	2·1	2·4	0·9–2·8
Reverse T3 (ng/L)	<50	70	50	120	<50	<50	80–250
Thyroglobulin (μg/L)	14·0	1·1	69·7	2·6	23·6	3·2	3·0–40·0
Total creatine kinase (U/L)†	364	178	385	242	184	295	26–192
SHBG (nmol/L)	45·5	45·7	28·2	32·8	14	13·6	Men 10–57, women 18–144
Total cholesterol (mmol/L)	7·9	7·2	5·2	4·3	4·2	4·0	<5 for a healthy adult in the UK
LDL cholesterol (mmol/L)	5·06	4·72	3·20	2·53	2·34	2·09	<3 for a healthy adult in the UK
IGF-1 (nmol/L)	9·9	11·4	24·3	26·9	32·9	31·7	Women 11·8–28·6, men 16·3–39·3
Markers of bone turnover‡							
Formation							
Bone-specific alkaline phosphatase (ng/mL)	12·6	14·8	17·4	15·4	10·7	11·8	Women (post-menopausal) 3·8–22·6, men 5·7–32·9
Osteocalcin (ng/mL)	9·7	10·7	13·0	16·8	12·7	12·9	Women (post-menopausal) 15–46, men (age 18–30 years) 24–70
P1NP (ng/mL)	26·3	27·3	55·2	56·3	52·8	46·6	Women (post-menopausal) 20·25–76·31
Resorption							
CTx (ng/mL)	0·389	0·339	0·700	0·839	0·585	0·525	Women (post-menopausal) 0·104–1·008, men (age 30–50 years) 0·096–0·584
NTx:Cr	24·5	30·8	25·3	24·7	19·5	28·2	Men 21–83
Red blood cell mass (10^{12}/L)	3·74	3·70	4·23	4·27	4·17	4·16	Women 3·8–5·3 × 10^{12}/L, men 4·20–5·80 × 10^{12}/L
Mean corpuscular volume (fL)	94·8	95·5	92·5	92·6	88·0	88·2	80–100
Haemoglobin concentration (g/L)	120	120	129	129	125	124	Women 115–160, men 130–170

CK-MM=skeletal muscle isoenzyme of creatine kinase. P1NP=procollagen type 1 N-propeptide. CTx=C-terminal cross-linking telopeptide of type 1 collagen. NTx:Cr=N-terminal cross-linking telopeptide of type 1 collagen:creatinine ratio. SHBG=sex hormone-binding globulin. T_3=tri-iodothyronine. TSH=thyroid-stimulating hormone. *From 148 healthy volunteers. †Only CK-MM isoenzyme detected. ‡When taking chronic thyroxine treatment.

Table: **Biochemical and metabolic characteristics of patients when or when not receiving thyroxine**

motor dyspraxia. He continued to take thyroxine throughout childhood and adulthood, except for an interval (26–29 years), during which time he noted constipation, weight gain, lethargy, and low mood.

A second son (patient 3, age 26 years) was delivered by elective caesarean section. He had a large tongue and similar facial appearance to patient 2, and had drowsiness and delayed linear growth, speech and motor development, which improved after treatment with thyroxine from age 3 years. He also has significant motor incoordination, for which he needed specialist schooling. A third son is unaffected, with normal growth and development.

We took serial measurements of biochemical characteristics (thyroid function, sex-hormone-binding globulin, creatine kinase, lipids, bone turnover markers) and physiological characteristics (sleeping heart rate, resting energy expenditure) when patients were and were not taking thyroxine, as described previously,[7,10] and compared these data with reference measurements from age and sex-matched people from a healthy volunteer cohort recruited by our clinical research facility. We did molecular genetic analysis of *THRA* and functional characterisation of mutant TRα1 and TRα2 (appendix).[7,10] We compared differences in normal and mutant receptor function with a two-tailed *t*-test using Excel (version 14.3.9). We did structural modelling of mutant TRα1, TRα2 and TRβ proteins using MacPyMOL Molecular Graphics System (version 1.5.0.4). All investigations were part of an ethically approved protocol or were clinically indicated. All patients gave informed written consent.

See Online for appendix

Role of the funding source

The funders had no role in study design, collection, analysis or interpretation of the data, writing the report, or the decision to submit the report for publication.

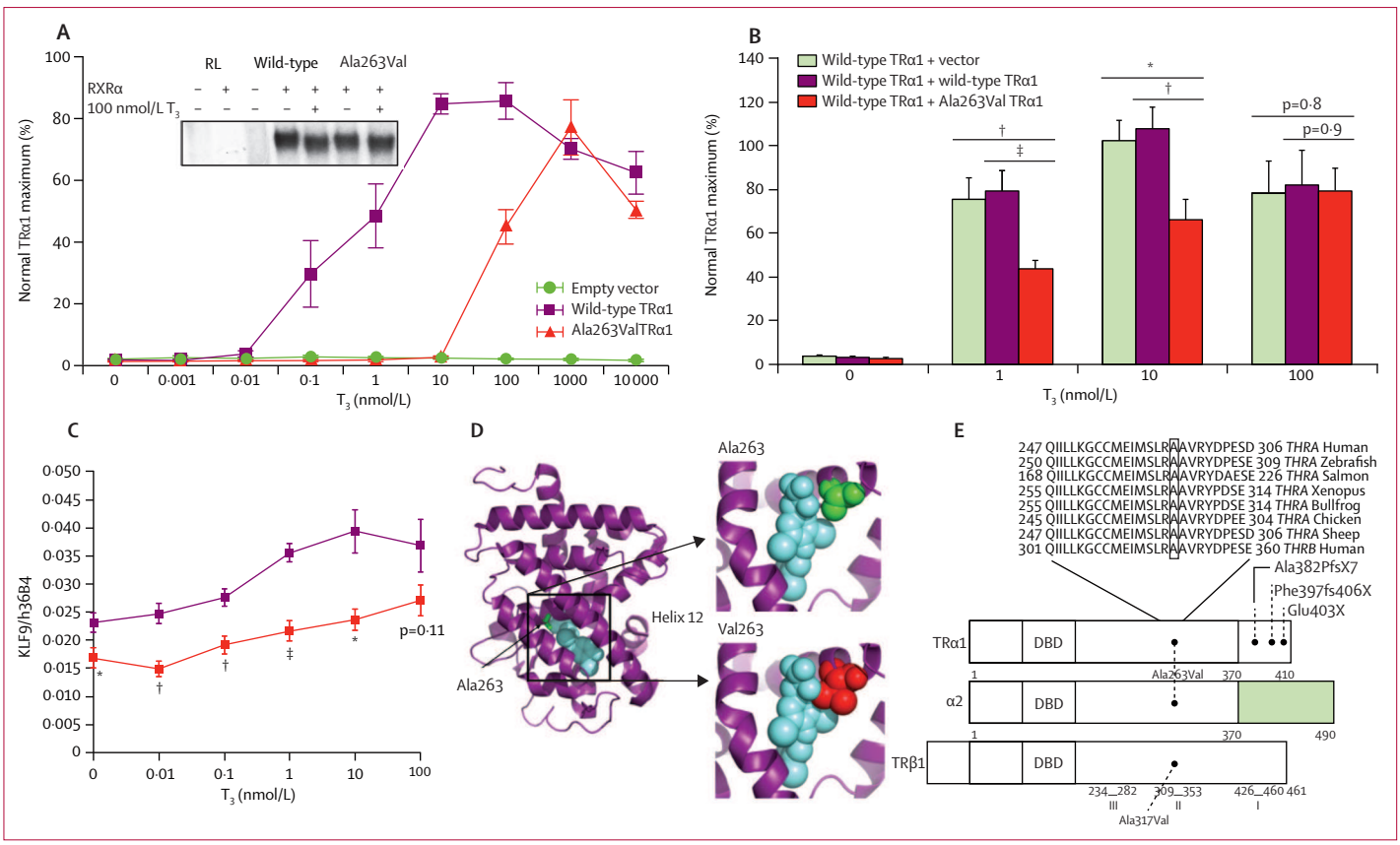

Figure 2: **Functional properties and molecular modelling of Ala263Val TRα1**
(A) Results of transfection assay for T_3-dependent activation in JEG-3 cells transfected with empty, wild-type, or Ala263Val TRα1 expression vectors together with a thyroid hormone-responsive reporter gene; the inset shows an electrophoretic mobility shift assay with comparable interaction of unliganded or hormone-bound normal TRα1 and RXR, and Ala263Val mutant TRα1 and RXR heterodimers with a direct repeat thyroid response element from the malic enzyme gene. (B) We tested dominant-negative inhibition in cells cotransfected with reporter gene and equal combinations of expression vectors. (C) Quantitative real-time PCR (internal control: 36B4, acidic ribosomal phosphoprotein) showing expression of *KLF9* in peripheral blood mononuclear cells from the patients (Ala263Val) or control individuals with changing T_3 concentrations. (D) Crystallographic modelling of the TRα1 ligand binding domain bound to tri-iodothyronine (blue). (E) Structures of TRα1, TRα2 and TRβ proteins. *p<0·05. †p<0·01. ‡p<0·001. DBD=DNA-binding domain. T_3=tri-iodothyronine. RXR=retinoid X receptor. RL=reticulocyte lysate.

Results

All patients were taking thyroxine when referred, and were assessed both while taking treatment and 6 weeks after discontinuation. The patients had a broad face, flattened nasal bridges, and prominent tongue (figure 1A). Patient 1's stature was normal (height 1·57 m, mid-parental height 1·69 m) and proportionate (sitting height 83·8 cm [*Z* score –1·3] subischial leg length 0·73 m [*Z* score –0·3], but head circumference was large (57·5 cm, >97th centile, figure 1B). Patient 2 was proportionately tall (height 1·86, mid-parental height 1·78 m, sitting height 0·97 m [*Z* score +1·1], subischial leg length 0·89 m [*Z* score +1·6]), with a substantially large head circumference (64·8 cm, >97th centile, figure 1B). Patient 3 was also proportionate (height 1·77 m mid-parental height 1·78, sitting height 0·95 m [*Z* score +0·3], subischial leg length 0·83 m [*Z* score +0·15]), but macrocephalic (head circumference 62 cm, >97th centile, figure 1B). Patients 2 and 3 have numerous skin tags and moles and their speech is dysarthric. None of the patients have had their IQ properly tested, but that of patient 3 was low average (88) at school and all have achieved an A level educational qualification (or equivalent).

All patients had a thickened skull vault (cranial hyperostosis; figure 1C). Patient 1's bone mineral density T scores were slightly low at the hip (by dual-energy x-ray absorptiometry –1·0; by quantitative CT –1·7) and at the femoral neck (by quantitative CT –0·5), but normal at lumbar spine (+1·9). By contrast, patients 2 and 3 both had high bone mineral density T scores at these sites (by dual-energy x-ray absorptiometry patient 2: hip +0·8, lumbar spine +1·9; patient 3: hip +1·4, lumbar spine +1·9) and quantitative CT (patient 2: total hip +1·52 [94th centile for age], femoral neck +1·7 [96th centile for age]; patient 3: total hip +1·9 [97th centile for age], femoral neck +2·8 [99th centile for age]).

When not taking thyroxine, all patients had normal concentrations of thyroid-stimulating hormone (TSH). Patient 1 and patient 3 had marginally low and patient 2 had low-to-normal free thyroxine concentrations; patient 1

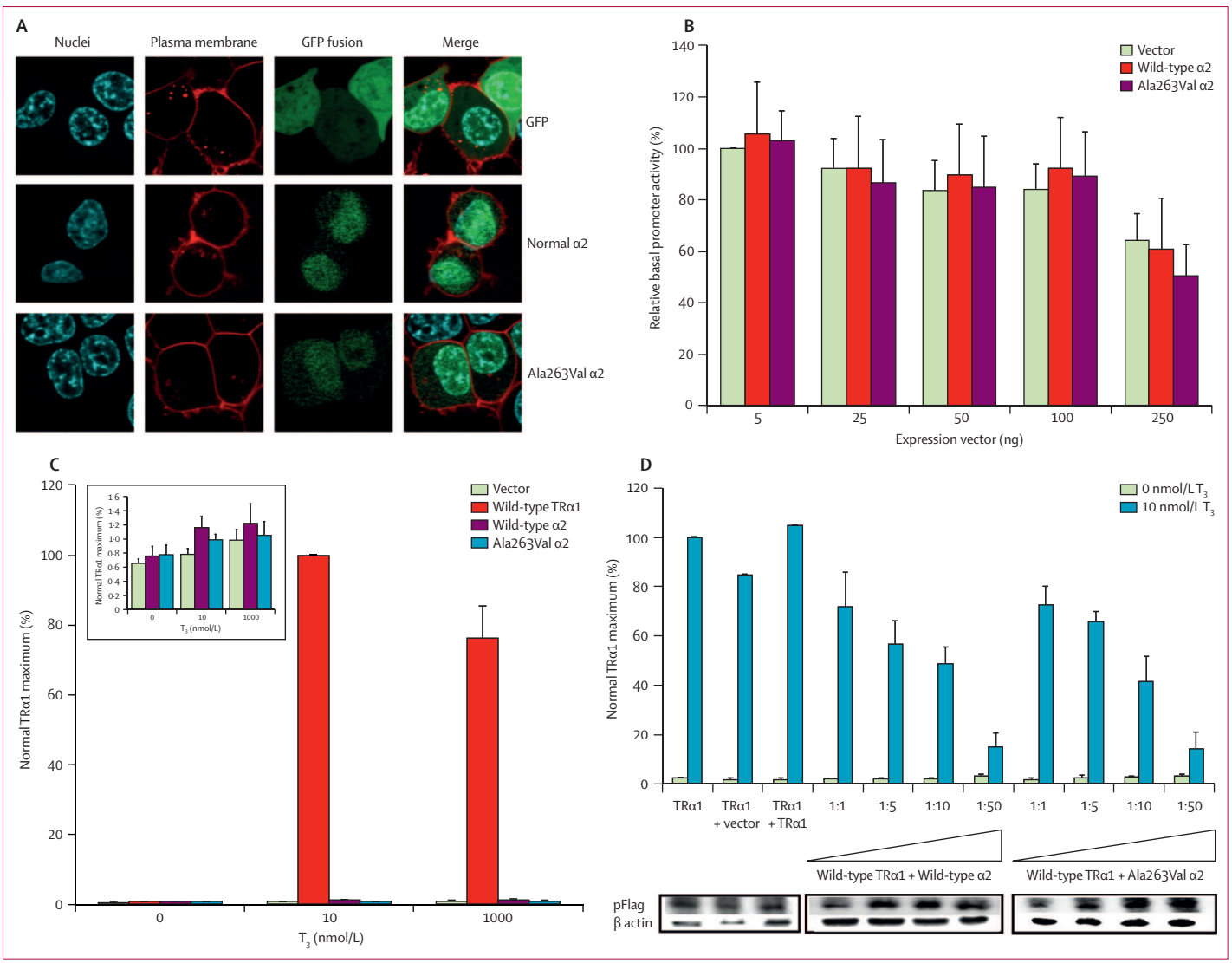

Figure 3: **Functional properties of Ala263Val TRα2**

(A) 293 cells transfected with either GFP, GFP-tagged normal TRα2, or Ala263Val mutant TRα2 expression vectors with visualisation of nuclei (blue), plasma membrane (red), and GFP fusion (green), and a composite merged image by immunofluorescence. We tested transcriptional function of wild-type TRα2 and Ala263Val mutant TRα2 proteins in JEG-3 cells cotransfected with reporter gene and increasing amounts (5–250 ng) of empty, normal, or Ala263Val TRα2 expression vectors in the absence of tri-iodothyronine (B), or a fixed amount of empty, TRα1, normal TRα2, and Ala263Val mutant TRα2 expression vectors with increasing T_3 concentrations (C; inset show magnified vector, α2, Ala263Val α2 responses), or a fixed amount of wild-type TRα1 and increasing ratio (1:1 to 1:50) of TRα2 expression vectors (D; inset shows western blot of flag epitope-tagged TR and control [β actin] proteins). T_3=tri-iodothyronine.

and patient 2 had normal and patient 3 had slightly high free tri-iodothyronine (T_3) concentrations (table). However, all patients had a low ratio of thyroxine:T_3 (figure 1D), with subnormal concentrations of reverse T_3 (table). Their resting energy expenditure was greatly reduced compared with healthy controls, with high concentration of skeletal muscle creatine kinase and mild, normocytic, anaemia (table). Sequencing of *THRA* showed that the patients were heterozygous for a nucleotide substitution (GCG to GTG), corresponding to an alanine to valine change at codon 263 in the sequence common to both TRα1 and TRα2; the mutation segregates with abnormal phenotype and thyroid biochemistry, being present in the three patients but absent in unaffected family members (sibling, father; appendix) and from normal genome databases (dBSNP, 1000 Genomes, NHLBI exome variant server).

Restriction fragment length polymorphism analysis and direct sequencing of TRα1 and TRα2 cDNAs derived from primary blood mononuclear cells from patient 1 confirmed that Ala263Val mutant TRα1 and TRα2 mRNAs are coexpressed together with normal variant transcripts in vivo (appendix). Lack of antibodies that reliably distinguish between TRα1 and TRα2 subtypes, and between normal and mutant TRs, precluded testing for expression at the protein level.

Binding of the Ala263Val mutant TRα1 to radiolabelled T_3 was greatly reduced compared with normal receptor (appendix), consistent with structural modelling, which predicted that the Ala263Val substitution would impair T_3 binding to the receptor owing to steric hindrance (figure 2D). In transfection assays, Ala263Val mutant TRα1 had little effect on transcriptional activation of a thyroid hormone-responsive target gene at low concentrations of T_3 (0·001–10 nM; figure 2A), but at higher concentrations (100–10 000 nM) mutant receptor function was similar to that of normal receptor. Ala263Val mutant TRα1 was able to bind to DNA (figure 2A) and, when coexpressed with wild-type TRα1, it inhibited the transcriptional activity of its normal counterpart in a dominant-negative manner (figure 2B, appendix). High concentrations of T_3 (100 nM) reversed such inhibition in vitro and also reversed reduced expression of a thyroid hormone-responsive target gene (*KLF9*) in patient-derived peripheral blood mononuclear cells studied ex vivo (figure 2B, C). Consistent with reversal of dominant-negative inhibition by mutant TRα1 at high T_3 concentrations, higher (1000 nM) concentrations fully dissociated Ala263Val mutant TRα1 from a corepressor (NCoR) and recruited a coactivator (TRAP220) in assays that measured receptor interactions with cofactors (appendix). Ala263Val mutant TRα1 fails to dissociate from NCoR or to associate fully with TRAP220 at concentrations that would be sufficient for normal receptor.

In comparison with its normal counterpart, the Ala263Val mutant TRα2 had similar cellular localisation, similarly negligible transcriptional function in either the absence or presence of T_3, and similarly weak dominant-negative activity when overexpressed (figure 3).

After thyroxine treatment at a replacement dose (1·1–1·8 μg/kg), free thyroxine and free T_3 increased in all patients, TSH concentrations and circulating thyroglobulin concentrations fell. Total reverse T_3 concentration rose in patients 1 and 2 but was unchanged in patient 3 (table). Resting energy expenditure rose in all patients during treatment, but remained subnormal, with a smaller increase for patient 1, who takes lower doses of thyroxine (75 μg vs 150 μg). Concurrently, LDL-cholesterol concentrations fell in all patients (table). Creatine kinase concentration fell in patient 1 and patient 2, but increased in patient 3 (table). Many, but not all, markers of bone turnover increased, but sleeping heart rate changed little (table). All patients noted that their symptoms improved after restarting thyroxine: paraesthesiae suggestive of carpal tunnel syndrome resolved in patient 1, while both patient 2 and patient 3 reported reduced motor incoordination and constipation.

Discussion

The patients had many clinical features that suggest hypothyroidism (growth retardation, developmental delay, constipation, macrocephaly, large tongue), despite normal concentrations of circulating thyroid hormones. However, they had a subnormal free thyroxine:free T_3 ratio, low reverse T_3 concentration, high muscle creatine kinase concentration, and mild anaemia. The clinical and biochemical features of our patients are similar to the phenotype of patients with defective TRα1 alone,[7–10] with no added characteristics attributable to any change to TRα2 function. Our observations accord with the absence of a phenotype linked specifically to TRα2 deficiency in a knockout mouse line.[16] A patient with a different, sporadic *THRA* mutation (asn359tyr) in both α1 and α2 subtypes,[17] had many dissimilar features (eg, clavicular agenesis, humeroradial synostosis, syndactyly, chronic diarrhoea, primary hyperparathyroidism), which are not present in TRα2 knockout mice,[16] and it is not clear whether these additional abnormalities are caused by the *THRA* mutation alone.[18]

The alanine at codon 263 of TRα is highly conserved in different species (figure 2E). The Ala263Val mutation is common to both TRα1 and α2, whereas previously described *THRA* mutations (glu403X, phe397fs406X, ala382PfsX7) are unique to TRα1. Three clusters of TRβ mutations (at amino acids 426–460, 309–353, and 234–282) are associated with resistance to thyroid hormone β and we have identified a TRβ mutation (ala317val) analogous to the Ala263Val mutation in TRα1 that localises to one of these hotspots (figure 2E). The dysfunction of ala317val mutant TRβ resembles that of Ala263Val mutant TRα1, with severely reduced thyroid hormone binding, impaired thyroid hormone-dependent transcriptional activation, and dominant-negative activity that is reversible at high concentrations of T_3 (appendix). Structural modelling shows how T_3 binding might be impaired (appendix). This amino acid change involves a residue that has previously been reported mutated to threonine in patients with resistance to thyroid hormone,[19] and is within a recognised mutation cluster affecting the thyroid hormone-binding domain of TRβ in patients with this disorder (figure 2E).

The index patient with the ala317val TRβ mutation presented at age 4 years, with a thyroglossal cyst, high thyroid hormone concentration, and unsuppressed thyroid-stimulating hormone. Affected family members (mother and two siblings) had the same biochemical profile, together with high reverse T_3 and thyroglobulin concentrations and these biochemical abnormalities segregate with heterozygosity for the ala317val mutation in TRβ (appendix). Each patient had features of resistance to thyroid hormone (proband: failure to thrive and increased appetite; sibling 1: frequent upper respiratory tract infections, hyperactivity, mild learning difficulties, and increased appetite; sibling 2: failure to thrive) and their resting energy expenditure was high (proband: 142%; sibling 1: 152%; sibling 2: 122%; mother: 127%; normal: 95–105% of predicted values). The differences between patients with the ala317val mutation in TRβ and those with the Ala263Val mutation in TRα1 underscores the importance of TRβ for mediating negative feedback within the hypothalamo-pituitary–thyroid axis and TRα

for mediating hormone action in the periphery (muscle, myocardium, gastrointestinal tract).

Our finding that the Ala263Val substitution inhibits binding of T_3 through steric hindrance provides a basis for the impaired hormone binding and transcriptional function of the Ala263Val mutant TRα1. By contrast, this aminoacid change is unlikely to have an effect on TRα2 function. Normal TRα2 does not bind T_3,[20] is devoid of intrinsic transcriptional activity, and is a weak dominant-negative inhibitor of TRα1 function,[21,22] perhaps because it interacts poorly with retinoid X receptor and corepressors,[22,23] making additional loss-of-function as a result of the Ala263Val mutation unlikely. Conversely, although blocking phosphorylation of aminoacids at the TRα2 carboxyterminus induces dominant-negative inhibitory function,[24] the Ala263Val mutation is located outwith this domain.

Our patients had numerous skin tags and moles, which we have also noted in other patients with resistance to thyroid hormone caused by mutations in TRα1 (appendix).[9] Although present in the general population, the universal occurrence of this feature in people with defective TRα—even in childhood—suggests that it might be an additional characteristic of the disorder, although its absence would not exclude diagnosis. The enzyme DIO3 is present in human skin[25] and its expression is regulated by TRα1,[26] such that people with defective TRα might have diminished DIO3 activity. Topical inhibition of DIO3 activity enhances keratinocyte proliferation in mice[25] and we speculate that cutaneous DIO3 deficiency in patients with defective TRα1 might mediate this phenotype. DIO3 deficiency or upregulation of hepatic DIO1 (as suggested by studies of TRα1 mutant mice[12]) might also mediate the low free thryoxine and high free T_3 concentrations, low ratios of free thryoxine:free T_3 and subnormal total reverse T_3 concentrations recorded in our patients.

Unlike previous reports of patients with highly deleterious TRα1 defects,[7,8,10] high concentrations of T_3 reversed Ala263Val mutant TRα1 dysfunction and dominant-negative activity in vitro. T_3 exposure restored subnormal expression of *KLF9* in mutation-containing primary blood mononuclear cells, suggesting that dominant-negative inhibition by mutant TRα1 can also be overcome in vivo. We correlate these observations with improvement in some peripheral markers of thyroid hormone action (resting energy expenditure, creatine kinase) after thyroxine treatment; moreover, thyroid hormone treatment at physiological dose raised T_3 concentrations and suppressed TSH concentrations in patients 1 and 2, suggesting that the pituitary-thyroid axis is still sensitive to thyroid hormones in these patients.

Starting thyroxine treatment in childhood improved growth and development, and alleviated symptoms in adulthood without abnormally increasing concentrations of markers of bone turnover, as recorded previously.[10] In transgenic mice harbouring a mutant TRα1 (arg384cys) with ten-fold reduced binding affinity towards T_3, increased concentrations of thyroid hormones can reverse neurological abnormalities.[27] Neurocognitive abnormalities might be less severe in these three patients because of early thyroid hormone treatment, but we cannot be certain of this possibility.

The identification of patients with equivalent defects in TRα1 and TRβ tempts the speculation that other patients with TRα1 mutations might exist. Roughly 125 different mutations in TRβ are known to cause resistance to thyroid hormone.[4,5] Furthermore, maternal inheritance of the TRα mutation in this family, and paternal inheritance of the TRα mutation in another family,[8] suggests that transmission of TRα mutations from parents to offspring may not be as impaired in people as in mice.[12] With thyroid hormone concentrations being almost normal in patients with resistance to thyroid hormone caused by mutations in TRα, the clinical and biochemical characteristics of this family (together with features of previous cases) define a phenotypic signature for this syndrome (panel). This signature should enable early identification and treatment of other patients, which would be of particular importance should thyroxine treatment prove to be widely beneficial for this disorder.

Panel: Research in context

Systematic review

We searched PubMed with the terms "THRA" and "mutation" for studies published in English between 2000 and 2014. Previously reported cases[7–10] of resistance to thyroid hormone caused by *THRA* mutations involve mutations that selectively disrupt thyroid hormone receptor (TR)α1 function, manifesting with typical features of hypothyroidism but paradoxically near-normal circulating thyroid hormone concentrations. A patient described in an abstract[17] had some hypothyroid features and near-normal hormone concentrations, but also many dissimilar characteristics that may not be caused by the *THRA* defect.

Interpretation

We describe the first patients with a mutation common to TRα1 and the variant TRα2 protein derived from the same gene. Their clinical (growth and developmental retardation, constipation, macrocephaly) and biochemical features (subnormal ratio of free thyroxine:free tri-iodothyronine [T_3], low reverse T_3) are similar to previous patients with resistance to thyroid hormone caused by mutation in TRα1, with no added phenotype attributable to the presence of mutant TRα2. T_3 reverses mutant receptor dysfunction in vitro and thyroxine therapy alleviates hormone resistance in vivo; starting treatment with thyroxine in childhood might have ameliorated their clinical phenotype. Future identification of other patients, based on common characteristics of these and previous patients that now define the syndrome, will be of clinical importance if early thyroxine treatment proves to be widely beneficial.

Contributors
CM, MA, WEV, ES, NS, MG, and KKC designed the study, collected, analysed, and interpreted data and wrote the report. ACO, KP, OR, GL, DH, DC, AE, CB, and SA collected, analysed, and interpreted data.

Declaration of interests
We declare no competing interests.

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