Resistance to Thyroid Hormone α- emerging definition of a disorder of thyroid hormone action

Carla Moran and Krishna Chatterjee

University of Cambridge Metabolic Research Laboratories, Wellcome Trust-MRC, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge CB2 0QQ, United Kingdom

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Address for correspondence and reprint requests:
V Krishna K Chatterjee, University of Cambridge, Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Level 4, Box 289, Addenbrooke’s Hospital, Cambridge, CB2 0QQ. Tel: (44)-1223-336842; Fax: (44)-1223-330598; Email: kkc1@medschl.cam.ac.uk

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Thyroid hormones (TH) act via nuclear receptors, encoded by separate genes (THRA, THRβ), with differing tissue distributions (TRα1: central nervous system, myocardium, skeletal muscle, gastrointestinal tract; TRβ1: liver, kidney; TRβ2: hypothalamus, pituitary, cochlea, retina). Alternative splicing of THRA generates a variant, non TH-binding, protein (TRα2), with a divergent carboxyterminal domain, whose function is not understood (1).

Resistance to thyroid hormone β (RTHβ), usually due to heterozygous mutations in THRβ, is characterised by raised T4 and T3 levels, non-suppressed TSH and a variable phenotype encompassing both hyperthyroid (e.g. failure to thrive, raised metabolic rate, tachycardia, low bone density, anxiety) and hypothyroid (e.g. dyslipidaemia) features (2). Consonant with its dominant mode of inheritance, dominant negative inhibition of wild type receptor function by mutant TRβ is central to the pathogenesis of this disorder (3). Although TRβ and α proteins exhibit marked aminoacid sequence similarity, such that introduction of a β receptor mutation causing RTHβ into the murine TRα gene is associated with an abnormal phenotype (e.g. growth retardation, locomotor abnormalities) (4), the homologous human disorder (Resistance to Thyroid Hormone α, RTHα), eluded discovery for over a decade probably because it was not associated with a readily-recognisable diagnostic signature. RTHα, due to a highly deleterious mutation (E403X), truncating the carboxyterminal region of TRα1, was first identified in a six yr old girl with hypothyroid features (e.g. skeletal dysplasia with growth retardation, neurodevelopmental delay, constipation) in selected target tissues, but associated with near-normal (borderline low or normal T4, borderline high or normal T3, normal TSH) thyroid function tests (5). Subsequently, further childhood plus adult male and female cases, harbouring frameshift/premature stop mutations also involving the carboxyterminus of TRα1 were found, with recognition of additional, potentially pathognomonic, features (macrocephaly, anaemia, dysmorphic, facies) (6,7). Indeed, recognition of a distinct, shared, phenotype encompassing macrocephaly and dysmorphic features in a single clinic, led to the identification of a further five cases with similar, carboxyterminal TRα1 mutations (8). Where functionally characterised, these TRα1 mutants exhibited negligible hormone binding and functional activity, with failure to dissociate from
transcriptional corepressor and recruit coactivator proteins – properties that are highly analogous to those of dominant negative TRβ mutants in RTHβ (3). From these reports, a biochemical signature for RTHα also seemed to emerge; although TH levels could be in the normal range, a computed T4/T3 ratio or reverse T3 levels (where ascertained) were subnormal (5,8,9). Following this, a missense THRA mutation (A263V), located in a receptor region common to both TRα1 and TRα2 proteins, was identified in three members of a family with similar clinical (growth and developmental retardation, constipation, macrocephaly) features. The A263V TRα1 mutant showed partial loss-of-function, being transcriptionally impaired at low TH concentrations in vitro, with reversal of this at higher T3 levels. It was tempting to speculate that such partial preservation of mutant TRα1 function accounted for a milder phenotype in this family. However, the possibility that thyroxine treatment of these cases since early childhood had also contributed to amelioration of the phenotype, could not be discounted. Concordant with no measurable gain- or loss-of-function of A263V mutant TRα2 compared with its normal counterpart, no added phenotypes attributable to mutated TRα2, could be discerned in these cases (10). In contrast, some unusual added clinical features (micrognathia, clavicular agenesis, metacarpal fusion and syndactyly of digits, hyperparathyroidism, chronic diarrhoea) were reported in an adult female harbouring a different mutation (N359Y), common to both TRα1 and TRα2 proteins (11).

In this context, the report by Demir and colleagues in the current issue of JCEM (12), describing three different THRA mutations (C380fs387X, R384H, A263S) in 10 RTHα patients from 3 different families, together with associated clinical features, clarifies several aspects of this disorder. The authors were able to discern a gradation in phenotype associated with different genotypes: a child, harbouring a highly deleterious mutation (C380fs387X) involving the carboxyterminus of TRα1, is growth retarded with cognitive and motor deficits that result in severe handicap; moderate cognitive deficit, motor abnormalities and growth retardation in infancy but less evident in adulthood, were recorded in a child and mother with a partial loss-of-function mutation (R384H) in TRα1; in a large family, harbouring A263S mutant TRα1 with slightly impaired function, mild growth retardation and
slight developmental delay in childhood culminated in average/low-average adult IQ, with constipation being a relatively conserved clinical feature. The A263S mutation in THRA reported by Demir et al, as well as another RTHα-associated mutation (D211G) reported recently by the same group, also involve the TRα2 protein (12,13). No added phenotypic characteristics were observed in nine affected individuals harbouring one or other of these mutated residues. Accordingly, it is conceivable that the unusual skeletal and other abnormalities unique to the patient with the N359Y THRA mutation, are attributable to a defect in another pathway; although whole exome sequencing was undertaken in this case, a pathogenic abnormality (for example in a non-coding gene region) has not been excluded (11). Alternatively, as depicted when RTHα mutations reported hitherto are mapped on the crystal structure of TRα1, the Asparagine to Tyrosine change at codon 359 (N359Y), involves a residue that is in an unusual location in the receptor protein compared to the position of other RTHα-associated mutations (Figure 2); therefore, the possibility of the N359Y substitution inducing a specific functional alteration in the receptor that is linked to unique, additional phenotypes, cannot be discounted. With the identification of a more functionally diverse repertoire of TRα1 mutations, a specific biochemical signature for RTHα seems less clearcut. Specifically, in some cases, particularly associated with mild TRα mutations (e.g. A263S, D211G), normal reverse T3 levels have been documented (12,13). The T4/T3 or T3/rT3 ratio remains abnormal in most cases, overlapping minimally with values in unaffected family members (12), but this abnormal thyroid function test pattern is a recognised feature of other disorders (e.g. MCT8 deficiency) (14); likewise, although mild anaemia is almost universal in RTHα, this parameter is unhelpfully nonspecific. Studies in a transgenic model of RTHα raised the possibility of circulating trace element (selenium) levels having diagnostic utility (15), but did not translate readily into the human context (7). In RTHβ, even subtle TRβ mutants exhibit impaired negative feedback regulation of hypothalamo-pituitary target genes (TRH, TSH) (16), reflected in pathognomonic, raised, TH levels. It has been suggested that a combination of raised type 1 deiodinase and reduced type 3 deiodinase enzyme activities accounts for thyroid biochemical abnormalities in RTHα (17); whether another TH metabolite that reflects such
altered deiodinase activity more consistently or a biomarker associated with another universally
dysregulated pathway in this disorder, can be identified, remains to be seen.

With the estimated prevalence of RTHβ being ~1 in 40,000, with over 160 different TRβ mutations
recorded hitherto, it is likely that RTHα is more common, but underdiagnosed. Both maternal and
paternal transmission of TRα mutations has now been recorded in several families (6, 8, 10, 12, 13),
suggesting that, unlike in a murine transgenic model (18), fertility in either gender is not unduly
compromised. However, screening for THRA defects based on current, documented, phenotypes may
artificially enrich for identification of particular TRα mutations (e.g. involving the carboxyterminal
receptor region). Supporting this notion, whole exome sequencing (WES), identified a TRα1 variant
(R384C), known to be pathogenic in transgenic mice (4), associated with autism spectrum disorder –
an unexpected phenotype (19). Indeed, interrogation of exome databases (e.g. ExAC;
http://exac.broadinstitute.org/gene/ENSG00000126351) reveals many rare, nonsynonymous THRA
variants including several in TRα1 predicted to be damaging, based either on homology to known
RTHβ mutations or structural modelling. However, in the absence of definitive diagnostic criteria
(clinical, biochemical) for RTHα, it may be necessary to develop functional assays to assess the
putative pathogenicity of receptor variants, as has been done for PPARG variants identified in WES of
type 2 diabetes cohorts (20). TRβ mutations causing RTHβ cluster within three regions of its hormone
binding domain and, as illustrated in Figure 1, many RTHα-associated TRα mutations have TRβ
counterparts, involving the same aminoacids, which localise to these clusters. As the repertoire of
RTHα-associated TRα mutations grows, it will be interesting to see whether they also localise to
particular receptor regions. One functional constraint on RTHβ-associated TRβ mutations is
preservation of the ability to exert dominant negative activity, such that receptor regions mediating
functions (DNA binding, interaction with corepressor and RXR) that are required for such activity are
devoid of naturally-occurring mutations (3); if dominant negative inhibition is also central to the
pathogenesis of RTHα, this property may also constrain localisation of RTHα mutations within TRα.

Seven out of fourteen TRα mutations recorded hitherto have occurred de novo, with mutations
involving some residues (e.g. Alanine 263, Arginine 384, Glutamic Acid 403) occurring more than
once in unrelated families; as has been shown in RTHβ (21), it is possible that nucleotide substitutions involving mutation-prone, CpG dinucleotides will occur more frequently, resulting in some aminoacid changes being overrepresented.

Although not identified via neonatal screening (in a program with both T4 and TSH measurement), thyroxine therapy from age 18 months has been beneficial for growth, motor development and constipation in a childhood case with a partial loss-of-function mutation (D211G) (13). Despite the first recorded patient harbouring a more deleterious E403X TRα mutation (5), we have observed similar benefit following five years of thyroxine therapy (Moran, Chatterjee and Dattani unpublished observations). Accordingly, despite the relative paucity of data, a trial of thyroxine therapy in RTHα cases harbouring both mild and severe loss-of-function TRα mutations may be indicated. However, careful surveillance to not only assess therapeutic benefit but also monitor for unwanted tissue toxicity (e.g. in liver, which expresses mainly TRβ), is warranted. In this context, as in RTHβ, reliable biomarkers of tissue responses to TH are needed. In the future, it also conceivable that genetic stratification may guide therapy of RTHα patients, with thyroxine or selective thyromimetics being used in patients harbouring TRα mutations with residual functional activity, with alternative approaches (e.g. blocking formation or activity of receptor-transcriptional corepressor complexes) in cases with severe, hormone-refractory, TRα defects.

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FIGURE LEGENDS

Figure 1. Schematic alignment of TRα1, TRα2 and TRβ1 showing functional regions (N-terminal, DNA binding domain DBD, hormone binding domains); the divergent carboxyterminus of TRα2 is cross-hatched. The location of recorded RTHα mutations, involving either TRα1 alone or both TRα1 and α2 proteins, is superimposed. Three regions of TRβ1 (aminoacids 234 to 282, 309 to 353 and 426 to 460), within which RTHβ mutations cluster are depicted. Within these clusters, published or our unpublished (D265A) RTHβ mutations, involving aminoacids homologous to residues mutated in RTHα, are shown.

Figure 2: The crystal structure of the hormone binding domain of human TRα1 bound to T3 (PDB 2H77, magenta) is shown. The location of aminoacids which are mutated (missense or premature stop or frameshift/premature stop) in RTHα is superimposed (red balls). The carboxyterminal region (distal helix 11 to helix 12) within which RTHα mutations occur, is highlighted in yellow.