Nuclear receptors and human disease: thyroid receptor \( \beta \), peroxisome-proliferator-activated receptor \( \gamma \) and orphan receptors

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Abstract

The nuclear receptor superfamily comprises a group of proteins that includes the molecular targets for classical steroid hormones such as glucocorticoids, androgens and vitamin D, together with a number of so-called ‘orphan’ receptors whose ligands and/or function remain to be determined. Many of the world’s most commonly prescribed drugs act via nuclear receptors, attesting to their importance as therapeutic targets in human disease (for example, the novel anti-diabetic thiazolidinediones rosiglitazone and pioglitazone are high-affinity ligands for peroxisome-proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \))). The study of transgenic mice harbouring global and tissue-specific alterations in nuclear receptor genes has greatly enhanced our understanding of the roles that these receptors play in mammalian physiology. In many cases, these findings have been complemented by the study of human subjects harbouring naturally occurring mutations within the corresponding receptor, whereas in others, such studies have served to highlight important differences that exist.

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between human and mouse physiology especially, for example, in relation to aspects of metabolism. Here we review the diverse clinical phenotypes that have been reported in subjects found to have germline mutations in thyroid hormone receptor β, PPARγ, hepatocyte nuclear factor 4α, small heterodimer partner, steroidogenic factor 1, DAX1, photoreceptor-specific nuclear receptor and NUR-related factor 1, and consider the molecular mechanisms through which aberrant signalling by mutant receptors might contribute to the pathogenesis of the associated disorders.

Introduction

Classical steroid hormones (e.g. glucocorticoids, mineralocorticoids, vitamin D) act principally within the cell nucleus to modulate target gene transcription through binding to specific receptors (e.g. glucocorticoid receptor, mineralocorticoid receptor, vitamin D receptor). These receptors are members of a broader nuclear receptor superfamily, which also includes proteins that are the targets for structurally unrelated ligands (e.g. thyroid hormone and retinoic acid), together with a large number of novel proteins, which were originally designated as ‘orphan’ receptors pending identification of their cognate ligand(s) [1]. However, the orphan status of several of these receptors has been challenged as evidence has begun to emerge of endogenous ligands that are capable of regulating receptor function at concentrations that approximate those found in vivo. Many of these molecules have also proved to be structurally distinct from steroid hormones, and bind with much lower affinities to receptors that are often more permissive. For example, a variety of fatty acid derivatives and eicosanoids have been shown to regulate peroxisome-proliferator-activated receptor γ (PPARγ) function.

As ligand-inducible transcription factors, nuclear receptors are organized in functional domains which are highly conserved among family members (Figure 1A): the N-terminal region often encodes an intrinsic transcriptional activation function (AF-1), a central DNA-binding domain (DBD) mediates receptor interaction with regulatory DNA sequences or response elements in target gene promoters, and the C-terminal region harbours the ligand-binding domain (LBD) and encompasses a powerful ligand-dependent transactivation function (AF-2). Inspection of the primary amino acid sequence encoding the central domain of virtually all nuclear receptors reveals the presence of two cysteine-rich motifs, each of which co-ordinates a zinc ion to form a ‘finger-like’ structure capable of directing sequence-specific DNA-binding (Figure 1A). The specificity of this interaction is mediated at least in part through the P-box, which lies at the base of the first zinc finger, with different amino acids within this region dictating DNA-response-element recognition. Residues within the P-box are encompassed within an α-helix that interacts directly with the major groove of DNA (see Chapter 5 in this volume). For some receptors, residues within an A-box form an additional α-helix which interacts with the minor
groove of DNA (Figure 1A). Several receptors [e.g. glucocorticoid receptor, mineralocorticoid receptor, androgen receptor and hepatocyte nuclear factor 4α (HNF4α)] form homodimeric complexes on response elements consisting of palindromic arrangements of two hexanucleotide motifs, whereas others [e.g. thyroid hormone receptor (TR), retinoic acid receptor, vitamin D receptor and PPAR] interact with a tandem repeat arrangement of hexanucleotide motifs as a heterodimer with the retinoid X receptor, another member of the nuclear receptor family [2]. A third type of receptor–DNA interaction is exhibited by some orphan receptors [e.g. nerve growth factor inducible factor I-B and steroidogenic factor 1 (SF1)], which bind monomerically to extended response elements that include additional nucleotides 5′ to a core hexanucleotide motif.

The hallmark of nuclear receptors is their ability to modulate transcription in response to ligand occupancy. Many undergo a conformational change upon hormone binding which facilitates the recruitment of a complex of ‘co-activators’ [e.g. steroid receptor co-activator-1 (SRC-1), cAMP-response-element-binding protein (CREB)-binding protein (CBP) and p300/CBP-associating factor (pCAF)], which modify the chromatin structure so as to permit transcriptional activation (Figure 1B; see Chapter 6 in this volume). In the absence of ligand, a subset of receptors, which includes TR and retinoic acid receptor, is capable of mediating transcriptional repression through recruitment of a distinct co-repressor complex [e.g. silencing mediator for retinoid and thyroid hormone receptors (SMRT)/nuclear receptor co-repressor (NCoR), Sin3a and histone deacetylase; see Chapter 7 in this volume].

Mutations in nuclear receptor genes form the basis of a number of inherited human diseases (Table 1). In the majority of cases the link between nuclear receptor defects and a particular human phenotype has been identified using one of two lines of investigation: first, the ‘candidate gene’ approach with direct sequence analysis of a gene of interest in affected subjects (as exemplified by the identification of mutations in human PPARγ in subjects with severe insulin resistance and partial lipodystrophy); secondly, the ‘reverse genetic’ approach in which linkage studies have associated a disease with a chromosomal locus, following which positional cloning has identified a gene encoding a nuclear receptor [e.g. mutations in HNF4α in maturity onset diabetes of the young type 1 (MODY1)].

Human nuclear receptor gene defects may be broadly categorized into germline (inherited or sporadic) or somatic. The latter class includes mosaic expression of a mutant receptor and gene rearrangements occurring in tumour cells, generating, for example, the promyelocytic leukaemia–retinoic acid receptor α oncoprotein in acute promyelocytic leukaemia (APML) [3], or the recently described PAX8–PPARγ1 fusion protein in thyroid follicular neoplasia [4]. For simplicity, this chapter will focus on germline mutations in a selected group of nuclear receptors [TRβ, PPARγ, HNF4α, small heterodimer partner (SHP), SF1, dosage-sensitive sex reversal-adrenal hypoplasia congenita (AHC) critical region on the X chromosome gene 1 (DAX1), photoreceptor-specific nuclear
(A)  

(B)  

Adipocyte differentiation  
Insulin sensitization  
Macrophage: inflammation, lipid trafficking  
Neoplasia
receptor (PNR) and NUR-related factor 1 (NURR1)], highlighting the advances in our understanding of nuclear receptor biology that have been made through the study of these comparatively rare human disorders.

**TRβ and the syndrome of resistance to thyroid hormone (RTH)**

**Background**

In mammals, the TR exists in two major isoforms, TRα (NR1A1) and TRβ (NR1A2), which are encoded by genes on chromosomes 17 and 3 respectively. Both gene loci give rise to two protein products each, TRα1 and TRα2 (which differ at the C-terminal end, with only the former able to bind ligand) and TRβ1 and TRβ2 (which differ at the N-terminal end, such that both retain the ability to bind T3 with high affinity). These isoforms exhibit differing tissue expression patterns: for example, TRα1 is the main species in the myocardium and skeletal muscle; TRα2, which may act as a functional antagonist of other TR isoforms, is expressed in a variety of tissues (e.g. brain and testis); TRβ1 predominates in the liver and kidney; both TRβ1 and TRβ2 are highly...
Table 1. Disorders associated with mutations in human nuclear receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Disorder</th>
<th>Clinical features</th>
<th>Mode of inheritance</th>
</tr>
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<tbody>
<tr>
<td>TRβ1</td>
<td>Resistance to thyroid hormone</td>
<td>Goitre, tachycardia, failure to thrive, ADHD</td>
<td>AD, AR, S</td>
</tr>
<tr>
<td>VDR</td>
<td>Hereditary vitamin D-dependent rickets (type II)</td>
<td>Hypocalcaemia, rickets, alopecia</td>
<td>AR</td>
</tr>
<tr>
<td>ERα</td>
<td>Oestrogen resistance</td>
<td>Tall stature, delayed epiphyseal fusion, osteoporosis (male)</td>
<td>AR</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid resistance</td>
<td>Fatigue, hypertension, hyperandrogenism, infertility</td>
<td>AD, AR, S</td>
</tr>
<tr>
<td>MR</td>
<td>Pseudohypoaldosteronism</td>
<td>Hypotension, salt loss</td>
<td>AD</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen-insensitivity syndrome</td>
<td>Partial or complete failure of masculinization</td>
<td>XL</td>
</tr>
<tr>
<td>PPARγ&lt;sup&gt;a&lt;/sup&gt;</td>
<td>PPARγ ligand resistance</td>
<td>Partial lipodystrophy, insulin resistance, hypertension</td>
<td>AD</td>
</tr>
<tr>
<td>HNF4α</td>
<td>Maturity onset diabetes of the young type I</td>
<td>Early-onset type 2 diabetes</td>
<td>AD</td>
</tr>
<tr>
<td>SHP</td>
<td>Maturity onset diabetes of the young type</td>
<td>Early-onset type 2 diabetes</td>
<td>AD</td>
</tr>
<tr>
<td>SFI</td>
<td>Adrenal hypoplasia congenita</td>
<td>Primary adrenal failure, XY sex reversal</td>
<td>AD, AR</td>
</tr>
<tr>
<td>DAX1</td>
<td>Enhanced S cone syndrome</td>
<td>Primary adrenal failure, hypogonadotrophic hypogonadism, impaired spermatogenesis</td>
<td>XL</td>
</tr>
<tr>
<td>PNR</td>
<td>(i) Familial Parkinson's syndrome</td>
<td>Resting tremor, bradykinesia, cogwheel rigidity</td>
<td>AD</td>
</tr>
<tr>
<td>NURR1</td>
<td>(ii) Schizophrenia/manic-depressive disorder</td>
<td>Increased sensitivity to blue light, visual loss</td>
<td>AR</td>
</tr>
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<sup>a</sup>A second variant P115Q has been reported in association with obesity in four subjects.
expressed in the hypothalamus and pituitary. Although studies in mice have greatly facilitated our understanding of the differing roles of these receptor subtypes in normal physiology and have therefore provided clues to the human phenotypes that one might anticipate in subjects harbouring mutant TRs, only one disorder consequent on aberrant TR signalling has been identified hitherto in humans, the syndrome of RTH. This condition, first described more than three decades ago by Refetoff and colleagues [5], is characterized by elevated circulating free thyroid hormone levels with a failure to suppress pituitary thyroid-stimulating hormone secretion and variable refractoriness to hormone action in the periphery [6]. In the majority of cases, affected individuals are heterozygous for diverse mutations in the LBD of TRβ (occurring within a region common to both TRβ1 and TRβ2 variants).

**Molecular genetics and mutant receptor properties in RTH**

Shortly after the cloning of the TRα and TRβ genes, Usala and colleagues [7] reported tight linkage between RTH and the TRβ gene locus in a kindred with generalized resistance to thyroid hormone (GRTH; see below). Subsequently, >100 different mutations have been described in more than 500 RTH kindreds worldwide, with the majority being inherited in an autosomal dominant manner, but with a significant minority (20–25%) arising sporadically. However, a small number of subjects exist in whom no mutation in TRβ can be demonstrated despite compelling clinical and biochemical evidence for the disorder (so-called ‘TRβ-negative RTH’), suggesting that abnormalities in other genes (e.g. transcriptional cofactors) can mimic the RTH phenotype. Data from animal studies support this notion, with mice harbouring deletion of SRC-1 exhibiting an RTH phenotype [8], as do animals doubly heterozygous for disruptions of SRC-1 and the homologous nuclear receptor co-activator transcriptional intermediary factor-2 (TIF-2) [9]. On the other hand, mice harbouring a mutation in TRα1 exhibit a phenotype dissimilar to RTH.

To date, virtually all of the mutations reported in RTH cluster within three regions of the LBD, often referred to as the ‘codon 200’ (residues 234–282), ‘codon 300’ (residues 310–353) and ‘codon 400’ (residues 429–461) mutations. In keeping with their location, mutant TRβ exhibit impaired hormone-binding and transcriptional activity. In addition, the mutant receptors are capable of inhibiting the action of their wild-type (WT) counterpart when co-expressed (Figure 1C) [10]. Clinical observations from two unusual cases of RTH provide evidence in support of the ‘dominant-negative’ inhibitory effects of mutant TRβ in vivo: in a unique family with recessively inherited RTH, individuals who were heterozygous for a deletion of one allele of the TRβ gene were clinically and biochemically unaffected [5], an observation which is consistent with findings in heterozygous TRβ-knockout mice [11]; conversely, a child exhibiting homozygosity for a dominant-negative TRβ mutant demonstrated a severe clinical phenotype with biochemical evidence of extreme resistance to thyroid hormone action [12]. Indeed, this critical requirement for
dominant-negative activity by mutant TRβ in the pathogenesis of the disorder may explain why other regions of the receptor appear to be devoid of naturally occurring mutations (so-called ‘cold areas’), reflecting the fact that a TRβ mutant must retain certain key properties (e.g. ability to heterodimerize with retinoid X receptor, bind to DNA and recruit transcriptional co-repressor) in order to induce the phenotype of RTH (Figure 1C).

Clinical features and management in RTH

A palpable goitre is the commonest presenting feature in RTH, and often triggers thyroid function tests, which may then reveal the distinctive biochemical signature of the disorder. Many of these individuals are otherwise asymptomatic and are deemed to have ‘generalized’ resistance (GRTH), a ‘euthyroid’ state in which high thyroid hormone levels are thought to compensate for global tissue resistance. However, the clinical manifestations of RTH are highly variable (Table 2), and other subjects may exhibit frank thyrotoxic features (e.g. failure to thrive in childhood, low body-mass index, tachycardia or dysrhythmia), suggesting predominant pituitary resistance to thyroid hormone (PRTH), a state in which peripheral tissues retaining ‘normal’ sensitivity to T₃ are exposed to high circulating hormone levels. Both GRTH and PRTH are associated with TRβ mutations, indicating that the two disorders represent phenotypic variants of a single genetic entity [13]. Moreover, whereas the clinical distinction between GRTH and PRTH remains useful in guiding management, there is significant overlap between these ‘states’. For example, tachycardia, hyperkinetic behaviour and anxiety have been documented in individuals deemed to have GRTH; conversely, serum-sex-hormone-binding globulin (a hepatic index of thyroid hormone action) is typically normal in PRTH, suggesting that resistance is not solely confined to the hypothalamic/pituitary/thyroid axis (HPG axis).

In general, treatment of RTH is governed by the prevailing symptoms. Thus subjects with ‘compensated’ GRTH rarely require intervention. Surgery or radio-iodine to treat the biochemical disturbance or goitre frequently fails, and may serve only to complicate the clinical picture by rendering the RTH patient hypothyroid [14]. In contrast, subjects with overt thyrotoxic features

<table>
<thead>
<tr>
<th>Table 2. Features of the syndrome of RTH</th>
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<tr>
<td>Elevated serum-free thyroid hormones</td>
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<tr>
<td>Unsuppressed TSH with enhanced bioactivity</td>
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<tr>
<td>Goitre</td>
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<tr>
<td>Tachycardia, atrial fibrillation, heart failure</td>
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<tr>
<td>Low body-mass index in childhood</td>
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<tr>
<td>Growth retardation, short stature</td>
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<tr>
<td>Attention-deficit hyperactivity disorder, low IQ</td>
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<tr>
<td>Ear, nose and throat infections, hearing loss</td>
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<td>Osteopenia</td>
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(PRTH) may benefit from attempts to reduce pituitary thyroid-stimulating hormone output; most easily achieved by the administration of a thyroid hormone analogue such as TRIAC (3,3,5-tri-iodothyroacetic acid), which preferentially binds TRβ and exerts a predominantly pituitary thyromimetic effect, thereby lowering circulating free thyroid hormone levels. However, the picture is complex and what is beneficial for one tissue (e.g. heart) may be deleterious to another. A relatively hypothyroid state within the liver may cause hypercholesterolaemia. Moreover, temporal variations in receptor sensitivity have been observed, and accordingly periodic review of the treatment strategy in any given individual is mandatory.

**PPARγ and the syndrome of PPARγ ligand resistance (PLR)**

**Background**

PPARγ (NR1C3) was first characterized as a transcription factor that regulates target gene expression in adipocytes and induces pre-adipocyte differentiation [15]. The PPARγ gene (on chromosome 3) undergoes alternative splicing to generate two protein products: a long PPARγ2 isoform (containing 28 additional residues in the N-terminal domain) with a restricted pattern of expression (high levels in adipose tissue), and a shorter PPARγ1 variant, which is more widely distributed. Although it is little more than a decade since the receptor was first cloned, there is already an extensive body of data implicating it in a diverse array of biological processes that extend beyond the adipocyte. For example, PPARγ mediates inhibition of inflammatory cytokine production (interleukin-6 and tumour necrosis factor-α) from monocytes [16], whereas receptor activation by oxidized low-density-lipoprotein-derived ligands promotes cholesterol trafficking in macrophages [17]. Recently, a somatic gene rearrangement, which results in the generation of a mutant PAX8–PPARγ fusion protein, has been reported to occur in 20–40% of human thyroid follicular tumours [4].

However, it is in relation to adipocyte biology and control of tissue insulin sensitivity that PPARγ has come to the fore, coinciding with the recent introduction of the thiazolidinediones (TZDs; e.g. rosiglitazone and pioglitazone), a novel class of anti-diabetic agent, for use in the management of Type II diabetes mellitus (T2DM). These agents are high-affinity ligands for PPARγ and reduce blood-glucose levels by enhancing tissue sensitivity to insulin action *in vivo* [18]. Recognition that PPARγ is the molecular target for these compounds strongly implicated the receptor in mammalian glucose homoeostasis and suggested that variation(s) in the human gene might be associated with alteration(s) in insulin sensitivity. Accordingly, abnormalities in glucose homoeostasis and adipogenesis have been the focus of attention for those studying aberrant PPARγ signalling in humans.
A polymorphism (Pro-12→Ala) within the unique N-terminus of PPARγ2 (allelic frequency up to 15% in certain populations), the most common human genetic variant reported to date, has been reported to afford protection against the risk of developing T2DM [19]. Indeed, it has been estimated that the global prevalence of T2DM might be reduced by as much as 25% if the entire population carried the allele containing Ala-12, thereby conferring a major population benefit for a relatively minor change in receptor function, with the less transcriptionally active Ala-12 variant promoting a lower rate of accretion of adipose tissue mass, leading to a preservation of insulin sensitivity [20]. However, there continues to be vigorous debate regarding both the extent of the proposed benefit and the mechanism through which it might occur, with recent evidence suggesting a complex interaction in which this genetic variant is influenced by environmental factors including dietary fatty acid intake [21].

A much rarer genetic mutation, Pro-115→Gln (P115Q in PPARγ2 nomenclature), which also occurs within the N-terminal domain of PPARγ, has been identified in a small number of obese subjects. In vitro studies suggest that the Pro-115 to Gln mutation renders the receptor more constitutively active than its WT counterpart (through disruption of receptor phosphorylation at an adjacent residue, Ser-114), and therefore predisposes to enhanced adipogenesis and obesity [22]. Moreover, preservation or even enhancement of insulin sensitivity in the face of such weight gain was also postulated as a possible component of this ‘gain-of-function’ phenotype, although the presence of diabetes in three of four reported subjects would seem to argue against the latter. The identification of further affected subjects with this mutation should help to clarify these observations.

Recently, we and others have reported a second class of genetic mutation in PPARγ, with the identification of loss-of-function mutations within the receptor LBD. These mutations are analogous to those previously reported in RTH, and we have therefore termed the clinical syndrome PLR.

**Molecular genetics and mutant receptor properties in PLR**

With the recognition that TZDs act via PPARγ, this candidate gene was screened in a cohort of 85 subjects with severe insulin resistance (defined by the co-existence of extreme hyperinsulinaemia and acanthosis nigricans). Initially we identified two different heterozygous missense mutations (Pro-467→Leu and Val-290→Met; P467L and V290M in PPARγ1 nomenclature) in the receptor LBD in three affected individuals [23]. Both mutations impaired receptor function through destabilization of helix 12, an amphipathic α-helix at the C-terminal end of the LBD which facilitates both ligand-binding and recruitment of transcriptional co-activators (Figure 2) [24]. Moreover, in a manner analogous to their TRβ counterparts in RTH, the mutant receptors inhibited WT function in a dominant-negative manner. Subsequently, other groups have identified additional loss-of-function mutations within the PPARγ LBD, Arg-425→Cys and Phe-388→Leu in
PPARγ2 (corresponding to Arg-397→Cys and Phe-360→Leu in PPARγ1; Figure 2), with all affected subjects exhibiting insulin resistance [25,26].

**Clinical features and management in PLR**

In keeping with the central role of PPARγ in adipogenesis, we now recognize that a stereotyped pattern of partial lipodystrophy is a cornerstone of the clinical phenotype of the PLR syndrome, with selective diminution of gluteal and limb fat but relative preservation of central adiposity. Whereas lipodystrophy itself is known to predispose to insulin resistance, detailed metabolic studies in one affected subject suggest that other factors may also...
contribute to its pathogenesis in this particular setting [27]. For example, subcutaneous adipocytes from a male harbouring the Pro-467→Leu mutation appear to be relatively metabolically inert, demonstrating both impaired lipogenesis and lipolysis. In addition, circulating levels of adiponectin, an adipokine that enhances insulin action in skeletal muscle and liver, were strikingly reduced in subjects harbouring PPARγ mutations when compared with healthy controls or subjects with severe insulin resistance not attributable to mutations in PPARγ [28].

Several other features of the human metabolic syndrome [including hypertension, dyslipidaemia (low high-density lipoprotein cholesterol, high triacylglycerols) and hepatic steatosis] are common findings in those with PLR, and affected females show a tendency to the polycystic ovarian syndrome. Another intriguing finding in the proband from the original Pro-467→Leu kindred was the occurrence of severe pre-eclampsia in both pregnancies. While this observation is potentially highly significant when coupled with knowledge that the murine PPARγ-gene knockout is lethal in utero due to combined cardiac and placental defects, its occurrence will need to be substantiated in further cases to determine whether this is truly an integral component of the human PLR syndrome (Table 3) [29].

Currently, the management of those with PLR is directed, where possible, at treatment of each individual component of the syndrome, i.e. insulin resistance/T2DM, hypertension and dyslipidaemia, together with their attendant complications. However, the availability of TZDs would appear to offer, at least in principle, a disease-specific therapy which should target the facets (lipodystrophy and insulin resistance) which underpin the syndrome. To date, we have had the opportunity to treat one individual from each original kindred with rosiglitazone. The subject from the Pro-467→Leu family exhibited a dramatic improvement in glycaemic control, which correlated with enhanced insulin sensitivity and accretion of adipose tissue. In marked contrast, the Val-290→Met proband showed very little clinical response. These findings appeared to correlate with the in vitro properties of the mutant receptors, with the Val-290→Met mutant exhibiting persistent dominant-negative activity even at the highest concentration of rosiglitazone, suggesting that a more potent receptor ligand might be needed to achieve a therapeutic effect [27].

Table 3. Features of the human PLR syndrome

| Partial lipodystrophy (especially limb and gluteal) |
| Insulin resistance with or without T2DM |
| Dyslipidaemia (high triacylglycerols, low high-density lipoprotein cholesterol) |
| Hypertension |
| Hepatic steatosis |
| Polycystic ovarian syndrome |
| (?) Pre-eclampsia |

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Digenic insulin resistance

Most recently, we have studied an unusual kindred in which five subjects exhibit severe insulin resistance [30]. Screening the PPARγ gene in this family revealed a novel heterozygous frameshift/premature stop mutation within the DNA-binding domain in seven individuals. As predicted, the truncated PPARγ mutant failed to bind DNA and regulate target gene transcription. In addition, it exhibited no detectable dominant-negative activity when co-expressed with the WT receptor. Remarkably, further candidate gene studies in this family identified a second heterozygous frameshift/premature stop mutation in protein phosphatase 1 regulatory subunit 3A (PPP1R3A), which encodes a protein expressed in cardiac and skeletal muscle that regulates activity of glycogen synthase, the rate-limiting enzyme in glycogen synthesis. Functional studies with the truncated PPP1R3A mutant protein indicated that it mis-localizes intracellularly, therefore suggesting that it is unlikely to function effectively in regulating glycogen synthase activity.

Correlation of genotypes with metabolic parameters within this kindred revealed that while either gene defect alone was insufficient to mediate the clinical phenotype, double heterozygosity for both PPARγ and PPP1R3A gene defects in five subjects resulted in severe insulin resistance. To our knowledge, there is no direct link between PPARγ and PPP1R3A action and the two genes are principally expressed in different tissues (adipose tissue and skeletal muscle, respectively). This kindred represents the first reported example of a digenic cause of human insulin resistance and suggests that a ‘metabolic dialogue’ between fat and skeletal muscle dictates insulin sensitivity.

HNF4α and MODY1

HNF4α (NR2A1) remains a true orphan nuclear receptor with no convincing endogenous ligand(s) identified to date. Its name derives from the fact that it was first cloned from rat liver, in a manner similar to HNF1α and β, HNF3α, β and γ, and HNF6. Although the nomenclature suggests that these proteins constitute a family of hepatic transcription factors, they are structurally quite distinct and their expression extends beyond the hepatocyte with, for example, HNF1α, HNF1β and HNF4α also being expressed in pancreatic islets [31]. Intriguingly, however, and perhaps to justify the original nomenclature, the functions of several of these proteins have been shown to be intimately related; for example, in pancreatic β-cells, HNF1α, HNF1β and HNF4α regulate the expression of the insulin and other genes involved in glucose transport and metabolism. Furthermore, mutations in HNF genes are associated with a specific subtype of early onset T2DM referred to as MODY [31]. This disorder, which is characterized by defective pancreatic β-cell function, is deemed to be present when T2DM occurs in at least two generations with at least one member being affected under the age of 25 years. MODY has long been recognized to be a heterogeneous entity, and it is now clear that this is reflected in a number of
different genes being involved (e.g. MODY type 1, HNF4α; type 2, glucokinase; type 3, HNF1α; MODY X, defective gene not yet identified).

In 1996, Bell and colleagues [32] were able to demonstrate linkage of MODY1 to a particular region of chromosome 20 (20q12–13) which encompasses the HNF4α gene locus [32]. Sequence analysis in a single large pedigree demonstrated that affected individuals were heterozygous for a nonsense mutation (Gln-268→Xaa) in the HNF4α gene, consistent with the dominant mode of inheritance of this disorder. To date, more than ten different missense, nonsense, frameshift and in-frame deletion/insertion mutations have been reported in the gene, predominantly in families with a MODY1 phenotype. However, in a small number of cases the possibility that the gene variation may not be responsible for MODY, but rather may represent an association with T2DM or a rare polymorphism, has not been excluded. The hyperglycaemia in subjects with HNF4α-related MODY tends to worsen with time, resulting in the need for treatment with oral hypoglycaemic agents or insulin in a significant proportion of patients [33].

It has been suggested that haploinsufficiency for HNF4α function in pancreatic islet β-cells is the pathogenic basis of the MODY1 phenotype. This hypothesis is based on two observations: first, many of the diverse mutations that have been reported result in truncated proteins with major functional deficits; secondly, HNF4α exhibits high constitutive transcriptional activity and was constitutively bound to fatty acids when crystallized [34]. However, others have raised the possibility of dominant-negative activity, and the lack of a MODY phenotype in HNF4α heterozygous-null mice might provide support for this alternative hypothesis.

SHP and obesity

SHP (NR0B2) is an atypical orphan nuclear receptor which, in comparison with other nuclear receptors, lacks N-terminal and DNA-binding domains, and consists solely of a C-terminal region corresponding to a putative LBD [35]. In humans it is expressed in liver, where it has been implicated in the regulation of cholesterol and bile acid homoeostasis, and at several other sites including the pancreatic β-cells, spleen, small intestine and adrenal glands. It is capable of modulating the activity of a number of other nuclear receptors, either inhibiting (e.g. HNF4α, pregnane X receptor, LRH-1 and liver X receptor α) or augmenting (e.g. PPARγ) their transcriptional activity [36].

Due to its expression in the pancreas and ability to regulate HNF4α, SHP was considered to be a plausible candidate gene in MODY. In 2001, Nishigori and colleagues [37] reported the identification of five mutations and a single polymorphism within the gene in a cohort of 173 unrelated Japanese subjects with early-onset, non-ketotic diabetes. Interestingly, five of the six mutations that were described in this study were associated with a mildly obese phenotype rather than diabetes, with affected individuals exhibiting birth weights.
that were at least 1 S.D. higher than the mean when corrected for gestational age. Accordingly, the authors went on to screen a second cohort of young obese subjects (n=101; body-mass index >25 kg/m²) and identified four SHP gene mutations, two of which differed from those found in the diabetic cohort. Combining these cohorts, 6.3% of Japanese subjects with early-onset obesity had SHP mutations. The SHP mutant proteins were impaired in their ability to inhibit HNF4α transactivation, leading the authors to speculate that such loss of SHP activity in utero might enhance HNF4α function leading to augmented insulin-stimulated adipogenesis. However, additional studies in other population-dependent cohorts have failed to confirm SHP mutations as a common association with obesity, although influences on birth weight have been observed [38].

**SF1 and DAX1 in disorders of gonadal and adrenal development**

SF1 (NR5A1) and DAX1 (NR0B1) are two orphan nuclear receptors that are expressed in the hypothalamus, pituitary gonadotrophs, gonads and adrenals and play a key role in both the development and function of the HPG axis at several levels [39].

**SF1**

The gene encoding SF1 is located at chromosome 9q33, and encodes a 461-amino-acid protein, which resembles other members of the nuclear receptor family. It binds to DNA as a monomer (the P-box within the first zinc-finger motif confers specificity for the hexanucleotide response element), and this interaction is stabilized through additional contacts between residues in the A-box part of the hinge region (Figure 1A) and the 5′-flanking nucleotide sequence upstream of the DNA-response element. Recently, naturally occurring SF1 mutations have been identified in two individuals with a male karyotype who exhibited complete sex reversal, with testicular dysgenesis, persistence of Mullerian structures and primary adrenal failure [40,41]. In the first subject a heterozygous missense mutation (Gly-35→Glu) was identified within the P-box of SF-1. Consistent with this, binding to and transactivation of a variety of target genes by the SF-1 mutant was impaired. In contrast, the second subject was found to be homozygous for a mutation (Arg-92→Gln) within the A-box of SF1, which weakens but does not abolish receptor binding to DNA. Heterozygous members of this second kindred were phenotypically normal. Taken together these observations suggest that, in humans, gene dosage of SF1 is critical in regulating adrenal and pituitary/gonadal axis development, with a ‘modest’ reduction in receptor function (as observed in the Arg-92→Gln heterozygotes) having no discernable consequences, while more ‘severe’ loss-of-function (as seen in the Gly-35→Glu heterozygote or the Arg-92→Gln homozygote) results in major developmental abnormalities.
A further heterozygous SF1 mutation (Arg-255→Leu) has been reported in a female (46XX) with primary adrenal failure. The presence of ovaries in this subject suggests that female gonadal development may be less dependent on SF1 function than adrenal and testis development [42].

DAX1

DAX1 is an unusual member of the nuclear receptor family, with an N-terminal domain containing tandem repeats of several Leu-Xaa-Xaa-Leu-Leu (LXXLL)-like amino acid motifs, similar to those found in transcriptional co-activators such as SRC-1. This is linked to a C-terminal putative LBD which resembles that of other nuclear receptors. Functional studies indicate that DAX1 is a transcriptional repressor, at least in part through inhibition of the activity of SF1 [43].

Human mutations in DAX1 cause X-linked AHC, a disorder of adrenal cortical development [44]. Males with this condition typically present in infancy or childhood with primary adrenal failure. Although the HPG axis appears to be intact in early life, hypogonadotrophic hypogonadism usually manifests at the time of puberty and reflects a combination of defects at both hypothalamic and pituitary levels. More than 80 different mutations have been reported in DAX1, mostly nonsense or frameshift mutations that cause premature truncation of the protein, thereby impairing its ability to function as a transcriptional repressor [39]. A small number of missense mutations have been identified in the putative LBD. As might be predicted, these mutations appear to be less deleterious, with the mutant proteins retaining some repressor activity, consistent with the milder phenotype of affected individuals. Other unusual phenotypic variants that have been reported include isolated hypogonadotrophic hypogonadism in a female homozygous for a truncation mutation in DAX1 through gene conversion [45], and delayed puberty in heterozygous female mutation carriers in one family [46]. Most recently, an unusually mild form of AHC was found in a male subject harbouring an apparently severe premature stop mutation, Gln-37→Xaa [47]. However, subsequent in vitro studies with this mutant revealed the unexpected generation of a partially functional N-terminally truncated DAX1 protein through the use of an alternative in-frame translation start site, thereby ameliorating the classical AHC phenotype. Taken together, these reports again emphasize the critical effects of DAX1 gene dosage in the development and function of both the HPG axis and adrenal glands.

PNR and enhanced S-cone syndrome (ESCS)

PNR (NR2E3) is a novel, recently identified orphan member of the nuclear receptor family that exhibits localized expression in retinal photoreceptor cells, suggesting that it might be involved in their differentiation and/or maintenance [48]. In support of this hypothesis, its human chromosomal location (15q24) has been independently identified as a susceptibility locus for retinal
Degenerative disorders [49]. Accordingly, Haider and colleagues [50] screened the PNR gene in families with Bardet–Biedl syndrome (a disorder characterized by retinitis pigmentosa, obesity, hypogonadism, renal dysfunction and mental retardation), including kindreds in whom there was clear evidence of linkage to chromosome 15, as well as others with unknown linkage [50]. No mutations in PNR were found in this original selective cohort and the authors therefore broadened their search to include a wider spectrum of retinal degenerative disease, including a small subset of individuals with the ESCS. Most hereditary human retinal degenerative disorders are associated with a reduction in the number of mature photoreceptors with consequent loss of visual function. However ESCS, an autosomal recessive retinopathy, is unusual and is characterized by a gain in photoreceptor function with affected individuals manifesting increased sensitivity to blue light mediated through the S (short-wavelength) blue cones, while simultaneously suffering visual loss (especially night blindness) to varying degrees of L (long red) and M (middle green) cone vision, and retinal degeneration. In a cohort of ESCS probands, 94% were found to harbour homozygous mutations (splice-acceptor site, deletions and missense) in PNR [50]. Although the precise mechanism through which aberrant PNR signalling leads to the phenotype of ESCS remains unclear, it has been suggested that the altered S- to L/M-cone sensitivity may reflect a return to a default pathway of cone differentiation, in which defects in PNR allow photoreceptor precursors to retain their S-cone commitment rather than switching to the L- or M-cone phenotype.

NURR1 and abnormalities of dopaminergic neurotransmission: familial Parkinson’s disease and schizophrenia/manic-depressive disorder

The NURR1 (NR4A2) gene is highly conserved and encodes a member of the nerve growth factor inducible factor I-B subfamily of nuclear receptors, which plays a critical role in mesencephalic dopamine neuronal development. Accordingly, NURR1 has been implicated as a potential candidate gene in conditions as diverse as Parkinson’s disease and schizophrenia/manic-depressive disorder.

Recently two nucleotide changes (-291Tdel and -245T to G), which map to the first non-coding exon of NURR1 (upstream of the transcriptional start site), were found to affect one in ten alleles from 107 individuals with familial Parkinson’s disease, but not subjects with sporadic Parkinson’s disease or unaffected controls [51]. In vitro studies demonstrated a dramatic reduction in mutant receptor mRNA compared with WT NURR1 transcript in cells transfected with expression vectors containing either the mutant or WT alleles, which correlated with a significant attenuation of receptor-mediated transactivation from a NuRE containing reporter gene in the presence of either mutant. Furthermore, expression of tyrosine hydroxylase, an enzyme mediating dopamine biosynthesis, was
markedly reduced in cells transfected with the mutant NURR1 allele. Studies in lymphocytes from two individuals with the -291Tdel mutation demonstrated a significant reduction in NURR1 mRNA to levels that were even lower than 50% (the level of transcripts that might be expected from the remaining WT allele), leading the authors to speculate that the mutant might inhibit expression of its WT counterpart in a dominant-negative manner. Further studies are awaited to confirm these findings and to determine the mechanisms through which aberrant NURR1 signalling induces the Parkinson’s disease phenotype.

In contrast with Parkinson’s disease, the precise role of the dopaminergic system in the pathogenesis of schizophrenia remains unclear, although the effectiveness of anti-dopaminergic agents therapeutically strongly implicates genes involved in dopamine cell development and maintenance. To date, only a handful of mutations in NURR1 (in exons 1 and 3) have been found in patients with schizophrenia and manic-depressive disorder, and larger-cohort studies are awaited to validate these findings [52,53]. In addition, it will be a challenge to explain how apparent loss-of-function mutations within NURR1 can be associated with disorders as divergent as Parkinson’s disease and schizophrenia/manic-depressive disorder, which are treated by enhancing or blocking dopaminergic neurotransmission respectively.

Conclusions

The study of human subjects harbouring naturally occurring mutations in various members of the steroid nuclear receptor superfamily has greatly enhanced our understanding of the diverse roles that these receptors play in normal mammalian physiology. Often these observations have complemented those derived from animal models, and in many cases they have served to emphasize the important differences that exist between the species. However, for a significant number of nuclear receptors that are well characterized, the human disorders or phenotypes associated with receptor defects remain to be elucidated. It is important that we continue to search for these ‘human receptor disorders’, not only to study the pathogenesis of the phenotype in affected individuals, but also to verify that the conclusions that have been drawn currently regarding the roles of such receptors in human physiology are correct.

Summary

- Mutations in human TRβ are associated with the syndrome of RTH, a disorder with a distinctive biochemical signature of elevated free thyroid hormones with an unsuppressed thyroid-stimulating hormone.
- Mutations in the LBD of human PPARγ are associated with a novel syndrome (PLR), which is characterized by a stereotyped pattern of partial lipodystrophy, insulin resistance, hypertension and other features of the human metabolic syndrome.
• Mutations in various orphan nuclear receptors are associated with a diverse group of human disorders involving aspects of metabolism (HNF4α, SHP), gonadal and adrenal development (SF1, DAX1), retinal function (PNR) and neuronal dopaminergic transmission (NURR1).

We thank J.W.R. Schwabe for undertaking the molecular modelling shown in Figure 2 and T.D. Wallman for secretarial assistance.

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