Genetic disorders of Nuclear Receptors

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ABSTRACT

Following the first isolation of their genes, genetic diseases of nuclear receptors were initially discovered by a candidate gene approach based on their known roles in endocrine pathways and physiological processes. Subsequently, the identification of disorders has been informed by phenotypes associated with gene disruption in animal models or by genetic linkage studies. More recently, whole exome sequencing has associated pathogenic genetic variants with unexpected, often multisystem, human phenotypes. To date, defects in 20 out of 48 human NR genes have been associated with human disorders, with different mutations mediating phenotypes of varying severity or several, distinct conditions being associated with different changes in the same gene. Studies of individuals with deleterious genetic variants can elucidate novel human roles of NRs, validating them as targets for drug development, or providing new insights into structure-function relationships. Importantly, human genetic discoveries enable definitive disease diagnosis and can provide opportunities therapeutically manage affected individuals. Here, we review germline changes in human NR genes associated with “monogenic” conditions, including a discussion of the structural basis of mutations that cause distinctive changes in nuclear receptor function and molecular mechanisms mediating pathogenesis.

INTRODUCTION

It has been almost 30 years since the first human nuclear receptor (NR) disorders were characterized at the molecular level (Figure 1). Since then, disorders associated with genetic defects in 20 out of the 48 known human NRs have been identified (Figure 1, Tables 1 & 2). In this review, we provide a brief overview of the range of human NR-associated conditions reported to date, and highlight some of the key pathogenic mechanisms involved (Figure 2A). Our focus is on well-established, germline, monogenic disorders. We will not cover the role of somatic NR variations or fusion genes in cancer, nor associations found in genome-wide association studies.

Thyroid hormone receptor α and -β (NR1A1 and -2)

Thyroid hormones (TH) regulate physiological processes (skeletal growth, maturation of the CNS, heart rate and contractility, energy expenditure) via receptors (TRα1, TRβ1, TRβ2) encoded by separate genes (*THRA/NR1A1, THBR/NR1A2*), with differing tissue distributions: TRα1 is highly expressed in the CNS, myocardium, gastrointestinal tract and skeletal muscle; TRβ1 is the predominant isoform in liver and kidney; TRβ2 expression is restricted principally to the hypothalamus, pituitary, retina and inner ear. Such divergence of receptor subtype expression likely mediates distinctive phenotypes associated with defective *THRB* or *THRA*.

Resistance to THβ (RTHβ), usually dominantly inherited, is recognized by a characteristic biochemical signature of elevated circulating TH and non-suppressed thyroid-stimulating hormone (TSH) levels, reflecting central (hypothalamic-pituitary) resistance to TH action, together with variable resistance in peripheral tissues. Approximately 160 different, heterozygous *THRB* mutations, which are localized in the ligand binding domain (LBD) and involve both TRβ2 and TRβ1 isoforms, have been identified in the disorder (1).

Affected individuals can have nonspecific symptoms or a goiter, prompting thyroid function tests that suggest the diagnosis; these individuals are deemed to have generalized resistance to TH (GRTH). In approximately 15% of cases the same biochemical picture can be associated with thyrotoxic features (e.g. weight loss, tremor, anxiety, tachycardia in adults; failure to thrive and hyperkinetic behaviour in children); disproportionate resistance to TH in TRβ-expressing hypothalamus and pituitary (PRTH), with relative retention of hormone sensitivity in TRα-expressing peripheral tissues, may account for this phenotype. GRTH and PRTH phenotypes can be associated with the same TRβ mutation and even coexist within a single family. Other recognized features of the disorder include attention-deficit hyperactivity disorder in childhood and dyslipidemia and reduced bone mineral density in adults (1).

Consonant with their location, most TRβ mutations impair hormone binding or (rarely) coactivator recruitment and inhibit action of their wild-type counterparts in a dominant-negative manner (Figure 2B). Receptor functional regions (DNA binding, dimerization, corepressor binding) are devoid of naturally occurring TRβ mutations, with RTHβ variants clustering within hotspots within the LBD (1). Homozygous *THRB* deletion mediates RTHβ in the first two recorded siblings with this disorder, who also had audiovisual abnormalities (2), and missense mutations in five other recessively inherited cases (3). In roughly 15% of people with typical biochemical features of RTHβ, no *THRB* defect can be identified; here, alterations in coregulators or other factors mediating TH action, have been postulated (1). Triiodothyroacetic acid (TRIAC) treatment, a centrally acting TH analogue that lowers TH levels, can control thyrotoxic features of the disorder.

Resistance to THα (RTHα**)**, characterised by features of hypothyroidism in selected tissues, eluded discovery probably because thyroid function tests are near-normal in the disorder. Most cases have been identified in childhood, with features including disproportionate (lower segment) growth retardation, macrocephaly, dysmorphic features, constipation, dyspraxia and intellectual deficit.

Biochemical abnormalities include low/low-normal thyroxine (T4) and high/high-normal triiodothyronine (T3) computing to a low T4/T3 ratio, variably reduced reverse T3, elevated muscle creatine kinase levels, and anemia (4, 5).

Heterozygous *THRA* mutations disrupt TRα1 function either markedly or partially and inhibit wild-type receptor action in a dominant-negative manner via a mechanism involving enhanced corepressor recruitment and target gene repression (Figure 2B; Figure 3A) (5). Some *THRA* defects also involve the carboxyterminally divergent, non-hormone-binding TRα2 isoform, with no discernible added clinical phenotype or gain- or loss-of-function attributable to the TRα2 variant (6). Consonant with resemblance of the RTHα phenotype with some features of conventional hypothyroidism, T4 therapy reverses metabolic abnormalities and also improves growth, constipation, dyspraxia and well-being.

Vitamin D receptor (NR1I1/VDR)

The principal role of the vitamin D receptor (VDR) is in regulation of calcium and phosphate metabolism with actions in the gastrointestinal tract, kidney and bone. Hypocalcemia and associated symptoms (skeletal and respiratory muscle weakness, seizures) in the early neonatal period or infancy due to lack of VDR-dependent intestinal calcium absorption dominates the phenotype of autosomal recessive, hereditary, vitamin D-resistant rickets (HVDRR) (also known as vitamin D-dependent rickets type II). Rickets manifests with bone pain, growth restriction and fractures; low circulating calcium and phosphate with raised alkaline phosphatase are associated with normal serum 25-hydroxy but very elevated 1,25 dihydroxyvitamin D3 (calcitriol) levels and secondary hyperparathyroidism and raised parathyroid hormone levels. Alopecia (patchy or total) affecting both scalp and body is a distinctive, non-osseous feature of the disorder.

Approximately 100 cases of HVDRR harboring ~45 different homozygous or compound heterozygous VDR mutations have been recorded: frameshift, premature-stop and DNA-binding domain (DBD) mutations lead to complete loss-of-function. Additionally, approximately 20 LBD variants exhibit either reduced ligand binding, failure to heterodimerize with RXR, or selective loss of coactivator recruitment (Figure 3B); a single HVDRR case lacking a VDR mutation has also been described (7). In one family, a missense VDR mutation (p. Glu420Ala), which abolished coactivator binding and exhibited dominant-negative activity, mediated HVDRR in the heterozygous state (8). Patients with HVDRR require oral or intravenous calcium therapy; high-dose vitamin D or calcitriol treatment can overcome the receptor defect in LBD mutation cases (7), raising the possibility of structure-guided design of synthetic analogues for treatment of a subset of HVDRR (9).

Interestingly, alopecia occurs in patients with VDR mutations that lead to loss of receptor expression, DNA binding or dimerization, but are not a feature in cases with ligand binding or coactivator recruitment defects. This abnormality is unresponsive to calcitriol therapy, leading to the hypothesis that inhibition of target genes by unliganded, wild-type VDR maintains normal cycling of hair follicles, with loss of such repression mediating hair loss (Figure 2C). Supporting this notion, mutations in Hairless (HR), a known component of NR repression complexes, also cause an alopecia syndrome (atrichia with papules) (10).

Glucocorticoid receptor α (NR3C1)

Disruption of glucocorticoid receptor α (GRα, encoded by *NR3C1*) is associated with familial glucocorticoid resistance (FGR, also known as generalized glucocorticoid resistance or Chrousos syndrome) (11, 12). This can be dominantly or recessively inherited, with a range of features depending on the severity of the defect or underlying molecular mechanism. Individuals with FGR often present with fatigue, but other signs of glucocorticoid insufficiency are rare. Because of reduced central feedback, adrenocorticotropic hormone (ACTH) is elevated, increasing cortisol and

partly compensating for glucocorticoid resistance. One consequence of elevated ACTH is an increase in adrenal mineralocorticoid and androgen production. Consequently, clinical and biochemical features of FGR can include hypertension, hypokalemia and metabolic alkalosis, as well as hirsutism, acne, male-pattern baldness, oligomenorrhea and infertility.

Most pathogenic missense variants in GRα are located in the LBD and affect glucocorticoid binding or transactivation. Patients or carriers with heterozygous changes tend to have a milder phenotype, although dominant-negative LBD variants have been reported (p.Ile559Asn, Ile747Met) (13, 14). LBD mutants often show variably reduced ligand binding affinity, as well as delayed nuclear translocation and altered interactions with coactivators (e.g. p160) (14). Clinical and biochemical features in individuals with homozygous mutations in *NR3C1* are usually more severe. No familial "activating" mutations in GRα have been reported, but a heterozygous variant (p.Asp410His) has been reported in a woman with features of tissue-selective glucocorticoid hypersensitivity (e.g. visceral obesity, dyslipidemia, type 2 diabetes (T2D), hypertension) (15). This mutant receptor increased transactivation of glucocorticoid-responsive genes.

Mineralocorticoid receptor (NR3C2)

The mineralocorticoid receptor (MR, encoded by *NR3C2*) plays a key role in renal sodium retention and cardiovascular endocrinology. Pathogenic loss-of-function variants in this receptor are associated with a renal form of mineralocorticoid resistance known as autosomal-dominant (or sporadic) pseudohypoaldosteronism type 1 (PHA I) (16, 17). Children typically present in early infancy with dehydration and failure to thrive, and have hyponatremia, hyperkalemia, and elevated aldosterone levels and plasma renin activity (PRA). Some infants with elevated aldosterone and PRA are asymptomatic. Sodium supplementation is usually required, but the condition improves in childhood. In contrast, the autosomal recessive form of PHA I, due to defects in the amiloride-

sensitive epithelial sodium channel (ENaC), is a more severe systemic condition that does not remit with age.

Pathogenic MR mutations include nonsense, frameshift, splice and missense mutations, with a potential hotspot at c.2839C>T (p.Arg947*). Missense mutations often affect key amino acids in the LBD and impair aldosterone binding and aldosterone-dependent transactivation (16, 18). Nuclear localization can sometimes be affected and different variants may have differential effects on MR-target genes (e.g. *SGK1*, *NDRG2, GILZ, SCNN1A*) (18, 19).

A gain-of-function MR variant was reported in 2000, in one family with early-onset hypertension (before the age of 20) (20). Females also exhibited marked hypertension during pregnancy. The heterozygous p.Ser810Leu variant identified in affected family members showed mild constitutive activity together with inappropriate responsiveness to progesterone. The leucine substitution at position 810 increases van der Waals interactions and lessens hydrogen bonding with steroid side groups, thereby enabling progesterone to bind and activate mutant MR (Figure 3C). Although this germline mutation is rare, it exemplifies how genetic variants in NRs can potentially alter ligand specificity; such altered ligand specificity is well recognized with somatic ERα variants in breast cancer or androgen receptor (AR) mutations in prostate cancer.

Estrogen Receptor α (NR3A1)

ERα (encoded by *ESR1*) is one of the best-studied NRs in human biology. To date, only three genomic pathogenic variants associated with a clear phenotype have been reported; however, these cases do provide important insight into the role of ERα in human development and health.

The first report of a pathogenic *ESR1* variant in 1994 involved a 28-year-old man who presented with tall stature (204cm), prolonged linear growth, delayed epiphyseal fusion and reduced bone mineral

density (z-score -3.1) (21, 22). He had normal puberty, but had elevated follicular stimulating hormone (FSH) and luteinizing hormone (LH) levels and reduced sperm viability. He also had impaired glucose tolerance, hyperinsulinemia and an abnormal lipid profile with evidence of early coronary atherosclerosis, although his BMI was elevated (30.5 kg/m^2). Genetic analysis revealed a homozygous stop gain variant (p.Arg157*). He had mildly elevated serum estradiol and resistance to estrogen treatment.

In 2013, the first female with estrogen resistance was reported (23). This 18-year-old woman presented with absent breast development, primary amenorrhea and abdominal pain due to hemorrhagic ovarian cysts. She had a small uterus with no endometrium, but did have evidence of androgenization. Her bone age was markedly delayed (> 4 years), she did not have a pubertal growth spurt, and her bone density was reduced (z-score -2.4) with elevated markers of osteoblastic activity. Her estradiol was very elevated (10-fold above normal) with elevated inhibin A and mildly raised gonadotropins, and she was resistant to estrogen treatment. Analysis of *ESR1* revealed a homozygous missense variant (p.Gln375His) in the LBD that impaired estrogen responsiveness in cell-based assays. Most recently, the first family with estrogen resistance (p.Arg394His) has been reported (24).

These reports provide important information about the role of ERα in humans. As expected, ERα mediates the main effects of estrogen on bone growth and mineralization, as well as breast and uterine development in females. As in ER knockout mice, gonadotropin concentrations are higher in males, possibly because very high estradiol and inhibin A levels in females partly mediate central feedback. Finally, the woman described above had no evidence of hyperinsulinemia or glucose intolerance, but she had a low BMI (16.6 kg/m2) and body fat (28%). Long-term monitoring is needed to see if metabolic abnormalities develop, although the difference in BMI between the two individuals may be a factor influencing insulin sensitivity.

Androgen Receptor (NR3C4)

Disruption of the AR results in androgen insensitivity syndrome (AIS) (25). This X-linked condition was first reported in 1953, and historically called “testicular feminization syndrome” (26). Women with complete AIS (CAIS) typically present during late adolescence with primary amenorrhea. Subsequent investigations show an absent uterus, 46,XY karyotype and elevated testosterone concentrations. Breast development usually occurs in adolescence due to the aromatization of androgens to estrogens, but androgen-dependent pubic hair is often absent or sparse. Occasionally the diagnosis is made when testes are found during hernia repair in childhood or with karyotype analysis for another indication. As with most conditions, a spectrum of phenotypes can occur. Partial AIS (PAIS) typically presents with atypical genitalia or hypospadias in the newborn period, and gynecomastia is a common feature at adolescence in boys with this condition (27). Mild AIS (MAIS) has also been reported in men with oligospermic infertility (28).

More than 800 different pathogenic variants in the *AR* have been reported. These changes include stop-gain, frameshift and missense variants that are distributed throughout the gene, and have been reviewed extensively elsewhere (http://androgendb.mcgill.ca/, 26, 29). Missense variants tend to affect important amino acids involved in DNA-binding or ligand interactions, but many different residues can be affected. Although some mutations associate more with complete or partial phenotypes, there can be overlap between the type and location of the change, its activity in *in vitro* assays and the degree of androgen insensitivity in affected individuals. Missense mutations in =the hydrophobic ligand-binding pocket of the LBD usually cause CAIS (26), while missense mutations in the large amino-terminal activation function domain (AF1) usually cause PAIS or MAIS. Of note, a subset of individuals thought to have AIS do not have variants in the AR gene, despite cultured genital fibroblasts showing androgen resistance in vitro (e.g. reduced dihydrotestosterone-induced

apolipoprotein D expression) (30). Disruption of AR-dependent co-factors or post-receptor signaling mechanisms have been proposed as the cause (AIS type II) (30).

The AR is unusual as it has a variable number of polyglutamine and polyglycine repeats in the amino-terminal region of the receptor. Expansion of the polyglutamine tract (to 38-65 CAG trinucleotide repeats) is associated with X-linked spinal and bulbar muscular atrophy (SBMA, also known as Kennedy disease) (31). This condition results from AR-polyglutamine toxicity; the mutant protein misfolds and aggregates in spinal cord motor neurons and muscle cells. SBMA can sometimes be associated with reduced androgen action, gynecomastia, low sperm count and testicular atrophy.
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Steroidogenic factor-1 (NR5A1)

Steroidogenic factor-1 (SF-1, *NR5A1*) was identified following the search for a common regulator of steroidogenic enzyme transcription. Complete deletion of *Nr5a1* in the mouse resulted in adrenal agenesis, gonadal (testicular) dysgenesis with persistent Müllerian structures (uterus, upper vagina) in XY animals and variable defects in gonadotropin release, confirming SF-1 as a key player in adrenal and gonad biology. Subsequently, other metabolic features such as late-onset obesity and ventromedial hypothalamic abnormalities were reported.

The first descriptions of pathogenic loss-of-function variants in *NR5A1* in humans were in 1999 and 2002 (32, 33). These reports included two 46,XY girls with testicular dysgenesis, Müllerian structures and salt-losing primary adrenal insufficiency. The first child had a *de novo*, heterozygous change (p. Gly35Glu) in the "P-box" region of the SF-1 DBD that affected binding to and transcriptional activation of target gene response elements (32). Functional studies suggested this was largely a gene dosage-dependent competitive effect, although partial dominant-negativity was reported in some systems. The second child had a homozygous pathogenic change (p. Arg92Gln) affecting the "A-box" region of SF-1 DBD (33). SF-1 belongs to a small subgroup of nuclear receptors that bind to DNA as monomers rather than as homo- or heterodimers. The A-box region is involved in stabilizing monomeric binding through an interaction with the DNA minor groove. Thus, a heterozygous P-box change and homozygous A-box change may have similar phenotypes.

The past decade has seen great increases in the number of reported pathogenic changes in *NR5A1* and also the spectrum of SF-1-associated conditions (34). More than 200 individuals and families are now described in the literature. Heterozygous loss-of-function variants in *NR5A1* occur in approximately 15% of individuals with testicular dysgenesis and reduced androgen production, resulting in 46,XY differences/disorders in sex development (DSD) (35). Phenotypes can range from females with a 46,XY karyotype to boys with penoscrotal hypospadias or undescended testes. Milder variants in *NR5A1* can be associated with a small subset of male factor infertility, sometimes with progressive endocrine dysfunction (36). Variants in *NR5A1* are also associated with primary ovarian insufficiency (POI) in 46,XX women, although the age of onset and natural time course are highly variable (37). Although many variants occur *de novo*, around 30% can be carried and maternally transmitted as a sex-limited dominant trait. Because multiple members of a family may have 46,XY DSD with 46,XX females being at risk of POI, careful family history and counseling is important. Very rarely, mutations in *NR5A1* can cause primary adrenal insufficiency in 46,XX girls.

Although true “gain-of-function” variants in SF-1 have not been reported, recent observations suggest that recurrent, heterozygous missense changes in codon 92 (p.Arg92Trp, p.Arg92Gln) of SF-1 are associated with ovotestes or testes in individuals with a 46,XX karyotype (38). Several individuals or families of diverse genetic ancestry have been reported. This particular amino acid change may interfere with expression of DAX-1 (see below) through WNT signaling in the developing gonad, but the exact mechanism “switching” the ovary into a testis remains unclear (Figure 3D).

DAX-1 (NR0B1)

Dosage-sensitive sex-reversal, adrenal hypoplasia congenita critical region on the X chromosome, gene 1 (DAX-1, encoded by *NR0B1*) is an orphan nuclear receptor that lacks the typical NR DBD, but has an amino-terminal region motif comprising three 66-67 amino acid tandem repeats. Similar to SF-1, DAX-1 plays a key role in adrenal and reproductive development.

DAX-1 disruption was first reported to cause X-linked adrenal hypoplasia congenita (AHC) in 1994 (39, 40). Since then, more than 200 individuals or families have been reported to have pathogenic variants in *NR0B1* (34). The classic features in males include primary adrenal insufficiency in early infancy or childhood, delayed or arrested puberty due to disordered gonadotropin release, and impaired spermatogenesis.

Nonsense or frameshift variants in *NR0B1* occur throughout the gene, whereas pathogenic missense variants tend to cluster in key areas of the ligand-like binding domain in regions that form the hydrophobic core of the protein (41). Few missense changes in the amino-terminal repeat structure of DAX-1 have been reported. Partial loss-of-function missense variants can be associated with a milder phenotype of delayed onset adrenal insufficiency in early adulthood, or partial hypogonadotropic hypogonadism (42). Surprisingly, milder phenotypes can also occur due to stop gain variants at the start of the protein (p. Trp37*, p.Trp39*); this effect is likely due to reinitiation of

translation of a truncated DAX-1 protein from a downstream methionine at codon 83 that remains partially functional (due to the repeat motif structure) and “rescues” the phenotype (43).

Although DAX-1-associated conditions are well established, the exact biological role of DAX-1 remains unclear. Many studies demonstrate that DAX-1 acts as a transcriptional repressor, potentially through a direct interaction with SF-1 (41). Indeed, duplication of the locus containing DAX-1 is associated with testicular dysgenesis, suggesting that it may act as an “anti-testis” gene. DAX-1 may play a role in regulating progenitor cell differentiation. Loss of DAX-1-dependent repression may result in premature differentiation of progenitor cells without appropriate expansion of cell numbers, ultimately resulting in tissue hypoplasia. Indeed, loss of DAX-1 can sometimes be associated with early puberty in humans, and transient adrenal hyper-responsiveness has been reported in *Dax1* knockout mice (44). Whether these phenomena reflect the true biological basis of DAX-1 function is still unclear.

Retinoic acid receptor-β (NR1B2)

Retinoic acid receptor-β (RARB) is expressed in many tissues during development and deletion of *Nr1b2* in mice causes multiple defects (e.g. CNS, vision, hearing, musculoskeletal, cardiovascular, gastrointestinal, pulmonary, renal) and high lethality.

In 2013, the first pathogenic *RARB* variants were reported in patients with *STRA6* mutation-negative syndromic microphthalmia and additional features such as diaphragmatic hernia, cardiac anomalies and pulmonary hypoplasia (PDAC syndrome). In one family, two siblings were found to be compound heterozygous for disruptive variants (p.Arg119*/p.Ile403SerfsTer15) (45). At least three other unrelated children with microphthalmia and one or more of these additional features have been found to carry *de novo*, heterozygous changes affecting an arginine hotspot at codon 387 (e.g. p.Arg387Ser, p.Arg387Cys), potentially mediating a gain-of-function mechanism (45, 46). Taken

together, these findings provide support for retinoic acid pathways in human eye development and organogenesis. Indeed, NR1B2 is highly expressed in the human retina, unlike NR1B1 or NR1B3 (FANTHOM5 dataset; http://www.proteinatlas.org/ENSG00000077092-RARB/tissue).

RAR-related orphan receptor-γ (NR1F3)

Retinoid-related orphan receptor γ (ROR-γ, encoded by *RORC*) plays a key role in thymocyte development and function, including differentiation of the Th17 cell subset. Recently, homozygous pathogenic variants in *RORC*, causing disruption of both the ROR-γ and ROR-γ-t isoforms, have been reported in seven individuals from three unrelated consanguineous pedigrees with immunodeficiency (47). These families – from Palestine, Chile and Saudi Arabia – have evidence of chronic mucocutaneous candidiasis (due to IL-17A and IL-17F deficiency) combined with susceptibility to mycobacterial disease and disseminated infections following Bacillus Calmette-Guérin (BCG) vaccines. Patients also all had a small thymus.

Photoreceptor-specific nuclear receptor (NR2E3)

Photoreceptor-specific nuclear receptor (PNR, encoded by *NR2E3*) is involved in retinal photoreceptor cell differentiation and degeneration, and its disruption results in retinal degeneration in the mouse (rd7). Pathogenic variants in *NR2E3* cause enhanced S-cone syndrome (ESCS) (48), an inherited retinal disorder characterized by increased visual function of the minority S (blue) cones and decreased L/M (red/green) cone and rod function. These findings likely represent increased S-cone proliferation at the expense of other cell types during cell fate determination. Patients typically develop night blindness and evidence of retinitis pigmentosa (RP). Autopsy studies have shown absence of rods and retinal disorganization and degeneration (49).

Amongst pathogenic variants in *NR2E3* reported to cause ESCS, homozygous p.Arg311Gln variants are most common and represent a hotspot for disease amongst the Crypto-Jewish population in

Portugal (50). The same change is also associated with Golgmann-Favre syndrome, a more severe form of ESCS (47). Variants in *NR2E3* are also found in autosomal-recessive and -dominant forms of RP (51, 52). Approximately 3% of dominantly inherited RP is due to a heterozygous PNR mutation (p.Gly56Arg) in the first zinc finger of the DBD, exhibiting dominant-negative activity (52, 53). Other variants cause altered cellular localization or homo- or hetero-dimerization with TLX/NR2E1 and RXRα/NR2C1 (54, 55). Another NR, RevErbα/NR1D1, has been used to potentially "rescue" disease progression in the rd7 mouse (56).

Chicken ovalbumin upstream promoter transcription factor I (NR2F1)

Chicken ovalbumin upstream promoter transcription factor I (COUPTF-I) is widely expressed in many tissues with strong expression in the brain and peripheral nervous system and has a potential role in regionalization of the neocortex and axonal projection. In humans, *NR2F1* haploinsufficiency and *de novo* heterozygous mutations in *NR2F1* have been reported in patients with Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS) (57, 58). Additional characteristics include developmental delay and variable, non-specific facial features. Most missense mutations (p.Arg112Lys, p.Ser113Arg, p.Arg115Pro) cluster in the DBD and may be associated with a more severe phenotype (58, 59). Other features reported recently include hypotonia, oromotor dysfunction, thinning of the corpus callosum, seizures, autistic spectrum disorder and hearing impairment (59).

COUPTF-II (NR2F2)

COUPTF-II plays a role in angiogenesis, vascular remodeling and heart development, as well as in more widespread regulation of cell fate during embryonic development. Recently, heterozygous variants in *NR2F2* have been reported in patients with a range of congenital cardiac disease phenotypes (60). In one family, a 3-bp duplication in *NR2F2* segregated with multiple cardiac defects (atrioventricular septal defect (AVSD), aortic stenosis/VSD, tetralogy of Fallot), whereas other heterozygous missense mutations or deletions of *NR2F2* have been associated with AVSD,

hypoplastic left heart syndrome or aortic coarctation (56). Congenital diaphragmatic hernia (CDH) may be an association in mice and humans (61).

Rev-erb-β (NR1D2)

Rev-erb-β has several proposed actions including being a potential repressor of gene transcription. Recently, a *de novo* heterozygous mutation in *NR1D2* was found in one individual with congenital heart disease (AVSD) (62). This variant (p.Arg175Trp) affects binding to the DNA minor groove and impairs transcriptional repression. Detailed analysis of *Nr1d2*$^{-/-}$ mice indicated a similar phenotype (62). As this is a very recent observation, the true contribution of Rev-erb-β to developmental heart defects is not yet known.

Estrogen-related receptor-β (NR3B2)

Estrogen-related receptor-β **(**ERR-β) has structural homology to the ERs and binds ER response elements, but is not activated by estrogens. ERR-β plays a role in placental development and is expressed in several tissues such as the inner ear during development and postnatal life. (Nucl Recept Signal. 2016 Jun 21;14:e002. doi: 10.1621/nrs.14002. eCollection 2016. Estrogen-related receptor β (ERRβ) - renaissance receptor or receptor renaissance? Divekar SD, Tiek DM, Fernandez A, Riggins RB). Homozygous disruption of *ESRRB* was first reported in a large consanguineous Turkish family with autosomal-recessive non-syndromic hearing loss (type 35) (63). Homozygous point mutations in the DBD and more often in the "ligand"-binding domain of ERR-β have also been reported as a rare cause of non-syndromic hearing loss, often in consanguineous families (63). A potential link between disruption of *ESRRB* and dental caries has also been proposed (64).

Farnesoid X receptor (NR1H4)

Farnesoid X receptor (FXR), a bile acid-activated NR, is a key mediator of bile acid homeostasis, regulating target genes that mediate hepatic export (e.g. *ABCB11*, bile salt export pump; *ABCB4*,

multidrug resistance protein 3), biosynthesis (e.g. *CYP7A*) or enterohepatic circulation (e.g. *NTCP*, *IBABP*) of bile acids, limiting their intrinsic hepatocellular toxicity.

Four different heterozygous variants in the FXR gene (-1g>t, p.Met1Val, p.Trp80Arg, p.Met173Thr) reducing its expression or transcriptional activity were identified from screening 92 women with intrahepatic cholestasis of pregnancy (ICP), a disorder characterized by late gestational pruritus and abnormal maternal and fetal liver function, predisposing to fetal distress and prematurity (65). The heterozygous -1G>T variant was subsequently identified in an unrelated ICP case (66).

Progressive familial intrahepatic cholestasis (PFIC), comprises three subtypes known to be associated with mutations in transport proteins (PFIC-1: *ATP8B1*; PFIC-2: *ABCB11/BSEP*; PFIC-3: *ABCB4*), but 30% of cases are idiopathic. FXR variants have recently been identified in four children with severe, neonatal cholestasis progressing to liver failure that was terminal or required transplantation. A homozygous, premature stop mutation (p.Arg176*) abrogating DNA binding and function was identified in one family and compound heterozygosity for an in-frame DBD insertion (pTyr139_Asn140insLys). A 31kB deletion encompassing the first two coding exons of *NR1H4* was identified in a second family. Similar to previous PFIC cases with defective bile salt export pump (BSEP, a known FXR target), cholestasis was associated with low/normal gamma-glutamyl transferase (GGT) levels and reduced BSEP expression. Severe vitamin K-independent coagulopathy, attributed to reduced FXR-dependent clotting factor levels, and reduced circulating levels of other FXR-dependent hormones and metabolites may represent other distinctive, diagnostically useful, biomarkers (67).

Hepatocyte nuclear factor 4α (NR2A1)

Hepatocyte nuclear factor 4α (HNF4α) controls gene expression in the liver (~40% of actively transcribed genes) and pancreas (11% of islet cell genes) and regulates pathways of hepatic

gluconeogenesis and pancreatic insulin secretion **[Editor's note: Please provide references for this statement.]**

Maturity-onset diabetes of the young (MODY), usually defined as diabetes mellitus (diagnosed before age 25 years) with negative islet cell autoantibodies, is most commonly (~50% of cases) due to mutations in HNF1α (MODY type 3), a homeobox family transcription factor, with *HNF4A* variants accounting for a further 10% of cases (MODY type 1). Approximately 100 different heterozygous mutations (58% missense; 20% frameshift or premature stop; 5% splice site), localizing to *HNF4A* coding exons have been recorded in this dominantly inherited disorder; a further 5% of variants localize to the pancreatic P2 promoter region of *HNF4A*, disrupting known tissue-specific transcription factor binding sites (68). Some HNF4α mutations, even those located outside the canonical DBD, compromise a protein interface in the HNF4α homodimer bound to DNA (69), with other variants disrupting transactivation, nuclear localization, or protein stability. Due to the large number of HNF4α-regulated target genes in liver and pancreas, it has been postulated that haploinsufficiency, with loss of even a fraction of functional receptor homodimers, mediates MODY (70).

In addition to young age of diagnosis and a family history of early-onset diabetes, reduced serum ApoA2 (known to be HNF4α-regulated) and triglyceride levels and exquisite sensitivity to sulfonylurea drug therapy may be useful markers of HNF4α MODY (71). *HNF4A* mutation carriage is also associated with excess insulin secretion, resulting in macrosomia and neonatal hyperinsulinemic hypoglycaemia (HH) in up to 50% of babies; the latter mandates neonatal surveillance of affected pregnancies because HH can be either mild and transient or more severe, requiring treatment with diazoxide (72). In addition to neonatal hyperinsulinism and macrosomia, renal proximal tubulopathy (Fanconi syndrome) with raised urinary calcium, phosphate and oxalate causing nephrocalcinosis has been recorded in patients with a specific HNF4α mutation (p.Arg76Trp) (73).

GWAS do show linkage of common variants around the *HNF4A* locus with T2D; a rare variant (p.Thr130Ile) in *HNF4A* confers modest (1.2 fold) risk of T2D and is associated with HDL cholesterol levels (68).

PPARγ (NR1C3)

PPARγ is essential for adipocyte differentiation but also regulates target genes that mediate triglyceride hydrolysis and fatty acid and glycerol uptake, together with genes involved in fatty acid re-esterification and lipid storage (74). Heterozygous, missense *PPARG* mutations (p. Pro467Leu, Val290Met), impairing its ligand-dependent transcriptional activity, were first identified in patients with severe insulin resistance (IR) and early-onset T2D (75); subsequently, the phenotype was recognized to encompass a distinctive pattern of partial lipodystrophy. Additional features (hepatic steatosis, dyslipidaemia) likely reflect impaired ability to buffer dietary lipid load, with tissue lipotoxicity mediating IR; the resulting hyperinsulinemia mediates polycystic ovarian dysfunction and acanthosis nigricans. Hypertension occurring independent of diabetic comorbidities is also a feature, suggesting a direct role for PPARγ in control of vascular tone (74).

Rare heterozygous *PPARG* variants associated with lipodystrophic IR localize to the LBD or DBD, disrupting either DNA binding or ligand-dependent transcription activation functions. Additionally, mutant receptors inhibit function of their wild-type counterparts in a dominant-negative manner (76). In a large, digenic kindred, PPARγ haploinsufficiency alone did not mediate IR, but acted in concert with a *PPP1R3A* mutation that affects muscle glycogen synthesis (77). Whole exome sequencing of around 9,000 individuals with T2D identified nine functionally deleterious, rare *PPARG* variants conferring substantial disease risk; however, it could not be ascertained whether adipose mass was reduced in these subjects (78). A common *PPARG* variant (p.Pro12Ala), occurring with varying frequency (2 to 18%) in different ethnic groups, is associated with a reduction in T2D risk (OR

0.86); conversely, the Pro12 allele is present in 80% of humans and can increase population T2D risk by up to 25%. Reduced target gene activation and induction of adipogenesis by the Ala12 PPARγ variant may lower adipose mass and improve insulin sensitivity in carriers, forming the basis of its protective effect (79). A rare variant (p.Pro113Gln) in the PPARγ amino-terminal domain, exhibiting gain of transcriptional function, was documented in four obese, but paradoxically diabetic, German subjects, but this or similar variants have not been found in other obese populations, suggesting a strong founder effect (76).

Small heterodimeric partner (NR02B)

Small heterodimer partner (SHP, *NR0B2*) is an atypical orphan NR that has a ligand-like binding domain with sequence homology to other NRs, but a truncated amino-terminal region lacking a true DBD. Heterozygous *NROB2* variants with diminished ability to inhibit HNF4α function were reported in 7% of Japanese patients with early-onset T2D, mild/moderate obesity and increased birth weight (80). A separate study documented loss-of-function *NR0B2* variants in 2.4% (19/805) of Japanese type 2 diabetics, but also in 0.8% (6/752) nondiabetic controls (81). In contrast, studies in different populations have not consistently found such high enrichment for rare SHP variants in cohorts with obesity or diabetes (82-85).

CONCLUSIONS

The identification of naturally occurring NR mutations has provided insights into their structure and function, but there are still many NRs where an associated disorder has not yet been discovered. Looking to the future, exome or genome sequencing may uncover association of NR gene variants with unexpected phenotypes or disorders not readily predicted from their known roles in physiological or developmental processes. In other situations, the phenotype might be subtle or even embryonic lethal. These technologies will also identify genetic variants whose functional consequences are uncertain, emphasizing the need to develop relevant, high-throughput assays of

variant NR function that can accurately predict their pathogenic significance, as has been described recently for *PPARG* (86).

With disorders of many classical NRs associated with changes in hormone levels linked to their cognate ligands, it is likely that defects in orphan receptors are also accompanied by distinctive changes in circulating metabolites or proteins. Metabolomic or proteomic profiling of case cohorts with defined NR gene defects may discern characteristic biochemical signatures, enabling better diagnosis of associated disorders or providing clues to the identification of unknown orphan receptor ligands.

A subset of individuals with typical clinical or biochemical features suggestive of disordered NR action do not have mutations in NR proteins and it is possible that variants in non-coding regions of the genome affecting function of enhancer regions or involving epigenetic modification of chromatin or non-coding RNAs account for such cases. Alternatively, it is possible that defects in genes encoding NR cofactor proteins could be associated with such phenotypes. With our increasing knowledge of the human genome and application of high throughput technologies to genome analysis and small molecule screening, the next 30 years are likely to be an equally exciting time for human NR research.

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FIGURE LEGENDS

Figure 1. A timeline of identification of defects in human NR genes.

The year when the first monogenic disorder associated with each nuclear receptor was published is shown on the x-axis. The cumulative number of human nuclear receptors associated with a disorder is shown by the line (y-axis).

Figure 2. Molecular mechanisms of disrupted NR action.

A. Mechanisms whereby NR gene and protein changes can alter function. Gene deletion or mutations causing mRNA instability or impairing key cellular functions can cause loss-of-function (left side, shown in red). Gain of receptor function due to duplication of an NR genomic locus (e.g. NR0B1) or ligand binding domain mutation (see also Fig 2A) is also recognized (right side, shown in black).

B. In several disorders, heterozygous receptor mutants (e.g. TRβ, TRα, PPARγ, VDR), inhibit the action of their wild type (WT) counterparts in a dominant negative manner. In contrast to WT receptor (upper panel), either defective binding of ligand (L) or recruitment of coactivator (CoA) by mutant (MUT) receptor impairs its dissociation of corepressor (CoR), mediating constitutive repression of target gene expression (lower panel).

C. Alopecia is not a universal feature of hereditary vitamin D resistance, being associated with VDR mutations that disrupt DNA binding, heterodimerization with RXR or cause loss of receptor expression, but not with variants exhibiting impaired ligand binding affinity or coactivator recruitment. Repression of target genes by unliganded wild type receptor maintains a normal hair growth cycle (upper panel), and loss of such inhibition with a subset of VDR mutants (lower panel), is thought to mediate this variable phenotype.

Figure 3. Structural modeling of NR mutations.

A. Modeling (PDB 2H77 TRα, PDB 1KKQ SMRT), showing that mutation of residues (red spheres) in the carboxyterminal region of TRα, which prematurely truncate helix 11 or 12 (left panel), facilitate its ability to accommodate corepressor (SMRT, blue) within a groove at the receptor surface (right panel).

B. Modeling (PDB 1RK3, VDR bound to DRIP 205 coactivator) showing that a glutamic acid residue (red sphere) (left panel) hydrogen bonds with the peptide backbone of coactivator (green; middle panel). Mutation to lysine abolishes this interaction (right panel).

C. Modeling (PDB 5HCV MR, PDB 5L7E S810L mutant MR), showing that wild type receptor, with serine at position 810, accommodates aldosterone via hydrogen bonding (red dotted line) with steroid (middle panel), whereas mutant MR with a leucine substitution accommodates progesterone via van der Waals (blue dotted line) interaction with steroid.

D. Modeling (PDB 2FF0) showing SF-1/NR5A1 bound to DNA. R92 makes an extensive hydrogen bond network in the minor groove to support monomeric binding. This interaction is disrupted by the R92W mutation due to the presence of an indole side-group.

Figure 1

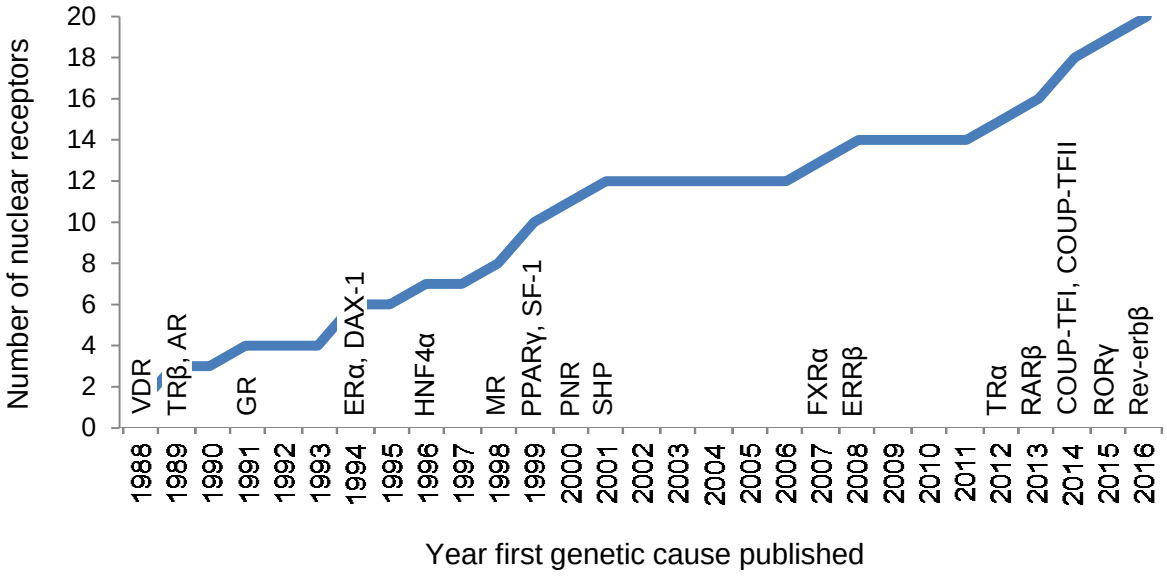

Number of nuclear receptors
0
2
4
6
8
10
12
14
16
18
20
VDR
TRβ, AR
GR
ERα, DAX-1
HNF4α
MR
PPARγ, SF-1
PNR
SHP
FXRα
ERRβ
TRα
RARβ
COUP-TFI, COUP-TFII
RORγ
Rev-erbβ
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Year first genetic cause published

Figure 2A

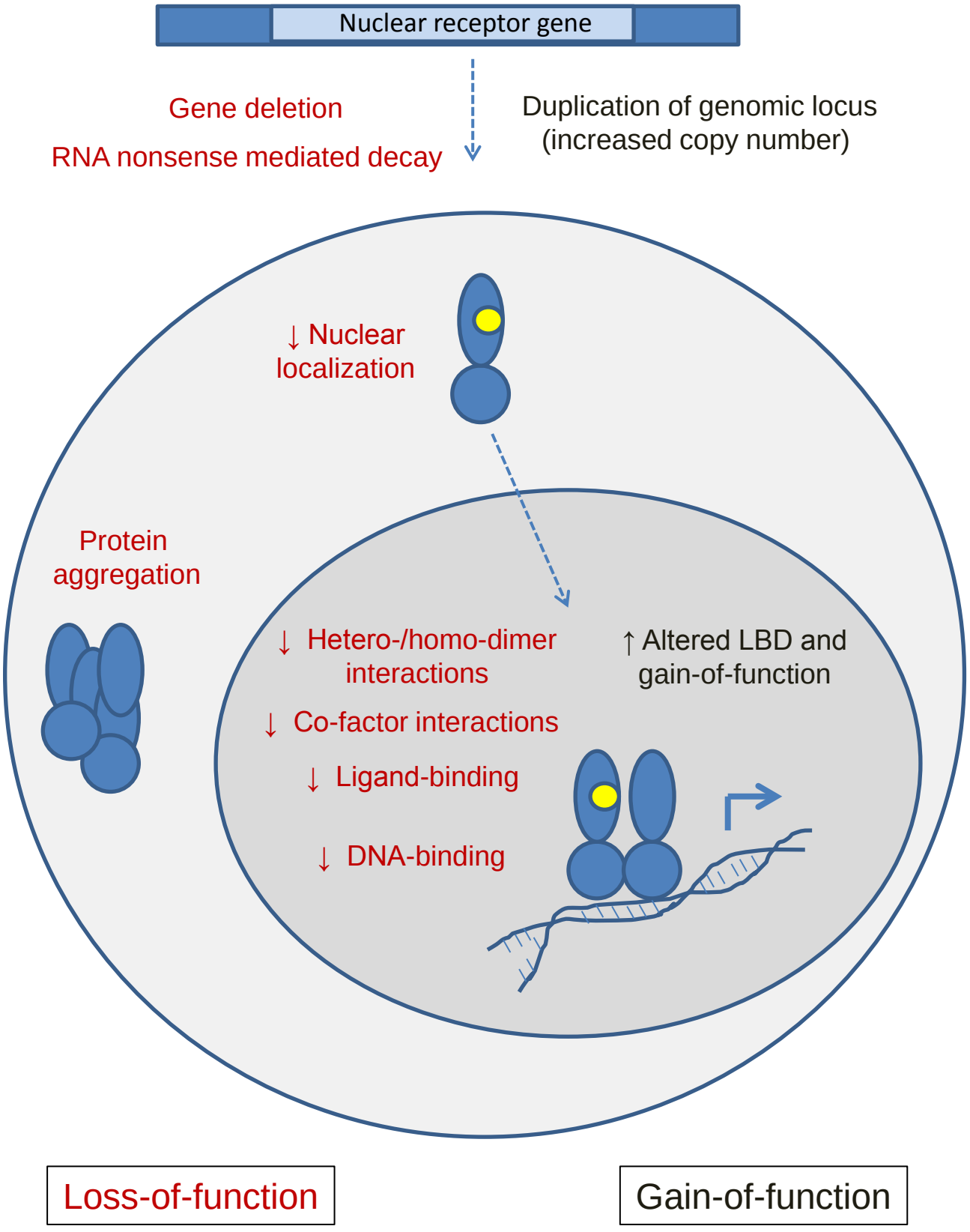

Nuclear receptor gene
Gene deletion
RNA nonsense mediated decay
Duplication of genomic locus
(increased copy number)
↓ Nuclear localization
Protein aggregation
↓ Hetero-/homo-dimer interactions
↑ Altered LBD and gain-of-function
↓ Co-factor interactions
↓ Ligand-binding
↓ DNA-binding
Loss-of-function
Gain-of-function

Figure 2B

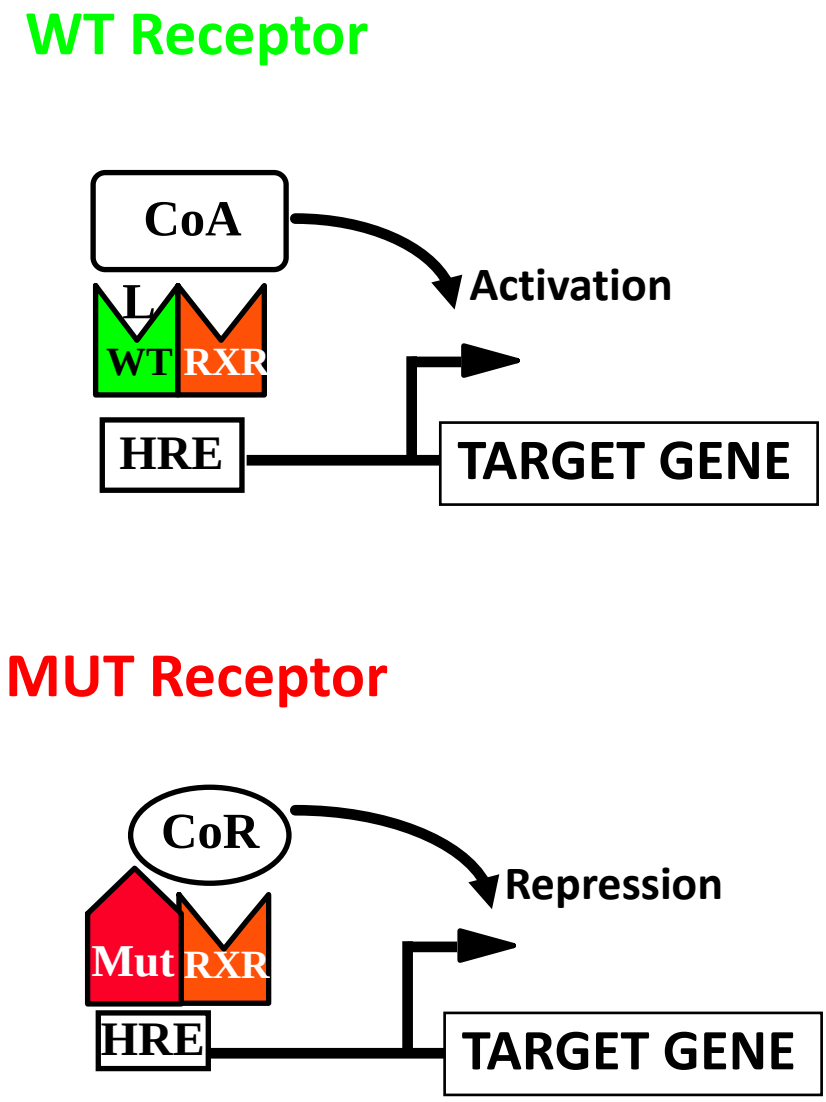
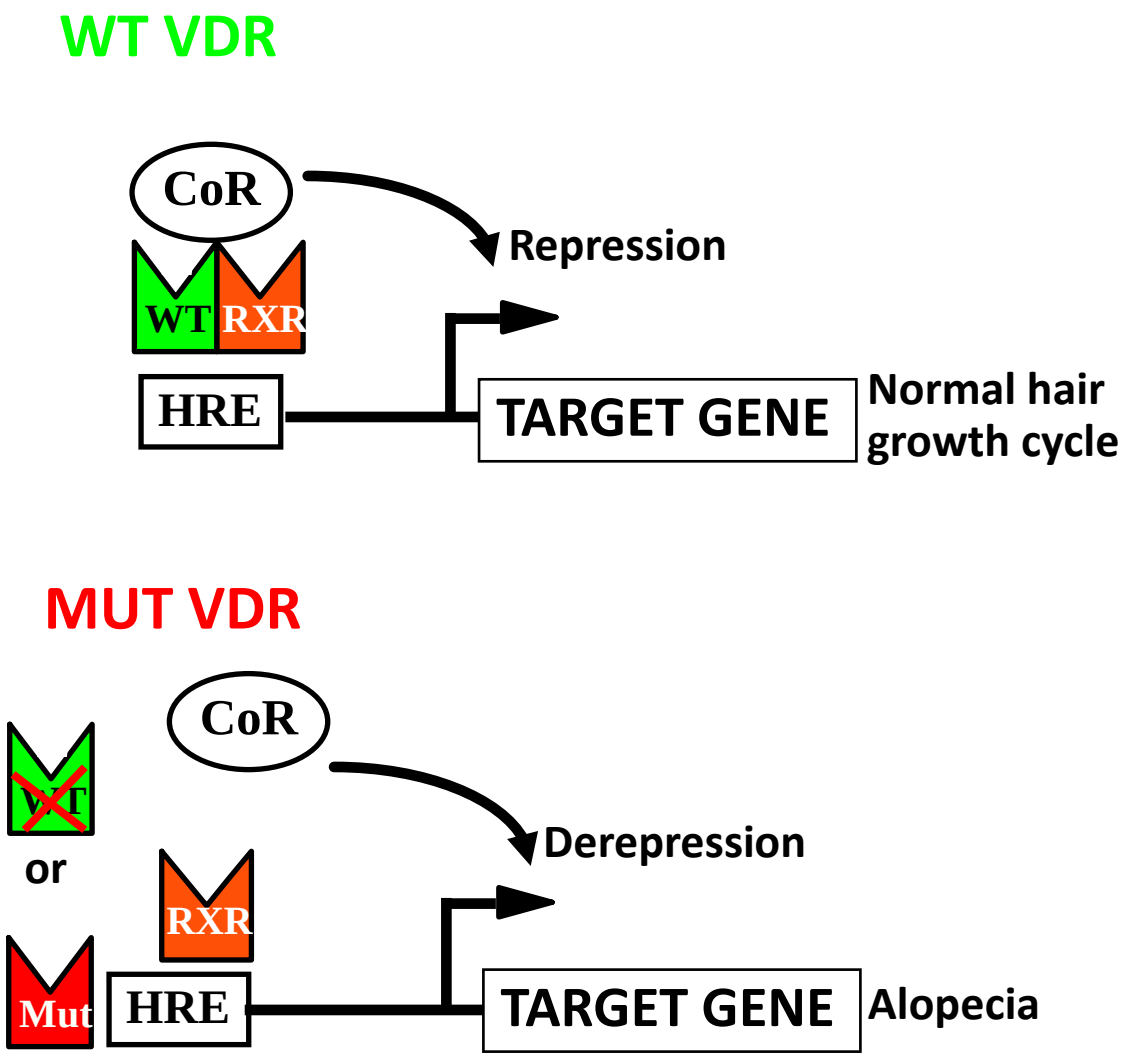
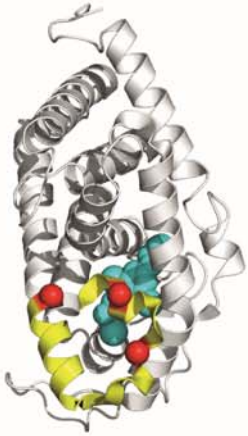

WT Receptor
CoA
L
WT
RXR
Activation
HRE
TARGET GENE
MUT Receptor
CoR
Mut
RXR
Repression
HRE
TARGET GENE

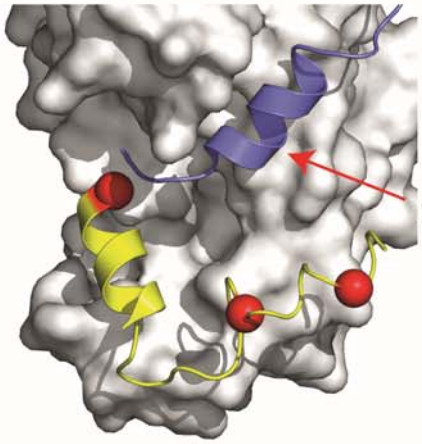
Figure 2C


WT VDR
CoR
Repression
WT
RXR
HRE
TARGET GENE
Normal hair growth cycle
MUT VDR
CoR
or
Derepression
RXR
Mut
HRE
TARGET GENE
Alopecia

A

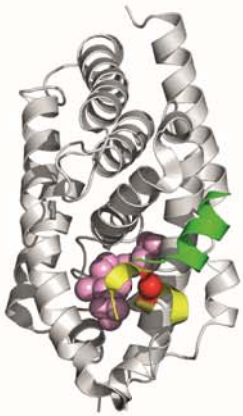
Thyroid receptor

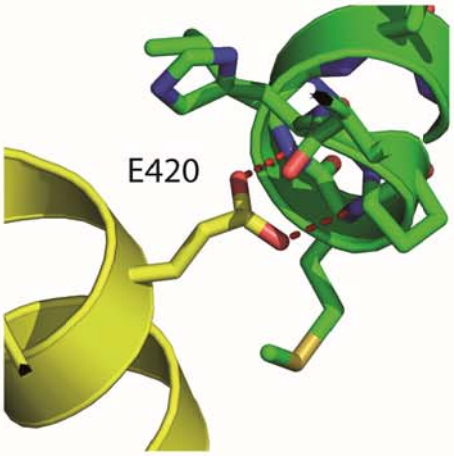


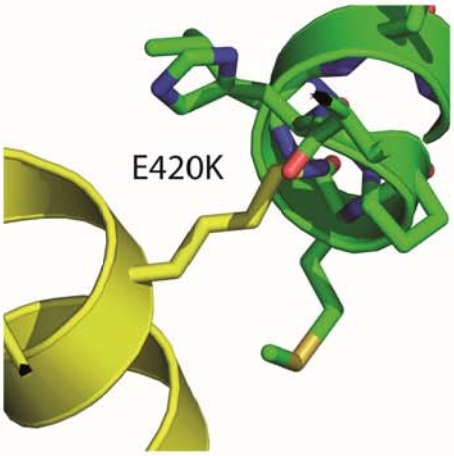


B

Vitamin D receptor

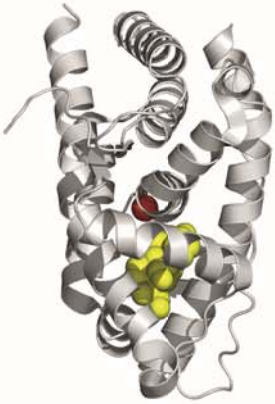


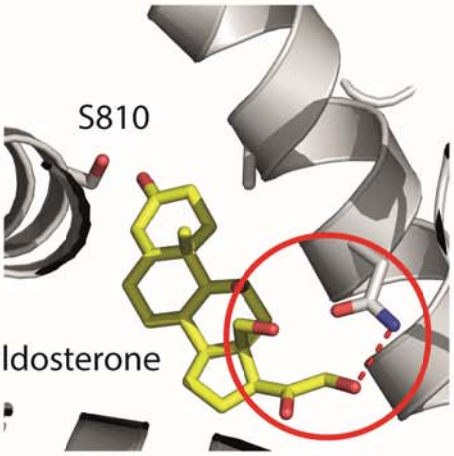


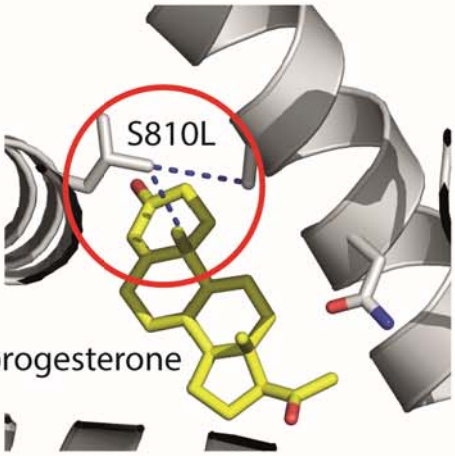


C

Mineralocorticoid Receptor

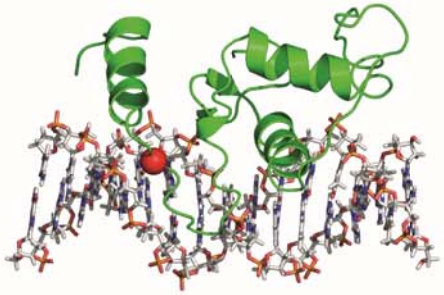


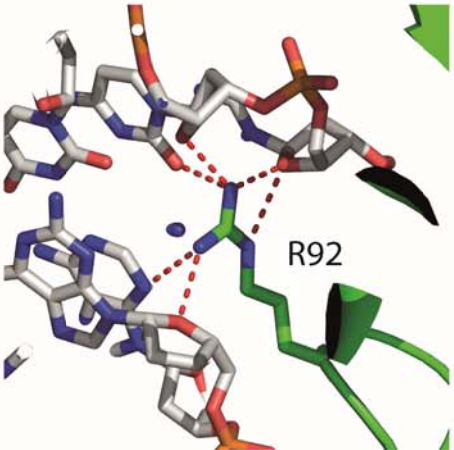




D

SF-1/NR5A1





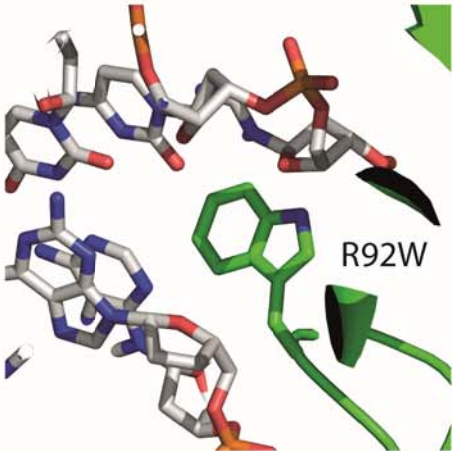


Table 1. Overview of pathogenic variants in classic ligand-dependent NRs associated with human genetic disorders

Receptor	Original name	Official name	HGNC gene	Ligand	OMIM	First report[A]	Number[B]	Inherited	Condition
TRα	Thyroid hormone receptor-α	*NR1A1*	*THRA*	Thyroid hormones	614450	2012	10	AD	Resistance to THα
TRβ	Thyroid hormone receptor-β	*NR1A2*	*THRB*	Thyroid hormones	188570	1989	100-200	AD	Resistance to THβ (dominant)
					274300	1992	5	AR	Resistance to THβ (recessive)
VDR	Vitamin D3 receptor	*NR1I1*	*VDR*	Vitamin D, 1,25-dihydroxy-vitamin D3	277440	1988	10 to 50	AR	Vitamin D-resistant rickets type IIA
GR	Glucocorticoid receptor	*NR3C1*	*NR3C1*	Cortisol	615962	1991	10 to 50	AD, AR	Glucocorticoid resistance
MR	Mineralocorticoid receptor	*NR3C2*	*NR3C2*	Aldosterone	177735	1998	50 to 100	AD	Pseudohypoaldosteronism (type I, autosomal dominant)
					605115	2001	1	AD	Hypertension with exacerbation in pregnancy
ERα	Estrogen receptor-α	*NR3A1*	*ESR1*	Estradiol	615363	1994	3	AR	Estrogen resistance
AR	Androgen receptor	*NR3C4*	*AR*	Testosterone	300068	1989	> 200	XLR	Androgen insensitivity
					312300	1991	> 200	XLR	Partial androgen insensitivity
					N/A	1994	50 to 100	XLR	Mild androgen insensitivity (infertility)
					313200	1994	> 200	XLR	Kennedy spinal and bulbar muscular atrophy

[A]First report shows the year that point mutations in the causative gene were first published. In some situations, clinical features of the condition were described previously.

Glucocorticoid resistance was first reported as a clinical syndrome in 1976 and studied further in relation to possible GR insensitivity throughout the 1980s.

Pseudohypoaldosteronism in infancy was first reported in 1958 and decreased aldosterone binding to patient cells, suggesting a defect in the MR, was shown in 1985.

Androgen insensitivity syndrome was first reported as “testicular feminization syndrome” in 1953 and identified as an X-linked condition likely due to androgen resistance in the 1960s and 1970s. In the 1980s decreased androgen binding to fibroblasts was shown for a subset of individuals with AIS.

[B]Number reflects either sporadic cases or families with the condition, not total number of affected individuals.

Abbreviations: OMIM, Online Mendelian Inheritance in Man; AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

Table 2. Overview of pathogenic variants in orphan or non-classic NRs associated with human genetic disorders

Receptor	Original name	Official name	HGNC gene	Ligand	OMIM	First report[A]	Number[B]	Inherited	Condition
SF1	Steroidogenic factor-1	*NR5A1*	*NR5A1*	Orphan	612965	1999	2	AD, AR	Primary adrenal insufficiency and gonadal dysgenesis (46,XY)
					612965	2003	100-200	AD, AR, SLD	46,XY DSD
					612964	2009	10 to 50	AD, AR	Primary Ovarian Insufficiency
					613957	2010	10 to 50	AD	Male factor infertility
					N/A	2016	10 to 50	AD	46,XX ovotesticular/testicular DSD
					N/A	2000	2	AD, AR	Primary adrenal insufficiency (46,XX)
DAX-1	Dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1	*NR0B1*	*NR0B1*	Orphan	300200	1994	> 200	XLR	X-linked adrenal hypoplasia (with hypogonadotropic hypogonadism , male infertility)
					300018	1991	10 to 50	dup	46,XY DSD
RARβ	Retinoic acid receptor-β	*NR1B2*	*RARB*	Retinoic acid	615524	2013	4	AD, AR	Syndromic microophthalmia (type 12), diaphragmatic hernia, pulmonary hypoplasia, cardiac defects
RORγ	RAR-related orphan receptor-γ	*NR1F3*	*RORC*	Orphan	616622	2015	3	AR	Immunodeficiency (type 42)
PNR	Photoreceptor-specific nuclear receptor	*NR2E3*	*NR2E3*	Orphan	268100	2000	50 to 100	AR	Enhanced S-cone disease (including Goldman-Favre syndrome)
					611131	2007	10 to 50	AR, AD	Retinitis pigmentosa (type 37)
COUP-TFI	Chicken ovalbumin upstream promoter transcription factor I	*NR2F1*	*NR2F1*	Orphan	615722	2014	10 to 50	AD	Bosch-Boonstra-Schaaf optic atrophy syndrome (developmental delay)
COUP-TFII	Chicken ovalbumin upstream promoter transcription factor II	*NR2F2*	*NR2F2*	Orphan	615779	2014	10 to 50	AD	Congenital heart defects, multiple (type 4)
Rev-erbβ[C]	Rev-erbβ	*NR1D2*	*NR1D2*	Orphan	N/A	2016	1	AD[D]	Congenital heart defects (AVSD)
ERRβ	Estrogen-related receptor-β	*NR3B2*	*ESRRB*	Orphan	608565	2008	< 10	AR	Autosomal recessive deafness (type 35)
FXRα	Farsenoid receptor-α	*NR1H4*	*NR1H4*	Bile acids	617049	2007	< 10	AR	Intrahepatic cholestasis of pregnancy Progressive familial intrahepatic cholestasis
HNF4α	Hepatocyte nuclear factor 4 receptor-α	*NR2A1*	*HNF4A*	Orphan	125850	1996	100	AD	MODY type 1 Hyperinsulinaemic hypoglycaemia
					616026	2014	1	AD[D]	Fanconi renotubular syndrome type 4 with MODY
PPARγ	Peroxisome proliferator activated receptor-γ	*NR1C3*	*PPARG*	Fatty acids, eicosanoids	604367	1999	10 to 50	??	Familial partial lipodystrophy (type 3) Digenic severe IR

					601665	1998	1	AD[D]	Severe Obesity
					125853	1998	1	AD[D]	Resistance to T2D
SHP	Small heterodimeric partner	*NR0B2*	*NR0B2*	Orphan	601665	2001	10 to 50	AD	Mild obesity, high birth weight (T2D)

[A]First report shows the year that point mutations in the causative gene were first published. JHH: Yes, ideally, they should have their own superscripted callouts. In some situations, clinical features of the condition were described previously. For example, X-linked adrenal hypoplasia congenita (X-linked AHC) causing "cytomegalic" adrenal hypoplasia was first reported in 1948 and the X-linked basis identified in the 1970s, followed by reports of gene deletion syndromes involving chromosome Xp21 in the 1980s. Enhanced S-cone syndrome was first described in 1990 and thought to be a disorder of photoreceptor determination and proliferation in 1995.

[B]Number reflects either sporadic cases or families with the condition, not total number of affected individuals.

[C]Association of mutations in Rev-erbβ (NR1D2) with congenital heart defects is currently based on a single case report.

[D]Inhertiance patterns are tentative, especially when only one individual or family has been reported.

Abbreviations: OMIM, Online Mendelian Inheritance in Man; N/A, not available; AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive; SLD, sex-linked dominant; MODY, maturity-onset diabetes of the young; DSD, disorders of/differences in sex development; IR, insulin resistance.