REVIEW

# Recent advances in understanding Cushing disease: resistance to glucocorticoid negative feedback and somatic USP8 mutations [version 1; referees: 2 approved]

Eleni Daniel, John Newell-Price

Department of Oncology and Metabolism, University of Sheffield Medical School, Sheffield, UK

**v1** **First published:** 02 May 2017, **6**(F1000 Faculty Rev):613 (doi: 10.12688/f1000research.10968.1)

**Latest published:** 02 May 2017, **6**(F1000 Faculty Rev):613 (doi: 10.12688/f1000research.10968.1)

## Abstract

Cushing's disease is a rare disease with a characteristic phenotype due to significant hypercortisolism driven by over-secretion of adrenocorticotropic hormone and to high morbidity and mortality if untreated. It is caused by a corticotroph adenoma of the pituitary, but the exact mechanisms leading to tumorigenesis are not clear. Recent advances in molecular biology such as the discovery of somatic mutations of the ubiquitin-specific peptidase 8 (*USP8*) gene allow new insights into the pathogenesis, which could be translated into exciting and much-needed therapeutic applications.

**Open Peer Review**

**Referee Status:** ✓ ✓

| | Invited Referees 1 | 2 |
|---|---|---|
| **version 1** published 02 May 2017 | ✓ | ✓ |

F1000 Faculty Reviews are commissioned from members of the prestigious F1000 Faculty. In order to make these reviews as comprehensive and accessible as possible, peer review takes place before publication; the referees are listed below, but their reports are not formally published.

1 **Richard Feelders**, Erasmus Medical Center Netherlands

2 **Marco Boscaro**, Padova University Hospital Italy

**Discuss this article**

Comments (0)

**Corresponding author:** John Newell-Price (j.newellprice@sheffield.ac.uk)

**How to cite this article:** Daniel E and Newell-Price J. **Recent advances in understanding Cushing disease: resistance to glucocorticoid negative feedback and somatic USP8 mutations [version 1; referees: 2 approved]** *F1000Research* 2017, **6**(F1000 Faculty Rev):613 (doi: 10.12688/f1000research.10968.1)



**Grant information:** The author(s) declared that no grants were involved in supporting this work.

**Competing interests:** The authors declare that they have no competing interests.

**First published:** 02 May 2017, **6**(F1000 Faculty Rev):613 (doi: 10.12688/f1000research.10968.1)

## Introduction

Cushing's disease (CD) is caused by a pituitary corticotroph adenoma over-secreting adrenocorticotropic hormone (ACTH) leading to excess cortisol secretion[1]. It is a rare disease, associated with high mortality and morbidity if untreated[2]. Morbidity is mainly due to the chronic metabolic adverse effects of hypercortisolism leading to the clinical features that include those due to protein wasting (skin thinning, myopathy), changes in fat distribution and glucose intolerance, osteoporosis, life-threatening infections, and psychiatric and cognitive changes, including depression and psychosis[3]. More than 90% of corticotroph adenomas present as microadenomas (that is, small tumors with a maximum diameter of less than 10 mm), but occasionally tumors can be macroadenomas of significant volume and cause pressure to surrounding structures such as the optic chiasm and the cavernous sinuses and may have invasive features. The only curative treatment is surgical resection of the tumor, usually performed through a transsphenoidal approach. Although initial remission is achieved in around 60% to 75% of patients, up to 30% experience recurrent disease on long-term follow-up. The treatment of recurrent disease is challenging, and options include radiotherapy, medical therapy, or additional surgery[4].

The pathogenesis of corticotroph adenomas is not clear. Corticotroph adenomas are monoclonal in origin, meaning that they arise from a single cell that multiplies to cause tumor growth[5–8]. This strongly points to a single somatic genetic defect in a corticotroph cell as the etiologic mechanism of disease[5] and implies that tumorigenesis occurs at the level of the pituitary. However, it is possible that other hypothalamic factors allow or facilitate this process. Genetic mutations have been identified in corticotroph adenomas; however, we are far from completely understanding the mechanisms leading to tumorigenesis, ACTH hyper-secretion, and invasiveness.

A better understanding of the pathogenesis could help identify new therapeutic targets. Existing medical treatments aim to control the hypercortisolemia associated with this condition and its devastating long-term effects but are not always effective. In this report, we present the current understanding of the pathogenesis of CD with an emphasis on molecular discoveries that have been reported in the last few years. These discoveries have created new possibilities for therapeutic targets, much needed for patients who cannot be cured by surgery. We will provide an overview of corticotroph tumorigenesis in the context of hypothalamic-pituitary-adrenal (HPA) axis regulation with an emphasis on the role of the glucocorticoid receptor in the resistance to the negative feedback of cortisol that occurs in CD, and we will explore the role of epidermal growth factor receptor (EGFR) signaling in ACTH hyper-secretion and corticotroph cell proliferation and the recent discovery of somatic ubiquitin-specific peptidase 8 (USP8) mutations in a significant number of patients with sporadic CD with an emphasis on therapeutic implications.

## Corticotroph adenoma pathogenesis and the hypothalamus-pituitary-adrenal axis

### Normal physiology

Corticotroph cell function is tightly regulated as part of the HPA axis. Hypothalamic corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) stimulate pituitary corticotroph cells to secrete ACTH[9]. CRH acts by binding to a G protein-coupled receptor on the cell surface of the corticotroph cells, CRH-R1. Ligand binding to CRH-R1 activates stimulatory G-protein alpha subunit ($G_{as}$), causing downstream intracellular signaling through cAMP and protein kinase A[10,11]. The final event of the signaling cascade is promotion of proopiomelanocortin gene (*POMC*) transcription and ACTH release[11,12]. POMC is a precursor polypeptide and upon cleavage produces ACTH[13]. In turn, ACTH binds to melanocortin 2 receptor (MC2R) on the surface of adrenocortical cells and stimulates the steroidogenesis pathway. The end product, cortisol, is released to the circulation and regulates the HPA axis by negative feedback through the glucocorticoid receptor type 2 (NR3C1) at the level of the hypothalamus and the pituitary. The NR3C1/cortisol complex translocates from the cytoplasm to the nucleus to bind to regulatory areas on the DNA and inhibits synthesis of POMC, CRH, and AVP mRNA and therefore reduces ACTH secretion. In theory, deregulation of any part of this complex process at the level of hypothalamus, pituitary, or negative glucocorticoid feedback mediated by NR3C1 could lead to tumorigenesis.

### The role of cortisol-negative feedback in corticotroph tumorigenesis

Loss of sensitivity to the negative cortisol feedback at the level of the pituitary and hypothalamus is a key feature of CD and is used for biochemical diagnosis[14]. Normally, *in vivo* administration of an exogenous glucocorticoid such as dexamethasone leads to suppression of endogenous cortisol and ACTH production, whereas in CD there is inadequate suppression of cortisol levels[3]. Incubation of primary cultures from corticotroph adenomas with cortisol causes a reduction in ACTH levels, indicating some response to negative feedback[15]; however, dexamethasone treatment causes less inhibition of ACTH release and POMC mRNA levels in cultured corticotroph adenoma cells than non-adenomatous pituitary cells[16]. Downregulation of the glucocorticoid receptor or mutations in its signaling pathway could be a plausible explanation of glucocorticoid resistance; however, in ACTH-producing corticotroph adenomas, the expression of the receptor has been found to be increased, and although NR3C1 mutations have been found in cases of CD, these are not frequent[17,18]. At the pre-receptor level, 11β-hydroxysteroid dehydrogenase type 2, a key enzyme that regulates cortisol activity in the tissues by converting active cortisol to inactive cortisone, may also be involved as it has been found to be highly expressed in corticotrophinoma cells but not normal corticotroph cells, indicating a mechanism through which the feedback of cortisol to the pituitary could be compromised in tumor cells[19,20]. Recent studies elucidate other mechanisms through which NR3C1 is implicated in the resistance to the negative glucocorticoid feedback seen in CD.

### Testicular orphan nuclear receptor 4

Testicular orphan nuclear receptor 4 (TR4) is a nuclear receptor encoded by the NR2C2 gene and acts as a regulator of transcription (activator or repressor) in various tissues, including the central nervous system and reproductive tissues[21]. A murine knockout for *TR4* exhibits growth retardation, weight loss, and reduced lipid accumulation[22–24]. TR4 is overexpressed in corticotroph adenomas and corticotroph tumor cell lines and activates *POMC* by binding to its promoter, an effect that is enhanced by

phosphorylation of TR4 through the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway. Overexpression of TR4 also induces ACTH secretion, cell proliferation, and tumor growth in a murine animal model harboring ACTH-secreting tumors[25]. Further studies showed that TR4 interacts with the glucocorticoid receptor, NR3C1, and overrides the negative regulation of NR3C1 on *POMC* transcription and ACTH secretion[26]. This indicates a pathway through which TR4 promotes resistance to the negative glucocorticoid feedback. Previous studies have shown that TR4 can be trans-activated by peroxisome proliferator-activated receptor-gamma (PPARγ) agonists such as rosiglitazone and polyunsaturated fatty acids[22]. In contrast to the mechanism described above, rosiglitazone has been shown to reduce ACTH secretion in cell lines, but clinical trials in patients with CD have had mixed results[27–30]. A possible explanation for this is that PPARγ agonists trigger multiple pathways in corticotroph cells, and further research into the role of TR4 may help identify other specific therapeutic targets for the treatment of CD.

### Heat shock protein 90

The *in vivo* function of the glucocorticoid receptor is heavily dependent on its interaction with the heat shock protein 90 (HSP90). HSP90 is a chaperone protein that stabilizes and activates proteins through induction of conformational changes. HSP90 protein interacts with the glucocorticoid receptor to facilitate ligand binding and aids its translocation to the nucleus, where NR3C1 binds to DNA and promotes transcription[31,32]. Corticotroph adenomas overexpress HSP90, and some inhibitors of HSP90 can enhance the transcriptional activity of the glucocorticoid receptor by inducing its release from HSP90 in a stable and high-affinity state for ligand binding[33]. Silibinin, a C-terminal HSP90 inhibitor, increased the transcriptional activity of NR3C1 in murine corticotroph cells and enhanced the suppression of ACTH secretion in primary corticotroph adenoma cell cultures, restoring glucocorticoid sensitivity *in vitro*. In keeping with the *in vitro* data, oral administration of silibinin in a murine CD model caused reductions in clinical features and tumor growth and in ACTH and endogenous glucocorticoid levels. Silibinin is a commercially available extract of milk thistle seeds and has been used in the treatment of prostate cancer and hepatotoxicity with a good safety profile and therefore is an interesting agent for assessment in the treatment of CD[34], and clinical trials using this agent have been proposed.

## Familial endocrine syndromes and Cushing's disease

Corticotroph adenomas are usually sporadic and only rarely have been observed as part of familial endocrine genetic syndromes. Germline mutations that cause familial endocrine syndromes affect different pathways, are seen infrequently in CD, and do not explain the majority of the corticotroph adenomas or indicate a common mechanistic explanation for the pathogenesis of CD. Familial CD has been reported in germline mutations of the tumor suppressor *MENIN* (causing multiple endocrine neoplasia type 1 syndrome, or MEN1), aryl-hydrocarbon receptor-interacting protein gene (*AIP*), and *CDKN1B* gene (or *p27/Kip1*) that encodes for p27, a cell cycle inhibitor and causes multiple endocrine neoplasia type 4 (MEN4)[35–41]. McCune-Albright syndrome is caused by post-zygotic somatic mutations in the *GNAS1* gene that encodes a stimulatory $G_{as}$ and causes a constitutive activation of the Gsα-cAMP signaling pathway. The disease is commonly associated with somatotrophinomas, and only three cases of CD have been described as harboring *GNAS* mutations in the tumor tissue[35,42,43].

## EGFR signaling

EGFR is a cell surface receptor with tyrosine kinase activity and is a member of the ErbB family of cell surface tyrosine kinase receptors. It is activated by ligand binding with peptide growth factors such as epidermal growth factor (EGF)[44]. Upon ligand binding, it forms homodimers, and the intracellular tyrosine kinase domain is activated. This promotes signal transduction through complex downstream phosphorylation pathways involving the MAPK pathway, the phosphoinositol kinase (PI3), phospholipase C gamma (PLCγ), and transcription factors, culminating in promoting cell proliferation and cell differentiation in various tissues[45–47]. Following signal transduction, the ligand-activated EGFR internalizes and is tagged with ubiquitin protein and degraded in the lysosomes[48]. EGFR signaling confers a powerful proliferation signal; overexpression of EGFR has been found in several cancers, and EGFR inhibitors are used in the treatment of these tumors[49–51].

EGFR signaling promotes proliferation and ACTH secretion in corticotroph cells. EGFR and its ligand EGF are highly expressed in corticotroph adenoma cells in up to 75% of corticotroph tumors as well as normal pituitary cells, gonadotroph, somatotroph, and lactotroph adenoma cells[52–55]. Higher levels of expression of EGFR/EGF in corticotroph adenoma cells are correlated with more aggressive tumors[52,54,56–58]. EGFR signaling in corticotroph cells could lead to proliferation by downregulation of *p27/Kip1* through a MAPK/ERK pathway[55]. Low expression of p27 has been found in CD, and a mouse knockout model for *p27/kip1* gene develops corticotroph tumors of the intermediate lobe and weight gain[59–61]. In humans, however, no somatic mutations of *p27/kip1* were found in 20 corticotroph adenomas[62]. Additionally to its proliferating effects, EGFR signaling promotes *POMC* expression and ACTH secretion through activation of the MAPK pathway[15,57,63]. In contrast, inhibition of EGFR signaling by gefitinib, an EGFR kinase inhibitor, inhibits *pomc* expression in mice and corticotroph cell proliferation in cell cultures, decreasing tumor growth and cortisol levels with improvement of clinical features[64].

## Somatic USP8 mutations in corticotroph adenomas

Sporadic corticotroph adenomas only rarely harbor somatic mutations in the genes that cause CD by germline mutations[65]. Recently, extremely elegant whole-exome sequencing and functional studies have shown that somatic mutations involving the *USP8* gene are found in 35% to 62% of sporadic corticotroph adenomas, providing significant insight into the mechanisms of disease and a direct link with EGFR signaling[66]. *USP8* is located on chromosome 15q21.2 and encodes a deubiquitinating enzyme, a protein member of the ubiquitin-specific processing protease family[67]. Ubiquitination is a reversible post-translational modification that targets proteins, including cell surface receptors, for degradation by the endosome-lysosome system through conjugation with a single or multiple ubiquitin proteins at lysine residues[68]. USP8 catalyzes the cleavage of ubiquitin tags (deubiquitination) and is involved

in tyrosine kinase receptor trafficking and endosome-lysosome function, leading to receptor recycling to the cell surface[69–72].

Further studies confirmed these findings; somatic *USP8* mutations were found in 35% of patients with CD (21 out of 60) in a series from Japan, 62% of corticotroph adenomas (75 out of 120) in a large series from China, and 36% (48 out of 134) in an international series[73–75]. These mutations seem to be specific to corticotroph adenomas since no *USP8* mutations were detected in 80 non-functioning pituitary adenomas, 80 prolactinomas, 84 growth hormone-secreting adenomas, and 58 pituitary adenomas of other etiologies[73,75,76]. USP8 has a 14-3-3 protein-binding site, and the *USP8* mutations found in corticotroph adenomas clustered to the 14-3-3 protein-binding motif encoded by exon 14 of the USP8 gene. 14-3-3 proteins are highly conserved regulatory proteins that bind to common recognition motifs and modify protein activity and interactions of the protein with other molecules; in the case of USP8, binding of 14-3-3 protein inhibits its deubiquitinating activity[77–79]. The *USP8* mutations found in CD caused impaired 14-3-3 protein binding which, in the majority of the mutations described, resulted in a proteolytic cleavage immediately upstream the 14-3-3 binding site because of an unidentified protease accessing the site. The cleavage created two fragments of USP8 protein, sized 40 and 90 KDa; the 40-KDa fragment possessed increased deubiquitinating activity and caused a significant inhibition of EGFR downregulation by degradation at the lysosomes, increased re-cycling of EGFR, and augmented EGFR-induced MAPK signaling leading to high POMC mRNA expression[66,74,75,80].

In corticotroph adenomas, *USP8* mutations were more likely in females, smaller-sized tumors, and microadenomas[66,74,75,80]. No difference in age, serum cortisol secretion, or Ki67 index was reported[74]. An international multicenter study in 134 secreting and 11 silent corticotroph adenomas in 105 adults and 29 pediatric cases showed that 36% of secreting corticotroph adenomas (48 out of 134) carry *USP8* mutations but that none of the silent corticotroph adenomas harbored mutations and that tumors carrying *USP8* mutations were more likely in adults[80].

These findings have clear implications that may translate into much-needed therapeutic applications since the identified signaling pathways could be targeted for treatment of CD. Inhibition of USP8, EGFR, or downstream signaling regulators and molecules involved in these pathways holds promise for treating *USP8*-mutated disease. In primary corticotroph cell cultures consisting of *USP8*-mutated tumor cells, USP8 small interfering RNA (siRNA) knockdown or blocking of EGFR attenuated ACTH secretion, an effect that was also achieved by treatment with the currently available EGFR inhibitor, gefitinib[75]. Furthermore, molecular characterization of tumors may help inform drug selection as *USP8*-mutated corticotroph tumors were more likely to express somatostatin receptor 5 (SSTR5), a receptor that can be targeted by the somatostatin analog pasireotide, and O6-methylguanine–DNA methyltransferase (*MGMT*) mRNA, indicating less favorable response to temozolomide, an alkylating chemotherapy agent used in aggressive CD[74]. More studies are needed to confirm these findings. However, clinical applications are already emerging; the EGFR inhibitor gefitinib is currently being assessed for the treatment of hypercortisolemia in CD in a phase 2 study in China[81].

## Conclusions

Understanding the mechanisms leading to the development of CD has been restricted by the low incidence of disease and limited tissue availability for research. Recent molecular developments give intriguing insights into the pathogenesis of CD in a significant number of corticotroph tumors. However, we are still far from understanding the neoplastic process completely. There has been progress in understanding the mechanism of glucocorticoid feedback resistance, a central feature in CD that allows tumors to escape the physiological regulatory mechanisms, through the identification of the interaction of the glucocorticoid receptor with transcription regulators TR4 and HSP90. The discovery of *USP8* mutations in a significant number of corticotroph adenomas (35% to 62%) highlighted the role of enhanced EGFR signaling in the pathogenesis of CD; untangling the interactions of downstream signaling molecules in this pathway (or these pathways) opens up a new area of research into CD pathogenesis. Molecules involved in USP8/EGFR and TR4 signaling pathways as well as selective inhibitors of HSP90 are emerging as attractive therapeutic targets, especially as no ideal treatment exists for treating corticotroph adenomas not cured by surgery, possibly paving the way for personalized medicine in the future. The outcomes of clinical trials using compounds that target these pathways are keenly awaited.

## Abbreviations

ACTH, adrenocorticotropic hormone; AVP, arginine vasopressin; CD, Cushing's disease; CDKN1B, cyclin-dependent kinase inhibitor 1B; CRH, corticotropin-releasing hormone; CRH-R1, corticotropin-releasing hormone receptor 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HPA, hypothalamic-pituitary-adrenal; HSP90, heat shock protein 90; MAPK, mitogen-activated protein kinase; NR3C1, glucocorticoid receptor; POMC, proopiomelanocortin; PPARγ, peroxisome proliferator-activated receptor-gamma; TR4, testicular orphan nuclear receptor 4; USP8, ubiquitin-specific peptidase 8.

### Competing interests

The authors declare that they have no competing interests.

### Grant information

The author(s) declared that no grants were involved in supporting this work.

## References

F1000 recommended


1. F Lacroix A, Feelders RA, Stratakis CA, *et al.*: **Cushing's syndrome.** *Lancet.* 2015; **386**(9996): 913–27.
PubMed Abstract | Publisher Full Text | F1000 Recommendation
2. Etxabe J, Vazquez JA: **Morbidity and mortality in Cushing's disease: an epidemiological approach.** *Clin Endocrinol (Oxf).* 1994; **40**(4): 479–84.
PubMed Abstract | Publisher Full Text
3. Newell-Price J, Trainer P, Besser M, *et al.*: **The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states.** *Endocr Rev.* 1998; **19**(5): 647–72.
PubMed Abstract | Publisher Full Text
4. Nieman LK, Biller BM, Findling JW, *et al.*: **Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline.** *J Clin Endocrinol Metab.* 2015; **100**(8): 2807–31.
PubMed Abstract | Publisher Full Text | Free Full Text
5. Biller BM, Alexander JM, Zervas NT, *et al.*: **Clonal origins of adrenocorticotropin-secreting pituitary tissue in Cushing's disease.** *J Clin Endocrinol Metab.* 1992; **75**(5): 1303–9.
PubMed Abstract | Publisher Full Text
6. Herman V, Fagin J, Gonsky R, *et al.*: **Clonal origin of pituitary adenomas.** *J Clin Endocrinol Metab.* 1990; **71**(6): 1427–33.
PubMed Abstract | Publisher Full Text
7. Alexander JM, Biller BM, Bikkal H, *et al.*: **Clinically nonfunctioning pituitary tumors are monoclonal in origin.** *J Clin Invest.* 1990; **86**(1): 336–40.
PubMed Abstract | Publisher Full Text | Free Full Text
8. Gicquel C, Le Bouc Y, Luton JP, *et al.*: **Monoclonality of corticotroph macroadenomas in Cushing's disease.** *J Clin Endocrinol Metab.* 1992; **75**(2): 472–5.
PubMed Abstract | Publisher Full Text
9. Vale W, Spiess J, Rivier C, *et al.*: **Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin.** *Science.* 1981; **213**(4514): 1394–7.
PubMed Abstract | Publisher Full Text
10. Aguilera G, Nikodemova M, Wynn PC, *et al.*: **Corticotropin releasing hormone receptors: two decades later.** *Peptides.* 2004; **25**(3): 319–29.
PubMed Abstract | Publisher Full Text
11. Chen R, Lewis KA, Perrin MH, *et al.*: **Expression cloning of a human corticotropin-releasing-factor receptor.** *Proc Natl Acad Sci U S A.* 1993; **90**(19): 8967–71.
PubMed Abstract | Publisher Full Text | Free Full Text
12. Giguere V, Labrie F, Cote J, *et al.*: **Stimulation of cyclic AMP accumulation and corticotropin release by synthetic ovine corticotropin-releasing factor in rat anterior pituitary cells: site of glucocorticoid action.** *Proc Natl Acad Sci U S A.* 1982; **79**(11): 3466–9.
PubMed Abstract | Publisher Full Text | Free Full Text
13. Raffin-Sanson ML, de Keyzer Y, Bertagna X: **Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions.** *Eur J Endocrinol.* 2003; **149**(2): 79–90.
PubMed Abstract | Publisher Full Text
14. Nieman LK, Biller BM, Findling JW, *et al.*: **The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline.** *J Clin Endocrinol Metab.* 2008; **93**(5): 1526–40.
PubMed Abstract | Publisher Full Text | Free Full Text
15. Grino M, Boudouresque F, Conte-Devolx B, *et al.*: ***In vitro* corticotropin-releasing hormone (CRH) stimulation of adrenocorticotropin release from corticotroph adenoma cells: effect of prolonged exposure to CRH and its interaction with cortisol.** *J Clin Endocrinol Metab.* 1988; **66**(4): 770–5.
PubMed Abstract | Publisher Full Text
16. Suda T, Tozawa F, Yamada M, *et al.*: **Effects of corticotropin-releasing hormone and dexamethasone on proopiomelanocortin messenger RNA level in human corticotroph adenoma cells *In vitro*.** *J Clin Invest.* 1988; **82**(1): 110–4.
PubMed Abstract | Publisher Full Text | Free Full Text
17. Karl M, Von Wichert G, Kempter E, *et al.*: **Nelson's syndrome associated with a somatic frame shift mutation in the glucocorticoid receptor gene.** *J Clin Endocrinol Metab.* 1996; **81**(1): 124–9.
PubMed Abstract | Publisher Full Text
18. Dahia PL, Honegger J, Reincke M, *et al.*: **Expression of glucocorticoid receptor gene isoforms in corticotropin-secreting tumors.** *J Clin Endocrinol Metab.* 1997; **82**(4): 1088–93.
PubMed Abstract | Publisher Full Text
19. Korbonits M, Bujalska I, Shimojo M, *et al.*: **Expression of 11 beta-hydroxysteroid dehydrogenase isoenzymes in the human pituitary: induction of the type 2 enzyme in corticotropinomas and other pituitary tumors.** *J Clin Endocrinol Metab.* 2001; **86**(6): 2728–33.
PubMed Abstract | Publisher Full Text
20. Tateno T, Izumiyama H, Doi M, *et al.*: **Differential gene expression in ACTH -secreting and non-functioning pituitary tumors.** *Eur J Endocrinol.* 2007; **157**(6): 717–24.
PubMed Abstract | Publisher Full Text
21. Lin SJ, Zhang Y, Liu NC, *et al.*: **Minireview: Pathophysiological roles of the TR4 nuclear receptor: lessons learned from mice lacking TR4.** *Mol Endocrinol.* 2014; **28**(6): 805–21.
PubMed Abstract | Publisher Full Text | Free Full Text
22. Xie S, Lee YF, Kim E, *et al.*: **TR4 nuclear receptor functions as a fatty acid sensor to modulate CD36 expression and foam cell formation.** *Proc Natl Acad Sci U S A.* 2009; **106**(32): 13353–8.
PubMed Abstract | Publisher Full Text | Free Full Text
23. Collins LL, Lee YF, Heinlein CA, *et al.*: **Growth retardation and abnormal maternal behavior in mice lacking testicular orphan nuclear receptor 4.** *Proc Natl Acad Sci U S A.* 2004; **101**(42): 15058–63.
PubMed Abstract | Publisher Full Text | Free Full Text
24. Kang HS, Okamoto K, Kim YS, *et al.*: **Nuclear orphan receptor TAK1/TR4-deficient mice are protected against obesity-linked inflammation, hepatic steatosis, and insulin resistance.** *Diabetes.* 2011; **60**(1): 177–88.
PubMed Abstract | Publisher Full Text | Free Full Text
25. F Du L, Bergsneider M, Mirsadraei L, *et al.*: **Evidence for orphan nuclear receptor TR4 in the etiology of Cushing disease.** *Proc Natl Acad Sci U S A.* 2013; **110**(21): 8555–60.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
26. F Zhang D, Du L, Heaney AP: **Testicular Receptor-4: Novel Regulator of Glucocorticoid Resistance.** *J Clin Endocrinol Metab.* 2016; **101**(8): 3123–33.
PubMed Abstract | Publisher Full Text | F1000 Recommendation
27. F Morcos M, Fohr B, Tafel J, *et al.*: **Long-term treatment of central Cushing's syndrome with rosiglitazone.** *Exp Clin Endocrinol Diabetes.* 2007; **115**(5): 292–7.
PubMed Abstract | Publisher Full Text | F1000 Recommendation
28. Pecori Giraldi F, Scaroni C, Arvat E, *et al.*: **Effect of protracted treatment with rosiglitazone, a PPARgamma agonist, in patients with Cushing's disease.** *Clin Endocrinol (Oxf).* 2006; **64**(2): 219–24.
PubMed Abstract | Publisher Full Text
29. Ambrosi B, Dall'Asta C, Cannavo S, *et al.*: **Effects of chronic administration of PPAR-gamma ligand rosiglitazone in Cushing's disease.** *Eur J Endocrinol.* 2004; **151**(2): 173–8.
PubMed Abstract | Publisher Full Text
30. F Heaney AP, Fernando M, Yong WH, *et al.*: **Functional PPAR-gamma receptor is a novel therapeutic target for ACTH-secreting pituitary adenomas.** *Nat Med.* 2002; **8**(11): 1281–7.
PubMed Abstract | Publisher Full Text | F1000 Recommendation
31. F Kirschke E, Goswami D, Southworth D, *et al.*: **Glucocorticoid receptor function regulated by coordinated action of the Hsp90 and Hsp70 chaperone cycles.** *Cell.* 2014; **157**(7): 1685–97.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
32. Echeverria PC, Picard D: **Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility.** *Biochim Biophys Acta.* 2010; **1803**(6): 641–9.
PubMed Abstract | Publisher Full Text
33. F Riebold M, Kozany C, Freiburger L, *et al.*: **A C-terminal HSP90 inhibitor restores glucocorticoid sensitivity and relieves a mouse allograft model of Cushing disease.** *Nat Med.* 2015; **21**(3): 276–80.
PubMed Abstract | Publisher Full Text | F1000 Recommendation
34. Ramasamy K, Agarwal R: **Multitargeted therapy of cancer by silymarin.** *Cancer Lett.* 2008; **269**(2): 352–62.
PubMed Abstract | Publisher Full Text | Free Full Text
35. Stratakis CA, Tichomirowa MA, Boikos S, *et al.*: **The role of germline *AIP*, *MEN1*, *PRKAR1A*, *CDKN1B* and *CDKN2C* mutations in causing pituitary adenomas in a large cohort of children, adolescents, and patients with genetic syndromes.** *Clin Genet.* 2010; **78**(5): 457–63.
PubMed Abstract | Publisher Full Text | Free Full Text
36. Matsuzaki LN, Canto-Costa MH, Hauache OM: **Cushing's disease as the first clinical manifestation of multiple endocrine neoplasia type 1 (MEN1) associated with an R460X mutation of the MEN1 gene.** *Clin Endocrinol (Oxf).* 2004; **60**(1): 142–3.
PubMed Abstract | Publisher Full Text
37. Simonds WF, Varghese S, Marx SJ, *et al.*: **Cushing's syndrome in multiple endocrine neoplasia type 1.** *Clin Endocrinol (Oxf).* 2012; **76**(3): 379–86.
PubMed Abstract | Publisher Full Text | Free Full Text
38. Raitila A, Georgitsi M, Karhu A, *et al.*: **No evidence of somatic aryl hydrocarbon receptor interacting protein mutations in sporadic endocrine neoplasia.** *Endocr Relat Cancer.* 2007; **14**(3): 901–6.
PubMed Abstract | Publisher Full Text
39. Cazabat L, Bouligand J, Salenave S, *et al.*: **Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients.** *J Clin Endocrinol Metab.* 2012; **97**(4): E663–70.
PubMed Abstract | Publisher Full Text
40. Georgitsi M, Raitila A, Karhu A, *et al.*: **Molecular diagnosis of pituitary adenoma predisposition caused by aryl hydrocarbon receptor-interacting protein gene mutations.** *Proc Natl Acad Sci U S A.* 2007; **104**(10): 4101–5.
PubMed Abstract | Publisher Full Text | Free Full Text
41. Georgitsi M, Raitila A, Karhu A, *et al.*: **Germline *CDKN1B*/p27$^{Kip1}$ mutation in**

**multiple endocrine neoplasia.** *J Clin Endocrinol Metab.* 2007; **92**(8): 3321–5.
PubMed Abstract | Publisher Full Text

42. Riminucci M, Collins MT, Lala R, *et al.*: **An R201H activating mutation of the GNAS1 (Gsalpha) gene in a corticotroph pituitary adenoma.** *Mol Pathol.* 2002; **55**(1): 58–60.
PubMed Abstract | Publisher Full Text | Free Full Text

43. Williamson EA, Ince PG, Harrison D, *et al.*: **G-protein mutations in human pituitary adrenocorticotrophic hormone-secreting adenomas.** *Eur J Clin Invest.* 1995; **25**(2): 128–31.
PubMed Abstract | Publisher Full Text

44. Normanno N, De Luca A, Bianco C, *et al.*: **Epidermal growth factor receptor (EGFR) signaling in cancer.** *Gene.* 2006; **366**(1): 2–16.
PubMed Abstract | Publisher Full Text

45. Carpenter G, Cohen S: **Epidermal growth factor.** *Annu Rev Biochem.* 1979; **48**: 193–216.
PubMed Abstract | Publisher Full Text

46. F Ogiso H, Ishitani R, Nureki O, *et al.*: **Crystal structure of the complex of human epidermal growth factor and receptor extracellular domains.** *Cell.* 2002; **110**(6): 775–87.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

47. Yarden Y: **The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities.** *Eur J Cancer.* 2001; **37**(Suppl 4): S3–8.
PubMed Abstract | Publisher Full Text

48. Levkowitz G, Waterman H, Ettenberg SA, *et al.*: **Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1.** *Mol Cell.* 1999; **4**(6): 1029–40.
PubMed Abstract | Publisher Full Text

49. Itakura Y, Sasano H, Shiga C, *et al.*: **Epidermal growth factor receptor overexpression in esophageal carcinoma. An immunohistochemical study correlated with clinicopathologic findings and DNA amplification.** *Cancer.* 1994; **74**(3): 795–804.
PubMed Abstract | Publisher Full Text

50. Kim MA, Lee HS, Lee HE, *et al.*: **EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number.** *Histopathology.* 2008; **52**(6): 738–46.
PubMed Abstract | Publisher Full Text

51. Mukohara T, Kudoh S, Yamauchi S, *et al.*: **Expression of epidermal growth factor receptor (EGFR) and downstream-activated peptides in surgically excised non-small-cell lung cancer (NSCLC).** *Lung Cancer.* 2003; **41**(2): 123–30.
PubMed Abstract | Publisher Full Text

52. LeRiche VK, Asa SL, Ezzat S: **Epidermal growth factor and its receptor (EGF-R) in human pituitary adenomas: EGF-R correlates with tumor aggressiveness.** *J Clin Endocrinol Metab.* 1996; **81**(2): 656–62.
PubMed Abstract | Publisher Full Text

53. Kontogeorgos G, Stefaneanu L, Kovacs K, *et al.*: **Localization of Epidermal Growth Factor (EGF) and Epidermal Growth Factor Receptor (EGFr) in Human Pituitary Adenomas and Nontumorous Pituitaries: An Immunocytochemical Study.** *Endocr Pathol.* 1996; **7**(1): 63–70.
PubMed Abstract | Publisher Full Text

54. Onguru O, Scheithauer BW, Kovacs K, *et al.*: **Analysis of epidermal growth factor receptor and activated epidermal growth factor receptor expression in pituitary adenomas and carcinomas.** *Mod Pathol.* 2004; **17**(7): 772–80.
PubMed Abstract | Publisher Full Text

55. Theodoropoulou M, Arzberger T, Gruebler Y, *et al.*: **Expression of epidermal growth factor receptor in neoplastic pituitary cells: evidence for a role in corticotropinoma cells.** *J Endocrinol.* 2004; **183**(2): 385–94.
PubMed Abstract | Publisher Full Text

56. Lubke D, Saeger W, Ludecke DK: **Proliferation Markers and EGF in ACTH-Secreting Adenomas and Carcinomas of the Pituitary.** *Endocr Pathol.* 1995; **6**(1): 45–55.
PubMed Abstract | Publisher Full Text

57. Childs GV, Rougeau D, Unabia G: **Corticotropin-releasing hormone and epidermal growth factor: mitogens for anterior pituitary corticotropes.** *Endocrinology.* 1995; **136**(4): 1595–602.
PubMed Abstract | Publisher Full Text

58. Xu M, Shorts-Cary L, Knox AJ, *et al.*: **Epidermal growth factor receptor pathway substrate 8 is overexpressed in human pituitary tumors: role in proliferation and survival.** *Endocrinology.* 2009; **150**(5): 2064–71.
PubMed Abstract | Publisher Full Text

59. Kiyokawa H, Kineman RD, Manova-Todorova KO, *et al.*: **Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of $p27^{Kip1}$.** *Cell.* 1996; **85**(5): 721–32.
PubMed Abstract | Publisher Full Text

60. Lidhar K, Korbonits M, Jordan S, *et al.*: **Low expression of the cell cycle inhibitor $p27^{Kip1}$ in normal corticotroph cells, corticotroph tumors, and malignant pituitary tumors.** *J Clin Endocrinol Metab.* 1999; **84**(10): 3823–30.
PubMed Abstract | Publisher Full Text

61. Fero ML, Rivkin M, Tasch M, *et al.*: **A syndrome of multiorgan hyperplasia with features of gigantism, tumorigenesis, and female sterility in $p27^{Kip1}$-deficient mice.** *Cell.* 1996; **85**(5): 733–44.
PubMed Abstract | Publisher Full Text

62. Dahia PL, Aguiar RC, Honegger J, *et al.*: **Mutation and expression analysis of the p27/kip1 gene in corticotrophin-secreting tumours.** *Oncogene.* 1998; **16**(1): 69–76.
PubMed Abstract | Publisher Full Text

63. Childs GV, Patterson J, Unabia G, *et al.*: **Epidermal growth factor enhances ACTH secretion and expression of POMC mRNA by corticotropes in mixed and enriched cultures.** *Mol Cell Neurosci.* 1991; **2**(3): 235–43.
PubMed Abstract | Publisher Full Text

64. F Fukuoka H, Cooper O, Ben-Shlomo A, *et al.*: **EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas.** *J Clin Invest.* 2011; **121**(12): 4712–21.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

65. Song ZJ, Reitman ZJ, Ma ZY, *et al.*: **The genome-wide mutational landscape of pituitary adenomas.** *Cell Res.* 2016; **26**(11): 1255–9.
PubMed Abstract | Publisher Full Text | Free Full Text

66. F Reincke M, Sbiera S, Hayakawa A, *et al.*: **Mutations in the deubiquitinase gene *USP8* cause Cushing's disease.** *Nat Genet.* 2015; **47**(1): 31–8.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

67. Faesen AC, Luna-Vargas MP, Sixma TK: **The role of UBL domains in ubiquitin-specific proteases.** *Biochem Soc Trans.* 2012; **40**(3): 539–45.
PubMed Abstract | Publisher Full Text

68. Clague MJ, Urbe S: **Ubiquitin: same molecule, different degradation pathways.** *Cell.* 2010; **143**(5): 682–5.
PubMed Abstract | Publisher Full Text

69. F Smith GA, Fearnley GW, Abdul-Zani I, *et al.*: **VEGFR2 Trafficking, Signaling and Proteolysis is Regulated by the Ubiquitin Isopeptidase USP8.** *Traffic.* 2016; **17**(1): 53–65.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

70. Jacomin AC, Bescond A, Soleilhac E, *et al.*: **The Deubiquitinating Enzyme UBPY Is Required for Lysosomal Biogenesis and Productive Autophagy in *Drosophila*.** *PLoS One.* 2015; **10**(11): e0143078.
PubMed Abstract | Publisher Full Text | Free Full Text

71. Mizuno E, Iura T, Mukai A, *et al.*: **Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes.** *Mol Biol Cell.* 2005; **16**(11): 5163–74.
PubMed Abstract | Publisher Full Text | Free Full Text

72. Niendorf S, Oksche A, Kisser A, *et al.*: **Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking *in vivo*.** *Mol Cell Biol.* 2007; **27**(13): 5029–39.
PubMed Abstract | Publisher Full Text | Free Full Text

73. F Perez-Rivas LG, Theodoropoulou M, Ferraù F, *et al.*: **The Gene of the Ubiquitin-Specific Protease 8 Is Frequently Mutated in Adenomas Causing Cushing's Disease.** *J Clin Endocrinol Metab.* 2015; **100**(7): E997–1004.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

74. F Hayashi K, Inoshita N, Kawaguchi K, *et al.*: **The USP8 mutational status may predict drug susceptibility in corticotroph adenomas of Cushing's disease.** *Eur J Endocrinol.* 2016; **174**(2): 213–26.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

75. F Ma ZY, Song ZJ, Chen JH, *et al.*: **Recurrent gain-of-function *USP8* mutations in Cushing's disease.** *Cell Res.* 2015; **25**(3): 306–17.
PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

76. F Ronchi CL, Peverelli E, Herterich S, *et al.*: **Landscape of somatic mutations in sporadic GH-secreting pituitary adenomas.** *Eur J Endocrinol.* 2016; **174**(3): 363–72.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

77. Tzivion G, Shen YH, Zhu J: **14-3-3 proteins; bringing new definitions to scaffolding.** *Oncogene.* 2001; **20**(44): 6331–8.
PubMed Abstract | Publisher Full Text

78. F Aghazadeh Y, Papadopoulos V: **The role of the 14-3-3 protein family in health, disease, and drug development.** *Drug Discov Today.* 2016; **21**(2): 278–87.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

79. Mizuno E, Kitamura N, Komada M: **14-3-3-dependent inhibition of the deubiquitinating activity of UBPY and its cancellation in the M phase.** *Exp Cell Res.* 2007; **313**(16): 3624–34.
PubMed Abstract | Publisher Full Text

80. F Perez-Rivas LG, Reincke M: **Genetics of Cushing's disease: an update.** *J Endocrinol Invest.* 2016; **39**(1): 29–35.
PubMed Abstract | Publisher Full Text | F1000 Recommendation

81. Zhao Y: **Targeted Therapy With Gefitinib in Patients With USP8-mutated Cushing's Disease.** NCT02484755. Phase 2 clinical trial. Accessed 3 Feb, 2017.
Reference Source

# Open Peer Review

## Current Referee Status: ✔ ✔

## Editorial Note on the Review Process

F1000 Faculty Reviews are commissioned from members of the prestigious F1000 Faculty and are edited as a service to readers. In order to make these reviews as comprehensive and accessible as possible, the referees provide input before publication and only the final, revised version is published. The referees who approved the final version are listed with their names and affiliations but without their reports on earlier versions (any comments will already have been addressed in the published version).

## The referees who approved this article are:

Version 1

1 **Marco Boscaro**, Endocrinology Unit, Department of Medicine, Padova University Hospital, Padova, Italy
***Competing Interests:*** No competing interests were disclosed.

1 **Richard Feelders**, Erasmus Medical Center, Rotterdam, Netherlands
***Competing Interests:*** No competing interests were disclosed.