Received: 18 March 2022 | Revised: 12 April 2022 | Accepted: 18 April 2022

DOI: 10.1111/jne.13148

INVITED REVIEW

Journal of Neuroendocrinology WILEY

Genetics of Cushing's disease

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Funding information
Supported by the Deutsche Forschungsgemeinschaft (DFG) (Project number: 314061271-TRR 205) to MT.

Abstract

Corticotroph tumours are primarily sporadic monoclonal neoplasms and only rarely found in genetic syndromes. Recurrent mutations in the ubiquitin specific protease 8 (USP8) gene are found in around half of cases. Mutations in other genes such as USP48 and NR3C1 are less frequent, found in less than ~20% of cases. TP53 and ATXR mutations are reported in up to one out of four cases, when focusing in USP8 wild type or aggressive corticotroph tumours and carcinomas. At present, USP8 mutations are the primary driver alterations in sporadic corticotroph tumours, TP53 and ATXR mutations may indicate transition to more aggressive tumour phenotype. Next generation sequencing efforts have identified additional genomic alterations, whose role and importance in corticotroph tumorigenesis remains to be elucidated.

KEYWORDS
corticotroph tumour, Cushing's disease, genetics, USP8

1 | INTRODUCTION

Cushing's disease is the most common form of endogenous hypercortisolaemia, which develops secondary to ACTH-secreting pituitary tumours (henceforth referred to as corticotroph tumours). It is mainly sporadic and is rarely seen in the context of endocrine tumour syndromes (reviewed in[1,2]). In the last few years, the advancement of next-generation sequencing technologies have brought a renaissance in the understanding of the genetic events underlying the pathogenesis of Cushing's disease. This article is part of a Special Issue on "Update of Cushing's Syndrome: 100 years after Minnie G" and summarizes the current knowledge on the genetics of corticotroph tumours.

2 | CUSHING'S DISEASE IN ENDOCRINE TUMOUR SYNDROMES

Multiple endocrine neoplasia (MEN) syndromes

MEN syndromes present with tumours in more than one endocrine organ and include MEN1 (loss of function mutation in *MEN1* gene encoding for menin), MEN2 and 2B (mutations in the *RET* oncogene) and MEN4 (mutations in the *CDKN1B* gene encoding for the cell cycle inhibitor p27/Kip1) (reviewed in[3]). Cushing's disease is rarely reported in paediatric or adult MEN1 patients[3–7] (reviewed in[3]). In a multicentre study, corticotroph tumours were found in 6/136 MEN1 patients presenting with pituitary tumours.[8] MEN2 (or MEN2A) and 2B rarely present with pituitary tumours and two cases were reported to present with Cushing's disease: an adult MEN2A and a paediatric MEN2B patient.[9,10] MEN4 is very rare, accounting for ~2% of MEN cases that do not carry *MEN1* mutations.[11–14] Most studies did not detect germline *CDKN1B* mutations in patients with corticotroph tumours[6,12,15,16]; Germline *CDKN1B* mutations were reported in two female patients with Cushing's disease, who also presented with primary hyperparathyroidism.[12,17] A recent study focusing on paediatric Cushing's disease patients with no known MEN history, reported heterozygous germline *CDKN1B* potential pathogenic variants in five out of 190 cases.[14] An association between p27 rs2066827 (V109G) polymorphism and corticotroph tumours was observed in a large Brazilian cohort of 447 patients who presented with different endocrine tumours, but no *CDKN1B* gene mutations.[18]

AIP

Germline mutations in the aryl hydrocarbon receptor (AHR) -interacting protein (*AIP*) are found in ~20% of families associated with non-syndromic familial isolated pituitary adenomas (FIPA).[19] Germline *AIP* mutations were found in one paediatric patient with an ACTH-secreting microadenoma[6] and in a 50-year-old male patient presenting with an aggressive corticotroph tumour.[20] Overall, germline *AIP* mutations in patients with Cushing's disease are very rare (1/74[21] and 3/44[22]). A germline mutation in the *AHR* gene was found in a male patient who presented with a giant corticotroph tumour.[23]

DICER1

Cushing's disease occurs in patients with DICER1 syndrome with very low penetrance. DICER1 syndrome is caused by germline heterozygous loss-of-function mutations in the gene encoding for the cytoplasmic endoribonuclease type III that is essential in microRNA biogenesis, and characterized by early-onset tumours that include among others pituitary blastoma.[24] Most patients with pituitary blastomas are younger than 3 years old and suffer from severe Cushing's disease and high mortality due to clinical complications.[24–29] In these cohorts, germline *DICER1* mutations were detected in 12 out of 13 patients. Germline heterozygous missense *DICER1* variants were also identified in seven unrelated paediatric patients with isolated Cushing's disease and no history of DICER1 syndrome.[30] Recently, a young adult patient with Cushing's disease was reported as having germline *DICER1* mutation inherited from her father.[31]

Carney complex

Carney complex (CNC) is a hereditary tumour syndrome, caused in the majority of cases by inactivating mutations in *PRKAR1A* gene that encodes for type 1 alpha regulatory subunit of the cAMP-dependent protein kinase A (PKA). CNC is characterized by myxomas, skin pigmentation, schwannomas as well as endocrine tumours, and although ACTH-independent Cushing's syndrome is a frequent manifestation of the disease especially in female patients,[32] Cushing's disease was not reported. At present, there are two case reports with germline *PRKAR1A* mutations: a male patient with Cushing's disease and clinical phenotype of CNC[33] and a male paediatric Cushing's disease patient with germline *PRKAR1A* mutation and LOH on the corticotroph tumour.[34] No germline *PRKAR1A* mutations were detected in the remaining 97 paediatric Cushing's disease patients included in the study, indicating that they are very rare in pituitary-dependent Cushing's syndrome.[34]

Lynch syndrome

Lynch syndrome results from germline mutations in mismatch repair genes such as *MSH2*, *MLH1*, *MSH6*, *PMS2* and *EPCAM*, and is associated with a hereditary cancer-predisposition disorder. An invasive corticotroph tumour was reported in a Lynch syndrome patient with germline mutation in *MLH1* gene, which also carried somatic mutations in the *MEN1* and *MSH6* genes.[35] In addition, germline MSH2 mutations were detected in two patients with an invasive corticotroph macroadenoma and a corticotroph carcinoma.[36,37]

3 | SPORADIC

Corticotroph tumours are mainly sporadic neoplasms that are monoclonal in origin.[38,39]

Corticotroph cell physiology relies on trophic signals from the hypothalamus, in the form of corticotrophin-releasing hormone (CRH) and vasopressin, and inhibitory glucocorticoid feedback from the adrenals. In addition, autocrine/paracrine loops that involve cytokines, growth and developmental factors control corticotroph cell growth and ACTH synthesis (reviewed in[40–42]). As discussed below, mutations in genes encoding for prominent - in corticotroph physiology - regulatory factors are uncommon. Instead, whole-exome sequencing efforts revealed previously unsuspected genetic events in sporadic corticotroph tumours.

3.1 | Trophic hypothalamic regulation

CRH and vasopressin receptors were found to be highly expressed in corticotroph tumours, but no mutations were found in the coding regions of the *CRHR1* and *V3R* genes.[43–47] CRH stimulates ACTH synthesis upon binding to a stimulatory G protein coupled receptor downstream to the cAMP/PKA signalling pathway in a crosstalk with the MAPK pathway.[48] As mentioned above, Cushing's disease is almost never seen in CNC and *PRKAR1A* mutations are extremely rare in corticotroph tumours. Somatic mutations in *GNAS* gene are rare in corticotroph tumours, and have previously been reported in 2/32 cases and in one paediatric patient with Cushing's disease.[49,50]

3.2 | Negative glucocorticoid feedback

Partial resistance to negative glucocorticoid feedback is a hallmark of Cushing's disease.[51] Initial reports suggested that mutations in the nuclear receptor subfamily 3 group C member 1 (*NR3C1*) gene that encodes for the glucocorticoid receptor are rare (reviewed in[52]). A somatic missense *NR3C1* mutation was found in a patient presenting with Nelson syndrome.[53] More recent whole-exome sequencing studies identified *NR3C1* mutations in a few more cases, with a meta-analysis calculating their presence in 6.2% of corticotroph tumours.[54] A study comprised of 49 Cushing's disease patients revealed *NR3C1* mutations in three patients with no differences regarding clinical parameter observed between the mutant and wild-type groups.[55] Therefore, although *NR3C1* mutations are not frequent in Cushing's disease, they are also not as extremely rare as previously considered.[56]

Search on the mechanisms facilitating glucocorticoid response, brought attention to regulatory factors such as heat shock protein 90 (HSP90), BRG1, HDAC2 and CABLES1. HSP90 is a chaperone that influences the folding of ligand-bound GR. BRG1 mediates the transcriptional repressor action of GR on the *POMC* promoter, while testicular receptor 4 (TR4 nuclear receptor subfamily 2, group C, member 2) blocks it. The loss of BRG1 and overexpression of HSP90 and TR4 observed in corticotroph tumours may be responsible for the impaired physiological response to the negative glucocorticoid feedback.[57–59]

In corticotroph cells, glucocorticoids mediate their antiproliferative action by inducing CABLES1 (CDK5 and ABL1 enzyme substrate 1). CABLES1 protein is downregulated/lost in more than half of corticotroph tumours.[60] Germline missense *CABLES1* variants were found in four out of 182 Cushing's disease patients (including 116 paediatric), all of which had large corticotroph tumours with high Ki67 and difficult to manage disease.[61]

In addition to regulating the inhibitory glucocorticoid action on *POMC* promoter, both BRG1 and CABLES1 affect the cell cycle proteins cyclin E and p27/Kip1. Both are deregulated in corticotroph tumours, with cyclin E being overexpressed and p27 being downregulated/lost at protein level.[62,63] No somatic *CDKN1B* mutations and no LOH or deregulated transcription were reported in sporadic corticotroph tumours.[64] BRG1 downregulates cyclin E transcription and low BRG1 levels are concomitant to increased cyclin E and loss of p27/Kip1 protein in corticotroph tumours.[57] We may therefore hypothesise that BRG1 loss releases cyclin E, which can trigger CDK2-induced p27/Kip1 phosphorylation marking it for proteasomal degradation. In parallel, CABLES1 stabilizes p27/Kip1, so its loss in corticotroph tumours may also contribute to reduction in p27/Kip1 protein levels.[60]

3.3 | Genes mutated in sporadic corticotroph tumours

3.3.1 | Ubiquitin specific protease 8 (USP8)

Whole-exome sequencing revealed a single mutational hotspot in the *USP8* gene in 40%–60% of corticotroph tumours.[65,66] Subsequent sequencing efforts in Caucasian and Asiatic populations identified *USP8* mutations in 35%–60% of cases as well as in ~50% of cases of progressive corticotroph tumour growth after bilateral adrenalectomy (Nelson's syndrome).[67–77] A somatic *USP8* mutation was found in the corticotroph tumour of a patient that presented with both adrenal Cushing's syndrome and central Cushing's disease and additionally carried somatic mutation in *NR3C1* in the corticotroph tumour and *CTNNB1* in the adrenal tumour.[78] A somatic *USP8* mutation was found in an adult patient with Cushing's disease, who also suffered from growth hormone deficiency due to *GH1* mutation.[79]

USP8 mutations were also detected in 13/45 paediatric patients with Cushing's disease, but not in a single centre study of 18 paediatric patients.[70,80] All *USP8* mutations reported are somatic, but a case of heterozygous germline *USP8* hotspot mutation in a paediatric patient with Cushing's disease has recently been reported.[81]

Exome sequencing reported *USP8* mutations exclusively in corticotroph tumours and not in other pituitary tumour types.[65,66,75,76,82] In addition, no mutations were found in ectopic ACTH producing tumours indicating a corticotroph tumour specific event.[83]

USP8 encodes for a deubiquitinase that removes ubiquitin molecules from client proteins, usually rescuing them from lysosome and changing their subcellular localization.[84] The *USP8* mutational hotspot is located in exon 14 in the 14-3-3 binding motif. One recently found mutation is also located in exon 14 upstream to the 14-3-3 binding motif.[85] In the wild-type protein, 14-3-3 binding causes conformational changes that enable USP8 to block its own catalytic activity.[86] Loss in the 14-3-3 binding motif in the *USP8* mutants enhances their deubiquitinase activity and enables access to proteases that cleave it to a C-terminal 40-KD fragment with high catalytic capacity.[65,87] Indeed, *USP8* mutants show loss of 14-3-3 binding and higher deubiquitinase activity in vitro compared to wild-type protein.[65]

The best characterized USP8 client is the epidermal growth factor receptor (EGRF).[88,89] EGF stimulates ACTH secretion without exerting a strong mitogenic action on corticotroph cells (reviewed in[90]). EGFR is highly expressed in corticotroph tumours and its overexpression stimulates *POMC* transcription and ACTH synthesis.[91,92] USP8 mutants rescue the receptor from lysosome and potentiate EGFR-induced *POMC* promoter activity.[65] In patients with Cushing's disease, *USP8* mutant tumours have higher *POMC* expression compared to wild-type.[69,93]

USP8 mutant tumours are more frequent in women and they tend to be smaller and noninvasive.[66,67] On the other hand, they are accompanied by higher postoperative cortisol levels and are more likely to recur after surgery.[67,71] *USP8* mutant tumours were observed to have increased expression of somatostatin receptor 5 (SSTR5) and O6-methylguanine DNA methyltransferase (MGMT), indicating favourable response to the SSTR5 ligand pasireotide and temozolomide.[69,76,94] In fact, a recent consensus suggested that the *USP8* mutational status may be useful as predictor of pasireotide response in patients with Cushing's disease.[95] Finally, USP8 could be a promising treatment target with small molecule inhibitors showing antiproliferative and antisecretory efficacy in vitro.[96–98]

3.3.2 | Ubiquitin specific protease 48 (USP48)

Next-generation sequencing in *USP8* wild-type tumours identified a second mutational hotspot in another deubiquitinase encoding gene, the *USP48*.[99,100] *USP48* mutations concentrate on a single amino acid (Met415) and are found in 4%–23% of *USP8* wild-type tumours.[77,85] *USP48* mutant tumours are more frequent in female patients and smaller compared to wild-type tumours.[99,100] One study suggested that they may be more invasive to the cavernous sinus.[77]

The *USP48* mutant has higher deubiquitinase activity.[99,100] In vitro experiments showed that the *USP48* mutant does not affect basal, but it enhances CRH-induced *POMC* promoter activity.[100] One of the USP48 clients is the transcription factor GLI1 that belongs to the sonic hedgehog (SHH) pathway, which plays an important role in pituitary development and tumorigenesis.[101–104] In vitro evidence

suggests that mutant USP48 acts via GLI1 to sensitize corticotroph tumour cells to the trophic action of CRH on ACTH synthesis.[100]

3.3.3 | Tumour protein P53 (TP53)

TP53 is the most commonly mutated tumour suppressor gene in human cancers. *TP53* mutations are assumed to be rare in corticotroph tumours with only a few reported cases.[105–107] Next-generation sequencing revealed that in selected populations (i.e., USP8 wild-type macroadenomas, aggressive corticotrophinomas) they are not as rare as previously thought with studies reporting somatic *TP53* mutations in up to 33% of cases.[100,108]

3.3.4 | Alpha thalassemia/mental retardation syndrome X-linked (ATXR)

A small fraction of *TP53* mutant corticotroph tumours were also found to carry somatic loss of function mutations in the *ATRX* gene.[100,109] *ATRX* mutations were observed more frequently (7/25) in corticotroph tumours including carcinomas compared to aggressive pituitary tumours and carcinomas of other histological subtypes (3/26[110]). Two corticotroph tumours from this study also carried mutations in the *PTEN* and *NF2* genes. Furthermore, two cases were reported of ACTH-secreting carcinomas with *TP53* and *ATXR* mutations, as well as *PTEN*.[109,111,112]

3.3.5 | Other

Whole-exome sequencing identified the V600E mutation of the *BRAF* proto-oncogene in 16% of *USP8* wild-type corticotroph tumours in a Chinese patient cohort.[99] Studies in other Caucasian and Asiatic cohorts showed this mutation to be either very rare (1/91[100];) or totally absent.[77,85]

Another whole-exome sequencing study reported missense mutations in the *CDH23* gene that encodes for a calcium dependent cell–cell adhesion glycoprotein member of the cadherin superfamily in four patients with sporadic corticotroph tumours.[113] It should be noted that the same study detected these mutations, which potentially affect protein folding or calcium binding, also in 0.8% of the control population.

Mutations in the *PIK3CA* proto-oncogene that encodes for the PI3K p110α catalytic subunit, which belongs to the PI3K survival pathway, were reported in one invasive corticotroph tumour,[114] as well as in a noninvasive ACTH-secreting microadenoma.[115]

4 | CONCLUSION

We have come a long way since the first report of Minnie G and characterization of "basophil adenomas of the anterior lobe of the pituitary" as the cause of Cushing's disease.[116] In the decades that followed, the mutational landscape of Cushing's disease remained empty, with genes involved in corticotroph physiology and tumorigenesis found to be rarely mutated. The advent of fast and high throughput technologies brought previously unsuspected genes like *USP8* and *USP48* into the spotlight. In addition, it prompted us to revisit genes that were previously considered to be extremely rarely mutated in Cushing's disease, like *NR3C1* and *TP53*, and reconsider their potential role in distinct corticotroph tumour populations. At present, *USP8* mutations are the primary driver alterations in sporadic corticotroph tumours. As we have seen, exome sequencing efforts have identified additional genomic alterations, whose role and importance in corticotroph tumorigenesis remains to be elucidated.[75,76,82]

This article is part of an update series on the diagnosis and treatment of Cushing's syndrome.[117–133]

AUTHOR CONTRIBUTIONS

Julia Simon: Data curation; writing – original draft; writing – review and editing. **Marily Theodoropoulou:** Conceptualization; data curation; funding acquisition; project administration; supervision; writing – review and editing.

ACKNOWLEDGMENTS

Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors report no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jne.13148.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Simon J, Theodoropoulou M. Genetics of Cushing's disease. *J Neuroendocrinol*. 2022;34(8):e13148. doi:10.1111/jne.13148