Gene mutations in Cushing's disease (Review)

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Received March 22, 2016; Accepted June 17, 2016

DOI: 10.3892/br.2016.729

Abstract. Cushing's disease (CD) is a severe (and potentially fatal) disease caused by adrenocorticotropic hormone (ACTH)-secreting adenomas of the pituitary gland (often termed pituitary adenomas). The majority of ACTH-secreting corticotroph tumors are sporadic and CD rarely appears as a familial disorder, thus, the genetic mechanisms underlying CD are poorly understood. Studies have reported that various mutated genes are associated with CD, such as those in menin 1, aryl hydrocarbon receptor-interacting protein and the nuclear receptor subfamily 3 group C member 1. Recently it was identified that ubiquitin-specific protease 8 mutations contribute to CD, which was significant towards elucidating the genetic mechanisms of CD. The present study reviews the associated gene mutations in CD patients.

Contents

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Key words: Cushing's disease, adrenocorticotropic hormone, pituitary adenomas, gene mutation

1. Introduction

Cushing's disease (CD) is a severe (and potentially fatal) disease caused by adrenocorticotropic hormone (ACTH)-secreting adenomas of the pituitary gland [often termed pituitary adenomas (PAs)], and constitutes 10-15% of all PAs worldwide (1). Excess secretion of ACTH in CD results in the following symptoms: Central obesity, hirsutism, glucose intolerance and osteoporosis. Since their first description in 1932 (2), the pathogenesis of tumors of the pituitary gland has not been elucidated, which has hindered the early diagnosis of many cases of PA.

Generally, PAs result from clonal expansion of somatic mutated cells (3). Studies have suggested that ~40% of sporadic PAs are associated with somatic mutations of genes (4). Germline mutations in genes also predispose individuals to PAs (5). Thus, tumorigenesis in the pituitary gland may be explained by gene mutation.

Recent studies have demonstrated that mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene, and Carney complex and multiple endocrine neoplasia type 1 (MEN1) are associated with sporadic PAs (6,7). Disruption in the balance of pituitary-secreting hormones as a result of gene mutation may result in severe-to-fatal consequences. Therefore, identification of relevant gene mutations is particularly useful in the early diagnosis of CD, and for genetic counseling of CD patients.

It has been suggested that CD may be a consequence of hereditary disease (8). Previous studies revealed genetic factors to be involved in CD (9,10). Recently, a review by Perez-Rivas and Reincke (11) specified the roles of mutations in ubiquitin-specific protease 8 (*USP8*) in CD. In the present study, the gene mutations that have been reported in CD patients are reviewed.

2. Menin 1 (*MEN1*) mutation

MEN1 is composed of 10 exons, is located in chromosome 11q13 and encodes a 610-amino acid menin protein. Bassett *et al* (12) identified 47 mutations in the coding exons

of *MEN1* as follows: 12 Nonsense mutations, 21 deletions, 7 insertions, 1 donor splice-site mutation and 6 missense mutations. The authors suggested that 80% of these mutations were likely to be inactivating (12). Mutations in *MEN1* have been found to be associated with pancreatic endocrine tumors (13). Furthermore, inactivating mutations of *MEN1* lead to a familial disorder termed MEN1, of which one common component is CD (14). Thus, it has been speculated that mutations in *MEN1* may also participate in CD. Stratakis *et al* (5) reported that two mutations of *MEN1* (a deletion mutation and p.Arg415X) were identified in CD patients. Matsuzaki *et al* (15) reported an R460X mutation in *MEN1* in a Brazilian subject with early-onset CD and his sister. A heterozygote C→T transition was detected at codon 460 in exon 10 in *MEN1*, which converts codon 460 CGA (Arg) to a stop codon TGA (15). Thus, mutations in *MEN1* lead to the early clinical manifestations of MEN1, and are involved in CD. Furthermore, these findings confirm the hereditary characteristics of CD.

3. Nuclear receptor subfamily 3 group C member 1 (*NR3C1*) mutation

Perfect balance in the requirement and secretion of glucocorticoid hormones (which is maintained by feedback from the hypothalamic-pituitary-adrenal-axis) is critical for the regulation of glucose metabolism and the feedback mechanism in the immune system.

CD patients share the characteristics of resistance to glucocorticoids and unresponsiveness to normal glucocorticoid negative feedback (16). However, somatic mutations of *NR3C1*, or dysfunction of genes associated with glucocorticoid receptor function, are rarely found in CD (5). However, Karl *et al* (10) reported a novel heterozygous missense mutation in *NR3C1* in a CD patient. The authors found that the mutation occurs in exon 5 of the coding region of *NR3C1* in lymphoblasts and fibroblasts, as well in 50% of sperm. As a result, the neutral and polar asparagine at codon 559 substitutes the neutral and hydrophobic amino acid, isoleucine. The authors also suggested that the mutation contributed to severe, sporadic, generalized glucocorticoid resistance. Further investigation demonstrated that the mutation was not detected in the patient's parents or seven siblings. Therefore, it was concluded that this novel mutation was *de novo* and present in the germline (10). That is, the mutation in *NR3C1* may be involved in CD.

4. Aryl hydrocarbon receptor-interacting protein (*AIP*) mutation

AIP is a protein of 330 amino acids and acts as a tumor suppressor (17). Studies have demonstrated that AIP combines with the aryl hydrocarbon receptor on the cell surface, and probably exerts its effects by regulating integrin function. More than 100 variants in *AIP* have been identified, of which the most frequent mutation occurs in the p.R304 locus (18). Approximately 15-30% of familial isolated PAs harbor germline mutations in *AIP* (19,20). PAs with mutations in *AIP* are predominantly somatotropinomas and prolactinomas; however, studies have revealed that *AIP* mutations may also occur in CD. Georgitsi *et al* (9) found a heterozygous c.696G>C (which leads to the silencing of p.P232P in exon 5) in a CD patient in Poland. Furthermore, Stratakis *et al* (5) reported a novel germline *AIP* mutation, c.308A>G/p.Lys103Arg, in the heterozygotic state in one pediatric patient with recurrent CD, although the authors suggested that the overall prevalence of *AIP* mutation was very low. These findings indicate that *AIP* mutations may be directly involved in the molecular pathogenesis of CD, but that screening for mutations may not be an effective method for the diagnosis of CD.

5. Tumor protein p53 (*TP53*) mutation

The p53 protein is encoded by a tumor-suppressor gene termed *TP53*, which is located on chromosome 17. p53 inhibits the $G_1 \rightarrow S$ transition of the cell cycle, and is significant in suppression of tumorigenesis. Studies have suggested that *TP53* mutations are associated with the pathogenesis of ~50% of human cancers, including those in the central nervous system (21,22). However, the role of p53 in tumors of the pituitary gland is controversial. Oliveira *et al* (23) demonstrated p53 protein to be positive in only two of 148 PA patients, suggesting that p53 may not be a biomarker for tumors of the pituitary gland. Other studies have indicated that p53 expression in tumors of the pituitary gland cannot be detected, including ACTH-secreting adenomas (24-26). By contrast, Buckley *et al* (27) found that abnormal expression of p53 was involved in the development of invasive pituitary tumors. The common alterations associated with *TP53* in human tumors are inactivating mutations, which occur between exon 5 to 8 (28). Levy *et al* (29) and Herman *et al* (30) failed to identify mutations in *TP53* in PAs, although Kawashima *et al* (31) reported that a somatic mutation of *TP53* contributed to a case of atypical PA that caused CD. The authors sequenced the region of exon 5 through to exon 8 of *TP53* and identified a missense mutation of CTG>CGG on codon 145 (L145R). The study indicated that the mutation was detected in tumor tissues, but not in peripheral blood (31). These studies imply that a somatic mutation of *TP53* may contribute to the pathogenesis of CD.

6. Nuclear receptor subfamily 0 group B member 1 (*NR0B1*) mutation

Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX-1) is critical in steroidogenic development and sex determination. Studies have demonstrated that DAX-1 suppresses the transcription of various genes expressed in the adrenal cortex and hypothalamic-pituitary-gonadal axis, such as steroidogenic factor-1 (32,33). DAX-1 is encoded by *NR0B1*, which comprises two exons separated by a 3.4-kb intron. Studies have shown involvement of mutations of *NR0B1* in hypogonadotropic hypogonadism and X-linked adrenal hypoplasia congenital (34,35). However, the role of DAX-1 in ACTH-secreting PAs remains poorly understood. Suzuki *et al* (36) analyzed the regulatory mechanisms of differentiation of pituitary cells in 89 corticotroph adenomas. DAX-1 was found to be positive in all subjects, indicating that DAX-1 is essential for the genesis of ACTH-secreting PAs. Furthermore, De Menis *et al* (37) described a novel mutation of *NR0B1* in a patient with ACTH-secreting PA and X-linked adrenal hypoplasia congenita and his mother [a 4-bp insertion

(AGCG) at nucleotide 259 in exon 1 of *NR0B1*] leading to premature termination of transcription. This evidence indicates that monitoring of mutations in *NR0B1* may be beneficial for early diagnosis in patients with CD and X-linked adrenal hypoplasia congenita.

7. Ubiquitin-specific peptidase 8 (*USP8*) mutation

USP8 is a member of the USP family. The specific roles of *USP8* mutations in CD have been reviewed by Perez-Rivas and Reincke (11). Here, three studies published recently, which reported mutations of *USP8* in CD, are reviewed.

Reincke *et al* (38) found that mutations of p.Ser718Cys, p.Pro720Arg and p.Ser718del in the 14-3-3 protein binding motif promoted the proteolytic cleavage and catalytic activity of USP8, which inhibited epidermal growth factor receptor (EGFR) degradation and prolonged EGF signaling, resulting in increased activity in the proopiomelanocortin (POMC) promoter and transcription, as well as causing CD. Perez-Rivas *et al* (39) demonstrated that somatic mutations comprising p.718Ser>Pro, Ser718del, p.720Pro>Gln, and p.720Pro>Arg in USP8 diminished EGFR ubiquitination and induced the activity of the POMC promoter. Ma *et al* (40) reported the significant clinical relevance of three somatic mutations (c.CTC2151-2153del/p.S718del, c.C2159G/p.P720R and c.T2152C/p.S718P) of *USP8* and CD. It was revealed that mutations in exon 14 of *USP8* disrupt the interaction between USP8 and 14-3-3 protein, leading to protection of the EGFR from lysosomal degradation. Mutations in USP8 sustain EGFR-mitogen-activated protein kinase signaling to promote ACTH production in CD (40). These findings clearly demonstrate that mutations in *USP8* contribute to CD.

8. Dicer 1, ribonuclease (RNase) III (*DICER1*) mutation

DICER1 is a highly conserved RNase III enzyme, the functions of which are predominantly associated with RNA interference pathways, including the processing of microRNA precursors into mature microRNAs (41,42). *DICER1* is associated with various tumors, such as pulmonary adenomas and pleuropulmonary blastomas (41,43). Hill *et al* (44) suggested that almost all patients with pleuropulmonary blastoma exhibit germline mutations of *DICER1*. In addition, mutations in *DICER1* are involved in the development of cystic nephroma (45). As mutated *DICER1* participates in diverse types of tumors, previous studies have indicated that *DICER1* mutations result in tumors of the pituitary gland. Wildi-Runge *et al* (46) reported a germline heterozygous *DICER1* mutation in a blastoma of the pituitary gland in an infant, suggesting a role for *DICER1* mutations in tumors of the pituitary gland. Furthermore, Sahakitrungruang *et al* (47) described two novel *DICER1* mutations in a one-year-old female with a blastoma of the pituitary gland presenting with CD. The results showed that a novel heterozygous c.3046delA (p.S1016VfsX1065) mutation in *DICER1* was identified by whole-exome sequencing of leukocytes and pituitary blastoma tumor tissues, and another somatic missense c.5538A->T (p.E1813V) mutation was identified in tumor tissues only (47). These findings indicate that *DICER1* mutations may facilitate with understanding the pathogenesis of CD.

9. Cytochrome P450 family 21 subfamily A member 2 (*CYP21A2*) mutation

CYP21A2 encodes active steroid 21-hydroxylase enzyme. Studies have suggested that 21-hydroxylase-deficient mice show failure of inhibition of the hypothalamic-pituitary-adrenal axis (48). Mutations in *CYP21A2* are responsible for congenital adrenal hyperplasia (CAH), which is associated with CD in certain cases. Haase *et al* (49) found that a homozygous mutation in exon 7 of *CYP21A2* (CTG>TTG, p.V281L) may have contributed to CD in a female patient with CAH. Boronat *et al* (50) reported that a 39-year-old female patient with an ACTH-producing PA carried two point mutations in *CYP21A2*: A severe splicing 655G mutation at intron 2 and a mild V28L mutation at exon 7. Concurrently, a severe 8-bp deletion mutation was found at exon 3 of the *CYP21A2* gene, which caused the 21-hydroxylase deficiency, in a 21-year-old CD patient (50). Although 21-hydroxylase deficiency is rarely observed in CD patients, *CYP21A2* mutations may (at least in part) contribute to CD.

10. GNAS complex locus (*GNAS*) mutation

GNAS1 (also termed gsp oncogene) comprises 13 exons and is located on chromosome 20q13. *GNAS* encodes various proteins, including the α subunit of the stimulatory G protein (Gsα), extra-large αs and 55-kDa neuroendocrine secretory protein. The activating and inactivating mutations of *GNAS* have previously been identified (51,52). *GNAS* mutations have been found to be involved in certain endocrine diseases. Patten *et al* (53) demonstrated that the A→G point mutation in *GNAS* (which causes reduced immunoactivity in the Gsα protein) is associated with Albright's hereditary osteodystrophy. Other studies have reported that ~40% of patients with functional PAs exhibit somatic mutations of GNAS, which often occur at codons R201 and Q227. Williamson *et al* (54) identified mutations of CAG→CGG and CAG→CAC/T at codon Q227 in only two of 32 ACTH-secreting PAs, and suggested that these mutations are an uncommon abnormality in CD. Riminucci *et al* (55) observed an R201H mutation of *GNAS* in a child with CD, thereby extending the disease spectrum of the R201 mutation of *GNAS*. Therefore, an association between *GNAS* mutations and CD may improve the understanding of CD pathogenesis.

11. Leukemia inhibitory factor (*LIF*) mutation and cyclin-dependent kinase inhibitor 1B (*CDKN1B*) mutation

LIF is the most pleiotropic member of the interleukin-6 family. LIF is essential in activation of the hypothalamo-pituitary-adrenal axis during inflammation (51). In LIF knockout mice, the ACTH response to stress is reduced, whereas LIF overexpression in transgenic mice leads to corticotroph cell hyperplasia and hypercortisolism (56,57). LIF exerts a regulatory function by binding to the LIF receptor (LIF-R) and gp130 (58). These studies indicate that LIF promotes ACTH secretion, and that mutations in LIF- or LIF-R-encoding genes may contribute to CD pathogenesis. However,

Table I. Gene mutations associated with CD.

Author, year	Gene	Mutation	Codon change	Mutation type	Location	Disease	Refs.
Stratakis, 2010	*MEN1*	-	-	Deletion mutation	-	Pediatric familial/syndromic CD	(5)
		p.R415X	-	Nonsense mutation	-	Pediatric familial/syndromic CD	
Matsuzaki, 2004		p. R460X	CGA>TGA	Nonsense mutation	Exon 10	MEN1 with CD	(15)
Karl, 1996	*NR3C1*	p.I559N	ATC>AAC	Missense mutation	Exon 5	CD	(10)
Georgitsi, 2007	*AIP*	c.696G>C/p.P232P	-	Silence mutation	Exon 5	CD	(9)
Stratakis, 2010		c.308A>G/ p.K103R	-	Missense mutation	-	Recurrent CD	(5)
Kawashima, 2009	*TP53*	p.L145R	CTG>CGG	Missense mutation	-	Atypical PA causing CD	(31)
De Menis, 2005	*NR0B1*	g.259_260insAGCG	-	Insertion mutation	Exon 1	ACTH-secreting PA and X-linked adrenal hypoplasia congenita	(37)
Reincke, 2015	*USP8*	p.S718C	-	Missense mutation	Exon 14	CD	(38)
		p.P720R		Missense mutation			
Perez-Rivas, 2015		p.S718del		Deletion mutation			(39)
Ma, 2015		p. S718P		Missense mutation			(40)
		p. P720Q		Missense mutation			
Sahakitrungruang, 2014	*DICER1*	c.3046delA/p.S1016VfsX1065	-	Frameshift mutation	-	Pituitary blastoma presenting with CD	(47)
		c.5538A>T/p.E1813V		Missense mutation	-		
Haase, 2011	*CYP21A2*	p.V281L	CTG>TTG	Missense mutation	Exon 7	CD with CAH	(49)
		-	-	Splicing mutation	Intron 2	ACTH-producing PA	
Boronat, 2004		-	-	Deletion mutation	Exon 3		(50)
Williamson, 1995	*GNAS*	p.R179G	CGC>GGC	Missense mutation	-	Corticotroph adenomas	(54)
Riminucci, 2002		p.Q227R	CAG>CGG	Missense mutation	-		(55)
		p.Q227H	CAG>CAC/T	Missense mutation	-		
		p.R201H	-	Missense mutation	Exon 8	CD	

MEN1, menin 1; *NR3C1*, nuclear receptor subfamily 3 group C member 1; *AIP*, aryl hydrocarbon receptor-interacting protein; *TP53*, tumor protein p53; *NR0B1*, nuclear receptor subfamily 0 group B member 1; *USP8*, ubiquitin-specific peptidase 8; *DICER1*, Dicer 1, ribonuclease III; *CYP21A2*, cytochrome P450 family 21 subfamily A member 2; *GNAS*, GNAS complex locus; -, not mentioned; CD, Cushing's disease; MEN1, multiple endocrine neoplasia type I; PA, pituitary adenomas; ACTH, adrenocorticotropic hormone; CAH, congenital adrenal hyperplasia.

Heutling *et al* (59) did not observe mutations in LIF-R in ACTH-secreting adenomas, and suggested that mutations in LIF-R were an unlikely cause for CD development (59).

CDKN1B, also known as MEN4 and p27/kip1, maps to chromosome 12p13 and encodes a CDK inhibitor, which restricts cell cycle progression at G_1. Lack of p27/kip1 function leads to the development of PAs (60). Furthermore, Liu *et al* (61) found that a selective inhibitor of CDK markedly suppressed ACTH levels and restrained growth of ACTH-secreting PAs in mice. One study revealed that germline *CDKN1B* mutations rendered individuals more susceptible to MEN1 (62). Thus, it is speculated that *CDKN1B* mutations may also participate in CD. However, Dahia *et al* (63) proposed that p27/kip1 mutations were not a feature of corticotroph tumors.

12. Conclusions

The majority of tumors of the pituitary gland appear to arise from a single mutated cell due to expansion of monoclonal cells. Therefore, distinct genetic changes are probably one of the most important events within tumorigenesis in the pituitary gland, including in ACTH-secreting corticotroph tumors. The current study reviewed previous investigations, which showed that various gene mutations are involved in CD (Table I). Cases are predominantly sporadic, therefore, the regulatory mechanisms of the gene mutations in CD are rarely investigated, although *USP8* mutations have been more extensively evaluated. Thus, current treatment of CD patients has not progressed as a result of the identification of gene mutations that are associated with CD. However, the findings of the present review offer potential benefits regarding genetic counseling and early diagnosis of CD.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant no. 81373150).

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