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The role of germline *AIP*, *MEN1*, *PRKAR1A*, *CDKN1B* and *CDKN2C* mutations in a large cohort of children and adolescents with pituitary adenomas

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Abstract

The prevalence of germline mutations in *MEN1*, *AIP*, *PRKAR1A*, *CDKN1B* and *CDKN2C* is unknown among pediatric patients with pituitary adenomas (PA). In this study, we screened children with PA for mutations in these genes; somatic *GNAS* mutations were also studied in a limited number of growth hormone (GH) or prolactin (PRL)-secreting PA. We studied 74 and 6 patients with either isolated Cushing disease (CD) or GH- or PRL-secreting PA, respectively. We also screened 4 pediatric patients with CD, and 4 with GH/PRL-secreting tumors who had some syndromic feature. There was one *AIP* mutation (p.Lys103Arg) among 74 CD patients. Two *MEN1* mutations that occurred in patients with recurrent or difficult to treat disease were found among syndromic CD patients. There was one *MEN1* and 3 *AIP* mutations (p.Gln307ProfsX104, p.Pro114fsX, p.Lys241X) among pediatric patients with isolated GH- or PRL-secreting PA and one additional *MEN1* mutation in a patient with positive family history. There were no mutations in the *PRKAR1A*, *CDKN1B*, *CDKN2C* or *GNAS* genes. Thus, germline *AIP* or *MEN1* gene mutations are frequent among pediatric patients with GH- or PRL-secreting PA but are significantly rarer in pediatric CD; *PRKAR1A* mutations are not present in PA outside of Carney complex.

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Keywords

AIP; Acromegaly; FIPA; multiple endocrine neoplasia; prolactinoma; tumor suppressor genes

Introduction

Pituitary adenomas occur frequently in the population (1,2) but clinically significant tumors are present in 1 of 1064 subjects in the general population (3,4). The molecular genetic processes that cause most pituitary adenomas are unknown despite the identification of a number of defects (5). Up to 40% of sporadic pituitary adenomas demonstrate somatic mutations in *GNAS* or other genes (6) but relatively few hereditary conditions have been associated with a predisposition to pituitary adenoma (7). Less than 5% of these tumors are associated with germline mutations of the *MEN1* and *PRKARIA* genes that cause multiple endocrine neoplasia (MEN) type 1 (MEN1) and Carney complex (CNC) (8-13). Mutations in additional genes have been identified more recently (14,15). The *aryl hydrocarbon receptor interacting protein* (*AIP*) gene accounts for approximately 15% of familial isolated pituitary adenomas (FIPA) cases (16), about 5% of cases of sporadic acromegaly, and an as yet unknown proportion of sporadic prolactinomas and Cushing disease (17,18). Defects in cyclin-dependent kinase inhibitor (*CDKI*) genes have been found in a small number of kindreds with MEN1-like features: *CDKN1B/p27Kip1* (14,19) and *CDKN1B (p27Kip1)*, *CDKN2C (p18INK4c)* and other *CDKI* genes (20). The majority of pituitary tumors caused by *MEN1*, *AIP* and *CDKI* gene mutations secrete growth hormone (GH) and/or prolactin (PRL) (14-16,21). Cushing disease, due to corticotropin (ACTH)-producing tumors, is rarely seen in the context of *MEN1*, *AIP* or *CDKI* mutations (18,22,23). Somatic mutations of the glucocorticoid receptor (*GR*) gene, dysfunction of genes related to *GR* function, and *TP53*-inactivating defects have rarely been found in aggressive or recurrent Cushing disease (24-26). Patients with pituitary adenomas and other syndromic features but no defined genetic abnormalities have also been reported (27,28).

We tested a large number of pediatric patients with pituitary adenomas for the prevalence of germline genetic defects in the *MEN1*, *AIP*, *PRKARIA*, *CDKN1B* and *CDKN2C* genes; the *GNAS* gene was also investigated in a small number of GH and/or PRL-secreting pituitary tumors that we had access to tissue. The data provide support for recommendations with regards to genetic testing and counseling for pediatric patients with pituitary tumors.

Subjects and Methods

Subjects

Our patients have been seen at a single center, the National Institutes of Health (NIH) Clinical Center, over the last 15 years under Institutional Review Board (IRB) approved protocols 95CH0059 and 97CH0076 of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). Diagnosis was confirmed after transsphenoidal surgery (TSS) and appropriate histology. The study population consisted of three main groups of pediatric patients that had been treated and followed up at the NIH: (a) those with isolated, sporadic Cushing disease (n=74); (b) pediatric patients with Cushing disease that occurred in the setting of a family history of neuro-endocrine disorder (n=4); (c) patients (n=6) with isolated GH and/or PRL-secreting pituitary adenomas and no family history of endocrine tumors. A group of 4 children and 7 adults with GH or PRL-secreting pituitary adenomas that occurred in a familial/syndromic setting with other endocrine disorders or tumors was also assessed genetically. Clinical and family features in some of the cases in this study have previously been described (29-32).

Tissue and DNA studies

Pituitary tumor tissue was collected at surgery. Whenever possible, tissue slices were snap frozen at -70°C ; the remainder was fixed in formalin. For light microscopy and immunocytochemistry (IHC), sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff, and the Gordon-Sweet silver reticulin stain. The avidin-biotin-peroxidase complex technique was used to identify GH and PRL-producing cells and to stain for corticotrophin (ACTH), thyrotropin (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and alpha subunit. Cytokeratin staining was obtained in cases where tissue was available.

DNA was obtained from peripheral blood lymphocytes for germline mutation testing (33,34); tumor DNA was extracted, when tissue was available for *GNAS* and loss-of-heterozygosity (LOH) studies. Direct bidirectional sequencing was employed to analyze all coding regions and the flanking exon/intron junctions of the *MEN1*, *AIP*, *PRKARIA*, *CDKN1B* and *CDKN2C* genes by standard methods; *GNAS* testing was obtained by sequencing for the common activating mutations in 3 GH- and/or PRL-producing tumors (15,20,33-35). All mutations were studied *in silico* and compared against databases (36). LOH studies were done in two *MEN1*-mutant tumors where tissue was available (29-32); all *MEN1* gene testing in negative-for-sequencing-defects cases with suggestive family history or other supportive for *MEN1* clinical signs (N=3) were screened for deletion by Multiplex ligation-dependent probe amplification (MLPA, data not shown) at GeneDx, Gaithersburg, MD (www.genedx.org). High quality DNA from peripheral blood was available in a very small number of patients to screen for *AIP* deletions (37).

Results

Pediatric isolated Cushing disease

Patients with sporadic, isolated Cushing disease included 74 children or adolescents. The mean age of the patients at diagnosis was 12.9 ± 3.1 years (range: 6-18 years). In three cases, patients had recurrent Cushing disease within six years post-TSS. In one of these recurrent cases (PT512.03), a novel germline *AIP* mutation, c.308A>G/p.Lys103Arg, was found in the heterozygotic state (Table 1). Clinically this patient was diagnosed aged six years with a 3×4 mm ACTH-secreting microadenoma, which despite remission after TSS, gradually grew back and required radiotherapy. The patient has now been in complete remission for more than 5 years but he has developed panhypopituitarism. The family was not available for genetic analysis for carrier status, but family history was negative. Most of the other 73 investigated patients presented with ACTH-secreting microadenomas and were cured after one TSS. No patient in the cohort was found to have germline mutations in *MEN1*, *CDKN1B* or *CDKN2C*. Unfortunately, we did not have high quality DNA from this group of patients to screen for *AIP* deletions (37).

Pediatric Cushing disease in a familial/syndromic setting

Four pediatric patients aged 11-14 years had Cushing disease that was associated with a family history of genetically confirmed *MEN1* (n=2), clinical features of *MEN1* (n=1) or a *TSC2* mutation-positive tuberous sclerosis (n=1). Genetic analyses in these cases demonstrated that the two patients were carriers of their familial *MEN1* mutations. One of them was diagnosed at the age of 15 years with an ACTH-producing microadenoma which was also positive for PRL by IHC, while the other one was an 18-year-old girl who presented with an ACTH-secreting adenoma that regressed spontaneously (and Cushing disease cured) after pituitary apoplexy. The other patients had no genetic cause diagnosed. Mutations in *AIP*, *PRKARIA*, *CDKN1B* and *CDKN2C* were not found and MLPA for *MEN1* did not show any 11q13 or *MEN1* gene deletions.

Pediatric isolated GH-secreting adenomas and prolactinomas

Six patients aged ≤ 18 years with a GH- or a PRL-producing tumor were studied, half of whom had genetic mutations identified. Three patients had previously unreported *AIP* mutations. The first male patient (PTMEN01.04), who had a large PRL-secreting tumor (50 mm), was treated successfully with a dopamine agonist; he had a c.721A>T/p.Lys241X *AIP* mutation. Interestingly, both he and his sister carried a t(9;16)(q32;p13) balanced translocation, inherited from their father. Neither his biological parents nor his sister carried the *AIP* mutation, indicating that it had occurred *de novo* in this patient. The second patient (PT33.05), a 14-year old female with a GH-secreting pituitary tumor and associated *café-au-lait* spots had a novel p.Pro114fsX germline *AIP* mutation. The mother did not carry the mutation and the father, reportedly normal, was not available for screening. The tumor in that patient was aggressive and required radiotherapy after 2 TSS. Histopathology showed a macroadenoma, with considerable nuclear pleomorphism and a moderately elevated proliferative index (MIB-1) of 6%. Cytokeratin was immunoreactive and showed typical dot-shaped intracytoplasmic fibrous bodies. The patient later developed panhypopituitarism.

Another 11-year old male patient (PT50.03) with a macroprolactinoma was found to have the same *MEN1* mutation as his father (c.655-7C>A). The *in silico* modeling (36) showed that c.655-7C>A leads to a decrease of the splice score from 0.435421 (wild type) to 0.155784, as an acceptor site. More molecular data on the effect of c.655-7C>A on menin were reported by Drori-Herishanu *et al.* (29).

Pediatric and adult GH-secreting adenomas and prolactinomas in a familial/syndromic setting

Only one of 11 patients with a “syndromic” presentation carried a single germline *MEN1* mutation. After his presentation at age 5 years, it became clear that patient PT526.03 (Table 1) had extensive family history that was consistent with MEN 1: his father had multiple kidney stones and enteropancreatic tumors and two paternal aunts had prolactinomas. His mixed GH and PRL-secreting adenoma was aggressive, did not respond to dopamine agonists and, after TSS, remission to *octreotide* was partial, requiring radiotherapy.

Another patient (PT105.03), diagnosed at age 11 years, had a paternal grandmother with a PRL-secreting macroadenoma. This patient had an aggressive and large tumor (30×20 mm) that was only partially responsive to *octreotide*® and he required 3 operations and radiotherapy. A novel *AIP* mutation was identified in this patient, and in his clinically asymptomatic (and radiologically and biochemically normal) father. The mutation (c.919dupC/p.Gln307ProfsX104insC) led to a shift of the stop codon 79 codons down-stream, generating a different carboxy terminal protein end after amino acid position 306. Histopathological analyses of the tumor revealed tightly compact cellularity of the adenoma with GH positivity (Fig. 1, Panels A, B). Interestingly the adenoma contained areas suggestive of hyperplasia on reticulin staining (Fig. 1, Panel C), a feature that has not been reported previously in association with *AIP* mutations.

Two kindreds (CAR556 and CAR590) with familial acromegaly due to pituitary hyperplasia had no mutations in any tested genes; there were no 11q13 deletions by cytogenetics and no *MEN1* gene deletions by MLPA testing. Similarly, no patients with multiple other syndromic/familial manifestations carried any mutations in *AIP*, *PRKARIA*, *CDKN1B* and *CDKN2C*.

Discussion

Few other large series have been published investigating the genetics of pediatric pituitary adenomas (38-40). In the present study, 8/88 (9.1%) children with hormone-secreting

pituitary adenomas had a germline genetic defect in a known gene. This is a much higher proportion than would be expected from the studies in adult patients (8,9). *AIP* or *MEN1* mutations were found in 5 of 11 (45.5%) cases with GH- or PRL-secreting tumors. In only 1 patient among these cases, there was positive family history. In addition, tumors with *AIP* or *MEN1* mutations were less frequently controlled by TSS; this echoes the findings of other groups (3,6). Georgitsi *et al.* studied a general cohort of pediatric/adolescent pituitary adenoma patients (38); they reported one novel *AIP* mutation (in a patient with a GH-secreting tumor) among 36 children.

Thus, it appears that screening for both *AIP* and *MEN1* mutations among pediatric patients with GH- and PRL-producing tumors, regardless of family history, may be of clinical value. This does not appear to be the case among pediatric patients with ACTH-producing pituitary tumors; in this sub-group genetic screening should be individualized, because only 1 of 73 patients with pediatric Cushing disease had an *AIP* mutation (1.4%), and this is the second only such patient described in the literature (18).

Interestingly, none of the patients with a GH- or PRL-secreting tumor that occurred in the setting of other syndromic features had mutations in the tested genes, which points to other genetic factors yet to be identified. *GNAS* mutations were also not present, although a small number was screened, and McCune-Albright syndrome patients were not part of this study. The lack of mutations in *PRKARIA* among all 18 patients with GH- or PRL-secreting tumors suggests that very rarely, if ever, patients with a germline *PRKARIA* mutation present with a pituitary tumors in the absence of other cardinal signs of CNC (37). This apparently is not the case for *MEN1* mutation-positive individuals, who may present in the pediatric age with a pituitary tumor without any other signs of MEN 1.

These data suggest an algorithm of genetic testing for pediatric pituitary tumors that is outlined in Figure 2.

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Figure 1.

Pedigree of the FIPA family with the p.Gln307ProfsX104insC *AIP* mutation. Magnetic resonance imaging shows a sagittal view of a pituitary adenoma in the proband (patient PT105.03, Table 1). Panels A, B and C show the hematoxylin and eosin, GH-staining and reticulin, respectively, in this aggressive pituitary tumor that was excised from patient PT105.03; the patient's father was an unaffected *AIP* mutation carrier, while the paternal grandmother had a prolactinoma (but declined genetic studies).

Figure 2.

A proposed algorithm for genetic testing in pituitary adenomas; *abbreviations*: FIPA = familial isolated pituitary adenomas; MEN 1= multiple endocrine neoplasia type 1.

Table 1
Patient information, clinical features and mutation status in the studied population of patients.

Patients	Age	Sex	Pituitary Tumor	Other Clinical Features	Gene defect
<i>Pediatric isolated Cushing's disease</i>					
PT512.03	6	M	ACTH / microadenoma	None	<i>AIPM</i> , p.Lys103Arg
<i>Pediatric familial/syndromic Cushing's disease</i>					
1. PT45.03	11	M	ACTH / microadenoma	Family history of MEN 1	None
2. PT87.03	14	M	ACTH / microadenoma	Family history of MEN 1	<i>MEN1</i> , p.Arg 415X
3. PT95.05	12	F	ACTH / microadenoma	Family history of MEN 1	<i>MEN1</i> deletion
4. PT131.01	12	M	ACTH / microadenoma	Tuberous sclerosis (TSC2 gene mutation)	None
<i>Pediatric isolated GH- and PRL-secreting adenomas</i>					
1. CAR602.01	8	M	GH	-	None
2. PTMEN01.04	18	M	PRL	Familial translocation : t(9;16)(q32;p13)	<i>AIP</i> , p.Lys241X
3. PT33.05	14	F	GH / macroadenoma	<i>Café-au-lait</i> spots	<i>AIP</i> , p.Pro114fsX
4. PT34.03	18	F	PRL / macroadenoma	-	None
5. PT50.03	11	M	PRL / macroadenoma	-	<i>MEN1</i> , c.655-7C>A
6. PT88.03	12	M	GH / microadenoma	-	None
<i>Pediatric familial/syndromic GH- and PRL-secreting adenomas</i>					
1. PT526.03	5	M	GH, PRL / macroadenoma	Family history of MEN-1	<i>MEN1</i> , p.His139Asp
2. CAR556.03	18	M	GH	Familial acromegaly	None
3. CAR590.02	3	M	GH	Familial acromegaly	None
4. PT105.03	11	M	GH, PRL / macroadenoma	<i>Café-au-lait</i> spots, hyperthyroidism	<i>AIP</i> , p.Gln307Pro fsX104
<i>Adult familial/syndromic GH- and PRL-secreting adenomas</i>					
1. CAR510.01	42	F	GH	Lentigo, meningioma	None
2. CAR598.01	50	M	GH	Lentigo, testicular germ cell tumor, renal cancer	None
3. CAR568.01	25	M	GH	Ehlers-Danlos syndrome	None
4. CAR623.02	30	F	GH	Thyroid cancer, lentigo	None
5. CAR16.02	32	F	GH	Thyroid cancer, cortisol-secreting adenoma, lentigo	None

Patients	Age	Sex	Pituitary Tumor	Other Clinical Features	Gene defect
6. CAR46.01	42	M	GH	Family history of schwannoma, lentigo	None
7. PT84.01	50	M	PRL	Ependymoma, HPP, other	None