Short Report

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


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 four pediatric patients with CD, and four with GH/PRL-secreting tumors who had some syndromic features. 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 patients with CD. There was one MEN1 and three 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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Key words: acromegaly – AIP – FIPA – multiple endocrine neoplasia – prolactinoma – tumor suppressor genes

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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 *PRKAR1A* genes that cause multiple endocrine neoplasia 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, PRKAR1A, 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 regard 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: (i) those with isolated, sporadic Cushing disease (*n* = 74); (ii) pediatric patients with Cushing disease that occurred in the setting of a family history of neuro-endocrine disorder (*n* = 4); and (iii) patients (*n* = 6) with isolated GH and/or PRL-secreting pituitary adenomas and no family history of endocrine tumors. A group of four children and seven 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, PRKAR1A, CDKN1B and CDKN2C genes by standard methods; GNAS testing was obtained by sequencing for the common activating mutations in three 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 negative-for-sequencing 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 6 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 at the age of 6 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) (31). Genetic analyses in these cases demonstrated that the patients were carriers of their familial MEN1 and TSC2 mutations. One of the MEN1 patients 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, PRKAR1A, 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 two 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.
Table 1. Patient information, clinical features and mutation status in the studied population of patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>Pituitary tumor</th>
<th>Other clinical features</th>
<th>Gene defect</th>
</tr>
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<tbody>
<tr>
<td>Pediatric isolated Cushing’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT512.03</td>
<td>6</td>
<td>M</td>
<td>ACTH/microadenoma</td>
<td>None</td>
<td>AIP, p.Lys103Arg</td>
</tr>
<tr>
<td>Pediatric familial/syndromic Cushing’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. PT45.03</td>
<td>11</td>
<td>M</td>
<td>ACTH/microadenoma</td>
<td>Family history of MEN1</td>
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</tr>
<tr>
<td>2. PT87.03</td>
<td>14</td>
<td>M</td>
<td>ACTH/microadenoma</td>
<td>Family history of MEN1</td>
<td>MEN1, p.Arg415X</td>
</tr>
<tr>
<td>3. PT95.05</td>
<td>12</td>
<td>F</td>
<td>ACTH/microadenoma</td>
<td>Family history of MEN1</td>
<td>MEN1 deletion</td>
</tr>
<tr>
<td>4. PT131.01</td>
<td>12</td>
<td>M</td>
<td>ACTH/microadenoma</td>
<td>Tuberosus sclerosis (TSC2 gene mutation); see Nandagopal et al. (31).</td>
<td>None</td>
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<tr>
<td>Pediatric isolated GH- and PRL-secreting adenomas</td>
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<td></td>
<td></td>
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<tr>
<td>1. CAR602.01</td>
<td>8</td>
<td>M</td>
<td>GH</td>
<td>–</td>
<td>None</td>
</tr>
<tr>
<td>2. PTMEN01.04</td>
<td>18</td>
<td>M</td>
<td>PRL</td>
<td>Familial translocation: t(9;16)(q32;p13)</td>
<td>AIP, p.Lys241X</td>
</tr>
<tr>
<td>3. PT33.05</td>
<td>14</td>
<td>F</td>
<td>GH/macroadenoma</td>
<td>Café-au-lait spots</td>
<td>AIP, p.Pro114fsX</td>
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<td>4. PT34.03</td>
<td>18</td>
<td>F</td>
<td>PRL/macroadenoma</td>
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<td>None</td>
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<td>5. PT50.03</td>
<td>11</td>
<td>M</td>
<td>PRL/macroadenoma</td>
<td>See Drori-Herishanu et al. (29)</td>
<td>MEN1, c.655-7C&gt;A</td>
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<tr>
<td>6. PT88.03</td>
<td>12</td>
<td>M</td>
<td>GH/microadenoma</td>
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<td>None</td>
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<tr>
<td>Pediatric familial/syndromic GH- and PRL-secreting adenomas</td>
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<tr>
<td>1. PT526.03</td>
<td>5</td>
<td>M</td>
<td>GH, PRL/macroadenoma</td>
<td>Family history of MEN-1</td>
<td>MEN1, p.His139Asp</td>
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<td>2. CAR556.03</td>
<td>18</td>
<td>M</td>
<td>GH</td>
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<tr>
<td>3. CAR590.02</td>
<td>3</td>
<td>M</td>
<td>GH</td>
<td>Familial acromegaly</td>
<td>None</td>
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<td>4. PT105.03</td>
<td>11</td>
<td>M</td>
<td>GH, PRL/macroadenoma</td>
<td>Café-au-lait spots, hyperthyroidism</td>
<td>AIP, p.Gln307Pro fsX104</td>
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<td>Adult familial/syndromic GH- and PRL-secreting adenomas</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>1. CAR510.01</td>
<td>42</td>
<td>F</td>
<td>GH</td>
<td>Lentigo, meningioma</td>
<td>None</td>
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<tr>
<td>2. CAR598.01</td>
<td>50</td>
<td>M</td>
<td>GH</td>
<td>Lentigo, testicular germ cell tumor, renal cancer</td>
<td>None</td>
</tr>
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<td>3. CAR568.01</td>
<td>25</td>
<td>M</td>
<td>GH</td>
<td>Ehlers-Danlos syndrome</td>
<td>None</td>
</tr>
<tr>
<td>4. CAR623.02</td>
<td>30</td>
<td>F</td>
<td>GH</td>
<td>Thyroid cancer, lentigo</td>
<td>None</td>
</tr>
<tr>
<td>5. CAR16.02</td>
<td>32</td>
<td>F</td>
<td>GH</td>
<td>Thyroid cancer, cortisol-secreting adenoma, lentigo</td>
<td>None</td>
</tr>
<tr>
<td>6. CAR46.01</td>
<td>42</td>
<td>M</td>
<td>GH</td>
<td>Family history of schwannoma, lentigo</td>
<td>None</td>
</tr>
<tr>
<td>7. PT84.01</td>
<td>50</td>
<td>M</td>
<td>PRL</td>
<td>Ependymoma, HPP, other</td>
<td>None</td>
</tr>
</tbody>
</table>

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 three 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.Gln307Pro fsX104insC) led to a
Role of \textit{AIP}, \textit{MEN1}, \textit{PRKAR1A}, \textit{CDKN1B} and \textit{CDKN2C} mutations in patients with PA

\textbf{Fig. 1.} Pedigree of the familial isolated pituitary adenomas (FIPA) family with the p.Gln307ProfsX104insC \textit{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, reticulin and GH-staining, respectively, in this aggressive pituitary tumor that was excised from patient PT105.03; the patient’s father was an unaffected \textit{AIP} mutation carrier, while the paternal grandmother had a prolactinoma (but declined genetic studies).

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. 1a, b). Interestingly, the adenoma contained areas suggestive of hyperplasia on reticulin staining (Fig. 1c), a feature that has not been reported previously in association with \textit{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 \textit{MEN1} gene deletions by MLPA testing. Similarly, no patients with multiple other syndromic/familial manifestations carried any mutations in \textit{AIP}, \textit{PRKAR1A}, \textit{CDKN1B} and \textit{CDKN2C}.

\textbf{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). \textit{AIP} or \textit{MEN1} mutations were found in 5 of 11 (45.5%) cases with GH- or PRL-secreting tumors. In only one patient among these cases, there was positive family history. In addition, tumors with \textit{AIP} or \textit{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 \textit{AIP} mutation (in a patient with a GH-secreting tumor) among 36 children.

Thus, it appears that screening for both \textit{AIP} and \textit{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 \textit{AIP} mutation (1.4%), and this is the second only such patient described in the literature (18).
Genetic testing for pituitary adenomas

Diagnosis of a pituitary adenoma under the age of 21 years

- Cushing disease (ACTH)
- Gigantism/Acromegaly (GH/PRL)
- Pituitary adenoma in a syndromic setting other than MEN 1 or FIPA

No genetic testing, unless family history is positive or other signs of a syndrome (MEN 1) or an AIP mutation exist

Genetic testing is recommended for a syndrome (i.e. MEN 1) or for an AIP mutation; up to half of these patients will have a positive test and genetic counseling should be offered

Genetic testing as indicated per syndrome (e.g. Carney complex, tuberous sclerosis, neurofibromatosis, other); no need for MEN 1 or AIP testing.

Fig. 2. A proposed algorithm for genetic testing in pituitary adenomas. FIPA, familial isolated pituitary adenomas; MEN1, multiple endocrine neoplasia type 1.

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 PRKAR1A among all 18 patients with GH- or PRL-secreting tumors suggests that very rarely, if ever, patients with a germline PRKAR1A mutation present with a pituitary tumor 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 MEN1 as suggested previously by Stratakis et al. (32).

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

Acknowledgements

We would like to thank Drs. Stephen J. Marx and Sunita Agarwal (NIH, Bethesda, MD) for extensive discussions, guidance and advice on several aspects of the study and a critical review of the manuscript. We thank our patients and the support and nursing staff of the NIH Clinical Center outpatient clinics, and the 1NW and 5NW wards. This work was supported by United States National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD) intramural project Z01-HD-000642-04 (Dr. C.A. Stratakis), Fonds d’Investissement pour la Recherche Scientifique 2007 (FIRS) du CHU de Liège, Belgium (Dr. A. Beckers). Finally, many thanks to Dr. Alex Vortmeyer (NCI, NIH) and Dr. Edward Oldfield (NINDS, NIH) for their pathology and surgical work, respectively, in our patients.

Conflict of interest

The authors have nothing to disclose.

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