**Abstract**

**Background:** Aryl hydrocarbon receptor interacting protein (AIP) mutations (AIPmut) cause aggressive pituitary adenomas in young patients, usually in the setting of familial isolated pituitary adenomas. The prevalence of AIPmut among sporadic pituitary adenoma patients appears to be low; studies have not addressed prevalence in the most clinically relevant population. Hence, we undertook an international, multicenter, prospective genetic, and clinical analysis at 21 tertiary referral endocrine departments.

**Methods:** We included 163 sporadic pituitary macroadenoma patients irrespective of clinical phenotype at diagnosis < 30 years of age.

**Results:** Overall, 19/163 (11.7%) patients had germline AIPmut; a further nine patients had sequence changes of uncertain significance or polymorphisms. AIPmut were identified in 8/39 (20.5%) pediatric patients. Ten AIPmut were identified in 11/83 (13.3%) sporadic somatotropinoma patients, in 7/61 (11.5%) prolactinoma patients, and in 1/16 non-functioning pituitary adenoma patients. Large genetic deletions were not seen using multiplex ligation-dependent probe amplification. Familial screening was possible in the relatives of seven patients with AIPmut and carriers were found in six of the seven families. In total, pituitary adenomas were diagnosed in 2/21 AIPmut-screened carriers; both had asymptomatic microadenomas.

**Conclusion:** Germline AIPmut occur in 11.7% of patients < 30 years with sporadic pituitary macroadenomas and in 20.5% of pediatric patients. AIPmut mutation testing in this population should be considered in order to optimize clinical genetic investigation and management.
Introduction

Clinically relevant pituitary adenomas have a prevalence of one case per 1064–12 393 of the population in Western Europe and an incidence of four cases per 100 000 (1–3). The majority of pituitary adenomas occur sporadically, while up to 5% of all cases occur against an inherited or familial background; more than half are due to multiple endocrine neoplasia type 1 (MEN1) (4). Familial isolated pituitary adenomas (FIPA) account for most of the remaining cases, along with rare conditions such as Carney’s complex (CNC) or MEN4 (5, 6). Aryl hydrocarbon receptor interacting protein (AIP) mutations (AIPmut) occur in 15–30% of FIPA kindreds (7–9). In a recent large international study of AIPmut-associated pituitary adenomas, Daly et al. (10) observed that AIPmut are associated with all types of pituitary adenomas; these tumors are large, occur at a young age, and demonstrate features of aggressiveness and treatment resistance. While somatotropinomas and prolactinomas predominate, other pituitary adenoma subtypes do occur in the setting of AIPmut, including Cushing disease and thyrotropinoma, and the silent somatotrope subtype of non-functioning adenomas has also recently been described (10, 11).

AIPmut occur infrequently in unselected populations of patients with sporadic pituitary tumors (0–3.0%), in children with sporadic pituitary adenomas (2.7%) or Cushing disease (1.4%) or in specific sporadic tumor types (e.g. acromegaly patients: 3.2%) (12–19). This suggests that AIPmut screening of large, unselected sporadic pituitary adenoma populations is impractical; similarly, limited screening performed only in children could miss cases that develop in patients in their late teens and twenties (10). In the largest study of pituitary adenoma patients with AIPmut (n = 96), the most clearly defined features at diagnosis were macroadenoma (93.3%) and young age at diagnosis (median: 23.0 years) (10). Combining this information, 81.2% of patients were diagnosed with a macroadenoma at <30 years of age. No study to date has used this existing knowledge on patient/tumor characteristics to determine whether focused AIPmut screening may be clinically informative. In order to explore the utility of focused genetic screening, we investigated the frequency of AIPmut among patients with sporadic pituitary macroadenomas that occurred before the age of 30 years, in whom no other known genetic cause or FIPA was present.

Methods

Patients

This genetic screening study was performed in sporadic pituitary adenoma patients with tumors ≥10 mm in maximal diameter on magnetic resonance imaging (MRI) that were diagnosed before the age of 30 years. Patients had to be free of known diagnosis of genetic causes or familial pituitary adenomas (e.g. MEN1, CNC, MEN4, McCune–Albright syndrome, or FIPA). The study was conducted in centers in Belgium, Brazil, Bulgaria, Czech Republic, France, Germany, Italy, Lebanon, and Spain.

A total of 163 patients provided informed consent in their own local language to undergo AIP gene sequencing (72.0% of patients meeting the criteria at the study centers agreed to undergo genetic testing). In cases where an AIPmut was found, genetic counseling and testing were offered to family members. After counseling, consenting AIPmut carriers underwent MRI and hormonal testing to assess for the presence of pituitary disease. Basic demographic, clinical, and therapeutic response data were collected for patients with AIPmut. All subjects gave their written informed consent for themselves or for their minor children, while assent of the child was also obtained if they were between 11 and 17 years (no child was under the age of 11 years at the time of genetic testing) and the study was approved by local ethics committees and the Ethics Committee of the University of Liège.

Genetic analysis

DNA extracted from peripheral blood was analyzed for AIPmut by direct sequencing. In patients with normal AIP sequencing, multiplex ligation-dependent probe amplification (MLPA) was used to search for extensive deletions, as described previously (12). The control DNA from 200 healthy individuals included 30 healthy controls of North African origin to cover this previously unaddressed population, which was represented in our European sample. The potential effect of each intronic or silent variation on AIP protein was evaluated in silico using a battery of different tools. For the in silico analyses, a 180–240 base portion of the AIP gene sequence surrounding each of the nucleotide variants was evaluated for potential splicing and/or functional effects using the following bioinformatic tools: Human Splicing Finder (http://www.umd.be/HSF/HSF.html), FANS (http://fans.nci.sinica.edu.tw/fans/input.do), and FastSNP (http://fastsnp.ibms.sinica.edu.tw). These tools generate predictions based on algorithms that compare consensus sequences for certain motifs (e.g. sites for intronic and exonic silencers and enhancers) within sequences of interest. As individual tools provide only an estimation of potential effect, it is generally recommended that more than one tool be used to evaluate genomic variations of interest (20). PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) was used to evaluate the potential effects of missense mutations on AIP structure. AIP sequence variants were compared with human single nucleotide polymorphism (SNP) databases (dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ sns_summary.cgi) and also against recently published results in genetically diverse populations (21).
Results

Patient characteristics

A total of 163 patients with sporadic pituitary macroadenomas diagnosed before the age of 30 years were included. Eighty-three (50.9%) patients had somatotropinomas (46 females; mean ± s.d. age at diagnosis: 23 ± 6.2 years, mean ± s.d. tumor diameter 29.2 ± 14.7 mm); 61 (37.4%) had prolactinomas (39 males; mean ± s.d. age at diagnosis: 19.6 ± 4.6 years, mean ± s.d. tumor diameter 34.5 ± 15.8 mm); 16 (9.8%) had non-functioning pituitary adenomas (NFPa; nine females; mean ± s.d. age at diagnosis: 22.3 ± 5.1 years, mean ± s.d. tumor diameter 29.5 ± 16 mm); two (1.2%) had Cushing disease (both females, ages at diagnosis: 30.0 and 12.0 years; tumor diameter 12.0 mm in both); and one patient had a thyrotropinoma (male, 15.0 years at diagnosis, tumor diameter 32 mm). Two patients had concomitant brain tumors, one somatotropinoma patient had a glioblastoma of which he later died, and a prolactinoma patient had a meningioma.

AIP sequencing

AIP sequence variations were observed in 28/163 patients (17.2%), of which 19 (11.7%) would likely result in altered AIP expression or function (Table 1). MLPA did not reveal any large deletions. The c.100–18C>T change in one patient is a previously reported rare SNP (rs117691341). Ten AIPmut were identified in 11/83 (13.3%) sporadic somatotropinoma patients. Eight patients were males and seven presented with invasive tumors. Six of the ten AIPmut led to premature stop codons (p.Arg22X, p.Glu82fsX7, p.Gly117AlafsX39, p.Gln184X, p.Tyr261X, and p.Arg304X (n=2 unrelated patients)). The previously described p.Arg271Trp mutation occurs at an amino acid with an established structure–function relationship (8, 10, 22, 23). Two other missense mutations were observed in silico to have a highly probable effect on protein structure (p.Glu84Lys and p.Ala277Pro). In silico analyses also strongly supported a pathological role for the synonymous p.Glu197Glu variant, via alternative splicing at a splicing enhancer site within an important structural domain. A c.993+60G>C variant in the 3’-untranslated region (UTR) had no predicted effect on splicing, but in silico analyses predicted a new transcription factor-binding site, resulting in a low-to-medium likelihood of a deleterious effect. The c.468+16C>T variant predicted a new intronic branch site and also the generation of a new transcription factor-binding site in silico, but of a low-to-moderate likelihood of a deleterious effect. The p.Arg16His variant in one patient has previously been reported (8, 15), but recently, it was noted clearly not to segregate with pituitary adenomas in a PIPA kindred (24). Other AIP sequence variants had no predicted effect on AIP structure/function (p.Arg128His, c.468+15G>T, and c.993+63C>T).

AIPmut were found in 7/61 (11.5%) sporadic prolactinoma patients, six of whom were males. One frameshift/truncation (p.Gly117AlafsX39) was pathogenic. Based on in silico analyses, four missense mutations predicted deleterious effects (high likelihood: p.Arg56Cys, p.Lys58Asn, and p.Tyr268Cys; moderate likelihood: p.Val195Ala). One synonymous change (p.Phe269Phe) was also deleterious as characterized by Igreja et al. (9). The p.Arg304Gln AIP variant did not strongly predict a deleterious effect on the protein in silico, but previous characterization in vitro suggests that it is indeed a mutation (9). The p.Arg16His variant was found in one patient (see above).

One AIPmut (c.88_89delGA and p.Asp30Trps14X) was found in 1/16 (6.25%) NFPa patients. No AIP variants were seen in the Cushing disease or thyrotropinoma patients.

Familial screening

Familial screening studies were possible in the relatives of seven patients with AIPmut. Mutation carriers were found in six of the seven families. In the family where no mutation carrier was identified, the asymptomatic mother of a somatotropinoma patient with a p.Gly82fsX7 AIPmut declined genetic analysis. Among a total of 21 AIPmut carriers, MRI revealed undiagnosed pituitary adenomas in two members of one family with a p.Arg304X mutation. In that family, three mutation carriers were identified and the patient’s mother and sister had pituitary microadenomas, neither of which was associated with pituitary hormonal abnormalities.

AIPmut in pediatric patients with pituitary adenomas

Considering only pediatric patients (i.e. under the age of 18 years), AIP variations were identified in ten of 39 patients (25.6%) diagnosed before 18 years of age (five somatotropinomas and four prolactinomas). Eight variations were likely to be pathogenic in this pediatric cohort (20.5%), which was a significantly higher rate than in the patients aged ≥18 years (11/124; P<0.01).

Therapeutic responses

In terms of responses to therapy, the results obtained are in line with those reported elsewhere in AIPmut-bearing patients with pituitary adenomas (10). In patients with somatotropinomas, 4/11 (36.4%) underwent two or more surgical interventions, while secondary (post-operative) somatostatin analog therapy achieved disease control in 1/9 (11%) treated patients. Tumor size before and after somatostatin analog therapy was
Table 1 Clinical characteristic of patients with different AIP gene mutations and variants. Pathological AIP gene mutations (AIPmut) are shown in bold, while variations of unlikely molecular impact and polymorphisms are in normal font.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>AIPmut/variant</th>
<th>AN or ref.*</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Hor. levels (ng/ml)</th>
<th>At diagnosis</th>
<th>Max. tum. diameter (mm)</th>
<th>Invasion (Y/N)</th>
<th>Evidence of del. effect</th>
<th>Familial study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrromegaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>p.Arg22X</td>
<td>(12)</td>
<td>M</td>
<td>10</td>
<td>GH 6.9</td>
<td>38</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>p.Glu82AsnX7</td>
<td>FJ514477.1</td>
<td>M</td>
<td>15</td>
<td>GH 48.6</td>
<td>60</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>p.Glu84Lys</td>
<td>GQ403801.1</td>
<td>F</td>
<td>20</td>
<td>GH 14.9</td>
<td>11</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>p.Gly117Alafs39X c.350delG</td>
<td>GQ847774.1</td>
<td>M</td>
<td>18</td>
<td>GH 566</td>
<td>17</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>p.Glu197Glu</td>
<td>JN561683</td>
<td>F</td>
<td>23</td>
<td>GH 0.5</td>
<td>26</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>p.Tyr261X</td>
<td>GQ403803.1</td>
<td>M</td>
<td>28</td>
<td>GH 77.0</td>
<td>38</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>p.Arg271Trp</td>
<td>(8)</td>
<td>M</td>
<td>18</td>
<td>GH 11.9</td>
<td>45</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>p.Arg304X</td>
<td>(7)</td>
<td>M</td>
<td>17</td>
<td>GH 9.0</td>
<td>27</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>p.Arg304X</td>
<td>(7)</td>
<td>F</td>
<td>25</td>
<td>GH 5.6</td>
<td>40</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>(17) N</td>
</tr>
<tr>
<td>10</td>
<td>p.Arg16His</td>
<td>(8)</td>
<td>F</td>
<td>29</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>p.Arg22His</td>
<td>(32)</td>
<td>M</td>
<td>27</td>
<td>GH 58.5</td>
<td>20</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>c.468+15C&gt;T</td>
<td>(15)</td>
<td>F</td>
<td>19</td>
<td>GH 34.0</td>
<td>13</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>c.468+16G&gt;T</td>
<td>(15)</td>
<td>F</td>
<td>15</td>
<td>N/A</td>
<td>23</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>c.993+60G&gt;C</td>
<td>(15, 29)</td>
<td>F</td>
<td>34</td>
<td>N/A</td>
<td>15</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>c.993+60G&gt;C</td>
<td>(15, 29)</td>
<td>M</td>
<td>22</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>16</td>
<td>c.993+63C&gt;T</td>
<td>(15, 29)</td>
<td>F</td>
<td>27</td>
<td>GH 26.0</td>
<td>25</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Prolactoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>p.Arg96Cys</td>
<td>GU969040</td>
<td>M</td>
<td>26</td>
<td>PRL 5240</td>
<td>70</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>p.Lys88Asn</td>
<td>GQ847773.1</td>
<td>M</td>
<td>20</td>
<td>PRL &gt; 4000</td>
<td>72</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>21</td>
<td>p.Gly117Alafs39X c.350delG</td>
<td>GQ847774.1</td>
<td>M</td>
<td>16</td>
<td>PRL 2478</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>22</td>
<td>p.Val115Ala</td>
<td>(32)</td>
<td>M</td>
<td>12</td>
<td>PRL 10560</td>
<td>40</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>23</td>
<td>p.Tyr268Cys</td>
<td>GQ412196.1</td>
<td>M</td>
<td>28</td>
<td>PRL 6340</td>
<td>85</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>(9) N</td>
</tr>
<tr>
<td>24</td>
<td>p.His269Phe</td>
<td>(9)</td>
<td>M</td>
<td>10</td>
<td>PRL 20000</td>
<td>N/A</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>26</td>
<td>p.Arg16His</td>
<td>(8)</td>
<td>M</td>
<td>20</td>
<td>PRL 3867</td>
<td>45</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>(9) N</td>
</tr>
<tr>
<td>27</td>
<td>c.100–18C&gt;T</td>
<td>rs117691341</td>
<td>M</td>
<td>15</td>
<td>PRL 459</td>
<td>28</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Non-secreting adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>p.Asp30TrpfsX14 c.88+8delG</td>
<td>GQ847775.1</td>
<td>M</td>
<td>19</td>
<td>Normal</td>
<td>39</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

N/A, not available; AN, Accession number; Ref., reference; Hor., hormone; tum., tumor; ES, extrasellar; del., deleterious.

*Reference of original mutation description.

aMother of affected patient declined genetic screening.

bAge at first symptoms is 28 years.
measurable in six cases; in one case, there was 60% tumor shrinkage, and in the remaining cases, there was no size change \((n = 4)\) or tumor growth \((n = 1)\). Among patients with prolactinomas, 3/6 of cases that received cabergoline did not achieve disease control with usual clinical doses \((3–4 \text{ mg/week})\); there were two cases of resistance to high-dose cabergoline \((7 \text{ mg/week})\), and in one of these cases, secondary resistance occurred after initial control. Five prolactinoma patients underwent neurosurgery (three patients had two interventions each; one required four interventions) and radiotherapy was used in three patients. The 19-year-old patient with a non-secreting pituitary adenoma underwent neurosurgery twice and radiotherapy thereafter; the tumor was negative for all pituitary hormones on immunohistochemistry.

**Discussion**

This is the first study to specifically investigate the prevalence of \(AIP\) sequence variations among patients thought to be at the greatest risk, namely those diagnosed with pituitary macroadenomas as children or young adults \((< 30\text{ years})\). The finding of \(AIPmut\) in 11.7% of the entire cohort and among 20.5% of patients aged < 18 years at diagnosis strongly supports the contention that screening for \(AIPmut\) among young sporadic patients with macroadenomas may be clinically useful. We noted no large \(AIP\) gene deletions using MLPA, which suggests that such changes occur in a minority of cases that are negative for \(AIPmut\) on sequencing \((9, 12, 25)\).

These results expand on those obtained in previous studies performed using different criteria. Studies in unselected sporadic pituitary adenoma populations demonstrated a very low rate of \(AIPmut\) status \((12–14, 16)\). Other studies undertook screening that was restricted to children/adolescents \((18, 26)\), to those with FIPA \((8, 9)\), to sporadic somatotropinoma patients \((7, 14, 15)\), or were limited to a subset of \(AIPmut\) \((19, 27)\). These studies identified many novel \(AIPmut\) mutations and, along with more recent work \((10)\), have characterized disease features and responses to therapy.

We identified \(AIPmut\) in 13.3% of sporadic somatotropinoma patients in our targeted cohort, including six distinct truncating mutations; an Italian acromegalic patient had a previously reported missense mutation \((p.Arg271Trp)\) \((8, 10, 23)\) that was shown to abolish the interaction of \(AIP\) with phosphodiesterase 4A5 \((17, 22)\). Two other missense mutations \((p.Glu84Lys\) and \(p.Ala277Pro)\) were predicted in silico to have probably damaging effects on \(AIP\) protein. Also, a new synonymous \(p.Glu197Glu\) mutation was strongly predicted to have a pathogenic effect via interrupting a splicing enhancer site. Notably, the frequency of \(AIPmut\) among prolactinoma patients \((11.5\%)\) was similar to that of somatotropinoma patients. \(AIPmut\) in prolactinoma patients were detected mostly in males and were relatively difficult to treat with dopamine agonists, which is in line with other recent work \((10)\). As males with prolactinomas can represent a particularly difficult group to treat \((28)\), it is possible that \(AIPmut\) status may partially contribute to this poorer disease control, although specific studies of \(AIPmut\) status in large prolactinoma cohorts are lacking. The silent variation, \(p.Phe269Phe\), was previously reported as pathogenic by disrupting splicing enhancer sites, causing a loss of exon 6 in a FIPA family with two somatotropinoma patients \((9)\). One novel truncating \(AIPmut\) \((p.Gly117AlafsX39)\) occurred in a 16-year-old patient with a prolactinoma and also in an unrelated 18-year-old unrelated acromegalic patient. The \(p.Lys58Asn, p.Arg56Cys, p.Tyr268Cys,\) and \(p.Val195Ala\) missense mutations scored as being likely to affect \(AIP\) protein on in silico analysis. A truncating \(AIPmut\) was found in 1/16 NFPA \((6.3\%)\) patients and is to our knowledge the first report of a pathogenic variation in sporadic NFPA. The patient was aged 19 years and presented with visual field alteration and panhypopituitarism due to mass effects. A previous study identified two \(AIP\) variations in a cohort of 55 NFPA patients, but these were found to be polymorphisms \((16)\). The number of NFPA patients included in our study is small, due to the relative rarity of NFPA in younger individuals. No \(AIPmut\) were found in Cushing disease or thyrotropinoma patients, but this is again attributed to the small sample size. Corticotropinomas are mainly microadenomas and recently, sporadic Cushing disease in the young has been shown to be very rarely associated with \(AIPmut\) \((18)\). Thyrotropinomas are very rare tumors in the general population, and to date, only one case associated with \(AIPmut\) has been reported \((10)\).

Among the \(AIP\) variants, a number were non-pathogenic on in silico analyses \((e.g. c.468+15 \text{ C}>\text{T}\) and \(c.993+63 \text{ C}>\text{T})\). With others, the evidence is less certain or is contradictory. The \(p.Arg16His\) variant occurs in an evolutionary conserved residue and is predicted to have a deleterious effect on \(AIP\) protein on in silico analysis. In contrast, lgreja et al. \((9)\) reported only a modest effect of this variant on \(AIP\) function in an in vitro assay. Also arguing strongly against it being a mutation is the recent publication from Guaraldi & Salvatori \((24)\) showing that this change did not segregate uniformly with pituitary adenomas in a large FIPA kindred. On balance, the evidence currently suggests that \(p.Arg16His\) is most likely a rare polymorphism. A \(p.Arg304Gln\) mutation was observed in a 15-year-old boy with a prolactinoma whose family screening revealed another seven mutation carriers. No carrier had evidence of pituitary tumor, but one carrier (his mother) was diagnosed with breast cancer and the other (his maternal aunt) has a history of stomach cancer. This mutation occurs at a CpG island hotspot and was previously reported in two families with GH-secreting adenomas and in two sporadic cases of corticotropinoma and one acromegaly patient \((13, 15, 17)\). The strong
clinical data (i.e. multiple affected subjects) and the experimental data are apparently divergent, with only modest effects being seen on in vitro AIP functional assays (9) and low potential for deleterious effects in silico. The c.993 + 60G>C variant in the 3′-UTR, while it predicts a low-to-moderate likelihood of a deleterious effect via a new transcription factor binding site and has been reported in colorectal cancer (29), was also present in normal control subjects and is possibly not a pathological variant. In vitro testing of AIP variants of uncertain nature using a more expanded range of assays of AIP function beyond phosphodiesterase 4A5-based constructs may be useful to confirm or rule out the pathogenicity of AIP variants.

As AIPmut are most commonly found either in a FIPA or familial setting (7–10, 18) and as pituitary adenomas can be diagnosed in asymptomatic AIPmut carriers via the screening process (30), we performed screening in families of AIPmut pituitary adenoma patients. The completeness of this particular data set is less than optimal for determining penetrance as family members in only seven of the 18 AIPmut patients consented to genetic screening. Mutation carriers were found in six families while in the seventh family the mother of the patient declined genetic studies. MRI revealed two microadenomas among the 21 (9.5%) carriers, which is in keeping with the relatively low disease penetrance of pituitary adenomas. While the tumors in these two AIPmut carriers are clinically silent at present, longer term follow-up is required similar to recent recommendations on incidentaloma surveillance (31). However, it is too early to determine whether these are AIPmut-related tumors or incidentalomas and a high clinical index of suspicion is warranted. Determining true disease penetrance in the setting of AIPmut requires studying a large number of kindreds to allow for detailed surveillance of a sizeable number of mutation carriers through childhood/adolescence and early adulthood.

In conclusion, screening for germline AIPmut in patients aged < 30 years with pituitary macroadenomas was positive in 11.7% of cases and in 20.5% of those aged < 18 years. Testing for AIPmut in children and young adults diagnosed with macroadenomas could be a useful addition to screening in FIPA kindreds considering the relatively high incidence of AIPmut in this well-defined and readily encountered sub-population (33).

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This study was supported by the Fonds d’Investissement pour la Recherche Scientifique 2008 (FIRS) du CHU de Liège, University of Liège, Belgium, and by the Oncogenetic Network of the French Ministry of Health, CNRS.

References


