BRIEF REPORT

Mutations in the Aryl Hydrocarbon Receptor Interacting Protein Gene Are Not Highly Prevalent among Subjects with Sporadic Pituitary Adenomas

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Context: Limited screening suggests that three germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene are not involved in sporadic pituitary tumorigenesis. Multiple novel mutations of this gene have since been identified in familial isolated pituitary adenoma cohorts.

Objective: The objective of the study was to undertake full AIP coding sequence screening to assess for the presence of germline and somatic mutations in European Union subjects with sporadic pituitary tumors.

Design: The study design was the analysis of DNA from peripheral blood lymphocytes and analysis of exons 1–6 and paraexonic intron sequences of AIP. Multiplex ligation-dependent probe amplification was used to screen separate sporadic pituitary tumor tissue samples for discrete and extensive deletions or mutations of the AIP gene.

Setting: The study was conducted in university tertiary referral Clinical Genetics, Molecular Biology, and Endocrinology Departments.

Results: In 107 patients [prolactinomas (n = 49), nonfunctioning tumors (n = 29), somatotropinomas (n = 26), ACTH-secreting tumors (n = 2), TSH-secreting tumors (n = 1)], no germline mutations of AIP were demonstrated. Among a group of 41 tumor samples from other subjects, a novel AIP mutation (R22X) was found in one sample in which the corresponding allele was deleted; follow-up screening of the patient demonstrated a germline R22X AIP mutation.

Conclusions: AIP mutations do not appear to play a prominent role in sporadic pituitary tumorigenesis in this population of European subjects.

MOLECULAR GENETIC STUDY of pituitary tumors has identified a variety of mutations or abnormal expression pattern that may play a role in tumorigenesis. Well-recognized examples include mutations in the Gsα gene, PTTG overexpression, and decreased MEG3 expression (1–4). MEN1 and PRKARIA gene mutations play a critical role in endocrine tumorigenesis in multiple endocrine neoplasmia-1 (MEN1) and Carney complex (CNC) (5, 6) but are of limited significance in sporadic pituitary tumors (7, 8). Thus, interest remains high in the identification of new molecular mechanisms to explain sporadic adenoma formation (9).

Aryl hydrocarbon receptor interacting protein (AIP), a 330-amino-acid protein present in the pituitary, interacts with the aryl hydrocarbon receptor and heat shock protein 90 dimer; AIP is involved in mediating cellular responses to environmental toxins like dioxin (10, 11). Mutations that remove conserved tetratricopeptide repeat domains at the carboxy terminal of AIP abrogate proper interaction with its receptor and heat shock protein 90, thereby disrupting nuclear localization (11). Vierimaa et al. (12) first reported the involvement of three AIP mutations in pituitary adenomas in non-MEN1, non-CNC families from Finland and Italy. Among the Finnish cohort, two AIP mutations were noted in germline and somatic DNA from sporadic pituitary ade-
Pituitary tumor samples

Forty-one frozen tissue samples from patients with sporadic pituitary tumors (different from the patients above) were collected as previously described (16) and studied for AIP gene mutations. These tumors, classified by immunohistochemical staining for pituitary hormones, included 23 somatotrophinomas (13 males, 10 females; mean (±sd) age at diagnosis: 56.6 ± 13.1 yr; 100% macroadenomas, 58.8% cavernous sinus invasion), two females with noninvasive ACTH-secreting microadenomas [mean (±sd) age at diagnosis: 32.5 ± 4.9 yr], and one male subject with a noninvasive TSH-secreting microadenoma diagnosed at age 48 yr.

None of the subjects had a history of MEN1, CNC, or FIPA. Twelve subjects (11.2%) had other endocrine tumors: parathyroid adenoma (n = 10), pancreatic endocrine tumor (n = 1), and thymoma (n = 1); MEN1 gene mutations were outruled by gene sequencing. All subjects gave their written informed consent to participate in the study, which was approved by the local ethics committee.

Genomic analysis of AIP and MEN1

Using DNA isolated from peripheral blood, exons of the AIP and MEN1 genes were amplified using exon flanking primers (12, 17). Apart from exons 4 and 5 of AIP, the same primers were used for gene sequencing using a CEQ8000 sequencer (Beckman Coulter, Fullerton, CA). The primers for AIP exons 4 and 5 were: GACGCAGCTGTGGTGTCC (AIP4F) and CTAGGGGGAGGATGGAT (AIP5F). The primers used for sequencing AIP exons 1, 2, 3, and 6 and the MEN1 gene are available on request.

AIP single-nucleotide polymorphism patterns were studied using a reference population of 86 normal individuals from Belgium (n = 31), France (n = 25), and Italy (n = 30). In addition, an international panel of reference populations were analyzed as follows: exons 2 and 5 [Applera Genomics Initiative (AGI) Caucasian and African American samples, Coriell Cell Repositories], exon 4 (CEU; Utah residents of northern/western European ancestry), and exon 6 [Centre d’Etude du Polymorphisme Humain (CEPH)].

Multiplex ligation-dependent probe amplification (MLPA) analysis

Loss of heterozygosity (LOH) within the AIP gene and along larger segments of chromosome 11q was assessed in tumor samples using MLPA. MLPA probe design has been described previously (18). Control probe-pairs that were specific to unrelated genes on chromosomes 7 and 17 were also designed. All probes had amplification products from 87 to 136 bp in length and had an annealing temperature higher than 70°C as per the RAW Probe program (MRK-Holland, Amsterdam, The Netherlands). PCR products were analyzed on an ABI3100 capillary electrophoresis apparatus (Applied Biosystems, Lennik, Belgium). Copy number quantification involved normalization of the peak area of the AIP-specific MLPA probe by dividing it by the combined areas of the control probes. This ratio was compared with the similar ratio obtained from control DNA. LOH was observed when the wild-type signal was reduced by 35–60% for each AIP-specific probe.

Results

AIP analysis in genomic DNA

Analysis of the coding sequence of AIP in genomic DNA from 107 subjects with sporadic pituitary adenomas revealed no mutations. The genotype frequencies of polymorphisms were similar to those of the reference populations (Table 1).

AIP analysis in pituitary tumor DNA

MLPA analysis revealed deletions at the AIP locus in two of 41 frozen pituitary tumor fragments [somatotrophinoma (n = 2)]. In one somatotrophinoma, a nonsense mutation p.Arg22X (CGA>TGA) at exon 1 of AIP was found, indicating a hemizygotic state with the loss of wild-type allele (GenBank accession no. EF158450). This male subject presented with acromegaly at a young age (24 yr) due to a macroadenoma that secreted high levels of GH and was resistant to somatostatin agonist therapy. Radiotherapy was required 1 yr postoperatively due to activity of the residual tumor. Analysis of peripheral blood DNA in the patient also revealed a germline p.Arg22X mutation. There was no family history of pituitary adenomas in this case. The other somatotrophinoma sample with a large deletion that included the AIP and MEN1 loci came from a young male with an invasive tumor. However, the corresponding AIP or MEN1 genes were intact in the other allele.

The genotype frequencies of all AIP polymorphisms were similar to those of the reference populations (Table 1).

Discussion

The involvement of AIP mutations in pituitary tumorigenesis was first suggested by Vierimaa et al. (12), who described three mutations (Q14X, R304X, and IVS3–1G>A) in subjects with somatotrophinomas and prolactinomas. Daly et al. (13) recently described a further nine AIP mutations (R16H, G47_R54del, Q142X, E174 frameshift, Q217X, Q239X, K241E, R271W, and Q285 frameshift) in a cohort of 73 families with FIIPA. Among these 12 described mutations, the majority lead to early stop codons and protein truncation, which would remove the third tetrapeptide repeat domain and the last five carboxy-terminal amino acids that are necessary for the biological activity of AIP (10). Q14X and IVS3–1G>A occurred in Finnish individuals with sporadic pituitary tumors; LOH at the AIP locus was shown in all
The clinical characteristics (young age, large/aggressive tumors) of the patient are similar to those of other patients with sporadic pituitary adenomas in continental Europe.创始人效应应考虑在与 pituitary adenomas, full family history of pituitary adenomas or other endocrine disease. A tumor sample from another patient with a somatotropinoma had a deletion that also included both AIP and MEN1, but both genes in the other allele were wild type. This raises the possibility that a mutation in another gene in this region of 11q13 could be involved in pituitary tumorigenesis in such patients.

This study confirms that germline mutations of AIP are infrequently found in patients with sporadic pituitary adenomas in continental Europe. Founder effects should be considered when extrapolating from AIP mutation prevalence among genetically delimited populations. Given that at least 13 AIP mutations have been described in association with pituitary adenomas, full AIP sequencing is needed to rule AIP involvement in sporadic pituitary tumorigenesis among other populations worldwide.

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**Table 1. Frequencies of AIP gene polymorphisms in subjects with sporadic pituitary adenomas**

<table>
<thead>
<tr>
<th>Exon</th>
<th>Sequence variation</th>
<th>Genotype</th>
<th>Controls (n = 86)</th>
<th>Genomic (n = 107)</th>
<th>Somatic (n = 41)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Frequency</td>
<td>Count</td>
<td>Frequency</td>
</tr>
<tr>
<td>2</td>
<td>p.Asp44Asp (rs11822907) c.132C&gt;T</td>
<td>C/C</td>
<td>1</td>
<td>86</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/T</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T/T</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>p.Asp172Asp (rs2276020) c.516C&gt;T</td>
<td>C/C</td>
<td>0.98</td>
<td>84</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/T</td>
<td>0.02</td>
<td>2</td>
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<tr>
<td></td>
<td></td>
<td>T/T</td>
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<td>0</td>
</tr>
<tr>
<td>5</td>
<td>p.Gln228Lys (rs641081) c.682C&gt;A</td>
<td>C/C</td>
<td>0</td>
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<td></td>
<td></td>
<td>C/A</td>
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<td>0</td>
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<tr>
<td></td>
<td></td>
<td>A/A</td>
<td>1</td>
<td>86</td>
<td>0.95</td>
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<tr>
<td>6</td>
<td>p.Ile327Thr (rs1049565) c.981C&gt;T</td>
<td>C/C</td>
<td>1</td>
<td>86</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/T</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
<td></td>
<td>T/T</td>
<td>0</td>
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</table>

The control population comprised normal subjects from Belgium (n = 31), France (n = 25), and Italy (n = 30), and the polymorphism frequencies were similar to those seen in the genomic and somatic populations. An additional 92 control samples from international sources were also studied (data not shown). These included AGI for single-nucleotide polymorphisms located in exons 2 and 5, CEU for exon 4, and CEPH in exon 6. The populations included: AGI (Caucasian and African-American samples from Coriell Cell Repositories Collection); CEU [CEPH (Utah residents with ancestry from northern and western Europe)]; CEPH [the genomic DNA was comprised of U.S. (Utah; 93%), French (4%), and Venezuelan (3%) samples from Coriell Cell Repository, which were pooled in equimolar amounts for use]. Polymorphism frequencies were similar to those seen in the Belgian-French-Italian control population and the genomic and somatic DNA samples from patients with sporadic pituitary adenomas.
References


