No evidence of somatic aryl hydrocarbon receptor interacting protein mutations in sporadic endocrine neoplasia

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Abstract
Germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene were recently observed in patients with pituitary adenoma predisposition (PAP). Though AIP mutation-positive individuals with prolactin-, mixed growth hormone/prolactin-, and ACTH-producing pituitary adenomas as well as non-secreting pituitary adenomas have been reported, most mutation-positive patients have had growth hormone-producing adenomas diagnosed at relatively young age. Pituitary adenomas are also component tumors of some familial endocrine neoplasia syndromes such as multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC). Genes underlying MEN1 and CNC are rarely mutated in sporadic pituitary adenomas, but more often in other lesions contributing to these two syndromes. Thus far, the occurrence of somatic AIP mutations has not been studied in endocrine tumors other than pituitary adenomas. Here, we have analyzed 32 pituitary adenomas and 79 other tumors of the endocrine system for somatic AIP mutations by direct sequencing. No somatic mutations were identified. However, two out of nine patients with prolactin-producing adenoma were shown to harbor a Finnish founder mutation (Q14X) with a complete loss of the wild-type allele in the tumors. These results are in agreement with previous studies in that prolactin-producing adenomas are component tumors in PAP. The data also support the previous finding that somatic AIP mutations are not common in pituitary adenomas and suggest that such mutations are rare in other endocrine tumors as well.

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Introduction
Germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene were recently reported in patients with pituitary adenoma predisposition (PAP; Vierimaa et al. 2006). Typically, individuals with germline AIP mutations display a young age at disease onset and growth hormone (GH)-producing pituitary adenomas, though patients with prolactin (PRL)- or adrenocorticotropic hormone (ACTH)-producing adenomas and non-secreting pituitary adenomas have been identified as well (Vierimaa et al. 2006, Barlier et al. 2007, Daly et al. 2007, Georgitsi et al. 2007b, Iwata et al. 2007, Toledo et al. 2007). Although many cases show family history of pituitary adenoma, in some cases the family history is weak or absent indicating incomplete penetrance (Vierimaa et al. 2006, Barlier et al. 2007, Daly et al. 2007, Georgitsi et al. 2007b). However, the highest AIP mutation frequencies have been observed in patients with family history of pituitary adenomas (15% in familial isolated pituitary adenoma (FIPA) patients and up to 50% in those with familial GH-producing adenomas) when compared with patients with sporadic tumors (0–7.4%) (Vierimaa et al. 2006, Yu et al. 2006, Barlier et al. 2007, Daly et al. 2007, Georgitsi et al. 2007b). Interestingly, the same AIP mutation can
associate with different tumor types in different families, and the phenotype can vary even between family members (Vierimaa et al. 2006, Daly et al. 2007). Heterogeneity in immunohistochemical staining has also been observed; for instance, tumors from GH-producing adenoma patients have shown only GH, GH and PRL, or GH and follicle-stimulating hormone (FSH) staining (Vierimaa et al. 2006, Daly et al. 2007). Tumor diameter has also been suggested to be larger in AIP mutation carriers when compared with non-carriers (Daly et al. 2007), although this was not detected in Finnish patients with PAP (Vierimaa et al. 2006).

Pituitary adenomas are component tumors in well-characterized endocrine neoplasia syndromes multiple endocrine neoplasia type 1 (MEN1) and Carney complex (CNC; Thakker 1998, Stratakis et al. 2001). The major predisposition genes for these syndromes are MEN1 and the protein kinase A regulatory subunit-1-α (PRKAR1A) respectively (Chandrasekharappa et al. 1997, Lemmens et al. 1997, Casey et al. 2000, Kirschner et al. 2000). Besides pituitary adenomas, MEN1 and CNC patients have tumors also in other endocrine glands. MEN1 lesions include parathyroid adenomas, entero-pancreatic islet tumors, adrenal cortical lesions, and foregut carcinoids, whereas in CNC heart and skin myxomas, primary pigmented nodular adrenocortical disease (PPNAD), testicular neoplasms, thyroid follicular adenomas or carcinomas, and ovarian cysts are common (Thakker 1998, Stratakis et al. 2001). Interestingly, MEN1 and PRKAR1A are only rarely mutated in sporadic pituitary adenomas, but instead mutations have been detected in some of the other contributing lesions (Leotlela et al. 2003, Kaltsas et al. 2004, Boikos & Stratakis 2006).

We have recently examined the occurrence of somatic AIP mutations in common cancers, with negative results (Georgitsi et al. 2007a). Thus far, no somatic mutations have been identified in pituitary adenomas either when studied by direct sequencing. Instead, loss of heterozygosity (LOH) has been detected in pituitary tumors as a second hit, the first being a germline mutation in the other allele (Barlier et al. 2007, Iwata et al. 2007, Georgitsi et al. 2007b). In this work, we sought for somatic AIP mutations also in non-pituitary endocrine tumors. The whole coding region of AIP was analyzed in 32 sporadic pituitary adenomas and 79 other sporadic endocrine tumors.

**Subjects and methods**

**Patient characteristics**

Patient information and samples were obtained either after informed consent or with the permission from the National Authority for Medicolegal Affairs. The study was approved by the appropriate ethics review committee.

Altogether 32 pituitary adenomas, of which nine were PRL-producing adenomas and 23 GH-producing adenomas, were included in the study (Table 1). The PRL-producing adenomas were from patients diagnosed at the Department of Pathology, Oulu University Hospital, Northern Finland. Immunohistochemistry for hypophyseal hormones was performed as a routine diagnostic procedure. All adenomas were positive for PRL and three adenomas stained positively also for GH. Tumor percentage, i.e. the percentage of tumor tissue in the sample, was determined according to a pathologist’s histological evaluation and ranged between 70 and 90%. Age at operation ranged between 35 and 64 years (mean age 49 years ±10.4). Sex distribution was five females and four males.

Out of the 23 GH-producing adenomas in the study, 21 belong to the population-based series of 54 Finnish acromegaly patients diagnosed at five university hospitals in Finland during 1980–1999 (Kauppinen-Makelin et al. 2005, Vierimaa et al. 2006). In addition, adenomas from two unselected Finnish sporadic acromegaly patients from Oulu region were included in this study. We had previously performed germline AIP mutation analysis in 20 patients from whom normal tissue was available; the results were negative (Vierimaa et al. 2006). Tumor percentage of the 22 tumors was determined according to a pathologist’s histological evaluation, ranging between 75 and 90%, except one with 20%. For one case, information on tumor percentage was not available. Age at diagnosis ranged between 17 and 66 years (mean age 40 years ±12.4). Age was not available for one patient. Ten patients were females and 13 were males.

A total of 79 other tumors of the endocrine system were available for this study (Table 1). Out of these, 61 tumors from 60 individuals were provided by the Department of Pathology, Helsinki University Central Hospital, Finland. Tumor percentage was determined according to a pathologist’s histological evaluation, 56 tumors with 60–100% and five tumors with 30–40%. Age at diagnosis ranged between 22 and 87 years (mean age 51 years ±15.5). Information on sex was available for 59 cases, of which 37 were females and 22 were males. One male individual had two tumors of the thyroid gland: one follicular adenoma and one papillary carcinoma. Corresponding normal tissue was available from 59 out of 61 cases. The remaining 18 endocrine tumors were provided by the Molecular Diagnostics Laboratory of the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark. Corresponding
normal tissue, adjacent to the tumor, was available in 7 out of 18 cases. Age at diagnosis ranged between 18 and 77 years (mean age 53 years ± 17.7). Sex distribution was 13 females and five males.

**Mutation analysis**

DNA was extracted from blocks of paraffin-embedded tissue according to standard procedures. The coding region and flanking intronic sequences of AIP were amplified by PCR and analyzed by genomic sequencing as previously described (Vierimaa et al. 2006). Poor quality DNA was amplified in shorter fragments, for which the primer sequences and protocols are available upon request. The PCR products were purified using ExoSAP-IT PCR purification kit (USB Corporation, Cleveland, OH, USA), and the sequencing reactions were performed using the Big Dye 3.1 Termination chemistry on an ABI3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Mutation analysis was initially performed on tumor samples, and the presence of all the observed changes was studied in the respective normal tissue, whenever available.

**Results**

Altogether 111 tumors were analyzed for somatic AIP mutations. In total, 1080 out of 1110 (97%) fragments were successful in the analysis.

The Finnish founder mutation (Q14X, 40CT) was detected in two PRL-producing adenomas. Both tumors showed complete loss of the wild-type allele (Fig. 1). One mutation carrier was diagnosed with PRL-producing adenoma at the age of 35 years. The mutation was also detected in the germline. This male patient did not have family history of endocrine tumors. The other Q14X-positive individual was a female patient diagnosed with PRL-producing adenoma at the age of 35 years. Though secretion of PRL predominated the clinical phenotype, she also displayed features somewhat compatible with early signs of acromegaly.

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**Table 1** Pituitary adenomas and other endocrine tumors studied for somatic mutations in the aryl hydrocarbon receptor-interacting protein (AIP) gene, and identified changes

<table>
<thead>
<tr>
<th>Endocrine tumors</th>
<th>Number of tumors analyzed</th>
<th>AIP change&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number of patients with AIP change (%)</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary adenomas</td>
<td>32</td>
<td>Q14X, 40C&gt;T</td>
<td>2 (22.2%)</td>
<td>0/532&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Prolactin producing</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Growth hormone producing</td>
<td>23</td>
<td></td>
<td></td>
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<tr>
<td>Other endocrine tumors</td>
<td>79</td>
<td></td>
<td></td>
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<tr>
<td>Paragangliomas</td>
<td>4</td>
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<tr>
<td>Pancreatic endocrine tumors</td>
<td>4</td>
<td></td>
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<tr>
<td>Parathyroid gland</td>
<td>8</td>
<td></td>
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<tr>
<td>Adenomas</td>
<td>6</td>
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<tr>
<td>Carcinomas</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>Adrenal gland</td>
<td>19</td>
<td></td>
<td></td>
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<tr>
<td>Pheochromocytomas</td>
<td>8</td>
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<tr>
<td>Adrenal cortical adenomas</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal cortical carcinomas</td>
<td>4</td>
<td></td>
<td></td>
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<tr>
<td>Thyroid gland</td>
<td>26</td>
<td></td>
<td></td>
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<tr>
<td>Follicular adenomas</td>
<td>5</td>
<td>G23E, 68G&gt;A</td>
<td>1 (3.8%)</td>
<td>5/532&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Follicular carcinomas</td>
<td>7</td>
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<tr>
<td>Papillary carcinomas</td>
<td>11</td>
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<tr>
<td>Medullary carcinomas</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Neuroendocrine tumors (carcinoids)</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lung</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cecum</td>
<td>2</td>
<td></td>
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<td>Appendix</td>
<td>4</td>
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<tr>
<td>Small intestine</td>
<td>9</td>
<td></td>
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<tr>
<td>Mixed endocrine–exocrine tumors (adenocarcinoids)</td>
<td>2</td>
<td></td>
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<tr>
<td>Appendix</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>Cecum</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<sup>a</sup>All present also in germline.

<sup>b</sup>Controls have been analyzed in our previous studies (Vierimaa et al. 2006, Georgitsi et al. 2007a,b).
Immunohistochemistry confirmed a mixed PRL/GH-producing tumor. Unfortunately, no normal tissue or information on the family history was available. Mutation screening of the 23 GH-producing adenomas did not reveal any changes in the \textit{AIP} gene. The only \textit{AIP} change detected in the other endocrine tumors was a missense heterozygous change (G23E, 68G>A) in one individual. This female patient was diagnosed with follicular adenoma of the thyroid gland at the age of 60 years. No LOH was observed in the tumor sample, and the change was present also in the corresponding normal tissue. We have previously observed this change in 6 out of 373 (1.6%) Finnish colorectal cancer samples and also in 5 out of 532 (0.9%) unrelated, anonymous control samples of different origin (Vierimaa et al. 2006, Georgitsi et al. 2007). Although the G23 is highly conserved among species, the presently available data are more compatible with a germline polymorphism than with a pathogenic mutation.

Discussion

Germline mutations in the \textit{AIP} gene have been associated with genetic predisposition to pituitary adenomas (Vierimaa et al. 2006). Most mutation-positive patients have had GH-producing tumors, but patients with PRL- and ACTH-producing adenomas as well as non-secreting tumors have also been reported (Vierimaa et al. 2006, Barlier et al. 2007, Daly et al. 2007, Georgitsi et al. 2007b, Toledo et al. 2007, Iwata et al. 2007). To date, no somatic \textit{AIP} mutations have been identified in pituitary adenomas or in any other tumor type studied (Iwata et al. 2007, Barlier et al. 2007, Georgitsi et al. 2007a,b). In addition to pituitary adenomas, the present work focused on analyzing somatic \textit{AIP} mutations also in other types of endocrine tumors.

No somatic \textit{AIP} mutations were observed in any of the tumors studied. However, one pathogenic mutation (Q14X) was detected in two patients with PRL-producing adenomas (two out of nine, 22%). This mutation is a Finnish founder mutation that has been detected in both familial setting as well as in patients with seemingly sporadic tumors (Vierimaa et al. 2006, Georgitsi et al. 2007b). Haplotype analysis has revealed that all the individuals studied share a common haplotype, confirming a common origin of the mutation (Vierimaa et al. 2006, unpublished data). In this study, the mutation was confirmed to be in the germline in the male mutation carrier patient. Both mutation-positive individuals have been diagnosed at the age of 35 years and the patient from whom information was available has no family history of endocrine tumors. Our results support previous observations that individuals with \textit{AIP} mutations are diagnosed at a relatively early age. These individuals usually do not display a strong family history of pituitary adenomas, and that besides GH-producing adenomas, \textit{AIP} mutations predispose also to PRL-producing adenomas which thus are part of the PAP phenotype (Vierimaa et al. 2006, Daly et al. 2007, this study).

Although PRL-producing adenomas are the most common pituitary adenomas (Arafah & Nasrallah 2001), only a few tumors have thus far been analyzed for somatic \textit{AIP} mutations. One obvious reason for this is that these tumors are only infrequently surgically removed as they have a low growth rate and a good response to drug treatment (Spada et al. 2005). Therefore, further studies are needed to clarify the possible role of somatic \textit{AIP} mutations in PRL-producing adenomas.

No somatic mutations were detected in any of the 23 GH-producing pituitary adenomas studied. This is in agreement with the previous studies, suggesting that somatic \textit{AIP} mutations do not have a major contribution in the genesis of these tumors (Barlier et al. 2007, Iwata et al. 2007). Overall, this is not surprising, as many tumor susceptibility genes are only rarely somatically mutated in the respective sporadic tumors; for instance, somatic mutations in the two major breast

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Electropherograms showing the \textit{AIP} mutation are seen in two prolactin-producing adenomas. (A) The corresponding wild-type sequence (B) The \textit{AIP} germline mutation (Q14X, 40C>T) found in two prolactin-producing adenomas with loss of the wild-type allele in the tumor tissue. The mutated base is indicated by arrows.}
\end{figure}
and ovarian cancer susceptibility genes BRCA1/2 are rare in breast tumors (Khoo et al. 1999, Yang et al. 2002).

No AIP mutations were detected in any of the 79 non-pituitary endocrine tumors studied here. Thus far, pituitary adenomas are the only tumors known to associate with AIP mutations. In contrast, in MEN1 syndrome pituitary neoplasia affects only ~30% of patients, whereas primary hyperparathyroidism (~95%) and pancreatic islet cell tumors (~40%) are the major clinical manifestations (Thakker 1998). Additionally, in CNC pituitary adenomas are relatively rare (~10% of patients) and the most common features are myxomas (~45%), PPNAD (~26%), schwannomas, testicular neoplasms, and thyroid adenomas or carcinomas (Stratakis et al. 2001). MEN1 and PRKAR1A are not frequently mutated in sporadic pituitary adenomas, but somatic mutations have been detected in some other endocrine lesions, such as MEN1 mutations in sporadic foregut endocrine tumors, parathyroid tumors, endocrine tumors of the pancreas, and lung carcinoids, and PRKAR1A mutations in thyroid and adrenal neoplasms (Leotlela et al. 2003, Kaltsas et al. 2004, Boikos & Stratakis 2006).

Our results are in agreement with previous studies in that somatic mutations seem rare in pituitary adenomas (Barlier et al. 2007, Georgitsi et al. 2007, Iwata et al. 2007). According to this study, such mutations appear uncommon also in non-pituitary endocrine tumors. We have also examined the contribution of AIP in common cancers with negative results (Georgitsi et al. 2007a). Taken together, this suggests that somatic AIP mutations are rare, and that thus far the only tumors associated with AIP mutations are pituitary adenomas.

Acknowledgements

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