Germline mutations of AIP gene in somatotropinomas resistant to somatostatin analogues

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Abstract

Objective: Most cases of familial isolated pituitary adenomas with mutated aryl hydrocarbon receptor-interacting protein (AIP: HGNC:358) gene develop somatotropinomas. They are characterised by an aggressive clinical phenotype including early age at diagnosis, large tumours and frequent invasiveness. There is little information on AIP gene mutations’ prevalence in isolated somatotropinomas characterised by poor response to somatostatin analogue treatment. The aim of this study was to investigate the prevalence of AIP mutations in non-familial cases of somatotropinomas with poor response to conventional treatment.

Design and methods: Fifty patients with acromegaly (22 males/28 females, age 51 ± 18 years) and 60 controls were included in this study performed at eight University Hospitals in Spain. None had family history of pituitary adenomas or other endocrine tumors. All patients failed to respond to conventional treatment including surgery and somatostatin analogues. Some patients received adjuvant radiotherapy and most cases required pegvisomant (PEG) treatment for normalisation of IGF1. AIP analysis was performed in DNA extracted from peripheral leucocytes, using standardised PCR protocol in which the coding regions of exons 1, 2, 3, 4, 5 and 6 were amplified. Possible deletions/duplications were studied using multiplex ligation-dependent probe amplification.

Results: Sequence changes of potential different significance that could be considered as mutations or variations of unknown significance (VUS) of the AIP gene were found in four patients (8%). In two cases, two different mutations previously described were found: p.Arg9Gln and p.Phe269Phe. Two other VUS were also found: c.787+24C>T in intron 5 and c.100-18C>T in intron 1. Age at diagnosis ranged from 21 to 50 years old, and in all patients, the tumor was a macroadenoma depicting IGF1 normalisation under PEG treatment.

Conclusions: AIP germline mutations show a low, but non-negligible, prevalence in non-familial acromegaly patients with tumors resistant to treatment with somatostatin analogues.

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Introduction

Pituitary adenomas are benign tumors with good therapeutic surgical outcomes when the adenoma is not large and the neurosurgical team has experience. During the last decade, great interest has been focused on the genes responsible or predisposing the development and progression of these tumors (1, 2). Although most of the pituitary adenomas are sporadic, few are included in well-defined familial syndromes such as MEN1. Furthermore, interesting data have been recently published on genes potentially participating in both the development of MEN1-like syndromes and the predisposition for pituitary adenomas (3, 4). Up to now, these findings seem to involve a reduced number of cases among the total population of patients with pituitary adenomas, although new data with more patients included in ongoing research programmes may change the present figures. Also, the potential correlation of the genotype and the phenotype in certain mutations associated with an aggressive clinical behaviour may open the option of designing new tools for a more individualised therapy.

Loss of heterozygosity of 11q13 has been found in up to 30% of sporadic pituitary adenomas, although new data with more patients included in ongoing research programmes may change the present figures. Also, the potential correlation of the genotype and the phenotype in certain mutations associated with an aggressive clinical behaviour may open the option of designing new tools for a more individualised therapy.

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identified the aryl hydrocarbon receptor-interacting protein (AIP) gene. This gene encodes the protein AIP, which has a role in metabolic clearance of dioxin and other toxic carcinogenetic agents. AIP possibly acts as a tumor suppressor gene, and germline inactivating mutations have been related to the development of pituitary tumors, but the mechanism by which AIP suppresses pituitary tumorigenesis has not been clarified. All these new data have increased the interest in studying germline mutations of AIP gene in different groups of patients with pituitary tumors and have revealed an association with multiple cases of single pituitary adenoma in family individuals (familial isolated pituitary adenoma (FIPA)) (6, 7, 8). Although AIP mutations may be present in virtually all subtypes of pituitary adenomas, for unknown reasons, there is a predominance of such mutations in adenomas of the somatolactotroph lineage and, particularly, with those having an aggressive biological behaviour and a lower response to different treatment modalities, including somatostatin analogues (9). We aimed to study whether the screening of mutations in patients presenting GH- or GH/PRL-secreting tumors without a familial pituitary disease background and characterised by having an aggressive clinical evolution – in particular those showing a failure to respond to conventional treatment with somatostatin analogues – was justified or not according to the prevalence obtained.

Materials and methods

Patients

Fifty patients and 60 controls were included in this study performed at eight University tertiary care Hospitals in Spain. The Ethic Committees of each hospital approved the protocol and a written informed consent was obtained from all the patients and controls before inclusion. Anonymous patient information on demographics, diagnosis, genetics, hormonal profiles at diagnosis and radiological criteria were collected. All patients had acromegaly according to current diagnostic guidelines. Paediatric cases (defined as age at diagnosis <18 years) were not included in this study. There were no antecedents of pituitary disorders or MEN1, MEN4, Carney complex, McCune–Albright syndrome or FIPA in the first-degree relatives of any of the patients. The family members of the patients were not offered to be studied unless an index case was identified as having an AIP gene mutation. The patients’ demographic data and treatments used were retrospectively recorded: mean age was 51 ± 18 years; 22 were males; 19 out of 50 were diagnosed before the age of 40 years. There were no gigantism cases in this series.

Controls

DNA samples from 60 individuals previously participating in a population-based health study in which lack of consanguinity and healthy condition were assessed were anonymised and served as controls.

Therapeutic interventions

All patients studied had failed to respond to conventional therapy including surgery and medical treatment. Somatostatin analogues were used in all cases, either lanreotide or octreotide, before and after surgical treatment, due to persistence of active disease after intervention, with a mean duration of this treatment of 56 ± 44 months. Unresponsiveness to somatostatin analogues was defined after 6 months of treatment, with the maximum usual dose of either compound (40 mg octreotide or 120 mg lanreotide autogel). Insulin-like growth factor 1 (IGF1) values were >2 SDS according to age and sex in all cases. Thirty-one (62% of the cases) received radiotherapy as adjuvant treatment. Pegvisomant (PEG) treatment was used to control residual GH hypersecretion in 90% of cases and patients were maintained with it for 37 ± 22 months. PEG monotherapy was initiated by daily s.c. injection of 10 mg without using loading and dose was titrated in 5 mg/day increments or decrements to maintain the IGF1 concentration below 2 SDS of IGF1 of each local laboratory. All but four patients received PEG daily; in those four patients, combined treatment with long-acting somatostatin analogues was used.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes by standard procedures QIAmp DNA (Qiagen). AIP gene (HGNC:358; ENSG00000110711) analysis was performed using standardised PCR protocol according to Vierimma et al. (5) in which amplifications of coding regions of exons 1, 2, 3, 4, 5 and 6 were performed. Thereafter, PCR products were sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and purification with Millipore system (96-well plates Multiscreen PCRu96 and Montage seq96), followed by analysis with ABI Prism Genetic Analyzer 3130.xl (Applied Biosystems). Possible extensive deletions/duplications were studied using multiplex ligation-dependent probe amplification (MLPA; SALSA MLPA KIT P244 AIP-MEN1, MRC, Amsterdam, The Netherlands). AIP sequence variants were compared with human single nucleotide polymorphism databases (dbSNP: http://www.ncbi.nlm.nih.gov/snp).

In order to detect potential splicing effects of each intronic variation or exonic silent or non-synonymous variation on AIP protein, we performed in silico analysis using ESEfinder Tool (http://rulai.cshl.edu/cgi-bin/tools/ESF3/esefinder.cgi) searching for differences in SRPs or snRNAs according to the nucleotide change.
Furthermore, in order to evaluate the effects of potential missense mutations, we analysed them using polyphen (http://genetics.bwh.harvard.edu/pph/) and MutPred (http://mutpred.mutdb.org/).

**Results**

In the analysis of the genetic material obtained from the 50 patients with a somatotropinoma with poor response to somatostatin analogues, MLPA showed no alterations in the electropherogram, indicating that no large AIP gene deletions/duplications were present in this group of patients. Sequence changes of potential different significance were found in four patients (8%). Two previously described mutations were found: c.26G>A (p.Arg9Gln) in exon 1 in one case and c.807C>T (p.Phe269Phe) in exon 6 in the other case. Mutation c.26G>A (p.Arg9Gln) was found in a female diagnosed at age 21 years and bearing a macroadenoma showing a complete resistance to somatostatin analogues, but good response to PEG treatment; mutation c.807C>T (p.Phe269Phe) in exon 6 was found in a 67-year-old female with diagnosis of acromegaly performed at 50 years of age, which required surgery and radiotherapy and presented a partial resistance to somatostatin analogues with good control with PEG.

Two other variations of unknown significance (VUS) were found: c.787+24C>T in intron 5 in a 44-year-old female with a macroadenoma partially resistant to somatostatin analogues, which was successfully controlled with combined treatment of somatostatin analogues and PEG, and c.100-18C>T in intron 1 in a 35-year-old male with a macroadenoma tumour requiring surgery and radiotherapy, who presented an absolute resistance to somatostatin analogues and achieved hormonal control while on maximal doses of daily PEG. All the cases achieved normal IGFI under PEG treatment. No mutations or VUS were found in any of the 60 controls studied.

**Discussion**

AIP mutations may be associated with any sort of pituitary adenoma, either functioning or non-functioning, although the most frequent association reported is with adenomas of somatotropic lineage and with a much higher frequency in FIPA kindreds rather than in sporadic cases. In our study of 50 acromegaly patients without FIPA antecedents and with poor response to somatostatin analogues treatment, we found a non-negligible prevalence of 8% of sequence changes in the AIP gene. Some of those changes may be VUS, like c.787+24C>T in intron 5 and c.100-18C>T in intron 1. The c.787+24C>T variation, found in one case, has not been previously described. The in silico analysis showed that T variant removes the putative binding sites for SF2/ASF and SF2/ASF (IgM-BRCA1) SRPs, suggesting that this variation could possibly be damaging. No significant variations were found when snRNA analysis was performed. The second VUS (c.100-18C>T) has been previously reported as a rare polymorphism (rs117691341), but it is not clearly known whether it may have a pathologic effect.

Reported data in different series of acromegalic patients indicate that the prevalence of AIP mutations is usually no higher than 11% in selected cases and lower in unselected patients (10, 11). In sporadic acromegaly, AIP mutations seem to be associated with a young age at diagnosis and to a large tumour, as reported by different investigators and, in particular, in a recent European multicentre study dealing with paediatric cases, which described a prevalence of 20% (12). This latter prevalence has been claimed as a sufficient justification for recommending the screening in such young cases. In contrast, as far as we know, our study is the first one in which the specific criteria for active screening was the lack of response to pharmacological treatment of acromegaly, and specially to somatostatin analogues. In a large international collaborative study recently published, including 75 patients with acromegaly, 30% sporadic, Daly et al. (9) showed that AIP mutations were associated with a poorer response to somatostatin analogues when compared with those without AIP mutations. In addition, in this series, the few patients treated with PEG did not show a satisfactory response (three out of four), in contrast to our study in which all individuals with any kind of AIP sequence change were controlled under PEG treatment. The results of this study do not solve the question of whether unresponsiveness to somatostatin analogues may be directly related to AIP mutations, and this issue deserves further investigation. Although one of the main determinants of hormonal control during somatostatin analogues treatment in acromegaly is the ratio of sst5/sst2 receptors in the tumour tissue, other different biological and clinical factors may influence or determine unresponsiveness to this kind of treatment (13, 14, 15). With respect to this, AIP mutations may contribute, at least in part, to the poor response to this

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SSA, somatostatin analogues.
particular pharmacological treatment just because of its stimulating tumour growth effect, leading to the development of macroadenomas, and it is well known that large somatotropinomas have lower response rates to somatostatin analogues (16). In the most recent paper published in young acromegalic subjects bearing AIP mutations, the response rate to somatostatin analogues treatment was only 11.7% and tumour shrinkage was virtually not observed (12).

More detailed data of previous studies performed in acromegalic patients without any special selection criteria for analysing AIP mutations are shown in Table 1. Overall, when no special criteria are applied when deciding to study AIP mutations, in sporadic acromegaly cases, the prevalence found is no higher than 5% (Georgitsi et al. (8), Barlier (10), Cazabat et al. (11), Occhi (17) and lwata (18)).

In contrast to unselected cases, the large series of familial cases published by Daly et al. (7) found a much higher prevalence of AIP mutations of about 15%, mostly corresponding to somatotropinomas, and in those, mean age at diagnosis was 25±11 years and mean tumour diameter was 24 mm. A young age at diagnosis seems particularly suspicious of bearing AIP mutations, as reported by this latter group of investigators, when they assessed the prevalence of mutations in young unselected non-familial cases (12), which turned out to be 13.3% for somatotropinomas. In a very young or paediatric population (<18 years), the percentage was as high as 20.5%, and if the cut-off age was <30 years, 11.7% for any pituitary adenoma. Considering the published data, it seems highly recommendable to always screen for AIP mutations in pituitary adenomas in patients aged <18 years, and it may also be reasonable in subjects <30 years, and specifically in acromegaly if the response to conventional treatment is poor, as shown in our series. In this study, the majority of the patients were older than 40 years, indicating that sequence variants of AIP gene may also be found in a significant number of older cases, provided that the tumour shows aggressive behaviour. The relatively high age at diagnosis, as well as its sporadic presentation, suggests that the patients in our series were carrying mutations with reduced pathogenicity.

In conclusion, AIP gene involvement may be found with a relevant prevalence in non-familial cases of acromegaly, and in particular in somatotropinomas with aggressive tumour behaviour not adequately responding to somatostatin analogues, not just at young ages but also in adult patients.

Declaration of interest

M Puig-Domingo and M Marazuela have received research grants from Pfizer, M Marazuela, M Puig-Domingo, T Lucas, I Bernabeu, I Halperin and C Alvarez-Escola have given lectures in meetings organized by Novartis and Pfizer.

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Author contribution statement

J Oriola supervised the mutational analyses; M José Perales, Celsa Quinteiro and Lourdes Loidi performed the technical laboratory work. M Puig-Domingo and J Oriola wrote the manuscript. M Molina, I Salinas, I Bernabeu and M Marazuela contributed to the manuscript elaboration. The rest of authors collected the samples and the clinical information.

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