Clinic study

Genetic analysis in young patients with sporadic pituitary macroadenomas: besides AIP don't forget MEN1 genetic analysis

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Abstract

Context: Germline mutations in the aryl hydrocarbon receptor interacting protein gene (AIP) have been identified in young patients (age ≤ 30 years old) with sporadic pituitary macroadenomas. Otherwise, there are few data concerning the prevalence of multiple endocrine neoplasia type 1 (MEN1) mutations in such a population.

Objective: We assessed the prevalence of both AIP and MEN1 genetic abnormalities (mutations and large gene deletions) in young patients (age ≤ 30 years old) diagnosed with sporadic and isolated macroadenomas, without hypercalcemia and/or MEN1-associated lesions.

Design: The entire coding sequences of AIP and MEN1 were screened for mutations. In cases of negative sequencing, multiplex ligation-dependent probe amplification was performed for the detection of large genetic deletions.

Patients and settings: One hundred and seventy-four patients from endocrinology departments of 15 French University Hospital Centers were eligible for this study.

Results: Twenty-one out of 174 (12%) patients had AIP (n = 15, 8.6%) or MEN1 (n = 6, 3.4%) mutations. In pediatric patients (age ≤ 18 years old), AIP/MEN1 mutation frequency reached nearly 22% (n = 10/46). AIPmut and MEN1mut were identified in 8/79 (10.1%) and 1/79 (1.2%) somatotropinoma patients respectively; they each accounted for 4/74 (5.4%) prolactinoma (PRL) patients with mutations. Half of those patients (n = 3/6) with gigantism displayed mutations in AIP. Interestingly, 4/12 (33%) patients with non-secreting adenomas bore either AIP or MEN1 mutations, whereas none of the eight corticotroph adenomas or the single thyrotropinoma case had mutations. No large gene deletions were observed in sequencing-negative patients.

Conclusion: Mutations in MEN1 can be of significance in young patients with sporadic isolated pituitary macroadenomas, particularly PRL, and together with AIP, we suggest genetic analysis of MEN1 in such a population.
Introduction

Familial cases of pituitary adenomas (PA) represent up to 5% of all PA, with 2.7% related to multiple endocrine neoplasia type 1 (MEN1) (1) and nearly 2.5% related to the clinical entity familial isolated pituitary adenomas (FIPA) (2). Together the two syndromes comprise the most common causes of hereditary conditions predisposing to PA (3). In 2006, Vierimaa et al. (4) identified mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene in the familial setting of PA. In FIPA kindreds, AIP mutations occur in 15–20% of cases (5), whereas they occur at a very low frequency in sporadic cases, between 0 and 4% (6, 7, 8, 9). Because patients mutated for AIP (AIPmut) have typically early onset disease and larger PA compared with controls (10), Tichomirowa et al. (11) performed AIP screening in young patients with isolated sporadic macroadenomas and identified that nearly 12% of patients had germline AIP mutations.

Mutations in the tumor suppressor gene MEN1 predispose to multiple endocrine and non-endocrine diseases including PA (12) that occur in ~40% of MEN1 cases (13). Whereas hyperparathyroidism is frequently reported as the first manifestation of MEN1 syndrome, pituitary disease can be the first lesion diagnosed in about 15% of patients with mutations in MEN1 (MEN1mut) (13, 14). Nevertheless, unlike AIP, there is limited data concerning the prevalence of MEN1 mutations in the specific subset of young patients diagnosed with isolated macroadenomas without any other disease of the MEN1 spectrum.

Therefore, we conducted a prospective study of a large cohort of patients in France that had been diagnosed with sporadic macroadenomas between January 2007 and December 2011 to determine the prevalence of both AIP and MEN1 gene abnormalities (point mutations and large deletions). We enrolled only patients diagnosed before 30 years old.

Materials and methods

Subjects

This genetic screening was performed in 174 patients with sporadic pituitary macroadenoma (maximal diameter ≥10 mm on pituitary MRI), diagnosed before 30 years of age and without hypercalcemia (corrected for serum albumin). Patients were enrolled from endocrinology departments of 15 French University Hospital Centers. All subjects provided informed written consent for the genetic screening. A subgroup of 59 patients had previously undergone AIP studies as part of an international collaborative study (11).

There were 79 (45.4%) subjects with somatotropinomas (49 males and 30 females, mean age at diagnosis 24.2±5.9 years), 74 (42.5%) with prolactinomas (PRL; 39 males and 35 females, mean age 20.3±5.2 years), 12 (6.8%) with clinically non-functioning PA (NFPAs, six males and six females, mean age 20.7±6 years), eight (4.6%) with corticotroph adenomas (two males and six females, age 22±5.4 years), and one female, aged 25 years, with a thyrotropinoma.

None of the subjects had a family history of MEN1 or FIPA. Family members of MEN1mut or AIPmut patients were contacted whenever possible and underwent genetic screening, followed by pituitary MRI and hormonal testing in case of positive genetic analysis.

Genomic analysis of AIP and MEN1

Genomic DNA from peripheral blood leukocytes was extracted and the coding exons and exon-intron boundaries of the AIP and MEN1 genes (NM_130799.2, NM_003977.2) were PCR amplified and screened by direct sequencing. Genomic DNA was also analyzed for large deletion in both genes by multiplex ligation-dependent probe (Salsa MLPA probe-mix P244-B1 AIP-MEN1, MRC-Holland, Amsterdam, The Netherlands). A 4.8 Mb region (from 11q13 to 11q13.3) was analyzed using probes localized on the MEN1 gene (exon 11, 10, 5 to 2), SNX15, FAM89B, RELA, SART1, and BRM51 genes; AIP gene (exons 1–6); and CCND1 gene. The potential effect of each missense or silent variation on AIP or menin protein was evaluated in silico using a battery of tools: Polyphen2 (http://genetics.bwh.harvard.edu), UMD-predictor (15), and Alamut 2.2.0 software (including SpliceSiteFinder, MaxEntScan, MNSPLICE, GeneSplicer, Human Splicing Finder, RESCUE-ESE).

Haplotype analysis

The AIP p.Gly117Alafs*39 mutation carriers were genotyped using 14 microsatellite markers surrounding both AIP and MEN1 genes; located at 64.3 and 67.0 Mb respectively. Markers were PCR amplified from genomic DNA, separated on an ABI 3730XL DNA sequencer, and analyzed with Peak Scanner v1.0 software (Applied Biosystems). Genetic markers’ primers sequences and amplification condition were reported elsewhere (16).

Statistical analysis

The Mann–Whitney U test was used for statistical analysis. P values below 0.05 were considered to denote statistical significance in this study. The mean age at diagnosis in each group of patients is referred to with s.d. (mean age ± s.d.).

Results

Genomic DNA AIP and MEN1 mutations among the study cohort

AIP and MEN1 genetic analysis in the study cohort identified 21 patients bearing mutations (21/174,
12%). Fifteen had AIP (8.6%) and six had MEN1 (3.4%) mutations (Table 1). No large genetic deletion was observed in any case using MLPA. In the cohort, the mean age at diagnosis was significantly lower in AIPmut patients compared with MEN1mut and non-mutated groups (18.7 ± 5 years (AIP) vs 22.2 ± 7.6 years (MEN1) and 22.7 ± 5.7 years (non-mutated group) respectively, P < 0.05).

In the pediatric population (i.e. age ≤ 18 years at diagnosis, n = 46), ten patients (21.7%, patients 3-6-7-9-10-13-14-15-16-19) bore mutations (seven in AIP and three in MEN1). The pediatric population included 30 PRL (65%), 11 somatotropinomas (24%), three NFP As (6.5%), and two ACTH-secreting adenomas (4.5%). There were 28 females (61%) and 18 males (39%), and the high proportion of females observed is due to the macroprolactinoma subgroup. The age at diagnosis of patients with AIP or MEN1 mutations was similar to their non-mutated pediatric counterparts (mean age 14.7 ± 2.8 years for AIPmut group, 15.3 ± 2.1 years for MEN1mut group, and 14.6 ± 3.6 years in non-mutated group, NS).

Overall, 11 different AIP variants were identified: seven of them led to a premature codon stop (Table 1), suggesting that they are deleterious. The variant p.Gly117Alafs*39 was found in five unrelated patients originating from two geographically close regions (two from Reunion, three from Comoros Islands, all of whom are of African origin). To address the issue of a possible founder effect, we genotyped 14 microsatellite markers surrounding the AIP gene. Although the lack of information on pedigrees and allele frequencies do not consent to draw final conclusions, our data are strongly suggestive for a common ancestor at least for four out five subjects sharing a genomic region on chromosome 11 ranging from 4.4 to 7.5 Mb (Table 2).

The four AIP remaining variants included two missense variants (p.Lys58Asn and p.Arg304Gln), previously reported as deleterious in the literature (11, 17), and two previously undescribed variants. The variant p.Leu294Pro is localized on exon 6, in the third tetratricopeptide repeat (TPR) domain, known as a key domain for protein–protein interactions and scored as being likely to affect AIP protein on in silico analyses. The deletion of three bases (c. 735_737 del) in exon 5 induces the loss of glutamine 246 in the second TPR domain (18), therefore supporting a strong pathological role of this mutant.

Genetic screening of family members of affected mutation carriers was possible in three different families (overall 11 subjects tested) and was positive in three subjects, the mother (aged 45) and the paternal grand father (aged 80) of patient 11 (p.Lys58Asn) and the mother (aged 51) of patient 12 (p.Arg304Gln). In all these carriers, pituitary MRI was normal.

Among the six MEN1 variants identified in our cohort, three of them led to premature stop codons, suggesting a deleterious effect (Table 1). The intronic mutation (c.655-6C>T) induces a deletion of exon 3 causing a frameshift and a premature stop codon 13 triplets further downstream (19). The two other variants included one missense mutation (p.Pro540Ser), already described but without demonstration of pathogenic effect in the original report (20), and one novel missense variant (p.Asp231His). In silico analysis showed a moderate to strong likelihood of a deleterious effect for these two variants (Table 1).

Genetic screening has been conducted in three family members (mother, father, and brother) of patient 16. The genetic analysis was positive in the asymptomatic father and allowed the diagnosis of asymptomatic primary hyperparathyroidism, hitherto unknown, with a normal pituitary MRI. The genetic screening of MEN1 has also been done in the mother of patient 15 and was negative.

Analysis by phenotype

Nine out of 79 (11.4%) somatotropinoma patients had AIP (six males and two females) or MEN1 mutations (one male, Table 1). Three out of six patients with gigantism were identified with AIP mutations (two males and one female) (Table 1). Patient 7, with the novel AIP missense variant p.Leu294Pro, was diagnosed at 10 years of age and was resistant to somatostatin analog therapy.

Eight out of 74 (10.8%) PRL patients bore AIP (three males and one female) or MEN1 (two males and two females) mutations (Table 1). The new missense MEN1 mutant (p.Asp231His) was identified in a 29-year-old male, who was affected by an aggressive PRL that was resistant to dopamine agonist therapy.

Only 12 patients (6.9%) from our whole cohort were affected by NFPAs. Four of them (33%) were identified as having either AIP or MEN1 mutations (Table 1). The deletion of a glutamine (p.Glu246del) in AIP protein was found in a young male, aged 20, who had a macroadenoa with partial immunoreactivity for GH (50%) but without any pituitary hormonal hypersecretion in vivo.

No mutation was identified in the eight patients diagnosed with corticotroph adenoma and in the female with a thyrotropinoma.

Discussion

Until 2006, mutations in the MEN1 gene were the main molecular abnormalities seen in cases of familial PA, particularly in association with other endocrine diseases. The implication of germline mutations in the AIP gene has since significantly extended the field of genetic analysis in apparent familial predisposition to PA (4).

While the data on AIP mutation status in the current study largely supports the emerging profile of the ideal
Table 1 Characteristics of pituitary adenomas in patients with AIP and MEN1 mutations.

<table>
<thead>
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<th>Region</th>
<th>Chr position</th>
<th>AIP mut/variant (NM_003977.2)</th>
<th>MEN1 mut/variant (NM_130799.2)</th>
<th>Polyphen scorea</th>
<th>UMD scoreb</th>
<th>Pathogenicityc</th>
<th>Reference for mutationd</th>
<th>Gender</th>
<th>Age at diagnosis (years)</th>
<th>Gigantism</th>
<th>GH (mUI/l)/IGF1 (ng/ml)</th>
<th>Maximal tumor diameter (mm)</th>
<th>CSI, SSE</th>
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<td>c.655-6 C &gt; T</td>
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<td>30</td>
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Underlined patients belong to pediatric population. CSI, cavernous sinus invasion; SSE, suprasellar extension; NA, not available; Y, yes/N, no.

aPolyphen score ranges from 0 to 1.
bUMD score ranges from 0 to 100.
cPathogenicity is estimated based on in silico predictions, clinical data, and data from the literature. Five different categories classified allelic variants: pathogenic (yes), likely pathogenic (likely), of unknown significance, unlikely pathogenic, and not pathogenic (32).
dThe number refers to the original publication of the first description of the mutation.
ePatients previously described in the study by Tichomirowa et al. (11), with updated clinical data.
fDespite the low score of in silico predictions, this variant was classified as pathogenic considering the numerous publications (8, 11, 17, 25, 29, 33).

fTheinserted sequence of 26 nucleotides was GAAAGGGGGTGTCAGCGCCGTAGAC.

The number refers to the original publication of the first description of the mutation.

fThis variant has been found in a patient of African origin. In this population, the allelic frequency may range from 0.7 to 1.4% according to EVS (Exome Variant Server from Exome Sequencing Project) and dbSNP (NCBI’s Variant database). On the other side, this variant induces a deletion of exon 3 causing a frameshift and a premature stop codon 13 triplets further downstream according to Roijers et al. (19). Moreover, we have found c.655-6C>T in other unrelated index cases with MEN1 lesion (personal not published data). Consequently, we have classified this variant as ‘likely pathogenic’.

hThis variant has been found in a patient of African origin. In this population, the allelic frequency may range from 0.7 to 1.4% according to EVS (Exome Variant Server from Exome Sequencing Project) and dbSNP (NCBI’s Variant database).
screening candidates, the major new finding relates to MEN1 screening in young sporadic pituitary macroadenoma patients. There are few data assessing the prevalence of MEN1 mutations in the specific case of isolated and sporadic macroadenoma. Stratakis et al. (21) reported one MEN1 mutation in a 11-year-old male with macroprolactinoma among six patients with isolated GH- or PRL-secreting adenoma. In our study, MEN1 mutations were identified in 3.4% of cases and this frequency reaches 6.5% in the pediatric population \((n = 3/46)\). No large genomic deletion was identified among MEN1-sequencing-negative patients; such findings have been reported previously in 1% of MEN1 families (22). In contrast to AIP mutation carriers, MEN1 mutations in our series had the same age at diagnosis as the population without mutation, in agreement with the data from Verges et al. (13) on micro- and macroadenomas. In invasive adenoma group from the study by Trouillas et al. (23), MEN1 mutation carriers tended to be younger than their non-mutated counterparts. In the oncogenic field, and particularly for MEN1 and hyperparathyroidism, it is well known that tumors arise earlier in mutated patients than in their non-mutated counterparts. In MEN1 pituitary tumors, data are missing to claim it. Consistent with the literature (13), PRL are over-represented in our MEN1 mutation patients \((4/6, 66\% \text{ in our cohort})\). Consequently, our results suggest that MEN1 mutations should be strongly considered in the young sporadic pituitary macroprolactinoma population, as we found an equal frequency of AIP and MEN1 mutations in our cohort (Fig. 1).

In our cohort, only one MEN1 mutation patient (patient 15) has developed primary hyperparathyroidism to date. Moreover, this hyperparathyroidism was completely asymptomatic and diagnosed about 10 years after the first symptoms of pituitary tumor. In addition, one family member of MEN1 proband (patient 16) was subsequently diagnosed with occult hyperparathyroidism thanks to positive genetic screening. Therefore, the genetic screening performed in family members of MEN1 mutation patients could result in a contributive diagnosis and therapeutic intervention. This is in line with the high penetrance of the MEN1 syndrome estimated near 90% at the age of 50 years (12).

By definition, FIPA families are free of mutations in the MEN1 gene. While our results show that both AIP and MEN1 contributed to sporadic macroadenomas in young patients, we have not found that MEN1 mutations can lead to isolated PA in a familial setting. We still have not identified any MEN1 mutations in the unrelated patients from our FIPA cohort (personal communication). This might be the consequence of the high frequency and penetrance of hyperparathyroidism (24). Hyperparathyroidism should be actively searched in cases of family members with isolated PA to focus genetic analysis either on MEN1 or on AIP. Subsequently, MEN1 genetic screening may now be not necessary a priori before designating kindred with multiple related members with isolated PA as having FIPA (Fig. 1).

The prevalence of AIP mutations in patients with sporadic pituitary adenoma without considering the age at diagnosis is low, between 0–4\% \((6, 9, 25, 26)\), including in those with sporadic macroadenoma (27). Strikingly, this frequency reaches 12\% in young patients \((\leq 30\text{ years})\) with sporadic macroadenoma (11), strongly supporting the idea that young patients should be the primary targets of genetic screening. Accordingly, our study identifies an overall mutation prevalence of AIP of 8.6\% in a similarly selected population. Two studies on patients diagnosed before 40 years have previously reported an AIP mutation frequency.
near 7% (7, 8). This prevalence reaches 14.3% in patients with GH-secreting tumors diagnosed before 25 years old (28). All these data lead us to propose to limit the genetic screening to subjects younger than 30 years old (Fig. 1).

In our pediatric population, AIP mutation prevalence reaches nearly 15% and as high as 40% (n = 4/10) in cases of acromegaly. In the literature, the frequency of AIP mutations in such populations varied from 2% (1/36) (28) to 20–23% (11, 25). Not only the age at diagnosis but also the size of the tumor is an important criterion that modifies the frequency of AIP mutation in isolated sporadic PA. The tumors from AIPmut patients in a FIPA cohort were overwhelmingly macroadenomas (10). However, among 74 children with Cushing’s disease, one AIP mutation was found in a patient diagnosed at the age of 6 years with a 3 x 4 mm ACTH-secreting adenoma (21). Subsequently, data are missing in the pediatric population to support the exclusion of children with microadenoma from the AIP genetic screening that we suggest in Fig. 1.

In our study, the majority of AIP mutations were found in somatotropinoma patients (8/15 mutated patients) as previously known (10). No mutation was found in corticotroph adenoma patients and in the single thyrotropinoma patient. However, AIP mutations have already been reported in several young patients with isolated sporadic corticotropinomas (7, 21), justifying AIP screening in such populations (Fig. 1). There is a variable penetrance of FIPA in AIPmut families with around 20–30% in the largest cohort studied (5, 29). Indeed, in our study, the positive genetic screening in the three family members was not accompanied by the identification of any PA on MRI. In five AIPmut carriers from the study of Cazabat et al. (7), no adenoma was identified by MRI, and of the AIPmut carriers (n = 2/21) from the study conducted by Tichomirowa et al. (11), two family members (not included in the current study) were diagnosed with a microadenoma and without associated hormonal oversecretion. Whether there are specific AIP mutants associated with higher penetrance of the disease.

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remains unknown. On the one hand, these observations are in agreement with the low penetrance of the FIPA syndrome; on the other hand, it asks the question of substantial benefits of AIP screening for asymptomatic family members. A long prospective study is clearly required to assess the impact of AIP screening in family members of AIPmut patients in order to clarify the natural history of asymptomatic AIPmut carriers.

In mutation-negative patients, we did not identify any large genomic deletion of AIP by MLPA in agreement with three other previous studies (6, 7, 11). This molecular abnormality could account for 9.5% of AIP-negative FIPA kindreds (30), and among 64 unrelated patients from a FIPA cohort of our laboratory, we identified one family member of homogenous FIPA with acromegaly, with large genomic deletion of AIP in exon 1 (A Barlier, personal communication). Considering the cost and the difficulties of this analysis, MLPA analysis should be considered primarily in cases of FIPA that are first shown to be negative on AIP sequencing.

Finally, our study identified an overall mutation prevalence of 12% for AIP and MEN1. Surprisingly, this frequency reaches 33% for patients diagnosed with NFPA (n=4/12). However, among the four patients, one of them (AIP mutated) was a silent somatotroph macroadenoma, whereas the remaining three others were non-reactive on immunostaining experiments. Excluding the silent somatotroph case, the mutation frequency of AIP becomes 20% (2/10), still higher than that observed in the study conducted by Tichomirowa et al. (6.5%, (11)). NFPA are very rare tumors in the population aged under 30 years old. Therefore, further studies are needed to clarify the mutation prevalence in young NFPA patients.

Although the MEN1 mutation prevalence was only to 3.4% in our series, taking into account the high penetrance of MEN1 syndrome, together with the possibility of up to 10% of de novo mutations (31) and the strong impact of MEN1 mutations in terms of genetic counseling and therapeutic management, we suggest to include not only AIP but also MEN1 genetic analysis in young patients with sporadic PA (Fig. 1). Nevertheless, in the current guidelines for MEN1 management, the genetic analysis of MEN1 is not specifically recommended in this kind of population (24). But until now, there were no data on the prevalence of MEN1 mutations in young patients with sporadic and isolated PA, particularly macroprolactinomas. Further investigations are required before including MEN1 genetic screening in clinical practice in such a population. Even if the phenotypes induced by a point mutation or a large deletion of the gene are not different (22, 30), the latter group might be associated with increased penetrance (30). Therefore, according to our results and those of the literature (7, 11), seeking large AIP and/or MEN1 deletions seems unjustified as a routine measure in cases of isolated sporadic PA even in young patients (Fig. 1).

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.

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