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AIP mutations in young patients with acromegaly and the Tampico Giant: the Mexican experience

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Abstract Although aryl hydrocarbon receptor-interacting protein (*AIP*) mutations are rare in sporadic acromegaly, their prevalence among young patients is nonnegligible. The objectives of this study were to evaluate the frequency of *AIP* mutations in a cohort of Mexican patients with acromegaly with disease onset before the age of 30 and to search for molecular abnormalities in the *AIP* gene in teeth obtained from the "Tampico Giant". Peripheral blood DNA from 71 patients with acromegaly (51 females) with disease onset <30 years was analysed (median age of disease onset of 23 years) and correlated with clinical, biochemical and imaging characteristics. Sequencing was also carried out in DNA extracted from teeth of the Tampico Giant. Five patients (7 %) harboured heterozygous, germline mutations of the *AIP* gene. In two of them (a 9-year-old girl with gigantism and a young man with symptoms of GH excess since age 14) the c.910C>T (p.Arg304Ter), well-known truncating mutation was identified; in one of these two cases and her identical twin sister, the mutation proved to be a de novo event, since neither of their parents were found to be carriers. In the remaining three patients, new mutations were identified: a frameshift mutation (c.976_977insC, p.Gly326AfsTer), an in-frame deletion (c.872_877del, p.Val291_Leu292del) and a non-sense mutation (c.868A > T, p.Lys290Ter), which are predicted to be pathogenic based on in silico analysis. Patients with *AIP* mutations tended to have an earlier onset of acromegaly and harboured larger and more invasive tumours. A previously described genetic variant of unknown significance (c.869C > T, p.Ala299Val) was identified in DNA from the Tampico Giant. The prevalence of *AIP* mutations in young Mexican patients with acromegaly is similar to that of European cohorts. Our results support the need for genetic evaluation of patients with early onset acromegaly.

Keywords Acromegaly · Gigantism · *AIP* · FIPA

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Introduction

Acromegaly is a systemic disorder caused in over 98 % of cases by a GH-secreting pituitary adenoma [1]. Although the majority of pituitary adenomas occur sporadically, a number of genetic conditions, most of them inheritable, are associated with the development of such lesions [2]. These

genetic conditions include type 1 multiple endocrine neoplasia (MEN1) [3], the Carney complex [3] and the McCune-Albright syndrome [4], as well as emerging syndromes such as familial isolated pituitary adenoma (FIPA) [5], the recently characterized X-linked acro gigantism [6], the syndrome of familial pheochromocytoma/paraganglioma/pituitary adenoma [7] and type 4 multiple endocrine neoplasia (MEN4) [8]. Most, if not all of these conditions, result from molecular abnormalities that either activate oncogenes or inactivate tumour suppressor genes and usually affect key signalling pathways involved in the control of cellular proliferation [2, 9].

Familial isolated pituitary adenoma is a condition diagnosed when more than one member of a family harbours a pituitary tumour, having excluded the other genetic syndromes mentioned above [5]. In approximately 17–20 % of patients from FIPA families, germ line mutations in the aryl hydrocarbon receptor-interacting protein (*AIP*) gene can be identified, showing an autosomal dominant inheritance pattern with incomplete penetrance [5, 10–12]. Such genetic abnormalities include deletions, insertions, missense, nonsense, and splice-site mutations that usually result in a truncated or missing protein; thus, *AIP* appears to function as a tumour suppressor gene [5, 9, 12–14].

Although *AIP* mutations are rare among patients with sporadic acromegaly, the prevalence among subjects diagnosed before the age of 30 is nonnegligible [11, 12, 15–17]. In the present study, we explored the presence of *AIP* germline mutations in a large population of Mexican patients with apparently sporadic acromegaly with disease onset before the age of 30. In addition, we looked for such mutations in teeth from José Calderón Torres, a Mexican giant who was one of the tallest human beings alive in the 1970s [18].

Patients and methods

Cohort of young patients with sporadic acromegaly

Patients were recruited from the Acromegaly Clinic of Hospital de Especialidades, Centro Médico Nacional Siglo XXI, and from the Endocrinology Section of the Instituto Nacional de Neurología y Neurocirugía, both in Mexico City. The evaluation took place from January 2013 to August 2015. The majority of the patients of this cohort have been included in a recent report by the International FIPA Consortium, including the five patients who were found to have *AIP* gene mutations [11]. Our local scientific and ethics committees approved the study and all subjects signed the corresponding informed consent.

The diagnosis of acromegaly was established based on the presence of the classical symptoms and signs of the disease as well as on a glucose-suppressed GH $>$ 1 ng/mL and an elevated age-adjusted IGF-1 level. The main inclusion criterion was an onset of symptoms before the age of 30 years. We defined gigantism when any of the following were present in a patient with a pituitary adenoma: (1) abnormally high growth velocity in children or teenagers with an elevated age-adjusted IGF-1 and a glucose-suppressed GH $>$ 1 ng/mL; (2) height $>$3 SDS above the mean for age and (3) height $>$2 SDS above the calculated mid-parental height, using country-specific growth charts [19].

A complete clinical history was obtained in all cases, focusing on age of onset and type of symptoms and the presence of acromegalic co-morbidities such as diabetes, hypertension, arthropathy, sleep apnea and malignant neoplasms. None of the patients were known to have a personal or familial history of MEN1 or 4, Carney complex or McCune-Albright syndrome. Information regarding hormonal profile, magnetic resonance imaging (MRI) results, therapeutic interventions, treatment outcome as well as histopathological diagnosis was retrieved from the patients' charts. Tumour volume was calculated according to the modified de Chiro and Nelson formula [20]. Acromegaly was considered cured or in remission when both the postoperative glucose-suppressed GH was $<$0.4 ng/mL and the IGF-1 concentrations were $<$1.2 $\times$ ULN (upper limit of normal). For those subjects on pharmacological treatment, biochemical control was defined as a fasting GH $<$ 2.5 ng/mL and an IGF-1 level $<$1.2 $\times$ ULN.

Hormonal assays

GH was determined using the Diasorin-Liaison assay (Salugia, Italy), which has a detection limit of 0.009 ng/mL and intra-and inter-assay CVs of 2.5 and 5.8 %, respectively; the IRP used in the calibration of this GH assay was WHO second 95/574.

IGF-1 was separated from its binding proteins by means of an acid–ethanol extraction step, prior to immunoassay. IGF-1 was measured by means of the Diasorin-Liaison chemiluminescent assay (Salugia, Italy); the IRP used in the calibration of this IGF-1 assay is WHO second 02/254. We established our own normative IGF-1 data analysing serum samples from 340 healthy individuals and thus calculated the real intra- and inter-assay CVs as 3 and 4 %, respectively. The remaining hormonal measurements were carried out using different commercially available assays.

Genetic analysis

Young patients with sporadic acromegaly

Genomic DNA was isolated from peripheral blood mononuclear cells using the QIAamp Blood Mini Kit

(QIAGEN Gmbh, Manheim, Germany). Sanger sequencing and dosage analysis of the *AIP* gene were performed at the Molecular Genetics Laboratory, Royal Devon Exeter, NHS Foundation Trust, as previously described [12, 21]. Briefly, the six coding exons and the corresponding exon–intron junctions of the *AIP* gene were real-time PCR amplified using specific forward and reverse primers. Each amplicon was tagged with M13 tails to allow subsequent sequencing. Unidirectional sequencing was carried out on an ABI 3730 Sequencer (Applied Biosystems Foster City, CA, USA). The sequences were compared with the published template (accession number NM_003977.2) using the Mutation Surveyor software, version 3.95 (Soft Genetics, State College, PA, USA). If no mutations were detected by sequencing multiplex ligation-dependent probe amplification (MLPA), dosage analysis was carried out, looking for partial or whole-gene deletions using the P244-B1 MLPA Kit (MRC-Holland, Amsterdam, The Netherlands). The Alamut Mutation Interpretation software, version 2.1 (Interactive Biosoftware, Rouen, France) was used to analyse the pathogenicity of the mutations. In silico analysis was carried out using the Exome Variant Server and the 1000 Genome databases (http://evs.gs.washington.edu/EVS/ and http://.www.1000genomes.org/).

Powerplex 16 (Sigma) analysis was performed on patient two and her family members (unaffected twin sister and both of her parents). Microsatellite analysis using a panel of chromosome 11 markers located around the *AIP* gene was performed on the genomic DNA samples from patients one and two, and the results were compared to previous data [22, 23]. Genomic DNA obtained from the pituitary adenoma of the giant twin was also processed for sequencing of the *AIP* gene to identify loss of heterozygosity.

Samples from the Tampico Giant

Two teeth samples were processed at the Institute of Anthropology and Palaeogenetics of the University of Mainz, Germany. DNA was extracted by means of a phenol–chloroform-based method, as previously described [24]. Real-time PCR was carried out using specific primers. PCR products were sequenced forward and reverse, and the sequences were aligned against the reference *AIP* sequence (accession number NC_018922.3) and analysed using the Lasergene SeqMan Pro software (DNASTAR inc. Madison, WN, USA).

Statistical analysis

Quantitative variables are presented either as mean ± SD or medians with interquartile ranges (IQR), depending on data distribution, which, in turn, was determined by means of the Shapiro-Wilks test. Proportions and frequencies were used for categorical variables. Differences in categorical variables among the groups were analysed using the X^2 or exact Fisher test. Quantitative variables were analysed using Student *t*, Mann–Whitney U, or Wilcoxon tests. For the comparison of continuous variables, we used the Wilcoxon rank-sum test and Kruskal–Wallis test to evaluate differences between groups. Statistical significance was considered to exist when the value of p was <0.05. Statistical software consisted of Stata version 11.2 (StataCorp, College Station, TX) and SPSS version 17 (SPSS inc).

Results

Clinical, biochemical and imaging characteristics of the general cohort

The selected cohort consisted of 71 patients of whom 51 (71.8 %) were women. The median age of onset of symptoms was 23 years (IQR 18–26). Four met the criteria for gigantism, and 18 (25.3 %) developed symptoms before the age of 18 years. The median adult height for women was 1.65 m (IQR 1.58–1.69) and 1.79 m (IQR 1.72–1.89) for men. Median follow-up time was 41 months (IQR 9–85). Besides acral enlargement, the most frequent symptoms were headaches (66.7 %), hyperhidrosis (45.7 %), fatigue (41.4 %), snoring (41.4 %) and arthralgiae (35.7 %). Erectile dysfunction was found in 27.3 % of men and menstrual abnormalities in 60.4 % of women. Diabetes and hypertension were present in 20 and 21 % of the patients, respectively.

Median basal and glucose-suppressed GH levels upon diagnosis of acromegaly were 26.4 ng/mL (IQR 8.68–51.2) and 22 ng/mL (IQR 7.2–40), respectively; median IGF-1 concentrations were 2.05 × ULN (IQR 1.78–3.0). Hyperprolactinaemia (range 49–7000 ng/mL) was present in 17 patients (26.5 %). All patients with PRL levels >150 ng/mL had clinical and biochemical evidence of hypogonadotropic hypogonadism. Fifty-five patients (84 %) harboured macroadenomas; 20 % had invasive tumours with extension into the cavernous sinus and/or compression of the optic chiasm; and 10 % had giant tumours larger than 4 cm. Median tumour volume at diagnosis was 355 mm^3 (IQR 128–794).

Primary treatment consisted of pituitary surgery in 87.2 %, in almost all instances via a microscopic transsphenoidal approach, whereas octreotide LAR, alone or in combination with cabergoline, was used in 12.8 %. Secondary treatment with octreotide LAR and cabergoline was used in 52.9 and 38.6 % of the patients, respectively; in 18.6 %, conventional radiation therapy was used

adjunctively. Upon last follow-up, 48.7 and 25.7 % had achieved GH concentrations below 2.5 ng/mL and 1 ng/mL, respectively, whereas 37.1 % had reached IGF-1 levels below 1.2 × ULN. A combined biochemical goal of GH < 2.5 ng/mL and an IGF-1 <1.2 × ULN was reached by 30 %.

Characteristics of the patients with *AIP* mutations

Out of the 71 screened patients, five (7 %) harboured *AIP* mutations. The prevalence of *AIP* mutations among patients developing symptoms at age 18 or younger was 16.6 %. Table 1 shows the main characteristics of the patients with *AIP* mutations, and Table 2 summarizes the findings of genetic testing.

Patient 1, currently a 27-year-old male, developed headaches and accelerated growth at age 14. He was evaluated at age 18, when he had reached a final adult height of 1.85 m (midparental height 1.66 m) and found to have PRL levels above 7000 ng/mL, and basal and post-glucose GH concentrations of 7.6 and 8 ng/mL, respectively, along with an IGF-1 twice the ULN. MRI revealed a giant pituitary lesion, measuring 6 cm in its largest diameter, compressing the optic chiasm and invading the right cavernous sinus. During a transsphenoidal attempt for surgical removal of the mass, a fibrotic lesion that bled easily was found; only a small intrasellar portion of the tumour was resected. Pathology reported a pituitary adenoma that immunostained for both GH and PRL, with a Ki-67 proliferative index of 3 %. Surgery was complicated by permanent diabetes insipidus and the eventual development of panhypopituitarism. He remained biochemically active and was started on octreotide LAR and cabergoline, normalizing both his GH and IGF-1 levels and reducing his PRL concentrations to 5 ng/mL after 6 months of treatment; at this point, the MRI showed 95 % shrinkage of the tumour, with evidence of a complete empty sella and herniation of the optic chiasm. Over the subsequent 3 years, the dosages of the somatostatin analogue (SSA) and the dopamine agonist (DA) were progressively reduced and he was able to discontinue treatment by the fourth year. Shortly after, he developed extreme fatigue and depression and an insulin-induced hypoglycaemia test revealed a totally absent GH response along with very low IGF-1 levels; recombinant GH was added to his hormone replacement treatment with good clinical response. Genetic testing of this patient revealed that he is heterozygous for the well-known c.910C>T nonsense, truncating mutation of the *AIP* gene, that results in a premature stop codon and the consequent loss of 26 amino acids at the C-terminal seventh α-helix of the molecule, with impaired interactions with client proteins [25]. No *AIP* mutations were found in DNA available from his mother and two half siblings. His father, a normal-height (1.67 m), healthy 58-year-old male, was found to be heterozygous for the same c.910C>T mutation.

Patient 2 is a 9-year-old identical twin girl who developed accelerated growth and acanthosis nigricans at age 6. Upon diagnosis at age 8, she was already 1.59 m tall (midparental height 1.55 m). Hormonal evaluation revealed a basal and glucose-suppressed GH of 264 and 229 ng/mL, respectively, and an IGF-1 concentration of 1448 ng/mL (6 × ULN). MRI of the sellar region showed a 4-cm pituitary adenoma with suprasellar and right parasellar extension. She underwent transsphenoidal surgery with resection of the intra- and suprasellar

Table 1 Characteristics of the five patients with *AIP* mutations

	Case 1	Case 2	Case 3	Case 4	Case 5
Gender	Male	Female	Female	Male	Female
Onset of symptoms	14	6	15	20	21
Age at Dx	18	9	17	21	26
Final adult height (metres)	1.85	–	1.72	1.64	1.60
GH at Dx (ng/mL)	7.6	269	8.2	51.3	39
IGF-1 at Dx (x ULN)	2	5	2	1.8	1.5
PRL at Dx (ng/mL)	>7000	2	28	77	23
Tumour size (mm)[a]	48 × 23 × 45	45 × 40 × 35	15 × 20 × 17	24 × 25 × 22	25 × 31 × 15
Tumour IHC	GH+, PRL++	GH++	GH++	GH++	GH++
Treatment[b]	TSS ↓ SSA + CBG	TSS ↓ CBG	SSA + CBG ↓ TSS ↓ SSA + CBG	TSS b↓ SSA + CBG	TSS ↓ SSA
Current status	GH deficient	Active	Active but improved	Active but improved	Controlled

[a] Tumour diameters: cephalo-caudal × transverse × anterior–posterior

[b] Treatment: TSS transsphenoidal surgery, SSA somatostatin analogue, CBG cabergoline

Table 2 Summary of *AIP* mutations and their potential pathogenicity

Case	DNA	Protein	Mutation type/MAF	Consequence
1 and 2	c.910C>T	p.Arg304Ter	Nonsense/0.00001715	Known mutation, premature stop codon, loss of 26 amino acids at the C-terminus
3	c.976_977insC	p.Gly326AfsTer	Frameshift/not reported	New, changes in the last 4 amino acids crucial for protein activity
4	c.872_877del	p.Val291_Leu292del	In-frame deletion/not reported	New, may theoretically disrupt the packaging of the C-terminal 7th α-helix
5	c.868A>T	p.Lys290Ter	Nonsense/not reported	New, premature stop codon, loss of 40 amino acids at the C-terminus
Tampico Giant	c.896C>T	p.Ala299Val	Missense/0.0004275	Known variant, uncertain pathogenicity

MAF Minor allele frequency (ExAc Database)

components of the lesion but the right parasellar portion could not be removed. The removed lesion proved to be a sparsely granulated somatotropinoma. Three months post-operatively, her GH levels decreased to 54 ng/mL, and her IGF-1 to 1008 ng/mL and a follow-up MRI revealed the presence of a right cavernous sinus remnant; she was started on cabergoline, as no SSA are available at the Institution where she receives medical care. This patient is heterozygous for the c.910C>T (p.Arg304Ter) *AIP* mutation, which was found in DNA extracted from both, peripheral blood and from the surgically resected pituitary lesion (loss of heterozygosity) (Fig. 1). Her so far unaffected identical twin (post-glucose GH 0.075 ng/mL, normal IGF-1 and normal pituitary MRI) was also heterozygous for the same mutation. Both parents were available for genetic analysis and neither of them harboured this *AIP* mutation. Microsatellite analysis, amplifying 16 different short tandem repeats (STR), was therefore carried out in genomic DNA obtained from the patient, her unaffected twin sister and both her parents. The results of such analysis confirmed that the twins are identical and that both the mother and the father are in fact the biological parents. These data suggest that this is in fact a de novo mutation occurring before the cells of the twins separated. In addition, analysis of microsatellite markers located around the *AIP* gene established that the mutational events affecting the c.910 locus in patients 1 and 2 are independent from each other—i.e. they did not originate from the same founder. No common haplotypes were found to be shared upon comparison of the microsatellite analysis from patients 1 and 2 with that from previously studied p.Arg304Ter cases [22, 23] from Ireland, England, Romania, India and the USA (Italian ancestry) (Fig. 2).

Patients 3, 4 and 5 all developed acral growth, headaches and fatigue before age 21 (Table 1). They all had documented elevated GH levels and mild hyperprolactinemia as well as pituitary macroadenomas with variable degrees of suprasellar extension. Surgical treatment was unsuccessful in all of them; however, they are well controlled on pharmacological treatment. These three patients have previously unknown *AIP* gene variants, which based on in silico analysis are predicted to be of pathogenic significance. No family members were available for genetic testing; however, there was no history of pituitary adenomas in any of them. In patient 3, *AIP* gene sequencing revealed a heterozygous frameshift mutation (c.976_977insC) affecting the last five residues of the protein leading to the loss of the normal stop codon. Patient 4 has an in-frame deletion of valine at codon 291 and leucine at codon 292 (c.872_877del), occurring in the third tetratricopeptide repeat (TPR) domain of the *AIP* protein, which may theoretically disrupt the packaging of the C-terminal seventh α-helix. Patient 5 harboured a heterozygous, nonsense mutation (c.868A>T; p.Lys290Ter) that results in a premature stop codon and the loss of 40 amino acids at the C-terminus of the *AIP* molecule; there is little doubt that this is a disease-causing mutation.

The five patients with *AIP* mutations had an earlier onset of acromegalic symptoms, tended to be younger and had larger and more invasive tumours than those without *AIP* mutations, although these differences did not reach statistical significance. Other features such as the prevalence of hyperprolactinemia, the GH and IGF-1 levels at diagnosis, and the prevalence of acromegalic co-morbidities were not different between patients with and without *AIP* mutations. In the five patients with *AIP* mutations, surgery was unsuccessful in curing or controlling acromegaly. Adequate biochemical control with pharmacological therapy was achieved in two patients with mutations (Patients 1 and 5), while this was the case in less than one-third of the patients without mutations. Patients 3 and 4 are clinically improved with pharmacological therapy and their GH and IGF-1 levels have decreased; however, they have not

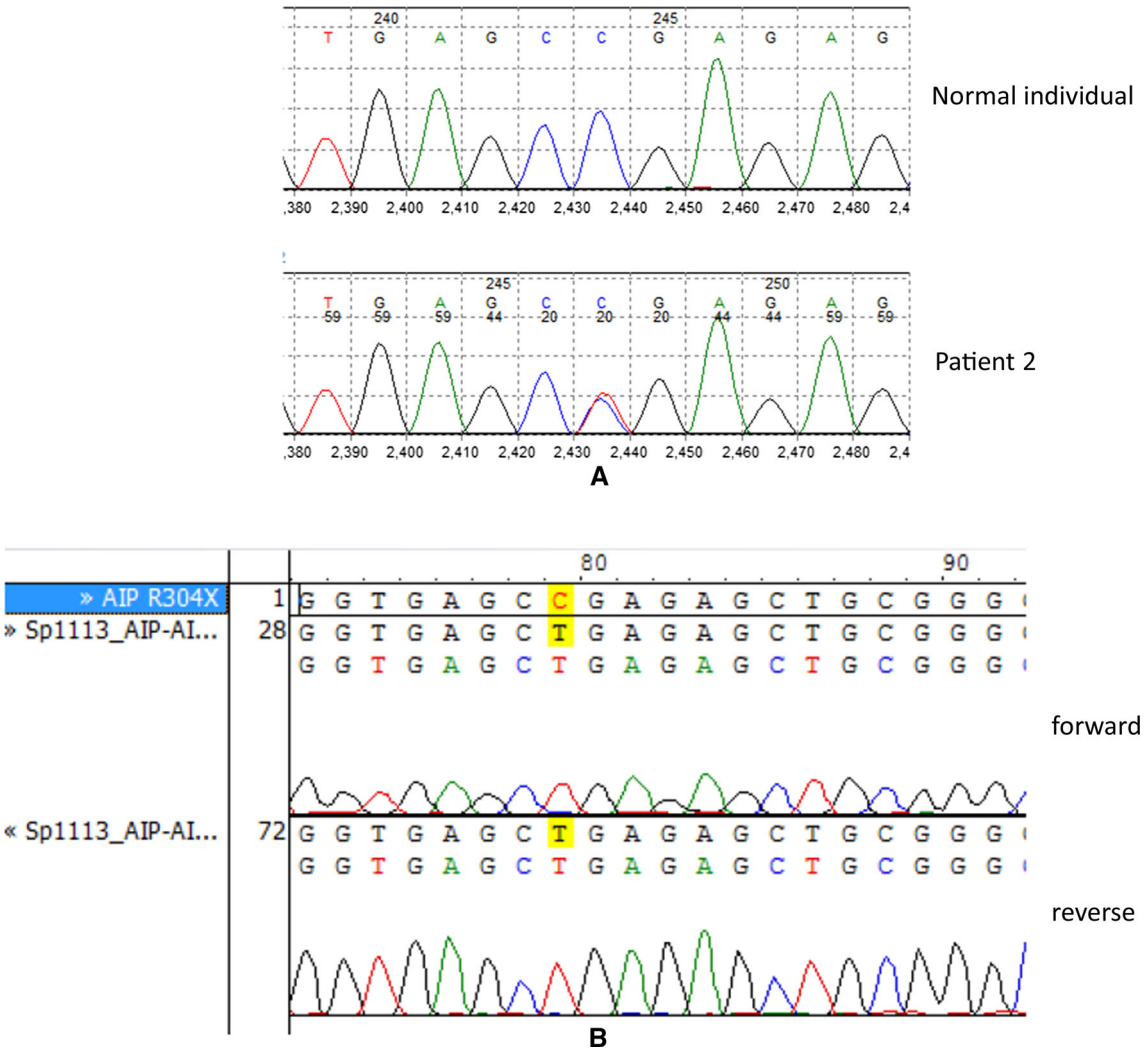

Fig. 1 **a** Patient 2 germline DNA showing a heterozygous mutation at locus 910 (c.910C>T). **b** Tumour DNA from this patient showing the loss of "C" at the 910 locus, which results in the presence of only the "T" allele, probably due to the loss of this region of the normal copy of chromosome 11 (loss of heterozygosity)

reached the usual hormonal targets indicative of biochemical control (GH < 2.5 ng/mL and IGF-1 < 1.2 × ULN).

The Tampico Giant

José Calderón Torres (JCT) was born in 1915 to normal-height parents in the city of Tampico, State of Tamaulipas, Mexico; he had a brother and a sister, also of normal height. He developed accelerated linear growth after he turned 13, and by age 18, he was known to be over 2 m tall. He worked as a stevedore in the port, carrying heavy loads on his back; his contemporaries remember him as being exceptionally strong but also of a depressive and melancholic mood. He quit his job at the port after 15 years, apparently because of abdominal hernias, severe joint pains and a poorly specified pleural condition. He died at his home on October 1973 and of natural but unknown causes at the age of 58 years. Shortly before his death, his recorded height was 2.35 m.

In October 2013, we were granted an official permission from the City of Tampico to exhume the remains of JCT (Fig. 3). A portion of the right femur was clearly identified. The jawbone was almost intact and was still holding several teeth; it was significantly enlarged. Several skull fragments, corresponding to the temporal and occipital bones were found, all showing a thickened diploe. The skull base was identified and it showed an enlarged and eroded pituitary fossa. Several teeth were removed for genetic analysis. The Tampico Giant harboured a previously described [25], heterozygous, c.896C>T missense

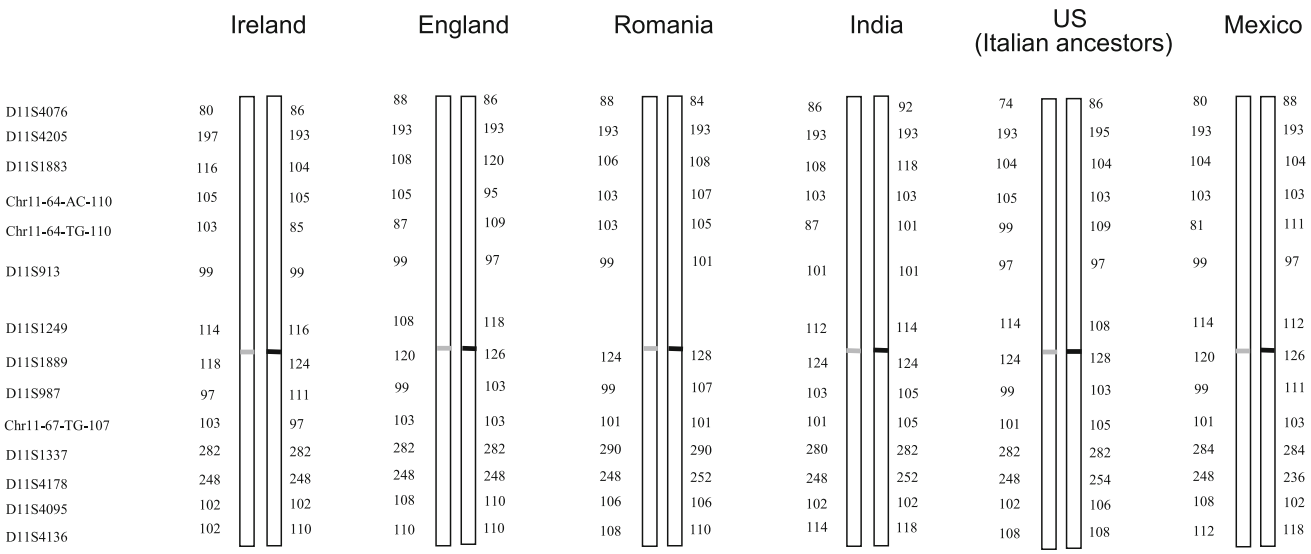

Fig. 2 Microsatellite analysis of patients with the p.Arg304Ter mutation from different countries showing that these patients do not share a common haplotype and therefore arise from an independent, recurring mutational events. Codes on the *left* represent the microsatellite markers and the numbers, the length of the microsatellites. The *grey bars* represents the mutant allele

Fig. 3 Exhumed remains of the Tampico Giant, showing fragments of the skull, including an enlarged maxillary holding several teeth and an enlarged and eroded pituitary fossa (*arrows*)

variant that results in an alanine for valine substitution at codon 299, located at the crucial third TPR domain of the *AIP* molecule.

Discussion

AIP mutations can be identified in 17–20 % of patients with acromegaly occurring in the setting of FIPA [10, 11]. Perhaps due to incomplete penetrance—rather than as a result of de novo mutations, as very few of these have been identified so far—they can also be found in some patients with apparently sporadic acromegaly with disease onset at a young age. The prevalence of these mutations in unselected acromegaly populations is 4 % [15–17], but it is considerably higher among young patients [15, 16, 26, 27]. In large cohorts, the reported prevalence among patients with disease onset before the age of 30 is 13 % [16, 27], although the figure varies in smaller cohorts: 10.1 % in French patients [15], 6 % in Turkish patients [27], 16 % in a study from Oxford [17], 2.3 % in a German study [26] and 17.24 % in Han Chinese patients [28]. In populations with disease onset before the age of 18, the reported prevalence ranges from 14 to 40 % [11, 29, 30]. Among patients with gigantism, the prevalence varies depending on the criteria used to diagnose the condition: 46.7 % when using the definition used in the present study [11] and 29 % when defining gigantism as an abnormally accelerated growth for age (>97th percentile) or as a final height >2 SD above the mean along with an elevated GH/IGF-1 and imaging evidence of a pituitary lesion [31]. Founder mutations identified in locations such as Finland [13], Ireland [22], Italy [32], Comoros Island [29] and England [33] could influence these results.

Besides a recently published Chinese report [28], the present study constitutes the first systematic evaluation of *AIP* mutations in a non-European population. The prevalence of *AIP* mutations in our genetically homogeneous Mexican Mestizo patients with young-onset sporadic acromegaly is similar to that reported in Caucasian populations [11, 15, 17, 26, 29, 30]. Five out of 71 (7 %) of our patients with acromegaly developing before the age of 30 harboured heterozygous, germline mutations of the *AIP* gene. In two of our patients, the well-known hot spot p.Arg304Ter mutation was found occurring independently,

whereas the remaining three, had novel molecular alterations not reported in any of the previously published reports or in the available data bases (ExAc, Exome Variant Server). Based on in silico analysis, these variants are predicted to yield a significantly altered *AIP* protein. Patient 3 has a frameshift mutation (c.976-977insC, p.Gly326AfsTer) which is predicted to result in changes in the final residues of the C-terminal seventh α-helix and loss of the normal stop codon, yet mRNA stability and protein expression have not been experimentally tested. The *AIP* mutation found in patient 4, a previously unknown in-frame deletion at c.872_877 (p.Val291_Leu291del), may theoretically disrupt the packaging of the third TPR repeat, and it affects a hydrophobic core necessary for the interaction with client proteins [24, 34]. Two missense variants affecting this residue (Val291) have been found in patients with pituitary tumours and in one of them, the amino acid substitution leads to an unstable protein due to enhanced proteosomal degradation [15, 35, 36]. Patient 5 has a new truncating mutation, which is also located in the third TPR domain and is expected to result in an unstable protein with impaired molecular interactions although we lack experimental proof to this.

Although they all had apparently sporadic acromegaly, of the 5 patients with *AIP* mutations, two had relatives that proved to be carriers of the same genetic defect albeit without any evidence of a pituitary lesion (the father of patient 1 and the twin sister of patient 2). Both of these patients had the p.Arg304Ter mutation, the most common and best-characterized molecular abnormality of the *AIP* gene [5, 11, 21]. Interestingly, neither of patient's 2 parents had any *AIP* mutations, whereas her unaffected identical twin proved to be a heterozygous carrier of the p.Arg304Ter mutation. Since paternity testing demonstrated that both were indeed the biological parents, our findings are consistent with a de novo genetic defect, occurring at a stage before the cells of the twins separated. Close surveillance of these asymptomatic carriers of the p.Arg304Ter mutation will facilitate the early diagnosis of pituitary adenomas in these family members, as previously shown [37].

Sporadic acromegaly occurring in the setting of *AIP* mutations not only is known to develop at an early age but is also characterized by a somewhat more aggressive phenotype [10, 11, 21]. Although this is indeed the case in patients who present with the typical acromegalic phenotype, those subjects whose mutations are detected through family screening usually have tumours that behave less aggressively [11]. Although our five patients with *AIP* mutations could not be cured by surgery, all except the young twin with the p.Arg304Ter mutation are currently well controlled with SSA therapy, and their general clinical, imaging and biochemical characteristics are not different from the rest of the subjects in our cohort without *AIP* gene abnormalities.

José Calderón Torres, better known as the Tampico Giant, was in the early 1970s the tallest man on earth with a documented height of 2.35 m. As a giant, he developed symptoms and signs of GH excess before puberty; however, he was never treated for his condition [18]. His clinical history would be typical of an *AIP* mutation positive patient, yet the only molecular abnormality found in this case was the infrequent p.Ala299Val variant which is located in the C-terminal seventh α-helix and may theoretically disrupt packaging with Leu292 [38, 37]. This variant, present in 0.043 % of the general population, was first described in a woman with sporadic acromegaly diagnosed at age 16 [38], and it has also been found in two late-middle age affected and unaffected members of an Irish p.Arg304Ter FIPA family, with the two variants located on different alleles [21]. In this Irish family 3 subjects carried it as a single variant; one of these 3 subjects had a microprolactinoma and is likely to be a phenocopy (i.e. not related to the *AIP* variant). Based on our studies evaluating genotype-phenotype correlations for the truncating *AIP* mutations [11], we have established that some of them have more severe biological consequences and some others have a milder impact. Since in animal models, the homozygous deletion of the *AIP* gene results in death during embryonic development [39, 40], we could speculate that composite heterozygosity, consisting of the inheritance of two different pathogenically significant mutations, would be incompatible with life. Therefore, although we cannot rule out that the p.Ala299Val variant might be somehow involved in the development of pituitary adenomas, the existence of asymptomatic composite heterozygotes [21] argues against a more than marginal or minor role.

We conclude that the prevalence of *AIP* mutations in young Mexican acromegaly patients is similar to that found in European cohorts. We have identified two patients with the hot spot p.Arg304Ter mutation, as well as three patients with previously unknown disease-causing mutations. The only *AIP* gene abnormality found in the Tampico Giant, one of the tallest men of the twentieth century, was a molecular variant of uncertain pathogenicity. Our results support performing genetic evaluations in all patients with acromegaly/gigantism developing at a young age.

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