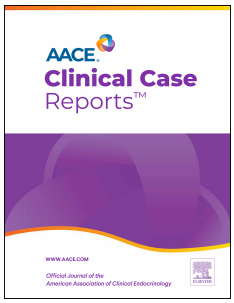

Complicated clinical course in incipient gigantism due to a treatment resistant, *AIP*-mutated pediatric somatotropinoma

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Complicated clinical course in incipient gigantism due to a treatment resistant, *AIP*-mutated pediatric somatotropinoma

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Complicated clinical course in incipient gigantism due to a treatment resistant, *AIP*-mutated pediatric somatotropinoma

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ABSTRACT

Background/objective: Our objective was to describe the clinical course and treatment challenges in a very young patient with a pituitary adenoma due to a novel *aryl hydrocarbon-receptor interacting protein* (*AIP*) gene mutation, highlighting limitations of somatostatin receptor immunohistochemistry to predict clinical responses to somatostatin analogs in acromegaly.

Case report: We report the case of a 7-year-old boy presenting with headache, visual field defects and accelerated growth following failure to thrive. Laboratory results showed high IGF-I (SDS +3.49) and prolactin levels (0.5 nmol/l), and magnetic resonance imaging identified a pituitary macroadenoma. Tumoral/hormonal control could not be achieved despite three neurosurgical procedures, each time with apparent total resection, or with lanreotide or pasireotide. IGF-I levels decreased with the GH-receptor antagonist pegvisomant. Loss of somatostatin receptor 5 was observed between the second and third tumor resection. *In vitro*, no effect on tumoral GH release by pasireotide (+/-cabergoline) was observed. Genetic analysis revealed a novel germline *AIP* mutation: p.Tyr202* (pathogenic; class 4).

Discussion: *In vitro* response of tumor tissue to somatostatin may better predict tumoral *in vivo* responses of somatostatin analogues than somatostatin receptor immunohistochemistry.

Conclusion:

We identified a novel pathological *AIP* mutation that was associated with incipient acrogigantism at an extremely young age and with a complicated course of disease. Growth acceleration can be masked due to failure to thrive. Tumoral growth hormone release *in vivo* may be predicted with *in vitro* exposure to somatostatin receptor

analogues, as it cannot be assumed that all *AIP* mutated somatotropinomas respond well to pasireotide.

Keywords: acrogigantism, acromegaly, AIP, pituitary adenoma, somatostatin receptor

Introduction

Pituitary adenomas (PAs) have a prevalence of 1 clinically-relevant case per 1000 in adults (1). Most PAs are sporadic, but 5% have a familial background (1, 2), the most common being familial isolated pituitary adenomas (FIPA). In FIPA, ~15-30% of cases are associated with pathological germline variants in the *aryl hydrocarbon receptor-interacting protein* (*AIP*) gene, a tumor suppressor gene located on chromosome 11q13 (2-6). Germline *AIP* mutations are particularly associated with growth hormone- (GH) or mixed GH-prolactin -secreting pituitary adenomas (3-6). Patients with *AIP* mutations are often male and have an aggressive clinical phenotype due to large invasive tumors, while *AIP* mutations are the most frequent genetic cause of pituitary gigantism (29%) (2, 5, 7, 8). In large case series, *AIP*-mutated pituitary adenomas usually present in adolescence or early adulthood (9). Early pediatric presentations of patients with *AIP* mutations and GH-secreting PAs are rarely described and responses to medical and surgical management in this challenging population are not well understood. Here, we report the challenges faced in the presentation, diagnosis and management of a very young child with a novel *AIP* mutation that led to a recurrent and resistant GH secreting macroadenoma.

Case description

A seven-year-old boy was hospitalized for evaluation of multiple progressive complaints over the previous two years, including frontal headache, fatigue, tics, leg pain, nocturnal sweating, constipation and poor food intake. He had a normal birthweight/height following an unremarkable pregnancy and his family history was normal. The growth curve showed normal growth until the age of three, followed by a marked decrease to about -2SDS at the age of six (Figure 1). Thereafter, his growth increased rapidly compared to Dutch national standards. His parents were of modest stature (father: 170.0 cm; mother 164.0 cm) by current Dutch median height standards (males: 182.9 cm); females 169.3 cm). A sellar tumor with an enlarged sella turcica was discovered (Figure 2). Laboratory analysis showed an IGF-I of 56.5 nmol/L(normal range (NR) 8.3-38.2; SDS +3.49) (10), prolactin of 0.5 nmol/l (NR <0.36), TSH of 1.07 mU/l (NR 0.6-5.6), fT4 20.2 of pmol/l (NR 13-26), afternoon cortisol of 270 nmol/l (NR<700), and undetectable LH and FSH (normal for prepubertal state). Over time, the growth rate accelerated further (Figure 1), in parallel with rising IGF-1 (71.2 nmol/l; +4.53 SDS), a random GH of 30.8 mcg/l (NR <4.0) and the prolactin increased to 0.75 nmol/l. The nadir GH value during an oral glucose tolerance test was 26.7 mcg/l. He complained of vomiting and loss of appetite. Treatment of the GH secreting macro-adenoma was initiated with lanreotide 120 mg four-weekly, which resulted in neither a biochemical response (IGF-I 76.6 nmol/l, GH 28.0 mcg/l), nor in inhibition of tumor growth after four doses. Lanreotide was switched to pasireotide LAR 60 mg four-weekly. One month after switching he developed new onset of bitemporal field defects and headaches that indicated symptomatic optic chiasmal compression, and he underwent transsphenoidal surgery for the first time. Two months post-operatively (three months after initiation of

pasireotide LAR) IGF-I (70.3 nmol/l) and GH (23.4 mcg/l) remained elevated. Pasireotide LAR showed no hormonal or tumoral effects and the GH receptor antagonist pegvisomant was started with a weekly dose of 40 mg. Although IGF-I levels dropped to 34.1 nmol/L (NR 10.9-47.3; 0.87 SDS); the local GH assay, which does not detect pegvisomant, continued to show an elevated random GH (48.6 mcg/l). After one month of pegvisomant, severe headaches returned, and bitemporal hemianopsia reoccurred due to increase in tumor volume (Figure 2). Pegvisomant was stopped and a second transsphenoidal resection followed (Figure 2). The histopathological report revealed a pituitary adenoma staining positive for GH and negative for prolactin (Figure 3). One month after the second transsphenoidal surgery, IGF-I level had declined to 29.3 (0.4 SDS), the GH level was 2.7 mcg/L and prolactin fell from 0.60 to 0.38 nmol/l (NR 0.1-0.5). Five months after surgery, the headaches returned and an MRI one month thereafter showed a small remnant lateral to the right internal carotid artery (Figure 2). IGF-I increased again to +2.9 SDS. A third transsphenoidal surgery was performed, leading to normalization of GH and IGF-I levels. Thirteen months after his last surgery, he received stereotactic radiotherapy (54 Gy) and 4 months after the radiotherapy his last IGF-1 was -0.8 SDS.

Due to the presentation with a macroadenoma at a young age, germline genetic testing for sequence variants and deletions in *AIP* and *MEN1* genes was performed. A novel heterozygotic truncating variant in the *AIP* gene was discovered (c.606C>G; p.Tyr202*; GnomAD database mean allele frequency (MAF: 0) which was accompanied by a second missense variant (c.695C>T: p.Pro232Leu; MAF: 0.00002502), both paternally inherited. Screening by MRI and hormone evaluation of his 37-year-old father was negative.

Histopathological analysis revealed a loss of SSTR5 expression between the second and third operations (Figure 3). *In vitro* characterization of the second surgical sample showed no statistically significant inhibition of GH secretion to incubation with pasireotide (10nM) or co-incubation with pasireotide plus cabergoline (both 10nM; Figure 4). Other compounds could not be tested due to the limited amount of available tissue. These interesting findings should be confirmed in a wider series of tumors from patients with AIP mutations and in appropriate wild-type acromegaly controls.

Given the lack of tumor size control with first and second generation SSAs and the unresectable remnant, which required radiotherapy at the age of ten, he will require intensive (endocrinological) follow-up, although no pituitary deficiencies have occurred to date. If needed, excessive GH can be controlled by pegvisomant, albeit with high vigilance for tumor regrowth.

Discussion

This case report describes a complicated somatotropinoma leading to accelerated longitudinal growth which was masked by an unexpected initial period of failure to thrive that was likely due to poor feeding because of nausea. The disease was diagnosed at the very young age of seven years and was found to be due to a previously undescribed *AIP* mutation that was inherited from his unaffected father. Decreased clinical SSA sensitivity may be related to the evolving tumor biology between surgeries, particularly the loss of tumoral somatostatin receptor 5 expression, while *in vitro* there was no tumoral response of GH to pasireotide and cabergoline.

Somatotropinomas are primarily treated with (transsphenoidal) neurosurgery, SSAs, dopamine agonists or GH receptor antagonists (11). Overall, in acromegaly long-acting SSAs can achieve biochemical normalization of GH and IGF-I in 50-60% and often lead to modest tumor shrinkage (11-14). Patients with *AIP* mutations have, however, significantly less tumor shrinkage and lower hormonal responses to first-generation SSAs (7).

SSAs act via somatostatin receptors (SSTRs1-5) (6). The first-generation SSAs octreotide and lanreotide have the highest affinity for SSTR2, and have a low affinity to SSTR3 and SSTR5 (6), while the second-generation SSA, pasireotide, has the highest affinity for SSTR5, followed by SSTR2, SSTR3 and SSTR1 (15). As we reported previously, pasireotide resistance is possibly more related to SSTR2 expression than to SSTR5 in the general acromegaly population (16, 17). SSA resistance may occur if the tumor is lacking SSTR2 (6). Daly et al. recently reported two *AIP*-mutated acromegaly patients with resistance to first-generation SSA, in which pasireotide treatment led to marked tumor shrinkage and persistent hormonal

control (18). In one case, very low to absent SSTR2 levels were seen and the efficacy of pasireotide must have been through other SSTRs like SSTR5 (18). Due to this, we initially expected that our patient would respond better to pasireotide despite resistance to first-generation SSAs, but this was not the case.

(18). The resistance to pasireotide probably relates in part to the low SSTR5 expression, since SSTR2 expression remained present. Nevertheless, the signaling via SSTR2 may be affected while leaving receptor expression unaffected. Possible factors in this phenomenon include ZAC1 and miR-34a, which both influence SSTR2 signaling (11, 19). In these cases, it may be preferable to test *in vitro* response of tumor tissue assessed by decreases in GH secretion (17). In the study of Coopmans *et al* (20) including 45 acromegaly patients who were previously treated with first-generation somatostatin analogues, combined with pegvisomant, SSTR2 immunoreactivity scores were found to be related to significant tumor shrinkage in patients treated with pasireotide, which was not the case for SSTR5. Muhammad *et al.* (16) found in the same cohort that IGF-1 lowering effects of pasireotide correlated with SSTR2 instead of SSTR5. However, the timing of the change in responsiveness and change in SSTR5 expression occurred simultaneously in the current case. In the study of Iacovazzo et al. including 39 patients with somatotropinomas, SSTR5 expression predicted responsiveness to pasireotide (21).

This case exemplifies the many challenges that can be faced in the recognition of acromegaly can be, especially when occurring at extremely young age. Acro-gigantism can occur with increased growth velocity in young patients, even without extremely elevated height compared to age/sex-matched references. An appreciation of the totality of the abnormal growth characteristics is important when assessing children with aberrant growth. In this case, a novel *AIP* mutation, p.Tyr202*, was

found. The unresponsiveness of the tumor to pasireotide could better be assessed by in vitro responsiveness, as opposed to somatostatin receptor evaluation. Future studies are necessary to test this hypothesis in cohorts with more patients and with a control group.

Conclusion

This informative case of incipient gigantism in a 7-year old child with a novel *AIP* mutation, p.Tyr202*, was associated with a highly-treatment resistant somatotropinoma. Although previous literature suggests a favorable response to pasireotide in some patients with *AIP* mutations and acromegaly (18), pasireotide had only limited effect in our patient, possibly related to decreasing SSTR5 expression of the tumor. *In vitro* GH suppression in cultured tumor tissue may predict *in vivo* treatment response better than assessing SSTR. Genetic testing of the *AIP* gene should be advocated in all patients with GH-secreting pituitary adenomas occurring in childhood and/or (incipient) pituitary gigantism (22).

Disclosure

The authors have no relevant disclosures.

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Figure legends

Figure 1 Growth chart of the patient with incipient gigantism.

The initially normal growth of the patient had been declining from three to six years of age, but then deflected markedly upwards. He was diagnosed at the age of seven years. The blue arrow corresponds with treatment with lanreotide (“LAN”); the purple arrow corresponds with treatment of pasireotide (PAS) and the red arrow PEGV surgery) with pegvisomant and surgery. One month after switching to pasireotide, the first transsphenoidal resection was performed. Two months thereafter, pegvisomant was started. After one month of pegvisomant, tumor volume increased; pegvisomant was stopped and a second resection followed. Six months thereafter, the third surgery took place.

Figure 2 Sequential magnetic resonance imaging over the clinical course of the patient between 2018 and 2020.

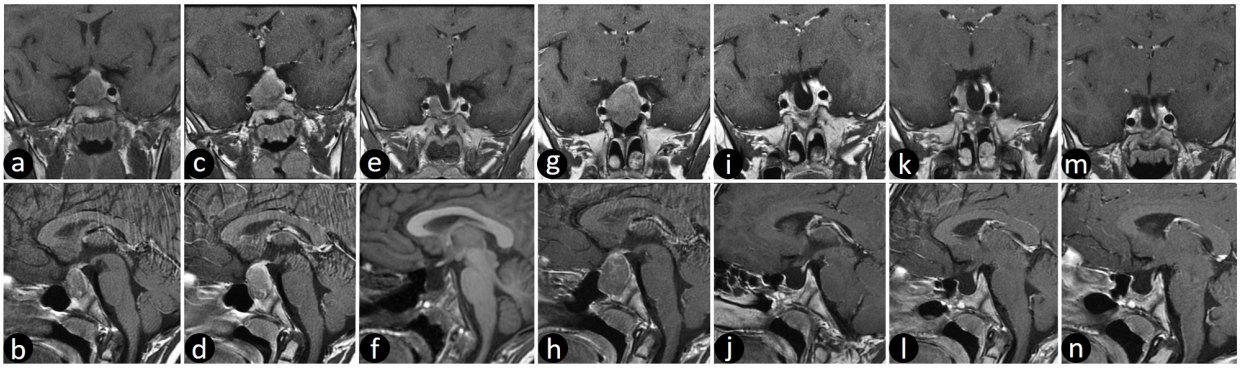
Contrast-enhanced T1-weighted sequences in coronal (a, c, e, g, I, k, m) and sagittal (b, d, f, h, j, l, n) planes were chosen and corrected for grey scale and magnification. The timing of the scans are as follows: at clinical presentation (a, b):before the first operation (lanreotide was switched to pasireotide after this MRI because of tumor growth and surgery was performed because of visual field defects due to chiasmic compression) (c,d);postoperatively after the first operation (e, f); before the second operation (g, h); postoperatively after the second operation (one month after intiating pegvisomant treatment) (i, j); before the third operation (k, l); postoperatively after the third operation (m, n). There was no inhibition of tumor growth after SSA use in terms of tumor size and extent. Correspondingly, growth hormone secretion was normalized after the respective tumor resections. The tumor is medial to the intracavernous intercarotid line (Knosp status Grade II); however, upon direct vision during the last surgery, there was invasion of the cavernous sinus wall.

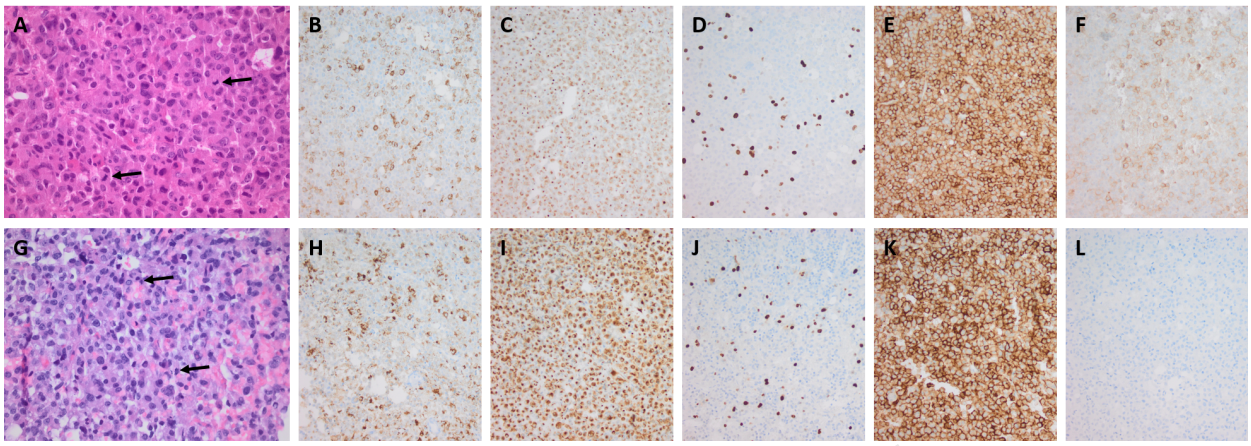
Figure 3 Histopathological features of the tumor at the second and third surgery

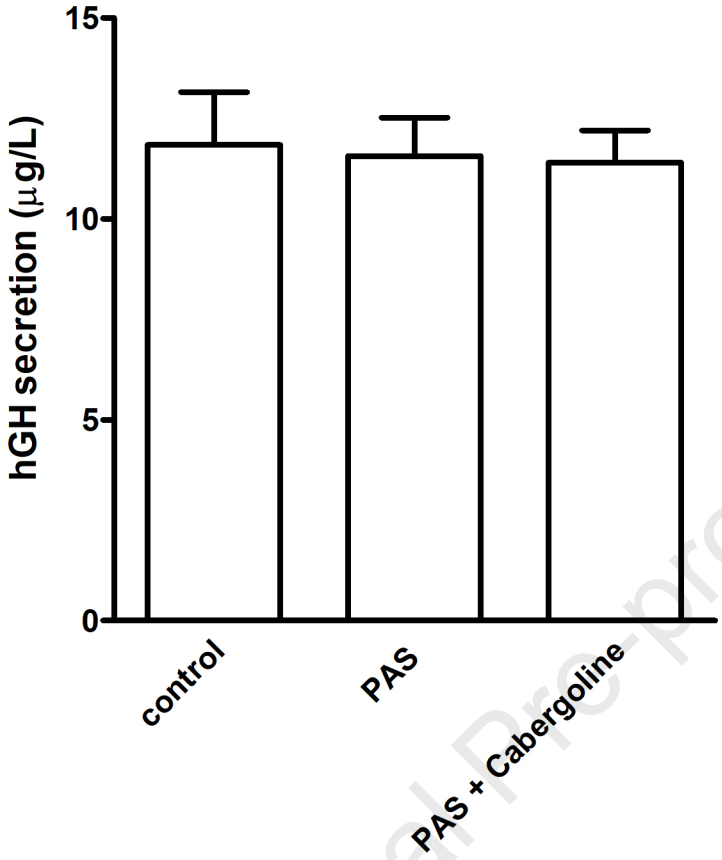
Tissue from the first surgery was unavailable.
A-F: second surgery; **G-L:** third surgery.
A & G: H&E staining show a pituitary adenoma with interspersed mitoses in both surgeries (black arrows).
B & H: GH expression. **C & I:** PanCK immunohistochemistry shows only a few fibrous bodies in both specimens.
In both specimens there is increased proliferation activity (Ki67 staining in **D & J**).
Tissue from both surgeries with homogeneous expression of SSTR2 (**E & K**), while SSTR5 is moderately expressed in the specimen of the second surgery (**F**) and absent in the tissue of the third surgery (**L**).

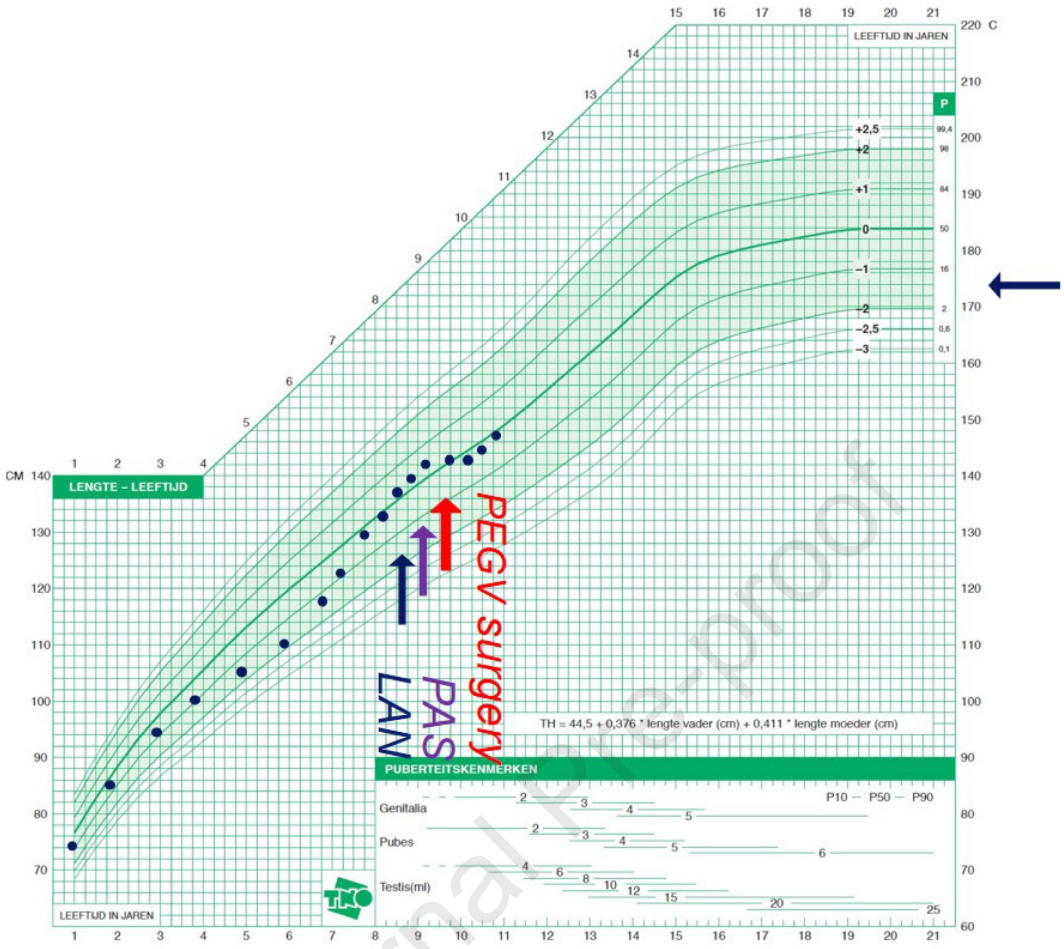
Figure 4 *In vitro* sensitivity of cultured tumor cells to pasireotide and cabergoline.

GH-secretion by primary cultured adenoma cells of the patient did not respond to incubation with pasireotide (PAS; 10nM) or pasireotide (10nM) plus cabergoline (10nM); there was no statistically significant change in growth hormone secretion after a 72 hr incubation with the drugs. Cells were cultured as a monolayer in 250 µl medium in a 48-well culture plate. Tumor cell isolation and culture conditions were as described in (17). Medium GH concentrations are expressed in µg/L and are the mean ± SD (n=3 wells per group). Data were analyzed by one-way ANOVA and multiple comparison between groups using Newman-Keuls test, using GraphPad Prism (San Diego, CA).









Highlights

- A 7-year-old boy with acromegaly had accelerated growth, headaches and a high IGF-1
- Pituitary macro-adenoma with a novel truncating *AIP* mutation, p.Tyr202*
- Multiple treatment modalities were needed to achieve tumoral & biochemical control
- *In vitro* tissue response to SSA may better predict *in vivo* response than SSTR immunohistochemistry
- Increased growth (velocity) can be masked by failure to thrive and parents' height

Clinical Relevance

We report a case of acromegaly at an extremely young age of 7 years. This was associated with a not earlier described *AIP* mutation (p.Tyr202*). This is an example where the prediction of biochemical response to SSA could better be estimated by *in vitro* response to SSA than SSTR immunohistochemistry.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: