CLINICAL STUDY

Granulation pattern, but not GSP or GHR mutation, is associated with clinical characteristics in somatostatin-naïve patients with somatotroph adenomas

Sarah Larkin¹², Raghava Reddy³, Niki Karavitaki³, Simon Cudlip⁴, John Wass³ and Olaf Ansorge¹²

¹Nuffield Department of Clinical Neurosciences and ²Department of Neuropathology, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK.
³Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Old Road, Headington, Oxford OX3 7LE, UK and ⁴Department of Neurosurgery, John Radcliffe Hospital, Headley Way, Oxford OX3 9DU, UK.

(Correspondence should be addressed to O Ansorge at Nuffield Department of Clinical Neurosciences, John Radcliffe Hospital; Email: olaf.ansorge@ndcn.ox.ac.uk; J Wass; Email: john.wass@nhs.net)

Abstract

Objective: Somatotroph adenomas causing acromegaly are histologically classified into densely granulated (DG) and sparsely granulated (SG) subtypes with different morphology, clinical characteristics and treatment outcomes. Granulation pattern has been reported to co-segregate with a recurrent mutation at codon 49 in growth hormone receptor (GHR) and GSP oncogene. This study examines response to the octreotide suppression test (OST) in relation to granulation pattern and mutation in GHR and GSP.

Design: This is a retrospective, single-centre study of 52 patients with pathologically confirmed somatotroph adenoma who were naïve to medical therapy presenting between January 2001 and October 2010.

Methods: Clinical, radiological and hormonal data at diagnosis were recorded. GHR and GSP were genotyped, granulation pattern determined and response to the OST measured.

Results: SG adenomas were larger (P = 0.038), occurred in younger patients (P = 0.029), were more common in females (P = 0.026) and were more invasive (P < 0.0001 and P = 0.001), with diminished responses to the OST (P = 0.007) compared with DG adenomas. GSP mutation was unrelated to granulation pattern but associated with smaller tumours (P = 0.027), producing more GH (P = 0.048) that responded better to the OST (P = 0.022). Codon 49 of GHR was not mutated.

Conclusions: Adenoma histological phenotype, not genotype, corresponds to clinical and biochemical characteristics and response to the OST. SG adenomas constitute a clinically more unfavourable subtype but are not associated with GHR mutations in our series. Ascertainment of the adenoma subtype may become an important consideration in the management of acromegaly.

European Journal of Endocrinology 168 491–499

Introduction

GH-secreting adenomas exist as distinct morphological subtypes

Pituitary somatotroph adenomas are the underlying cause of acromegaly in more than 99% of cases. Somatotroph adenomas arising from GH-secreting cells account for 10–15% of pituitary adenomas and are histologically classified into two subtypes: densely granulated (DG) and sparsely granulated (SG) with an intermediate mixed subtype displaying both densely and sparsely granulated regions (mixed granulation pattern, MG; reviewed in (1)).

DG somatotroph adenomas are the most common subtype, comprising large, round, strongly acidophilic cells that appear similar to normal somatotrophs. There is strong, diffuse cytoplasmic GH immunoreactivity. Electron microscopy reveals a well-developed rough endoplasmic reticulum, a prominent Golgi complex and numerous large (300–600 nm) secretory granules containing GH, distributed throughout the cytosol (2, 3, 4, 5).

SG GH-secreting pituitary adenomas are less common and have poorly cohesive cells, forming sheet-like structures. Cells often show nuclear pleomorphism with weak, focal GH immunoreactivity and do not resemble normal somatotrophs. Electron microscopy reveals small uniform granules of GH, aligned along the plasma membrane (2, 3, 4, 5). The most distinctive feature of SG adenomas is the presence of fibrous bodies. These are paranuclear, dense aggregates of cytokeratin (predominantly cytokeratin 8) and endoplasmic reticulum
fragments. Differences in the distribution of cytokeratin co-segregate with differences in number, size and distribution of GH-containing granules, so cytokeratin distribution is often used as a marker of the SG subtype (see Fig. 1).

**Mutations in stimulatory G-protein alpha subunit and GH receptor are associated with pituitary adenomas**

Genetic analysis of somatotroph adenomas revealed mutations at the GNAS complex locus (known as GSP). This locus contains four alternative promoters and 5′ exons and has a complex imprinted expression pattern. Alternative splicing gives rise to different forms of the G-protein alpha subunit (Gsα), which couples seven-transmembrane receptors to adenylyl cyclase (6). A missense mutation at Arg201 or Gln227 results in ablation of GTPase activity, resulting in constitutive activation of Gsα (7) and elevated cAMP. The effect of GSP mutations on the clinical presentation of acromegaly is unclear but studies have reported that tumours with a GSP mutation (GSP+) are smaller than wild-type tumours (GSP−) (2, 8, 9, 10). The association of GSP with a difference in acute or long-term response to treatment with somatostatin analogues (SSAs) remains equivocal, although larger and more recent studies suggest that there is no association (2, 8, 9, 10, 11, 12, 13, 14).

Mutations of GHR (H49L) have been reported exclusively in SG adenomas (15) where they impair glycosylation-mediated GHR processing, maturation, ligand binding and signalling. Treatment with the GH antagonist pegvisomant caused fibrous body formation in DG cells, implicating functional GHR in DG subtype morphology. Reports are conflicting concerning the association of GSP mutation with adenoma subtype, but larger and more recent studies found no significant association (2, 9, 15, 16).

**Tumour size, proliferation index, invasiveness and response to SSAs vary according to subtype**

Previous studies found that SG adenomas were larger, more common in females, more likely to show suprasellar extension, cavernous sinus invasion and a reduced response to SSA treatment than DG adenomas (2, 9, 13, 17, 18). Reduced expression of somatostatin receptors and E-cadherin is associated with the SG phenotype, but the phenotypic consequences remain unclear (19). On the basis of baseline clinical characteristics, the DG and MG subtypes have been previously grouped together, while the SG subtype is identified as a distinct entity with different clinical features (2, 16, 18). This difference in behaviour has been attributed to the presence of the fibrous body associated with the SG subtype (18), but these studies are largely qualitative and a more thorough, quantitative study of the influence of the fibrous body on clinical features is required (2, 16, 18). Despite differences in morphology and clinical behaviour, adenoma subtype is not currently considered when making treatment decisions for acromegaly.

**The octreotide suppression test as a predictor of response to long-term SSA treatment**

Poorly controlled acromegaly is associated with a two- to threefold increase in mortality and significant morbidity (20, 21). With adequate control (post-therapy GH <2.5 μg/l, ‘safe’ levels), mortality is comparable to that of the general population (22). After surgery, if unsuccessful, long-term treatment with SSAs is the most common therapy and around half of patients achieve ‘safe’ GH levels (23) (reviewed in (24)). Various factors have been reported to predict patient response to long-term SSA therapy, including tumour size, pretreatment serum GH levels, somatostatin receptor density and response to the octreotide suppression test (OST) (16, 23, 25, 26, 27, 28). A GH nadir of <1.75 μg/l upon acute administration of 100 μg octreotide during the OST had a positive predictive value of 94% and a negative predictive value of 100% for achievement of ‘safe’ mean GH levels upon therapy with long-acting octreotide (23). This finding was supported by a similar study in which GH nadir <1.67 μg/l during the OST predicted achievement of ‘safe’ GH levels after long-term depot SSA.
treatment with 80% sensitivity and 83% specificity (29). A better response to SSA treatment has been reported with the DG subtype (2, 13, 14). A recent study has also identified T2-weighted signal intensity in somatotroph adenoma on magnetic resonance imaging (MRI) scan as a significant predictive factor of response to treatment with long-acting SSAs. A hyperintense signal was shown to predict a poorer response to long-term SSA treatment and to be associated with a SG histological subtype (30).

This study investigates factors affecting the preoperative response to the OST in patients naïve to SSA and radiotherapy treatment. It also examines for the first time whether somatic mutations in GSP radiotherapy treatment. It also examines for the first time whether somatic mutations in GSP

Materials and methods

Patients

Fifty-two patients with pathologically confirmed somatotroph adenoma with a defined histological subtype presenting between January 2001 and October 2010 were included. Those who received medical treatment for their acromegaly before surgery were excluded. Preoperatively, all patients were evaluated clinically, biochemically and radiologically. Age at surgery, sex, tumour size, fasting GH, age-adjusted insulin-like growth factor 1 (IGF1), GH response to OST and the anterior pituitary hormone profile were established. Fifty-two patients with pathologically confirmed somatotroph adenoma with a defined histological subtype were included. Those who received medical treatment for their acromegaly before surgery were excluded.

Histopathology

Immunohistochemical analyses were performed on 5 μm sections from FFPE tumour specimens. After de-waxing, endogenous peroxidase activity was blocked (10% (v/v) H2O2 in PBS, pH 7.3, 30 min). Antigen retrieval was achieved by autoclaving in sodium citrate (10 mmol/l; pH 6.0, 10 min; MIB-1) or incubation with protease (Menarini, Wokingham, UK), 10 min; Cam 5.2). Sections were incubated with primary antibody Cam 5.2 (mouse, pre-diluted, Becton-Dickinson (Oxford, UK)) or MIB-1 (mouse, DAKO (Ely, UK)) diluted 1 in 100 with antibody diluent (DAKO REAL). Sections were incubated at 25 °C for 35 min with secondary antibody and at 25 °C for 35 min with secondary antibody (DAKO REAL EnVision/HRP, Rabbit/Mouse (ENV) Kit). DAKO REAL DAB+chromagen was applied for 10 min and sections were counterstained with Harris’s haematoxylin.

Tumours were categorised as SG, DG or of MG. A mixed phenotype was assigned when more than 30% of tumour cells deviated from the dominant Cam5.2 pattern. This approach has been used in other studies (16, 18) and allows cross-study comparison of our data. Proliferative activity of the tumour was determined by calculating the percentage of cells expressing the Ki-67 antigen as determined by staining with the MAB MiB-1. This was done on a single section adjacent to those used for H&E, Cam 5.2 and GH assessment. The whole tumour area was taken into account and the percentage estimated by a single neuropathologist. Where a range of values was reported, the uppermost value in the range was used for analysis (e.g. 'MiB-1 of 3–5%' = MiB-1 of 5% for this study).

Biochemical evaluation

GH and IGF Fast serum GH and IGF1 levels were available in 51 patients and were recorded at the time of the OST. Serum GH was measured by a two-site chemiluminescent immunometric assay (Immulite/Immulite 1000 hGH Kit, EURO/DPC Ltd., Gwynedd, UK); intra-assay coefficient of variation (CV), 5.3–6.5%; inter-assay CV, 5.7–6.2%; calibration range, up to 40 ng/ml (104 mIU/l); sensitivity, up to 0.01 ng/ml (0.026 mIU/l)). Serum IGF1 was measured by a two-site immunoenzymometric assay (OCTEIA IGF1 Kit, Immnodiagnostic Systems Ltd., Boldon, UK). Values were age-normalised by calculating the IGF1 s.d., i.e. the number of standard deviations from the mean of the normal population adjusted for age. The range for the normal population is assumed to be equivalent to the mean ±2 s.d. (31) ((≤20 years, 16–118 nmol/l; ≤30 years, 11.7–36.1 nmol/l; ≤40 years, 2.9–25.8 nmol/l; ≤50 years, 6.4–19.3 nmol/l; ≤60 years, 4.6–27.5 nmol/l; ≤70 years, 3.9–25.7 nmol/l; >70 years, 6.6–25.0 nmol/l); intra-assay CV, 2.3–3.5%; inter-assay CV, 7.0–7.1%; sensitivity, 0.25 nmol/l).

Other pituitary hormone tests Gonadotrophin (LH and FSH) deficiency was diagnosed on the basis of low or ‘inappropriately normal’ LH and FSH levels combined with serum testosterone below reference values in adult men and with low serum oestradiol and oligo-/amenorrhoea in adult premenopausal women or low gonadotrophins in postmenopausal women. ACTH deficiency was defined by a peak cortisol level <580 nmol/l at 30 min on the short Synacthen test; TSH deficiency was defined by low or ‘inappropriately normal’ TSH with free thyroxine (FT4) levels below the reference range. All results were classified as normal or abnormal according to the above criteria.

Octreotide suppression test OST was performed after an overnight fast (23). Octreotide (100 μg, Sandostatin, Novartis) was administered s.c. following baseline blood sampling. Blood was subsequently sampled at hourly intervals for 6 h. GH concentration was determined at each timepoint and the nadir response calculated (23).

www.eje-online.org
MRI of pituitary

A standard protocol of T2-weighted axial and T1-weighted coronal and sagittal high-resolution spin echo sequences was performed. Tumours were classified as microadenoma (<10 mm) or macroadenoma (≥10 mm) and assessed for cavernous sinus invasion and suprasellar extension. Actual tumour volume was available for 39 patients, calculated using the formula (0.5 × width × length × height (mm³)) (32).

GHR and GSP sequencing

DNA was extracted from 5×10 μm sections of FFPE tissue from archival surgical specimens (QiAmp FFPE DNA Kit, Qiagen). PCR was performed to generate amplicons including GHR exon 4 and codons 201 and 227 of GSP. Primers were GHR sense (5'-AGGATCACATATGACTCAGC-CC-3') and antisense (5'-AGGAATTCAGAAAGGCATG-3') (15). For GSP, codons 201 and 227 were amplified separately, within short amplicons (<300 bp). Codon 201 sense (5'-GGACTCTGAGCCCTCTTTCCAAAC-3') and antisense (5'-CAGAGGGGACTGGGTTAAGTCAA-3') and codon 227 sense (5'-TCCAGTGGAACAGTCACTTCAA-3') and antisense (5'-GGAGCCCTGATCCCTAACAACACA-3').

DNA template (50 ng) was added to 10×PCR buffer solution (10% v/v), dNTPs (200 μM) (Promega) 0.5 U of Taq polymerase (HotStarTaq Plus, Qiagen) and 400 nM each of forward and reverse primers. GSP reactions also contained MgCl₂ (final concentration 4 mM). Cycling conditions were 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s (GHR) or 20 s (GSP); 59 °C (GHR) or 55 °C (GSP) for 30 s and 72 °C for 1 min (GHR) or 30 s, followed by a single 5-min extension (GSP) in a total volume of 20 μl. Products were examined by agarose gel separation and purified (MinElute PCR Purification Kit (Qiagen)). Sequencing reactions were performed using BigDye Terminator chemistry and an ABI-3730 sequencer.

GHR restriction endonuclease digestion

GHR amplicons were screened for H49L mutations by restriction endonuclease digestion using BsrI (New England Biolabs, Hitchin, UK), which recognises the wild-type sequence at codon 49 (5'-ACTGGA-3'). PCR amplicons digested with BsrI yielded two fragments (125 and 92 bp). DNA (1 μg) was added to 5 μl of 10× reaction buffer (10% v/v), BsrI (10 U) and water (total volume 50 μl) and incubated at 65 °C for 1 h. Products were examined by agarose gel separation. A positive control that contained a BsrI recognition site was included, as was a no-enzyme negative control.

Statistical analysis

Data are expressed as mean (± s.d.) and were analysed by the Kruskal–Wallis test. Mann–Whitney U test, χ² test or Fisher’s exact test where appropriate. A value of P < 0.05 was considered significant. Data were recorded and analysed using Microsoft Excel, SPSS 20 and GraphPad Prism 5. (La Jolla, CA, USA)

Results

Patient characteristics, radiological characteristics, OST responses and histological characteristics are summarised in Tables 1 and 2.

Patient characteristics

Fifty-two patients (31 females) were included in the study. Age at surgery was 48 ± 14 years (mean ± s.d.; range 19–80 years). There were 23 patients with DG subtype, 10 with MG and 19 with SG. Women were more likely to develop a SG subtype than MG or DG, whereas DG was more common in men (P = 0.026; χ² test). Age at surgery also differed with subtype: DG 52 ± 14 years (mean ± s.d.), MG 54 ± 10 years and SG 41 ± 15 years (P = 0.029; Kruskal–Wallis test). There was no association between GSP mutation status and patient age at surgery, but more males were GSP+ and more females were GSP− (P = 0.0421, Fisher’s exact test).

Radiological characteristics

The SG subtype was associated with a higher frequency of suprasellar extension and cavernous sinus invasion than other subtypes (P = 0.0014 and P < 0.0001 respectively, χ² test). The SG subtype also had a larger maximum tumour dimension (21.3 ± 10.9 mm; mean ± s.d.) than the MG (13.2 ± 5.7 mm) or DG subtype (11.8 ± 6.6 mm; P = 0.0417, Kruskal–Wallis test), but there was no such significant association with tumour volume. GSP status did not differ according to the presence of suprasellar extension, cavernous sinus invasion or tumour size (as determined by maximum dimension, tumour volume or classification as macro- or microadenoma).

Hormone profile

IGF1 levels were available for 51 patients. IGF1 s.d. ratios did not differ significantly between subtypes (P = 0.881, Kruskal–Wallis test). GSP status also had no significant effect on IGF1 s.d. values (P = 0.624, Mann–Whitney U test). Gonadotrophin levels were not available in one patient (MG, GSP−). When macroadenomas and microadenomas were considered separately, there was no significant difference in the profile of prolactin or thyroid function between granulation subtypes or GSP status. In macroadenomas, gonadotrophin deficiency was more common in the SG subtype (P = 0.002, χ² test), as was ACTH deficiency (P = 0.032, χ² test).
**Table 1** Comparison of adenoma subtype and GSP mutation status with clinicopathological features.

<table>
<thead>
<tr>
<th>Granulation pattern</th>
<th>DG</th>
<th>MG</th>
<th>SG</th>
<th>P value</th>
<th>GSP+</th>
<th>GSP−</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at surgery (mean ± s.d.)</td>
<td>52 ± 14</td>
<td>54 ± 10</td>
<td>41 ± 15</td>
<td>0.029&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50 ± 15</td>
<td>46 ± 14</td>
<td>0.4093&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14 (66.7%)</td>
<td>2 (9.5%)</td>
<td>5 (23.8%)</td>
<td>0.026&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14 (70%)</td>
<td>6 (30%)</td>
<td>0.0421&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female</td>
<td>9 (29.0%)</td>
<td>8 (28.5%)</td>
<td>14 (46.2%)</td>
<td></td>
<td>11 (36.7%)</td>
<td>19 (63.3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Radiological characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cavernous sinus invasion present</td>
<td>4 (21.1%)</td>
<td>2 (10.5%)</td>
<td>13 (68.4%)</td>
<td>0.0014&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9 (47.4%)</td>
<td>10 (52.6%)</td>
<td>1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Suprasellar extension present</td>
<td>5 (20.8%)</td>
<td>3 (12.5%)</td>
<td>16 (66.7%)</td>
<td>&lt;0.0001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10 (41.7%)</td>
<td>14 (58.3%)</td>
<td>0.100&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maximum dimension (mm; mean ± s.d.; n=39)</td>
<td>11.8 ± 6.6</td>
<td>13.2 ± 5.7</td>
<td>21.3 ± 10.9</td>
<td>0.0417&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.6 ± 7.8</td>
<td>17.1 ± 9.8</td>
<td>0.245&lt;sup&gt;c,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tumour volume (mm&lt;sup&gt;3&lt;/sup&gt;; mean ± s.d.; n=18)</td>
<td>863 ± 2577</td>
<td>370 ± 679</td>
<td>1299 ± 2474</td>
<td>0.501&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1706 ± 3134</td>
<td>292 ± 740</td>
<td>0.093&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Macroadenoma</td>
<td>15 (46.9%)</td>
<td>3 (9.4%)</td>
<td>14 (43.8%)</td>
<td>0.0633&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18 (51.6%)</td>
<td>13 (41.9%)</td>
<td>0.2436&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Microadenoma</td>
<td>8 (40%)</td>
<td>7 (35%)</td>
<td>5 (25%)</td>
<td></td>
<td>7 (38.9%)</td>
<td>11 (61.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Histological characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGf1 SDS (mean ± s.d.)</td>
<td>35.1 ± 19.8</td>
<td>36.4 ± 13.7</td>
<td>40.4 ± 28.2</td>
<td>0.881&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.6 ± 17.4</td>
<td>38.0 ± 26.8</td>
<td>0.624&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Octreotide suppression test (n=36)</td>
<td>30.98 ± 53.88</td>
<td>10.45 ± 5.44</td>
<td>18.97 ± 17.83</td>
<td>0.822&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.91 ± 10.8</td>
<td>30.63 ± 49.08</td>
<td>0.6169&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GH nadir (µg/l; mean ± s.d.)</td>
<td>4.42 ± 10.53</td>
<td>1.28 ± 0.84</td>
<td>4.66 ± 4.01</td>
<td>0.0398&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97 ± 2.56</td>
<td>6.46 ± 9.76</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GH absolute acute reduction (Δ µg/l; mean ± s.d.)</td>
<td>26.56 ± 43.67</td>
<td>9.17 ± 5.27</td>
<td>14.32 ± 15.45</td>
<td>0.9533&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.85 ± 9.43</td>
<td>26.1 ± 40.99</td>
<td>0.7862&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GH percentage acute reduction (mean ± s.d.)</td>
<td>88.1 ± 6.4</td>
<td>85.0 ± 10.4</td>
<td>67.2 ± 20.8</td>
<td>0.0073&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84.2 ± 15.6</td>
<td>73.9 ± 17.6</td>
<td>0.0217&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Histological characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mil-1 proliferation index (mean ± s.d.)</td>
<td>2.2 ± 1.1</td>
<td>2.7 ± 0.5</td>
<td>4.1 ± 2.5</td>
<td>0.0014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 1.4</td>
<td>3.3 ± 2.3</td>
<td>0.6076&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

DG, densely granulated; MG, mixed granulation pattern; SG, sparsely granulated. Bold numerals, *P<0.05, n=37.
<sup>a</sup>Kruskal–Wallis test.
<sup>b</sup>Mann–Whitney U test.
<sup>c</sup>x<sup>2</sup> test.
<sup>d</sup>Fisher’s exact test.

**Somatostatin response (OST)**

Factors affecting response to the OST are summarised in Table 2. Preoperative OST results were available for 36 patients. There was no significant association between adenoma subtype or GSP status and fasting GH values. However, the number of patients achieving a satisfactory response to the OST (GH nadir <1.75 µg/l) and the percentage acute reduction in GH varied according to both subtype and GSP status. Percentage acute reduction in GH was much greater in DG (88.13 ± 6.39%) and MG (85.03 ± 10.42%) than in the SG subtype (67.17 ± 20.84) and also in GSP+ (84.2 ± 15.59%) compared with GSP− (73.94 ± 17.61%, P=0.0217, Mann–Whitney U test) cases (Table 1). Of the patients who showed a satisfactory OST response (18 of 36 patients tested), the most common finding was a DG adenoma (nine patients, 50%), with fewer responders exhibiting the MG subtype (five patients, 27.8%) and SG subtype (four patients, 22.2%; P=0.058; x<sup>2</sup> test). Other characteristics significantly associated with an unsatisfactory OST response included presence of suprasellar extension and a macroadenoma (Table 2).

Logistic regression analysis with suprasellar extension, size of adenoma and granulation subtype as covariables did not identify any of these factors as significantly affecting OST response.

**Histological characteristics**

Ki67 values were available for 51 patients. Higher proliferation indices were associated with the SG subtype (4.1 ± 2.5) compared with the MG (2.7 ± 0.5) and DG (2.2 ± 1.1) subtypes, but there was no difference in proliferation index between GSP + and GSP− tumours.

**Adenoma subtype, GHR and GSP status**

Forty-nine of 52 cases were sequenced at codon 201 or 227 of GSP (three cases had insufficient tissue). Twenty-six cases (53%) had a mutation in GSP (24 R201C, one R201S and one Q227R), and of these cases, 13 (50%) were DG, seven (27%) were MG and six (23%) were SG. GSP mutation did not co-segregate with adenoma subtype (P=0.163, x<sup>2</sup> test; Table 3). In addition to
the cases described in this study, a further 21 cases were examined for mutation at codon 49 of GHR, and of these, eight were DG and 13 were SG. No mutations were found by either sequencing or restriction endonuclease digestion in a total of 30 SG tumours (Fig. 2).

Discussion

Adenoma subtype correlates with clinical variables, but does not predict OST response in naïve patients

Patients were included in this study only if they had received no previous medical treatment for their acromegaly. Although this criterion may represent a selection bias, it ensures that the response to the OST is not altered by previous exposure to SSA. Pretreatment with octreotide may alter expression of SSTR2a (11, 33) affecting response to the OST. Although tumour subtype was not associated with a difference in the absolute GH nadir achieved during the OST, patients with DG adenomas showed a greater percentage reduction in GH compared with other subtypes and a greater proportion of patients achieving a satisfactory response (GH nadir < 1.75 μg/l) exhibited DG adenomas. The influence of GSP mutation on response to the OST is less clear. GSP− tumours were associated with a greater percentage reduction in GH in response to the OST, but there was no difference in the number of patients achieving a satisfactory response, which has been shown to be the most reliable predictor of long-term response to octreotide LAR treatment (23).

The OST has been demonstrated to provide favourable positive (94%) and negative (100%) predictive values for achievement of safe GH levels with long-term octreotide LAR treatment (23). The findings of this study suggest that patients with DG adenomas may respond better to long-term octreotide LAR treatment than those with the MG or SG subtype as measured by GH levels. Granulation pattern was not found to be predictive of OST response in this cohort; however, the size of the cohort (n = 36) was small and further studies are required to determine whether granulation subtype can predict response to the OST of long-term SSA treatment. The differences between the morphological subtypes that could account for a difference in response are unclear. It has been reported previously that somatostatin receptor distribution is an important factor in the response to SSA and that this distribution differs between adenoma subtypes (33). It has also been suggested that different somatostatin receptor subtypes may perform different functions; reduction of GH release may be mediated predominantly by SSTR2a, and

<p>| Table 2 Comparison of OST outcome with GSP mutation status and clinicopathological features. |</p>
<table>
<thead>
<tr>
<th>OST outcome</th>
<th>Satisfactory (n=18)</th>
<th>Unsatisfactory (n=18)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at surgery (mean±s.d.)</td>
<td>57±13</td>
<td>44±12</td>
<td>0.28a</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (54%)</td>
<td>6 (46%)</td>
<td>0.50b</td>
</tr>
<tr>
<td>Female</td>
<td>11 (48%)</td>
<td>12 (52%)</td>
<td></td>
</tr>
<tr>
<td>GSP status (n=33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSP−</td>
<td>5 (31%)</td>
<td>11 (69%)</td>
<td>0.11b</td>
</tr>
<tr>
<td>GSP+</td>
<td>10 (59%)</td>
<td>7 (41%)</td>
<td></td>
</tr>
<tr>
<td>Granulation pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG</td>
<td>9 (69%)</td>
<td>4 (31%)</td>
<td>0.06c</td>
</tr>
<tr>
<td>MG</td>
<td>5 (63%)</td>
<td>3 (37%)</td>
<td></td>
</tr>
<tr>
<td>SG</td>
<td>4 (27%)</td>
<td>11 (73%)</td>
<td></td>
</tr>
<tr>
<td>Radiological characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macroadenoma</td>
<td>8 (33%)</td>
<td>16 (67%)</td>
<td>0.01b</td>
</tr>
<tr>
<td>Microadenoma</td>
<td>10 (83%)</td>
<td>2 (17%)</td>
<td></td>
</tr>
<tr>
<td>Suprasellar extension</td>
<td>4 (20%)</td>
<td>16 (80%)</td>
<td>0.00b</td>
</tr>
<tr>
<td>Cavernous sinus invasion</td>
<td>5 (33%)</td>
<td>10 (67%)</td>
<td>0.09b</td>
</tr>
</tbody>
</table>

DG, densely granulated; MG, mixed granulation pattern; SG, sparsely granulated. Bold numerals, P<0.05, n=36.

aMann–Whitney U test.
bFisher’s exact test.
c2 test.

<p>| Table 3 Summary of adenoma subtype and GSP mutation status. |
| GSP mutation status does not segregate with adenoma subtype. No mutations in GHR were found. |</p>
<table>
<thead>
<tr>
<th>Granulation pattern</th>
<th>DG</th>
<th>MG</th>
<th>SG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSP−</td>
<td>9</td>
<td>3</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>GSP+</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>10</td>
<td>17</td>
<td>49</td>
</tr>
</tbody>
</table>
tumour shrinkage by SSTR5, a position supported by the higher affinity of SSTR2a than SSTR5 for octreotide and lanreotide (27, 28, 34, 35). The prevalence of SSTR2a was found by Kato et al. (35) to be greater in DG adenomas compared with other subtypes, which may partially explain their increased responsiveness to the OST and long-term SSA treatment.

**There is no association between adenoma subtype and mutations in GHR or GSP**

We found a GSP mutation in 53% of adenomas that did not correlate with adenoma subtype, in agreement with previous findings (incidences ranging from 40–64%) (2, 7, 8, 9, 10, 16, 36). No mutations at codon 49 of GHR were found, suggesting that these mutations are not associated with the SG subtype in this population (75 cases sequenced, 30 of which were SG adenomas). This is commensurate with previous findings in a cohort of 18 somatotroph adenomas for which the coding region of GHR was sequenced and no mutations were found at codon 49 or other loci (37). However, no distinction was made based on adenoma subtype in this study.

**Mutation status of GSP does not affect tumour proliferation**

Mutation in GSP was not associated with a difference in proliferation index, suprasellar extension, cavernous sinus invasion or incidence of macroadenomas compared with microadenomas. Constitutive Gsα activation might be expected to increase cellular cAMP, which acts both to increase GH expression and as a mitogenic signal (38, 39). However, its levels are tightly regulated by the action of phosphodiesterases (PDEs) that hydrolyse it to AMP. Regulation by PDEs could, at least partially, counter the effect of GSP mutation. Intracellular cAMP levels have been shown not to differ between GSP+ and GSP− adenomas, whereas PDE activity was sevenfold higher in GSP+ tumours, an increase that was largely attributable to PDE4 (40).

**SG adenomas represent a distinct subgroup with larger, more invasive tumours and a reduced acute response to octreotide**

The SG subtype was associated with larger tumour volume and higher incidence of suprasellar extension and cavernous sinus invasion and was more common in females. It was also associated with a lower age at surgery. It is unknown whether age at surgery in this study reflects age at onset of symptoms, so this may not reflect age at onset of disease. SG adenomas also had a higher proliferation index, as reported by others (2, 17, 18). The SG subtype responded less well to the OST, and fewer patients with SG adenomas achieved ‘safe’ GH levels. These findings are in agreement with previously published studies suggesting that SG adenomas represent a more proliferative and invasive subtype of tumour that may respond less well to SSA therapy (2, 13, 16, 18). The mechanism underlying this behaviour of SG adenomas is unknown. Studies investigating the role of cell–cell adhesion protein E-cadherin and its associated proteins α-, β- and γ-catenin have shown that their expression is reduced in SG compared with DG adenomas (16, 19) and that their expression correlates with that of SSTR2a (41, 42). The consequences of reduced E-cadherin expression have not been extensively investigated, but a reduction in expression of components of the adherens junction could be partially responsible for the increased proliferative and invasive properties of this subtype as both these processes require disruption of cell–cell adhesion. Reduction in expression of E-cadherin has been associated with the epithelial-to-mesenchymal transition in other cancers of epithelial origin, which could contribute to a more proliferative and invasive phenotype (43, 44).

**Conclusions**

Adenoma subtype, rather than mutations in GSP or GHR, affects tumour size, proliferation index and parasellar invasiveness. SG adenomas present as larger tumours in younger patients, with a greater incidence of suprasellar and cavernous sinus invasion and a higher frequency in females. Neither adenoma subtype nor GSP mutation is predictive of the OST response in somatostatin-naive patients; however, DG adenomas are more common in patients with a satisfactory OST response and a greater percentage acute reduction in GH. GHR mutations are unlikely to play a significant role in somatotroph adenoma pathogenesis.

**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.
Funding
This work was supported by the NIHR Oxford Biomedical Research Centre.

References


39 Ham J, Ivan M, Wynford-Thomas D & Scanlon ME GH3 cells expressing constitutively active Gαz(Q227L) show enhanced hormone secretion and proliferation. *Molecular and Cellular Endocrinology* 1997 127 41–47. (doi:10.1016/S0303-7207(96)03987-1)


Received 2 October 2012
Revised version received 27 November 2012
Accepted 3 January 2013