IS RECEPTOR PROFILING USEFUL FOR PREDICTING PITUITARY THERAPY?

Journal:	*European Journal of Endocrinology*
Manuscript ID	EJE-18-0549.R1
mstype:	Debate
Date Submitted by the Author:	n/a
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Keywords:	pituitary, Somatostatin receptors, dopamine receptors, receptor profiling

IS RECEPTOR PROFILING USEFUL FOR PREDICTING PITUITARY THERAPY?

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SHORT TITLE: pituitary receptor profiling & treatment

KEY WORDS: Pituitary; somatostatin receptors; receptor profiling; dopamine receptors

ARTICLE WORD COUNT: 4520

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ABSTRACT

Medical treatment of pituitary tumours may present important challenges in the presence of resistance to first line therapy. In this setting, the availability of specific markers of responsiveness/resistance could be helpful to provide tailored patients' treatment. Pituitary receptor profiling has emerged as a potentially useful tool for predicting the response to specific pituitary-directed medical therapy, mainly somatostatin analogues and dopamine agonists. However, its utility is not always straightforward. In fact, agonist-receptor coupling to the consequent biological response is complex and sometimes jeopardizes the understanding of the molecular basis of pharmacological resistance. Defective expression of pituitary receptors, genetic alterations, truncated variants, impaired signal transduction or involvement of other proteins, such as cytoskeleton proteins or the Aryl hydrocarbon receptor interacting protein amongst others, have been linked to differential tumour phenotype or treatment responsiveness with conflicting results, keeping the debate on the utility of pituitary receptor profiling open. Why does this occur? How can we overcome the difficulties? Is there a true role for pituitary receptor profiling in the near future?

All authors of this debate article agree on the need of prospective studies using standardized methods in order to assess the efficacy of receptor profiling as a reliable clinical predictive factor.

ABSTRACT WORD COUNT: 199

INTRODUCTION

Functioning and non-functioning pituitary tumours (PT) entail a challenging set of diseases with intrinsic diagnostic and treatment difficulties, frequently associated with increased morbidity and mortality. Tumour resection is the first-line treatment for many PT; however, surgery is not always feasible or curative and adjuvant therapy is attempted (1). Approximately 10 to 30% of patients will not respond to the currently recommended medical therapy in terms of PT bulk reduction and/or hormonal secretion inhibition. In this setting, biochemical, radiological, histological, immunological, genetic or molecular markers would be truly helpful for the individualization of patients' treatment and would enable a relevant shift in the therapeutic decision-making process towards an individualized and personalized medicine. In addition, this strategy would allow to establish realistic prognostic and predictive values of specific biomarkers, for a more efficient and cost-effective approach, especially in the long-term.

In this setting, in the era of personalized medicine, pituitary receptor profiling has emerged as a potentially useful tool for predicting the response to specific pituitary-directed treatments, mainly somatostatin analogues (SSA) and dopamine agonists (DA). This approach could allow to optimize patients' management and, consequently, reduce the burden of health care costs, as well as of side effects. However, its utility is not always straightforward. We here review and discuss the pros and cons of somatostatin (SSTR) and dopamine receptor (DR) profiling in PT and suggest future investigational perspectives.

SOMATOSTATIN AND DOPAMINE RECEPTORS IN PITUITARY TUMOURS

Pathological findings in pituitary cells have proved the existence of several cell membrane receptors, including SSTR and DR, which modulate pituitary cell proliferation and hormonal secretion. In this regard, five SSTR subtypes have been described (SSTR1-5), which are encoded by genes localized on different chromosomes, with two SSTR2 isoforms, SSTR2A and SSTR2B, generated via alternative splicing. Furthermore, two non-canonical truncated SSTR5– splice variants have been revealed and termed sst5TMD4 and sst5TMD5, due to the fact that they exhibit four and five transmembrane domains, respectively (2). The five canonical SSTRs

share 40-60% homology (3), but the elicited effects are different according to the SSTR profile on the cell surface. SSTR can form heterodimers with dopamine, opioid, epidermal growth factor (EGF) receptors or other SSTR subtypes (4, 5, 6, 7), which generate receptor oligomers with unique pharmacological profiles. Similarly to other G-protein coupled receptors, SSTRs regulate their responsiveness to continued agonist exposure by showing different degrees of receptor internalization and degradation (8), as also demonstrated in other neuroendocrine neoplasms (9). In addition, two main subtypes of DR, DR1 and DR2, are expressed in pituitary tissues (10).

PT retain SSTR and DR expression, whose activation elicits different effects, depending on the specific pituitary cell type (11). sst5TMD4, on the other hand, has been found to be rarely expressed in normal tissues, whilst it is over-expressed in PT, breast and thyroid cancer (12, 13, 14). Thus, presumably, targeted pharmacological treatments may be developed, allowing control of tumour cell metabolism. Long-acting SSA, Octreotide (OCT) and Lanreotide (LAN), have a high binding affinity for SSTR2 and, to a lesser extent, to SSTR5 and SSTR3 (3, 15), whilst the new-generation drug, Pasireotide, is a multi-receptor-targeted SSTR ligand, which binds with high affinity both SSTR2 and SSTR5 (16). In addition, Pasireotide induces rapid SSTR2 recycling to the plasma membrane after endocytosis, reducing the desensitization effect (17). Preclinical and clinical studies demonstrated the efficacy of long acting SSA in the treatment of PT, particularly GH (18) and TSH-secreting tumours (19). Moreover, international multi-center studies demonstrated the efficacy of Pasireotide in the medical management of Cushing's disease (20, 21) and GH-secreting pituitary tumours (22). Cabergoline, on its side, is an ergot-derived DA with high affinity for DR2 and lower affinity for DR1, α1- and α2-adrenergic, and 5-HT1- and 5-HT2-serotonin receptors (23, 24). Cabergoline has proved to be effective for the medical management of prolactinomas, as it allows control of clinical symptoms, prolactin (PRL) level reduction, and tumour volume shrinkage. In addition, this drug could be useful also for selected ACTH and GH-secreting PT and non-functioning PT (25, 26, 27).

However, the tandem mediator – receptor – response is not always as simple and straightforward as we would desire from a clinical point of view. In fact, there is sometimes a

dissociation between the presence of a receptor and the observed response. For instance, some tumours may express the receptor but do not respond to specific therapy, whilst some patients whose tumours do not express a particular receptor may adequately respond to targeted treatment. To complicate things even more, there have been differences in the techniques used for analyzing SSTRs across the literature; some studies detected mRNA with quantitative polymerase chain reaction (qPCR) or reverse-transcription (RT)-qPCR, others quantified the amount of protein using immunohistochemistry (IHC) or Western blot techniques, and some studies were performed *in vivo* using scintigraphy.

PRO: THE CASE FOR PITUITARY RECEPTOR PROFILING

THE SOMATOSTATIN RECEPTOR FAMILY IN THE AID OF PITUITARY TUMOUR TREATMENT

SSTRs are normally expressed in the adult pituitary, except for SSTR4. SSTR5 is the predominant subtype in normal human pituitary, followed by SSTR2, SSTR1, SSTR3 and SSTR4. Canonical SSTRs are heterogeneously expressed in PT, whereas the sst5TMD4 splice variant has been found to be overexpressed in PT, breast and thyroid cancer (12, 13, 14). Several studies focused on SSTR characterization with the use of different techniques, from qPCR (11, 28, 29, 30) to IHC (31, 32, 33) and *in vivo* scintigraphy (31, 34, 35). Some reports have characterized SSTR3 as the predominant SSTR expressed in certain acromegaly cases resistant to first-generation SSA which could explain the lack of response (36). Nonetheless, SSTR2 has proved to be the most frequently expressed SSTR reported in acromegaly studies (29). In these settings, SSTR2 expression has been the focus of many studies attempting to identify a pattern to predict SSA response. Besides SSTR heterogeneous expression, low SSTR levels or reduced receptor density may explain the proportion of patients partially or completely resistant to SSA and, therefore, pituitary receptor profiling could help predict the response to specific treatments.

SSTR2 and SSTR5: role in response to SSA

First generation SSA, OCT and LAN, are considered the first-line medical treatment in acromegaly when surgery fails to control the disease. However, 20-25% of patients present resistance to OCT and LAN treatment. Resistant patients are defined as those who show a reduction <50% in GH and IGF1 levels, a decrease of <20% in tumour mass or an increase in size during treatment (37). In general, 9 to 12 months are necessary to assess treatment outcome. Apart from an inefficient high-cost therapy, poor responsiveness leads to patient exposure to the deleterious effects of excessive GH and IGF1 levels for several months. Studies concerning PT and the possibility to predict tumour response to first generation SSA started in the late 90s. Using different techniques, such as ^{111}In-pentetreotide scintigraphy, magnetic resonance imaging, Northern blot, PCR, researchers have found a correlation between SSTR positivity and hormonal response to OCT and/or LAN (35, 38, 39, 40). For instance, the ability to visualize GH-secreting PT with ^{111}In-pentetreotide scintigraphy positively correlated with the ability to reduce GH secretion by OCT (38). A clear correlation has also been found between SSTR2 expression levels, assessed by Northern blot, and *in vivo* and *in vitro* sensitivity to OCT (39). In the following years, several studies investigated SSTR2 expression as a biomarker to predict PT response to first generation SSA. Indeed, several studies have shown that SSTR2 strongly correlates with hormonal suppression and, hence, tumour responsiveness to SSA therapy. With the advent of qPCR, SSTR expression has become accessible and has been used by the majority of studies attempting to define a correlation between SSTR expression and medical outcome. Specifically, OCT efficacy in decreasing hormonal secretion has been positively correlated with SSTR2 mRNA levels, mainly in GH-secreting tumours (11, 29, 41). The fact that SSTR2 could help predict PT response to first generation SSA has also been described in IHC reports. The use of IHC for SSTR2A detection in specimens obtained by surgery has been recommended since the results were useful in identifying patients with acromegaly who could benefit from SSA treatment (32, 33, 42, 43).

The list of evidences regarding the usefulness of SSTR profiling is long and still growing. In a series of 22 somatotropinomas, SSTR1-5 mRNA absolute copy numbers were assessed by RT-

PCR and response to OCT long-acting repeatable (LAR) was evaluated by measuring hormone levels (GH and IGF1) and tumour volume. In this study, SSTR5 was the predominantly expressed SSTR, followed by SSTR2, SSTR3, SSTR1 and SSTR4. A positive correlation was found between SSTR2 expression levels and decreased hormone secretion at 3 months, as well as tumour volume reduction after 6 months of treatment. On the other hand, SSTR5 negatively correlated with the decrease in hormone levels. Furthermore, a higher SSTR2/SSTR5 ratio was observed in controlled patients with OCT LAR when compared with those uncontrolled (29). Even though final SSTR presence on cell membrane was not evaluated in this study, the reported results support the evidence that mRNA levels may still be useful in predicting SSA responsiveness in GH-secreting tumours. In another study considering 88 somatotropinomas, Wildemberg et al. found that a low SSTR2A expression is a strong negative predictive factor for biochemical response to first generation SSA. In this study, SSTR expression was assessed by IHC and there was a positive correlation between SSTR2 mRNA and protein levels (44). Since IHC is routinely used to assess PT, IHC SSTR2 analysis could help predict responsiveness to first generation SSA and improve patient management. Indeed, another study by Gatto et al. demonstrated that the results of IHC, performed with a SSTR2A rabbit monoclonal antibody, overlap those of RT-PCR. In this study, the authors found a strong correlation between SSTR2A immunostaining and IGF1 normalization after SSA treatment (45). Interestingly, densely granulated tumours, which are better responders to SSA, have higher SSTR2A expression levels as compared to sparsely granulated tumours (46). In addition, sst5TMD4 has been reported to have a dominant-negative effect on SSTR2 signalling and could explain the resistance to first generation SSA in patients with PT displaying high SSTR2 expression (47). In a comparison study between OCT and Pasireotide, somatotropinomas that exhibited low SSTR2 and lower SSTR2/SSTR5 ratio at mRNA level were better responders to this novel SSTR ligand (48). A similar result has been described in an IHC study, where SSTR5 was found to be a predictor of response to Pasireotide in patients whose disease was not controlled by treatment with first generation SSA. None of the patients lacking SSTR5 was responsive to the treatment, whereas cases with higher SSTR5 expression showed a greater reduction in IGF1 levels. In addition, a

positive correlation between SSTR2A immunostaining, assessed with the same IHC approach, and first generation SSA response was confirmed. As expected, only cases with membranous receptor expression were responsive to the treatment, confirming that SSTR presence on cell membrane is a prerequisite for the responsiveness to these drugs (49). High SSTR5 and low SSTR2 expression has also been reported in ACTH-secreting tumours (50), where low SSTR2 levels are probably due to high circulating cortisol levels, that are not found in acromegaly. Indeed, the imbalance in SSTR2/SSTR5 expression may deeply influence corticotroph tumour responsiveness to Pasireotide, indicating that disease activity, mainly depending on circulating cortisol levels, may profoundly impair SSA efficacy in Cushing's disease (51). On the contrary, there is no evidence that GH circulating levels may affect SSTR expression, thereby influencing the responsiveness of GH-secreting pituitary tumours to SSTR ligands. Cushing's disease usually benefits from Pasireotide therapy and, in fact, this drug has been approved for the medical treatment of corticotropinomas (20, 21, 52, 53, 54).

The results of the studies reported here indicate that in GH-secreting PT high SSTR2 levels and SSTR2/SSTR5 ratio associate with responsiveness to first generation SSA, while low SSTR2 levels and high SSTR/SSTR5 ratio favours Pasireotide efficacy. In ACTH-secreting PT, on the other side, low SSTR2 levels may account for the scant efficacy of first generation SSA, supporting the use of Pasireotide, which may also act by binding SSTR5.

Other SSTR involved?

SSTR family comprises receptors different from SSTR2 and SSTR5, which could play an important role in patients poorly responsive to SSA. An *in vitro* study has demonstrated that activation of SSTR1 by a SSTR1-selective ligand decreased GH and PRL secretion, as well as reduced cell-viability, in tumour cells derived from acromegalic patients. In this study, SSTR1 mRNA levels correlated with the extent of hormone secretion inhibition induced by the SSTR1-selective agonist (55). In another *in vitro* study, the same SSTR1-selective agonist was able to inhibit chromogranin A secretion and reduce cell viability in non-functioning PT (NFPT) (56), confirming a possible role for this SSTR in SSA response. Another example is SSTR3, for

213 which an important role has been proposed in mediating tumour shrinkage in somatotropinomas
214 (36), NFPT (11) and gonadotropinomas (57).
215 Overall, these findings regarding SSTR family support the proposal that receptor pituitary
216 profiling could be a useful tool for PT patient stratification. A standardized evaluation of SSTR
217 expression, which is feasible and relatively low-cost, could represent a valid option that could
218 lead to personalized medicine in PT, allowing to optimize patient management and,
219 consequently, reduce the burden of health care costs, as well as of side effects.
220
221 IS THE DOPAMINE RECEPTOR PATHWAY USEFUL?
222 Systematic and meta-analysis studies regarding DR expression profile in the different tumour
223 cell populations are scarce, though it has been demonstrated that DR2 is present in nearly 90%
224 of all PT including PRL-, GH-, ACTH-secreting PT and NFPT (58, 59, 60). In PRL-secreting
225 tumours, DA such as Cabergoline and Bromocriptine, are the gold-standard treatment for both
226 micro- and macro-adenomas according to the Endocrine Society Clinical Practice Guidelines
227 (61, 62). A small number of patients (5 to 10%) is defined as non-responder due to resistance to
228 DA (63). It has been reported that one strategy to improve treatment in these resistant patients is
229 to gradually increase DA dose, although adverse effects should be accurately evaluated. In a
230 prospective study using high Cabergoline doses, normalization of PRL levels was achieved in
231 96.2% of patients after increasing the dose up to 12 mg per week (64). Possible predictors of
232 resistance in prolactinomas have been suggested, such as male gender and tumour diameter >1
233 cm (65, 66). Men usually harbour macroprolactinomas with symptoms of sellar mass effect at
234 presentation while microprolactinomas are usually diagnosed in women aged 20-50 years
235 presenting symptoms of hypogonadism. Some Authors have proposed that these differences in
236 presentation could be in part explained by the fact that hypogonadism in males is usually
237 neglected and medical attention is sought later, possibly allowing the development of a DA
238 resistant phenotype (i.e. due to decreased DR2 expression) (67, 68). On the contrary, other
239 Authors provide evidence for the lack of a gender prevalence in D -resistant prolactinomas (69).

Previous reports have found that prolactinomas that are Bromocriptine-resistant have a 4-fold decrease in DR2 expression when compared with responsive prolactinomas (70). In addition, a polymorphism causing a cytosine to thymine transition at position 957 in *DR2* gene has been associated with faster DR2 mRNA decay which could explain reduced receptor expression (71) and, consequently, DA resistance. Fusco et al. reported a comparison study between the efficacy of SSA, Cabergoline and a chimeric SSA-DA compound (BIM-23A760) in suppressing PRL secretion in DA-resistant prolactinomas. The results of this study confirmed that compounds targeting DR2 remain the best option in the treatment of prolactinomas. Moreover, DR2 mRNA levels in DA-resistant tumours were significantly lower as compared to those found in DA-sensitive prolactinomas, indicating a possible correlation between DR status and DA response (72). Failure of medical treatment usually leads to transsphenoidal surgery, the second line treatment, in particularly resistant subjects. Pituitary receptor profiling could help predict receptor target status, aiding the selection of the best therapeutic approach.

The results of the studies reported here indicate that DR2 status could influence PRL-secreting tumours responsiveness to DA. DR decreased expression levels may account for the limited DA efficacy in resistant patients and other medical therapies, such as chimeric SSA-DA compounds, should be considered.

AGAINST: THE CASE AGAINST PITUITARY RECETOR PROFILING

THE SOMATOSTATIN RECEPTOR PATHWAY: A CUMBERSOME ROUTE

The occasional lack of correlation observed between the use of SSA and its associated pathological and clinical responses may be explained by several reasons. First, there is a variable expression of SSTR in different tumours. Second, the expression and methods for detection of SSTRs have been variable across studies. In addition, the treatment used in different studies has not always been the same; some studies were performed using first generation SSA, whilst others used the newly-available Pasireotide. Another point of heterogeneity is whether patients had been pretreated or not with SSA. In addition, it is also important to know which outcome was measured, that is, if they were evaluating the decrease in

GH and IGF1 levels and/or if they were studying a possible decrease in tumour volume. All these heterogeneities encumber trustworthy and comparable conclusions.

Variability in the expression of SSTRs

Pituitary tumours may be heterogeneous regarding the expression of specific receptors, both from a qualitative and quantitative point of view. For instance, some tumours express low levels of SSTR2, but high levels of SSTR5 (46, 73, 74); other tumours express low levels of both SSTR2 and SSTR5. The former situation could lead to an incomplete resistance to first generation SSA that mainly exert their action by bibding SSTR2, and the latter could still be sufficient for an adequate response to SSA because of an additive effect mediated by a functional interaction between SSTR2 and SSTR5.

Heterogeneity in the techniques used for analyzing SSTRs

Concerning studies in the setting of acromegaly, there have been differences in the techniques used for analyzing SSTRs; some studies detected mRNA with qPCR or RT-qPCR, others quantified the amount of protein using IHC or Western blot techniques, and some studies were performed *in vivo* using scintigraphy. It would be truly interesting if this latter technique could predict SSA response, but large studies have failed to prove its relevance (34, 38).

Although many studies have reported a correlation between SSTR measurement and response to SSA, this has not been always the case. In this regard, Corbetta et al. (75) did not find any significant correlation between SSTR, measured by mRNA, and *in vivo* responsiveness in 19 somatotropinomas. Park et al. (28) could not prove a correlation between SSTR2 and SSTR5 mRNA expression and GH values in 16 somatotropinomas. Gonzalez et al. (76) reported that neither SSTR2 nor SSTR5 expression correlated with baseline or post OCT GH or IGF1 levels or tumour volume by qPCR or IHC in 60 somatotropinomas. Takei et al. (42) studied 22 somatotropinomas using IHC and found that SSTR2 protein levels correlated positively with GH suppression, but, in contrast, SSTR5 did not show any correlation with GH levels. Ibañez-Costa et al. (77) evaluated IHC and function in 32 somatotropinomas and described that OCT

and LAN significantly and similarly decreased GH secretion and cell viability, without evident correlation between the response and SSTR expression pattern. Moreover, analysis of the differences reported in studies that used IHC denote a wide range of heterogeneity, mainly because of the employed antibody (monoclonal vs. polyclonal antibodies), the studied cell fraction (cell membrane fraction or both cytoplasm plus cell membrane fraction), and the scoring system (intensity, amount or the Remmele IRS score, etc..) (42, 44, 45, 78, 79, 80). A universal scoring system has been proposed (45) to try to overcome some of these heterogeneities when comparing different SSA actions.

Preoperative treatment and specific SSA used

While in some studies patients were treated with first generation SSA (OCT and LAN), some patients received therapy with the newly-available Pasireotide. As first generation and the new SSTR agonist interact with different SSTR, results are not always overlapping.

Regarding pre-surgical therapy with SSA, SSTR2 expression is probably consequently reduced, thus encumbering a reliable interpretation on the relationship between SSTR expression and GH- IGF1 reduction, which becomes even more difficult to interpret. In the study by Casar-Borota et al. (78) of 65 somatotropinomas treated with OCT, SSTR2 expression levels were reduced in the pretreated group and positively correlated with GH and IGF1 reduction. However, no correlation was found between tumour volume reduction and SSTRs. Fougner et al. (81), on their part, evaluated 71 somatotropinomas also treated with OCT and found that SSTR2 was positively correlated with GH reduction after an acute OCT test in patients who did not receive SSA pre-treatment but, in contrast, they did not find any correlation in patient who were pre-treated with OCT. Moreover, SSTR2 protein level assessed by Western blot did not correlate with the response to OCT.

Outcome evaluation: GH/IGF1 reduction and/or tumour volume reduction

Many studies have evaluated GH and IGF1 response to SSA. However, there are less studies that have evaluated SSA efficacy regarding tumour volume reduction. In this regard, although

there are some studies that found a relationship between tumour volume reduction and SSTR2 expression (29), no relationship was found in others.

All the above-mentioned findings regarding SSTR expression jeopardize trustworthy interpretation of studies and elaboration of definitive conclusions. In fact, measurement of SSTR expression may be promising, but it is difficult, and its utility for the prediction of clinical outcomes is not so straightforward. The presence of SSTR does not unequivocally ensure an adequate response to SSA.

Impaired signal transduction

Several mechanisms are involved in the impairment of signal transduction mediated by SSTRs, which concern the SHP1-PI3k-Akt, SHP2-MAPK, PLC, PKA A, RKIP, gsp, AIP-ZAC1, e-cadherin, B-arrestin and filamin A pathways. These pathways may be defective, so the corresponding migration, angiogenesis, secretion, proliferation and apoptosis functions may be impaired, leading to potential variability in the efficacy of SSA (45, 82, 83).

Specifically, the presence of the SSTR5-truncated isoforms has been correlated with a reduced response to SSA in GH-secreting tumours; for instance, sst5TMD4 was reported as particularly abundant in OCT-resistant somatotropinomas, suggesting its potential role in the attenuated response to SSA observed in some PT (84). The aryl hydrocarbon receptor interacting protein (AIP) is another issue of interest, since several studies have observed how its low expression may be also associated with a worse response to SSA (82, 85). B-arrestins, on their part, seem to affect the desensitization–internalization process of G-protein-coupled receptors, including SSTR, and they have been involved, for instance, in the recycling of SSTRs in GH- and PRL-secreting PT. Thus, low expression of B-arrestin in GH- and PRL-secreting tumours, in comparison to NFPT, has been associated with reduced recycling rate of SSTR2, a higher amount of biologically active receptor exposed on the cell membrane, and a better response to SSA in terms of GH suppression, both *in vitro* and *in vivo* (86). In addition, low levels of filamin A, which is required for both DR2 and SSTR2 intracellular signalling, have been

associated to a worse response to SSA (86). In addition, the expression of E-cadherin in somatotroph PT was related to tumour size, invasiveness, and SSA response (87).

Therefore, other variables different from SSTR expression levels may influence SSA responsiveness and should be taken into account when trying to explore predictive factors of PT therapeutic response.

THE DOPAMINE RECEPTOR PATHWAY: ANOTHER CHALLENGE TO OVERCOME.

It has been extensively demonstrated, with various techniques, that DR are expressed in the large majority of PT, including GH-, PRL-, ACTH-secreting, and NFPT. In this setting, DA seem a relevant targeted treatment, mediated through their interaction with DR. We know less about DR expression in prolactinomas, since only around 10% of patients with these tumours finally undergo surgery, in most cases due to DA failure to normalize hormone secretion and reduce tumour volume. However, findings up to date do not seem to aid unequivocally in the prediction of clinical outcomes in prolactinomas. In fact, although the majority of reports show an apparent association between DR2 expression and DA response, some studies have not corroborated this correlation. A possible bias that we must consider is precisely the fact that only those prolactinomas which behave more aggressively and are resistant to medical treatment are the ones that have provided molecular information. In this regard, resistance to DA has been linked to a reduction or loss of DR2, to variations in the ratio between the short and long isoforms, which are thought to activate distinct intracellular pathways and mediate differential effects following ligand activation, or to post-receptor mechanisms. Caccavelli et al. (70) indeed found an association between response to Bromocriptine and DR2 expression in prolactinomas and observed that resistant tumours showed a reduced binding to DR2. In the same line, Fusco et al. (72) described an apparent association between lower DR2 levels and reduced response to DA, with no relationship with SSTR status. However, Shimazu et al. (88) did not find an association between DR2 status and resistance to Cabergoline in 12 surgically treated resistant prolactinomas that were studied at mRNA and IHC level. They described that resistance to Cabergoline was correlated with a reduction in DR2 long isoform levels.

If we consider the setting of acromegaly, Ferone et al. (43) found that SSTR2 and DR2 were positively correlated with *in vitro* and *in vivo* percent GH suppression by OCT in 24 somatotropinomas. In contrast, Neto et al. (58), who evaluated 39 somatotropinomas and observed a predominant expression of DR2, could not prove a significant difference in the expression of this receptor between controlled and uncontrolled acromegalic patients.

In light of these reports, where there is not a complete agreement regarding the relationship between receptor expression and DA response, we cannot assure that knowledge of pituitary DR status will allow straightforward predictions on medical outcome. Moreover, aberration of subsequent molecular pathways involved in the DA-DR interaction, including the ones related to NGF receptors, filamin A and Gαi2, may contribute to heterogeneities in the observed response (63, 89). DR status may probably help to predict response to DA, but evidence is still not sufficient to establish fully accurate conclusions.

CONCLUSIONS

Based on the available information up to date, we cannot conclude in favour or against profiling pituitary receptors as a useful tool to predict PT treatment response. Indeed, the evidence that SSTR profiling predicts response to SSA treatment in GH-secreting PT is supported by some studies but not by others. Many other variables should be taken into account, including variations in other proteins, such as AIP, ZAC, B-arrestin, filamin or E-cadherin, that may lead to dissociated responses. Moreover, heterogeneity in measuring techniques may disguise the true relevance of SSA/DA receptor profiling and differences across studies in the treatments used or the criteria considered may bias the resulting conclusions (Figure 1).

There is a lack of systematic reviews and meta-analyses regarding receptor subtyping and definition of medical outcome. In addition, most studies lack an analysis of sensitivity and specificity. We need prospective studies of patients after surgery using standardized methods. It would be very important to assess the quality of antibodies in IHC studies, as well as the development of a uniform and standardized scoring system, to facilitate routine evaluation of SSTR and DR expression in PT and allow reliable clinical predictions.

DECLARATION OF INTEREST

The Authors declare they have no conflict of interest concerning the subject of this debate.

FUNDING

This contribution did not receive specific funding.

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743

744 FIGURE LEGENDS

745 Figure 1: Summary of the main concerns regarding the advantages and disadvantages of
746 pituitary receptor profiling for the management of pituitary tumours. “SSTR”: somatostatin
747 receptors; “SSA”: somatostatin analogues; “DR”: dopamine receptors”.

Figure 1

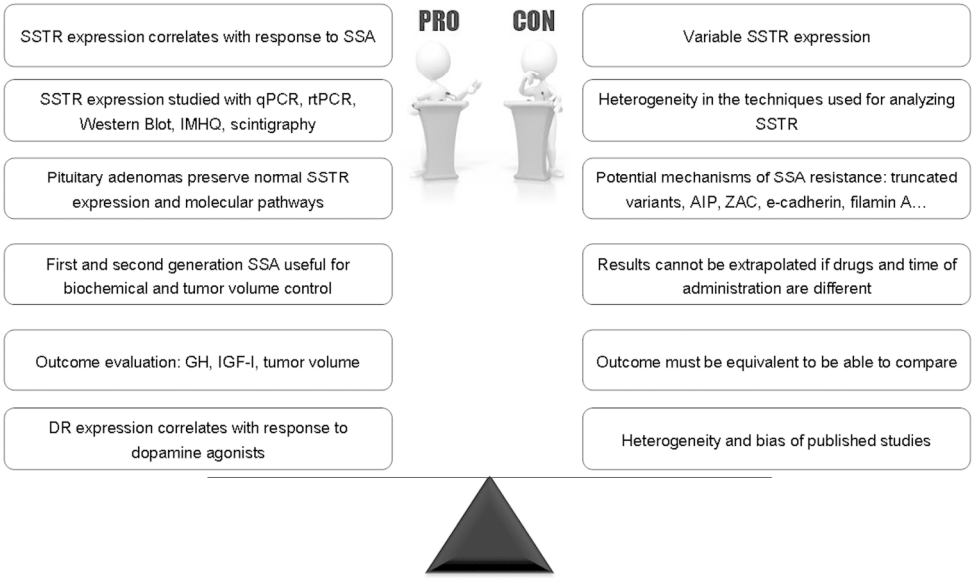

Figure 1

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