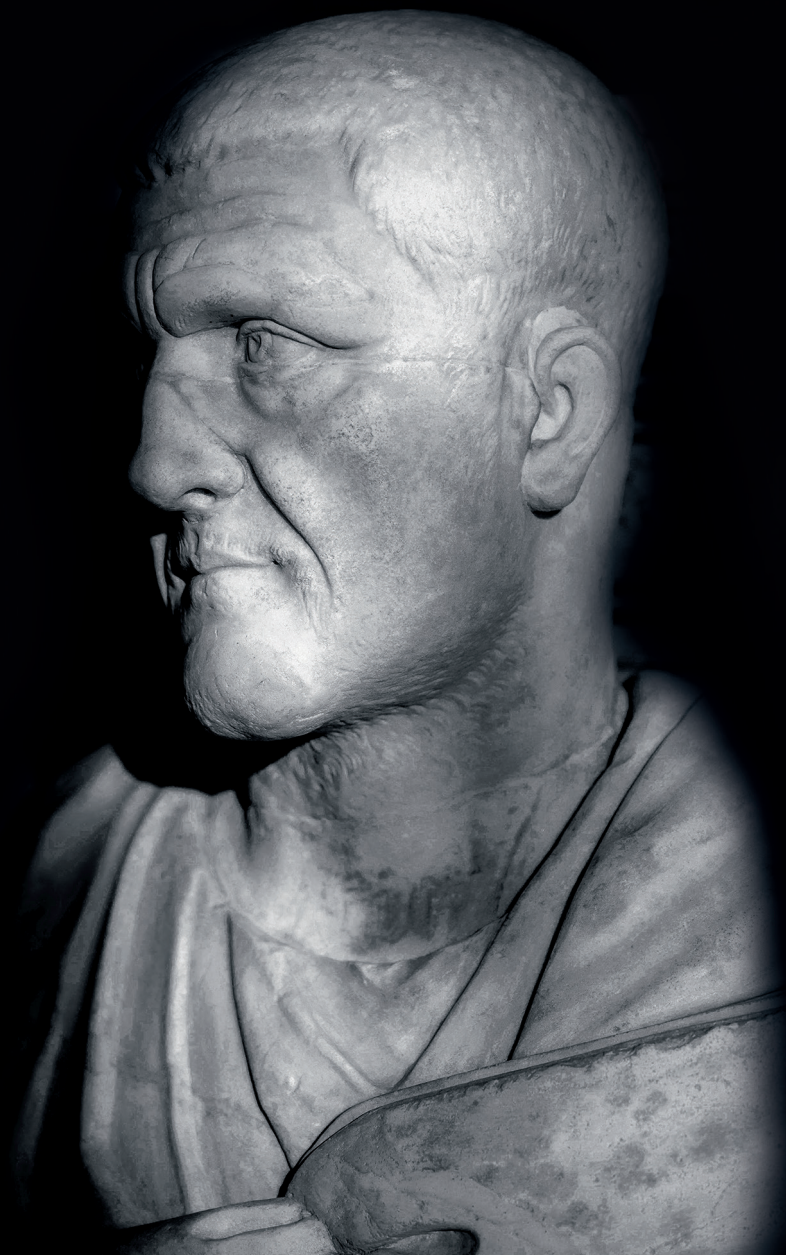


Acromegaly: current and emerging treatment options

Eva C. Coopmans



Acromegaly: current and emerging treatment options

Eva Christine Coopmans

Printing of this thesis was supported by:

Recordati B.V.

Pfizer B.V.

IPSEN Farmaceutica B.V.

Eurocept Homecare

Chipsoft

ApotheekZorg

Erasmus University Medical Center

Colofon

Author:	Eva C. Coopmans
Cover design en lay-out:	Miranda Dood, Mirakels Ontwerp
Printing:	Gildeprint - The Netherlands
ISBN:	978-94-6419-268-1

Copyright © 2021 Eva C. Coopmans, Rotterdam, The Netherlands

All rights reserved. No part of this thesis may be reproduced, distributed, or transmitted in any form or by any means, electronic or mechanical, without the prior written permission of the author, or where appropriate, of the publisher of the article.

Acromegaly: Current and Emerging Treatment Options

Acromegalie: Huidige en toekomstige behandelopties

Thesis

to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus

Prof.dr. F.A. van der Duijn Schouten

and in accordance with the decision of the Doctorate Board.

The public defence shall be held on
Wednesday 8 september 2021
at 15.30 hrs
by
Eva Coopmans
born in Zwijndrecht, Netherlands.

Doctoral Committee:

Promotor: prof.dr. A.J. van der Lelij

Copromotor: dr. S.J.C.M.M. Neggers

Other members: prof.dr. N.R. Biermasz
prof.dr. L.J. Hofland
prof.dr. M.W. Vernooij
prof.dr. P.H.L.T. Bisschop
prof.dr. C.M.F. Clemens
dr. A.F. Daly
prof.dr. P.M. van Hagen
prof.dr. W.W. de Herder

Paranymphs: drs. A. Blažević
drs. E.S. van der Valk

To Nico and my parents, without whom this thesis would not be.

Table of contents

Chapter 1	General introduction and outline of the thesis.	08
<hr/>		
Part I: Determinants of response to somatostatin analogues		
<hr/>		
Chapter 2	Multivariable prediction model for biochemical response to first-generation somatostatin receptor ligands treatment in acromegaly.	44
Chapter 3	Pasireotide responsiveness in acromegaly is mainly driven by somatostatin receptor subtype 2 expression.	68
Chapter 4	T2-signal intensity, SST receptor expression, and somatostatin analogs efficacy predict response to pasireotide in acromegaly.	94
<hr/>		
Part II: Pasireotide: mechanism of action and clinical applications		
<hr/>		
Chapter 5	Efficacy and safety of switching to pasireotide in acromegaly patients controlled with pegvisomant and somatostatin analogues: PAPE extension study.	120
Chapter 6	Potential antitumour activity of pasireotide on pituitary tumours in acromegaly.	140
<hr/>		
Part III: Emerging treatment option: ketogenic diet in acromegaly		
<hr/>		
Chapter 7	Eucaloric very-low-carbohydrate ketogenic diet in acromegaly treatment.	154

Part IV: Role of AIP variants in pituitary adenomas

Chapter 8	The role of AIP variants in pituitary adenomas and concomitant thyroid carcinomas in the Netherlands: a nationwide pathology registry (PALGA) study.	162
------------------	--	-----

Part V: General discussion and future perspectives

Chapter 9	General discussion and future perspectives.	186
------------------	---	-----

Part VI: Summary, Samenvatting

Summary	224
Samenvatting	227

List of abbreviations	232
Erasmus MC PhD Portfolio	234
Publications not in this thesis	239
Word of thanks	240
About the author	246



Chapter 1

General introduction
and outline of the thesis

1

Acromegaly

An introduction to the disease

Acromegaly (enlargement (*megaly*) of the extremities (*acral*)) is a rare endocrine disorder characterized by increased growth hormone (GH) and, consequently, insulin-like growth factor I (IGF-I), usually as a result of a GH-producing pituitary tumour⁽¹⁾. Both acromegaly and gigantism are rare disorders that are caused by excessive secretion of GH and IGF-I; however, gigantism occurs prior to epiphyseal closure before the end of puberty and leads to increased linear growth, whereas acromegaly occurs when GH excess is present after epiphyseal closure⁽²⁾.

Historical context

From ancient history throughout the modern age, individuals of abnormal stature have figured prominently in legends, myths and origin stories. Examples of extraordinary physical proportions are Goliath, Hercules, or Bigfoot. The figure of the giant is typical a warlike character with superhuman strength and size. These figures consequently inspired a sense of awe and enthrallment, leading to a public fascination with giant acromegalic and acromegaly patients who were objects of spectacle in circuses or toured for money (Figure 1). At the end of the 19th-century medical science was concerned with understanding the pathogenesis, and the stage was set for further research into the pathological mechanisms underlying the disease of gigantism and acromegaly.

Although the Dutch physician Johannes Wier (1515?-1588) was in 1567 the first to clearly describe the characteristic clinical picture of a patient with the disease, the term ‘acromegaly’ was in 1886 coined by the French neurologist Pierre Marie (1853–1940). Although Pierre Marie was aware of the enlarged pituitary gland he did not describe this as cause of the disease.

Pierre Marie wrote (in French): “*Il existe une affection caractérisée surtout par une hypertrophie des pieds, des mains et du visage, que nous proposons d'appeler acromégalie, c'est-à-dire hypertrophie des extrémités (non pas qu'en réalité, les extrémités soient seules atteintes pendant toute la durée de la maladie, mais parce que leur augmentation de volume est un phénomène initial et constitue le trait le plus caractéristique de cette affection). L'acromégalie est tout à fait distincte du myxœdème et de la maladie de Paget (ostéite déformante), ainsi que de la leontiasis ossea de Virchow*”⁽³⁾

[A condition characterized by hypertrophy of the hands, feet and the face exist which we propose to be called ‘acromegaly’ which means hypertrophy of the extremities. In reality the extremities are swollen during the disease course and their increase in volume is the most characteristic feature of this disease. Acromegaly is different from myxoedema, Paget’s disease or leontiasis ossea of Virchow’.]

However several others had described the clinical condition before him ⁽⁴⁾, using different names for the disorder, such as ‘prosopectasia’ (widening of the face) or ‘macrosomia’ (abnormally large body size). This period was followed by the publication of large bodies of work on acromegaly, including the discovery of Minkowski ⁽⁵⁾ of the link between the pituitary gland and acromegaly: pituitary enlargement (*i.e.* caused by a pituitary adenoma) was the cause and not the consequence of acromegaly, as initially thought. Moreover, it became clear that gigantism and acromegaly were part of the same coin and not two entirely different disorders ⁽⁶⁾. Subsequently, the cause of acromegaly could be further determined after the discovery of GH and IGF-I and its association with pituitary hyperfunction and not hypofunction of the pituitary tumour ⁽⁷⁾.

FIGURE 1. A historical patient with stage name giant Constatin with pituitary gigantism.



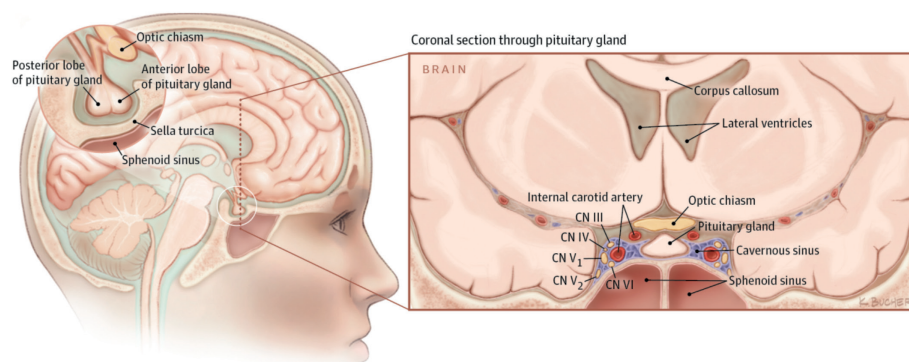
Left picture source: <https://wellcomecollection.org/works/jpy3unnm>, CC-BY-4.0. Right picture source: Beckers AB et al, Paleogenetic study of ancient DNA suggestive of X-linked acrogigantism, *ERC*. 2017; 24(2):17-20. Image reproduced with permission of the rights holder, *ERC*.

Anatomy

The pituitary gland is pea-sized and is located in the sphenoid bone (sella turcica), a recess in the sphenoid bone, below the hypothalamus (Figure 2). The pituitary gland is composed of the anterior lobe (adenohypophysis) and the posterior lobe (neurohypophysis). The anterior lobe is composed of three parts:

- Pars distalis, which secretes anterior pituitary hormones;
- Pars intermedia, which is considered rudimental in humans;
- Pars tuberalis, which surrounds the infundibulum.

FIGURE 2. Anatomy of the pituitary gland and surrounding structures.



Source: Molitch ME, *Diagnosis and treatment of pituitary adenomas: a review*, JAMA. 2017; 317:516-524. Image reproduced with permission of the rights holder, JAMA.

The optic chiasma (where the optic nerves intersect) is located on top of the superior part of the pituitary gland (Figure 2). Lesions compressing the chiasm produce bitemporal hemianopia (visual field deficit), where there is loss of the temporal fields. The internal carotid artery supplies the infundibulum, median eminence, pars tuberalis and posterior lobe, whereas mainly venous drainage from the infundibulum and the posterior lobe of

the pituitary gland supplies the pars distalis. The anterior pituitary gland is especially vulnerable to disturbances in blood supply because of the venous blood supply.

The cavernous sinuses are paired dural venous sinuses and constitute a thin-walled venous network (Figure 2). They receive blood from the superficial middle cerebral vein, inferior and superior ophthalmic veins, and the intercavernous and the sphenoparietal sinus. The cavernous sinus transmits multiple cranial nerves (CN) to the superior orbital fissure and foramen rotundum and includes in the lateral wall from superior to inferior the oculomotor nerve (CN III), trochlear nerve (CN IV) and two trigeminal nerves (CN V; ophthalmic and maxillary division). A structure that is crossing through the cavernous sinuses is the abducens nerve (CN VI) together with the internal carotid artery (carotid siphon).

The posterior lobe of the pituitary gland consists of mainly axons of hypothalamic neurons that produce oxytocin and antidiuretic hormone (vasopressin). The anterior lobe of the pituitary gland integrates hormonal signals that control adrenal, thyroid, growth, metabolic and reproductive functions. Five distinct cellular compartments within the pituitary gland secrete specific hormones (GH, LH, FSH, TSH, ACTH and prolactin) in response to hypothalamic as well as intra-pituitary and peripheral hormonal and growth factor signals.

Growth hormone

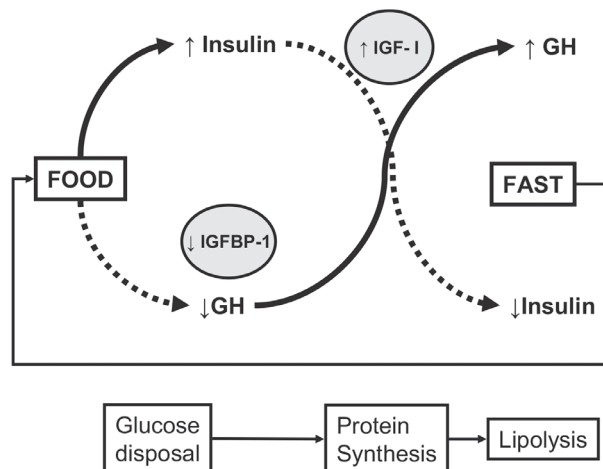
Growth hormone is secreted as a 191-amino-acid polypeptide and is entering the circulatory system in a pulsatile fashion. Endogenous somatostatin was identified by the research group of Roger Guillemin and Andrew Schally, both Nobel Prize winners in Physiology or Medicine for their discoveries concerning “the peptide hormone production of the brain” in 1977. Somatostatin was described as a hypothalamic-inhibiting peptide, which inhibited GH secretion of the pituitary⁽⁸⁾. To date we know that the central control of the secretion of GH is under dual hypothalamic control by hypothalamic-releasing (GH releasing hormone (GHRH)) and hypothalamic-inhibiting hormones (somatostatin (SST)/somatotropin release inhibitory factor (SRIF)). These hormones traverse the hypophyseal portal system and impinge directly the somatotrophs (*i.e.* pituitary cells that produce GH). However, the peripheral action of GH is mediated by a GH receptor, which is predominantly expressed in the liver and cartilage. GH acts directly on target tissue, but also induces the synthesis of peripheral insulin-like growth factor I (IGF-I) from

the liver. IGF-I is a 70-aminoacid polypeptide with endocrine, paracrine, and autocrine effects, which shares structural similarity with proinsulin⁽⁹⁾. It is mainly produced by the liver (accounting for around 75% of circulating IGF-I) secondary to GH and insulin endocrine stimulation in the liver.

Effect of GH on glucose, lipid, and protein metabolism

GH has both catabolic and anabolic actions. GH is mainly a catabolic hormone during fasting, stress or in the absence of IGF-I stimulation whereas it becomes an anabolic hormone during feeding or in the presence of IGF-I stimulation. The effects of GH on substrate metabolism in humans are simple: GH switch from carbohydrate utilization during conditions of food surplus to lipolysis and lipid oxidation when food is sparse (Figure 3).

FIGURE 3. Schematic integration of the feast and famine cycle.



The "feast and famine cycle" by Rabinowitz and Zierler⁽¹⁰⁾, according to which substrate metabolism cycles between feast and famine in three phases. In the immediate postprandial period, insulin acts alone to build up glycogen and fat. In the intermediate phase, insulin and GH act in synergy to promote IGF-I production and bioactivity and subsequent building up protein. In the remote postabsorptive or fasting state, GH acts alone and is triggering fat mobilization and utilization. Source: Møller N et al, Effects of Growth Hormone on Glucose, Lipid, and Protein Metabolism in Human Subjects, *Endocrine Reviews*. 2009;30(2):152-77. Image reproduced with permission of the rights holder, *Endocrine Reviews*.

Insulin and IGF-I synthesis and secretion are also coordinately regulated by changes in food intake (Figure 3). When inadequate carbohydrates are ingested during fasting or a very-low-carbohydrate ketogenic diet (*i.e.* ketogenic diet) the body is deprived of dietary sugars and starches and it will decrease its portal insulin levels. In this metabolic state, the liver converts fatty acids into ketone bodies that can provide fuel to the body. Portal insulin is the driver of the GH receptor (GHR) expression on hepatocytes (*i.e.* the cells secreting most of circulating IGF-I). Because reduced portal insulin secretion down-regulates hepatic GHR⁽¹¹⁾, and therefore reduces hepatic IGF-I synthesis^(9, 12). This mechanism is used by nature to survive prolonged fasting to reduce the unwanted hypoglycemic effects of IGF-I and to increase GH secretion that stimulated lipolysis and gluconeogenesis. Therefore, a ketogenic diet can be considered a fasting-mimicking diet.

In healthy participants, GH levels increase and IGF-I levels decrease during fasting or a carbohydrate restriction^(13, 14). Previous studies in acromegaly patients indicate that fasting decreases the portal insulin concentration, leading to a reduced hepatic IGF-I synthesis^(9, 12). More about this topic is written in the outlines of this thesis and Chapter 7.

Epidemiology

The estimated prevalence of acromegaly is 2.8–13.7 cases per 100,000 individuals with an annual incidence rate of 0.2–1.1 cases per 100,000 individuals^(15–17). The diagnosis is usually made in the fifth decade of life with still a considerable duration of active disease until diagnosis of median five years. This diagnostic delay duration of active disease until the diagnosis is the main determinant of the severity of most acromegaly complications^(18, 19). These data emphasize that further education and enhanced awareness of acromegaly among clinicians is important.

Pathophysiology

Acromegaly develops in the majority of cases ($\geq 95\%$) when anterior pituitary somatotroph tumours proliferate and oversecrete GH into the peripheral circulation. However, in rare cases acromegaly may arise from a familial tumour or be due to pituitary hyperplasia or ectopic secretion of GH or GHRH from a pancreatic tumour, lymphoma or carcinoid⁽²⁰⁾. Overall, these tumours are benign tumours arising from glandular epithelial tissue and do not metastasize. However, they may impinge on critical parasellar neural structures and act aggressively. GH and IGF-I both dependently and independently impact the peripheral tissues to increase somatic growth and metabolic dysfunction⁽¹⁾.

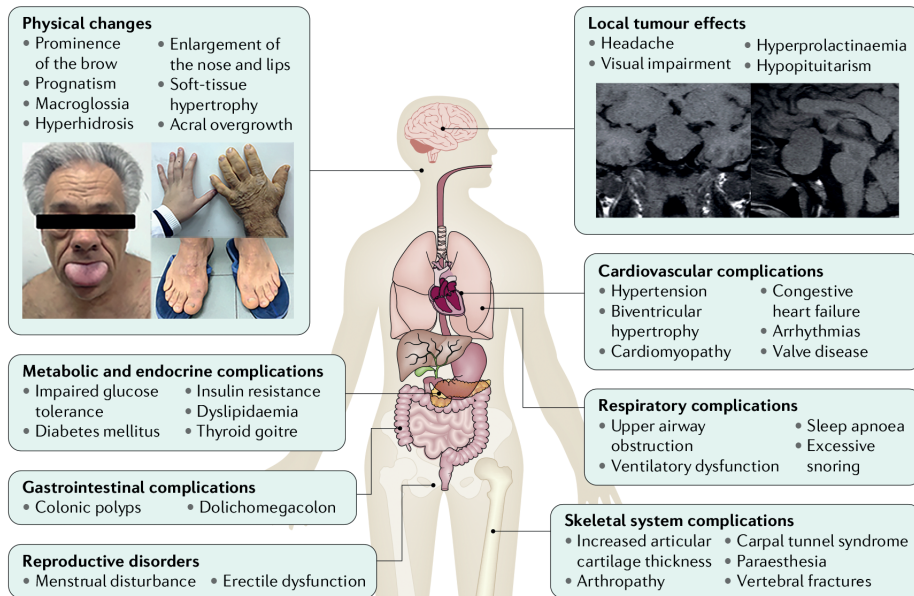
Based on the largest diameter of the tumour mass on a magnetic resonance imaging (MRI), pituitary tumours are classified into microadenomas (<10 mm), macroadenomas (≥10 mm), and giant adenomas (≥40 mm). Most GH-secreting pituitary tumours are macroadenomas⁽²¹⁾.

The vast majority of the GH-secreting pituitary tumours occur sporadically, however, their pathogenesis remains elusive. Novel germline mutations and somatic mutations have been identified that disrupt intracellular pathways in GH-secreting pituitary tumours associated with some forms of gigantism and familial acromegaly. For example, X-linked acro-gigantism (X-LAG) is a pediatric disorder caused by an Xq26.3 genomic duplication and characterized by early-onset gigantism syndrome resulting from an excess of G^H⁽²²⁾. Hereditary acromegaly is rare and may occur in association with Carney complex, McCune-Albright syndrome and multiple endocrine neoplasia type 1^(23, 24) or may occur as isolated familial pituitary adenomas.

Rare cases of familial acromegaly have been reported in families with a predisposition for GH-secreting pituitary tumours in childhood or young adulthood, and up to 30% of these tumours have been linked to germline mutations in *AIP* (the gene for aryl hydrocarbon receptor-interacting protein)^(24, 25). Low penetrance of *AIP* mutations with an autosomal dominant predisposition has been reported in approximately 5% of sporadic acromegaly. Tumours in acromegaly patients with germline *AIP* mutations show aggressive behaviour and are known to have a poor response to first-generation somatostatin receptor ligands (SRLs)^(23, 26). In contrast, the common somatic mutation in guanine nucleotide-binding protein α -stimulating polypeptide (*GNAS*, ~40%) are more frequently found in patients with less aggressive tumours that respond well to first-generation SRLs^(27, 28).

Clinical symptoms and co-morbidities

Prolonged exposure to excess GH or IGF-I leads to enlargement of the extremities and facial features and a wide range of systemic manifestations that are associated with increased mortality. The most common manifestations of acromegaly are acral and facial overgrowth, including exaggerated growth of the hands and feet, prognathism; and soft tissue hypertrophy⁽²⁹⁻³¹⁾ (Figure 4). The presence of a pituitary tumour can result in tumour mass effects, including headache, bitemporal hemianopia and decreased acuity⁽³⁰⁾.

FIGURE 4. Clinical manifestations of acromegaly.

Source: Colao A, Acromegaly, *Nature Reviews*. 2019; 4; 5(20):1-17. Image reproduced with permission of the rights holder, *Nature Reviews*.

Acromegaly is also associated with nonspecific symptoms, including headache, hyperhidrosis (excessive sweating), fatigue, headache, carpal tunnel syndrome, colon polyps, sleep apnea, reproductive disorders and osteoarthritis. If untreated, acromegaly leads to systemic manifestations that are associated with increased mortality, such as type 2 diabetes or glucose intolerance, hypertension, OSAS, cardiovascular diseases, hypopituitarism as well as malignant neoplasms^(19, 29-31). For example, the frequency of differentiated thyroid carcinomas (DTCs) is increased in patients with acromegaly, with papillary thyroid carcinoma (PTC) being the most frequently reported type (up to 25% with a standardized mortality ratio of 3.99)⁽³²⁻³⁸⁾. Given the frequency of malignant thyroid tumours in these patients, the potential for a common mechanism behind both tumours remains valid. However, it is more likely that the higher frequency of malignant thyroid tumours in these patients derived from increased screening programs.

Diagnosis

When acromegaly is suspected based on the presence of typical clinical symptoms of acromegaly, a biochemical diagnosis is confirmed by detection of high serum levels of GH that are not suppressed during an oral glucose tolerance test (OGTT) and increased serum IGF-I concentrations⁽³⁹⁾. Large inter- and intra-individual variability exists between the different IGF-I assays. When monitoring IGF-I levels over time, it is recommended that the same immunoassay is used that adhere to the WHO international standards for IGF-I and use the appropriate normative data⁽⁴⁰⁾. Following biochemical diagnosis, contrast-enhanced MRI of the sellar region is required for accurate tumour localization and assessment of local compressive mass effects. Diagnostic workup must focus on tumour mass effects (*e.g.* visual field and acuity), evaluation of pituitary-reserve function and in young patients (<30 years of age) with acromegaly or gigantism on genetic analysis of the *AIP* gene⁽⁴¹⁾.

Treatment of acromegaly

Treatment modalities should aim at normalizing GH and IGF-I levels, controlling tumour volume, improving clinical signs and symptoms, preventing systemic comorbidities, thereby reducing mortality^(39, 42). Depending on patient characteristics and tumour size and localization the possibilities range from surgery, medical treatment, radiotherapy or a combination of these. Treatment of acromegaly is complex and most cases require a stepwise, multidisciplinary approach to control the disease.

Surgery

Transsphenoidal surgery (TSS) remains a cornerstone treatment for most patients with acromegaly^(39, 43, 44), especially in those harbouring a microadenoma or well-defined intrasellar macroadenoma^(45, 46). Surgery is the only treatment that can provide cure and results in lower lifetime treatment costs⁽⁴⁷⁾. Biochemical remission is assessed three months after surgery according to the latest consensus criteria on acromegaly therapeutic outcomes⁽⁴²⁾:

- An age- and sex normalized IGF-I level (standard deviation score of ≤ 2);
- A random GH <1.0 ug/L or a nadir GH after OGTT <0.4 ug/L.

Both transsphenoidal techniques, microscopic vs endoscopic, provide comparable results in acromegaly, regardless of the size or degree of invasion⁽⁴⁸⁾. The transsphenoidal approach is effective with biochemical remission rates up to 80% when performed by an experienced surgeon. However, postoperative remission rates are much lower (about 50%) in patients with macroadenomas, often with suprasellar extension. Therefore, in patients for whom surgery is contra-indicated, in whom prefer medical treatment, or in whom postoperative remission is not achieved, adjuvant treatment is needed. This is primarily in the form of medical treatment with radiotherapy generally reserved as a third-line treatment option^(44, 49)

Radiotherapy

Radiotherapy is indicated as a third-line therapy after unsuccessful surgery and if medical therapy is unavailable, unsuccessful or not tolerated⁽³⁹⁾. Conventional fractionated radiotherapy is administered in at least 20 fractions, eventually reaching a total dose of 40-50 Gray (Gy)⁽⁹¹⁾. Stereotactic radiosurgery has been developed to provide more precise targeting of the tumour with a high-dose radiotherapy. Another irradiation technique is stereotactic radiosurgery using gamma knife, which delivers a single tumour-focused radiation fraction and allows exact focusing with minimal surrounding tissue exposure, especially to the optic tract. Stereotactic radiosurgery is preferred over conventional radiation therapy unless there is substantial residual tumour burden or the tumour is too close to the optic chiasm resulting in an exposure of more than 8 Gy^(49, 50). Stereotactic radiosurgery may be associated with a better biochemical remission (52% vs 48% remission at 10 years) and lower risk of radiation-induced hypopituitarism with at least one deficient axis^(50, 51). Tumour growth is usually arrested over several years, and pituitary tumour-derived GH-hypersecretion may persist during the initial years⁽⁵²⁾.

Medical treatment

In the most recent consensus statement by Melmed *et al.*, it is recommended that medical therapy in acromegaly patients is advised for patients with persistent disease activity despite surgical resection of the tumour as well as patients in whom surgery is not appropriate⁽⁴²⁾. The medical treatment options are as follows: dopamine agonist (*e.g.* cabergoline), long-acting first-generation SRLs, the GH receptor antagonist pegvisomant and the second-generation SRL pasireotide long-acting release (LAR).

First-line medical therapy

First-generation somatostatin receptor ligands

First-generation SRLs monotherapy with lanreotide Autogel (LAN) or octreotide long-acting release (LAR) represents the mainstay of medical therapy for acromegaly⁽⁴²⁾.

First-generation SRLs act predominantly by binding and activating the somatostatin receptor subtype 2a (SST_{2a}) and SST₅ receptor, and to a lesser extent SST₁ receptor⁽⁵³⁻⁵⁵⁾. A high SST₂ protein expression of the pituitary tumour is the main pathophysiological rationale for its efficacy in acromegaly. Besides direct inhibition of pituitary GH secretion, which leads to a lower hepatic IGF-I production, first-generation SRLs can indirectly suppress IGF-I production by the liver by reducing portal insulin levels, which leads to a reduction in hepatic GH receptor expression⁽¹¹⁾. Thus, first-generation SRLs have also a non-pituitary action on the IGF-I production.

Overall, the effect of first-generation SRLs on biochemical control is considered to be equivalent⁽⁶⁾ and patients have biochemical response rates varying from 25 to 45%⁽⁵⁶⁻⁶⁰⁾. However, these studies employing different definitions of biochemical response over time and varying study entry criteria (*e.g.* post-surgery, medically naïve, or preselected sensitivity to first-generation SRLs)⁽⁶¹⁾.

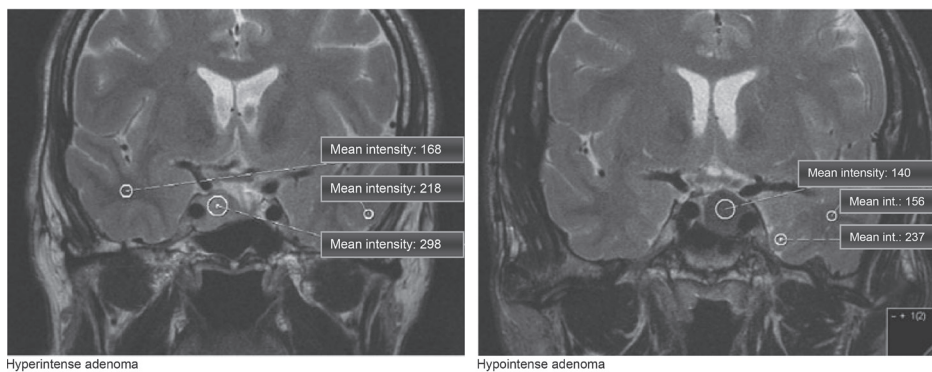
The effect of first-generation SRL on clinically relevant tumour volume reduction is considered to be equivalent^(62, 63), but compared with octreotide LAR, there are fewer data on lanreotide ATG on tumour volume reduction. Various studies mentioned that first-line treatment with first-generation SRLs induces a variable degree of tumour volume reduction up to 70% of patients, while up to 2% of somatostatin analogue-treated tumours continue to grow^(60, 64, 65).

The visual assessment of T2-weighted MRI signal can categorize pituitary tumours into hypointense, isointense, and hyperintense. In particular, the presence of hypointense T2-weighted tumour MRI images, which correlate with dense tumour granularity, predicts a favourable first-generation SRL response⁽⁶⁶⁻⁶⁸⁾. T2-weighted MRI (Figure 5) showing a hyperintense pituitary tumour (left) and a hypointense pituitary tumour (right). T2-weighted MRI signal of the pituitary tumour has been recently recognized as a non-invasive predictor of response to first-generation SRL therapy in acromegaly.

Antitumour effects (*i.e.* cell degeneration or tumour cell necrosis) have not been documented over the past three decades of clinical experience with first-generation SRL treatment. Although first-generation SRL treatment does not significantly change the

mean T2-signal intensity ratio on the MRI in GH-secreting pituitary tumours⁽⁶⁹⁾, a study from Bonneville et al., observed that tumours can shift to T2-hyperintensity during first-generation SRL treatment in some (7 out of 68) treatment-naïve patients⁽⁶⁸⁾. More about T2-weighted MRI signal of the pituitary tumour as predictor of this class of drugs (*i.e.* first-generation SRL and pasireotide LAR) is written in Chapter 4.

FIGURE 5. Examples for measurement of T2-weighted MRI intensity.



Source: Heck A et al, *Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly*, *Clinical Endocrinology*. 2012, 7:72-78. Image reproduced with permission of the rights holder, *Clinical Endocrinology*.

Overall, first-generation SRLs are well tolerated with only mild side-effects. Side-effects of these class of drugs are mostly explained by the physiological action of somatostatin. These include gastro-intestinal related complaints such as nausea and diarrhoea, and abdominal pain or distension. More severe adverse effects may include hair loss, cholelithiasis and bradycardia^(56, 59). Cozzolino and colleagues showed in a large meta-analysis of 47 prospective interventional trials that both octreotide LAR and lanreotide ATG significantly reduced insulin secretion and therefore a slight deterioration in glucose tolerance in a minority of patients can be observed⁽⁷⁰⁾. In general, the net effect of SRLs on glucose metabolism is considered marginal. Glucose can increase in up to 42% of patients, although in a few patients improvement of hyperglycemia may be seen after GH and IGF-I reduction.

1 Although first-generation SRLs can effectively control hormonal hypersecretion in acromegaly, most patients are (partly) resistant to this treatment. If biochemical control is not achieved after administering the maximal dose of first-generation SRLs, the consensus criteria ⁽⁴²⁾ recommend that treatment should be individualized based on the presence or absence of clinically relevant residual tumour and impaired glucose tolerance.

Dopamine agonists

Dopamine agonists, in particular cabergoline, suppress GH hypersecretion not only in mixed GH-PRL-secreting pituitary tumours but also in solely GH-secreting pituitary tumours. Dopamine agonists are acting on dopamine receptor subtype 2 receptors. Currently, two different molecules are available in the market: cabergoline and bromocriptine. The former one is currently the most used, due to better patient compliance (weekly administration versus daily administration of bromocriptine), and better tolerated, due to reduced adverse effects. Cabergoline is orally administered usually twice or three times per week, starting with 0.5–

1 mg each week, but dose up-titration is possible up to 3.5 mg each week (*i.e.* 0.5 mg every day), if well tolerated. They can be considered as first-line medical therapy post-surgery only for patients with modestly elevated GH and IGF-I levels (IGF-I <2.5 x upper limit of normal (ULN) and is effective in around 35% of patients ^(39, 42, 71-73). In particular, the chances of achieving a normal IGF-I level on cabergoline are clearly better (up to 50%) when IGF-I is less than 1.5 x ULN than when it is over 1.5 x ULN (up to 30%) ⁽⁷³⁾.

The addition of cabergoline to continued first-generation SRLs treatment has also a limited role in the treatment of acromegaly and is indicated in patients with modestly elevated IGF-I levels during first-generation SRL administration ^(42, 73). Co-treatment with cabergoline is also recommended for patients who have no access to pegvisomant. Regarding the efficacy, in a meta-analysis consisting of 77 patients with modestly elevated IGF-I levels, up to 50% achieved normalized IGF-I levels after adding cabergoline therapy ⁽⁷³⁾. Data on a combination treatment of cabergoline with pegvisomant are limited, but this may be an option in patients who experience side effects or are intolerant to first-generation SRLs.

Dopamine agonists may exert antiproliferative and proapoptotic effects on the pituitary tumour cells ⁽⁷⁴⁾. In a novel study ⁽⁷⁵⁾, in ten first-generation SRL-resistant patients total

tumour shrinkage went from $33.4 \pm 40.6 \text{ mm}^3$ to $20.9 \pm 20.8 \text{ mm}^3$ uL ($p=0.009$) after the addition of cabergoline ($0.25\text{--}2 \text{ mg/week}$) for six months. A meta-analysis examined the effect of cabergoline on tumour volume in five studies⁽⁷³⁾. Tumour shrinkage was associated with higher baseline prolactin and IGF-I concentrations. However, information on tumour volumes is scarce, which could underestimate tumour shrinkage. For patients who are biochemically resistant to first-generation SRLs, the addition of cabergoline may be useful as SRLs could maintain long-term effects on tumour mass, while cabergoline may lower IGF-I levels.

The adverse effects include nausea, headache, dizziness, orthostatic hypotension, and mood disorders⁽⁷³⁾. High cabergoline dosages used in Parkinson's patients have valvulopathy as a known side effect⁽⁷⁶⁾. Lower dosages, short-term use of dopamine agonists have not been proven to induce valvulopathy in acromegaly patients⁽⁷⁷⁾.

Second-line and alternative therapies

There are multiple options for second-line therapy as recommended by the consensus criteria⁽⁴²⁾: pegvisomant combined with or substituted for first-generation SRLs or pasireotide LAR monotherapy.

Pegvisomant

Pegvisomant is a pegylated GH-receptor antagonist that interferes with the signalling of the GH receptor and indicated as second-line or third-line therapy⁽⁴²⁾. Pegvisomant monotherapy has a reported efficacy rate of 89–97%^(78, 79). Lower efficacy rates have been reported in observational registries such as the German Pegvisomant Observational Study (GPOS) and ACROSTUDYTM^(80, 81). However, the lower efficacy rates in the observational registration study might likely be explained by inadequate dosing by the prescribing physicians.

Similarly, pegvisomant in combination with first-generation SRL has shown high efficacy rates with IGF-I normalization in >60% of patients, provided that the appropriate dose has been used^(82, 83). Co-treatment with first-generation SRL and pegvisomant was the most effective treatment in a recent meta-analysis of 90 studies⁽⁸⁴⁾. According to the consensus statement⁽⁴²⁾, combination therapy with first-generation SRLs and pegvisomant is recommended if patients are not controlled on first-generation SRLs, have an impaired glucose tolerance and a clinically relevant residual tumour.

Pegvisomant monotherapy cannot decrease tumour size. In the ACROSTUDY, a global surveillance study, from 936 included patients only 3.2% had a significant increase by MRI at mean of 2-year follow-up⁽⁸⁰⁾. Thus, we do not expect pegvisomant to decrease or control tumours that are growing, however, we do not expect a significant increase in tumour size either. Neither has there been a report of tumour increase in a combination treatment with pegvisomant⁽⁸⁵⁾. The addition of first-generation SRL to pegvisomant can achieve relevant tumour shrinkage in about 20% of patients⁽⁸³⁾. Therefore, the synergistic effects of the combination therapy could influence both tumour volume as well as IGF-I reduction. The first-generation SRLs could cause tumour shrinkage, whereas, pegvisomant lowers IGF-I levels, which is favourable for blood glucose levels. In a study assessing the long-term safety of combination therapy with first-generation SRL and pegvisomant in 14 (16.3%) patients the size of the tumour significantly decreased above the 20%, whereas tumour size increase was observed in one patient⁽⁸⁵⁾.

Regarding the safety aspects of pegvisomant monotherapy, in a recent update of the ACROSTUDY⁽⁸⁶⁾, hepatobiliary-related side effects were found for 9.8% of 2,090 patients, from which 4.2% are considered related to treatment. Lipodystrophy at the injection site of pegvisomant is also a common side-effect, which is preventable by frequent rotation of the injection site and regressed in most patients after discontinuing pegvisomant⁽⁸⁷⁾. In a subgroup of patients, the combination therapy of first-generation SRL and pegvisomant has favourable effects on quality of life (QoL) compared with first-generation SRL monotherapy, including the patients whose disease is biochemically controlled⁽⁸⁸⁾.

Pasireotide

In 2014, pasireotide (LAR) has been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of acromegaly^(8,9). Pasireotide LAR is a long-acting somatostatin multi-receptor ligand with a unique receptor binding profile. Compared with first-generation SRLs, which show the highest affinity to SST₂ receptor, pasireotide binds with higher affinity to SST₅ receptor, followed by 2, 3 and 1⁽¹⁰⁾. Pasireotide LAR has a slightly lower affinity to SST₂ receptor than classical first-generation SRLs⁽¹⁰⁾. Besides its broader SST receptor binding profile, pasireotide LAR has unique functional post-receptor effects such as reduced activation of SST₂ receptor internalization, phosphorylation and less β -arrestin mobilization than

octreotide^(89, 90). The rationale for the use of pasireotide LAR in the medical treatment of acromegaly is the higher affinity for SST₅ or the differential activation of SST₂ receptor.

Biochemical response

The first reports of preclinical and clinical phase II studies have shown promising data on the efficacy of pasireotide LAR therapy in achieving normal GH and IGF-I levels⁽⁹¹⁻⁹⁷⁾. Three phase III studies [C2305, C2404 and PAPE study] reported the clinical efficacy of pasireotide LAR therapy in patients with acromegaly^(56, 98, 99). The first two studies were performed in medically naïve acromegaly patients and patients with acromegaly who were inadequately controlled despite ≥6 months treatment on maximum doses of first-generation SRLs.

Pasireotide LAR therapy demonstrated in the C2305 and C2404 study superior efficacy first-generation SRLs therapy in achieving normal serum IGF-I levels^(56, 98). Efficacy of pasireotide LAR are higher as first-line therapy option than those achieved with octreotide LAR in medically-naïve patients⁽⁵⁶⁾. Biochemical control rates after the maximum dose of first-generation SRL is up to 20% higher than with continuation of the agent⁽⁹⁸⁾. Also, during the extension phase of both studies, the response rates were comparable^(100, 101). Among the 81 patients inadequately controlled with octreotide LAR who switched to pasireotide LAR as second-line therapy option, about 17% achieved normal serum IGF-I levels⁽¹⁰²⁾. During the extension up to approximately 5.8 years, 37.0% of patients achieved GH <1.0 µg/L and normal IGF-I at some point during the core or extension phase⁽¹⁰³⁾. The effects of pasireotide LAR and octreotide LAR therapy on GH levels reduction were, however, superimposable in the C2305 study⁽⁵⁶⁾. This suggests a predominant role of somatostatin receptor subtype₂ (SST₂) receptor in mediating the inhibitory effect of pasireotide LAR on GH secretion.

To address its mechanism of action and clinical application alone or in combination with pegvisomant, our group conducted the PAPE study (*i.e.* Pasireotide and Pegvisomant Study in Acromegaly)⁽⁹⁹⁾. In this study we investigated a cohort of patients partially responsive to first-generation SRLs, who were well controlled on a combination of first-generation SRLs and pegvisomant. Our data indicate that switching to pasireotide LAR (as second- or third-line therapy option), either as monotherapy or in combination with pegvisomant, results in control of IGF-I concentrations in the vast majority (77%) of patients⁽⁹⁹⁾. Stratified by treatment group; 93% of patients on pasireotide

LAR monotherapy achieved IGF-I normalization within three months⁽⁹⁹⁾. Of patients on pegvisomant and pasireotide LAR combination therapy, 67% achieved IGF-I normalization within three months.

The current consensus criteria⁽⁴²⁾ advocate pasireotide LAR monotherapy as second-line treatment for patients without biochemical response to first-generation SRLs if a clinically relevant residual tumour that is unsuitable for resection is present.

Tumour response

It has been reported that pasireotide LAR shows similar inhibitory effects on the growth of cultured tumour specimens compared with first-generation SRLs⁽¹⁰⁴⁾. Moreover, as mentioned before, antitumour effects (*i.e.* cell degeneration or tumour cell necrosis) have not been documented over the past three decades of clinical experience with first-generation SRLs.

In the phase II study of SOM230 (pasireotide), clinically significant tumour volume reduction of more than 20% with pasireotide LAR was achieved in 39% of patients, while a clinically significant increase in tumour volume was not observed⁽⁹⁶⁾. Among patients previously treated with first-generation SRLs (C2404 study), tumour volume reduction occurred more in patients on pasireotide LAR 40 mg (19%) and pasireotide LAR 60 mg (11%), than in patients inadequately controlled on optimal doses of first-generation SRLs (2%)⁽⁹⁸⁾. However, in the C2305 study pasireotide LAR and octreotide LAR therapy had a similar effect on tumour volume reduction, even though pasireotide LAR was superior to octreotide LAR in providing biochemical control⁽⁵⁶⁾. The mean decrease in tumour volume during the extension study was 25% for crossover to pasireotide LAR and 18% for crossover to octreotide LAR, while a greater proportion of patients receiving pasireotide LAR achieved clinically significant ($\geq 20\%$) tumour volume reduction (54% vs 42% patients receiving octreotide LAR)⁽¹⁰²⁾. Taken together, these clinical studies suggest that pasireotide LAR might exert a greater effect on tumour control, in particular in patients that show no tumour response during first-generation SRL treatment.

Antitumour effects (*i.e.* cell degeneration or tumour cell necrosis) have not been documented over the past three decades of clinical experience with first-generation SRL treatment. Although first-generation SRL treatment does not significantly change the mean T2-signal intensity ratio on the MRI in GH-secreting pituitary tumours⁽⁶⁹⁾, a study from Bonneville et al., observed that tumours can shift to T2-hyperintensity during first-

generation SRL treatment in some (7 out of 68) treatment-naïve patients⁽⁶⁸⁾. More about T2-weighted MRI signal of the pituitary tumour and an antitumour effect of pasireotide LAR is written in Chapter 6.

Quality of life and symptoms

In medically-naïve patients (the C2305 study), pasireotide LAR and octreotide LAR showed similar improvements in the five symptoms of acromegaly score (*i.e.* headache, fatigue, perspiration, paresthesia, and osteoarthritis)⁽⁵⁶⁾. Moreover, the Acromegaly Quality of Life (AcroQoL) questionnaire was used to assess health-related QoL and showed less symptom severity scores in both treatment groups⁽⁵⁶⁾. Although significantly more pasireotide LAR patients achieved biochemical control than octreotide LAR patients, they did not observe that pasireotide LAR therapy may have a favourable effect on QoL compared to first-generation SRL therapy. In the C2404 study, they noted more improvements in acromegaly symptom scores in patients given pasireotide LAR therapy than in patients inadequately controlled on optimal doses of first-generation SRLs therapy^(98, 103).

Safety

In Phase II and III studies in acromegaly pasireotide LAR therapy is well tolerated and has a comparable safety profile to first-generation SRLs, except for a greater frequency and degree of hyperglycaemia-related adverse events^(56, 97-100, 102, 103, 105, 106). Clinical studies have consistently shown that after pasireotide LAR initiation fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA1c) levels rise the first three months, and remain stable without further antidiabetic therapy^(98, 101, 105, 107, 108). In the ACCESS study, designed to assess the safety of Pasireotide LAR therapy in patients with acromegaly, hyperglycaemia-related adverse events were reported in 46% of patients. However, pasireotide LAR withdrawal for this reason was reported in only 9% of patients⁽¹⁰⁵⁾. Among patients in the PAPE study, the frequency of type 2 diabetes doubled from 33% at baseline to 69% at 24 weeks⁽⁹⁹⁾. Pasireotide LAR, which potentially can induce QTc prolongation, should be used with caution in acromegaly patients who are taking, either as prophylaxis or treatment, medications for COVID-19 (azithromycin, hydroxychloroquine), which can also affect QTc interval⁽¹⁰⁹⁾.

1

Outlines of the thesis

Part I. Determinants of response to somatostatin analogues

First-generation SRLs remain the mainstay of medical therapy in acromegaly. Although first-generation SRLs can control hormonal hypersecretion in GH-secreting pituitary tumours, many patients are at least partially resistant to treatment and will be exposed to high GH and IGF-I concentrations. Previous studies have reported several clinical predictors of biochemical response to first-generation SRL monotherapy but used widely differing definitions of biochemical response over time and varying study entry criteria. More importantly, previous studies lack to identify patient-specific clinical predictors for different biochemical response groups, including partial- as well as non-responders to first-generation SRL therapy. In **Chapter 2**, prediction models were created incorporating patient-specific clinical predictors and categorize patients into groups by biochemical response to investigate whether we can individually predict the initial first-generation SRL treatment response.

It is assumed that the efficacy of a given somatostatin analogue is directly correlated to the SST receptor subtype binding profile and expression in the GH-secreting tumour^(5,6). However, although guidelines do not report specific recommendations, it has been suggested that first-generation SRLs (*i.e.* lanreotide or octreotide) are more effective when SST₂ receptor is predominantly expressed, whereas SST₅ receptor expression is better at predicting the response to pasireotide LAR treatment than SST₂ receptor expression^(110, 111). In the follow-up analysis of patients from the PAPE study up to nine months of pasireotide LAR treatment, we investigate in **Chapter 3** whether response to first-generation SRLs correlates to pasireotide LAR treatment. Secondly, we assessed to what extent SST₂ and SST₅ receptor expression in GH-secreting pituitary tumours are correlated to the response to pasireotide LAR treatment. In this chapter, we aimed to confirm whether the biochemical response to pasireotide LAR treatment is indeed driven by SST₅ receptor.

In **Chapter 4** we address the anti-proliferative effects of pasireotide LAR treatment in patients from the PAPE study up to nine months of pasireotide LAR treatment. We investigate the relationship between the T2-weighted signal of the adenoma and the hormonal and tumoural responses to pasireotide LAR, alone or in combination with

pegvisomant therapy, including the SST₂ and SST₅ receptor expression in the tumour. Secondly, we investigate to what extent the T2-weighted signal of the tumour and its response to pasireotide LAR correlates with responsiveness to first-generation SRL treatment.

Part II. Pasireotide: mechanism of action and clinical applications

Taken together, previous clinical studies indicate that even in acromegaly patients in whom (biochemical) disease activity can't be controlled by first-generation SRLs, pasireotide LAR (as second- or third-line therapy option), either as monotherapy or in combination with pegvisomant, can achieve biochemical control in most patients^(56, 98, 99). The follow-up efficacy and safety analysis of patients from the PAPE study up to nine months of pasireotide LAR are presented in **Chapter 5**. We aimed to identify the optimal pegvisomant and pasireotide LAR dose to achieve IGF-I control within the references ranges. In addition, we assessed the relation between baseline insulin secretion as measured with the OGTT and pasireotide-induced hyperglycaemia.

We were struck by the peculiar finding of increased T2-weighted MRI signal of the adenoma in a single patient with a prolactinoma after receiving pasireotide LAR therapy^{y(112)}. In general, a hyperintense T2-signal indicates cystic degeneration, tumour cell necrosis, or both, which suggests an antitumour effect of pasireotide LAR. That peculiar finding provides us with an incentive to investigate in **Chapter 6** the T2-weighted signal by MRI in the follow-up analysis of patients from the PAPE study.

Part III. Emerging treatment option: very-low-carbohydrate ketogenic diet

Previous clinical studies^(98, 113) and our PAPE study⁽¹⁰⁶⁾ suggest that the superior efficacy in lowering IGF-I of pasireotide LAR compared with first-generation SRL in first-generation SRL resistant patients is mediated by its stronger inhibition of insulin secretion. Because reduced portal insulin secretion down-regulates hepatic GHR⁽¹¹⁾, and therefore reduces hepatic IGF-I synthesis^(9, 12), we proposed and ran a 2-week trial using a eucaloric ketogenic diet in acromegaly patients (**Chapter 7**)⁽¹¹⁴⁾. By using a combined approach, making the liver GH-resistant via the ketogenic diet and by keeping GH concentrations low via first-

generation SRL therapy, we postulated that we can achieve normal IGF-I levels in acromegaly patients previously uncontrolled with first-generation SRL therapy alone.

This study is particularly relevant since it might provide evidence that insulin is a very important permissive factor for IGF-I production and it even overrides GH in importance in controlling IGF-I in acromegaly patients. And as outlined in the preceding sections, many patients are at least partially resistant to first-generation SRL treatment and need additional (medical) treatment, which includes *e.g.* the expensive treatment with pegvisomant or pasireotide LAR. These results could affect the clinical management of acromegaly as a ketogenic diet might be used as an effective treatment option and could reduce the cost of expensive drugs.

Part IV. Role of AIP variants in pituitary tumours and thyroid carcinomas

To finish this thesis, we investigate in **Chapter 8** the prevalence of *AIP* gene mutations and mutations in genes that have been associated with neuroendocrine tumours in series of tumours from patients presenting with both pituitary adenomas and DTCs. Given the increased frequency of DTCs in patients with GH-secreting pituitary tumours, the potential for a common mechanism behind both tumours remains valid. Since these features are relatively rare, a nationwide pathology registry (PALGA) study was performed in The Netherlands.

Part V. General discussion

Chapter 9 serves as a general discussion on the previous chapters and conclusion of this thesis.

References

1. Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119(11):3189-202.
2. Hannah-Shmouni F, Trivellin G, Stratakis CA. Genetics of gigantism and acromegaly. *Growth Horm IGF Res.* 2016;30-31:37-41.
3. P. M. Sur deux cas d'acromegalie; hypertrophie singuliere non congenitale des extremités superieures, inferieures et cephalique. *Rev Med Liege.* 1886(6):297-333.
4. de Herder WW. The History of Acromegaly. *Neuroendocrinology.* 2016;103(1):7-17.
5. Minkowski O. Uber einem Fall von Akromegalie. *Berliner klinische Wochenschrift.* 1887;21:371-4.
6. Massalongo R. Sull'acromegalia. *Riforma Med.* 1892;8:74-7.
7. de Herder WW. Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). *Pituitary.* 2009;12(3):236-44.
8. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, et al. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science.* 1973;179(4068):77-9.
9. Clemmons DR. Involvement of insulin-like growth factor-I in the control of glucose homeostasis. *Curr Opin Pharmacol.* 2006;6(6):620-5.
10. Rabinowitz D, Zierler KL. A Metabolic Regulating Device Based on the Actions of Human Growth Hormone and of Insulin, Singly and Together, on the Human Forearm. *Nature.* 1963;199(4896):913-5.
11. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab.* 2000;85(12):4712-20.
12. Ho PJ, Friberg RD, Barkan AL. Regulation of pulsatile growth hormone secretion by fasting in normal subjects and patients with acromegaly. *J Clin Endocrinol Metab.* 1992;75(3):812-9.
13. Ho KY, Veldhuis JD, Johnson ML, Furlanetto R, Evans WS, Alberti KG, et al. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *J Clin Invest.* 1988;81(4):968-75.
14. Hartman ML, Veldhuis JD, Johnson ML, Lee MM, Alberti KG, Samojlik E, et al. Augmented growth hormone (GH) secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *J Clin Endocrinol Metab.* 1992;74(4):757-65.

15. Lavrentaki A, Paluzzi A, Wass JA, Karavitaki N. Epidemiology of acromegaly: review of population studies. *Pituitary*. 2017;20(1):4-9.
16. Burton T, Le Nestour E, Neary M, Ludlam WH. Incidence and prevalence of acromegaly in a large US health plan database. *Pituitary*. 2016;19(3):262-7.
17. Dal J, Feldt-Rasmussen U, Andersen M, Kristensen LO, Laurberg P, Pedersen L, et al. Acromegaly incidence, prevalence, complications and long-term prognosis: a nationwide cohort study. *Eur J Endocrinol*. 2016;175(3):181-90.
18. Zarool-Hassan R, Conaglen HM, Conaglen JV, Elston MS. Symptoms and signs of acromegaly: an ongoing need to raise awareness among healthcare practitioners. *J Prim Health Care*. 2016;8(2):157-63.
19. Esposito D, Ragnarsson O, Johannsson G, Olsson DS. Prolonged diagnostic delay in acromegaly is associated with increased morbidity and mortality. *Eur J Endocrinol*. 2020;182(6):523-31.
20. Petrossians P, Daly AF, Natchev E, Maione L, Blijdorp K, Sahnoun-Fathallah M, et al. Acromegaly at diagnosis in 3173 patients from the Liege Acromegaly Survey (LAS) Database. *Endocr Relat Cancer*. 2017;24(10):505-18.
21. Petrossians P, Tichomirowa MA, Stevenaert A, Martin D, Daly AF, Beckers A. The Liege Acromegaly Survey (LAS): a new software tool for the study of acromegaly. *Ann Endocrinol (Paris)*. 2012;73(3):190-201.
22. Trivellin G, Daly AF, Faucz FR, Yuan B, Rostomyan L, Larco DO, et al. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med*. 2014;371(25):2363-74.
23. Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab*. 2010;95(11):E373-83.
24. Rostomyan L, Beckers A. Screening for genetic causes of growth hormone hypersecretion. *Growth Horm IGF Res*. 2016;30-31:52-7.
25. Caimari F, Korbonits M. Novel Genetic Causes of Pituitary Adenomas. *Clin Cancer Res*. 2016;22(20):5030-42.
26. Marques P, Caimari F, Hernandez-Ramirez LC, Collier D, Iacovazzo D, Ronaldson A, et al. Significant Benefits of AIP Testing and Clinical Screening in Familial Isolated and Young-onset Pituitary Tumors. *J Clin Endocrinol Metab*. 2020;105(6).

27. Gadelha MR, Kasuki L, Korbonits M. The genetic background of acromegaly. *Pituitary*. 2017;20(1):10-21.
28. Neou M, Villa C, Armignacco R, Jouinot A, Raffin-Sanson ML, Septier A, et al. Pangenomic Classification of Pituitary Neuroendocrine Tumors. *Cancer Cell*. 2020;37(1):123-34 e5.
29. Colao A, Ferone D, Marzullo P, Lombardi G. Systemic Complications of Acromegaly: Epidemiology, Pathogenesis, and Management. *Endocrine Reviews*. 2004;25(1):102-52.
30. Melmed S. Medical progress: Acromegaly. *N Engl J Med*. 2006;355(24):2558-73.
31. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. *J Clin Endocrinol Metab*. 2008;93(1):61-7.
32. Gasperi M, Martino E, Manetti L, Arosio M, Porretti S, Faglia G, et al. Prevalence of thyroid diseases in patients with acromegaly: results of an Italian multi-center study. *J Endocrinol Invest*. 2002;25(3):240-5.
33. Gullu BE, Celik O, Gazioglu N, Kadioglu P. Thyroid cancer is the most common cancer associated with acromegaly. *Pituitary*. 2010;13(3):242-8.
34. Dagdelen S, Cinar N, Erbas T. Increased thyroid cancer risk in acromegaly. *Pituitary*. 2014;17(4):299-306.
35. Kim HK, Lee JS, Park MH, Cho JS, Yoon JH, Kim SJ, et al. Tumorigenesis of papillary thyroid cancer is not BRAF-dependent in patients with acromegaly. *PLoS One*. 2014;9(10):e110241.
36. Dal J, Leisner MZ, Hermansen K, Farkas DK, Bengtsen M, Kistorp C, et al. Cancer Incidence in Patients With Acromegaly: A Cohort Study and Meta-Analysis of the Literature. *J Clin Endocrinol Metab*. 2018;103(6):2182-8.
37. Lai NB, Garg D, Heaney AP, Bergsneider M, Leung AM. No Benefit of Dedicated Thyroid Nodule Screening in Patients with Acromegaly. *Endocr Pract*. 2020;26(1):16-21.
38. Terzolo M, Reimondo G, Berchialla P, Ferrante E, Malchiodi E, De Marinis L, et al. Acromegaly is associated with increased cancer risk: a survey in Italy. *Endocr Relat Cancer*. 2017;24(9):495-504.
39. Katznelson L, Laws ER, Jr., Melmed S, Molitch ME, Murad MH, Utz A, et al. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2014;99(11):3933-51.
40. Clemmons DR. Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. *Clin Chem*. 2011;57(4):555-9.
41. Lecoq AL, Kamenicky P, Guiochon-Mantel A, Chanson P. Genetic mutations in sporadic pituitary adenomas--what to screen for? *Nat Rev Endocrinol*. 2015;11(1):43-54.

42. Melmed S, Bronstein MD, Chanson P, Klibanski A, Casanueva FF, Wass JAH, et al. A Consensus Statement on acromegaly therapeutic outcomes. *Nat Rev Endocrinol*. 2018;14(9):552-61.
43. Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A, et al. Expert consensus document: A consensus on the medical treatment of acromegaly. *Nat Rev Endocrinol*. 2014;10(4):243-8.
44. Katznelson L, Atkinson JLD, Cook DM, Ezzat SZ, Hamrahian AH, Miller KK. American Association of Clinical Endocrinologists Medical Guidelines for Clinical Practice for the Diagnosis and Treatment of Acromegaly-2011 Update. *Endocr Pract*. 2011;17:1-+.
45. Fahlbusch R, Honegger J, Buchfelder M. Surgical management of acromegaly. *Endocrinol Metab Clin North Am*. 1992;21(3):669-92.
46. Ross DA, Wilson CB. Results of transsphenoidal microsurgery for growth hormone-secreting pituitary adenoma in a series of 214 patients. *J Neurosurg*. 1988;68(6):854-67.
47. Biermasz NR, Roelfsema F, Pereira AM, Romijn JA. Cost-effectiveness of lanreotide Autogel in treatment algorithms of acromegaly. *Expert Rev Pharmacoecon Outcomes Res*. 2009;9(3):223-34.
48. Starke RM, Raper DM, Payne SC, Vance ML, Oldfield EH, Jane JA, Jr. Endoscopic vs microsurgical transsphenoidal surgery for acromegaly: outcomes in a concurrent series of patients using modern criteria for remission. *J Clin Endocrinol Metab*. 2013;98(8):3190-8.
49. Hannon MJ, Barkan AL, Drake WM. The Role of Radiotherapy in Acromegaly. *Neuroendocrinology*. 2016;103(1):42-9.
50. Abu Dabrh AM, Asi N, Farah WH, Mohammed K, Wang Z, Farah MH, et al. Radiotherapy Versus Radiosurgery in Treating Patients with Acromegaly: A Systematic Review and Meta-Analysis. *Endocr Pract*. 2015;21(8):943-56.
51. Knappe UJ, Petroff D, Quinkler M, Schmid SM, Schoff C, Schopohl J, et al. Fractionated radiotherapy and radiosurgery in acromegaly: analysis of 352 patients from the German Acromegaly Registry. *Eur J Endocrinol*. 2020;182(3):275-84.
52. Lee CC, Vance ML, Xu Z, Yen CP, Schlesinger D, Dodson B, et al. Stereotactic radiosurgery for acromegaly. *J Clin Endocrinol Metab*. 2014;99(4):1273-81.
53. Cuevas-Ramos D, Fleseriu M. Somatostatin receptor ligands and resistance to treatment in pituitary adenomas. *J Mol Endocrinol*. 2014;52(3):R223-40.
54. Hofland LJ, Lamberts SW. The pathophysiological consequences of somatostatin receptor internalization and resistance. *Endocr Rev*. 2003;24(1):28-47.

55. Taboada GF, Luque RM, Neto LV, Machado Ede O, Sbaffi BC, Domingues RC, et al. Quantitative analysis of somatostatin receptor subtypes (1-5) gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. *Eur J Endocrinol*. 2008;158(3):295-303.
56. Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab*. 2014;99(3):791-9.
57. Melmed S, Cook D, Schopohl J, Goth MI, Lam KS, Marek J. Rapid and sustained reduction of serum growth hormone and insulin-like growth factor-1 in patients with acromegaly receiving lanreotide Autogel therapy: a randomized, placebo-controlled, multicenter study with a 52 week open extension. *Pituitary*. 2010;13(1):18-28.
58. Murray RD, Melmed S. A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. *J Clin Endocrinol Metab*. 2008;93(8):2957-68.
59. Mercado M, Borges F, Bouterfa H, Chang TC, Chervin A, Farrall AJ, et al. A prospective, multicentre study to investigate the efficacy, safety and tolerability of octreotide LAR (long-acting repeatable octreotide) in the primary therapy of patients with acromegaly. *Clin Endocrinol (Oxf)*. 2007;66(6):659-68.
60. Caron PJ, Bevan JS, Petersenn S, Flanagan D, Tabarin A, Prevost G, et al. Tumor shrinkage with lanreotide Autogel 120 mg as primary therapy in acromegaly: results of a prospective multicenter clinical trial. *J Clin Endocrinol Metab*. 2014;99(4):1282-90.
61. Colao A, Auriemma RS, Pivonello R, Kasuki L, Gadelha MR. Interpreting biochemical control response rates with first-generation somatostatin analogues in acromegaly. *Pituitary*. 2016;19(3):235-47.
62. Giustina A, Mazziotti G, Torri V, Spinello M, Floriani I, Melmed S. Meta-analysis on the effects of octreotide on tumor mass in acromegaly. *PLoS One*. 2012;7(5):e36411.
63. Mazziotti G, Giustina A. Effects of lanreotide SR and Autogel on tumor mass in patients with acromegaly: a systematic review. *Pituitary*. 2010;13(1):60-7.
64. Bevan JS. Clinical review: The antitumoral effects of somatostatin analog therapy in acromegaly. *J Clin Endocrinol Metab*. 2005;90(3):1856-63.
65. Besser GM, Burman P, Daly AF. Predictors and rates of treatment-resistant tumor growth in acromegaly. *Eur J Endocrinol*. 2005;153(2):187-93.
66. Heck A, Ringstad G, Fougner SL, Casar-Borota O, Nome T, Ramm-Petersen J, et al. Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly. *Clin Endocrinol (Oxf)*. 2012;77(1):72-8.

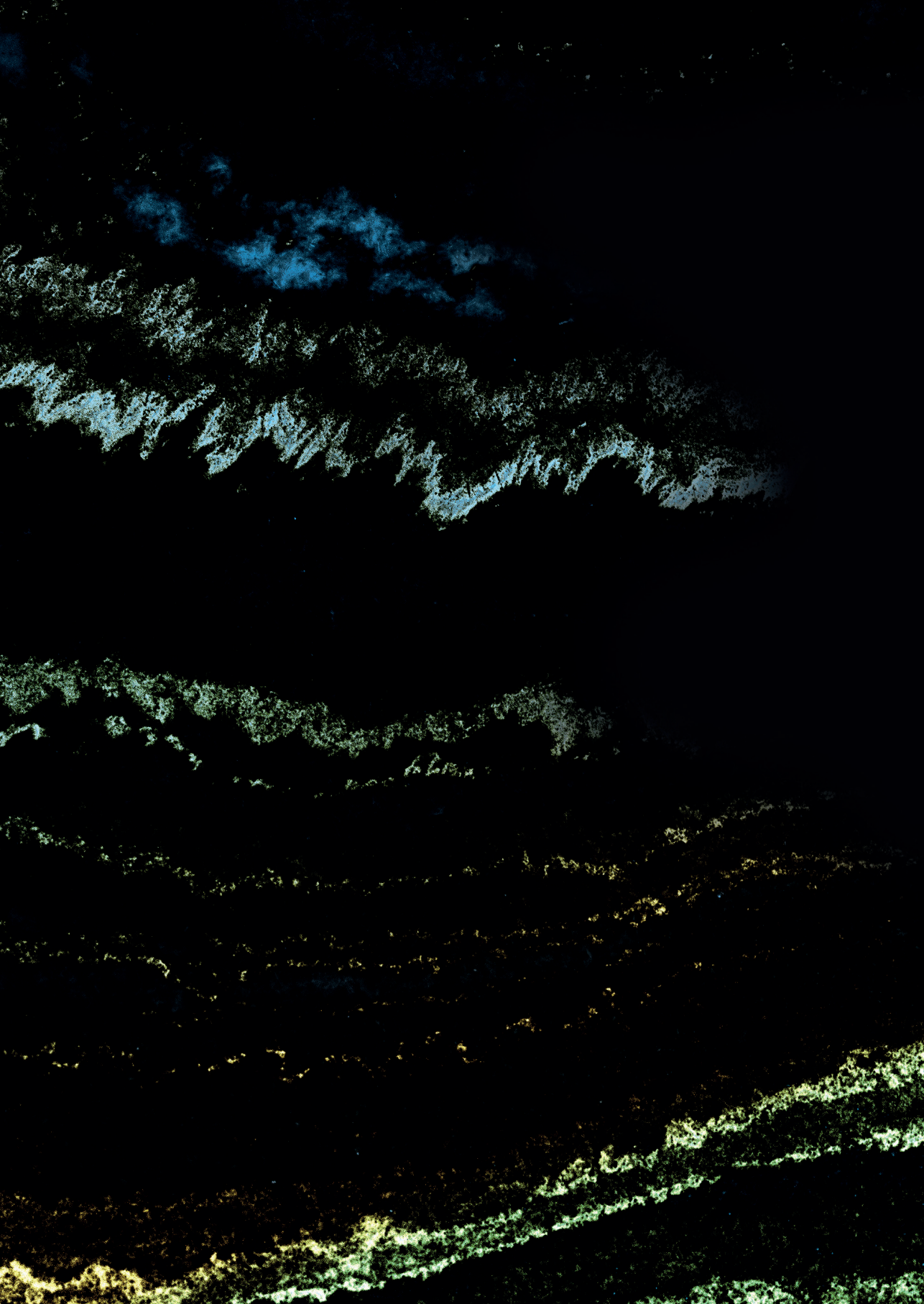
67. Potorac I, Petrossians P, Daly AF, Alexopoulou O, Borot S, Sahnoun-Fathallah M, et al. T2-weighted MRI signal predicts hormone and tumor responses to somatostatin analogs in acromegaly. *Endocr Relat Cancer*. 2016;23(11):871-81.
68. Bonneville F, Riviere LD, Petersenn S, Bevan J, Houchard A, Sert C, et al. MRI T2 signal intensity and tumor response in patients with GH-secreting pituitary macroadenoma: PRIMARYS post-hoc analysis. *Eur J Endocrinol*. 2018.
69. Heck A, Emblem KE, Casar-Borota O, Ringstad G, Bollerslev J. MRI T2 characteristics in somatotroph adenomas following somatostatin analog treatment in acromegaly. *Endocrine*. 2016;53(1):327-30.
70. Cozzolino A, Feola T, Simonelli I, Puliani G, Pozza C, Giannetta E, et al. Somatostatin Analogs and Glucose Metabolism in Acromegaly: A Meta-analysis of Prospective Interventional Studies. *J Clin Endocrinol Metab*. 2018;103(3):2089-99.
71. Diez JJ, Iglesias P. Current management of acromegaly. *Expert Opin Pharmacother*. 2000;1(5):991-1006.
72. Katznelson L, Atkinson JL, Cook DM, Ezzat SZ, Hamrahian AH, Miller KK, et al. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of acromegaly--2011 update. *Endocr Pract*. 2011;17 Suppl 4:1-44.
73. Sandret L, Maison P, Chanson P. Place of cabergoline in acromegaly: a meta-analysis. *J Clin Endocrinol Metab*. 2011;96(5):1327-35.
74. An JJ, Cho SR, Jeong DW, Park KW, Ahn YS, Baik JH. Anti-proliferative effects and cell death mediated by two isoforms of dopamine D2 receptors in pituitary tumor cells. *Mol Cell Endocrinol*. 2003;206(1-2):49-62.
75. Suda K, Inoshita N, Iguchi G, Fukuoka H, Takahashi M, Nishizawa H, Yamamoto M, Yamada S, Takahashi Y. Efficacy of combined octreotide and cabergoline treatment in patients with acromegaly: a retrospective clinical study and review of the literature. *Endocr J*. 2013;60(4):507-15.
76. Caputo C, Prior D, Inder WJ. The need for annual echocardiography to detect cabergoline-associated valvulopathy in patients with prolactinoma: a systematic review and additional clinical data. *Lancet Diabetes Endocrinol*. 2015;3(11):906-13.
77. Vallette S, Serri K, Rivera J, Santagata P, Delorme S, Garfield N, et al. Long-term cabergoline therapy is not associated with valvular heart disease in patients with prolactinomas. *Pituitary*. 2009;12(3):153-7.

78. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, et al. Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *N Engl J Med.* 2000;342(16):1171-7.
79. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, et al. Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. *Lancet.* 2001;358(9295):1754-9.
80. van der Lely AJ, Biller BM, Brue T, Buchfelder M, Ghigo E, Gomez R, et al. Long-term safety of pegvisomant in patients with acromegaly: comprehensive review of 1288 subjects in ACROSTUDY. *J Clin Endocrinol Metab.* 2012;97(5):1589-97.
81. Schreiber I, Buchfelder M, Droste M, Forssmann K, Mann K, Saller B, et al. Treatment of acromegaly with the GH receptor antagonist pegvisomant in clinical practice: safety and efficacy evaluation from the German Pegvisomant Observational Study. *Eur J Endocrinol.* 2007;156(1):75-82.
82. Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poublon RM, Feelders RA, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. *J Clin Endocrinol Metab.* 2014;99(10):3644-52.
83. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ. Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. *J Clin Endocrinol Metab.* 2007;92(12):4598-601.
84. Qiao N, He M, Shen M, Zhang Q, Zhang Z, Shou X, et al. Comparative Efficacy of Medical Treatment for Acromegaly: A Systematic Review and Network Meta-Analysis of Integrated Randomized Trials and Observational Studies. *Endocr Pract.* 2020;26(4):454-62.
85. Neggers SJ, de Herder WW, Janssen JA, Feelders RA, van der Lely AJ. Combined treatment for acromegaly with long-acting somatostatin analogs and pegvisomant: long-term safety for up to 4.5 years (median 2.2 years) of follow-up in 86 patients. *Eur J Endocrinol.* 2009;160(4):529-33.
86. Buchfelder M, van der Lely AJ, Biller BMK, Webb SM, Brue T, Strasburger CJ, et al. Long-term treatment with pegvisomant: observations from 2090 acromegaly patients in ACROSTUDY. *Eur J Endocrinol.* 2018;179(6):419-27.
87. Bonert VS, Kennedy L, Petersenn S, Barkan A, Carmichael J, Melmed S. Lipodystrophy in patients with acromegaly receiving pegvisomant. *J Clin Endocrinol Metab.* 2008;93(9):3515-8.
88. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, et al. Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. *J Clin Endocrinol Metab.* 2008;93(10):3853-9.

89. Lesche S, Lehmann D, Nagel F, Schmid HA, Schulz S. Differential effects of octreotide and pasireotide on somatostatin receptor internalization and trafficking in vitro. *J Clin Endocrinol Metab.* 2009;94(2):654-61.
90. Cescato R, Loesch KA, Waser B, Macke HR, Rivier JE, Reubi JC, et al. Agonist-biased signaling at the SST₂ A receptor: the multi-somatostatin analogs KE108 and SOM230 activate and antagonize distinct signaling pathways. *Mol Endocrinol.* 2010;24(1):240-9.
91. Bruns C, Lewis I, Briner U, Meno-Tetang G, Weckbecker G. SOM230: a novel somatostatin peptidomimetic with broad somatotropin release inhibiting factor (SRIF) receptor binding and a unique antisecretory profile. *Eur J Endocrinol.* 2002;146(5):707-16.
92. Weckbecker G, Briner U, Lewis I, Bruns C. SOM230: a new somatostatin peptidomimetic with potent inhibitory effects on the growth hormone/insulin-like growth factor-I axis in rats, primates, and dogs. *Endocrinology.* 2002;143(10):4123-30.
93. Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, et al. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. *J Clin Endocrinol Metab.* 2004;89(4):1577-85.
94. Murray RD, Kim K, Ren SG, Lewis I, Weckbecker G, Bruns C, et al. The novel somatostatin ligand (SOM230) regulates human and rat anterior pituitary hormone secretion. *J Clin Endocrinol Metab.* 2004;89(6):3027-32.
95. van der Hoek J, de Herder WW, Feelders RA, van der Lely AJ, Uitterlinden P, Boerlin V, et al. A single-dose comparison of the acute effects between the new somatostatin analog SOM230 and octreotide in acromegalic patients. *J Clin Endocrinol Metab.* 2004;89(2):638-45.
96. Petersenn S, Schopohl J, Barkan A, Mohideen P, Colao A, Abs R, et al. Pasireotide (SOM230) demonstrates efficacy and safety in patients with acromegaly: a randomized, multicenter, phase II trial. *J Clin Endocrinol Metab.* 2010;95(6):2781-9.
97. Tahara S, Murakami M, Kaneko T, Shimatsu A. Efficacy and safety of long-acting pasireotide in Japanese patients with acromegaly or pituitary gigantism: results from a multicenter, open-label, randomized, phase 2 study. *Endocr J.* 2017;64(7):735-47.
98. Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol.* 2014;2(11):875-84.

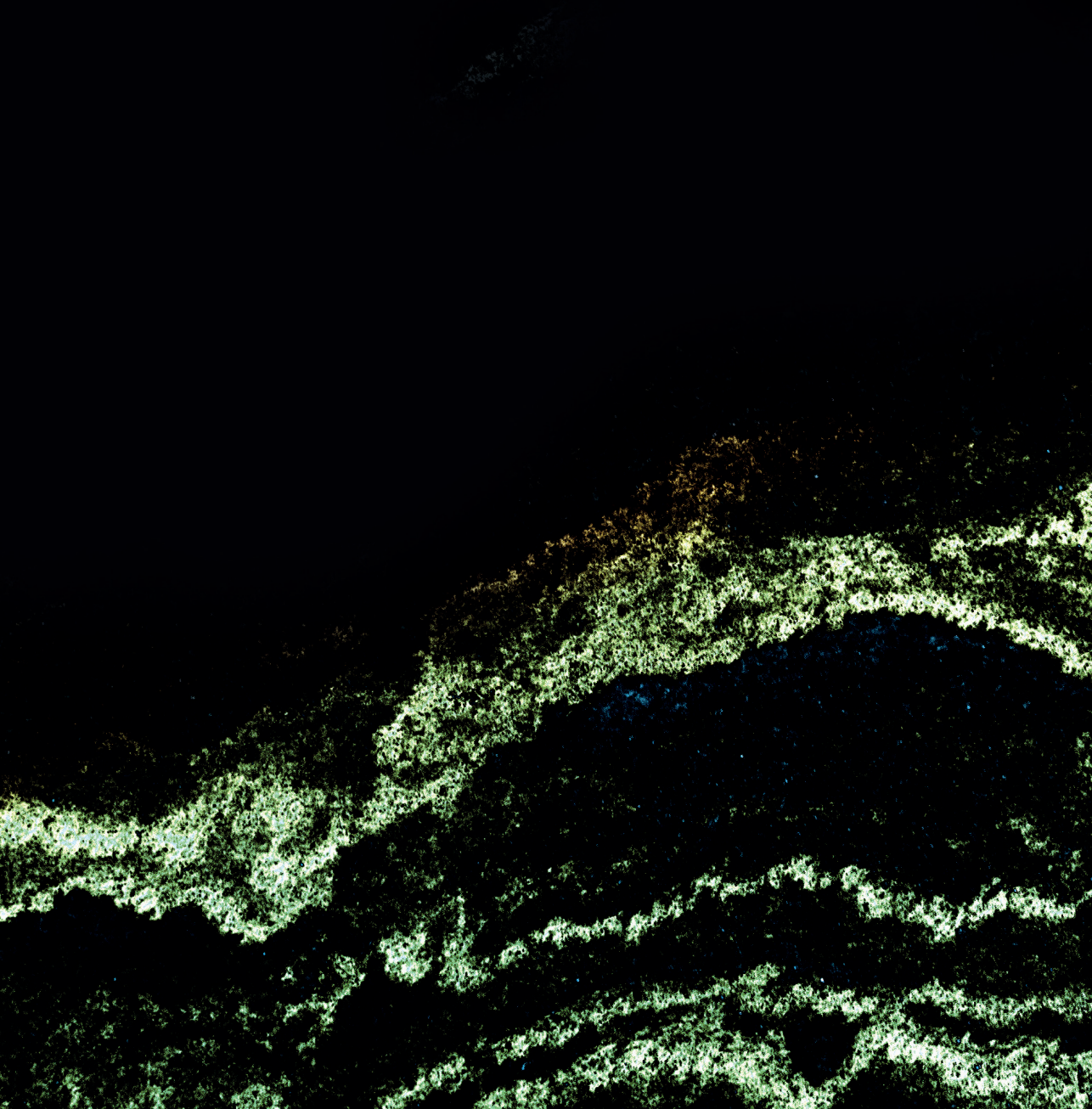
99. Muhammad A, van der Lely AJ, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of Switching to Pasireotide in Patients With Acromegaly Controlled With Pegvisomant and First-Generation Somatostatin Analogues (PAPE Study). *J Clin Endocrinol Metab.* 2018;103(2):586-95.
100. Gadelha MR, Bronstein MD, Brue T, De Marinis L, Fleseriu M, Guitelman M, et al. Long-acting pasireotide provides clinical benefit in patients with acromegaly: Results from a 30-month analysis of the phase 3 PAOLA study. *Endocrine Reviews.* [Abstract]. In press Volume 38, Issue 3, Supplement 1, June 2017.
101. Sheppard M, Bronstein MD, Freda P, Serri O, De Marinis L, Naves L, et al. Pasireotide LAR maintains inhibition of GH and IGF-1 in patients with acromegaly for up to 25 months: results from the blinded extension phase of a randomized, double-blind, multicenter, Phase III study. *Pituitary.* 2015;18(3):385-94.
102. Bronstein MD, Fleseriu M, Neggers S, Colao A, Sheppard M, Gu F, et al. Switching patients with acromegaly from octreotide to pasireotide improves biochemical control: crossover extension to a randomized, double-blind, Phase III study. *BMC Endocr Disord.* 2016;16:16.
103. Colao A, Bronstein MD, Brue T, De Marinis L, Fleseriu M, Guitelman M, et al. Pasireotide for acromegaly: long-term outcomes from an extension to the Phase III PAOLA study. *Eur J Endocrinol.* 2020;182(6):583.
104. Ibanez-Costa A, Rivero-Cortes E, Vazquez-Borrego MC, Gahete MD, Jimenez-Reina L, Venegas-Moreno E, et al. Octreotide and pasireotide (dis)similarly inhibit pituitary tumor cells in vitro. *J Endocrinol.* 2016;231(2):135-45.
105. Fleseriu M, Rusch E, Geer EB, Investigators AS. Safety and tolerability of pasireotide long-acting release in acromegaly-results from the acromegaly, open-label, multicenter, safety monitoring program for treating patients who have a need to receive medical therapy (ACCESS) study. *Endocrine.* 2017;55(1):247-55.
106. Muhammad A, Coopmans EC, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study. *Eur J Endocrinol.* 2018;179(5):269-77.
107. Franck SE, Korevaar TIM, Petrossians P, Daly AF, Chanson P, Jaffrain-Rea ML, et al. A multivariable prediction model for pegvisomant dosing: monotherapy and in combination with long-acting somatostatin analogues. *Eur J Endocrinol.* 2017;176(4):421-31.

108. Schmid HA, Brue T, Colao A, Gadelha MR, Shimon I, Kapur K, et al. Effect of pasireotide on glucose- and growth hormone-related biomarkers in patients with inadequately controlled acromegaly. *Endocrine*. 2016;53(1):210-9.
109. Fleseriu M, Buchfelder M, Cetas JS, Fazeli PK, Mallea-Gil SM, Gurnell M, et al. Pituitary society guidance: pituitary disease management and patient care recommendations during the COVID-19 pandemic-an international perspective. *Pituitary*. 2020;23(4):327-37.
110. Amarawardena W, Liyanarachchi KD, Newell-Price JDC, Ross RJM, Iacovazzo D, Debono M. Pasireotide: successful treatment of a sparsely granulated tumour in a resistant case of acromegaly. *Endocrinol Diabetes Metab Case Rep*. 2017;2017.
111. Iacovazzo D, Carlsen E, Lugli F, Chiloiro S, Piacentini S, Bianchi A, et al. Factors predicting pasireotide responsiveness in somatotroph pituitary adenomas resistant to first-generation somatostatin analogues: an immunohistochemical study. *Eur J Endocrinol*. 2016;174(2):241-50.
112. Coopmans EC, van Meyel SWF, Pieterman KJ, van Ipenburg JA, Hofland LJ, Donga E, et al. Excellent response to pasireotide therapy in an aggressive and dopamine-resistant prolactinoma. *Eur J Endocrinol*. 2019;181(2):K21-k7.
113. Henry RR, Ciaraldi TP, Armstrong D, Burke P, Ligueros-Saylan M, Mudaliar S. Hyperglycemia associated with pasireotide: results from a mechanistic study in healthy volunteers. *J Clin Endocrinol Metab*. 2013;98(8):3446-53.
114. Coopmans EC, Berk KAC, El-Sayed N, Neggers S, van der Lely AJ. Eucaloric Very-Low-Carbohydrate Ketogenic Diet in Acromegaly Treatment. *N Engl J Med*. 2020;382(22):2161-2.



Part I

Determinants of response to somatostatin analogues





Chapter 2

Multivariable prediction model for biochemical response to first-generation somatostatin receptor ligands treatment in acromegaly

Eva C. Coopmans, Tim I.M. Korevaar, Sebastiaan W.F. van Meyel,
Adrian F. Daly, Phillipe Chanson, Thierry Brue, Brigitte Delemer,
Václav Hána Jr., Annamaria Colao, Davide Carvalho,
Marie-Lise Jaffrain-Rea, Günter K. Stalla, Claudia Fajardo-Montañana,
Albert Beckers, Aart J. van der Lely, Patrick Petrossians
and Sebastian J.C.M.M. Neggers

J Clin Endocrinol Metab 2020; 105(9): 2964-74.

Abstract

CONTEXT: First-generation SRLs represent the mainstay of medical therapy for acromegaly, but they provide biochemical control of disease in only a subset of patients. Various pre-treatment biomarkers might affect biochemical response to first-generation SRLs.

OBJECTIVE: To identify clinical predictors of the biochemical response to first-generation SRLs monotherapy defined as biochemical- (IGF-I $\leq 1.3 \times$ upper limit of normal; ULN), partial- ($>20\%$ relative IGF-I reduction without normalization) and non-response ($\leq 20\%$ relative IGF-I reduction), and IGF-I reduction.

DESIGN: Retrospective multicenter study.

SETTING Eight participating European centers.

METHODS: We performed a meta-analysis of participant data from two cohorts (Rotterdam and Liège acromegaly survey (LAS), 622 out of 3520 patients). Multivariable regression models were used to identify predictors of biochemical response to first-generation SRL monotherapy.

RESULTS: Lower IGF-I concentration at baseline (odds ratio (OR) = 0.82, 95% confidence interval (CI) 0.72–0.95 IGF-I ULN, $P = .0073$) and lower bodyweight (OR = 0.99, 95% CI 0.98–0.99 kg, $P = .038$) were associated with biochemical response. Higher IGF-I concentration at baseline (OR = 1.40, (1.19–1.65) IGF-I ULN, $P \leq .0001$), the presence of type 2 diabetes (oral medication OR = 2.48, (1.43–4.29), $P = .0013$; insulin therapy OR = 2.65, (1.02–6.70), $P = .045$), and higher bodyweight (OR = 1.02, (1.01–1.04) kg, $P = .0023$) were associated with achieving partial response. Younger patients at diagnosis are more likely to achieve nonresponse (OR = 0.96, (0.94–0.99) year, $P = .0070$). Baseline IGF-I and growth hormone concentration at diagnosis were associated with absolute IGF-I reduction ($\beta = 0.90$, standard error (SE) = 0.02, $P \leq .0001$ and $\beta = 0.002$, SE = 0.001, $P = .014$, respectively).

CONCLUSION: Baseline IGF-I concentration was the best predictor of biochemical response to first-generation SRL, followed by bodyweight, while younger patients are more likely to achieve non-response.

Introduction

First-generation SRLs represent the mainstay of medical therapy for acromegaly^(1, 2). Medical treatment is usually indicated for patients for whom transsphenoidal surgery is not an option or is not curative. Long-acting first-generation SRLs act mainly by binding and activating SST₂, which is together with SST₅ receptor the most frequently expressed SST subtypes of GH-secreting pituitary adenomas⁽³⁻⁵⁾. Overall, the efficacies of first-generation SRLs, such as lanreotide ATG and octreotide LAR, seem to be similar⁽⁶⁾ and patients have biochemical response rates varying from 25 to 45%⁽⁶⁻⁹⁾.

Although first-generation SRLs can control hormonal hypersecretion in GH-secreting pituitary adenomas, many patients are at least partially resistant to treatment and will be exposed to high GH and IGF-I concentrations. Excess of serum GH and/or IGF-I leads to cardiovascular, metabolic and musculoskeletal comorbidities, which, in turn, increase mortality as a result of cardiovascular-, cerebrovascular- and respiratory morbidities^(10, 11). However, mortality in patients with acromegaly significantly declined over time and could be explained by the availability of new medical treatment options^(11, 12).

Previous studies have reported several clinical predictors of biochemical response to first-generation SRL monotherapy, including age at diagnosis, sex, bodyweight, height, serum (nadir) GH and IGF-I at diagnosis and baseline, tumor size, genetic mutations (*e.g.* *AIP*, T2-weighted MRI signal intensity, pathological features (granulation pattern and SSTs receptors of the adenoma) and the presence of type 2 DM (anti-diabetic medication use) as well as previous treatment modalities (surgery and medical therapy)^(3-5, 13-26). Overall, these studies have used widely differing definitions of biochemical response over time and varying study entry criteria (de novo, post-surgery, or preselected sensitivity to first-generation SRLs)⁽²²⁾. Although no validated criteria for first-generation SRL therapy failure exist, only a small minority (<10%) should be considered to be fully resistant to first-generation SRLs⁽²⁷⁾. More importantly, previous studies have not been able to identify patient-specific clinical predictors for different biochemical response groups, including partial- as well as non-responders to first-generation SRL therapy.

Given the importance of biochemical control in acromegaly but the lack of studies investigating predictors for the different biochemical response groups, we aim to identify clinical predictors of the biochemical response to initial treatment with the maximum

dose of first-generation SRLs monotherapy for at least six months. We categorized patients into groups by biochemical response (biochemical-, partial- and non-response) and by IGF-I reduction criteria (absolute and relative). These prediction models can be used to guide and individualize treatments and could avoid the consequences with ineffective treatment with first-generation SRLs such as prolonged biochemical and metabolic disease.

Patients and methods

Cohort description

Patients were included from two retrospective cohorts: ⁽¹⁾ the Rotterdam cohort and ⁽²⁾ the Liège Acromegaly Survey (LAS) cohort ^(21, 28, 29). The Rotterdam cohort contains data from acromegaly patients using first-generation SRL monotherapy collected at the outpatient clinic of the Pituitary Center Rotterdam, Erasmus University Medical Center in Rotterdam between 1977 and 2018. The LAS cohort (n=3194 from 10 centers) was created using a software tool that enables hospitals throughout Europe to include acromegaly patients and report patient, biochemical and tumor characteristics ⁽²¹⁾. For this study, only patients using first-generation SRL monotherapy were enrolled from additional seven European centers. The inclusion period was between 1990 and 2018. Patients receiving first-generation SRL monotherapy could have previously undergone surgery, however, patients that had undergone radiotherapy were excluded from the study.

Rotterdam cohort

Clinical and biochemical data were collected from acromegaly patients initially treated with the maximum dose of first-generation SRL monotherapy (*i.e.* lanreotide ATG 120 mg or octreotide LAR 30 mg every 28 days) for at least six months. We excluded 26 patients because they were not initially treated with the maximum dose of first-generation SRL monotherapy for at least six months. We were able to select 326 potential patients using first-generation SRL monotherapy. We excluded 132 patients for two reasons: ⁽¹⁾ IGF-I normalization was achieved (defined as $\leq 1.3 \times$ upper limit of normal (ULN)) at baseline (*i.e.* before initiating first-generation SRL monotherapy, n=44), and ⁽²⁾ follow-up data during SRL monotherapy were missing (n=88). In total (n=194) patients remained in the

cohort. The subjects then visited our outpatient clinic between every 16 and 24 weeks. At each visit to our outpatient clinic, standard measurements were performed including assessments of IGF-I and GH concentrations. From all patients written informed consent was obtained prior to inclusion, and the study was approved by the Medical Ethics Committee of the Erasmus University Medical Center in Rotterdam.

LAS cohort

Acromegaly patients from the LAS database treated with initially the maximum dose of first-generation SRL monotherapy for at least six months were selected. We excluded 197 patients because they were not initially treated with the maximum dose of first-generation SRL monotherapy for at least six months. From the LAS database, we were able to select 590 potential patients using first-generation SRL monotherapy. We excluded 162 patients for two reasons: ⁽¹⁾ IGF-I normalization was achieved at baseline (defined as $\leq 1.3 \times \text{ULN}$ before initiating first-generation SRL monotherapy, $n=91$), and follow-up data during SRL monotherapy were missing ($n=71$). In total ($n=428$) patients remained in the cohort. The medical ethics committee from the Liège University hospital approved the protocol and was covering the other European centers.

Outcome

The primary endpoint was the biochemical response to treatment with first-generation SRLs classified as: 1) biochemical response: defined as a normalized IGF-I ($\text{IGF-I} \leq 1.3 \times \text{ULN}$) irrespective of normalized GH ($\text{GH} \leq 2.5 \text{ } \mu\text{g/L}$) concentration were achieved after at least six months of treatment; 2) partial resistance: defined as a $>20\%$ relative reduction of IGF-I without normalization; and 3) non-response: defined as a failure to decrease IGF-I concentration by $>20\%$, which represent the intra-assay variability. The primary endpoint was independent of GH values. The secondary endpoints were absolute and relative IGF-I reduction (between serum IGF-I at baseline vs lowest IGF-I during first-generation SRL monotherapy).

Blood measurements

In the Rotterdam cohort, total IGF-I and GH concentrations were measured with different assays. IGF-I concentration before and after first-generation SRL treatment were analyzed using different assays: Immulite 2000 assay, a solid-phase, validated

enzyme-labelled chemiluminescent immunometric assay (DPC Biermann GmbH/Siemens, Fernwald, Germany; intra-assay variability of 2–5%, inter-assay variability of 3–7%) and two different radioimmunoassays (Diagnostic Systems Laboratories, Webster, Tex., USA, intra-assay coefficients of variation (CV) 3.9%, interassay CV 4.2%, and Medgenix Diagnostics, Fleurus, Belgium; intra-assay CV 6.1%, interassay CV 9.9%). GH concentration was initially measured by immunoradiometric assay (IRMA; CIS Bio International, Gif-sur-Yvette, France, intra-assay CVs 2.8%, inter-assay CVs 4.4%). Since February 2013, IGF-I and GH concentrations were analyzed using the immunometric assay (IDS-iSYS, Boldon, UK), which is free of interference from pegvisomant⁽¹⁾. Inter-assay CVs for GH and IGF-I were <5% (GH; n=190) and (IGF-I; n=190) in serum based internal quality control measurements over a period of 1 year.

In the LAS cohort, containing acromegaly patients from seven European centers, GH and IGF-I serum measurements were assessed locally and consequently performed with different assays. In this study, the IGF-I concentration was chosen to be expressed as the ULN of the reference ranges used in the local center (the IGF-I divided by the age- and sex-specific ULN). GH concentration was measured as a single random sample and expressed as absolute value.

Candidate predictors

Variables that were considered as possible pre-treatment predictors required to achieve disease control during first-generation SRL monotherapy were selected based on previous studies^(4, 5, 13-21, 25, 26, 30-33), biological plausibility and availability of robust data ascertainment in both cohorts and included: age at diagnosis and baseline, sex, bodyweight, height, serum (nadir) GH and IGF-I at diagnosis and baseline, tumor size (micro-, macroadenoma and non-visible at diagnosis) and the presence of type 2 DM at baseline as well as previous treatment modalities (surgery and medical therapy with a (partial) dopamine agonist). Bodyweight, GH and IGF-I concentrations were collected between six months before and at the time of first-generation SRL initiation. Other data were collected at baseline (as indicated), were fixed data in the patient's record or were established during the disease process.

Statistical analysis

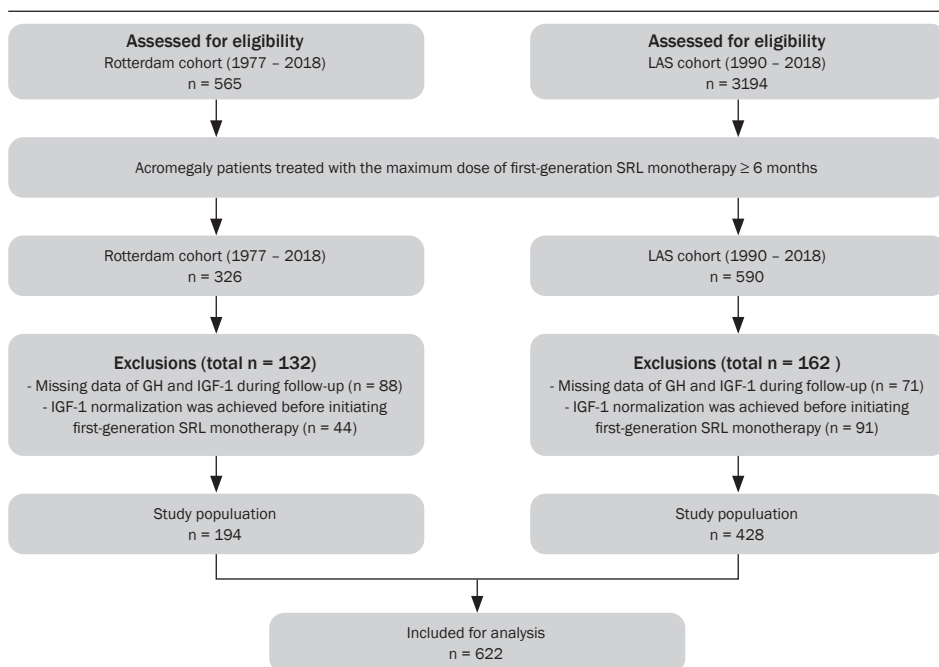
Data are expressed as median (interquartile range). Differences between two subgroups were analyzed using an unpaired *t*-test or the Mann-Whitney *U* test (in case of a non-parametric distribution). Nominal variables were analyzed using Fisher's exact test. For all regression models, the residuals were normally distributed, final models were not affected by multicollinearity, and non-linearity was assessed utilizing restricted cubic splines with 3 – 4 knots. We used univariable linear regression models to assess the association between each candidate predictor and the outcome. The decision for linear regression models instead of multiple models for the identification of predictors was based on Akaike information criteria and log-likelihood tests comparing multilevel models with random intercepts and/or slope per cohort vs standard linear regression correcting for cohort. To allow for optimal generalizability of effect estimates that predict the outcome, we performed standard linear regression correcting for cohort. We selected useful predictors using forward selection based on the change in regression coefficients and residual explained variability of the model, with a *P* value <0.20 as to keep predictors liberally in the model. To cope with (differentially) missing values of the candidate predictors, missing data on candidate predictors were multiple imputed (fifty times). The imputation model included most candidate predictor variables (missing values Rotterdam/LAS cohort as percentage: bodyweight 27.8/19.9%, height: 8.3/1.4%, GH at diagnosis 17.5/11.2%, tumor size 2.1/5.1%, panhypopituitarism 0/69.6%, age at baseline 0/0.5%, GH at baseline 13.9/11.7%) and the outcome variables. The candidate predictors age at diagnosis, gender, IGF-I (x ULN) at diagnosis and baseline, the presence of type 2 DM and previous treatment modalities (surgery and medical therapy) had no missing values. There was no difference between the original or any of the imputed datasets. All analyses were performed in each of the completed datasets, and final results were pooled using Rubin's rules. We investigated the predictive discriminative ability of significant predictors from the multivariable analyses by performing receiver-operating-characteristics (ROC) analysis. A *P* value lower than 0.05 (two-tailed) was used as a cut-off for statistical significance. All statistical analyses were performed using Statistical Package of Social Sciences, version 25.0 for Windows (SPSS) or using R statistical software, version 3.5.2 (packages *foreign*, *mice*, *pROC* and *rms*).

Results

Cohort characteristics

After exclusions, the final study population comprised 662 patients (Figure 1), the characteristics and previous treatment modalities of which are depicted in Table 1. Patients included in the LAS cohort were younger at diagnosis (44.0 vs 49.0 years) and more likely to be diagnosed with a macroadenoma (81.5 vs 74.2%). Patients from the LAS cohort more often underwent surgery and received medical treatment (*i.e.* (partial) dopamine agonists therapy) before the start of first-generation SRL and had a lower baseline IGF-I concentration. In the total cohort the median duration of first-generation SRL therapy is 132.4 months (IQR 36.4–215.3) and 284 (45.7%) patients received first-generation SRL as primary treatment. Observed biochemical response occurred in 80% of patients and a partial- or non-significant response occurred in 13% and 7%, respectively. No significant differences were observed in the Rotterdam and LAS cohort between excluded and included patients.

FIGURE 1. Flowchart of the selection procedure.



IGF-I, insulin-like growth factor 1; first-generation SRL, first-generation somatostatin receptor ligand; ULN, upper limit of normal.

TABLE 1. Cohort demographics and clinical characteristics of the total group, Rotterdam cohort and LAS cohort.

	Total cohort (n = 622)	Rotterdam cohort (n = 194)	LAS cohort (n = 428)
Age at diagnosis (years)	46.0 (36.0 – 55.0)	49.0 (39.8 – 58.0)	44.0 (34.0 – 53.0)*
Sex (female)	295 (47.4%)	95 (49.0%)	200 (46.7%)
Bodyweight (kg)	82.8 (71.0 – 96.4)	90.0 (75.2 – 100.1)	80.0 (70.0 – 95.0)*
Height (cm)	172.0 (166.0 – 180.0)	176.0 (168.8 – 182.3)	172.0 (165.0 – 179.3)*
Nadir GH at diagnosis (µg/L)	5.8 (2.4 – 15.3)	4.3 (1.9 – 10.6)	6.0 (2.5 – 18.0)*
GH at diagnosis (µg/L)	8.5 (3.9 – 21.8)	12.2 (5.9 – 28.3)	7.3 (3.6 – 19.8)*
IGF-I at diagnosis (x ULN)	2.7 (2.0 – 3.6)	2.9 (2.4 – 4.1)	2.6 (1.9 – 3.5)*
Tumor size			
Microadenoma	103 (16.7%)	46 (23.7%)	57 (13.5%)*
Macroadenoma	493 (80.2%)	144 (74.2%)	349 (81.5%)*
Non-visible adenoma	19 (3.1%)	4 (2.1%)	15 (3.5%)
Presence of type 2 DM	227 (36.5%)	76 (39.2%)	151 (35.3%)
Oral medication	196 (31.5%)	66 (34.0%)	130 (30.4%)
Insulin therapy	44 (7.1%)	10 (5.2%)	34 (7.9%)
Previous treatment			
Surgery	205 (33.0%)	38 (19.6%)	167 (39.0%)*
Surgical re-intervention	22 (3.5%)	8 (4.1%)	14 (3.3%)
Medical treatment	111 (17.8%)	12 (6.2%)	99 (23.1%)*
Panhypopituitarism	44 (7.1%)	27 (13.9%)	17 (4.0%)*
Age at baseline (years)	47.0 (37.0 – 56.5)	50.0 (40.0 – 59.0)	46.0 (36.0 – 55.0)*
GH at baseline (µg/L)	7.6 (3.5 – 20.1)	8.1 (3.2 – 21.8)	7.3 (3.6 – 19.9)
IGF-I at baseline (x ULN)	2.7 (1.9 – 3.6)	2.9 (2.2 – 4.1)	2.6 (1.9 – 3.5)*
Octreotide LAR	311 (50%)	123 (63.4%)	188 (43.9%)*
Treatment response			
Biochemical response	498 (80.1%)	114 (58.8%)	384 (89.7%)*
Partial response	79 (12.7%)	50 (25.8%)	29 (6.8%)*
Non-response	45 (7.2%)	30 (15.5%)	15 (3.5%)*
Duration first-generation SRL therapy (months)	132.4 (36.4 – 215.3)	17.7 (8.5 – 66.7)	177.2 (116.7 – 243.3)*

Data are mean (SD), median (IQR) or number (%). Asterisk represents $p \leq 0.05$ for the comparisons between Rotterdam and LAS cohort and are derived from the Student's *t*-test (continuous variables) and Pearson's χ^2 test (categorical variables).

IGF-I, insulin-like growth factor 1; first-generation SRL, first-generation somatostatin receptor ligand; Octreotide LAR, Octreotide long-acting release; ULN, upper limit of normal.

Univariable predictors of the biochemical response

All univariable analyses of the candidate predictors of biochemical response to first-generation SRL monotherapy, including predictors of IGF-I reduction, are depicted in Table 2.

TABLE 2. Univariable analysis of the predictors of the biochemical response during first-generation SRL monotherapy.

	Biochemical response	Partial response	Non-response	Absolute IGF-I reduction	Relative IGF-I reduction
Age at diagnosis (years)	-	-	Younger *	Non-linear *	Non-linear ^b
Sex	-	-	-	Male *	-
Bodyweight (kg)	↓ Bodyweight**	↑ Bodyweight**	↓ Bodyweight ^b	-	
Height (cm)	Smaller *	Taller ^b	Taller ^a	Non-linear ^b	Smaller **
Nadir GH at diagnosis (µg/L)	Non-linear ^b	Non-linear *	-	Non-linear **	Non-linear *
GH at diagnosis (µg/L)	↓ GH *	Non-linear *	↑ GH ^a	Non-linear **	-
IGF-I at diagnosis (x ULN)	↓ IGF-I ***	↑ IGF-I ***	-	↑ IGF-I ***	↑ IGF-I ***
Tumor size					
Microadenoma	-	-	-	-	-
Macroadenoma	-	-	Macroadenoma ^b	Macroadenoma ^b	-
Non-visible adenoma	-	-	-	-	-
Presence of type 2 DM					
Oral medication	-	Yes ^b	-	-	-
Insulin therapy	-	Yes ^b	-	-	-
Previous treatment					
Surgery	Yes **	Yes ***	No *	No **	Yes ^b
Surgical re-intervention	-	-	-	-	-
Medical treatment	Yes **	Yes ***	No ^b	No **	-
Panhypopituitarism	No **	Yes *	Yes **	-	No **
Age at baseline (years)	-	Older *	Younger *	-	-
GH at baseline (µg/L)	Non-linear ^a	Non-linear ^b	-	↓ GH **	Non-linear **
IGF-I at baseline (x ULN)	↓ IGF-I ***	↑ IGF-I ***	-	↑ IGF-I ***	↑ IGF-I ***
Octreotide LAR	-	-	-	-	-

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ^a a borderline significance at $p < 0.07$, ^b trend towards significance at $p < 0.20$. IGF-I, insulin-like growth factor 1; SRL, somatostatin receptor ligand; Octreotide LAR, Octreotide long-acting release; ULN, upper limit of normal.

Predictors of biochemical response

In multivariable analyses, baseline IGF-I concentration and bodyweight were determinants of biochemical response (Table 3). A lower IGF-I concentration at baseline was associated with a higher chance of achieving biochemical response (OR 0.82, 95% CI 0.72–0.95 IGF-I ULN, $P = 0.0073$, Table 3). Moreover, a lower bodyweight was associated with a higher chance of a biochemical response (OR 0.99, 95% CI 0.98–0.99 kg, $P = 0.038$, Table 3). The combined discriminative ability of IGF-I concentration and bodyweight combined to predict biochemical response was adequate (AUC 0.77, 95% CI 0.72–0.81, Fig. S1⁽³⁴⁾).

Predictors of partial response

In the multivariable analyses, baseline IGF-I concentration, the presence of type 2 DM and bodyweight were determinants of partial response (Table 3). A higher IGF-I concentration at baseline was associated with a higher chance of achieving partial response (OR 1.40, 95% CI 1.19–1.65 IGF-I ULN, $p \leq 0.0001$, Table 3). The presence of type 2 DM was associated with a higher chance of a partial response (oral medication OR 2.48, 95% CI 1.43–4.29, $P = 0.0013$; insulin therapy OR 2.65, 95% CI 1.02–6.70, $P = 0.045$, Table 3). Moreover, a higher bodyweight was associated with a higher chance of a partial response (OR 1.02, 95% CI 1.01–1.04 kg, $P = 0.0023$, Table 3). The combined discriminative ability of the IGF-I concentration, the presence of type 2 DM and bodyweight to predict partial response was good (AUC 0.80, 95% CI 0.76–0.86, Fig. S2⁽³⁴⁾).

Predictors of non-response

In multivariable analyses, age at diagnosis, surgery and tumor size at diagnosis were determinants of non-response (Table 3). Younger patients at diagnosis had a higher chance of achieving non-response (OR 0.96, 95% CI 0.94–0.99 year, $P = 0.0070$, Table 3). Surgery at baseline tended to be inversely associated with a higher chance of a non-response (OR 0.48, 95% CI 0.22–1.05, $P = 0.067$, Table 3). In other words, non-response patients underwent surgery less often. In addition, the presence of a macroadenoma (vs microadenoma) at baseline tended to be associated with a higher chance of a non-response but failed to reach significance (OR 2.64, 95% CI 0.89–7.84, $P = 0.081$, Table 3). Age at diagnosis, surgery and tumor size combined had an adequate discriminative ability to predict non-response (AUC of 0.78, 95% CI 0.69–0.85, Fig. S3⁽³⁴⁾).

TABLE 3. Multivariable analysis to predict biochemical, partial and non-response.

	OR	95% CI	P value
Biochemical response			
IGF-I at baseline (x ULN)	0.82	[0.72 – 0.95]	0.0073
Bodyweight (kg)	0.99	[0.98 – 0.99]	0.0379
Partial response			
IGF-I at baseline (x ULN)	1.40	[1.19 – 1.65]	<0.0001
Type 2 DM and oral medication †	2.48	[1.43 – 4.29]	0.0013
Type 2 DM and insulin therapy †	2.65	[1.02 – 6.70]	0.0451
Bodyweight (kg)	1.02	[1.01 – 1.04]	0.0023
Non-response			
Age at diagnosis (years)	0.96	[0.94 – 0.99]	0.0070
Surgery at baseline	0.48	[0.22 – 1.05]	0.0666
Macroadenoma ‡	2.64	[0.89 – 7.84]	0.0814
Non-visible adenoma ‡	<0.01	[<0.01 – ∞]	0.9868

DM, diabetes mellitus; IGF-I, insulin-like growth hormone factor 1; SE, standard error; SRL, somatostatin receptor ligand.

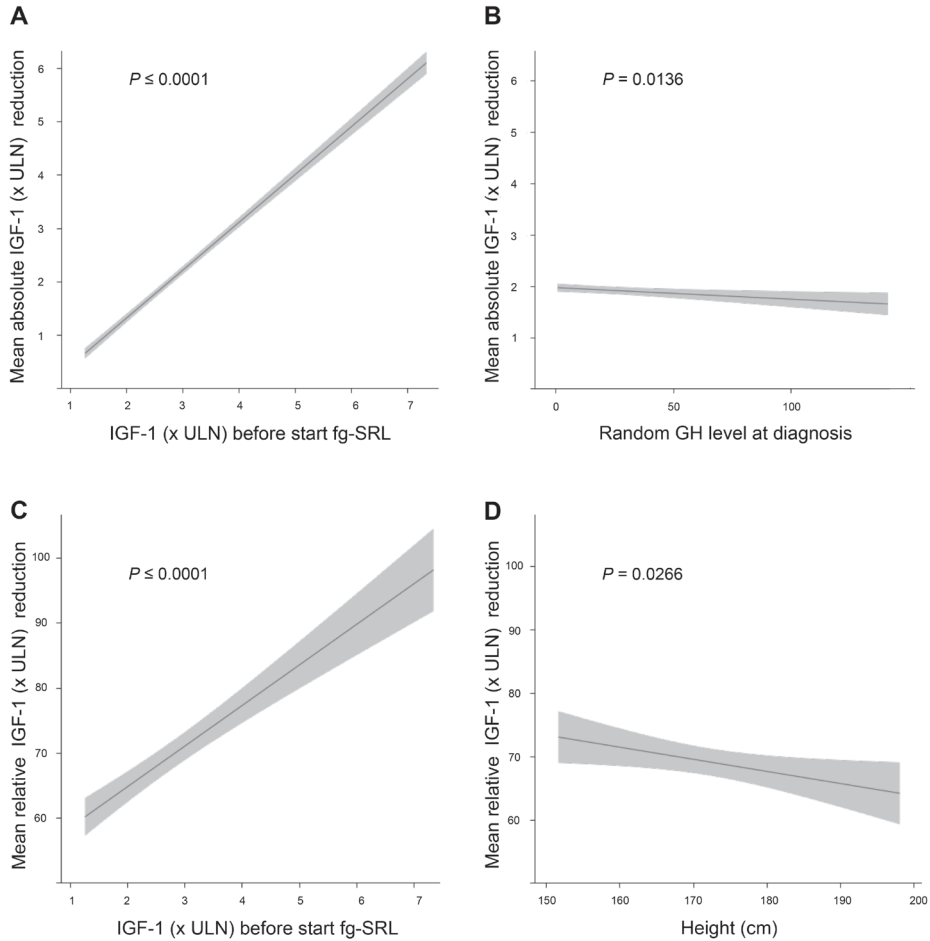
† = Compared with patients without type 2 DM.

‡ = Compared with patients harbouring a microadenoma.

Predictors of IGF-I reduction

Multivariable analyses to predict absolute IGF-I reduction detected a positive association for baseline IGF-I concentration (β 0.90, SE 0.02, $p \leq 0.0001$; Figure 2A and Table 4). A significant inverse association was seen between GH concentration at diagnosis and absolute IGF-I reduction (β > -0.002 , SE 0.001, $P = 0.014$; Figure 2B and Table 4).

With regards to relative IGF-I reduction, the multivariable analyses include a positive association for baseline IGF-I concentration (β 6.24, SE 0.66, $p \leq 0.0001$; Figure 2C and Table 4). In addition, height was inversely associated with relative IGF-I reduction (β -0.1912 , SE 0.0860, $P = 0.027$; Figure 2D and Table 4).

FIGURE 2. Predictors of IGF-I reduction.

Association between absolute IGF-I reduction and (A) IGF-I (xULN) before start first-generation SRL therapy and (B) GH concentration at diagnosis. Association between relative IGF-I reduction and (C) IGF-I (x ULN) and (D) height before start first-generation SRL therapy. The grey shade represents the 95% confidence interval of the predicted mean. IGF-I, insulin-like growth factor 1; fg-SRL, first-generation somatostatin receptor ligand; ULN, upper limit of normal.

TABLE 4. Multivariable analysis to predict IGF-I reduction.

	β	SE	P value
Absolute IGF-I reduction			
IGF-I at baseline (x ULN)	0.90	0.02	<0.0001
GH at diagnosis ($\mu\text{g/L}$)	-0.002	0.001	0.0136
Relative IGF-I reduction			
IGF-I at baseline (x ULN)	6.24	0.66	<0.0001
Height (cm)	-0.19	0.09	0.0266

IGF-I, insulin-like growth hormone factor 1; SE, standard error.

Discussion

This is the first large cohort study to focus on identifying clinical predictors of the biochemical response to treatment with first-generation SRLs by categorizing patients into groups by response (biochemical-, partial- and non-response) and by IGF-I reduction criteria (absolute and relative). The main findings of this study are that: ⁽¹⁾ baseline IGF-I concentration, bodyweight and the presence of type 2 DM can contribute in distinguishing complete biochemical from partial response and ⁽²⁾ that non-response during first-generation SRLs occurred more in patients that were younger at diagnosis and tended to harbour larger tumors and underwent surgery less often.

In our study, baseline IGF-I concentration was the best predictor of first-generation SRL treatment response. A lower IGF-I concentration at baseline was associated with a higher chance of a complete biochemical response, while a higher IGF-I concentration was associated with a higher risk of only partial response and higher IGF-I (absolute and relative) reduction to first-generation SRLs. Previously it has been identified that lower circulating IGF-I and GH levels correspond with better biochemical response to first-generation SRLs ^(4, 21, 35). Thus, lower baseline IGF-I concentrations could identify patients that will achieve biochemical control, while higher baseline IGF-I concentrations could identify those that will achieve partial responses while at the same time having a

greater absolute and relative reduction in circulating IGF-I levels. While the latter seems counterintuitive, a greater reduction even if IGF-I levels will not decrease below the set threshold is still of clinical value in the multimodel therapy setting.

We observed that a lower bodyweight was associated with a higher chance of biochemical control, while a higher bodyweight was associated with partial response to first-generation SRLs. This finding is in accordance with previous findings from the somatoline depot for acromegaly (SODA) registry⁽¹³⁾, that identified that patients with a lower BMI (BMI <30 kg/m²) tended to have better biochemical control of IGF-I than patients with a higher BMI (BMI ≥30 kg/m²) after 24 months of first-generation SRL therapy.

The presence of concomitant type 2 DM, besides baseline IGF-I concentration and bodyweight, is a pre-treatment predictor of partial response to first-generation SRL monotherapy, is also expected. The SODA study⁽¹³⁾, the same study as mentioned before, showed that after 24 months of first-generation SRL therapy, normalization of IGF-I concentration was achieved less frequently in diabetic patients. In a previous study of the LAS database, a significant relationship between glucose levels and IGF-I at baseline was shown for acromegaly patients, even in the absence of type 2 DM⁽²¹⁾. Overall, the prevalence of insulin sensitivity alterations has been found to correlate with higher BMI in patients with acromegaly as observed in the general population⁽³⁶⁾. Taking into account that type 2 DM patients likely had higher bodyweight at enrollment, both findings may be explained by the presence of hyperinsulinemia. Hyperinsulinemia may counteract possible SRL-induced pituitary-independent mechanisms of biochemical control by enhancing the synthesis of IGF-I through upregulation of hepatic GH receptors^(37, 38). Combined with the fact that first-generation SRL therapy may impair glucose metabolism, largely owing to inhibition of insulin leading to further increase of glucose secretion^(39, 40), type 2 DM patients might benefit from initial therapy with pegvisomant instead of first-generation SRLs.

Age at diagnosis appears to be the most important determinant of achieving non-response during first-generation SRL therapy, likely reflecting (indirectly) the exposure to high circulating levels of GH and IGF-I^(18, 21, 23, 28, 41). This observation confirms and builds upon previous studies^(21, 42) proposing age at diagnosis to be a clinical marker of tumor size and aggressiveness. Younger patients (aged <40 years) tend to have larger and potentially harbour more aggressive tumors than older patients; the reason for this

is unknown, although the increased prevalence of genetic mutations like *AIP* in younger acromegaly-gigantism patients with first-generation SRL resistance may play a role^(25, 26).

Knowing that larger tumors secrete more GH, while older patients generally have lower GH concentrations at diagnosis^(18, 21, 28), it becomes apparent why older patients have been shown to be more sensitive to first-generation SRLs therapy⁽¹⁸⁾. Subsequent, surgical total tumor resection or debulking decreases the basal GH secretion, and increases the likelihood of achieving biochemical disease control with first-generation SRLs. This observation is consistent with long-term trials^(20, 43, 44) and our data that show a better biochemical control of IGF-I with first-generation SRLs after surgery.

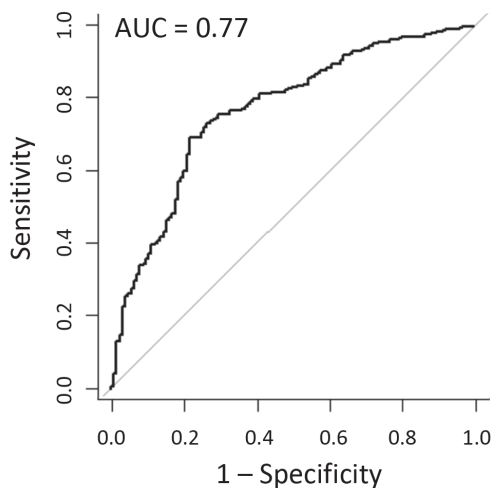
A strength of our study is the relatively large number of patients in which the biochemical response to first-generation SRL treatment were systematically investigated, while remaining to be treated with a single agent, with a stable high dose for a long period of treatment (*i.e.* exceeding the median period of 12 months) when compared to previous literature⁽²⁷⁾. The LAS database provides some specific advantages in that it is not limited to a national dataset nor does it deal with patients managed with only a single treatment modality, and therefore, may better reflect the general population of acromegaly patients and overcome selection bias. In our study, we utilize a more liberal definition of biochemical response (defined as $\text{IGF-I} \leq 1.3 \times \text{ULN}$). To overcome the limitation of a cutoff validity, we confirmed our data by using the cutoff of $\leq 1.2 \times \text{ULN}$ in the multivariable analyses, which did not affect the effect estimates of the final models. The main limitations of our study lie in the retrospective nature of this study. Due to the retrospective collection of data on IGF-I concentration during first-generation SRL treatment, performed on patients followed-up for at least six months, a selection bias cannot be ruled out. It is conceivable that patients unresponsive to first-generation SRLs were switched to alternative treatment options, if available, and did not reach the six months follow-up period required for admission in the study. However, before considering first-generation SRL resistance, at least six months are generally assumed to be necessary to assess treatment outcome^(45, 46). Finally, we are limited by the use of different IGF-I assays during first-generation SRL treatment.

SRLs remain the mainstay of medical therapy in acromegaly, however, a proportion of patients are at least partially resistant to treatment. Patient-specific clinical predictors of first-generation SRL response in patients with acromegaly include baseline IGF-I concentration as the best predictor: lower baseline IGF-I concentrations could identify

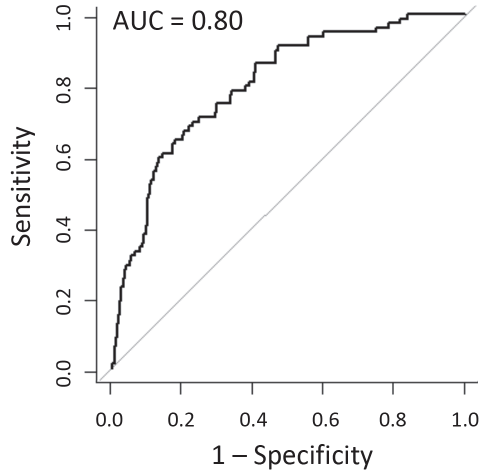
patients that will achieve biochemical control, while higher baseline IGF-I concentrations could identify those that will achieve partial responses while at the same time having a greater absolute and relative reduction in circulating IGF-I levels. In the latter group, a greater reduction in IGF-I levels even it does not decrease below the set threshold is still of great clinical value in the multimodal therapy setting. Other patient-specific clinical predictors of first-generation SRL response are bodyweight, the presence of type 2 DM, age at diagnosis and a trend towards tumor size and previous surgery. These prediction models can be used to guide and individualize treatments and could avoid the negative consequences of ineffective treatment with first-generation SRLs such as prolonged active biochemical and metabolic disease.

Appendix

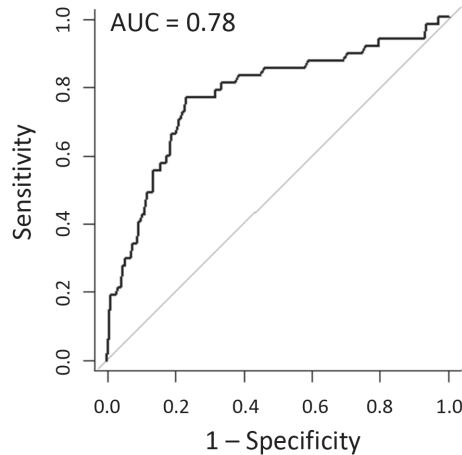
SUPPLEMENTAL FIGURE 1. Distinctive ability of IGF-I (x ULN) level and weight before start in identifying patients that will achieve biochemical response to treatment (defined as IGF-I levels ≤ 1.3 (x ULN) with or without GH levels ≤ 2.5 $\mu\text{g/L}$) utilizing a receiver-operating-characteristics analysis.



SUPPLEMENTAL FIGURE 2. Distinctive ability of type 2 DM, and IGF-I (x ULN) level and weight before start first-generation SRL therapy in identifying patients that will achieve partial response to treatment (defined as significant (>20%) relative reduction of IGF-I, without normalization, independent of GH values) with utilizing a receiver-operating-characteristics analysis.



SUPPLEMENTAL FIGURE 3. Distinctive ability of age at diagnosis, surgery before start SRL and tumor size in identifying patients that will achieve non-response to treatment (relative IGF-I reduction $\leq 20\%$) utilizing a receiver-operating-characteristics analysis.



References

1. Katznelson L, Laws ER, Jr, Melmed S, Molitch ME, Murad MH, Utz A, et al. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2014;99(11):3933-51.
2. Melmed S, Bronstein MD, Chanson P, Klibanski A, Casanueva FF, Wass JAH, et al. A Consensus Statement on acromegaly therapeutic outcomes. *Nat Rev Endocrinol.* 2018;14(9):552-61.
3. Hofland LJ, Lamberts SW. The pathophysiological consequences of somatostatin receptor internalization and resistance. *Endocr Rev.* 2003;24(1):28-47.
4. Cuevas-Ramos D, Fleseriu M. Somatostatin receptor ligands and resistance to treatment in pituitary adenomas. *J Mol Endocrinol.* 2014;52(3):R223-R40.
5. Taboada GF, Luque RM, Neto LV, Machado Ede O, Sbaffi BC, Domingues RC, et al. Quantitative analysis of somatostatin receptor subtypes (1-5) gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. *Eur J Endocrinol.* 2008;158(3):295-303.
6. Murray RD, Melmed S. A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. *J Clin Endocrinol Metab.* 2008;93(8):2957-68.
7. Caron P, Petersenn S, Flanagan D, Tabarin A, Prevost G, Maisonnobe P, et al. Tumor responses to lanreotide Autogel/Depot 120 Mg after 12 and 24 weeks are correlated with tumor responses after 48 weeks in treatment-naïve acromegalic patients: Post Hoc analyses of the primary study. *Endocrine Reviews.* 2014;35((Caron P) CHU Larrey, Toulouse, France).
8. Melmed S, Cook D, Schopohl J, Goth MI, Lam KS, Marek J. Rapid and sustained reduction of serum growth hormone and insulin-like growth factor-1 in patients with acromegaly receiving lanreotide Autogel therapy: a randomized, placebo-controlled, multicenter study with a 52 week open extension. *Pituitary.* 2010;13(1):18-28.
9. Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab.* 2014;99(3):791-9.
10. Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119(11):3189-202.
11. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. *J Clin Endocrinol Metab.* 2008;93(1):61-7.
12. Esposito D, Ragnarsson O, Granfeldt D, Marlow T, Johannsson G, Olsson DS. Decreasing mortality and changes in treatment patterns in patients with acromegaly from a nationwide study. *Eur J Endocrinol.* 2018;178(5):459-69.

13. Gordon MB, Molitch ME, Woodmansee WW, Ioachimescu AG, Gambetti OV, Carver D, et al. Body mass index as one of determinants of biochemical control in acromegaly: 2-year data from the soda registry. *Endocrine Reviews*. 2017;38(3).
14. Gadelha MR, Kasuki L, Korbonits M. Novel pathway for somatostatin analogs in patients with acromegaly. *Trends Endocrinol Metab*. 2013;24(5):238-46.
15. Giustina A, Mazziotti G, Torri V, Spinello M, Floriani I, Melmed S. Meta-analysis on the effects of octreotide on tumor mass in acromegaly. *PLoS One*. 2012;7(5):e36411.
16. Sherlock M, Fernandez-Rodriguez E, Alonso AA, Reulen RC, Ayuk J, Clayton RN, et al. Medical therapy in patients with acromegaly: Predictors of response and comparison of efficacy of dopamine agonists and somatostatin analogues. *J Clin Endocrinol Metab*. 2009;94(4):1255-63.
17. Mazziotti G, Giustina A. Effects of lanreotide SR and Autogel on tumor mass in patients with acromegaly: a systematic review. *Pituitary*. 2010;13(1):60-7.
18. van der Lely AJ, Harris AG, Lamberts SW. The sensitivity of growth hormone secretion to medical treatment in acromegalic patients: influence of age and sex. *Clin Endocrinol (Oxf)*. 1992;37(2):181-5.
19. Freda PU, Katznelson L, Van Der Lely AJ, Reyes CM, Zhao S, Rabinowitz D. Long-acting somatostatin analog therapy of acromegaly: A meta-analysis. *J Clin Endocrinol Metab*. 2005;90(8):4465-73.
20. Petrossians P, Borges-Martins L, Espinoza C, Daly A, Betea D, Valdes-Socin H, et al. Gross total resection or debulking of pituitary adenomas improves hormonal control of acromegaly by somatostatin analogs. *Eur J Endocrinol*. 2005;152(1):61-6.
21. Petrossians P, Tichomirowa MA, Stevenaert A, Martin D, Daly AF, Beckers A. The Liege Acromegaly Survey (LAS): a new software tool for the study of acromegaly. *Ann Endocrinol (Paris)*. 2012;73(3):190-201.
22. Colao A, Auriemma RS, Pivonello R, Kasuki L, Gadelha MR. Interpreting biochemical control response rates with first-generation somatostatin analogues in acromegaly. *Pituitary*. 2016;19(3):235-47.
23. Suliman M, Jenkins R, Ross R, Powell T, Battersby R, Cullen DR. Long-term treatment of acromegaly with the somatostatin analogue SR-lanreotide. *J Endocrinol Invest*. 1999;22(6):409-18.
24. Potorac I, Petrossians P, Daly AF, Alexopoulou O, Borot S, Sahnoun-Fathallah M, et al. T2-weighted MRI signal predicts hormone and tumor responses to somatostatin analogs in acromegaly. *Endocr Relat Cancer*. 2016;23(11):871-81.

25. Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab.* 2010;95(11):E373-83.
26. Beckers A, Petrossians P, Hanson J, Daly AF. The causes and consequences of pituitary gigantism. *Nat Rev Endocrinol.* 2018;14(12):705-20.
27. Colao A, Auriemma RS, Lombardi G, Pivonello R. Resistance to somatostatin analogs in acromegaly. *Endocrine Reviews.* 2011;32(2):247-71.
28. Petrossians P, Daly AF, Natchev E, Maione L, Blijdorp K, Sahnoun-Fathallah M, et al. Acromegaly at diagnosis in 3173 patients from the Liege Acromegaly Survey (LAS) Database. *Endocr Relat Cancer.* 2017;24(10):505-18.
29. Franck SE, Korevaar TIM, Petrossians P, Daly AF, Chanson P, Jaffrain-Rea ML, et al. A multivariable prediction model for pegvisomant dosing: monotherapy and in combination with long-acting somatostatin analogues. *Eur J Endocrinol.* 2017;176(4):421-31.
30. Gatto F, Feelders RA, van der Pas R, Kros JM, Waaijers M, Sprij-Mooij D, et al. Immunoreactivity score using an anti-SST_{2A} receptor monoclonal antibody strongly predicts the biochemical response to adjuvant treatment with somatostatin analogs in acromegaly. *J Clin Endocrinol Metab.* 2013;98(1):E66-71.
31. Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, et al. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. *J Clin Endocrinol Metab.* 2004;89(4):1577-85.
32. Venegas-Moreno E, Vazquez-Borrego MC, Dios E, Gros-Herguido N, Flores-Martinez A, Rivero-Cortés E, et al. Association between dopamine and somatostatin receptor expression and pharmacological response to somatostatin analogues in acromegaly. *J Cell Mol Med.* 2018;22(3):1640-9.
33. Wildemberg LE, Neto LV, Costa DF, Nasciuti LE, Takiya CM, Alves LM, et al. Low somatostatin receptor subtype 2, but not dopamine receptor subtype 2 expression predicts the lack of biochemical response of somatotropinomas to treatment with somatostatin analogs. *J Endocrinol Invest.* 2013;36(1):38-43.
34. Coopmans EC, Korevaar TIM, S.W.F. vM, Daly AF, Chanson P, Brue T, et al. Data from: Multivariable prediction model for biochemical response to first-generation somatostatin receptor ligands in acromegaly. Figshare Repository. Deposited 31 March 2020. <https://doi.org/10.6084/m9.figshare.12053280.v2>.

35. Newman CB, Melmed S, Snyder PJ, Young WF, Boyajy LD, Levy R, et al. Safety and efficacy of long-term octreotide therapy of acromegaly: results of a multicenter trial in 103 patients--a clinical research center study. *J Clin Endocrinol Metab.* 1995;80(9):2768-75.
36. Fieffe S, Morange I, Petrossians P, Chanson P, Rohmer V, Cortet C, et al. Diabetes in acromegaly, prevalence, risk factors, and evolution: data from the French Acromegaly Registry. *Eur J Endocrinol.* 2011;164(6):877-84.
37. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab.* 2000;85(12):4712-20.
38. Ciresi A, Amato MC, Pivonello R, Nazzari E, Grasso LF, Minuto F, et al. The metabolic profile in active acromegaly is gender-specific. *J Clin Endocrinol Metab.* 2013;98(1):E51-9.
39. Ferone D, Colao A, van der Lely AJ, Lamberts SW. Pharmacotherapy or surgery as primary treatment for acromegaly? *Drugs Aging.* 2000;17(2):81-92.
40. Colao A, Ferone D, Marzullo P, Cappabianca P, Cirillo S, Boerlin V, et al. Long-term effects of depot long-acting somatostatin analog octreotide on hormone levels and tumor mass in acromegaly. *J Clin Endocrinol Metab.* 2001;86(6):2779-86.
41. Melmed S, Braunstein GD, Horvath E, Ezrin C, Kovacs K. Pathophysiology of Acromegaly. *Endocr Rev.* 1983;4(3):271-90.
42. Besser GM, Burman P, Daly AF. Predictors and rates of treatment-resistant tumor growth in acromegaly. *Eur J Endocrinol.* 2005;153(2):187-93.
43. Bevan JS, Atkin SL, Atkinson AB, Bouloux PM, Hanna F, Harris PE, et al. Primary medical therapy for acromegaly: an open, prospective, multicenter study of the effects of subcutaneous and intramuscular slow-release octreotide on growth hormone, insulin-like growth factor-I, and tumor size. *J Clin Endocrinol Metab.* 2002;87(10):4554-63.
44. Colao A, Attanasio R, Pivonello R, Cappabianca P, Cavallo LM, Lasio G, et al. Partial surgical removal of growth hormone-secreting pituitary tumors enhances the response to somatostatin analogs in acromegaly. *J Clin Endocrinol Metab.* 2006;91(1):85-92.
45. Caron PJ, Bevan JS, Petersenn S, Flanagan D, Tabarin A, Prévost G, et al. Tumor Shrinkage With Lanreotide Autogel 120 mg as Primary Therapy in Acromegaly: Results of a Prospective Multicenter Clinical Trial. *J Clin Endocrinol Metab.* 2014;99(4):1282-90.
46. Freda PU, Katznelson L, van der Lely AJ, Reyes CM, Zhao S, Rabinowitz D. Long-acting somatostatin analog therapy of acromegaly: a meta-analysis. *J Clin Endocrinol Metab.* 2005;90(8):4465-73.



Chapter 3

**Pasireotide responsiveness in acromegaly is
mainly driven by somatostatin receptor
subtype 2 expression**

Ammar Muhammad, Eva C. Coopmans, Federico Gatto,
Sanne E. Franck, Joseph A.M.J.L. Janssen, Aart J. van der Lely,
Leo J. Hofland and Sebastian J.C.M.M. Neggers

J Clin Endocrinol Metab 2018; 104(3): 915-24.

Abstract

BACKGROUND: The response to first-generation SRLs treatment in acromegaly correlates with expression of SST₂ receptor. However, pasireotide shows the highest binding affinity for SST₅ receptor. It has been suggested that in acromegaly, SST₅ receptor expression is better at predicting the response to pasireotide LAR treatment than SST₂ receptor expression.

AIM: To investigate in patients with active acromegaly whether response to first-generation SRL treatment correlates to pasireotide LAR treatment and to what extent SST₂ and SST₅ receptor expression are correlated to the response to pasireotide LAR treatment.

METHODS: We included 52 patients from a cohort that initially received SRL treatment, followed by SRL and pegvisomant combination treatment, and finally pasireotide LAR treatment. The long-term response to pasireotide LAR was evaluated using a pasireotide LAR score. In 14 out of 52 patients, somatotroph adenoma tissue samples were available to evaluate SST₂ and SST₅ receptor expression using a previously validated immunoreactivity score (IRS).

RESULTS: The percentage IGF-I (times the ULN) reduction, which was observed after first-generation SRL treatment, correlated with pasireotide LAR response score during follow-up ($r = 0.40$; $P = 0.003$; $n = 52$). After exclusion of first-generation SRL-pretreated patients, SST₂ IRS was positively correlated to pasireotide LAR score ($r = 0.58$; $P = 0.039$; $n = 9$), whereas SST₅ receptor IRS showed no relation ($r = 0.35$; $P = 0.36$; $n = 9$).

CONCLUSIONS: In a cohort of patients partially responsive to SRLs, the IGF-I-lowering effects of pasireotide LAR treatment correlated with the effect of SRL treatment and seemed to be mainly driven by SST₂ receptor expression instead of SST₅ receptor.

Introduction

Acromegaly is a severe systemic condition most commonly caused by a somatotroph adenoma that secretes excessive levels of growth hormone (GH) and insulin-like growth factor I (IGF-I), leading to increased mortality and morbidity⁽¹⁾. Treatment modalities that normalize GH and IGF-I levels restore normal life expectancy⁽²⁾. This goal can be achieved pharmacologically, both by inhibiting pituitary GH secretion and blocking peripheral GH action.

First-generation long-acting somatostatin receptor ligands (SRLs, octreotide and lanreotide) represent the cornerstone for medical treatment of acromegaly. The biochemical response to SRL treatment has been consistently shown to be positively correlated to somatostatin receptor subtype 2 (SSTR₂) protein expression on the adenoma⁽³⁻⁷⁾. These compounds inhibit pituitary GH secretion by preferential binding with high affinity to SSTR_{2a}. However, biochemical normalization of GH and IGF-I can only be achieved in about 40% of patients. Therefore, the majority of patients are partially or even completely resistant to SRLs. An effective treatment option to normalize IGF-I levels in partially resistant patients is the addition of the GH receptor antagonist pegvisomant to SRLs. A recent study from our group showed that patients using SRLs and pegvisomant combination treatment had a lower SSTR₂ expression at the time of surgery compared with medically naïve patients⁽⁸⁾. The required pegvisomant dose to achieve IGF-I normalization was inversely correlated to SSTR₂ expression, but not to SSTR₅ expression⁽⁸⁾.

Pasireotide long-acting release (pasireotide LAR) is a novel multireceptor somatostatin analogue, which binds with high affinity to all SSTR subtypes but SSTR₄. In contrast to octreotide, pasireotide shows high subnanomolar affinity to SSTR₅⁽⁹⁾. *In vitro* studies have shown that pasireotide modulates somatostatin receptor trafficking and phosphorylation in a distinct manner from octreotide^(10, 11), inducing less SSTR₂ internalization, phosphorylation and β -arrestin recruitment than octreotide. In medically naïve acromegaly patients pasireotide LAR has demonstrated superior efficacy in reducing IGF-I levels over octreotide LAR, while the effect on GH reduction was superimposable⁽¹²⁾. This latter observation was also recently confirmed *in vitro*⁽¹³⁾. We recently reported the 24-weeks results of the pasireotide LAR and pegvisomant (PAPE) study⁽¹⁴⁾. This prospective open-label conversion study assessed the efficacy and safety of pasireotide

LAR alone or in combination with pegvisomant in acromegaly patients controlled with SRLs and pegvisomant combination treatment⁽¹⁴⁾. Switching to pasireotide LAR resulted in a significant pegvisomant dose reduction, but also a higher incidence of diabetes mellitus⁽¹⁴⁾.

It is assumed that the efficacy of a given somatostatin receptor ligand is directly correlated to the SSTR subtype binding profile and the pattern of SSTR expression in the somatotroph adenoma^(5, 6). However, although guidelines do not report specific recommendations so far, it is generally assumed that octreotide and lanreotide are more effective when SSTR₂ is highly and predominantly expressed, while pasireotide is more effective when SSTR₅ is the predominant subtype and SSTR₂ is absent or poorly expressed⁽¹⁵⁾. The aims of the present study were therefore: 1) to investigate whether the IGF-I response after SRL treatment correlates to the IGF-I response after pasireotide LAR treatment; 2) to investigate to what extent SSTR₂ and SSTR₅ immunoreactivity are correlated to responsiveness to pasireotide LAR treatment in somatotroph adenomas.

Materials and methods

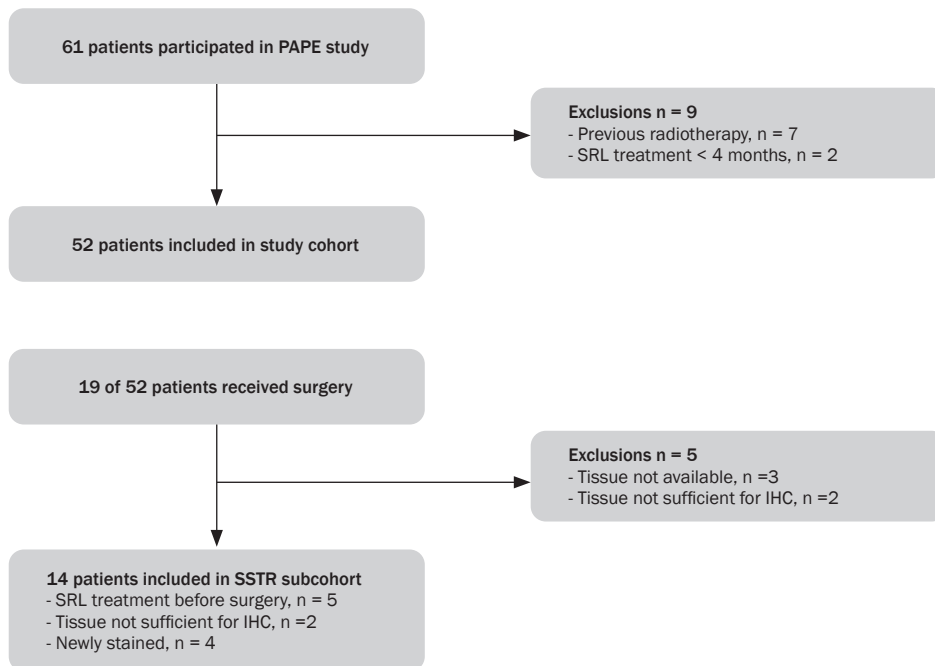
Patients and somatotroph adenoma tissue selection

Data collection of acromegaly patients was performed at the Erasmus MC Pituitary Center in Rotterdam. We initially started with a cohort of 61 acromegaly patients who received pasireotide LAR treatment during their participation in the PAPE study (Figure 1)⁽¹⁴⁾. All these patients have previously been treated with SRLs, followed by SRL and pegvisomant combination therapy. Cabergoline was used in 7 patients in combination with SRLs, and in two patients during the PAPE study⁽¹⁴⁾. After exclusion of patients that received postoperative radiotherapy ($n = 7$), and patients that received SRL treatment less than 4 months ($n = 2$), 52 patients remained and were finally included in the study cohort. In total 19 out of these 52 patients previously underwent neurosurgery. Reasons for surgery included adenomas with reasonable chance for cure such as (intrasellar) microadenomas, or macroadenomas with risk of visual impairment.

We selected only those patients with sufficient adenoma tissue available to perform immunohistochemistry (IHC). One patient underwent a second surgery during follow-up. For clarity, in this latter case we analyzed only the tissue sample of the first surgery.

From the 14 remaining somatotroph adenoma tissue samples included for IHC analysis (SSTR subcohort), 10 tissue samples have been stained previously⁽⁸⁾, while 4 cases were newly stained (figure 1). We retrospectively collected data on medical history and clinical response to first-generation SRLs. Prospective data on the pegvisomant dose and IGF-I levels were used from the PAPE study⁽¹⁴⁾. The PAPE study was registered with ClinicalTrials.gov, number NCT02668172. All patients were included after written informed consent.

FIGURE 1. Flowchart of the selection procedure for the study cohort and the somatotroph adenoma tissue samples included in the SSTR subcohort. All patients eventually received SRL and pegvisomant combination treatment, and were switched to pasireotide LAR treatment during the PAPE study⁽¹⁴⁾.



Outcomes

Response to SRL treatment was defined as IGF-I \times age-adjusted upper limit of normal (IGF-I \times ULN), and as percentage of IGF-I suppression after at least 4 months SRL treatment. In patients that underwent surgery, post-operative IGF-I levels after at least 3 months were considered. Response to pasireotide LAR during the PAPE study was divided into short-term and long-term response. Short-term treatment response was defined as IGF-I levels (\times ULN) at 24 weeks (*i.e.* after 3 injections of pasireotide LAR 60 mg). During the extension phase from 24 until 48 weeks, both the pasireotide LAR dose and pegvisomant dose were titrated according to a protocol to achieve IGF-I levels within the normal range. Therefore, the long-term response to pasireotide LAR was based on a composite “Pasireotide LAR treatment response score” (pasireotide LAR score) in order to fully capture the effect of pasireotide LAR taking into account pegvisomant dose reduction, discontinuation and eventually pasireotide LAR dose reduction. The pasireotide LAR score comprised five categories representing the difference in pegvisomant dose and pasireotide LAR dose at week 48 versus baseline (week number 0): 0 = pegvisomant dose reduction 0-33% (in combination with pasireotide LAR 60 mg), 1 = pegvisomant dose reduction 33-66% (+ pasireotide LAR 60 mg), 2 = pegvisomant dose reduction 66-100% (+ pasireotide LAR 60 mg), 3 = pegvisomant treatment discontinued and pasireotide LAR dose reduced to 40 mg, 4 = pegvisomant treatment discontinued and pasireotide LAR dose reduced to 20 mg every 4 weeks. 100% pegvisomant dose reduction corresponds to pasireotide LAR 60 mg monotherapy. Higher pasireotide LAR score corresponds to a better response to pasireotide LAR treatment.

IGF-I assays

Total IGF-I serum concentrations during the PAPE study were measured by the immunometric IDS-iSYS assay (Immunodiagnostic Systems Limited; Boldon, United Kingdom; intraassay coefficient of variation (CV) 8.1%, interassay CV 2.1%)⁽¹⁴⁾. Total IGF-I serum concentrations before and after SRL treatment were measured using different assays: Immulite 2000 assay, a solid-phase, validated enzyme-labelled chemiluminescent immunometric assay (DPC Biermann GmbH/Siemens, Fernwald, Germany; intraassay variability of 2 - 5%, interassay variability of 3 - 7%), the immunometric IDS-iSYS assay (Immunodiagnostic Systems Limited; Boldon, United Kingdom; intraassay coefficient of variation (CV) 8.1%, interassay CV 2.1%), and two different radioimmunoassays

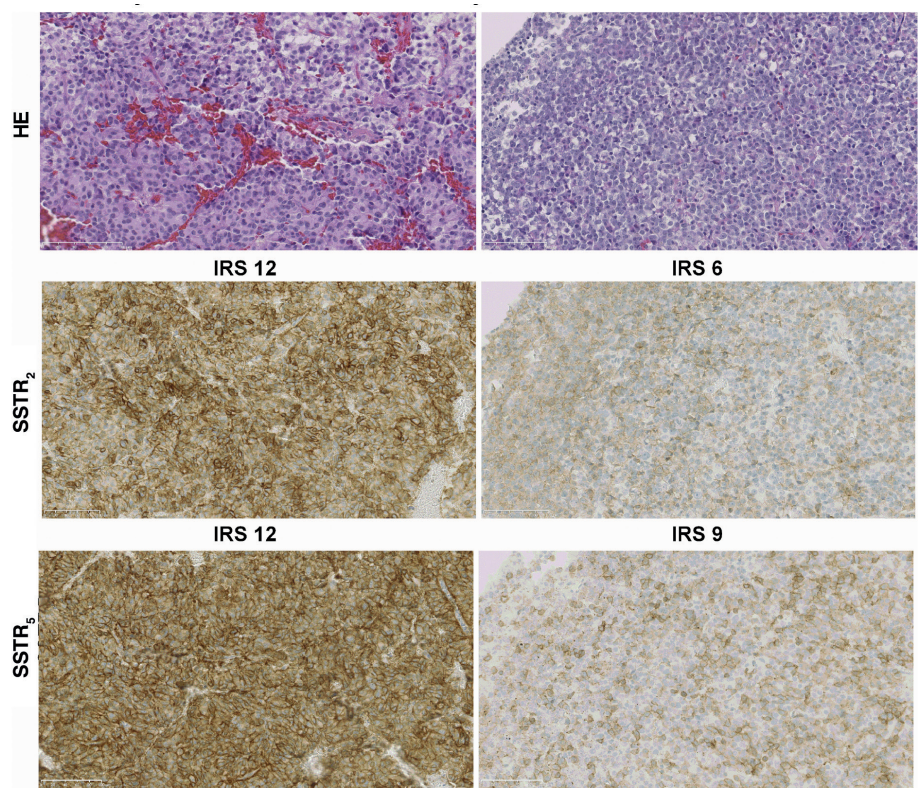
(Diagnostic Systems Laboratories, Webster, Tex., USA, intraassay CV 3.9%, interassay CV 4.2%), and Medgenix Diagnostics, Fleurus, Belgium; intraassay coefficient of variation (CV) 6.1%, interassay CV 9.9%). Total IGF-I was interpreted according to the sex and age-dependent ranges used in accordance with previous reports^(16,17). Because different IGF-I assays were used over time, IGF-I levels were expressed as upper limits of normal (ULN), and not as the absolute values.

Immunohistochemistry

Somatotroph adenoma tissues were stained for hematoxylin and immunostained for SSTR₂ and SSTR₅.

Formalin-fixed paraffin-embedded tumor samples were cut into sequential 4-mm-thick sections, deparaffinized, and stained using a fully automated Ventana BenchMark ULTRA stainer (790-2208; Ventana, Tucson, AZ) according to the manufacturer's instructions at the Pathology Department. Binding of peroxidase-coupled antibodies was detected using 3,39-diaminobenzidine as a substrate, and the sections were counterstained with hematoxylin. The rabbit monoclonal anti-SSTR₂ antibody clone UMB-1 (SS-8000; BioTrend, Köln, Germany) was used at a dilution of 1:50 and the rabbit monoclonal anti-SSTR₅ antibody clone UMB-4 at a dilution of 1:400 (ab109495; Abcam, Cambridge, United Kingdom). Normal pancreatic tissue served as a positive control for both SSTR₂ and SSTR₅ staining. For negative controls, the primary antibody was omitted. Immunostaining of the adenomas was scored semiquantitatively based on an immunoreactivity scoring (IRS) system. The IRS is calculated by the product of the percentage of positive-stained cells (0 is 0%; 1 is 10%; 2 is 10% to 50%; 3 is 51% to 80%; and 4 is 80%) and the staining intensity (0 is no staining; 1 is weak staining; 2 is moderate staining, and 3 is strong staining)⁽¹⁸⁾. The IRS ranges between 0 and 12. The newly stained somatotroph adenoma tissue samples were scored by two independent investigators (A.M. and E.C.C.) based on the histopathological description of the sample provided by the pathologist. Both investigators were blinded to each other's findings and the patients' characteristics. Figure 2 shows two representative cases.

FIGURE 2. SSTR₂ and SSTR₅ protein expression of somatotroph adenomas scored by the IRS. HE, hematoxylin and eosin.



Statistical methods

Categorical data were represented as observed frequencies and percentages. Continuous data were represented as mean and 95% confidence interval (CI) or median and range. The Kolmogorov-Smirnov and the Shapiro-Wilk test were used to test normality of variables. If assumption of normal data distribution was met, the paired t-test was used. For non-normally distributed variables the Wilcoxon signed-rank test was used. Results of correlation analyses were represented as Spearman's Rank correlation coefficients (*r*). We considered *P*-values < 0.05 (two-tailed) to be statistically significant. Statistical analyses

were performed with SPSS version 24 (IBM SPSS Statistics for Windows, Armonk, N.Y., USA) and graphs were drawn using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, California, USA).

Results

Patient characteristics and treatment modalities

Characteristics of the 52 patients included in the study cohort are presented in Table 1. After initial treatment with SRLs, most patients (84.6%) had IGF-I levels above 1.2 x the ULN. All patients continued to receive SRL and pegvisomant combination treatment with a median pegvisomant dose of 100 mg/week (IQR 60 - 160).

Table 2 reports the characteristics of patients that were included in the SSSTR subcohort (n = 14). All these patients harboured a macroadenoma. 5 out of 14 patients had received SRL treatment prior to surgery, [4 achieved initial IGF-I normalization ($< 1.2 \times \text{ULN}$)] and the remaining nine patients were drug-naïve before surgery. During follow-up, all 14 patients received SRL and pegvisomant combination therapy (median pegvisomant dose 100 mg/week (IQR 80 - 145)). After 24 weeks, the median pegvisomant dose was 45 mg/week (IQR 23 - 75) and decreased to 0 mg/week (IQR 0 - 70) after 48 weeks. Three out of 14 (21.4%) patients were on pasireotide LAR monotherapy after 24 weeks, increasing to 8 patients (57.1%) after 48 weeks. More in detail, in two patients pasireotide LAR dose was reduced to 40 mg and in two other patients to 20 mg monotherapy every 4 weeks. In the study cohort, at 24 weeks, the median pegvisomant dose was 45 mg/week (IQR 30 - 80) and 10 of 52 (19.2%) patients were on pasireotide LAR monotherapy. At 48 weeks, the median pegvisomant dose decreased to 40 mg/week (IQR 0 - 90) and 25 of 52 (48.1%) patients were on pasireotide LAR monotherapy.

TABLE 1. Patient characteristics entire cohort. IGF-I levels are shown before initiation of SRL monotherapy and after ≥ 4 months SRL monotherapy. Pegvisomant dose during SRL and pegvisomant combination therapy at baseline of PAPE study.

Characteristics	Patients (n = 52)
Age (median, range)	53.5 (26 – 80)
Female patients (n, %)	22 (42.3%)
Macroadenomas (n, %)	44 (84.6%)
Previous surgery (n, %)	19 (34.6%)
SRL treatment prior to surgery (n, %)	5 (9.6%)
SRL treatment duration (months, mean, 95% CI)	11.2 (8.2 – 14.2)
IGF-I before SRL therapy (x ULN, mean, 95% CI)	3.14 (2.80 – 3.49)
IGF-I after SRL therapy (x ULN, mean, 95% CI)	2.13 (1.82 – 2.45)
IGF-I ≤ 1.2 x ULN after SRL monotherapy (n, %)	13 (27.1%)
Weekly pegvisomant dose (mg, mean, 95% CI)	137 (101 – 172)

Protein expression of somatostatin receptor subtype 2 and 5

The median $SSTR_2$ IRS was 9 (IQR 5 – 12), the median $SSTR_5$ IRS was 12 (IQR 5.5 – 12), and the median $SSTR_2/SSTR_5$ ratio was 1.0 (IQR 0.6 – 1.8). We did not find a statistically significant difference in $SSTR_2$ and $SSTR_5$ expression between medically naïve (n = 9) and SRL pre-treated (n = 5) patients ($P = 0.31$ and $P = 0.25$, respectively). More in detail, median $SSTR_2$ IRS was 12 (IQR 5 – 12) in pre-treated patients and 8 (4 – 10.5) in the naïve ones, while median $SSTR_5$ IRS was respectively 6 (2– 12) and 12 (8.5 – 12) in SRL pre-treated and naïve patients.

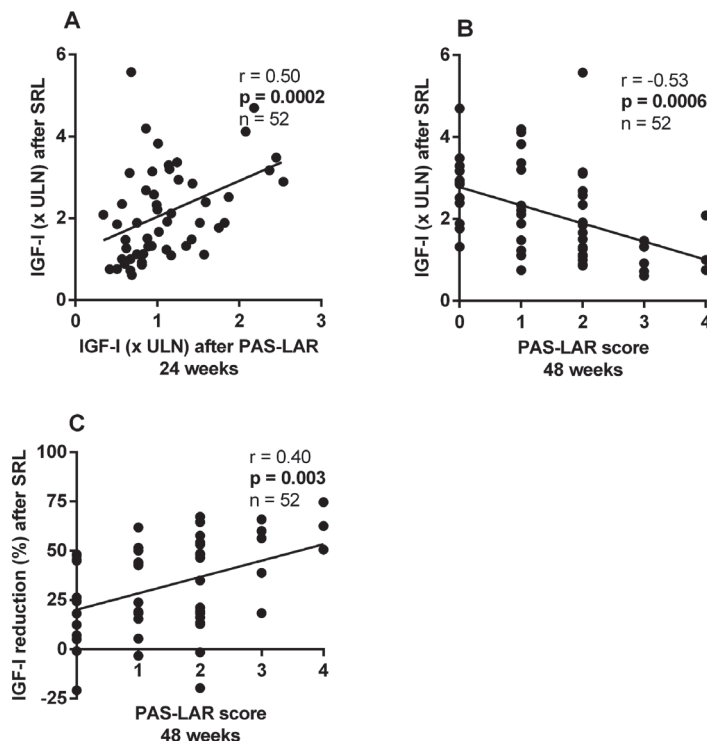
The relation between $SSTR$ immunoreactivity and response to SRL treatment is shown in the supplemental data⁽¹⁹⁾. In line with previous findings, the percentage IGF-I reduction after SRL treatment was positively correlated to $SSTR_2$ IRS, while an inverse trend was observed between $SSTR_2$ IRS and IGF-I (x ULN) levels after SRL treatment. The pegvisomant dose at baseline during the PAPE study was inversely correlated to the $SSTR_2$ IRS.

TABLE 2. Patient characteristics of the SSTR subcohort.

P. number	Sex, age (yrs)	First-generation SRL pretreatment	IGF-I (ULN) after first-generation SRL	% IGF-I reduction after first-generation SRL	IGF-I (ULN) after PAS-LAR 24 weeks	Baseline PEGV dose (mg/week)	48 weeks PEGV dose (mg/week)	48 weeks % PEGV dose reduction	48 weeks PAS-LAR dose (mg)	48 weeks PAS-LAR score	SSTR ₂ (IRS)	SSTR ₅ (IRS)
1	M, 80	No	0,72	65,9	,90	80	0	100	40	3	6	12
2	F, 37	No	1,67	48,5	1,05	80	0	100	60	2	12	12
3	M, 57	No	1,48	42,7	1,28	80	50	37,5	60	1	8	1
4	M, 38	No	1,77	18,2	1,78	700	540	22,9	60	0	1	12
5	F, 36	Yes	3,83	15,5	1,11	100	50	50	60	1	1	0
6	F, 36	No	3,17	7,1	2,91	400	400	0	60	0	6	9
7	M, 61	Yes	1,51	54,2	,79	80	0	100	60	2	12	12
8	F, 71	No	2,21	5,5	1,24	120	60	50	60	1	2	12
9	M, 51	No	3,14	21,2	1,16	120	0	100	60	2	9	12
10	M, 53	Yes	1,89	44,9	1,14	100	100	0	60	0	12	12
11	F, 56	No	0,76	74,6	,39	20	0	100	20	4	12	8
12	F, 46	Yes	1,00	62,6	,49	100	0	100	20	4	9	4
13	M, 46	Yes	1,85	19,4	,49	70	0	100	60	2	12	6
14	F, 69	No	0,61	60,0	,72	220	0	100	40	3	9	12

Detailed description of patients' general characteristics, IGF-I levels after first-generation SRL and PAS-LAR treatment, and the SSTR₂ and SSTR₅ immunoreactivity score (IRS). In addition, PEGV doses are shown during first-generation SRL and PEGV combination treatment at baseline and after switching to PAS-LAR treatment at 48 weeks. The PAS-LAR score takes into account both the achieved PEGV dose reduction and PAS-LAR dose reduction at 48 weeks compared with baseline.

FIGURE 3. Relation between response to SRL treatment and response to pasireotide LAR treatment in the study cohort.



(A) IGF-I (x ULN) levels after SRL treatment were correlated to IGF-I (x ULN) levels after pasireotide LAR treatment at 24 weeks and (B) inversely correlated to the pasireotide LAR score at 48 weeks. (C) The percentage of IGF-I (x ULN) reduction after SRL treatment was positively correlated to the pasireotide LAR score at 48 weeks.

Relationship between response to SRL treatment and pasireotide LAR treatment in study cohort

We observed a significant positive correlation between IGF-I (x ULN) levels after SRL treatment and IGF-I levels after 24 weeks pasireotide LAR treatment ($r = 0.50$, $P = 0.0002$, $n = 52$, Figure 2A). However, no relation was observed between the percentage IGF-I reduction after SRL treatment and after 24 weeks pasireotide LAR treatment ($r = 0.026$, $P = 0.85$, $n = 52$) (Supplemental Figure 1A)⁽¹⁹⁾. With respect to response to

pasireotide LAR after 48 weeks, IGF-I (x ULN) levels after SRL treatment showed a strong inverse correlation with the pasireotide LAR score ($r = -0.53$, $P = 0.0006$, $n = 52$, Figure 2B). Moreover, the percentage IGF-I (x ULN) reduction after SRL treatment was positively correlated to the pasireotide LAR score ($r = 0.40$, $P = 0.003$, $n = 52$, Figure 2C) as well. We also observed a significant relationship between IGF-I (x ULN) levels after SRL treatment and after 48 weeks pasireotide LAR treatment ($r = 0.30$, $P = 0.028$, $n = 52$) (Supplemental Figure 1B)⁽¹⁹⁾.

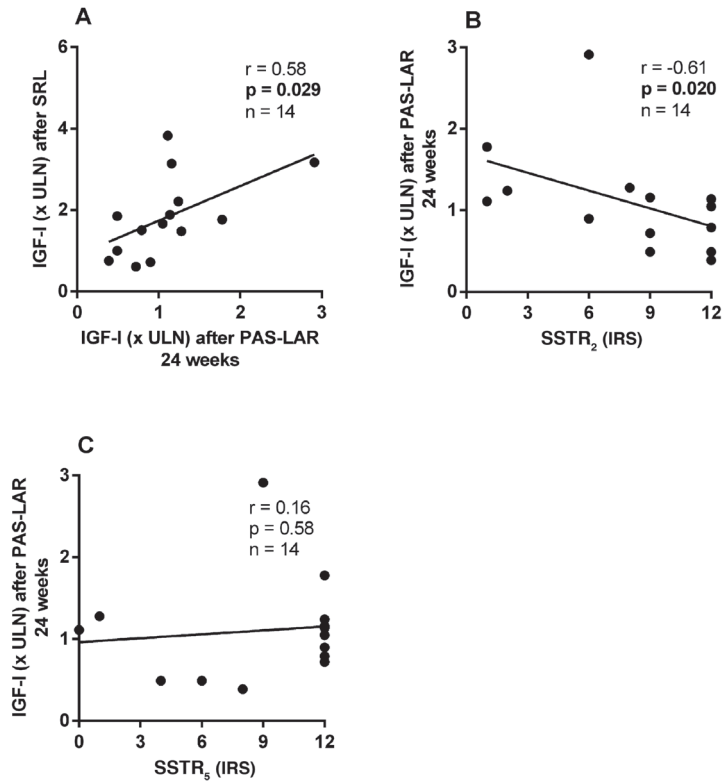
Relationship between response to SRL treatment and pasireotide LAR treatment in SSTR subcohort

In the SSTR subcohort, we observed a positive correlation between IGF-I (x ULN) levels after SRL treatment and after 24 weeks pasireotide LAR treatment ($r = 0.58$, $P = 0.029$, $n = 14$, Figure 3A), while no correlation was observed with percentage IGF-I reduction (Supplemental Figure 1C)⁽¹⁹⁾. Higher SSTR₂ IRS was correlated with lower IGF-I levels (x ULN) after 24 weeks pasireotide LAR treatment ($r = -0.61$, $P = 0.020$, $n = 14$, Figure 3B), while SSTR₅ IRS did not show any relation to IGF-I levels ($r = 0.16$, $P = 0.58$, $n = 14$, Figure 3C).

With respect to the 48 weeks pasireotide LAR response, IGF-I (x ULN) levels after SRL treatment were also inversely correlated to the pasireotide LAR score ($r = -0.71$, $P = 0.005$, $n = 14$, Figure 4A). Furthermore, IGF-I (x ULN) levels after SRL treatment were correlated to IGF-I (x ULN) levels after pasireotide LAR treatment at 48 weeks ($r = 0.58$, $P = 0.031$, $n = 14$, Supplemental Figure 1D)⁽¹⁹⁾.

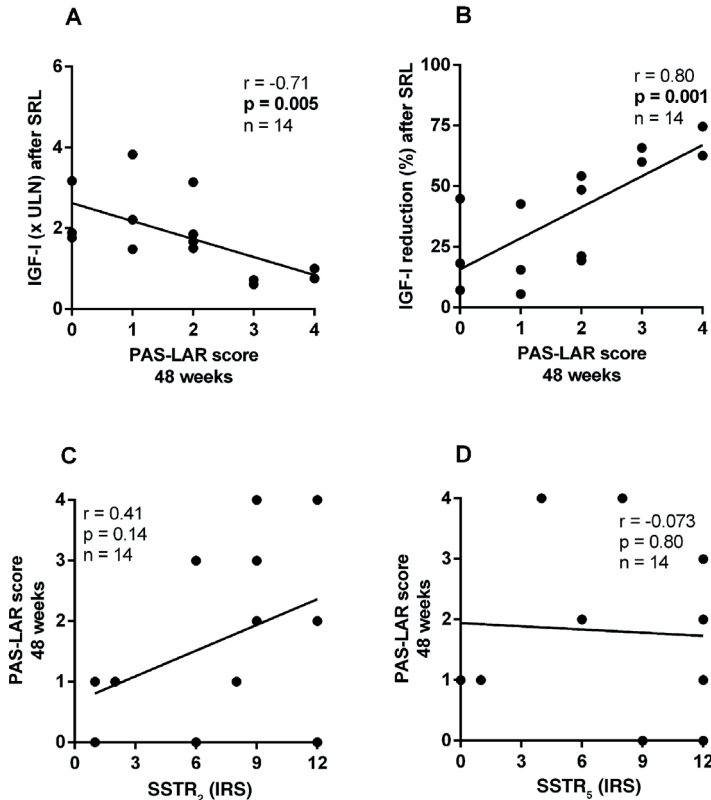
We observed a trend, although not statistically relevant, for a direct correlation between SSTR₂ IRS and the pasireotide LAR score ($r = 0.41$, $P = 0.14$, $n = 14$, Figure 4C), and no relation was found between SSTR₅ IRS and pasireotide LAR score ($r = -0.073$, $P = 0.80$, $n = 14$, Figure 4D). Interestingly, considering only those patients naïve to SRL treatment before surgery ($n = 9$), the correlation between SSTR₂ IRS and the pasireotide LAR score ($r = 0.69$, $P = 0.039$, $n = 9$) was statistically significant, while this was not the case for SSTR₅ IRS.

FIGURE 4. Relation between response to SRL treatment and the response to pasireotide LAR treatment at 24 weeks in the SSTR subcohort.



(A) IGF-I (x ULN) levels after pasireotide LAR treatment at 24 weeks were correlated to IGF-I levels after SRL treatment and (B) inversely correlated to SSTR₂ expression, (C) but not to SSTR₅ expression.

FIGURE 5. Relation between response to SRL treatment and the response to pasireotide LAR treatment at 48 weeks in the SSTR subcohort.



(A) The pasireotide LAR score at 48 weeks was inversely correlated to IGF-I (x ULN) levels after SRL treatment and (B) positively correlated to the percentage of IGF-I reduction after SRL treatment. (C) The pasireotide LAR score showed a trend for a relation with $SSTR_2$ expression, but (D) $SSTR_5$ expression did not show any relation.

Discussion

Our results suggest that in acromegaly patients the responsiveness to pasireotide LAR treatment is mainly correlated to SSTR₂ expression, and not to SSTR₅. This observation is further supported by the finding that after 48 weeks treatment the percentage IGF-I reduction after SRL treatment was correlated to the pasireotide LAR treatment response score. Our study provides the novel finding that the *in vivo* response to pasireotide LAR is directly correlated to both the clinical response to first-generation SRL treatment and SSTR₂ expression on adenoma tissue.

However, these results are not unexpected. Indeed, our data confirm previous *in vitro* studies, carried out in primary cultures of GH-secreting adenomas, showing that the efficacy of naïve somatostatin (SRIF-14), octreotide and pasireotide in the reduction of GH secretion was positively correlated with SSTR₂ mRNA expression, but not with SSTR₅ ^(13, 20, 21). According to these findings, our group and other authors have reported that the effect of octreotide and pasireotide on GH suppression is almost superimposable both *in vitro* and *in vivo* ^(12, 13, 22).

The observed positive relation between SSTR₂ protein expression and the percentage IGF-I reduction after SRL treatment, is in line with previous studies ^(5, 6). Furthermore, an inverse relation between the pegvisomant dose and SSTR₂ expression was recently reported, suggesting that the required pegvisomant dose to normalize IGF-I levels in patients with partial response to SRLs is a surrogate marker for the degree of SRL resistance ⁽⁸⁾. In our cohort, the SRL pretreated patients had a trend for a higher SSTR₂ expression than medically naïve patients. This finding is in contrast to previous studies ^(5, 6), and it is probably correlated to a lack of statistical power in our study (5 vs 9 adenoma samples)

Our results show that IGF-I levels after pasireotide LAR treatment were directly correlated to SSTR₂ expression, and not to SSTR₅ expression. After exclusion of SRL pretreated patients, SSTR₂ IRS was also significantly correlated to the pasireotide LAR score, a tool designed to uncover the impact of switching to pasireotide LAR treatment in patients using SRLs and pegvisomant combination treatment. While there is no evidence that SSTR₅ expression is affected by SRL presurgical treatment, it has been widely demonstrated that patients receiving SRL treatment prior to neurosurgery show

significantly lower SSTR₂ protein expression compared to medically naïve patients^(4-6, 13). Although this is not evident in our cohort, a pooled analysis of SRL pretreated and medically naïve patients can introduce bias. Exclusion of the SRL pretreated patients from our analysis ($n = 5$) resulted in a stronger relationship between SSTR₂ IRS and the response to pasireotide LAR treatment.

A strength of our study lies in the relatively large number of patients in which the clinical efficacy of SRLs, SRL/pegvisomant combination treatment and pasireotide LAR treatment were systematically investigated in combination with data on SSTR expression of somatotroph adenomas in a well characterized subgroup of patients. The main limitation of our study lies in the retrospective collection of data on IGF-I levels during SRL treatment and the use of different IGF-I assays during follow-up. The IGF-I levels measured after 24 weeks pasireotide LAR treatment may be partly influenced by the carry-over effect of withdrawal of SRLs after 12 weeks. While the carry-over effect of SRLs may have influenced the short-term response to pasireotide LAR after 24 weeks, the response to pasireotide LAR treatment after 48 weeks is probably not affected. In our cohort, the pasireotide LAR score at 48 weeks might therefore be the most informative marker for responsiveness to pasireotide LAR treatment.

Two studies have previously investigated the relationship between the immunohistochemical expression of SSTR₂ and SSTR₅ in somatotroph adenomas and the clinical response to first-generation SRL and pasireotide LAR treatment in acromegaly⁽²³⁾. Iacovazzo et al suggested that SSTR₅ expression drives the responsiveness to pasireotide LAR treatment in patients resistant to first-generation SRLs. These authors investigated a cohort of 39 acromegaly patients requiring post-operative SRL treatment, of which 11 patients were resistant to SRL and were switched to pasireotide LAR treatment. They observed that none of the patients lacking SSTR₅ expression was responsive to pasireotide LAR, whereas 5 out of 7 patients with membranous expression of SSTR₅ were responsive to pasireotide LAR. Furthermore, patients with a higher SSTR₅ score had a greater reduction in IGF-I levels. However, they found no difference in SSTR₂ expression between pasireotide responders and non-responders.

These results are in contrast with our findings, which suggest that SSTR₂ expression, and not SSTR₅ expression, is more important for the clinical response to pasireotide LAR. The main difference between our study and the study from Iacovazzo et al.⁽²³⁾ is that we included mainly patients who were partially responsive to SRLs, while Iacovazzo

et al included only SRL resistant patients. Secondly, the patients in our cohort all received pegvisomant treatment before switching to pasireotide LAR during the PAPE study, whereas the patients in the Iacovazzo study did not receive pegvisomant treatment, and were directly switched to pasireotide LAR (Table 2).

Furthermore, in our cohort we cannot rule out a direct effect of pegvisomant treatment on SSTR expression⁽⁸⁾. Pegvisomant is known to increase serum GH levels⁽²⁴⁾, which could result in reduced hypothalamic GHRH secretion, which in turn may lead to a downregulation of SSTR expression. Although the impact of pegvisomant on SSTR expression is plausible⁽²⁵⁾, there is no evidence that pegvisomant treatment plays a major role in the modulation of SSTR expression via the activation of GH-IGF-I axis.

In addition, these differences are unlikely to be explained by the use of a different SSTR expression scoring system. Although the other authors used a scoring method proposed by *Volante* et al which takes into account both subcellular localization and extent of staining⁽²⁶⁾, the method we used from *Remmele* et al is a semiquantitative score which takes into account both intensity and percentage of positive cells⁽¹⁸⁾. Interestingly, both scoring systems have been recently found to show high inter-laboratory and inter-observer agreement for SSTRs expression in neuroendocrine tumors⁽²⁷⁾.

In conclusion, our results suggest that SSTR₂ expression of somatotroph adenomas is more important than SSTR₅ in driving the responsiveness to pasireotide LAR treatment in a peculiar subset of acromegaly patients (*e.g.* partial responders). It is plausible that the enhanced efficacy of pasireotide LAR compared to first-generation SRLs is mediated by its stronger inhibition of insulin secretion, rendering the liver less sensitive to GH action⁽²⁹⁸⁾. The enhanced efficacy of pasireotide LAR could also be the consequence of a differential activation of SSTR₂ by the different compounds (*e.g.* reduced activation of SSTR₂ internalization and faster recycling on the cell membrane)^(29,30), rather than by the higher binding affinity of pasireotide LAR for SSTR₅. Future studies should investigate whether this is indeed the case.

Acknowledgements

We are grateful for the assistance of Hans Stoop from the pathology department for the immunohistochemistry. We also acknowledge the study nurses who contributed to the study, and the patients for their participation in the PAPE study.

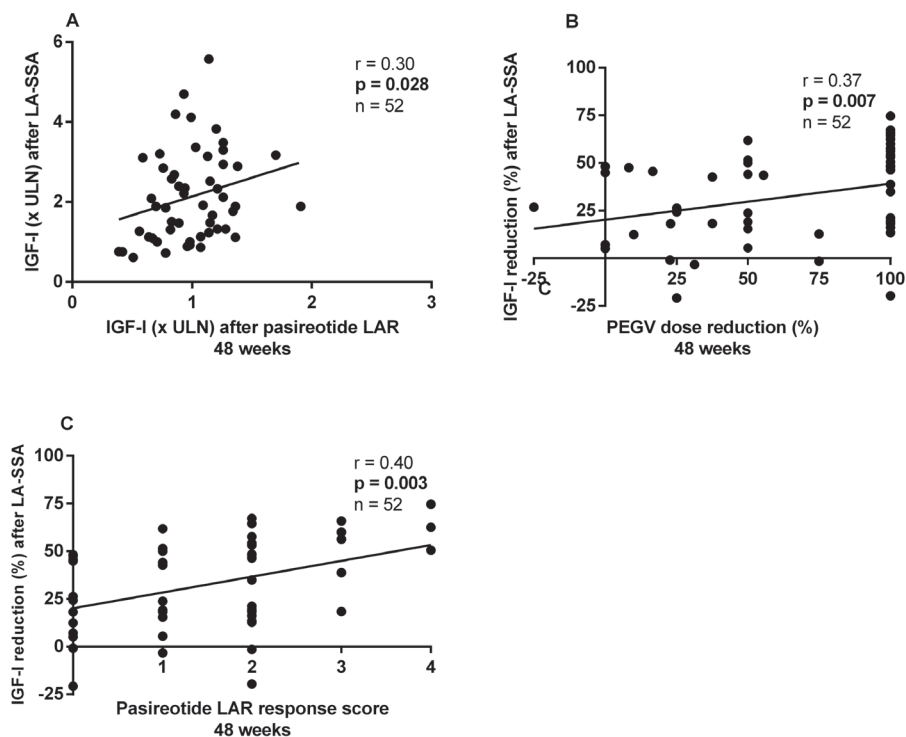
Author contributions

AM, LH, and SN conceived and designed the study. AM was responsible for data management and statistical analyses after discussion with all authors. All authors participated in data interpretation and in writing of the report.

Appendix

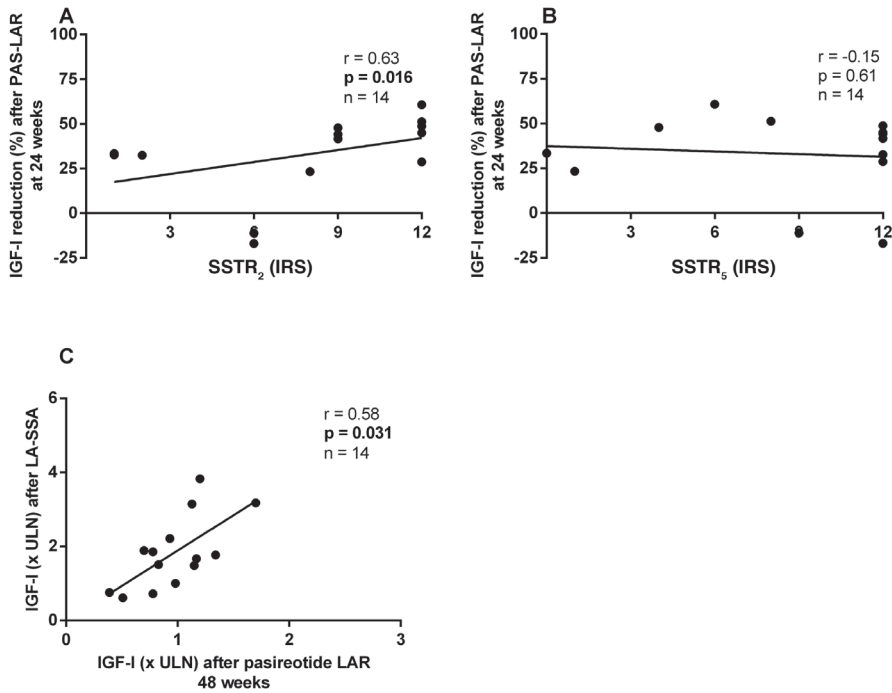
Supplemental material

SUPPLEMENTARY FIGURE 1. Relationship between response to first-generation SRL treatment and SSTR immunoreactivity score.



(IRS) The percentage IGF-I reduction after first-generation SRL treatment was positively related to $SSTR_2$ IRS ($r = 0.54$, $P = 0.046$, $n = 14$), but no correlation was found with $SSTR_5$ IRS ($r = 0.090$, $P = 0.76$, $n = 14$). IGF-I (x ULN) levels after first-generation SRL treatment showed an inverse trend with $SSTR_2$ IRS ($r = -0.31$, $P = 0.28$, $n = 14$), while no correlation was observed with $SSTR_5$ IRS ($r = -0.12$, $P = 0.69$, $n = 14$) (Figure 2A and 2B). Exclusion of first-generation SRL pretreated patients did not change the relation to $SSTR_2$ ($r = -0.34$, $P = 0.37$, $n = 9$) and $SSTR_5$ IRS ($r = 0.10$, $P = 0.98$, $n = 9$).

SUPPLEMENTARY FIGURE 2. Relationship between baseline pegvisomant dose during combination treatment and SSTR protein IRS.



We observed an inverse correlation between the pegvisomant dose at baseline and SSTR₂ IRS ($r = -0.61$, $P = 0.020$, $n = 14$), but SSTR₅ IRS was not correlated with the pegvisomant dose ($r = 0.32$, $P = 0.27$, $n = 14$).

References

1. Melmed, S., Medical progress: Acromegaly. *N Engl J Med*, 2006. 355(24): p. 2558-73.
2. Holdaway, I.M., M.J. Bolland, and G.D. Gamble, A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. *Eur J Endocrinol*, 2008. 159(2): p. 89-95.
3. Taboada, G.F., et al., Quantitative analysis of somatostatin receptor subtype (SSTR₁₋₅) gene expression levels in somatotropinomas and non-functioning pituitary adenomas. *Eur J Endocrinol*, 2007. 156(1): p. 65-74.
4. Plockinger, U., et al., Selective loss of somatostatin receptor 2 in octreotide-resistant growth hormone-secreting adenomas. *J Clin Endocrinol Metab*, 2008. 93(4): p. 1203-10.
5. Gatto, F., et al., Immunoreactivity score using an anti-SST_{2A} receptor monoclonal antibody strongly predicts the biochemical response to adjuvant treatment with somatostatin analogs in acromegaly. *J Clin Endocrinol Metab*, 2013. 98(1): p. E66-71.
6. Casar-Borota, O., et al., Expression of SSTR_{2a}, but Not of SSTRs 1, 3, or 5 in Somatotroph Adenomas Assessed by Monoclonal Antibodies Was Reduced by Octreotide and Correlated With the Acute and Long-Term Effects of Octreotide. *J Clin Endocrinol Metab*, 2013.
7. Fougner, S.L., et al., The clinical response to somatostatin analogues in acromegaly correlates to the somatostatin receptor subtype 2a protein expression of the adenoma. *Clin Endocrinol (Oxf)*, 2008. 68(3): p. 458-65.
8. Franck, S.E., et al., Somatostatin Receptor Expression in GH-Secreting Pituitary Adenomas Treated with Long-Acting Somatostatin Analogues in Combination with Pegvisomant. *Neuroendocrinology*, 2016.
9. Ma, P., et al., Pharmacokinetic-pharmacodynamic comparison of a novel multiligand somatostatin analog, SOM230, with octreotide in patients with acromegaly. *Clin Pharmacol Ther*, 2005. 78(1): p. 69-80.
10. Lesche, S., et al., Differential effects of octreotide and pasireotide on somatostatin receptor internalization and trafficking in vitro. *J Clin Endocrinol Metab*, 2009. 94(2): p. 654-61.
11. Poll, F., et al., Pasireotide and octreotide stimulate distinct patterns of SST_{2A} somatostatin receptor phosphorylation. *Mol Endocrinol*, 2010. 24(2): p. 436-46.
12. Colao, A., et al., Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab*, 2014: p. jc20132480.

13. Gatto, F., et al., In vitro head-to-head comparison between octreotide and pasireotide in GH-secreting pituitary adenomas. *J Clin Endocrinol Metab*, 2017.
14. Muhammad, A., et al., Efficacy and Safety of Switching to Pasireotide in Patients With Acromegaly Controlled With Pegvisomant and First-Generation Somatostatin Analogues (PAPE Study). *J Clin Endocrinol Metab*, 2018. 103(2): p. 586-595.
15. Chalabi, M., et al., Somatostatin analogs: does pharmacology impact antitumor efficacy? *Trends Endocrinol Metab*, 2014. 25(3): p. 115-27.
16. Bidlingmaier, M., et al., Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *J Clin Endocrinol Metab*, 2014. 99(5): p. 1712-21.
17. Elmlinger, M.W., et al., Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). *Clin Chem Lab Med*, 2004. 42(6): p. 654-64.
18. Remmele, W. and H.E. Stegner, [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. *Pathologe*, 1987. 8(3): p. 138-40.
19. Muhammad A, et al., Supplemental material: Pasireotide responsiveness in acromegaly is mainly driven by somatostatin receptor subtype 2 expression. 2018, Figshare.
20. Hofland, L.J., et al., The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. *J Clin Endocrinol Metab*, 2004. 89(4): p. 1577-85.
21. Jaquet, P., et al., Human somatostatin receptor subtypes in acromegaly: distinct patterns of messenger ribonucleic acid expression and hormone suppression identify different tumoral phenotypes. *J Clin Endocrinol Metab*, 2000. 85(2): p. 781-92.
22. Ibanez-Costa, A., et al., Octreotide and Pasireotide (dis)similarly inhibit pituitary tumor cells in vitro. *J Endocrinol*, 2016.
23. Iacovazzo, D., et al., Factors predicting pasireotide responsiveness in somatotroph pituitary adenomas resistant to first-generation somatostatin analogues: an immunohistochemical study. *Eur J Endocrinol*, 2016. 174(2): p. 241-50.
24. Madsen, M., et al., Circulating levels of pegvisomant and endogenous growth hormone during prolonged pegvisomant therapy in patients with acromegaly. *Clin Endocrinol (Oxf)*, 2014. 80(1): p. 92-100.

25. Murray, R.D., et al., Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. *J Clin Invest*, 2004. 114(3): p. 349-56.
26. Volante, M., et al., Somatostatin receptor type 2A immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. *Mod Pathol*, 2007. 20(11): p. 1172-82.
27. Kasajima, A., et al., High interlaboratory and interobserver agreement of somatostatin receptor immunohistochemical determination and correlation with response to somatostatin analogs. *Hum Pathol*, 2017.
28. Leung, K.C., et al., Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab*, 2000. 85(12): p. 4712-20.
29. Cescato, R., et al., Agonist-biased signaling at the SST₂A receptor: the multi-somatostatin analogs KE108 and SOM230 activate and antagonize distinct signaling pathways. *Mol Endocrinol*, 2010. 24(1): p. 240-9.
30. Kao, Y.J., M. Ghosh, and A. Schonbrunn, Ligand-dependent mechanisms of SST₂A receptor trafficking: role of site-specific phosphorylation and receptor activation in the actions of biased somatostatin agonists. *Mol Endocrinol*, 2011. 25(6): p. 1040-54.

Pasireotide responsiveness in acromegaly is mainly driven by somatostatin receptor subtype 2 expression



Chapter 4

**T2-signal intensity, SST receptor expression,
and somatostatin analogs efficacy predict
response to pasireotide in acromegaly**

Eva C. Coopmans, Joppe J. Schneiders, Nour El-Sayed, Nicole S. Erler,
Leo J. Hofland, Aart J. van der Lely, Patrick Petrossians, Julia Potorac,
Ammar Muhammad and Sebastian J.C.M.M. Neggers

Eur J Endocrinol 2020; 182(6): 595-605.

Abstract

OBJECTIVE: T2-signal intensity and somatostatin (SST) receptor expression are recognized predictors of therapy response in acromegaly. We investigated the relationship between these predictors and the hormonal and tumoral responses to long-acting pasireotide (pasireotide LAR) therapy, which were also compared with responsiveness to first-generation somatostatin receptor ligands (SRLs).

DESIGN: The PAPE study is a cohort study.

METHODS: We included 45 acromegaly patients initially receiving SRLs, followed by combination therapy with pegvisomant, and finally pasireotide LAR. We assessed tumor volume reduction ($\geq 25\%$ from baseline), IGF-I levels (expressed as the upper limit of normal), and T2-weighted MRI signal and SST receptor expression of the adenoma.

RESULTS: Patients with significant tumor shrinkage during pasireotide LAR showed higher IGF-I levels during pasireotide LAR (mean (S.D.): 1.36 (0.53) vs 0.93 (0.43), $P = 0.020$), less IGF-I reduction after first-generation SRLs (mean (S.D.): 0.55 (0.71) vs 1.25 (1.07), $P = 0.028$), and lower SST₂ receptor expression (median (IQR): 2.0 (1.0-6.0) vs 12.0 (7.5-12.0), $P = 0.040$). Overall, T2-signal intensity ratio was increased compared with baseline (mean (S.D.): 1.39 (0.56) vs 1.25 (0.52), $P = 0.017$) and a higher T2-signal was associated with lower IGF-I levels during pasireotide LAR (β : -0.29, 95% CI: -0.56 to -0.01, $P = 0.045$). A subset of pasireotide LAR treated patients with increased T2-signal intensity achieved greater reduction of IGF-I (mean (S.D.): 0.80 (0.60) vs 0.45 (0.39), $P = 0.016$).

CONCLUSIONS: Patients unresponsive to SRLs with a lower SST₂ receptor expression are more prone to achieve tumor shrinkage during pasireotide LAR. Surprisingly, tumor shrinkage is not accompanied by a biochemical response, which is accompanied with a higher T2-signal intensity.

Introduction

Acromegaly is a rare endocrine disease characterized by growth hormone (GH) hypersecretion and elevated insulin-like growth factor 1 (IGF-1) levels, generally as a result of a GH-producing pituitary tumor⁽¹⁾. Treatment modalities are aimed at normalizing IGF-1 levels, reducing GH levels below 1.0 µg/L, decreasing tumor volume and improving clinical symptoms⁽²⁾. Transsphenoidal surgery is considered the gold standard in acromegaly management⁽²⁾, but medical therapy has an increasingly important role. Pasireotide long-acting release (pasireotide LAR) is a somatostatin multi-receptor ligand approved for second-line medical therapy for patients with acromegaly for whom surgery is not an option or is not curative^(3, 4).

Previous clinical studies have indicated that pasireotide LAR can achieve control of GH and IGF-1 levels in a subset of acromegaly patients not responding adequately to first-generation somatostatin receptor ligands (SRLs) and might reduce tumor size in patients uncontrolled with first-generation SRLs^(5, 6). The PAPE study^(7, 8) investigated acromegaly patients well-controlled on a combination treatment of first-generation SRLs and the GH receptor antagonist pegvisomant (pegvisomant). Switching to pasireotide LAR with or without pegvisomant, resulted in control of IGF-1 levels in most (77.0%) patients.

T2-weighted MRI signal of the adenoma has been recently recognized as a non-invasive predictor of response to first-generation SRL therapy in acromegaly. Somatotroph adenomas with lower T2-signal intensity are frequently smaller and less invasive than adenomas with a higher T2-signal intensity⁽⁹⁻¹²⁾, but the correlation with first-generation SRL-induced tumor shrinkage was inconsistent among studies^(9, 11, 13, 14). Although T2-hypointense adenomas are associated with higher GH levels at diagnosis⁽¹⁰⁾, these patients have greater GH and IGF-1 reductions after a median of six months of pre-surgical first-generation SRLs treatment than patients with T2-iso- or -hyperintense adenomas^(9, 11, 14).

There is evidence that biochemical response to somatostatin analogs can be predicted by the SST receptor subtype binding profile in the somatotroph adenoma^(15, 16). The first-generation SRLs show a high preferential binding affinity for SST₂ receptor, while pasireotide LAR exhibits particularly high affinity for SST₅ receptor⁽¹⁷⁾. The *in vitro* anti-proliferative⁽¹⁸⁾ and anti-secretory⁽¹⁹⁾ effects of first-generation SRLs and pasireotide LAR

therapy were superimposable. Although the latter suggests a predominant role for the SST₂ receptor in mediating the inhibitory effect of pasireotide LAR on GH secretion in adenomas, results of the same *in vitro* study⁽¹⁹⁾ indicated that somatotroph adenomas with lower SST₂ receptor expression were better responders to pasireotide LAR compared to first-generation SRLs. To our knowledge, there are no studies investigating the correlation between anti-proliferative effects of pasireotide LAR and the SST receptor profile in acromegaly patients.

The aims of this prospective open-label conversion study were: 1) to investigate the relationship between the T2-weighted signal of the adenoma and the hormonal and tumoral responses to pasireotide LAR, alone or in combination with pegvisomant therapy, including the SST₂ and SST₅ receptor expression in adenomas; 2) to investigate to what extent this correlates with responsiveness to first-generation SRLs.

Materials and Methods

Cohort Description

Data collection of acromegaly patients was performed at the outpatient clinic of the Pituitary Center Rotterdam, Erasmus University Medical Center in Rotterdam. We initially started with a cohort of 61 acromegaly patients who received pasireotide LAR treatment during their participation in the PAPE study (Figure 1); details of the study design have been reported^(7, 8). Briefly, all included patients have previously been treated with first-generation SRLs, followed by pegvisomant and SRL combination therapy. At baseline, the pegvisomant dose was reduced by 50% up to three months, while first-generation SRLs were continued. When IGF-1 remained $\leq 1.2 \times \text{ULN}$ after three months, patients were switched to pasireotide LAR 60 mg monotherapy for three months. When IGF-1 was $> 1.2 \times \text{ULN}$, patients were switched to pasireotide LAR 60 mg, and they continued with the 50% reduced pegvisomant dose for three months. During the extension phase up to nine months of pasireotide LAR treatment, the goal was to achieve IGF-1 normalization (IGF-1 $\leq 1.2 \times \text{ULN}$) through protocol-based dose titration of pegvisomant⁽⁷⁾.

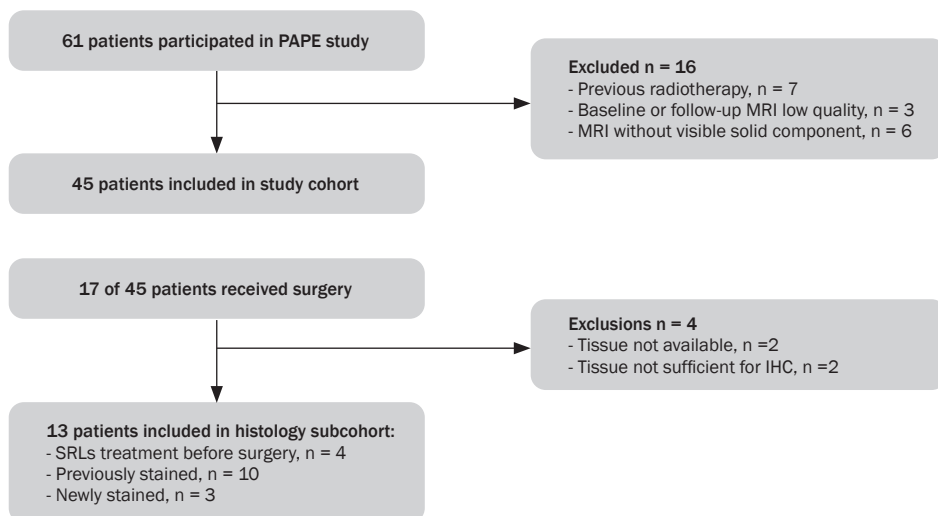
We prospectively collected data on pasireotide LAR, either as monotherapy or in combination with pegvisomant, while data on medical history and clinical response

in patients during first-generation SRLs treatment were collected retrospectively. The PAPE study was registered with ClinicalTrials.gov, number NCT02668172. The study was approved by the independent Medical Ethics Committee of the Erasmus University Medical Center in Rotterdam, and the appropriate data use agreements were in place for the database. Written informed consent was obtained from all study participants prior to inclusion.

Patients who received postoperative radiotherapy (n=7), patients with low quality baseline or follow-up MRI during pasireotide LAR, alone or in combination with pegvisomant (n=3), and patients with a MRI without a visible solid component (n=6) were excluded. In total 45 patients remained and were finally included in this study cohort. 17 out of these 45 patients underwent neurosurgery before the PAPE study.

Immunohistochemistry (IHC) was assessed in patients with sufficient adenoma tissue available (n=13). One patient underwent a second surgery, but we analyzed only the tissue sample of the first surgery. From the 13 remaining tissue samples included for IHC analysis, 10 tissue samples were stained previously and three were newly stained (Figure 1).

FIGURE 1. Flowchart of the selection procedure for the study cohort and the somatotroph adenoma tissue samples. All patients eventually received first-generation SRL and pegvisomant combination treatment, and were switched to pasireotide LAR treatment alone or in combination with pegvisomant during the PAPE study.



IHC = immunohistochemistry; MRI = Magnetic resonance imaging; PAPE = pasireotide and pegvisomant study; SRL = first-generation somatostatin receptor ligand; SST = somatostatin.

Biochemical measurements

Random GH serum concentrations were analyzed using the IDS-iSYS assay (IDS-iSYS, Immunodiagnostic Systems Limited, Boldon, UK), which is free of interference from pegvisomant⁽²⁰⁾. Total IGF-1 serum concentrations during the PAPE study were analyzed by the commercially available immunometric assay (IDS-iSYS, Immunodiagnostic Systems Limited, Boldon, UK). Inter-assay coefficients of variation (CVs) for GH and IGF-1 were <5% (GH; n=190) and (IGF-1; n=190) in serum based internal quality control measurements over a period of 1 year. Total IGF-1 serum concentrations before and after first-generation SRL treatment were analyzed using different assays: Immulite 2000 assay, a solid-phase, validated enzyme-labelled chemiluminescent immunometric assay (DPC Biermann GmbH/Siemens, Fernwald, Germany; intra-assay variability of 2–5%, inter-assay variability of 3–7%), the immunometric IDS-iSYS assay (IDS-iSYS, Boldon, UK; intra-assay CV 8.1%, interassay CV 2.1%), and two different radioimmunoassays (Diagnostic Systems Laboratories, Webster, Tex., USA, intra-assay CV 3.9%, interassay CV 4.2%, and Medgenix Diagnostics, Fleurus, Belgium; intra-assay CV 6.1%, interassay CV 9.9%).

IGF-1 concentrations are expressed as the upper limit of normal (ULN; measured IGF-1 divided by the age- and sex-specific ULN)⁽²¹⁾.

Response to pasireotide LAR, alone or in combination with pegvisomant during the PAPE study was divided into short- and long-term treatment response. Short-term treatment response was evaluated as IGF-1 levels (x ULN) and as absolute IGF-1 reduction during three months of pasireotide LAR treatment (*i.e.* after three injections of pasireotide LAR 60 mg). Absolute IGF-1 reduction was calculated by subtracting IGF-1 (x ULN) level at baseline (*i.e.* the start of the study) from IGF-1 (x ULN) level at follow-up. During the extension phase until nine months, the pegvisomant dose was titrated according to a protocol⁽⁷⁾ to achieve normalized IGF-1 levels. Therefore, the long-term treatment response to pasireotide LAR was based on a composite “Pasireotide LAR treatment response score” (pasireotide LAR score) in order to fully capture the effect of pasireotide LAR taking into account pegvisomant dose reduction, discontinuation and finally pasireotide LAR dose reduction⁽⁸⁾. The pasireotide LAR score comprised five categories (0–4) representing the difference in pegvisomant dose and pasireotide LAR dose during nine months of treatment versus baseline (*i.e.* the start of the study). The highest pasireotide LAR score reflects the best response to pasireotide LAR treatment.

MRI evaluation

Gadolinium-enhanced pituitary MRI was performed at baseline (*i.e.* the start of the study), or maximum 1 year before study entry, and during nine months of pasireotide LAR, either as monotherapy or in combination with pegvisomant. The MRI examinations of each patient were collected for centralized, blinded reading. A single experienced neuroradiologist (J.S) evaluated the MRIs in the Picture Archive Communication System (PACS, Sectra®, Linköping, Sweden). According to previous literature the T2-weighted signal on the baseline MRI was visually compared to the normal pituitary tissue and if not visible, to the gray matter of the temporal lobe^(9, 10). Moreover, quantification of the T2-weighted MRI signal by Region Of Interest (ROI) measurement of the adenoma, normal pituitary tissue and gray matter of the temporal lobe was performed and used as ROI-derived T2 intensity ratio of the adenoma versus grey matter.

Tumor volume was calculated according to the formula: height x width x length x $\pi/6$ ⁽²²⁾. Relative tumor shrinkage was calculated by subtracting the tumor volume at baseline from the follow-up measurement, divided by the tumor volume at baseline: a tumor volume change of $\geq 25\%$ from baseline was considered significant. Absolute tumor shrinkage was calculated by subtracting tumor volume at baseline from tumor volume at follow-up.

Reviewing the tumoral response to first-generation SRLs, diagnostic MRIs as well as follow-up MRIs performed at least after three months of first-generation SRL treatment were assessed. In our cohort, we were unable to assess T2-signal intensity of these diagnostic MRIs visually or by ROI measurement due to the overall low resolution. For evaluation of tumor response to first-generation SRL treatment, tumor shrinkage of $\geq 25\%$ was considered significant.

Immunohistochemistry

Somatotroph adenoma tissues were stained for hematoxylin and immunostained for SST₂ and SST₅ receptor as described previously⁽²³⁾. Immunoreactivity of the adenomas for SST₂ and SST₅ receptor was scored semi quantitatively using a validated immunoreactivity scoring system (IRS)⁽²⁴⁾. Two independent investigators (E.C. and A.M.) – blinded to each other's findings and the patient's characteristics – scored the protein expression of the newly stained adenoma tissue samples taking into consideration the histopathological description of the sample provided by the pathologist.

Outcomes

The primary endpoints of the study were tumor volume reduction of $\geq 25\%$ from baseline and the prediction of the T2-weighted MRI signal for biochemical response and tumor volume reduction during pasireotide LAR, alone or in combination with pegvisomant therapy. Additional endpoints were ⁽¹⁾ SST receptor IHC scores and ⁽²⁾ absolute IGF-1 reduction achieved after at least four months of high dose first-generation SRL treatment. The biochemical response to pasireotide LAR, either as monotherapy or in combination with pegvisomant, was divided into short-term (until three months) and long-term (until nine months) response. The secondary endpoints were absolute tumor shrinkage and random GH serum concentrations.

Statistical Analysis

Continuous data were expressed as mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate. Categorical data were represented as observed frequencies and percentages. The empirical distributions of continuous variables were plotted and assessed for normality using the Kolmogorov-Smirnov test. Natural logarithmic transformation of the data was done when required. We compared categorical variables between two or more groups with the χ^2 test, and we compared continuous variables between groups with either Student's *t* test or Mann-Whitney *U* test (for two groups, as appropriate). We tested correlations between continuous variables with either Pearson's (*r*) or Spearman's rank correlation coefficient (*r_s*), as appropriate. A multivariable linear regression model was used to investigate associations between candidate predictors (*e.g.* age, sex, T2-signal intensity ratio and tumor shrinkage) for the outcome hormonal response during pasireotide LAR treatment. Statistical analyses were performed with R statistical software, version 3.4.1 (packages *rms*) and graphs were drawn using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, California, USA). All tests were two-sided and α was set at 0.05 without any multiplicity correction.

Results

Cohort Demographics and Clinical Characteristics

Cohort demographics and clinical characteristics of the 45 included patients receiving pasireotide LAR treatment are summarized in Table 1. After three months, 10 (22.2%) out of 45 patients were on pasireotide LAR 60 mg monotherapy every four weeks, increasing to 31 (68.9%) patients during nine months of pasireotide LAR treatment. The remaining 14 (31.1%) patients with elevated IGF-1 levels continued with their reduced dose of pegvisomant treatment in combination with pasireotide LAR 60 mg monthly. In this combination group, the average total reduction in pegvisomant dose was 32.9% (median 30 mg/week [IQR 0–95] vs 80 mg/week [60–160], $P = <0.0001$) in combination with pasireotide LAR after nine months of treatment.

We confirmed that quantitative measurement defined as ROI-derived T2 intensity ratio of the adenoma versus pituitary tissue on MRI corresponded well with the visual assessment at baseline. At the end of the study, the mean T2-signal intensity ratio of the adenoma in the total cohort during pasireotide LAR treatment was significantly higher compared with baseline (mean 1.39 [SD 0.56] vs 1.25 [0.52], $P = 0.017$).

Tumoral Response

Tumoral response was evaluated after a mean of 11.8 months of pasireotide LAR treatment. In 33 patients (73.3%) tumor volume decreased from baseline; this shrinkage was significant ($\geq 25\%$) in only 15 cases (33.3%) (Figure 2). Tumor volume increased from baseline in 12 patients (26.7%); of which 6 (13.3%) had a significant ($\geq 25\%$) tumor volume increase. Three patients harbored microadenomas without clinically relevant tumor size increase. The other three harbored macroadenomas and the observed significant increase in tumor size was only clinically relevant in one patient (*e.g.* surgical intervention and/or stereotactic radiosurgery is needed). With regard to the tumoral response to first-generation SRL treatment, tumor shrinkage was significant ($\geq 25\%$) in 13 cases (28.9%). In a previous study our group observed in approximately the same cohort a significant tumor shrinkage ($\geq 20\%$) in 13 (16.9%) out of 77 patients during combination treatment with first-generation SRLs and pegvisomant (25). Interestingly, an additional significant decrease in tumor volume was observed during pasireotide LAR in two cases (15.4%).

TABLE 1. Cohort demographics and clinical characteristics of the total group, non-hyperintense group and hyperintense adenomas subgroup. Data are reported as mean (SD), median (IQR) or number (%). Asterisk represents $p \leq 0.05$ for the comparisons between non-hyperintense and hyperintense adenoma group and are derived from the Student's t test (continuous variables) and Pearson's χ^2 test (categorical variables).

	All patients	Non-increased T2-signal intensity subgroup	Increased T2-signal intensity subgroup
	n = 45	n = 33	n = 12
Age (years)	52.0 (11.7)	51.7 (11.8)	52.8 (11.9)
Female patients	19 (42.2%)	15 (45.5%)	4 (33.3%)
Previous surgery	17 (37.8%)	15 (45.5%)	2 (16.7%)
SRL treatment duration (months)†	9.9 (10.5)	7.9 (6.2–10.1)	6.5 (4.5–7.9)
IGF-1 after SRL therapy (x ULN)	2.22 (1.06)	2.20 (1.08)	2.29 (1.07)
IGF-1 absolute reduction after SRL therapy (x ULN)	1.01 (1.01)	1.03 (0.97)	0.95 (1.16)
Pegvisomant dose at baseline (mg/week)	80 (60–160)	80 (20–120)	100 (63–175)
IGF-1 during pasireotide LAR 3 months (x ULN)	1.12 (0.49)	1.17 (0.51)	0.97 (0.43)
IGF-1 absolute reduction during asireotide LAR 3 months (x ULN)	0.50 (0.52)	0.45 (0.39)	0.80 (0.60)*
Pasireotide LAR score 9 months	1.5 (1.1)	1.5 (1.2)	1.5 (1.1)
Macroadenomas	36 (80.0%)	24 (72.7%)	12 (100.0%)
Tumor volume (mm ³)	1608 (439–4504)	779 (333–3041)	3687 (1919–7178)*
T2-signal adenoma/pituitary or GM at baseline	1.24 (0.50)	1.26 (0.52)	1.19 (0.47)
Mean time between MRIs (months)	18.8 (7.3)	19.0 (7.8)	18.1 (6.0)
SST ₂ receptor††	9.0 (4.0–12.0)	9.0 (6.0–12.0)	6.5 (1.0–12.0)
SST ₅ receptor†††	12.0 (7.0–12.0)	12.0 (8.0–12.0)	9.0 (6.0–12.0)
SST ₂ /SST ₅ receptor ratio†††	1.0 (0.5–1.4)	1.0 (0.6–1.1)	1.0 (0.1–2.0)

GM=grey matter, IGF-1=insulin-like growth factor 1, pasireotide LAR=pasireotide long-acting release, SRL=first-generation somatostatin receptor ligand, ULN=upper limit of normal.

†One patient received first-generation SRL treatment less than 4 months; ††Obtained from 13 tissue samples;

†††Obtained from 12 tissue samples.

At baseline, tumor size was correlated with random GH levels ($r = 0.85$, $P = <0.0001$). Moreover, larger adenomas correlated with greater absolute tumor shrinkage during pasireotide LAR treatment ($r = 0.51$, $P = 0.00038$). Microadenomas tended to present more often with higher T2-signal intensity at baseline, compared with macroadenomas (1.58 [SD 0.60] vs 1.18 [0.47], $P = 0.06$). T2-signal intensity at baseline had no effect on significant tumor shrinkage (mean 1.12 [SD 0.35] vs 1.31 [0.57], $P = 0.23$).

With regard to the hormonal responsiveness, patients with significant tumor shrinkage during pasireotide LAR treatment had less IGF-1 reduction after first-generation SRL treatment when compared to the total cohort (mean 0.55 x ULN [SD 0.71] vs 1.25 [1.07], $P = 0.028$; Figure 3A). After three months of pasireotide LAR treatment patients with significant tumor shrinkage had higher IGF-1 levels than patients without significant tumor shrinkage (mean 1.36 x ULN [SD 0.53] vs 0.93 [0.43], $P = 0.020$; Figure 3B).

T2-Signal Intensity of the Adenoma

The visual assessment of T2-weighted MRI signal categorized 12 (26.7%) adenomas as hypointense, 13 (28.9%) as isointense and 20 (44.4%) as hyperintense at baseline.

We observed an inverse correlation between IGF-1 (x ULN) levels during three months of pasireotide LAR treatment and T2-signal intensity of adenomas at baseline ($r = -0.39$, $P = 0.0075$; Figure 4A). In other words, higher T2-signal intensity adenomas at baseline are correlated with a better hormonal response (*i.e.* meaning lower IGF-1 (x ULN) levels during three and nine months of pasireotide LAR treatment).

FIGURE 2. Change in tumor volume from baseline to follow-up (%). The dashed line represents 25% reduction for tumor volume. Figure shows relative change for tumor volume in individual patients with available data at baseline and follow-up.

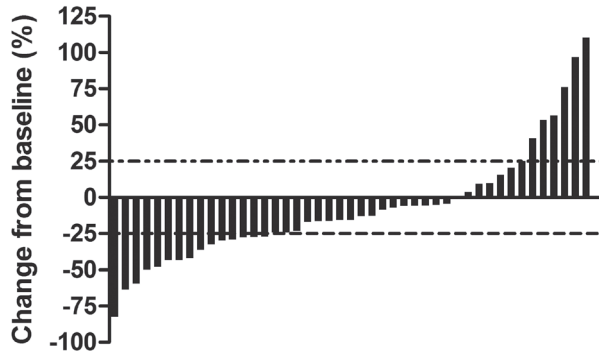
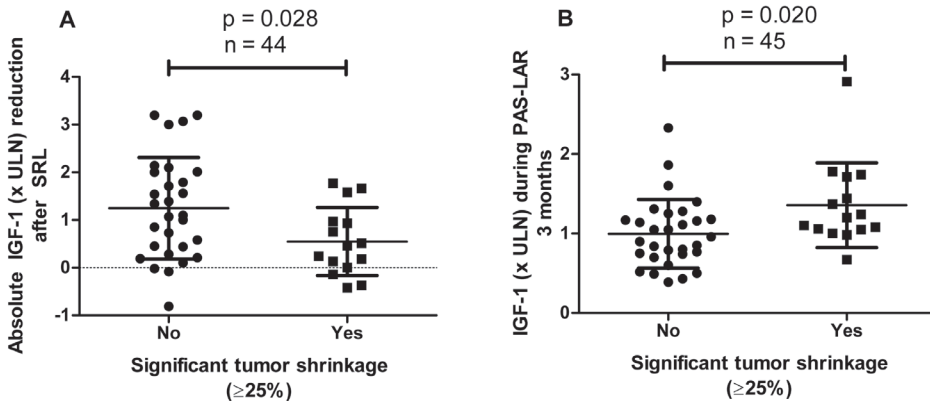
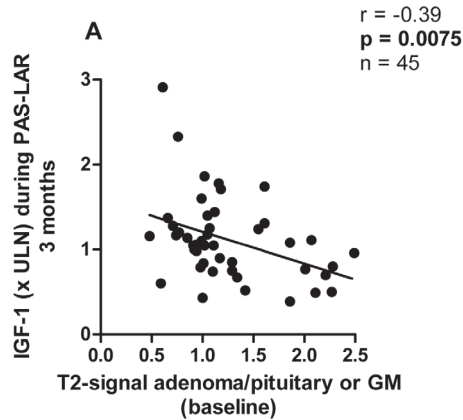


FIGURE 3. Relation between significant tumor shrinkage and hormonal response to SRL treatment and pasireotide LAR treatment. Data are presented as scatter dot plots depicting the mean (SD). (A) The absolute IGF-1 (x ULN) reduction after SRL treatment was greater in patients without significant tumor shrinkage and (B) The IGF-1 (x ULN) levels during three months of pasireotide LAR are lower in patients without significant tumor shrinkage.



IGF-1=insulin-like growth factor 1, pasireotide LAR=pasireotide long-acting release, SRL=first-generation somatostatin receptor ligand, ULN=upper limit of normal.

FIGURE 4. Relation between T2-weighted MRI signal intensity of the adenomas and hormonal response to pasireotide LAR treatment. Data is presented as a scatter plot with Pearson correlation coefficient (r). (A) T2-signal intensity adenomas at baseline were inversely correlated to IGF-1 (x ULN) levels during three months of pasireotide LAR treatment.



GM=grey matter, IGF-1=insulin-like growth factor 1, pasireotide LAR=pasireotide long-acting release, ULN=upper limit of normal.

Increased T2-Signal Intensity of the Adenoma

As recently reported, in 14 patients T2-weighted MRI signal intensity of the adenoma was increased during pasireotide LAR treatment⁽²⁶⁾. Two out of 14 patients with increased T2-weighted MRI signal intensity of the adenoma received postoperative radiotherapy and were excluded from the following analysis.

At baseline, we did not observe any significant differences in age, sex and biochemical response after first-generation SRL treatment or T2-signal intensity ratio between patients with increased T2-signal intensity and patients with non-increased T2-signal intensity (Table 1). Patients with increased T2-signal intensity had larger adenomas at baseline compared to the total cohort (median 2687 mm³ [IQR 1919–7178] vs 779 mm³ [333–3041], $P = 0.026$; Table 1). Furthermore, we found no significant differences in absolute IGF-1 reduction after first-generation SRL treatment in patients with increased

T2-signal intensity adenomas (mean 0.95 x ULN [SD 1.16] vs 1.03 [0.97]; $P = 0.83$; Table 1). These patients further showed greater reduction of IGF-1 levels during three months of pasireotide LAR treatment (mean 0.80 x ULN [SD 0.60] vs 0.45 [0.39], $P = 0.016$; Table 1). However, they did not present more often with significant tumor shrinkage (4 patients (33.3%) vs 11 patients (33.3), $\chi^2 P = 1.00$) nor with greater absolute tumor shrinkage during pasireotide LAR (median 146 mm³ [IQR -25–1199] vs 110 mm³ [5–449]; $P = 0.79$).

Immunohistochemistry of the Adenoma

We observed that patients with significant tumor shrinkage had lower SST₂ receptor (median 2.0 [IQR 1.0–6.0] vs 12.0 [7.5–12.0], $P = 0.040$; Figure 5A) as well as lower SST₂/SST₅ receptor ratio expression (median 0.2 [IQR 0.1–0.7] vs 1.0 [0.9–1.8], $P = 0.024$; Figure 5C). SST₅ receptor expression did not differ significantly between the groups (median 12.0 [IQR 9.0–12.0] vs 12.0 [4.8–12.0], $P = 0.63$; Figure 5B).

From the 13 tissue samples included for IHC analysis, two tissue samples (14%) were derived from patients with increased T2-signal intensity. We did not observe any significant difference in SST₂, SST₅ receptor expression, and SST₂/SST₅ receptor ratio in patients with increased T2-signal intensity, compared to the total cohort (Table 1).

Hormonal Response

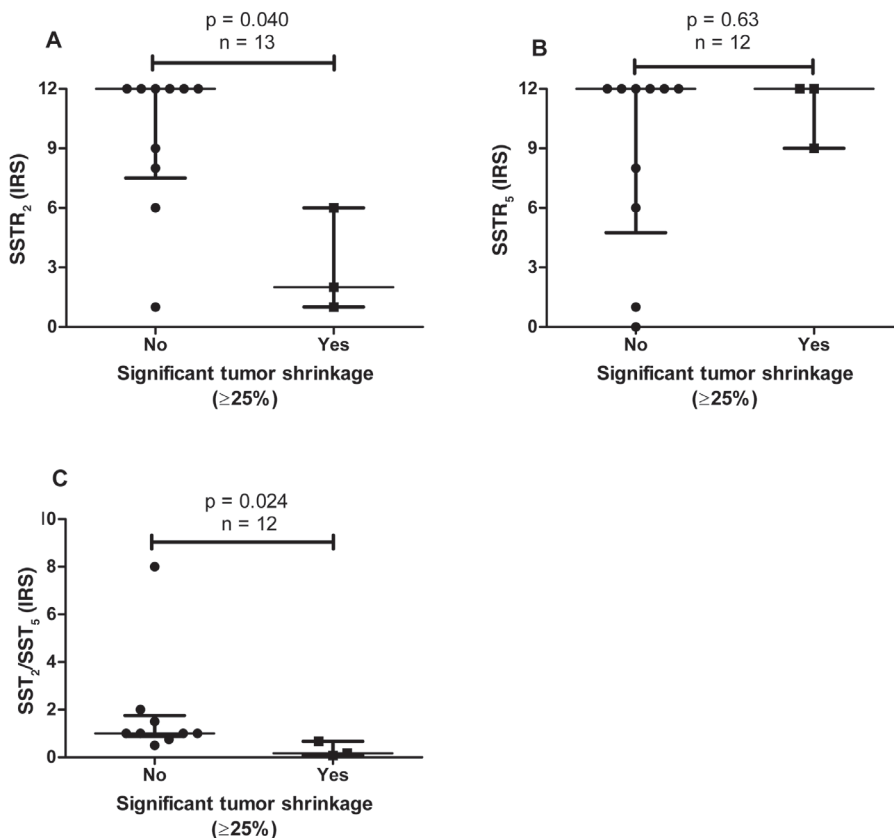
Table 2 shows the regression coefficients and 95% confidence intervals from the multivariable linear regression model for IGF-1 (x ULN) levels during three months of pasireotide LAR. A significant inverse association was seen between T2-signal intensity of adenomas at baseline and IGF-1 (x ULN) levels during three months of pasireotide LAR (β -0.29, 95% CI -0.56–0.01, $P = 0.045$; Table 2). Moreover, we detected a positive association between significant tumor shrinkage and IGF-1 (x ULN) levels during three months of pasireotide LAR (β 0.34, 95% CI 0.02–0.65, $P = 0.035$; Table 2). In other words, higher T2-signal intensity adenomas at baseline and less tumor shrinkage were associated with lower IGF-1 (x ULN) levels during three months of pasireotide LAR.

TABLE 2. Determinants of IGF-1 (x ULN) during pasireotide LAR 3 months.

Characteristic	Multivariable Beta [95% CI]	P value
Gender (female)	-0.06 [-0.36–0.23]	0.66
T2-signal adenoma/pituitary or GM (baseline)	-0.29 [-0.56–0.01]	0.045
Significant tumor shrinkage ($\geq 25\%$)	0.34 [0.02–0.65]	0.035

GM=grey matter, IGF-1=insulin-like growth factor 1, pasireotide LAR=pasireotide long-acting release, ULN=upper limit of normal.

FIGURE 5. Relation of significant tumor shrinkage and expression of SST receptors. Data are presented as scatter dot plots depicting the median (IQR). p values are for the comparisons between groups and are derived from the Mann-Whitney U test. (A) Patients with significant tumor shrinkage had lower $SSTR_2$ receptor expression (C) as well as a lower $SSTR_2/SST_5$ receptor ratio, (B) while SST_5 receptor expression was not significant different between the groups.



Discussion

Our study provides the novel finding that in acromegaly patients the tumoral responsiveness to pasireotide LAR treatment is directly correlated to hormonal unresponsiveness to both first-generation SRL and pasireotide LAR treatment and a lower SST₂ receptor expression. A higher baseline T2-signal intensity was associated with a better biochemical response to pasireotide LAR after three months. Interestingly, as previously shown in our cohort we are the first to observe a substantial increase in T2-signal intensity of the adenoma in response to pasireotide LAR⁽²⁶⁾. Increased T2-signal intensity indicates cystic degeneration, tumor cell necrosis, or both, suggesting an anti-tumor effect of pasireotide LAR. This increase was particularly substantial (>50%) in eight patients.

This potential antitumor activity of pasireotide LAR emerged when we observed a change in mean T2-signal intensity ratio in somatotroph adenomas after pasireotide LAR treatment in our cohort. In contrast, we did not observe any significant changes in the mean T2-signal intensity ratio after first-generation SRL treatment⁽¹⁴⁾. Compared to the total cohort, patients with increased T2-signal intensity in whom (biochemical) disease activity could not be controlled by SRLs, greater reduction of IGF-1 levels was observed during three months of pasireotide LAR treatment. These patients had larger adenomas but did not present more often with significant tumor shrinkage. However, we consider it necessary to differentiate tumor shrinkage from cell degeneration, tumor necrosis, or both (*e.g.* anti-tumor effects). While pasireotide LAR shows similar inhibitory effects on tumor growth as octreotide in GH secreting adenoma cell cultures⁽¹⁸⁾, it additionally showed anti-tumor effects (cell degeneration or tumor necrosis) in a subgroup of patients, which have not been observed with first-generation SRLs. Long-term data collection is required to further understand the anti-tumor effects of pasireotide LAR. Among the SST receptor subtypes only SST₂ and SST₃ might be responsible for the pasireotide LAR-induced cystic degeneration, tumor cell necrosis, or both, and may reduce disease activity and might result in long-term remission. Studying the effects of pasireotide LAR on pituitary histology might help unravel the differences in SST receptors specific signaling pathways and should be further explored.

Although previous clinical studies^(5, 6) and our study suggest that pasireotide LAR might exert a greater effect on tumor control, especially in patients whose disease is inadequately controlled on first-generation SRLs, we observed a significant tumor volume

increase in six patients. The magnitude of the observed tumor volume increase is similar to previous studies^(5, 6, 27). This observation was not clinically relevant in most (5/6, 83.3%) patients, of which three harbored microadenomas. We considered tumor growth to be clinically relevant only in those with emerging growth that affects the treatment approach (*e.g.* surgical intervention and/or stereotactic radiosurgery) within two years of study entry. However, one of the 6 patients experienced a substantial (>50%) increase in tumor size during nine months of pasireotide LAR treatment (26270 mm³ vs 12495 mm³) and underwent surgery. This patient was diagnosed during adolescence, and investigations for germline mutations in the *aryl hydrocarbon receptor-interacting protein (AIP)* and *multiple endocrine neoplasia type 1 (MEN1)* genes detected no abnormalities. It is noteworthy that this patient showed aggressive and treatment-resistant pituitary tumor behavior throughout previous treatment modalities, and exhibited patient and tumor characteristics predictive of aggressive tumor behavior in the future (*e.g.* young age at diagnosis, large tumor size and high GH secretion)⁽²⁸⁾. These young patients with large tumors and/or those with high pretreatment levels of GH particularly warrant close monitoring for continued tumor progression, regardless of treatment modality.

Hormone responsiveness (*i.e.* lower IGF-1 levels) during pasireotide LAR treatment in the total cohort was associated with higher T2-signal intensity. This finding is contrary to previous studies on first-generation SRLs, reporting greater IGF-1 reduction during SRL treatment in T2-hypointense adenomas^(9, 11, 13, 14). Thus, T2-weighted MRI signal intensity may provide complementary predictive information in relation to the responses to both kinds of drugs. However, these results are not unexpected considering that patients with higher T2-signal intensity adenomas may have lower IGF-1 levels at diagnosis⁽¹⁰⁾. Consequently, these patients require less IGF-1 reduction in order to obtain biochemical control.

Most studies have reported hormone secretion and cell growth to be synchronous, as tumors responding to first-generation somatostatin analogs with GH inhibition also exhibit tumor shrinkage. However, we observed a dissociation between achieved IGF-1 levels and the presence of significant tumor shrinkage during pasireotide LAR treatment. Two mechanisms may account for this dissociation.

Firstly, the dissociation between anti-secretory and anti-proliferative effects can be based on the different roles of SST receptor subtypes in GH secreting tumors. To our knowledge, this is the first reported incidence of additional (significant) decrease in tumor

volume after switching from first-generation SRL to pasireotide LAR treatment. Our results give a clear indication that this dissociation can be based on the different roles of SST receptor subtypes. Previous *in vitro* studies^(19, 29, 30) and our data⁽²³⁾ as previously reported by Muhammad et al., indicate that, overall, pasireotide exerts its anti-secretory activity mainly by the activation of SST₂. Patients with significant tumor shrinkage had significantly lower SST₂ receptor expression, as well as a lower SST₂/SST₅ ratio compared to patients without significant tumor shrinkage, which probably accounts for the lack of biochemical control. Yet, we have to point out that we analyzed the tissue samples from only 13 patients. Although there is no evidence that SST₅ receptor expression is affected by presurgical first-generation SRL treatment, it has been widely demonstrated that patients receiving SRL treatment prior to surgery show significantly lower SST₂ receptor protein expression compared to medically naive patients^(15, 19, 31, 32). Even though this is not evident in our cohort, a pooled analysis of SRL-pretreated and medically naive patients can introduce bias. Exclusion of the SRL-pretreated patients (n=4) from our analysis did not affect our observation since the difference in SST₂ receptor expression between patients with or without significant tumor shrinkage remained significant.

Secondly, the tumor size may explain why significant tumor shrinkage is not accompanied by lower IGF-1 levels. In our cohort absolute tumor shrinkage during pasireotide LAR treatment was positively correlated with larger tumors and higher GH levels at baseline. These results confirm and build upon a previous study stating that IGF-1 levels remain unaffected above a certain threshold of GH concentrations⁽³³⁾. Knowing that larger tumors secrete more GH, it becomes apparent that pasireotide LAR can affect tumor size and thereby GH levels, without affecting IGF-1 levels. In fact, we observed significantly decreased GH levels during pasireotide LAR therapy in subjects from the PAPE study as previously reported by Muhammad et al.^(7, 8), which can be explained by the suppressive effect of pasireotide LAR on GH secretion. This finding is also compatible with reports of increased GH levels during pegvisomant treatment⁽³⁴⁾ and the lower GH levels can be explained by the discontinuation of or further dose reduction in pegvisomant.

A strength of our study lies in the relatively large number of patients in which the hormone and tumor response to different treatment options were systematically investigated including data on T2-signal intensity and SST receptor expression of somatotroph adenomas. The main limitations of our study lie in the retrospective

collection of data on IGF-1 levels during first-generation SRL treatment, and the use of different IGF-1 assays during SRL treatment. Another limitation of our study is the lack of generalizability, as most selected patients did not received surgery and may not reflect the general population of acromegaly patients. However, the fact that our patients had a high degree of resistance to first-generation SRL therapy fits with the second-line indication of pasireotide LAR in most non-US countries. At last, we should mention that most previous studies expressed IGF-1 differently to evaluate efficacy; with most studies using the achieved reduction in IGF-1 level at any point^(9-11,35), while we used IGF-1 levels expressed as ULN during three and nine months in our study.

In conclusion, patients showing no biochemical response to first- or second-generation somatostatin analogs with particularly large tumors with a lower SST₂ receptor expression are prone to achieve tumor shrinkage during pasireotide LAR treatment. On the contrary, lower IGF-1 levels during pasireotide LAR are associated with a higher SST₂ receptor expression and a higher T2-signal intensity. In patients with increased T2-signal intense adenomas, pasireotide LAR-induced cystic degeneration, tumor cell necrosis, or both, might reduce disease activity by achieving greater IGF-1 reduction in patients with relatively larger adenomas. This poses the question whether possibly SST₃ or other SST receptors may be relevant as well during pasireotide LAR treatment in mediating tumor shrinkage or inducing cell degeneration, tumor cell necrosis, or both. Future research should aim at elucidating differences in the hormone and tumor responsiveness in response to pasireotide LAR treatment. To predict which subgroup of patients will benefit from treatment with this multi-receptor ligand and the consequences for the clinical management of acromegaly remains to be elucidated.

Acknowledgments

We acknowledge the clinical chemist (Sjoerd A. A. van den Berg) and neurosurgeons (Alof H.G. Dallenga and Ian K. Haitsma). All contributed to the study.

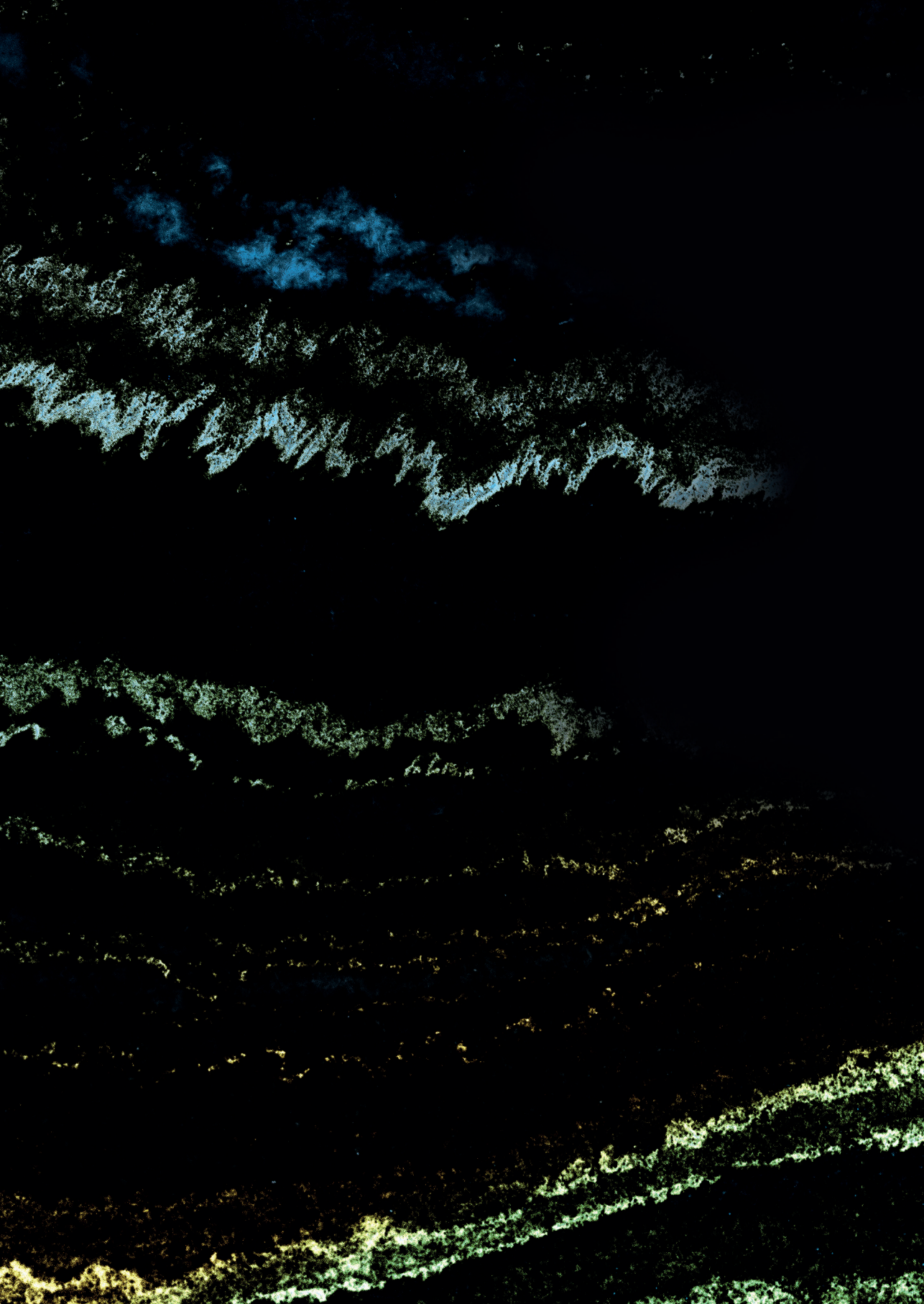
References

1. Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119(11):3189-202.
2. Melmed S, Bronstein MD, Chanson P, Klibanski A, Casanueva FF, Wass JAH, et al. A Consensus Statement on acromegaly therapeutic outcomes. *Nat Rev Endocrinol.* 2018;14(9):552-61.
3. Signifor LAR [package insert]. East Hanover: Novartis Pharmaceuticals Corporation; 2014 [
4. Signifor LAR [summary of product characteristics]. Basel: Novartis Pharma AG; 2014 [
5. Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab.* 2014;99(3):791-9.
6. Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol.* 2014;2(11):875-84.
7. Muhammad A, van der Lely AJ, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and safety of switching to pasireotide in acromegaly patients controlled with pegvisomant and first-generation somatostatin analogues (PAPE study). *J Clin Endocrinol Metab.* 2017.
8. Muhammad A, Coopmans EC, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study. *Eur J Endocrinol.* 2018;179(5):269-77.
9. Potorac I, Petrossians P, Daly AF, Alexopoulou O, Borot S, Sahnoun-Fathallah M, et al. T2-weighted MRI signal predicts hormone and tumor responses to somatostatin analogs in acromegaly. *Endocr Relat Cancer.* 2016;23(11):871-81.
10. Potorac I, Petrossians P, Daly AF, Schillo F, Ben Slama C, Nagi S, et al. Pituitary MRI characteristics in 297 acromegaly patients based on T2-weighted sequences. *Endocr Relat Cancer.* 2015;22(2):169-77.
11. Heck A, Ringstad G, Fougner SL, Casar-Borota O, Nome T, Ramm-Petersen J, et al. Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly. *Clin Endocrinol (Oxf).* 2012;77(1):72-8.

12. Hagiwara A, Inoue Y, Wakasa K, Haba T, Tashiro T, Miyamoto T. Comparison of growth hormone-producing and non-growth hormone-producing pituitary adenomas: imaging characteristics and pathologic correlation. *Radiology*. 2003;228(2):533-8.
13. Shen M, Zhang Q, Liu W, Wang M, Zhu J, Ma Z, et al. Predictive value of T2 relative signal intensity for response to somatostatin analogs in newly diagnosed acromegaly. *Neuroradiology*. 2016;58(11):1057-65.
14. Bonneville F, Riviere LD, Petersenn S, Bevan J, Houchard A, Sert C, et al. MRI T2 signal intensity and tumor response in patients with GH-secreting pituitary macroadenoma: PRIMARYS post-hoc analysis. *Eur J Endocrinol*. 2018.
15. Gatto F, Feelders RA, van der Pas R, Kros JM, Waaijers M, Sprij-Mooij D, et al. Immunoreactivity score using an anti-SST_{2A} receptor monoclonal antibody strongly predicts the biochemical response to adjuvant treatment with somatostatin analogs in acromegaly. *J Clin Endocrinol Metab*. 2013;98(1):E66-71.
16. Casar-Borota O, Heck A, Schulz S, Nesland JM, Ramm-Petersen J, Lekva T, et al. Expression of SSTR2a, but not of SSTRs 1, 3, or 5 in somatotroph adenomas assessed by monoclonal antibodies was reduced by octreotide and correlated with the acute and long-term effects of octreotide. *J Clin Endocrinol Metab*. 2013;98(11):E1730-E9.
17. Schmid HA. Pasireotide (SOM230): development, mechanism of action and potential applications. *Mol Cell Endocrinol*. 2008;286(1-2):69-74.
18. Ibanez-Costa A, Rivero-Cortes E, Vazquez-Borrego MC, Gahete MD, Jimenez-Reina L, Venegas-Moreno E, et al. Octreotide and pasireotide (dis)similarly inhibit pituitary tumor cells in vitro. *J Endocrinol*. 2016;231(2):135-45.
19. Gatto F, Feelders RA, Franck SE, van Koetsveld PM, Dogan F, Kros JM, et al. In Vitro Head-to-Head Comparison Between Octreotide and Pasireotide in GH-Secreting Pituitary Adenomas. *J Clin Endocrinol Metab*. 2017;102(6):2009-18.
20. Manolopoulou J, Alami Y, Petersenn S, Schopohl J, Wu Z, Strasburger CJ, et al. Automated 22-kD Growth Hormone-Specific Assay without Interference from Pegvisomant. *Clinical chemistry*. 2012;58(10):1446-56.
21. Bidlingmaier M, Friedrich N, Emeny RT, Spranger J, Wolthers OD, Roswall J, et al. Reference intervals for insulin-like growth factor-1 (igf-i) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *J Clin Endocrinol Metab*. 2014;99(5):1712-21.

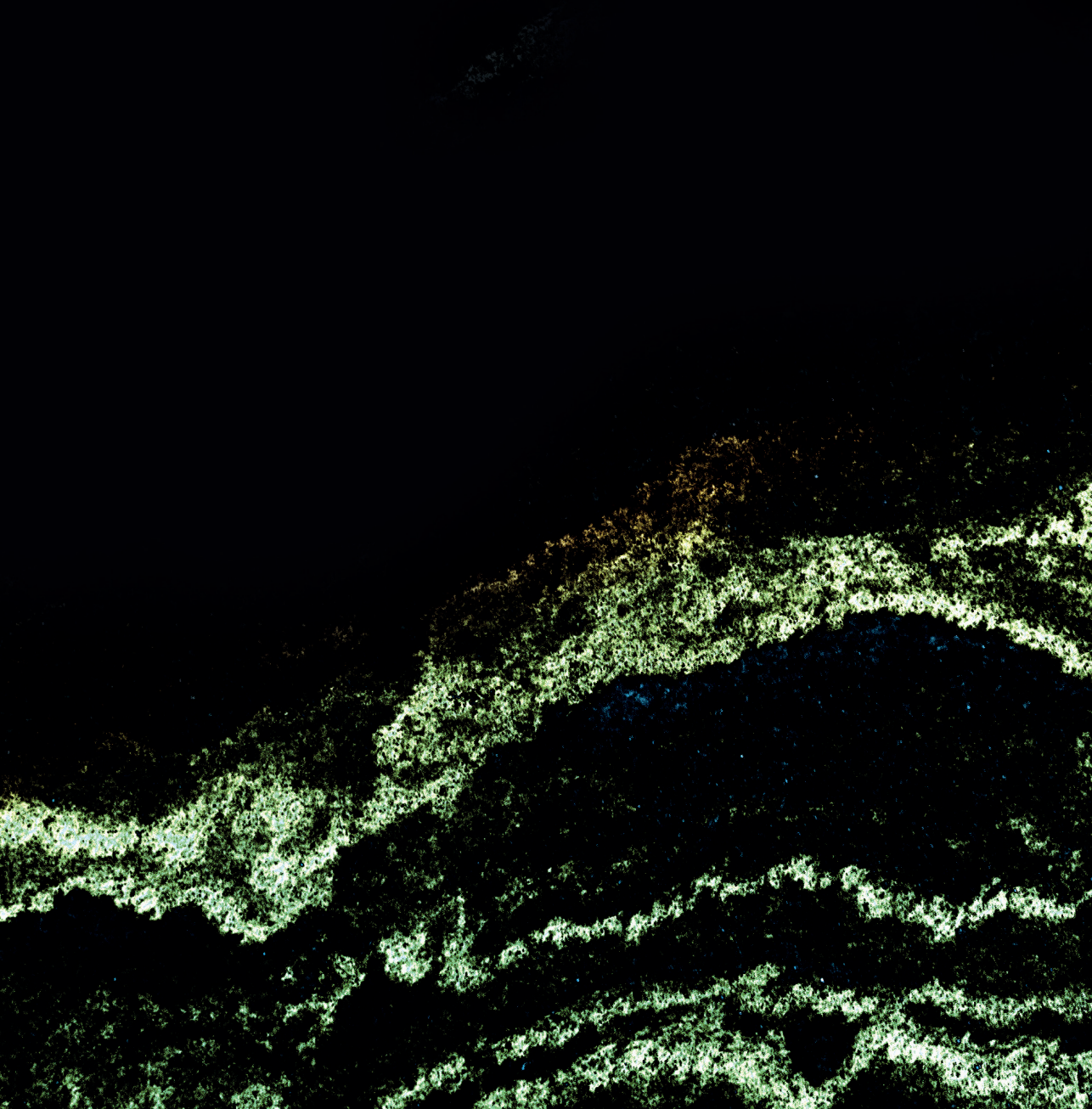
22. Lundin P, Pedersen F. Volume of pituitary macroadenomas: assessment by MRI. *J Comput Assist Tomogr.* 1992;16(4):519-28.
23. Muhammad A, Coopmans EC, Gatto F, Franck SE, Janssen J, van der Lely AJ, et al. Pasireotide responsiveness in acromegaly is mainly driven by somatostatin receptor subtype 2 expression. *J Clin Endocrinol Metab.* 2018.
24. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue *Pathologe.* 1987;8(3):138-40.
25. Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poulblon RM, Feelders RA, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. *J Clin Endocrinol Metab.* 2014;99(10):3644-52.
26. Coopmans EC, van der Lely AJ, Schneiders JJ, Neggers S. Potential antitumour activity of pasireotide on pituitary tumours in acromegaly. *Lancet Diabetes Endocrinol.* 2019.
27. Bronstein MD, Fleseriu M, Neggers S, Colao A, Sheppard M, Gu F, et al. Switching patients with acromegaly from octreotide to pasireotide improves biochemical control: crossover extension to a randomized, double-blind, Phase III study. *BMC Endocr Disord.* 2016;16:16.
28. Besser GM, Burman P, Daly AF. Predictors and rates of treatment-resistant tumor growth in acromegaly. *Eur J Endocrinol.* 2005;153(2):187-93.
29. Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, et al. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. *J Clin Endocrinol Metab.* 2004;89(4):1577-85.
30. Jaquet P, Saveanu A, Gunz G, Fina F, Zamora AJ, Grino M, et al. Human somatostatin receptor subtypes in acromegaly: distinct patterns of messenger ribonucleic acid expression and hormone suppression identify different tumoral phenotypes. *J Clin Endocrinol Metab.* 2000;85(2):781-92.
31. Plockinger U, Albrecht S, Mawrin C, Saeger W, Buchfelder M, Petersenn S, et al. Selective loss of somatostatin receptor 2 in octreotide-resistant growth hormone-secreting adenomas. *J Clin Endocrinol Metab.* 2008;93(4):1203-10.
32. Casar-Borota O, Heck A, Schulz S, Nesland JM, Ramm-Petersen J, Lekva T, et al. Expression of SSTR2a, but not of SSTRs 1, 3, or 5 in somatotroph adenomas assessed by monoclonal antibodies was reduced by octreotide and correlated with the acute and long-term effects of octreotide. *J Clin Endocrinol Metab.* 2013;98(11):E1730-9.

33. Barkan AL, Beitins IZ, Kelch RP. Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. *J Clin Endocrinol Metab.* 1988;67(1):69-73.
34. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, et al. Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *N Engl J Med.* 2000;342(16):1171-7.
35. Heck A, Emblem KE, Casar-Borota O, Ringstad G, Bollerslev J. MRI T2 characteristics in somatotroph adenomas following somatostatin analog treatment in acromegaly. *Endocrine.* 2016;53(1):327-30.



Part II

**Pasireotide: mechanism of
action and clinical applications**





Chapter 5

**Efficacy and safety of switching to pasireotide
in acromegaly patients controlled with
pegvisomant and somatostatin analogues:
PAPE extension study**

Ammar Muhammad, Eva C. Coopmans, Patric Delhanty,
Alof H.G. Dallenga, Ian K. Haitsma, Joseph A.M.J.L. Janssen,
Aart J. van der Lely and Sebastian J.C.M.M. Neggers

Eur J Endocrinol 2018; 179(5): 269-77.

Abstract

OBJECTIVE: To assess the efficacy and safety after 48 weeks of treatment with pasireotide LAR alone or in combination with pegvisomant in patients with acromegaly. In addition, we assessed the relation between insulin secretion and pasireotide-induced hyperglycemia.

DESIGN: The PAPE extension study is a prospective follow-up study until 48 weeks after the core study of 24 weeks.

METHODS: Fifty-nine out of 61 patients entered the extension study. Efficacy was defined as the percentage of patients achieving IGF-I normalisation ($\leq 1.2 \times$ the upper limit of normal (ULN)) at 48 weeks through protocol-based adjustment of pegvisomant and pasireotide LAR doses. At baseline, insulin secretion was assessed by an oral glucose tolerance test (OGTT).

RESULTS: At the end of the study, median IGF-I was $0.98 \times$ ULN, and 77% of patients achieved normal IGF-I levels with a mean pegvisomant dose of 64 mg/week, and an overall cumulative pegvisomant dose reduction of 52%. Frequency of diabetes mellitus increased from 68% at 24 weeks to 77% at 48 weeks, and nine patients discontinued pasireotide LAR treatment, mainly because of severe hyperglycemia. Pasireotide-induced hyperglycemia was inversely correlated with baseline insulin secretion ($r = -0.37$, $P < 0.005$).

CONCLUSIONS: Pasireotide LAR normalises IGF-I levels in most acromegaly patients, with a 50% pegvisomant-sparing effect. However, pasireotide LAR treatment coincided with a high incidence of diabetes mellitus. The risk for developing diabetes during pasireotide LAR treatment seems inversely related to insulin secretion at baseline.

Introduction

Acromegaly is a systemic condition most commonly caused by GH secreting pituitary adenomas leading to elevated GH and IGF-I levels, and are associated with increased mortality and morbidity⁽¹⁾. The main goals of the present treatment for acromegaly are to normalise GH and IGF-I levels, reduce or control tumour size and to improve QoL and multisystem comorbidities^(2, 3).

First-generation SRLs are considered the mainstay medical treatment of acromegaly. First-generation SRLs suppress GH secretion by preferential binding to SST_{2a} receptor. First-generation SRLs have favourable safety profiles and a clinically neutral impact on glucose homeostasis^(4, 5). In clinical practice only about 40% of patients treated with monotherapy First-generation SRLs achieve biochemical normalisation of GH and IGF-I. Therefore, most patients are refractory to treatment with first-generation SRLs, and require additional therapies⁽⁶⁻⁸⁾.

The competitive GH receptor antagonist pegvisomant is currently the most effective treatment to normalise circulating IGF-I levels in acromegaly, as monotherapy or in combination with first-generation SRL⁽⁹⁻¹⁴⁾. Pegvisomant has as advantage that it improves insulin sensitivity⁽¹⁵⁻¹⁸⁾. In combination with first-generation SRL, a lower necessary pegvisomant dose is required to normalise IGF-I levels in acromegaly than compared with pegvisomant monotherapy^(19, 20).

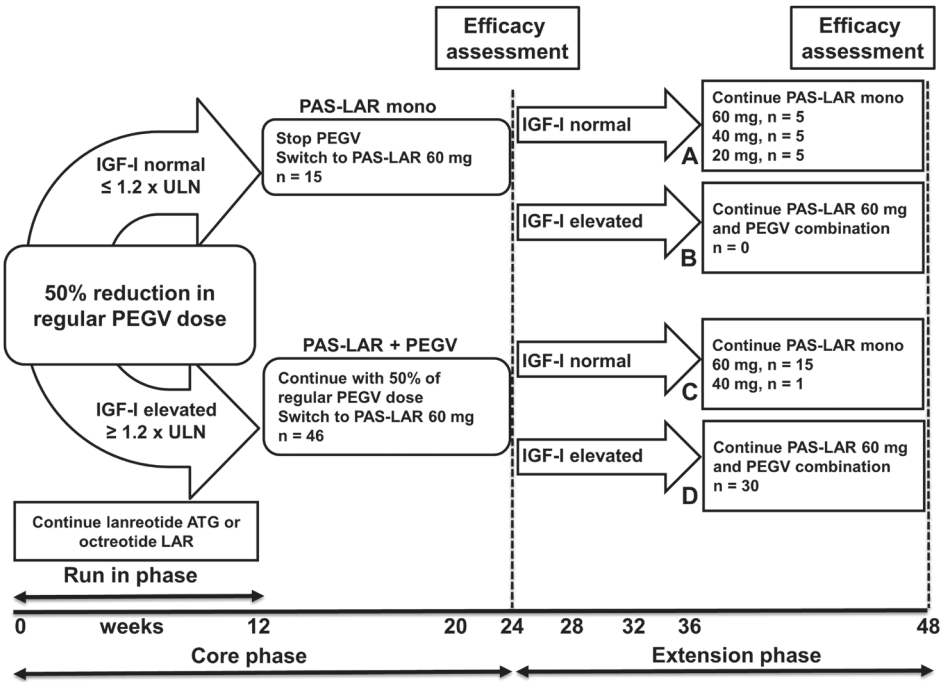
Pasireotide LAR is a second-generation multi-receptor somatostatin analogue designed with a broader binding somatostatin receptor profile than first-generation SRLs⁽²¹⁾. Pasireotide LAR has been shown to provide superior clinical efficacy over first-generation SRL in treatment-naïve acromegaly patients and in patients inadequately controlled with first-generation SRLs^(22, 23). Pasireotide LAR has a similar safety profile to first-generation SRLs, with the exception of a higher incidence of hyperglycemia, and this incidence has been reported to occur in about 60–88% of patients during treatment with pasireotide LAR⁽²²⁻²⁴⁾. Recently, we have reported the efficacy and safety of pasireotide LAR alone or in combination with pegvisomant in acromegaly patients up to 24 weeks treatment (PAPE core study)⁽²⁴⁾. Switching to pasireotide LAR treatment resulted in a 66% reduction in pegvisomant dose, but was simultaneously associated with a higher incidence of diabetes mellitus. Here, we present the long-term 48-week results of the efficacy and safety of pasireotide LAR alone or in combination with pegvisomant treatment in acromegaly.

Subjects and methods

Study design

The PAPE study is a prospective, open-label, single-center study in acromegaly patients, designed to assess the efficacy and safety of pasireotide LAR alone, or in combination with pegvisomant⁽²⁴⁾ (ClinicalTrials.gov, number Nbib2668172). The primary endpoint was to assess efficacy at 24 weeks. Here, we report the following secondary endpoints: efficacy and safety from 24 up to 48 weeks. During this follow-up, the goal was to achieve IGF-I normalisation ($\text{IGF-I} \leq 1.2 \times \text{ULN}$) through protocol-based dose titration of pegvisomant and pasireotide LAR (Fig. 1). In the core study, two groups of patients continued in the extension phase: the pasireotide LAR 60 mg monotherapy group and the pasireotide LAR/pegvisomant combination group⁽²⁴⁾.

FIGURE 1. PAPE study design. The number of participants in each study arm were based on the intention-to-treat population ($n = 61$).



Patients in the pasireotide LAR 60mg monotherapy group continued with the 60mg dose until 48 weeks, unless their IGF-I levels decreased below the median sex- and age-adjusted IGF-I normal limits. In that case, the pasireotide LAR dose was decreased to 40mg every 4 weeks. Subsequently, if after at least two pasireotide LAR 40mg injections, IGF-I levels remained suppressed (below the median IGF-I), the pasireotide LAR dose was further decreased to 20 mg (ARM A). However, if IGF-I became elevated ($\geq 1.2 \times \text{ULN}$) during pasireotide LAR monotherapy, pegvisomant treatment was restarted in the same dose of the run-in phase (ARM B).

Patients in the pasireotide LAR/pegvisomant combination group who achieved IGF-I normalisation at 24 weeks using pegvisomant doses ≤ 80 mg/week, were after 24 weeks instructed to discontinue pegvisomant treatment and to continue with pasireotide LAR 60mg monotherapy during follow-up (ARM C) (Fig. 1). In patients using pegvisomant doses higher than 80mg/week, the dose was reduced by 50% every 4 weeks until their IGF-I was normalised ($\leq 1.2 \times \text{ULN}$). If IGF-I levels became elevated above $1.2 \times \text{ULN}$ during combination treatment, the pegvisomant dose was further increased every 4 weeks depending on the measured IGF-I levels after adjusting treatment (ARM D):

- IGF-I between 1.2 and $1.5 \times \text{ULN}$, the pegvisomant dose was increased by 20%.
- IGF-I between 1.5 and $1.7 \times \text{ULN}$, the pegvisomant dose was increased by 30%.
- IGF-I between 1.7 and $2.0 \times \text{ULN}$, the pegvisomant dose was increased by 40%.
- IGF-I $\geq 2.0 \times \text{ULN}$, the pegvisomant dose was increased by 50%.

If monitoring revealed glucose levels in the diabetic range according to the American Diabetes Association (ADA) criteria ⁽²⁵⁾, metformin was initiated as first-line treatment option. If glycemic control was not achieved after metformin treatment, a DPP-4 inhibitor was added as second choice. When patients still did not achieve normoglycemia, they were switched to a treatment with sulfonylureas or GLP-1 receptor analogues. Finally, insulin was started in patients intolerant to GLP-1 analogues or when glycemic control was not achievable with GLP-1 analogues. All patients received intensive blood glucose monitoring after the start of treatment. Patients who developed rapid hyperglycemia received insulin treatment. The PAPE study was approved by the Medical Ethics Committee of the Erasmus University Medical Centre, and all patients provided written informed consent.

Patients

After 24 weeks, 59 out of 61 patients entered the extension phase (Supplementary Fig. 1). The inclusion and exclusion criteria have been reported previously⁽²⁴⁾. Briefly, key inclusion criteria were patients with good metabolic control of acromegaly ($\text{IGF-I} \leq 1.2 \times \text{ULN}$) for at least 6 months combination treatment of weekly pegvisomant and maximum doses of first-generation SRLs. Key exclusion criteria were pituitary surgery or radiotherapy within the 6 months prior to study entry and poorly controlled diabetes mellitus, defined as $\text{HbA1c} \geq 9.0\%$.

Study assessments

Laboratory measurements were described previously in the core study⁽²⁴⁾. Safety assessments included assessment of heart function by electrocardiogram at baseline, 24 weeks and 48 weeks. The severity of adverse events (AEs) was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE)⁽²⁶⁾. At baseline after an overnight fast, a standardised oral glucose tolerance test (OGTT) was performed with 1.75 g glucose per kg body weight (maximum 75 g while simultaneously glucose and insulin levels were measured at $t = -15 \text{ min}$, $t = 0 \text{ min}$, $t = 30 \text{ min}$, $t = 60 \text{ min}$, $t = 90 \text{ min}$, and $t = 120 \text{ min}$ ⁽²⁷⁾. We used published indexes of β -cell function to estimate the first phase and second phase insulin secretion during the OGTT⁽²⁸⁾.

Outcomes

The secondary efficacy endpoints were descriptive in nature and were based on the intention-to-treat population. For patients who discontinued the study before 48 weeks, imputation based on the principle of last observation carried forward (LOCF) was used. Main efficacy endpoint was defined as the percentage of responders at 48 weeks in the intention-to-treat population, and in each treatment arm (pasireotide LAR monotherapy and pasireotide LAR/pegvisomant combination treatment), with an exact 95% CI. Response was defined as $\text{IGF-I} \leq 1.2 \times \text{ULN}$. Other descriptive endpoints for efficacy included the percentage of patients who could stop pegvisomant treatment after 48 weeks, and the percentage cumulative pegvisomant dose reduction, which was calculated as the sum of all administered pegvisomant doses of all patients at 48 weeks compared with baseline. As reported previously⁽²⁴⁾, secondary endpoints for safety included the incidence of hyperglycemia and diabetes mellitus, vital signs and electrocardiogram.

Statistical analyses were performed using SPSS software (version 24 for Windows; SPSS Inc.) and GraphPad Prism version 6.04 (GraphPad Software). Categorical data are represented as observed frequencies and percentages. Continuous data are represented as mean and 95% CI or median and range. The Kolmogorov–Smirnov and the Shapiro–Wilk test were used to test normality of variables. If assumption of normality was met, the paired *t*-test was used. For non-normally distributed variables, the Wilcoxon signed-rank test was used. For data that did not meet the criteria for normality, logarithmic transformation was applied. Correlation analyses were performed using the Pearson correlation test. *P* values of <0.05 were considered statistically significant.

Results

Efficacy

The baseline characteristics of the study population have been published before in the core study⁽²⁹⁾. The percentage of patients achieving IGF-I and GH normalisation are shown in Table 1.

TABLE 1. Percentage of patients achieving normal IGF-I and GH levels during the study.

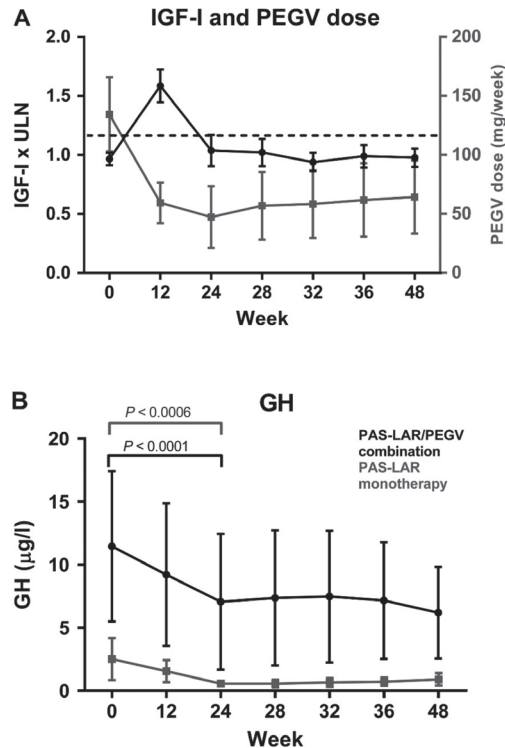
	Baseline	12 weeks	24 weeks	48 weeks
IGF-I $\leq 1.2 \times$ ULN				
Overall	53/61 (86.9%)	15/61 (24.6%)	45/61 (73.8%)*	47/61 (77.0%)*
Pasireotide LAR monotherapy	14/15 (93.3%)	15/15 (100%)	14/15 (93.3%)	14/15 (93.3%)*
Pasireotide LAR/pegvisomant combination	39/46 (84.8%)	0/46 (0%)	31/46 (67.4%)	33/46 (71.7%)*
GH ≤ 2.5 μg/L				
Overall	23/61 (37.7%)	29/61 (47.5%)	41/61 (67.2%)*	41/61 (67.2%)*
Pasireotide LAR monotherapy	10/15 (66.7%)	11/15 (73.3%)	15/15 (100%)*	14/15 (93.3%)*

At 48 weeks, 77.0% of patients had IGF-I levels within the reference range with a mean IGF-I of $0.98 \times \text{ULN}$ (95% CI: 0.90–1.06) (Fig. 2A and Table 1). Stratified by treatment group; 93.3% of patients using pasireotide LAR monotherapy achieved IGF-I normalisation at 24 weeks, which was sustained at 48 weeks. While 67.4% of patients using pasireotide LAR and pegvisomant combination therapy achieved IGF-I normalisation at 24 weeks, which increased to 71.7% at 48 weeks. Overall after 48 weeks, 12 of the 14 non-controlled patients had IGF-I levels between 1.2 and $1.4 \times \text{ULN}$. During the extension phase the mean pegvisomant dose had to be increased from 47 mg/week (95% CI: 21–73) to 64 mg/week (95% CI: 33–95). At 48 weeks the cumulative reduction in pegvisomant dose decreased to 52.0% after 48 weeks compared with baseline, and 50.8% (31/61) of patients were off pegvisomant treatment at the end of study.

In Fig. 2B GH serum levels are shown stratified by treatment group. Patients in the pasireotide LAR monotherapy group had significantly lower baseline GH levels (GH $2.5 \mu\text{g/L}$ (95% CI: 0.8–4.2)) than patients in the pasireotide LAR/pegvisomant combination group (GH $11.5 \mu\text{g/L}$ (95% CI: 5.5–17.4)). Both groups showed a significant decrease in GH serum levels after initiation of pasireotide LAR treatment, with subsequently stable suppressed GH levels in the pasireotide LAR monotherapy group until 48 weeks.

The 15 patients in the pasireotide LAR monotherapy group remained controlled throughout the study (Fig. 1). In fact, in 10 patients (66.7%) a dose reduction was possible to pasireotide LAR 40 mg and 20 mg (ARM A). No patients had to restart pegvisomant treatment in this group (ARM B). In patients using pasireotide LAR and pegvisomant combination therapy, 16 (34.8%) patients switched to pasireotide LAR monotherapy during the extension phase, and in one patient a further dose reduction was possible to pasireotide LAR 40 mg (ARM C). The remaining 30 patients required pasireotide LAR and pegvisomant combination treatment, of which 13 patients (21.3%) achieved less than 25% pegvisomant dose reduction (ARM D). In 12 patients (19.7%) between 25 and 50% pegvisomant dose reduction was achieved, and in 5 patients (8.2%) a reduction of more than 50% could be attained.

FIGURE 2. IGF-I serum levels and the weekly pegvisomant dose (2A). Dotted straight black line reflects the IGF-I 1.2 x ULN cut-off. GH serum levels (2B) are depicted in the pasireotide LAR monotherapy group in grey and in the combination Pasireotide LAR/pegvisomant group in black. Data are expressed as mean (95% CI).



Pasireotide LAR monotherapy

Among the 15 patients that were switched to pasireotide LAR 60 mg monotherapy at 12 weeks, 10 patients had a progressive decline in IGF-I during follow-up, with levels dropping below the median IGF-I reference range. In these patients, the pasireotide LAR dose was therefore reduced to 40 mg every 4 weeks. Subsequently, in five of those patients the dose was further decreased to 20 mg. At baseline, these patients used a relatively low median pegvisomant dose of 60 mg/week. In one additional patient who could stop pegvisomant after 24 weeks, the pasireotide LAR could be further reduced to 40 mg during the follow-up.

Partial and non-responders

We observed that 10 patients on combination treatment who achieved IGF-I normalisation after 24 weeks and therefore could stop pegvisomant, had to restart pegvisomant treatment during follow-up because their IGF-I levels again increased above $1.2 \times \text{ULN}$ (arm D). One patient required a higher dose of pegvisomant after 48 weeks (100 mg/week) than at baseline (80 mg/week). In three patients using very high doses of pegvisomant at baseline (mean baseline pegvisomant dose 580 mg/week), we could not attain a significant pegvisomant dose reduction (mean pegvisomant dose was remained 560 mg/week after 48 weeks).

Safety

Hyperglycemia and diabetes mellitus The most common AE related to pasireotide LAR treatment was hyperglycemia, and mainly recorded during the core study⁽²⁹⁾. In total 60 out of 61 patients had a hyperglycemia-related AE, of which 47 (77.0%) were grade 1 and 2 (Table 2). During the extension study grade 3 hyperglycemia was recorded in two patients, while no patients had a grade 4 hyperglycemia-related AE.

At 48 weeks, diabetes of most patients was managed with a combination of metformin and a DPP-4 inhibitor. 73.8% patients required at least one antidiabetic medication. Between 24 and 48 weeks, five patients developed mild diabetes which required additional treatment with metformin alone or combined with a DPP-4 inhibitor. Due to hypoglycemic symptoms related to sulfonylurea treatment, four patients were switched to a GLP-I analogue (Supplementary Table 1).

During the extension phase the incidence of diabetes mellitus increased slightly from 68.9% to 77.0%. Mean HbA1c and fasting plasma glucose levels increased significantly after pasireotide LAR treatment, but decreased slightly after initiation of antidiabetic treatment during follow-up (Fig. 3A and B). Mean HbA1c concentrations increased from 6.0% (5.8–6.1) at baseline to 7.3% (6.9–7.7) after 24 weeks, but decreased slightly to 7.0% (6.6–7.4) after 48 weeks. Stratified by treatment group; in the pasireotide LAR monotherapy group, mean HbA1c increased from 6.0% (95% CI: 5.5–6.5%) at baseline to 7.1% (95% CI: 6.3–7.9%) at 24 weeks, and decreased to 6.8% (95% CI: 6.0–7.7%) at 48 weeks. In the combination treatment group, HbA1c levels showed a similar pattern, increasing from 6.0% (95% CI: 5.9–6.1%) at baseline to 7.4% (95% CI: 6.9–7.9%) at 24 weeks, and decreasing to 7.1% (95% CI: 6.6–7.5%) after 48 weeks. No significant

difference was observed in HbA1c levels between patients using pasireotide LAR monotherapy and combination treatment at baseline ($P = 0.36$), 24 weeks ($P = 0.72$), and after 48 weeks ($P = 0.26$).

Fasting plasma glucose levels followed a similar profile with 6.2 mmol/L (5.8–6.5) at baseline to 9.2 mmol/L (8.1–10.3) at 24 weeks, and 8.7 mmol/L (95% CI: 7.7–9.6) after 48 weeks.

Excluding patients that were receiving insulin therapy at baseline ($n = 3$), fasting insulin levels rose from 67.5 mmol/L (95% CI: 48.8–86.2) at baseline to 161.4 mmol/L (95% CI: 113.5–209.3) after 12 weeks. Insulin levels dropped significantly after initiation of pasireotide LAR treatment. After 24 weeks, fasting insulin levels decreased significantly to 89.0 mmol/L (95% CI: 54.9–123.1). We observed a significant inverse relationship between the insulin area under the curve (AUC) during OGTT at baseline and the increase in HbA1c levels between baseline and 24 weeks ($r = -0.30$, $P = 0.03$). The estimates for β -cell function showed a stronger correlation with the increase in HbA1c; first phase insulin secretion (Stumvoll index, $r = -0.37$, $P = 0.005$) and second phase insulin secretion ($r = -0.38$, $P = 0.004$).

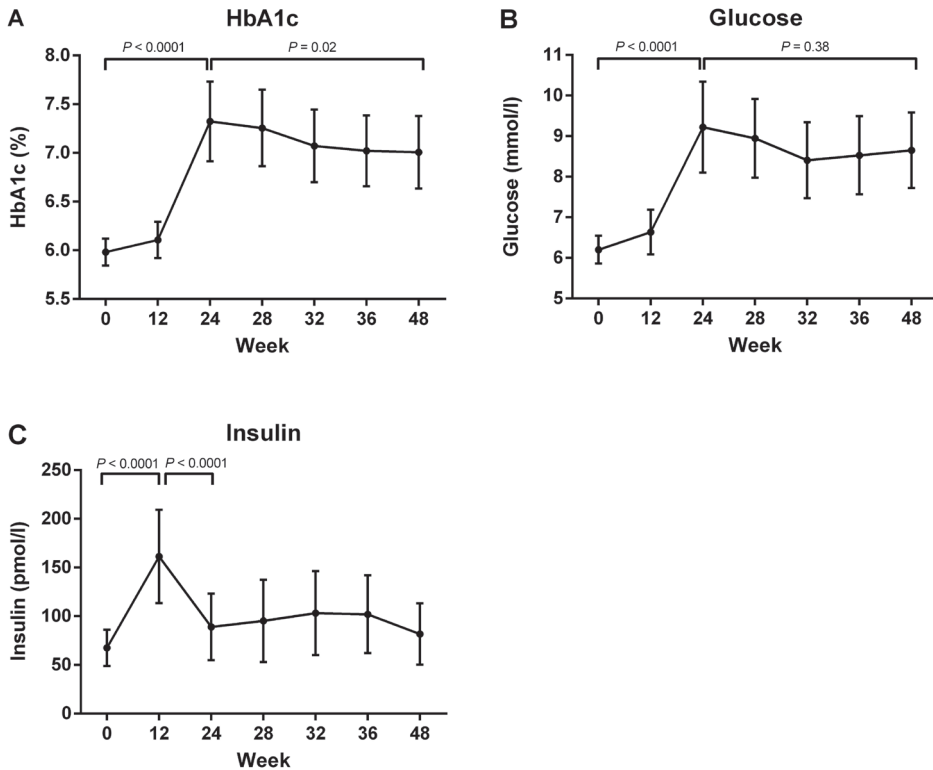
TABLE 2. Adverse events regardless of study-drug relationship (>5%) until 48 weeks.

Adverse events	Grade 1/2	Grades 3/4
Hyperglycemia	48 (78.7%)	14 (23.0%)
Diarrhea	21 (34.4%)	0
Fatigue	19 (31.1%)	0
Arthralgia	16 (26.2%)	0
Myalgia	16 (26.2%)	0
Headache	13 (21.3%)	0
Pain injection site	12 (19.7%)	0
Dizziness	11 (18.0%)	0
Hypoglycemia	9 (14.8%)	0
Arthralgia	16 (26.2%)	0
Nausea	7 (11.5%)	0
Alopecia	5 (8.2%)	0
Abdominal pain	4 (6.6%)	0

Non-hyperglycemia related adverse events

After hyperglycemia, diarrhoea (34.4%), and fatigue (31.1%) were the most common AEs (Table 2). Headache was reported by 13 (21.3%) patients and most frequently experienced in the first week after initiation of pasireotide LAR treatment, but resolved later after consecutive injections. One patient was diagnosed with sick sinus syndrome during pasireotide LAR treatment. After discontinuation of pasireotide LAR this AE resolved and she was successfully switched to pegvisomant monotherapy. Slightly more patients developed diarrhoea during the extension phase ($n = 21$) compared to core phase of the study ($n = 18$).

FIGURE 3. HbA1c (3A), fasting plasma glucose (3B), fasting plasma insulin (3C) concentrations are expressed as mean (95% CI).



Discussion

The results of this clinical study suggest that the efficacy of pasireotide LAR was long-term sustained, as after 48 weeks, 77% of patients achieved IGF-I normalisation while a cumulative pegvisomant dose reduction of 54.2% could be achieved, reconfirming the pegvisomant-sparing effect of pasireotide LAR. Hyperglycemia was the most important and most common adverse event during the follow-up until 48 weeks. The incidence of diabetes mellitus increased from 68.9% at 24 weeks to 77% at 48 weeks.

It is important to note that the extension phase of our study was not designed to detect a statistically significant difference between the treatment groups after 48 weeks, but it was undertaken to optimally titrate the pegvisomant and pasireotide LAR dose to achieve control of IGF-I within the reference ranges. To achieve IGF-I normalisation, we used a systematic pegvisomant and pasireotide LAR dose titration protocol.

We observed that pasireotide LAR elicited a wide range of clinical response in this study, ranging from patients that were very sensitive to very resistant. On one hand of the spectrum, patients could stop pegvisomant treatment and switch to a lower pasireotide LAR dose of 40 mg and even 20 mg/month. This IGF-I oversuppression to below median range during pasireotide LAR treatment has also recently been described in two patients with acromegaly that were uncontrolled with octreotide LAR 20–30 mg/month and, after crossover to pasireotide LAR 40 mg/month, achieved suppressed IGF-I levels below the normal range⁽³⁰⁾. Conversely, on the other end of the spectrum, a number of patients achieved less than 25% pegvisomant dose reduction. This heterogeneous clinical response to pasireotide LAR may be explained by the inclusion of a heterogeneous acromegaly population with a large variation in baseline pegvisomant dose.

It is unlikely that a carry-over effect of first-generation SRLs had a large impact in our study on the ultimately achieved pegvisomant dose reduction after 48 weeks. Nevertheless, it is possible that the initial response in IGF-I normalisation of the ten patients who had to restart pegvisomant treatment during the extension phase was related to a disappearing carry-over effect of the first-generation SRLs.

It is not clear why the three patients using the highest doses of pegvisomant could not reduce their pegvisomant dose after switching to pasireotide LAR. Theoretically, one could hypothesise that patients using combination treatment of first-generation SRL and high-dose pegvisomant have relatively low SST₂ and high SST₅ receptor expression

compared to patients using first-generation SRL monotherapy and that therefore these patients would be good responders to pasireotide LAR treatment. It remains to be determined whether SST₂ or SST₅ protein expression is responsible for driving the response to pasireotide LAR treatment in our study. Furthermore, it is still unknown how pegvisomant is metabolised in humans, and a wide inter-individual variation in pegvisomant serum levels have been observed when the same doses were administered (17, 31-33). Pegvisomant serum levels are increased during combination treatment of first-generation SRL and pegvisomant, but it is unknown whether this also occurs during combination treatment with pasireotide LAR. Therefore, theoretically patients using high doses of pegvisomant may benefit less from switching to pasireotide LAR.

The insulin-suppressive effect of pasireotide LAR may be related to the degree of pasireotide-induced hyperglycemia, as insulin AUC and (more pronounced) baseline β -cell function were inversely correlated with the observed increase in HbA1c between baseline and 24 weeks. These findings indicate that the lower the insulin secretion is at baseline, the greater will be the risk of pasireotide-induced hyperglycemia during follow-up, even in patients with well-controlled diabetes at baseline. Therefore, besides pre-treatment baseline glucose and HbA1c levels (34), pancreatic β -cell function is probably an additional and independent risk factor of pasireotide-induced hyperglycemia. Patients using pasireotide LAR and pegvisomant combination treatment did not have a lower HbA1c level than patients using pasireotide LAR monotherapy. This observation suggests that the pegvisomant insulin-sensitising effect does not work in pasireotide-induced hyperglycemia (34).

Our results show that pasireotide LAR treatment reduces IGF-I and GH levels after two injections, which parallels the reduction in insulin levels, which also occurs after two injections, suggesting that the early-onset effect of pasireotide LAR on IGF-I reduction may be (partly) mediated by suppressing insulin secretion. This is further supported by the observation that although the effects of pasireotide LAR on GH suppression were superimposable compared with octreotide LAR, pasireotide LAR treatment induced a greater suppression of IGF-I (22).

Conclusions

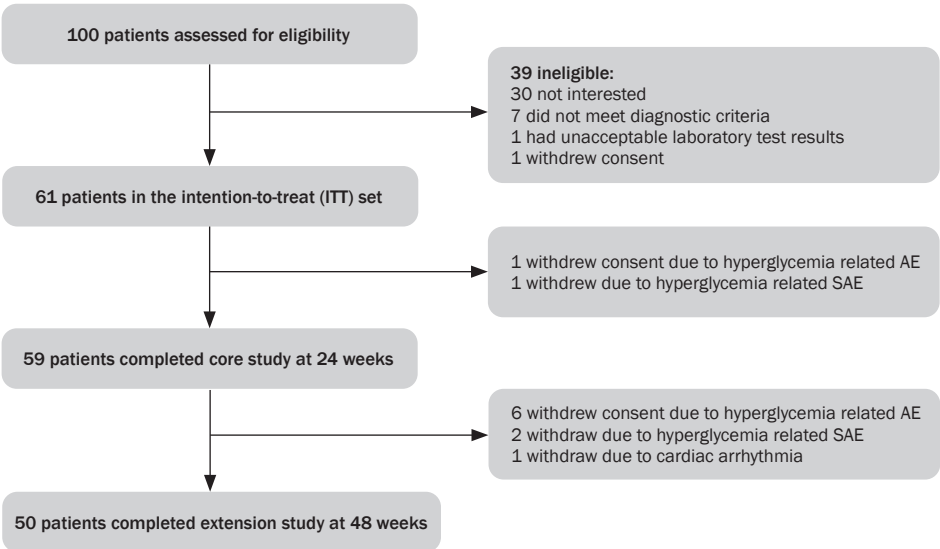
Pasireotide LAR monotherapy or in combination with pegvisomant normalises IGF-I levels in most acromegaly patients despite an about fifty percent reduction in cumulative pegvisomant doses. However, pasireotide LAR therapy coincides with a high incidence of diabetes mellitus, and the risk for developing diabetes during pasireotide LAR therapy seems inversely related to insulin secretion at baseline.

Appendix

Supplemental data

After 48 weeks, diabetes of most patients was managed with a combination of metformin and a DPP-4 inhibitor. 73.8% patients required at least one antidiabetic medication. Between 24 and 48 weeks, five patients developed mild diabetes which required additional treatment with metformin alone or combined with a DPP-4 inhibitor. Due to hypoglycemic symptoms related to sulfonylurea treatment, four patients were switched to a GLP-1 analogue (supplemental table 1). During the follow-up, eight patients discontinued pasireotide LAR treatment due to hyperglycemia-related adverse events (supplemental figure 1). During follow-up, two patients with insulin-dependent type 2 diabetes experienced worsening of glycemic control requiring higher insulin doses, one patient with pre-existent diabetes developed severe hyperglycemia requiring insulin treatment, and three patients with pre-diabetes at baseline needed multiple antidiabetic drugs. Furthermore, two patients who developed mild diabetes that was manageable with metformin and vildagliptin, subjectively experienced no improvement in QoL after start of pasireotide LAR treatment, and therefore decided to discontinue pasireotide LAR during follow-up.

SUPPLEMENTAL FIGURE 1. Flowchart of the selection procedure for the study cohort.



AE = adverse event, SAE = serious adverse event.

SUPPLEMENTAL TABLE 1. Antidiabetic medications initiated after pasireotide LAR initiation. Patients may have taken more than one antidiabetic medication. Data based on last observation carried forward (LOCF).

Antidiabetic medication	Baseline	24 weeks	48 weeks
None	46 (75.4%)	19 (31.1%)	16 (26.3%)
Metformin	15 (24.6%)	19 (31.1%)	25 (41.0%)
DPP-4 inhibitors	0	14 (23.0%)	17 (27.9%)
Sulfonylureas	3 (4.9%)	12 (19.7%)	8 (13.1%)
Insulin	3 (4.9%)	6 (9.8%)	6 (9.8%)
GLP-1 analogues	2 (3.3%)	3 (4.9%)	7 (11.5%)
Thiazolidinediones	1 (1.6%)	1 (1.6%)	1 (1.6%)

References

1. Melmed S. Medical progress: Acromegaly. *N Engl J Med*. 2006;355(24):2558-73.
2. Giustina A, Chanson P, Bronstein MD, Klibanski A, Lamberts S, Casanueva FF, et al. A consensus on criteria for cure of acromegaly. *J Clin Endocrinol Metab*. 2010;95(7):3141-8.
3. Giustina A, Chanson P, Kleinberg D, Bronstein MD, Clemmons DR, Klibanski A, et al. Expert consensus document: A consensus on the medical treatment of acromegaly. *Nat Rev Endocrinol*. 2014;10(4):243-8.
4. Grasso LF, Auriemma RS, Pivonello R, Colao A. Adverse events associated with somatostatin analogs in acromegaly. *Expert Opin Drug Saf*. 2015;14(8):1213-26.
5. Mazziotti G, Giustina A. Effects of lanreotide SR and Autogel on tumor mass in patients with acromegaly: a systematic review. *Pituitary*. 2010;13(1):60-7.
6. Annamalai AK, Webb A, Kandasamy N, Elkhawad M, Moir S, Khan F, et al. A Comprehensive Study of Clinical, Biochemical, Radiological, Vascular, Cardiac, and Sleep Parameters in an Unselected Cohort of Patients With Acromegaly Undergoing Presurgical Somatostatin Receptor Ligand Therapy. *J Clin Endocr Metab*. 2013;98(3):1040-50.
7. Carmichael JD, Bonert VS, Nuno M, Ly D, Melmed S. Acromegaly clinical trial methodology impact on reported biochemical efficacy rates of somatostatin receptor ligand treatments: a meta-analysis. *J Clin Endocrinol Metab*. 2014;99(5):1825-33.
8. Caron PJ, Bevan JS, Petersenn S, Flanagan D, Tabarin A, Prevost G, et al. Tumor shrinkage with lanreotide Autogel 120 mg as primary therapy in acromegaly: results of a prospective multicenter clinical trial. *J Clin Endocrinol Metab*. 2014;99(4):1282-90.
9. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, et al. Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. *N Engl J Med*. 2000;342(16):1171-7.
10. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, et al. Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. *Lancet*. 2001;358(9295):1754-9.
11. Schreiber I, Buchfelder M, Droste M, Forssmann K, Mann K, Saller B, et al. Treatment of acromegaly with the GH receptor antagonist pegvisomant in clinical practice: safety and efficacy evaluation from the German Pegvisomant Observational Study. *Eur J Endocrinol*. 2007;156(1):75-82.

12. van der Lely AJ, Biller BM, Brue T, Buchfelder M, Ghigo E, Gomez R, et al. Long-term safety of pegvisomant in patients with acromegaly: comprehensive review of 1288 subjects in ACROSTUDY. *J Clin Endocrinol Metab.* 2012;97(5):1589-97.
13. Freda PU, Gordon MB, Kelepouris N, Jonsson P, Koltowska-Haggstrom M, van der Lely AJ. Long-term treatment with pegvisomant as monotherapy in patients with acromegaly: experience from ACROSTUDY. *Endocr Pract.* 2015;21(3):264-74.
14. Grottoli S, Maffei P, Bogazzi F, Cannavo S, Colao A, Ghigo E, et al. ACROSTUDY: the Italian experience. *Endocrine.* 2015;48(1):334-41.
15. Droste M, Domberg J, Buchfelder M, Mann K, Schwanke A, Stalla G, et al. Therapy of acromegalic patients exacerbated by concomitant type 2 diabetes requires higher pegvisomant doses to normalise IGF1 levels. *Eur J Endocrinol.* 2014;171(1):59-68.
16. Higham CE, Rowles S, Russell-Jones D, Umpleby AM, Trainer PJ. Pegvisomant improves insulin sensitivity and reduces overnight free fatty acid concentrations in patients with acromegaly. *J Clin Endocrinol Metab.* 2009;94(7):2459-63.
17. Lindberg-Larsen R, Moller N, Schmitz O, Nielsen S, Andersen M, Orskov H, et al. The impact of pegvisomant treatment on substrate metabolism and insulin sensitivity in patients with acromegaly. *J Clin Endocrinol Metab.* 2007;92(5):1724-8.
18. Urbani C, Sardella C, Calevro A, Rossi G, Scattina I, Lombardi M, et al. Effects of medical therapies for acromegaly on glucose metabolism. *Eur J Endocrinol.* 2013;169(1):99-108.
19. Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poublon RM, Feelders RA, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. *J Clin Endocrinol Metab.* 2014;99(10):3644-52.
20. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ. Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. *J Clin Endocrinol Metab.* 2007;92(12):4598-601.
21. Bruns C, Lewis I, Briner U, Meno-Tetang G, Weckbecker G. SOM230: a novel somatostatin peptidomimetic with broad somatotropin release inhibiting factor (SRIF) receptor binding and a unique antiseecretory profile. *Eur J Endocrinol.* 2002;146(5):707-16.
22. Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab.* 2014;99(3):791-9.
23. Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol.* 2014;2(11):875-84.

24. Muhammad A, van der Lely AJ, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of Switching to Pasireotide in Patients With Acromegaly Controlled With Pegvisomant and First-Generation Somatostatin Analogues (PAPE Study). *J Clin Endocrinol Metab.* 2018;103(2):586-95.
25. American Diabetes Association. (2) Classification and diagnosis of diabetes. *Diabetes Care* 2015 38 (Supplement 1) S8–S16
26. National Cancer Institute: Common Terminology Criteria for Adverse Events (CTCAE). Version 4.0. 2010.
27. World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications – Report of A WHO Consultation. Part 1. Diagnosis and Classification of Diabetes Mellitus: World Health Organization; 1999.
28. Stumvoll M, Van Haefen T, Fritsche A, Gerich J. Oral glucose tolerance test indexes for insulin sensitivity and secretion based on various availabilities of sampling times. *Diabetes Care.* 2001;24:796-7.
29. Muhammad A, Coopmans EC, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study. *Eur J Endocrinol.* 2018;179(5):269-77.
30. Shimon I, Saeger W, Wildemberg LE, Gadelha MR. Somatotropinomas inadequately controlled with octreotide may over-respond to pasireotide: the importance of dose adjustment to achieve long-term biochemical control. *Hormones.* 2017;16(1):84-91.
31. Muhammad A, van der Lely AJ, O'Connor RD, Delhanty PJ, Dal J, Dallenga AH, et al. What is the efficacy of switching to weekly pegvisomant in acromegaly patients well controlled on combination therapy? *Eur J Endocrinol.* 2016;174(5):663-7.
32. Madsen M, Fisker S, Feldt-Rasmussen U, Andreassen M, Kristensen L, Orskov H, et al. Circulating levels of pegvisomant and endogenous growth hormone during prolonged pegvisomant therapy in patients with acromegaly. *Clin Endocrinol (Oxf).* 2014;80:92-100.
33. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, et al. Cotreatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. *J Clin Endocrinol Metab.* 2005;90(10):5627-31.
34. Schmid HA, Brue T, Colao A, Gadelha MR, Shimon I, Kapur K, et al. Effect of pasireotide on glucose- and growth hormone-related biomarkers in patients with inadequately controlled acromegaly. *Endocrine.* 2016;53(1):210-9.



Chapter 6

Potential antitumour activity of pasireotide on pituitary tumours in acromegaly

Eva C. Coopmans, Aart J. van der Lely, Joppe J. Schneiders
and Sebastian J.C.M.M. Neggers

Lancet Diabetes & Endocrinology 2019; 7(6): 425-6.

Shrinkage of pituitary adenomas with pasireotide – Authors' reply

Eva C. Coopmans, Aart J. van der Lely, Joppe J. Schneiders
and Sebastian J.C.M.M. Neggers

Lancet Diabetes & Endocrinology 2019; 7(7): 509-10.

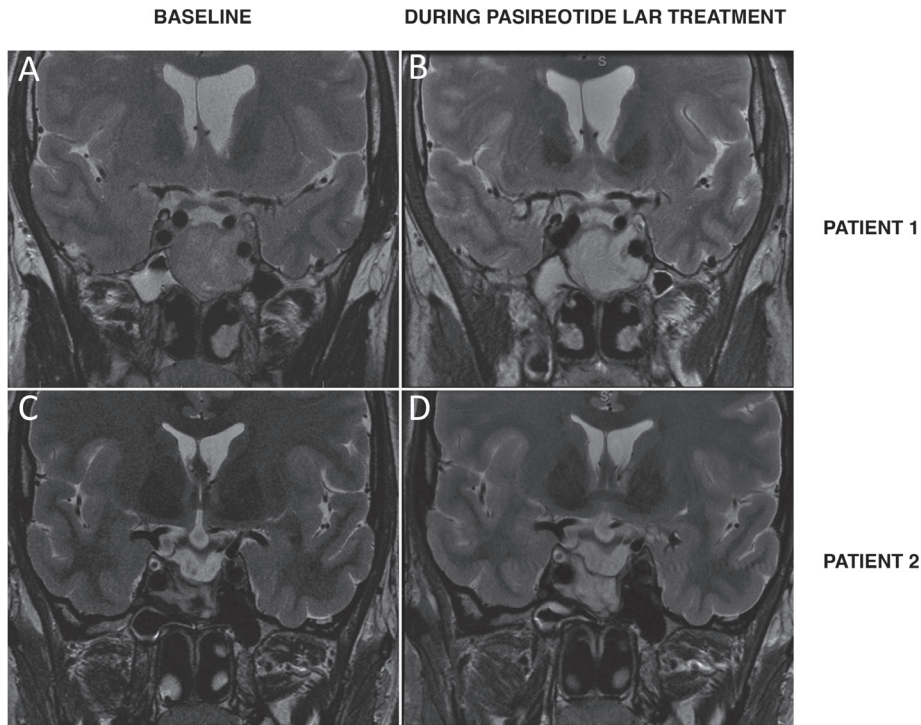
Letter to the editor

Pasireotide LAR is a second-generation somatostatin multireceptor ligand for the treatment of acromegaly. Previous studies indicate that pasireotide LAR can achieve control of growth hormone (somatotropin) and IGF-I concentrations, and might reduce tumour size in patients uncontrolled with first-generation somatostatin receptor ligands^(1, 2).

The PAPE study^(3, 4), which investigated patients with acromegaly who were well controlled on a combination of first-generation SRLs and the growth hormone receptor antagonist pegvisomant, indicated that switching to pasireotide LAR resulted in control of IGF-I concentrations in most patients. In this follow-up analysis of patients from the PAPE study, we assessed 47 patients for T2-weighted signal by MRI (appendix).

Surprisingly, the T2-weighted MRI signal of the adenoma was increased (mean increase 1.71 [SD 0.62]) in 14 (30%) of 47 patients^(3, 4) during pasireotide LAR treatment (appendix). This increase was particularly substantial (>50%) in eight patients, where the majority of the adenoma became T2-hyperintense (Figure 1). In general, a hyperintense T2 signal indicates cystic degeneration, tumour cell necrosis, or both, which suggests an antitumour effect of pasireotide LAR. T2 hyperintensity was found in ten of 47 patients within 9 months of pasireotide LAR treatment, and in four additional patients after the end of the study (median 29.5 months [IQR 11.4–35.4] of pasireotide LAR treatment). Post-study MRI (median 32.4 months [IQR 31.3–33.5] after study end) was also done in two of the first ten patients. In these patients, the T2-weighted MRI signal remained hyperintense in the adenoma of one patient (after 31 months of pasireotide LAR treatment) and increased further in the adenoma of the second (after 33 months of pasireotide LAR treatment).

FIGURE 1. Representative T2-weighted MRI scans from two patients, at baseline and during pasireotide LAR treatment. Region of interest adenoma to normal pituitary ratio at baseline was 1.05 for patient 1 and 1.34 for patient 2. This signal increased to 1.63 for patient 1 and 1.85 for patient 2 during pasireotide LAR treatment.



At the end of the study, the mean T2-signal intensity ratio in the total cohort ($n = 47$) during pasireotide LAR treatment was significantly higher than baseline (1.42 [SD 0.57] vs 1.26 [0.52], $P = 0.015$) (appendix). Although not biochemically controlled during first-generation SRL treatment, patients with T2-hyperintense adenomas ($n = 14$) had a significantly larger decrease in IGF-I concentrations after 3 months of treatment with pasireotide LAR, compared with the total cohort (43.6% [SD 26.5] vs 24.6% [24.3], $P = 0.0021$). Substantial decreases in IGF-I concentrations warranted treatment dosage reduction in two of 14 patients.

Despite pasireotide LAR showing inhibitory effects on the growth of cultured tumour specimens similar to first-generation SRLs⁽⁵⁾, as antitumour effects (*i.e.* cell degeneration or tumour cell necrosis) have not been documented over the past three decades of clinical experience with SRLs, pasireotide LAR treatment was not expected to have antitumour effects. Although first-generation SRL treatment does not change the mean T2-signal intensity ratio in somatotroph adenomas significantly⁽⁶⁾, in a 2018 study, Bonneville and colleagues⁽⁶⁾ can shift to T2-hyperintensity during first-generation somatostatin receptor ligand treatment in some (7 [10%] of 68) treatment-naïve patients. Hence, an antitumour effect of this class of drugs could be considered. In contrast, the change in mean T2-signal intensity ratio after pasireotide LAR treatment, and the 3-fold higher incidence of T2-hyperintense adenomas in our cohort, indicate that this effect is particular to pasireotide LAR.

Our results could affect the clinical management of acromegaly in two ways. First, preoperative treatment with pasireotide LAR might induce (partial) cystic degeneration, tumour cell necrosis, or both, which could improve surgical outcomes. Second, pasireotide LAR induced cystic degeneration, tumour cell necrosis, or both, might reduce disease activity and even alleviate symptoms or, detrimentally, induce anterior pituitary deficiencies. Although no new-onset anterior pituitary deficiencies were observed in our cohort, we recommend close monitoring of pituitary tumour status, alongside regular assessments of growth hormone and IGF-I concentrations. In case of substantial necrosis of the pituitary adenoma, it might be necessary to reconsider the treatment regimen.

To our knowledge this is the first reported incidence of significant cystic degeneration, tumour cell necrosis, or both during pasireotide LAR therapy in patients with acromegaly. Additional studies are needed to address the effects of pasireotide LAR on pituitary histology and its consequences for clinical practice.

Authors reply

We thank Adrian F Daly and colleagues for their comments on our Correspondence⁽⁷⁾ and appreciate the opportunity to further discuss our proposed antitumour activity of pasireotide LAR on pituitary tumours in acromegaly. Daly and colleagues raise the point that although increased T2-weighted signal intensity (T2WSI) was associated with improved hormonal control, a significant decrease in tumour volume did not occur with nine months of pasireotide LAR treatment. This could call into question the existence of the suggested antitumour effect of pasireotide LAR.

We agree with Daly and colleagues that besides T2WSI, there are more relevant ways to assess an antitumour effect of pasireotide LAR. We believe, however, that it is necessary to differentiate tumour shrinkage from cystic degeneration or tumour cell necrosis. It has been reported that pasireotide LAR shows similar inhibitory effects on the growth of cultured tumour specimens compared with first-generation SRLs⁽⁵⁾.

Additionally, pasireotide LAR seems to have an antitumour effect (cystic degeneration or tumour cell necrosis)⁽⁷⁾ that has not been observed with first-generation SRLs. The potential antitumour activity of pasireotide LAR emerged when we observed a change in mean T2WSI ratio and a 3-fold higher incidence of T2-hyperintense adenomas in our cohort, compared with patients receiving first-generation SRLs⁽⁷⁾.

Of note, apart from hormonal control we did observe and report clinically significant ($\geq 25\%$) tumour shrinkage in 5 of the 14 patients with adenomas in which we observed increased T2WSI during the first nine months of pasireotide LAR treatment. Furthermore, not all patients with increased T2WSI adenomas during pasireotide LAR treatment had a T2-hyperintense adenoma at baseline (of 14 adenomas, 7 adenomas had hyperintensity, 4 adenomas had T2-hypointensity, and 3 adenomas had T2-isointensity).

In the two patients with acromegaly due to *AIP* germline mutations reported by Daly and colleagues⁽⁸⁾, treatment with pasireotide LAR induced clinically significant tumour shrinkage in the adenoma of one patient (after at least 24 months of treatment) and a regression of the tumour remnant in the other (after 60 months of treatment). We tested nine (19%) of the 47 patients in our cohort for germline *AIP* mutations, but none were detected. Therefore, the available data are insufficient to draw any firm conclusions about the relationship between the observed increased T2WSI and the potential antitumour activity of pasireotide LAR. In other reports of treatment-naïve patients^(9, 10), clinically

significant tumour shrinkage was observed in one patient after about 12 months of pasireotide LAR treatment, and substantial tumour shrinkage in the other after 3 months of pasireotide LAR treatment. However, comparison of patients in previous reports⁽⁸⁻¹⁰⁾ with our cohort might be challenging; no T2WSI data was collected or observed and the two patients reported by Yamamoto and colleagues⁽⁹⁾ and Chiloire and colleagues⁽¹⁰⁾ are treatment-naïve patients, while our cohort as well as the patients reported by Daly and colleagues⁽⁸⁾ partially responded to first-generation SRLs. The patient with maintained regression of tumour remnant⁽⁸⁾ after 18 months off pasireotide LAR treatment might provide some evidence to support pasireotide LAR antitumour activity.

We have not yet published data on tumour shrinkage after long-term pasireotide LAR treatment in our cohort. Post-study MRIs were assessed in 10 of the 14 patients; interestingly, an additional decrease in tumour volume was observed in the adenomas of five patients (mean tumour volume was 2.9 cm³ [SD 3.0] at baseline, 2.3 cm³ [2.2] after 9 months of pasireotide LAR, and 1.9 cm³ [10.0] after 30.3 months of pasireotide LAR). We will obtain more conclusive data in the next few years.

We agree with Daly and colleagues that additional pituitary histology is needed to confirm whether cystic degeneration, tumour cell necrosis, or both leads to increased T2WSI. Moreover, the identification of genetic, molecular, and clinical characteristics associated with the effects of pasireotide LAR will improve clinical practice.

Appendix Supplementary methods

Patients selection

Data collection of acromegaly patients was performed at the outpatient clinic of the Pituitary Center Rotterdam, Erasmus University Medical Center in Rotterdam. We initially started with a cohort of 61 acromegaly patients who received pasireotide LAR treatment during their participation in the PAPE study^(3, 4). All of these patients have previously been treated with first-generation SRLs, followed by SRLs and pegvisomant combination therapy. After exclusion of patients that received postoperative radiotherapy less than 10 years ago (n=1), patients with low quality baseline or follow-up MRI during pasireotide LAR (n=5), and patients with a MRI without a visible solid component (n=8), 47 patients remained and were finally included in the study cohort. In total 19 out of these 47 patients previously underwent neurosurgery. Reasons for surgery included microadenomas with reasonable chance for cure and macroadenomas with risk of visual impairment.

Outcomes

Gadolinium-enhanced pituitary MRI was performed at screening and after a mean of 8.5 months of treatment with pasireotide LAR (*i.e.* end of the extension phase, SD 1.2). The MRI examinations of each patient were collected for centralised, blinded reading. One experienced neuroradiologist (J.S) evaluated MRIs in the Picture Archive Communication System (PACS, Sectra®, Linköping, Sweden). T2-weighted MRI signal of the adenoma was visually assessed and quantified by region of interest measurement. Tumour volume was calculated according to the formula: height x width x length x $\pi/6$ ⁽¹¹⁾. A pituitary tumour volume change of $\geq 25\%$ from screening was considered significant. We retrospectively collected data on medical history and clinical response to patients receiving maximum doses of SRLs treatment longer than four months, while data on pasireotide LAR were prospectively collected from the PAPE study^(3, 4).

Supplementary results

Results of patient and tumour characteristics

At baseline, the median age at baseline was 52·0 (range 26-80) years and 21 (42·9%) patients were females. Most patients (80·9%) harboured a macroadenoma and 19 out of 47 (40·4%) patients previously underwent neurosurgery. Two patients received radiotherapy 12 and 19 years prior to study entry. Table 1 shows tumour characteristics at baseline and during pasireotide LAR treatment of the affected patients and the total cohort.

During follow-up, 11 out of 47 patients were on pasireotide LAR 60 mg monthly as monotherapy after three months, increasing to 32 patients after nine months of pasireotide LAR treatment. The remaining 15 patients with elevated IGF-I levels continued with their reduced dose of pegvisomant treatment, but now in combination with pasireotide LAR 60 mg monthly. When compared to baseline, the cumulative reduction in pegvisomant dose was 34·6% in combination with pasireotide LAR after nine months of pasireotide LAR treatment.

At the end of the study, in 16 out of 47 (34·0%) patients the adenomas showed significant tumour shrinkage during pasireotide LAR treatment, five of these patients had T2-hyperintense adenomas. Patients with T2-hyperintense adenomas have a significant higher mean T2-signal intensity ratio ($P = 0·0069$), when compared to the total cohort.

SUPPLEMENTAL TABLE 1. Tumour characteristics at baseline and during pasireotide treatment of patients with cystic degeneration and/or tumour cell necrosis and the total cohort. Data are mean (SD) or median (IQR).

	Before pasireotide LAR treatment	During pasireotide LAR treatment	P value
Total cohort (n=47)			
T2-signal intensity (adenoma/pituitary) ratio	1·26 (0·52)	1·42 (0·57)	0·015
Adenoma volume (cm ³)	1·4 (0·4-4·3)	1·2 (0·3-3·8)	0·071
Patients with cystic degeneration and/or tumour cell necrosis (n=14)			
T2-signal intensity (adenoma/pituitary) ratio	1·23 (0·52)	1·71 (0·62)	0·0069
Adenoma volume (cm ³)	2·1 (0·8-5·3)	1·7 (1·1-4·6)	0·82

Pasireotide LAR = pasireotide long-acting release.

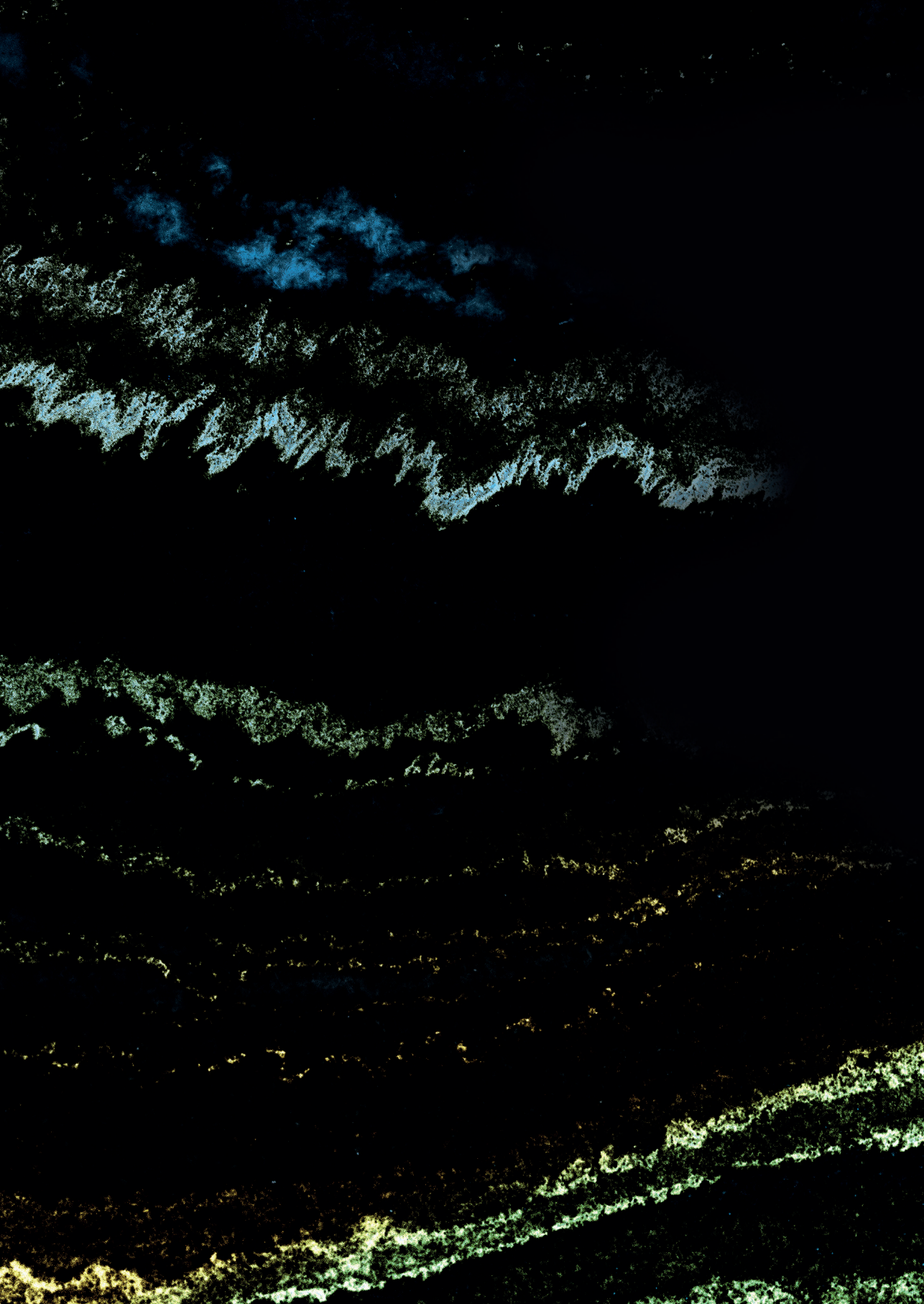
Ethics statement

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and reported in accordance with the International Conference on Harmonization Harmonized Tripartite Guideline: Guideline for Good Clinical Practice. The study protocol was approved by the Medical Ethics Committees of Erasmus Medical Centre. All patients provided written informed consent to participate in the PAPE study. This study was registered with ClinicalTrials.gov as NCT02668172, the Dutch Trial Register, and the World Health Organization International Clinical Trials Registry Platform as NTR5282.

References

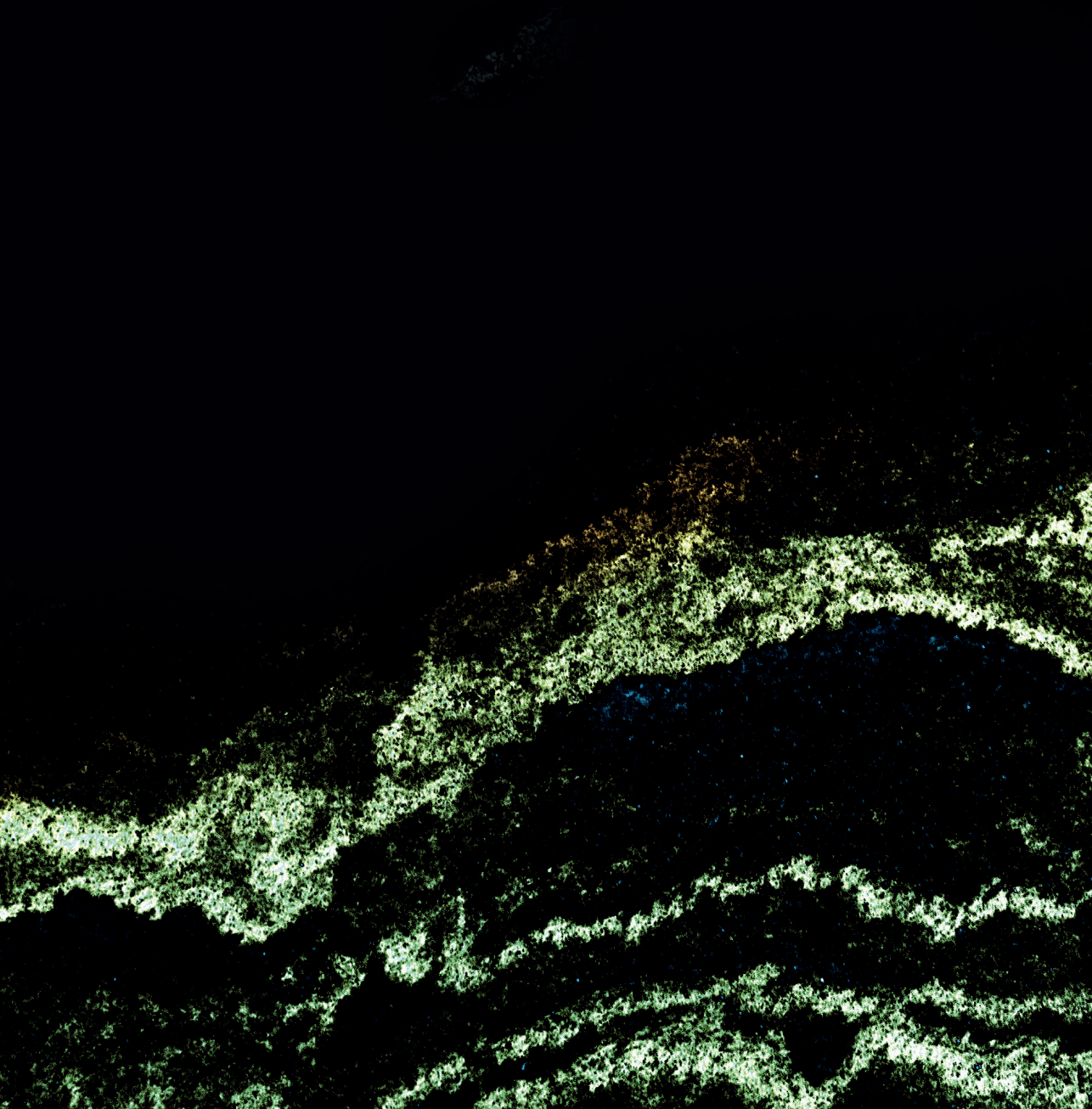
1. Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab.* 2014;99(3):791-9.
2. Gadelha MR, Bronstein MD, Brue T, Coculescu M, Fleseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol.* 2014;2(11):875-84.
3. Muhammad A, van der Lely AJ, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of Switching to Pasireotide in Patients With Acromegaly Controlled With Pegvisomant and First-Generation Somatostatin Analogues (PAPE Study). *J Clin Endocrinol Metab.* 2018;103(2):586-95.
4. Muhammad A, Coopmans EC, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study. *Eur J Endocrinol.* 2018;179(5):269-77.
5. Ibanez-Costa A, Rivero-Cortes E, Vazquez-Borrego MC, Gahete MD, Jimenez-Reina L, Venegas-Moreno E, et al. Octreotide and pasireotide (dis)similarly inhibit pituitary tumor cells in vitro. *J Endocrinol.* 2016;231(2):135-45.
6. Bonneville F, Riviere LD, Petersenn S, Bevan J, Houchard A, Sert C, et al. MRI T2 signal intensity and tumor response in patients with GH-secreting pituitary macroadenoma: PRIMARYS post-hoc analysis. *Eur J Endocrinol.* 2018.
7. Coopmans EC, van der Lely AJ, Schneiders JJ, Neggers S. Potential antitumour activity of pasireotide on pituitary tumours in acromegaly. *Lancet Diabetes Endocrinol.* 2019;7(6):425-6.
8. Daly AF, Rostomyan L, Betea D, Bonneville JF, Villa C, Pellegata NS, et al. AIP-mutated acromegaly resistant to first-generation somatostatin analogs: long-term control with pasireotide LAR in two patients. *Endocr Connect.* 2019;8(4):367-77.
9. Yamamoto R, Robert Shima K, Igawa H, Kaikoi Y, Sasagawa Y, Hayashi Y, et al. Impact of preoperative pasireotide therapy on invasive octreotide-resistant acromegaly. *Endocr J.* 2018;65(10):1061-7.
10. Chiloiro S, Giampietro A, Bianchi A, Tartaglione T, Bima C, Vita MG, et al. Acromegaly can be cured by first-line pasireotide treatment? *Endocrine.* 2019;64(1):196-9.

11. Lundin P, Pedersen F. Volume of pituitary macroadenomas: assessment by MRI. *J Comput Assist Tomogr*. 1992;16(4):519-28.



Part III

**Emerging treatment option:
ketogenic diet in acromegaly**





Chapter 7

Eucaloric very-low-carbohydrate ketogenic diet in acromegaly treatment

Eva C. Coopmans, Kirsten A.C. Berk, Nour El-Sayed,
Sebastian J.C.M.M. Neggers and Aart J. van der Lely

New England Journal of Medicine 2020; 382(22): 2161-2.

Correspondence

Acromegaly is caused by a growth hormone-secreting pituitary tumor, and its signs link to increased growth hormone-dependent hepatic insulin-like growth factor I (IGF-I) synthesis.⁽¹⁾ A eucaloric very low-carbohydrate ketogenic diet (euVLCK;<50 g/day) induces ketosis and reduces portal insulin concentrations, which down-regulate hepatic growth hormone receptors⁽²⁾ and reduce IGF-I synthesis.^(3,4) SRLs reduce pituitary growth hormone secretion, resulting in IGF-I normalization in approximately 50% of patients⁽⁵⁾. We hypothesized that in patients with acromegaly, a eucaloric very-low-carbohydrate ketogenic diet would exert insulin-induced IGF-I normalization without the unwanted increase in growth hormone, given that growth hormone-inhibiting somatostatin receptor ligand therapy would be continued.

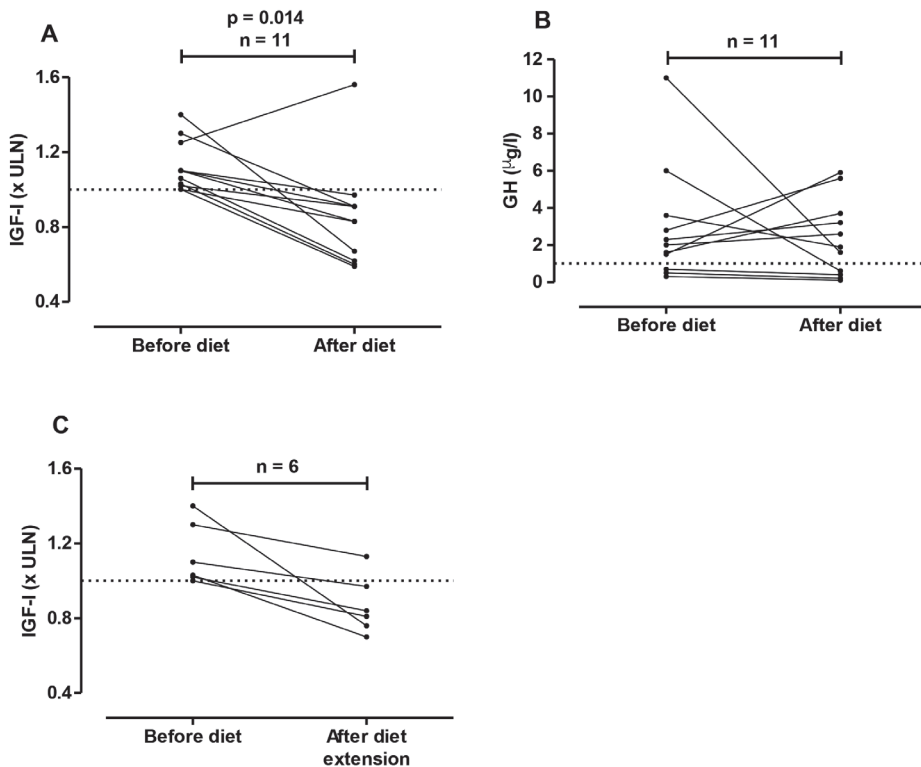
We conducted a proof-of-concept study involving 11 patients (6 of whom were women) to investigate whether a 2-week, eucaloric very-low-carbohydrate ketogenic diet (35 g of carbohydrate, approximately 155 g of fat, and approximately 115 g of protein per day) as adjuvant treatment to first-generation SRLs would reduce IGF-I concentrations in patients with uncontrolled acromegaly (Netherlands Trial Register number, NL7093). Details of the study design are provided in the protocol, available with the full text of this letter at NEJM.org.

During the diet, the patients' mean (\pm SD) carbohydrate intake decreased from 194.4 ± 143.1 g per day to 32.6 ± 14.7 g per day. The median IGF-I concentration decreased significantly, from 1.10 times the upper limit of the normal range (interquartile range, 1.02 to 1.25) to 0.83 times the upper limit of the normal range (interquartile range, 0.62 to 0.91) ($P = 0.01$), and normalized in all but one patient (Fig. 1A). There was not a concomitant increase in the growth hormone concentration (median, 2.0 μ g per liter [interquartile range, 0.7 to 3.6] before the diet vs. 1.9 μ g per liter [interquartile range, 0.4 to 3.7] after the diet; $P = 1.00$) (Fig. 1B). The one patient who did not have a normal IGF-I concentration after the diet did have a substantial decrease in the growth hormone concentration and had presented with the highest degree of insulin resistance. Overall, the glycated hemoglobin level decreased slightly (from 39.8 ± 5.2 to 38.6 ± 4.4 mmol per mole; mean difference, -1.2 mmol per mole; 95% confidence interval, -2.1 to -0.3). Although the diet was eucaloric, the mean body weight decreased by approximately 1 kg.

The association of weight loss and changes in IGF-I concentrations was not significant (Spearman's coefficient, -0.24).

Overall, the diet was well accepted; all the patients completed the study. A reduction in the somatostatin receptor ligand dose was possible in three of the six patients who continued following a low-carbohydrate ketogenic diet (80 g of carbohydrate per day) as adjuvant treatment to their initial somatostatin receptor ligand therapy. After a median of 3.0 months, the median IGF-I concentration among these patients was 0.83 times the upper limit of the normal range (interquartile range, 0.75 to 1.01) (Fig. 1C).

FIGURE 1. IGF-I and growth hormone concentrations in patients with acromegaly.

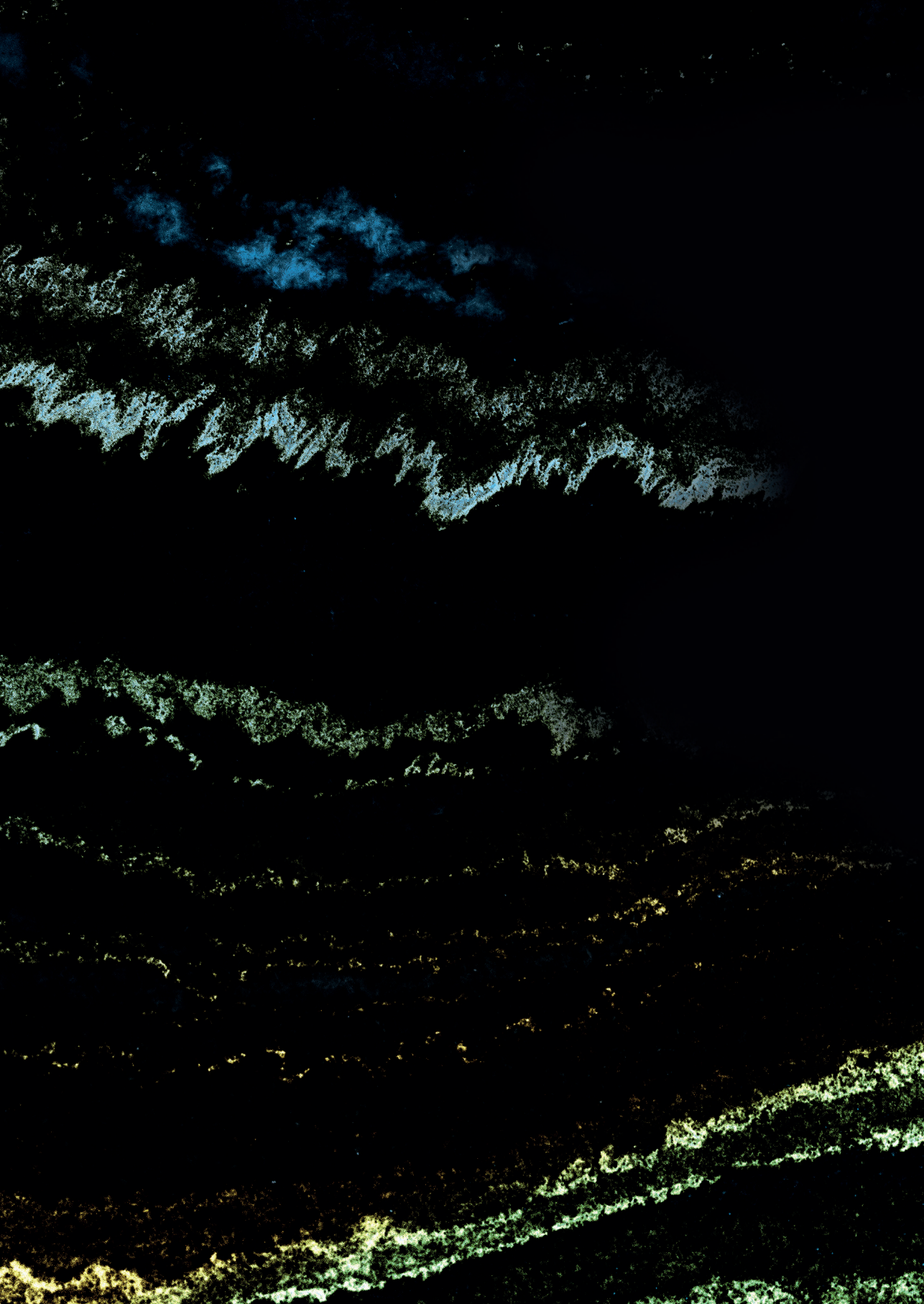


All the patients followed a eucaloric very-low-carbohydrate ketogenic diet (35 g of carbohydrate, approximately 155 g of fat, and approximately 115 g of protein per day) as an adjuvant to first-generation somatostatin receptor ligand therapy. Panels A and B show the changes in the concentrations of insulin-like growth factor I (IGF-I) and growth hormone, respectively, in all 11 patients. Panel C shows the changes in the IGF-I concentrations in the 6 patients who continued a low-carbohydrate diet (80 g of carbohydrate per day) for a median of 3.0 months. The dashed line in each panel indicates the normal value. ULN denotes the upper limit of the normal range.

Although none of the participants had type 2 diabetes or hypoglycemia-related adverse events, we recommend tight glycemic control in patients taking antidiabetic medication. Primary prevention of dehydration, a common side effect, can be managed by adequate fluid intake (≥ 2 liters per day). Our pilot study showed the ability of an adjuvant eucaloric very-low-carbohydrate ketogenic diet to result in IGF-I control in patients with acromegaly whose disease was uncontrolled with first-generation SRL therapy. Additional studies are needed to evaluate the longterm safety and efficacy of, as well as adherence to, a eucaloric very-low-carbohydrate ketogenic diet.

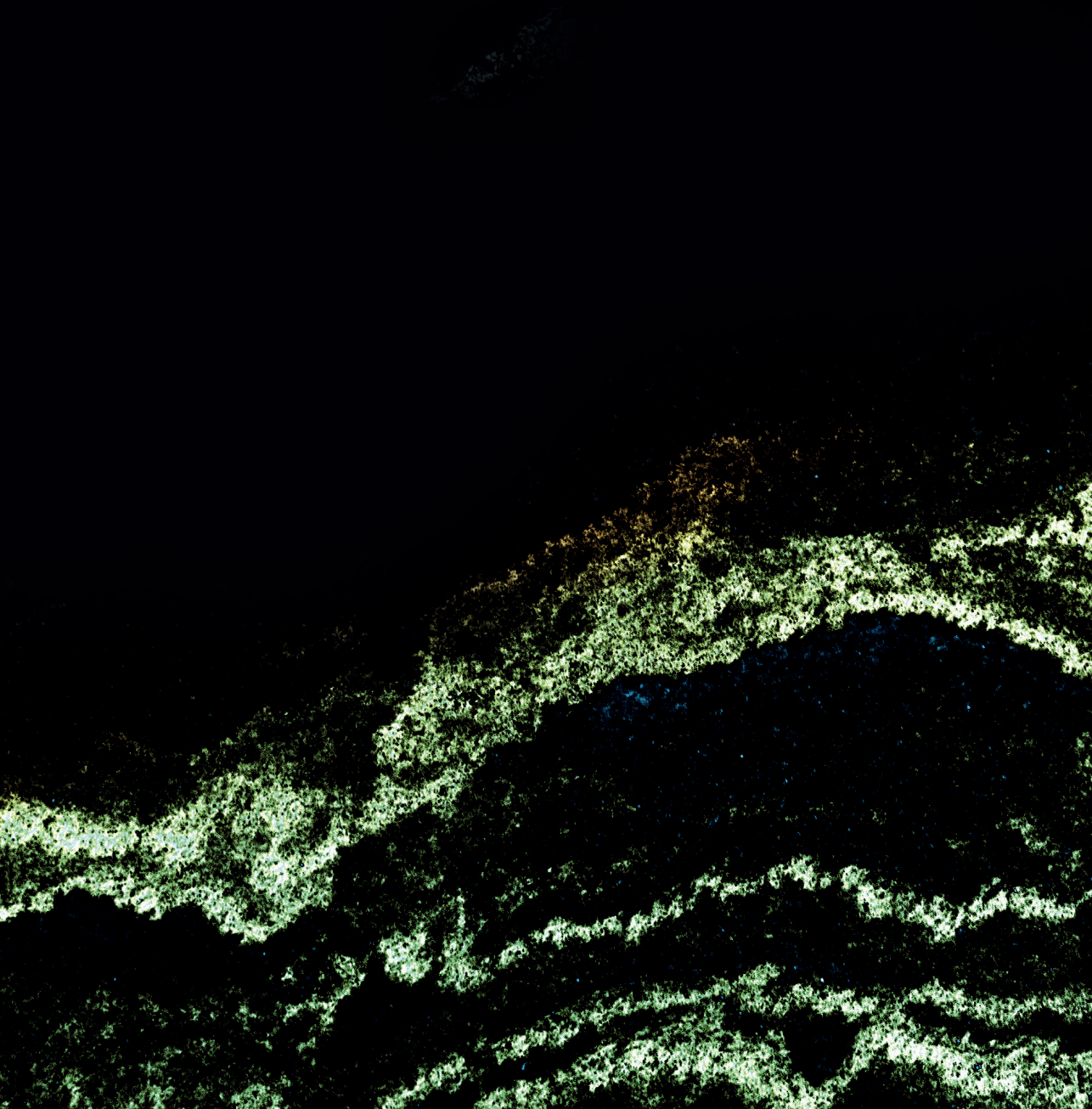
References

1. Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest* 2009;119:3189-202.
2. Leung K-C, Doyle N, Ballesteros M, Waters MJ, Ho KKY. Insulin Regulation of Human Hepatic Growth Hormone Receptors: Divergent Effects on Biosynthesis and Surface Translocation1. *J Clin Endocrinol Metab*. 2000;85:4712-20.
3. Clemmons DR. Involvement of insulin-like growth factor-I in the control of glucose homeostasis. *Curr Opin Pharmacol* 2006;6:620-5.
4. Ho PJ, Friberg RD, Barkan AL. Regulation of pulsatile growth hormone secretion by fasting in normal subjects and patients with acromegaly. *J Clin Endocrinol Metab* 1992;75:812-9.
5. Carmichael JD, Bonert VS, Nuno M, Ly D, Melmed S. Acromegaly clinical trial methodology impact on reported biochemical efficacy rates of somatostatin receptor ligand treatments: a meta-analysis. *J Clin Endocrinol Metab* 2014;99:1825-33.



Part IV

Role of AIP variants in pituitary adenomas





Chapter 8

**The role of AIP variants in pituitary
adenomas and concomitant
thyroid carcinomas in the Netherlands:
a nationwide pathology registry (PALGA) study**

Eva C. Coopmans, Ammar Muhammad, Adrian F. Daly,
Wouter W. de Herder, Folkert J. van Kemenade, Albert Beckers,
Marij de Haan, Aart J. van der Lely, Esther Korpershoek
and Sebastian J.C.M.M. Neggers

Endocrine 2020; 68: 640-9.

Abstract

PURPOSE: Germline mutations in the aryl-hydrocarbon receptor interacting protein (*AIP*) have been identified often in the setting of familial isolated pituitary adenoma (FIPA). To date there is no strong evidence linking germline *AIP* mutations to other neoplasms apart from the pituitary. Our primary objective was to investigate the prevalence of *AIP* gene mutations and mutations in genes that have been associated with neuroendocrine tumors in series of tumors from patients presenting with both pituitary adenomas and differentiated thyroid carcinomas (DTCs).

METHODS: Pathology samples were retrieved from all pituitary adenomas in patients with concomitant DTCs, including one with a known germline *AIP* variant. Subsequently, two additional patients with known germline *AIP* variants were included, of which one presented only with a follicular thyroid carcinoma (FTC).

RESULTS: In total, 17 patients (14 DTCs and 15 pituitary adenomas) were investigated by targeted next generation sequencing (NGS). The pituitary tumor samples revealed no mutations, while among the thyroid tumor samples BRAF (6/14, 42.9%) was the most frequently mutated gene, followed by NRAS (3/11, 27.3%). In one *AIP*-mutated FIPA kindred, the *AIP*-variant c.853C>T; p.Q285* was confirmed in the FTC specimen, including evidence of loss of heterozygosity (LOH) at the *AIP* locus in the tumor DNA.

CONCLUSIONS: Although most observed variants in pituitary adenomas and DTCs were similar to those of sporadic DTCs, we confirmed in one *AIP* mutation-positive case the *AIP*-variant and LOH at this locus in an FTC specimen, which raises the potential role of the *AIP* mutation as a rare initiating event.

Introduction

Pituitary adenomas are mostly benign monoclonal neoplasms that arise from any of the five hormone-secreting cell types of the anterior lobe of the pituitary gland, and cause disease due to hormonal hypersecretion and tumor mass effects. Most pituitary adenomas occur sporadically (95%). Although in the majority of these sporadic cases the exact molecular pathogenesis remains unknown, in a significant proportion of somatotropinomas (30%) and corticotropinomas (60%) activating somatic mutations have been found in the *GNAS* and *USP8* genes, respectively^(1, 2). In addition, germline mutations may predispose to pituitary tumorigenesis, which together represent about 5% of patients with pituitary adenomas⁽³⁾.

Germline mutations have been described in the *aryl-hydrocarbon receptor interacting protein* (*AIP*) gene in the setting of either familial isolated pituitary adenoma (FIPA) or in simplex, young-onset pituitary adenomas, such as pituitary gigantism⁽⁴⁻⁶⁾. The *AIP* gene encodes a 330-amino-acid co-chaperone involved in subcellular trafficking, nuclear receptor stability and transactivation potential^(4, 7). It is postulated that in *AIP*-mutated pituitary adenomas, *AIP* loses its activity as a tumor suppressor, which is supported by the association of loss-of-function mutations and the presence of loss of heterozygosity (LOH) at the *AIP* locus in the pituitary adenoma. To date there is no strong evidence linking germline *AIP* mutations to other neuro-endocrine neoplasms apart from the pituitary.

The frequency of differentiated thyroid carcinomas (DTCs) is increased in patients with somatotropinomas, with papillary thyroid carcinoma (PTC) being the most frequently reported type (up to 25%)⁽⁸⁻¹³⁾. As thyroid follicular epithelial cells express insulin-like growth factor 1 (IGF-1) receptors and IGF-1 is an important factor for promoting replication and reducing apoptosis of these cells⁽¹⁴⁾, IGF-1 could potentially be linked to the promotion of thyroid cancer in acromegalic patients. *BRAF* mutations have proved to be the most common genetic event (about 60% of cases) involved in the onset of PTC in the general population⁽¹⁵⁾; other frequently-identified genetic events include point mutations of the *RAS* genes and *RET/PTC* and *PAX8/PPAR γ* chromosomal rearrangements^(15, 16). Based on earlier reports, LOH of chromosome 22 is particularly common in follicular thyroid carcinomas (FTCs), and it is associated with the widely invasive type⁽¹⁷⁻¹⁹⁾.

Given the frequency of malignant thyroid tumors in somatotropinomas, the potential for a common mechanism behind both tumors remains valid. The role of an *AIP* mutation as an initiating event is open to question since the *AIP* protein may interact with the tyrosine kinase receptor, encoded by the *RET* protooncogene in the pituitary⁽²⁰⁻²⁴⁾. Coexistence of PTCs with somatotropinomas in *AIP*-mutated patients is very rare and has been described in three cases^(25, 26). Although only one case of LOH at the *AIP* locus (11q13) in FTCs is previously described⁽²⁷⁾, Daly and colleagues recently described an FTC in a teenaged *AIP* mutation-positive carrier in which decreased *AIP* staining was seen in the FTC tumor that was accompanied by LOH at the *AIP* locus in the tumor DNA⁽²⁸⁾. Thus, the finding of DTCs and pituitary adenomas in the same individuals or kindreds could represent a rare association of germline *AIP* mutations.

To date, there has only been one study that reported in 12 patients with somatotropinomas and concomitant DTC that *AIP* was not overexpressed in the thyroid tumor tissue using immunohistochemistry⁽²⁹⁾. Here we studied the presence of mutations in *AIP* in patients with DTCs and concomitant pituitary adenomas, including all five adenoma types. Subsequently, the available tumors from these patients were investigated using targeted next generation sequencing (NGS) for mutations in *AIP* and additional neuro-endocrine tumor-related genes. Since these features are relative rare, a nationwide survey was performed in the Netherlands.

Materials and Methods

Patients

From *Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief* (PALGA), the nationwide Dutch network and registry of histo- and cytopathology, all patient records of individuals included 1993-2016 were retrieved matching the following search criteria: pituitary adenoma (*i.e.* prolactinomas, non-functioning pituitary adenomas (NFPAs), somatotropinomas, corticotropinomas and thyrotropinomas) and DTC (*i.e.* FTC, follicular variant of papillary thyroid carcinoma (FVPTC) and PTC). The standardized records contain an encrypted patient identification number (allowing for identification of multiple samples of one patient), data on age at diagnosis and sex, date of arrival of the histological tissue, presence of metastasis and the diagnosis of the pathology report.

The PALGA search identified 15 patients with a history of thyroid carcinoma and pituitary adenoma with no known genetic background (*i.e.* sporadic), except for one with a known germline *AIP* variant from the Erasmus University Medical Center that was part of the PALGA search data range as well. Two additional patients from this center with known germline *AIP* variants were included in the study, of which one who presented only with an FTC. The latter has a familial history of pituitary adenomas (*i.e.* father was *AIP* mutation carrier and diagnosed with acromegaly), however, the pituitary gland was not affected in this patient. Therefore, this patient was not identified in the PALGA search data range. The second patient with a somatotropinoma and classical-variant PTC was successfully treated by total thyroidectomy in 1975, and therefore not part of the PALGA search date range, while the tumor sample showed well-preserved histomorphology. In total, we included 17 patients.

Approval from the Medical Ethical Committee of the Erasmus University Medical Center and informed consent to use the tumor tissues for research purposes were obtained. Tumor tissues from all Dutch medical centers were used according to the code of conduct, Proper Secondary Use of Human Tissue, established by the Dutch Federation of Medical Scientific Societies⁽³⁰⁾.

Data collection

Anonymized data were collected on age at diagnosis, sex, year of diagnosis, presence of metastasis, immunohistochemical staining results (adrenocorticotrophic hormone (ACTH), follicle-stimulating hormone (FSH), GH, luteinizing hormone (LH), PRL) and type of differentiated thyroid carcinoma (FTC, FVPTC or classical-variant PTC).

Genetic analysis of germline *AIP* mutation

As mentioned above, three patients from the Erasmus University Medical Center included in the study were previously investigated for the presence of *AIP* mutations. This was performed using leukocyte DNA extracted from peripheral blood as described by Vierimaa et al.⁽⁵⁾; multiplex ligation-dependent probe amplification studies were performed as described previously^(31, 32). Normal population genetic databases were assessed for the presence of *AIP* variant frequencies; *AIP* variant pathogenicity was assessed using Alamut (Interactive Biosoftware). In addition, classification of variants was also performed according reported guidelines⁽³³⁾. All patients provided informed written consent for genetic testing.

Tumor DNA samples

We excluded low quality tissue of pituitary adenoma (n = 2) or thyroid carcinoma (n = 2) from the study. As mentioned before, patient no. 17 presented only with an FTC. In total 29 tumor DNA samples from 17 index patients were studied; DNA obtained exclusively from thyroid tumor was available for 14 (82.4%) of the cases, and only pituitary tumor DNA for 15 (88.2%) of the cases.

DNA was isolated from representative tumor areas by microdissection, from approximately 10 hematoxylin stained sections from formalin-fixed, paraffin-embedded (FFPE) tumor tissue, using proteinase-K and 5% Chelex 100 resin. Selection of representative tumor areas was performed on a paraffin slide stained with hematoxylin and eosin by a pathologist (L.O. and F.G.). In addition, DNA was quantified with the Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA). All tumor DNAs that were used for mutation screening contained $\geq 60\%$ of tumor cells.

Targeted NGS and data analysis

A custom-made targeted gene panel (TGP) was designed using the TruSeq Custom Amplicon 1.5 kit system (Illumina, San Diego, CA) and the Ion AmpliSeq designer software (<https://ampliseq.com/>; Thermo Fisher Scientific, Breda, the Netherlands), to study DNA from FFPE tumor tissues (Table 1). The panel was designed specifically for FFPE-DNA use (amplicon range 125-175 bp). Targeting contained the entire coding sequences of *AIP* (coverage based on design: 92.11%), *CDKN1B* (96.84%), *GNAS* (82.93%), *GPR101* (97.46%), *HRAS* (63.08%), *KRAS* (82.28%), *MEN1* (84.73%), *NRAS* (100.00%), *PIK3CA* (96.55%), *PRKACB* (91.46%), *PRKAR1A* (100.00%), *RET* (86.34%), *SDHA* (93.48%), *SDHAF2* (100.00%), *SDHB* (98.65%), *SDHC* (91.93%), *SDHD* (77.95%), and the hotspot region *BRAF* (p.V600E). In addition, single nucleotide polymorphisms (SNPs) were selected on chromosome 11 and 22 to enable copy number variation (CNV) detection (Table 1). Mutation detection was performed using the S5-XL system (Ion Torrent) with manufacturer's materials and protocols (Thermo Fisher Scientific). Library preparations and sequencing was performed as described earlier⁽³⁴⁾. Data analysis was performed using SeqPilot version 4.2.2. (JSI medical systems). CNV detection was evaluated using SNPitty, which visualizes B-allele frequencies from NGS sequencing data (35). The American College of Medical Genetics and Genomics (ACMG) standards and guidelines were used for interpretation of sequence variants of unknown significance

(VUS)⁽³³⁾. When classifying and reporting a variant we used the online software prediction program Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>) and Align GVGD (http://agvgd.hci.utah.edu/agvgd_input.php) as well as the gnomAD database (<https://gnomad.broadinstitute.org>), cBioportal database (<https://www.cbioportal.org>) and the Cosmic database (<https://cancer.sanger.ac.uk/cosmic>).

In patient no. 17, we also examined the most common FTC driver gene alterations⁽³⁶⁾ by a targeted NGS designed to study *PTEN* and the *TERT* promoter. The panel included the entire coding sequences of *CDKN2A*, *KEAP1*, *PTEN*, *STK11*, and *TP53*, as well as hotspots: *AKT1* (exon 3), *AKT2*⁽³⁾, *AKT3*⁽²⁾, *ALK*^(20, 22-25), *APC*⁽¹⁶⁾, *ARAF*⁽⁷⁾, *BRAF*^(11, 12, 14, 15), *CDK4*^(2, 4, 7, 8), *CTNNB1*^(3, 7, 8), *DDR2*⁽¹⁴⁻¹⁹⁾, *EGFR*^(12, 18-21), *EIF1AX*^(1, 2), *ERBB2* (*HER2*)^(8, 17-21), *ERBB3*^(3, 6-10, 21, 23), *ESR1*^(4, 5, 7, 8), *EZH2*⁽¹⁶⁾, *FBWX7*^(9, 10), *FGFR1*^(4, 7, 12-14), *FGFR2*^(7, 9, 12), *FGFR3*^(7, 9, 14, 15), *FOXL2*⁽¹⁾, *GNAI1*^(4, 5), *GNAQ*^(4, 5), *GNAS*^(8, 9), *HRAS*⁽²⁻⁴⁾, *IDH1*⁽⁴⁾, *IDH2*⁽⁴⁾, *JAK2*⁽¹⁴⁾, *JAK3*^(4, 16), *KIT*^(8, 9, 11, 13-18), *KNSTRN*⁽¹⁾, *KRAS*⁽²⁻⁴⁾, *MAP2K1*⁽¹⁻⁶⁾, *MET*^(2, 14, 19, 20), *MTOR*^(30, 39, 40, 43, 47, 53, 56, 57), *MYD88*⁽⁵⁾, *NFE2L2*⁽²⁾, *NOTCH1*^(26, 27), *NRAS*⁽²⁻⁴⁾, *OXA1L*⁽¹⁾, *PDGFRA*^(12, 14, 18), *PIK3CA*^(2, 5, 8, 10, 14, 21), *POLD1*^(6, 8, 12, 15-17, 24), *POLE*^(9-14, 21, 25), *RAC1*⁽²⁾, *RAF1*⁽⁷⁾, *RET*^(11, 16), *RHOA*⁽²⁾, *RIT1*^(4, 5), *RNF43*⁽²⁻¹⁰⁾, *ROS1*⁽³⁶⁻⁴¹⁾, *SF3B1*^(14, 15) and *SMAD4*^(3, 9, 12). In addition, it also covers the known C228T, 242_243delinsTT and the C250T of the *TERT* promoter. To investigate the presence of driver fusions, the FTC tumor of patient no. 17 was investigated using Archer technology. RNA was isolated according manufactures instructions using the RNeasy kit (Qiagen). Subsequently, Archer was performed with the Archer FusionPlex CTL panel (Illumina) according manufacturer's instructions and analysed using the S5-XL system. Sequencing data was uploaded and analyzed using the Archer Analysis software (<https://analysis.archerdx.com>). If all quality criteria were met as indicated by the Archer's instructions, data was considered valid. Details are available on request.

TABLE 1. Characteristics of the custom made targeted gene panel

Characteristics	Panel I																												
Type of sample	FFPE-DNA																												
Amplicon length, bp	125 – 175																												
Amplicons designed	399 (X 2)																												
Common genes included (pituitary adenoma and DTC)	<ol style="list-style-type: none"> 1. – AIP (NM_003977): exon 1 to 6; 2. – BRAF (NM_004333): exon 15; 3. – CDKN1B (NM_004064): exon 1 to 2 4. – GNAS (NM_016592): exon 1 to 13 5. – GPR101 (NM_054021): exon 1; 6. – HRAS (NM_005343): exon 2 to 6; 7. – KRAS (NM_004985): exon 2 to 5; 8. – MEN1 (NM_000244): exon 1 to 10; 9. – NRAS (NM_002524): exon 3; 10. – PIK3CA (NM_006218): exon 2 to 21; 11. – PRKACB (NM_207578): exon 1 to 10; 12. – PRKAR1A (NM_212471): exon 2 to 11; 13. – RET (NM_020975): exon 2 to 20; 14. – SDHA (NM_004168): exon 2 to 15; 15. – SDHAF2 (NM_017841): exon 1 to 4; 16. – SDHB (NM_003000): exon 1 to 8; 17. – SDHC (NM_003001): exon 1 to 6; 18. – SDHD (NM_003002): exon 1 to 4 																												
SNPs target region chromosome 11	<table> <tr><td>– rs2513613</td><td>– rs10838307</td></tr> <tr><td>– rs34593780</td><td>– rs4267090</td></tr> <tr><td>– rs2631403</td><td>– rs7939803</td></tr> <tr><td>– rs330253</td><td>– rs2887046</td></tr> <tr><td>– rs73455029</td><td>– rs11233227</td></tr> <tr><td>– rs7949600</td><td>– rs6483324</td></tr> <tr><td>– rs1247726</td><td>– rs2851171</td></tr> <tr><td>– rs4943948</td><td>– rs736287</td></tr> <tr><td>– rs681017</td><td>– rs2510718</td></tr> <tr><td>– rs10750552</td><td>– rs1638585</td></tr> <tr><td>– rs1620333</td><td>– rs7110021</td></tr> <tr><td>– rs630172</td><td>– rs35787427</td></tr> <tr><td>– rs481303</td><td>– rs611697</td></tr> <tr><td>– rs1455113</td><td></td></tr> </table>	– rs2513613	– rs10838307	– rs34593780	– rs4267090	– rs2631403	– rs7939803	– rs330253	– rs2887046	– rs73455029	– rs11233227	– rs7949600	– rs6483324	– rs1247726	– rs2851171	– rs4943948	– rs736287	– rs681017	– rs2510718	– rs10750552	– rs1638585	– rs1620333	– rs7110021	– rs630172	– rs35787427	– rs481303	– rs611697	– rs1455113	
– rs2513613	– rs10838307																												
– rs34593780	– rs4267090																												
– rs2631403	– rs7939803																												
– rs330253	– rs2887046																												
– rs73455029	– rs11233227																												
– rs7949600	– rs6483324																												
– rs1247726	– rs2851171																												
– rs4943948	– rs736287																												
– rs681017	– rs2510718																												
– rs10750552	– rs1638585																												
– rs1620333	– rs7110021																												
– rs630172	– rs35787427																												
– rs481303	– rs611697																												
– rs1455113																													

SNPs target region chromosome 22	- rs1970640	- rs2017869
	- rs3747031	- rs1894252
	- rs5996639	- rs956548
	- rs2285206	- rs2038010
	- rs2294206	- rs2143695
	- rs62636244	- rs5769583
	- rs17003592	- rs1296750
	- rs3884944	- rs6010046

NM and ENST are both available at <http://www.ensembl.org>.

DTC, differentiated thyroid carcinoma; FFPE, formalin-fixed, paraffin-embedded; SNPs, single nucleotide polymorphisms.

Statistical analysis

We calculated proportions and rates for categorical variables, means \pm standard deviations, or medians and ranges for parametric or non-parametric variables. For statistical analysis, the Statistical Package for the Social Sciences (SPSS) version 23.0.0 (IBM Corp, Armonk, NY, USA) was used. The significance level was set at $P < 0.05$ for all tests.

Results

Cohort characteristics

In total, seventeen patients were included for pathology NGS analysis. Clinical characteristics are summarized in Table 2. In most patients, the onset of thyroid carcinoma was detected later than the onset of the pituitary adenoma (median 51.5 years (IQR 48.3 – 66.3) versus 57.0 years (44.0 – 69.0)). Thyroid carcinoma was diagnosed before the pituitary adenoma in five cases, from 1– 18 years before their pituitary adenoma had been diagnosed. Classical-variant PTC was reported in most patients ($n = 9$), following by FTC ($n = 5$) and FVPTC ($n = 3$). Thyroid carcinoma metastasis was found in five patients (29.4%); three had locoregional lymph node metastases, one had skeletal metastases and the other had lung metastases.

Regarding the pituitary adenomas, no pituitary hormonal staining was reported in most patients (*i.e.* NFPAs; $n = 5$), while others stained positively for ACTH ($n = 2$), GH ($n = 2$), LH ($n = 1$) and PRL ($n = 1$). Combined expression was reported in three patients: GH and PRL, and FSH with either LH or TSH. The staining data was not reported in two patients.

TABLE 2. Clinical characteristics of patients included in the study.

Characteristics	Value
Patients from PALGA search	$n = 14$
Type of sample available	Pituitary tumor DNA, $n = 13$ (92.9%) Thyroid tumor DNA, $n = 11$ (78.6%)
Patients with known AIP germline variants	$n = 3$
Type of sample available	Pituitary tumor DNA, $n = 2$ (66.7%) Thyroid tumor DNA, $n = 3$ (100.0%)
Sex	Female/male: $n = 15$ (88.2%)/2 (11.8%)
Age at onset pituitary adenoma (yrs)	Median, 51.5 (IQR 48.3 – 66.3)
Age at onset thyroid carcinoma (yrs)	Median, 57.0 (IQR 44.0 – 69.0)
No. and type of pituitary adenoma from available samples	Single, $n = 12$ (80.0%) Multiple, $n = 3$ (20.0%) Non-functioning, $n = 5$ (33.3%) FSH + LH, $n = 1$ (6.7%) ACTH, $n = 2$ (15.0%) FSH + TSH, $n = 1$ (6.7%) GH, $n = 2$ (15.0%) GH + PRL, $n = 1$ (6.7%) LH, $n = 1$ (6.7%) PRL, $n = 1$ (6.7%) Unknown, $n = 1$ (6.7%)
No. and type of thyroid carcinoma from available samples	Single, $n = 14$ (100.0%) PTC, $n = 7$ (50.0%) FTC, $n = 4$ (28.6%) FVPTC, $n = 3$ (21.4%)
Metastasis	$n = 5$ (29.4%)

ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone; FTC, follicular thyroid cancer; FVPTC, follicular variant of papillary thyroid carcinoma; GH, growth hormone; IQR, interquartile range; LH, luteinizing hormone; SS, Sanger sequencing; TSH, thyroid-stimulating hormone; PA, pituitary adenoma; PRL, prolactin; PTC, classical-variant papillary thyroid carcinoma; yrs, years.

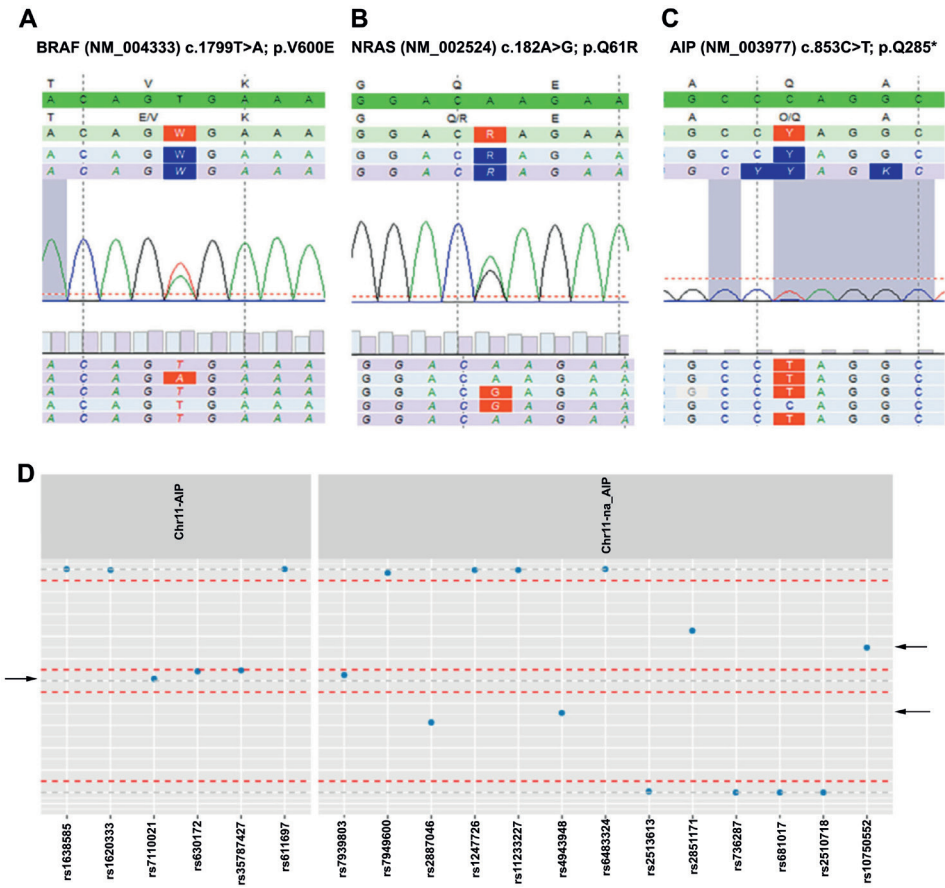
Genetic Characterization

Detection of variants in sporadic patients

NGS analysis of the 14 patients from the PALGA search revealed no known mutations in targeted genes in pituitary tumor DNA and eight mutations in thyroid tumor DNA. Table 3 summarizes the identified mutations and CNVs (*i.e.* LOH) of chromosome 11 and 22. The 13 pituitary tumor samples showed no gene mutations. Among the 11 thyroid tumor samples, *BRAF* (5/11, 45.5%) was the gene most frequently mutated, followed by *NRAS* (3/11, 27.3%). These classical *BRAF* (p.V600E) point mutations were found in 57.1% (n = 4) of classical-variant PTC specimen and once (33.3%) in FVPTC specimen (Figure 1 panel A). *NRAS* codon 61 point mutation is the most common among *RAS* mutations and this was only observed in FTC specimen: p.Q61R twice (50.0%) (Figure 1 panel B) and p.Q61K once (25.0%).

In addition, two VUSs were found in pituitary tumor DNA. These VUSs involved *AIP*-variant c.433C>T; p.P145S (n = 1) and *HRAS*-variant c.505C>T; p.R169W (n = 1) (Table 3). Prediction software to determine pathogenicity predicted the *AIP* p.P145S variant as benign (Align GVGD Class C0) to probably damaging (Polyphen-2 score of 0.978 (sensitivity: 0.76; specificity: 0.96)). The variant was never detected in the healthy population (gnomAD), nor is it found in large series of different tumor types from the cBioportal (n = 10967 tumor samples) and Cosmic (n = 92857 tumor samples) databases. Therefore, we considered *AIP* p.P145S as a VUS. The prediction software predicted *HRAS* p.R169W as probably damaging (GVGD Class C15 and a Polyphen-2 score of 0.988 (sensitivity: 0.73; specificity: 0.96)). However, the variant also appeared in the European and American population with an allele frequency of 0.01% (rs151229168; gnomAD). In addition, a TCGA PanCancer Atlas Studies search using the cBioportal database did not report the *HRAS* p.R169W variant in the 10967 tumor samples. Furthermore, the variant is also not reported by the Cosmic database in all tumor types, including thyroid tumors (cBioportal 500 and Cosmic 9985 thyroid samples). So, although the prediction software indicates the *HRAS* variant as probably damaging, we consider *HRAS* p.R169W as a VUS.

FIGURE 1. Direct sequencing of PCR antisense products in thyroid tumor samples obtained from 14 patients revealing the presence of (A: corresponding with patient no. 2) the *BRAF* p.V600E variant in six patients, (B: corresponding with patient no. 1) the *NRAS* p.Q61R variant in two patients, and (C: corresponding with patient no. 17) the *AIP* p.Q285 variant in one patient. LOH of chromosome 11 was identified in two of the 15 pituitary tumor samples; one was a partial chromosome 11 LOH deletion. In thyroid tumor samples, in 2 of the 14 samples chromosome 11 was identified; one was a partial chromosome 11 LOH deletion. Chromosome 22 was identified in two of the 14 thyroid tumor samples. (D, arrow: corresponding with patient no. 12) Demonstrates a representative example of LOH. LOH, loss of heterozygosity.



LOH of chromosome 11 was identified in two of 13 pituitary tumor samples (15.4%), both in 11q13; one had a partial chromosome 11 LOH deletion (Table 3). A representative example of LOH is demonstrated in Figure 1D. No pituitary tumor samples showed LOH of chromosome 22. Out of the 11 patients with thyroid carcinomas, two patients had LOH of chromosome 22. No LOH of chromosome 11 was identified in the thyroid carcinomas.

Detection of variants in patients with known germline *AIP* variants

Genetic screening of germline DNA from patients 15, 16 and 17 revealed several *AIP* variants. Patient no. 15 had two *AIP*-variants: c.787+25 G>A; p.? and *60 G>C; p.?. Variant prediction software noted *60 G>C as probably benign, whereas c.787+25 G>A was noted in 2/4 prediction models to lead to a new splice acceptor site at c.787+27. In the second patient (patient no. 16), two *AIP*-variants were detected: c.682C>A; p.Q288K, which is a known benign polymorphism, and c.920A>G; p.Q307R; considered a benign variant. In patient no. 17, a pathological *AIP*-variant c.853C>T; p.Q285* was identified.

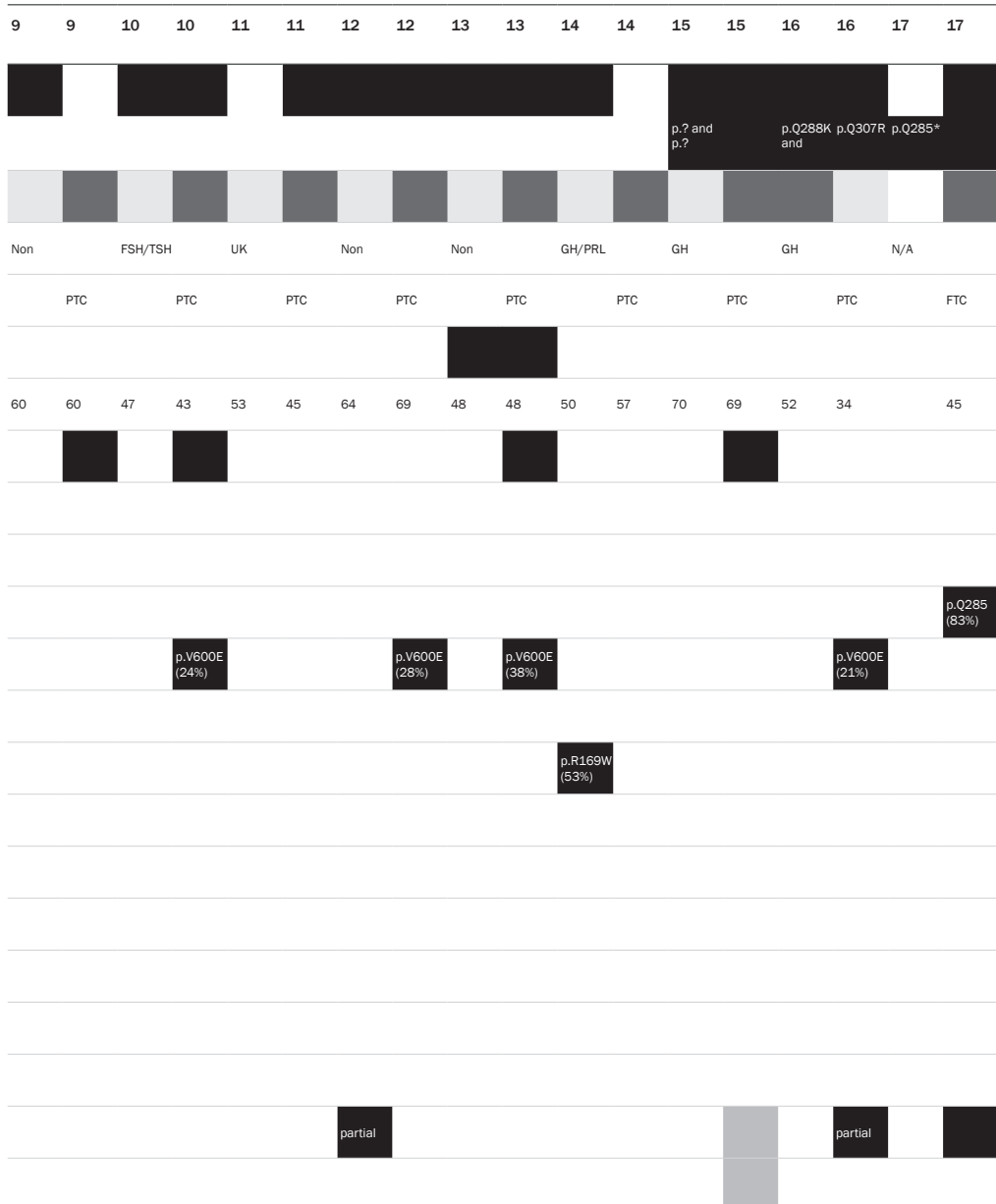
NGS analysis of the three patients with known germline *AIP* variants revealed no known mutations in the pituitary tumor DNA, however, two mutations were identified in the thyroid tumor DNA. In patient no. 17, the *AIP*-variant c.853C>T; p.Q285* was confirmed in FTC specimen (allele frequency 83%), while no mutations in other genes or translocations were observed (Figure 1 panel C). The *BRAF*(p.V600E) point mutation was found in patient no. 16. No pituitary tumor samples showed loss of heterozygosity of chromosome 11. LOH of chromosome 11 was identified in two (patient no. 16 and 17) of the three thyroid carcinomas (66.7%); one was a partial chromosome 11 deletion. No LOH of chromosome 22 was identified in both pituitary adenomas and thyroid carcinomas.

TABLE 3. Cluster of mutations and CNVs. Cases are categorized by pituitary adenoma and differentiated thyroid carcinoma.

Patient ID	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8
Tumor studied																
Known germline AIP variants																
Type of tumor																
Type of pituitary tumor	UK		PRL		Non		ACTH		ACTH		LH		Non		FSH/LH	
Type of thyroid carcinoma		FTC		FVPTC		FTC		PTC		FVPTC		FTC		FVPTC		FTC
Male																
Age at diagnosis (yrs)	25	37	51	58	50	42	70	70	68	69	67	74	49	59	48	45
Metastasis																
Type of mutation																
AIP									p.P145S (16%)							
BRAF				p.V600E (39%)				p.V600E (40%)								
GPR101																
HRAS																
MEN1																
NRAS		p.Q61R (41%)										p.Q61R (38%)			p.Q61K (32%)	
PIK3CA																
RET																
SDHA																
SDHB																
LOH chr. 11																
LOH chr. 22																

Yes
 No
 Unreliable
 Pituitary adenoma
 Thyroid carcinoma

ACTH, adrenocorticotrophic hormone; FSH, follicle-stimulating hormone; FTC, follicular thyroid cancer; FVPTC, follicular variant of papillary thyroid carcinoma; GH, growth hormone; LH, luteinizing hormone; LOH, loss of heterozygosity; Non, nonfunctioning pituitary adenomas; TSH, thyroid-stimulating hormone; PA, pituitary adenoma; PRL, prolactin; PTC, classical-variant papillary thyroid carcinoma; TC, thyroid carcinoma; UK, unknown; VUS, variant of unknown significance; yrs, years.



Discussion

To our knowledge, this is the first study to analyze the prevalence of *AIP* gene mutations and mutations in genes that have been associated with neuro-endocrine tumors in series of tumors from patients presenting with both pituitary adenomas and DTCs. We showed that genetic alterations were observed in 71.4% (10/14) of DTCs and in 13.3% (2/15) of pituitary adenomas tissues, while there was no overlap between genetic alterations within tissues from the same patient. Among patients with pathological germline *AIP* variants, one *AIP* variant c.853C>T; p.Q285* was confirmed in the FTC specimen (patient no. 17), including evidence of loss of the *AIP* wild-type allele, based on the relatively high allele frequency (83%) of the germline mutation in the tumor DNA. Unfortunately, we were unable to confirm this LOH based on the SNPs analysis, due to low quality of the FTC tissue. This patient came from an *AIP*-mutated FIPA kindred, however, her pituitary gland was unaffected. This supports that the finding of DTCs and pituitary adenomas are not totally fortuitous coexistence in an *AIP* mutation-positive FIPA kindred, thereby echoing a recent finding of FTC in an *AIP* mutation carrier by Daly et al. (28). In a second patient with a somatotropinoma with two benign *AIP*-variants (p.Q288K and p.Q307R), a somatic *BRAF* (p.V600E) mutation was detected in PTC specimen in combination with a partial chromosome 11 LOH deletion. Although the partial chromosome 11 LOH deletion could indicate a second hit in the thyroid tissue, the observed LOH concerns SNPs located downstream (3') of the *AIP* gene, while the SNPs located in the *AIP* gene did not indicate loss of heterozygosity.

It is noteworthy that although the most common mechanism to lose the wild-type copy of a tumor suppressor gene (*e.g.* *AIP*) in DTC specimen is a large deletion affecting the wild-type allele, other mechanisms could also play a role, such as another somatic mutation in other parts of the gene, or silencing of the wild-type copy with epigenetic mechanism-promoter methylation or microRNAs which are not covered by NGS. Moreover, we should emphasize that DTCs are more progressed in transformation since they are malignant when compared to pituitary adenomas. Therefore, it might be interesting to investigate the role of *AIP* mutation in thyroid adenomas (*i.e.* earlier in the transformation) in further studies.

In the total cohort, the most common oncotype in pituitary adenoma-related DTC was classical-variant PTC (9 out of 14 cases; see Table 1) with a high frequency (42.9%,

6/14) of *BRAF* (p.V600E) mutations, whereas none of these cases harbored *NRAS* mutations. These results confirm and build upon previous studies stating that among PTC, virtually all tumors that harbor a *RAS* mutation grow forming neoplastic follicles and no papillary structures and are, therefore, diagnosed as the FVPTC, while *BRAF* is the most frequent genetic alteration in classical-variant PTC^(15, 16). In line with this, the *NRAS* codon 61 point mutations were only observed in FTC specimen in three cases (21.4%)⁽³⁷⁾, which was the second most frequently mutated gene among the thyroid tumor samples. Although the limited number of DTCs in the present series prevents us from drawing any final conclusions on the prevalence of *BRAF* and *NRAS* mutations in DTCs in patients with versus those without pituitary adenomas, *BRAF* and *NRAS* seems the main genetic drivers of thyroid follicular epithelial cell transformation in our cases.

Our results are not in accordance with previous data, which suggested that *BRAF* mutation may not play a dominant role in development of DTC in patients with acromegaly^(11, 38). In these studies only one (9.1%)(11) or two (14.3%)⁽³⁸⁾ patients with concomitant PTC had the *BRAF* mutation, which in both studies was more frequently present in PTC patients without acromegaly. This discrepancy might be explained by (1) the inclusion of relative more FVPTC patients in the study from Aydin et al.⁽³⁸⁾ which is different to our cohort, or⁽²⁾ our distinct study population, as included patients had not only of GH-producing adenomas but all five hormone-secreting cell types. In fact, previous studies^(11, 29, 38) were carried out exclusively in acromegaly patients, while the patients we studied included only one patient with a GH-producing tumor. Therefore, direct comparison between our cohort and the acromegaly cohorts is limited.

In line with our findings, studying 12 DTC patients with acromegaly, Mian et al. reported that 70% of PTC patients with acromegaly were *BRAF* positive⁽²⁹⁾. Moreover, *AIP* expression was similar between neoplastic and normal tissue, while the aryl-hydrocarbon receptor (AHR) was expressed more in PTCs carrying *BRAF* mutations than in normal tissue, irrespective of acromegaly status⁽²⁹⁾. These data suggest that *BRAF* mutations and AHR overexpression may be associated with DTC risk in acromegaly, at least in patients with concomitant PTC.

Although there is no gender preponderance in pituitary adenoma patients, the vast majority of those with concomitant DTCs were female (15 out of 17) and is in accordance with previous literature, probably reflecting a trend seen in the general population. When comparing differences between patients with and without concomitant DTC, is

seems the former were relatively older. The mean age at onset and diagnosis of pituitary adenoma was mean 55 years [SD 12] in the cohort vs mean 44 years [SD 17] in the general population, with the mean age at diagnosis in female patients being younger; 34 years⁽³⁹⁾. The onset and diagnosis of DTC was median 57 years [IQR 44–69] in the cohort vs 46 years [IQR 10–85] in the general population⁽⁴⁰⁾, with the median age at diagnosis in female patients being younger; 45 years (40). In addition, in the two previously reported cases of acromegaly and concomitant PTC, and harboring a germline *AIP* variant, both patients were female and diagnosed with acromegaly at age 67 and 74, respectively.

This is in contrast to the clinical characteristics of patients bearing germline *AIP* mutations; the disease usually manifests in the second decade of life, almost all cases are diagnosed before the age of 30 years^(28, 41–44) and they are predominantly males⁽⁴⁵⁾. With this in mind, it should be stressed that after progress is made in the treatment of pituitary adenomas and its complications, these patients may live long enough to reach the age of increased cancer risk.

A strength of our study lies in the relatively large number of patients in which the pituitary adenoma and concomitant DTC tumor tissue were systematically investigated by targeted NGS. The main limitations of our study lie in the retrospective collection of tumor samples, and we had to exclude several tissues due to low quality. Another limitation is the lack of clinical data from the patients, including follow-up and family history data. Therefore, it should be stressed that we cannot rule out if patients from the PALGA search had additional risk factors for DTCs (*e.g.* received radiotherapy). At last, we should be borne in mind that the increased number of the diagnoses of thyroid cancer in these patients could be due to the fact that they are examined more accurately and more frequently than before (*i.e.* surveillance bias).

In conclusion, the absence of somatic *AIP* mutations observed in patients with pituitary adenomas and concomitant DTCs suggest that their contribution to tumoral pathogenesis is probably limited and seems unlikely the genetic cause predisposing to the higher DTC risk observed in these patients. Though the finding of DTCs and pituitary adenomas could represent a new variant of MEN syndrome with a *de novo* germline mutation in a not yet identified gene, we suggest that this may be a fortuitous coexistence based on our observed variants that were similar to those of sporadic DTCs. In view of this and in line with the clinical practice guidelines from Katznelson et al.⁽⁴⁶⁾, we

recommend including regularly thyroid examination and thyroid ultrasound only if there is a palpable thyroid nodularity. While the finding of the *AIP*-variant and LOH at this locus in FTC specimen in one *AIP* mutation-positive case, opens up a potential role for *AIP* mutation as an initiating event, further studies of *AIP* genetic status among DTCs in FIPA kindred cohorts are warranted to answer this question.

Acknowledgement

We are indebted to the many pathologists, scientists and other collaborators who have contributed to developing and maintaining PALGA. In addition, we acknowledge the neurosurgeons (Alof H.G. Dallenga and Ian K. Haitzma) and L. Oudijk (L.O.) and F.H. Groenendijk (F.G.) who performed histological evaluation of the tissues. All contributed to the study.

Statement of Ethics

Approval from the Medical Ethical Committee of the Erasmus University Medical Center and informed consent to use the tumor tissues for research purposes were obtained. Tumor tissues from all Dutch medical centers were used according to the code of conduct, Proper Secondary Use of Human Tissue, established by the Dutch Federation of Medical Scientific Societies⁽³⁰⁾.

All patients provided informed written consent for genetic testing.

Author Contributions

Design of the study, and acquisition, analysis, and interpretation of data for the work: E.C.C. and E.K. Targeted NGS and data analysis: M.H and E.K. Drafting the work: E.C.C. and E.K. Critical review of drafts for important intellectual content: A.M., A.F.D., W.W.H, F.J.K., A.B., A.J.L., S.J.C.M.M.N.

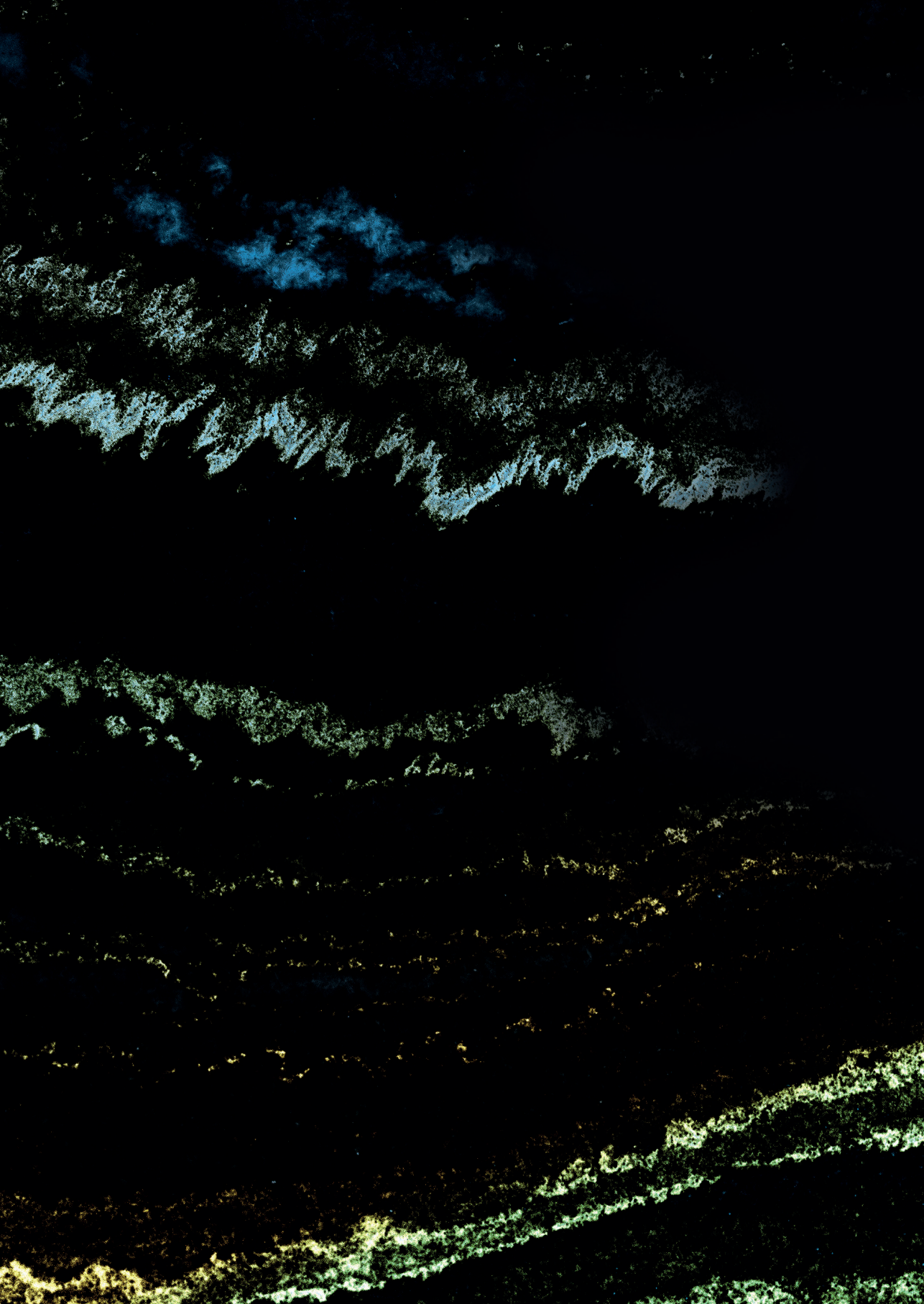
References

1. Caimari F, Korbonits M. Novel Genetic Causes of Pituitary Adenomas. *Clin Cancer Res*. 2016;22(20):5030-42.
2. Vandeva S, Daly AF, Petrossians P, Zacharieva S, Beckers A. GENETICS IN ENDOCRINOLOGY: Somatic and germline mutations in the pathogenesis of pituitary adenomas. *Eur J Endocrinol*. 2019.
3. Aflorei ED, Korbonits M. Epidemiology and etiopathogenesis of pituitary adenomas. *J Neurooncol*. 2014;117(3):379-94.
4. Beckers A, Aaltonen LA, Daly AF, Karhu A. Familial isolated pituitary adenomas (FIPA) and the pituitary adenoma predisposition due to mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene. *Endocr Rev*. 2013;34(2):239-77.
5. Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, et al. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. *Science*. 2006;312(5777):1228-30.
6. Rostomyan L, Daly AF, Petrossians P, Nachev E, Lila AR, Lecoq AL, et al. Clinical and genetic characterization of pituitary gigantism: an international collaborative study in 208 patients. *Endocr Relat Cancer*. 2015;22(5):745-57.
7. Trivellin G, Korbonits M. AIP and its interacting partners. *J Endocrinol*. 2011;210(2):137-55.
8. Gasperi M, Martino E, Manetti L, Arosio M, Porretti S, Faglia G, et al. Prevalence of thyroid diseases in patients with acromegaly: results of an Italian multi-center study. *J Endocrinol Invest*. 2002;25(3):240-5.
9. Gullu BE, Celik O, Gazioglu N, Kadioglu P. Thyroid cancer is the most common cancer associated with acromegaly. *Pituitary*. 2010;13(3):242-8.
10. Dagdelen S, Cinar N, Erbas T. Increased thyroid cancer risk in acromegaly. *Pituitary*. 2014;17(4):299-306.
11. Kim HK, Lee JS, Park MH, Cho JS, Yoon JH, Kim SJ, et al. Tumorigenesis of papillary thyroid cancer is not BRAF-dependent in patients with acromegaly. *PLoS One*. 2014;9(10):e110241.
12. Dal J, Leisner MZ, Hermansen K, Farkas DK, Bengtsen M, Kistorp C, et al. Cancer Incidence in Patients With Acromegaly: A Cohort Study and Meta-Analysis of the Literature. *J Clin Endocrinol Metab*. 2018;103(6):2182-8.

13. Lai NB, Garg D, Heaney AP, Bergsneider M, Leung AM. No Benefit of Dedicated Thyroid Nodule Screening in Patients with Acromegaly. *Endocr Pract.* 2019.
14. Onoda N, Ohmura E, Tsushima T, Ohba Y, Emoto N, Isozaki O, et al. Autocrine role of insulin-like growth factor (IGF)-I in a human thyroid cancer cell line. *Eur J Cancer.* 1992;28A(11):1904-9.
15. Fagin JA, Wells SA, Jr. Biologic and Clinical Perspectives on Thyroid Cancer. *N Engl J Med.* 2016;375(11):1054-67.
16. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. *Nat Rev Endocrinol.* 2011;7(10):569-80.
17. Roque L, Rodrigues R, Pinto A, Moura-Nunes V, Soares J. Chromosome imbalances in thyroid follicular neoplasms: a comparison between follicular adenomas and carcinomas. *Genes Chromosomes Cancer.* 2003;36(3):292-302.
18. Hemmer S, Wasenius VM, Knuutila S, Franssila K, Joensuu H. DNA copy number changes in thyroid carcinoma. *Am J Pathol.* 1999;154(5):1539-47.
19. Jung S-H, Kim MS, Jung CK, Park H-C, Kim SY, Liu J, et al. Mutational burdens and evolutionary ages of thyroid follicular adenoma are comparable to those of follicular carcinoma. *Oncotarget.* 2016;7(43):69638-48.
20. Cañibano C, Rodriguez NL, Saez C, Tovar S, Garcia-Lavandeira M, Borrello MG, et al. The dependence receptor Ret induces apoptosis in somatotrophs through a Pit-1/p53 pathway, preventing tumor growth. *EMBO J.* 2007;26(8):2015-28.
21. Ozfirat Z, Korbonits M. AIP gene and familial isolated pituitary adenomas. *Mol Cell Endocrinol.* 2010;326(1-2):71-9.
22. Vargiolu M, Fusco D, Kurelac I, Dirnberger D, Baumeister R, Morra I, et al. The tyrosine kinase receptor RET interacts in vivo with aryl hydrocarbon receptor-interacting protein to alter survivin availability. *J Clin Endocrinol Metab.* 2009;94(7):2571-8.
23. de Oliveira SK, Hoffmeister M, Gambaryan S, Muller-Esterl W, Guimaraes JA, Smolenski AP. Phosphodiesterase 2A forms a complex with the co-chaperone XAP2 and regulates nuclear translocation of the aryl hydrocarbon receptor. *J Biol Chem.* 2007;282(18):13656-63.
24. Kang BH, Altieri DC. Regulation of survivin stability by the aryl hydrocarbon receptor-interacting protein. *J Biol Chem.* 2006;281(34):24721-7.
25. Urbani C, Russo D, Raggi F, Lombardi M, Sardella C, Scattina I, et al. A novel germline mutation in the aryl hydrocarbon receptor-interacting protein (Aip) gene in an Italian family with gigantism. *J Endocrinol Invest.* 2014;37(10):949-55.

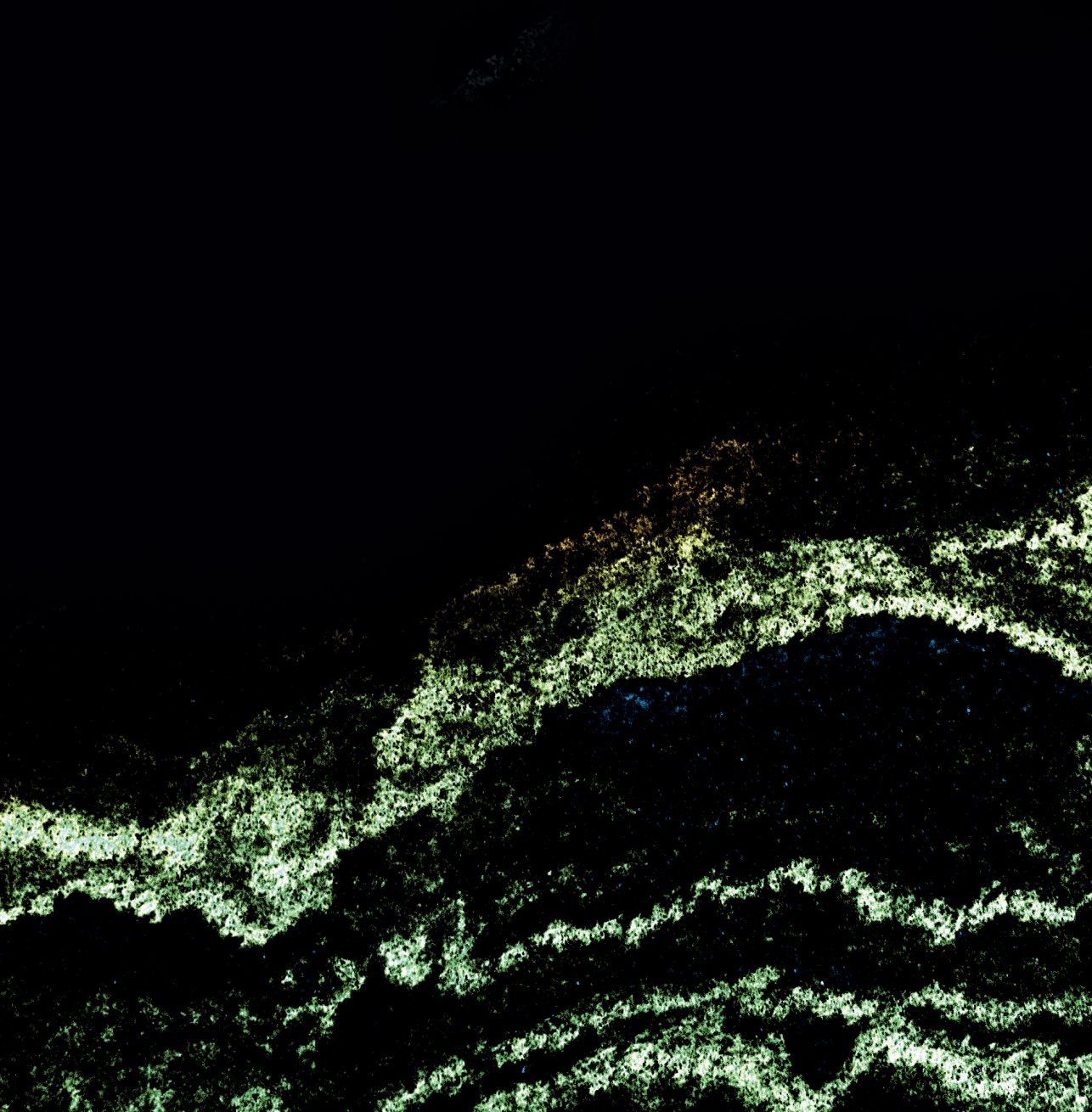
26. Occhi G, Trivellin G, Ceccato F, De Lazzari P, Giorgi G, Dematte S, et al. Prevalence of AIP mutations in a large series of sporadic Italian acromegalic patients and evaluation of CDKN1B status in acromegalic patients with multiple endocrine neoplasia. *Eur J Endocrinol.* 2010;163(3):369-76.
27. Nord B, Larsson C, Wong FK, Wallin G, Teh BT, Zedenius J. Sporadic follicular thyroid tumors show loss of a 200-kb region in 11q13 without evidence for mutations in the MEN1 gene. *Genes Chromosomes Cancer.* 1999;26(1):35-9.
28. Daly AF, Rostomyan L, Betea D, Bonneville JF, Villa C, Pellegata NS, et al. AIP-mutated acromegaly resistant to first-generation somatostatin analogs: long-term control with pasireotide LAR in two patients. *Endocr Connect.* 2019;8(4):367-77.
29. Mian C, Ceccato F, Barollo S, Watutantrige-Fernando S, Albiger N, Regazzo D, et al. AHR Over-Expression in Papillary Thyroid Carcinoma: Clinical and Molecular Assessments in a Series of Italian Acromegalic Patients with a Long-Term Follow-Up. *PLOS ONE.* 2014;9(7):e101560.
30. Vermeulen E, Geesink I, Schmidt MK, Steegers C, Verhue D, Brom FW, et al. Secondary use of human tissue: consent and better information required. *Ned Tijdschr Geneesk.* 2009;153:A948.
31. Barlier A, Vanbellinghen JF, Daly AF, Silvy M, Jaffrain-Rea ML, Trouillas J, et al. Mutations in the aryl hydrocarbon receptor interacting protein gene are not highly prevalent among subjects with sporadic pituitary adenomas. *J Clin Endocrinol Metab.* 2007;92(5):1952-5.
32. Georgitsi M, Heliovaara E, Paschke R, Kumar AV, Tischkowitz M, Vierimaa O, et al. Large genomic deletions in AIP in pituitary adenoma predisposition. *J Clin Endocrinol Metab.* 2008;93(10):4146-51.
33. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-24.
34. Geurts-Giele WR, Rosenberg EH, Rens Av, Leerdam MEv, Dinjens WN, Bleeker FE. Somatic mosaicism by a de novo MLH1 mutation as a cause of Lynch syndrome. *Mol Genet Genomic Med.* 2019;7(7):e00699.
35. van Riet J, Krol NMG, Atmodimedjo PN, Brosens E, van IWFJ, Jansen M, et al. SNPitty: An Intuitive Web Application for Interactive B-Allele Frequency and Copy Number Visualization of Next-Generation Sequencing Data. *J Mol Diagn.* 2018;20(2):166-76.
36. Giordano TJ. Genomic Hallmarks of Thyroid Neoplasia. *Annu Rev Pathol.* 2018;13(1):141-62.

37. Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn GW, 2nd, Tallini G, et al. RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab.* 2003;88(5):2318-26.
38. Aydin K, Aydin C, Dagdelen S, Tezel GG, Erbas T. Genetic Alterations in Differentiated Thyroid Cancer Patients with Acromegaly. *Exp Clin Endocrinol Diabetes.* 2016;124(3):198-202.
39. Day PE, Loto MG, Glerean M, Picasso MFR, Lovazzano S, Giunta DH. Incidence and prevalence of clinically relevant pituitary adenomas: retrospective cohort study in a Health Management Organization in Buenos Aires, Argentina. *Arch Endocrinol Metab.* 2016;60:554-61.
40. Sciuto R, Romano L, Rea S, Marandino F, Sperduti I, Maini CL. Natural history and clinical outcome of differentiated thyroid carcinoma: a retrospective analysis of 1503 patients treated at a single institution. *Ann Oncol.* 2009;20(10):1728-35.
41. Daly AF, Tichomirowa MA, Petrossians P, Heliövaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab.* 2010;95(11):E373-83.
42. Hernandez-Ramirez LC, Gabrovská P, Denes J, Stals K, Trivellin G, Tilley D, et al. Landscape of Familial Isolated and Young-Onset Pituitary Adenomas: Prospective Diagnosis in AIP Mutation Carriers. *J Clin Endocrinol Metab.* 2015;100(9):E1242-54.
43. Williams F, Hunter S, Bradley L, Chahal HS, Storr HL, Akker SA, et al. Clinical experience in the screening and management of a large kindred with familial isolated pituitary adenoma due to an aryl hydrocarbon receptor interacting protein (AIP) mutation. *J Clin Endocrinol Metab.* 2014;99(4):1122-31.
44. Korbonits M, Storr H, Kumar AV. Familial pituitary adenomas - who should be tested for AIP mutations? *Clin Endocrinol (Oxf).* 2012;77(3):351-6.
45. Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, et al. Germline AIP Mutations in Apparently Sporadic Pituitary Adenomas: Prevalence in a Prospective Single-Center Cohort of 443 Patients. *J Clin Endocrinol Metab.* 2012;97(4):E663-E70.
46. Katznelson L, Laws ER, Jr., Melmed S, Molitch ME, Murad MH, Utz A, et al. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab.* 2014;99(11):3933-51.



Part V

General discussion and future perspectives



General discussion

The first aim of this thesis was to define the position of pasireotide LAR in the current medical management of acromegaly. To meet this goal we investigated the determinants of response to somatostatin analogues, including the prediction of the gold standard first-generation SRL monotherapy (Chapter 2), and pasireotide LAR, alone or in combination with pegvisomant therapy in acromegaly (Chapter 3 and 4). Furthermore, we conducted the PAPE study to assess the clinical response and safety of switching to pasireotide LAR alone or in combination with pegvisomant treatment in patients controlled with first-generation SRL and pegvisomant combination treatment (Chapter 5 and 6). The second aim of this thesis was to elucidate the role of a ketogenic diet as an adjuvant emerging treatment option for acromegaly, because reduced portal insulin secretion down-regulates hepatic GHR, and therefore reduces hepatic IGF-I synthesis (Chapter 7). Last but not least, in Chapter 8 we undertook a nationwide PALGA study to examine the role of *AIP* variants in acromegaly patients with concomitant DTCs, the third aim of this thesis.

This chapter will focus on the main discussion points of the previous chapters presented in this thesis. The conclusion of this thesis and the future perspectives will be discussed at the end of this chapter.

1. Determinants of response to somatostatin analogues

1.1 Prediction of first-generation SRL treatment response in acromegaly

In Chapter 2 we incorporated patient-specific clinical predictors based on literature, which we used to develop a multivariable regression model in order to predict the initial biochemical response to treatment with first-generation SRLs. Baseline IGF-I concentration, bodyweight and the presence of type 2 diabetes can contribute in distinguishing complete biochemical from partial response. Non-response during first-generation SRLs occurred more in patients that were younger at diagnosis and tended to harbour larger tumours and underwent surgery less often.

As described in Chapter 2, baseline IGF-I concentration was the best predictor of first-generation SRL treatment response. Lower baseline IGF-I concentrations could identify patients that will achieve biochemical control, while higher baseline IGF-I concentrations

could identify those that will achieve partial responses while at the same time having a greater absolute and relative reduction in circulating IGF-I levels. Previously it has been identified that lower circulating IGF-I and GH levels correspond with better biochemical response to first-generation SRLs⁽¹⁻³⁾. Thus, lower baseline IGF-I concentrations could identify patients that will achieve biochemical control, while higher baseline IGF-I concentrations could identify those that will achieve partial responses while at the same time having a greater absolute and relative reduction in circulating IGF-I levels. While the latter seems counterintuitive, a greater reduction even if IGF-I levels will not decrease below the set threshold is still of clinical value in the multimodel therapy setting.

We observed that a lower bodyweight was associated with a higher chance of biochemical control, while a higher bodyweight was associated with partial response to fg-SRLs. This finding is in accordance with previous findings from the somutaline depot for acromegaly (SODA) registry⁽⁴⁾, which identified that patients with a lower BMI (BMI < 30 kg/m²) tended to have better biochemical control of IGF-1 than patients with a higher BMI (BMI ≥ 30 kg/m²) after 24 months of fg-SRL therapy.

One likely explanation is the hyperinsulinism in the higher bodyweight group which enhanced synthesis of IGF-I through upregulation of hepatic GH receptors. Another explanation is the higher volume of distribution in the higher bodyweight group and possibly decreasing serum concentrations of first-generation SRLs in these patients. However, this is less likely to explain the difference in weight since we did not observe an age difference between the biochemical and partial response group. To date, there have been no studies investigating the role of volume distribution in the serum concentration of first-generation SRLs in acromegaly.

We observed that the presence of concomitant type 2 diabetes, besides baseline IGF-I concentration and bodyweight, is a pre-treatment predictor of partial response to first-generation SRL monotherapy. This finding is in accordance with previous findings from the somutaline depot for acromegaly (SODA) study⁽⁴⁾, that showed that after 24 months of first-generation SRL therapy, normalization of IGF-I concentration was achieved less frequently in diabetic patients. In a previous study of the LAS database, a significant relationship between IGF-I and glucose levels at baseline was shown for acromegaly patients, even in the absence of type 2 diabetes⁽¹⁾. Overall, the prevalence of insulin sensitivity alterations has been found to correlate with higher BMI in patients with acromegaly as observed in the general population⁽⁵⁾.

This study is limited by the use of different IGF-I assays during first-generation SRL treatment. Strengths of the study are: 1) the relatively large number of patients in which the biochemical response to first-generation SRL treatment were systematically investigated, while remaining to be treated with a single agent, with a stable high dose for a long period of treatment when compared to previous literature⁽⁶⁾; 2) the LAS database since it is not limited to a national dataset nor does it deal with patients managed with only a single treatment modality, and therefore, may better reflect the general population of acromegaly patients and overcome selection bias. The results are therefore widely generalizable in clinical practice during the initiation of first-generation SRL treatment.

1.2 Prediction of pasireotide LAR treatment response in acromegaly

The aims of the studies reported in Chapter 3 and 4 were: 1) to investigate the relationship between the response to first-generation SRLs and the hormonal and tumoural responses to pasireotide LAR, alone or in combination with pegvisomant therapy, including the SST₂ and SST₅ receptor expression in tumours assessed by immunohistochemistry and ;2) to investigate to what extent this correlates with the T2-weighted MRI signal of the tumours.

We observed that the IGF-I lowering effects of pasireotide LAR treatment correlated to SST₂ receptor expression and not to SST₅ receptor. This observation is further supported by the finding that in this cohort, which include patients showing a partial response to first-generation SRLs, the IGF-I lowering effects of first-generation SRL treatment correlated with the effect of pasireotide LAR treatment after both three and nine months' treatment. Regarding the T2-weighted signal of the tumours, lower IGF-I levels during pasireotide LAR are associated with a higher T2-signal intensity in the tumour. Surprisingly, biochemical response is not accompanied by tumour shrinkage during pasireotide LAR treatment. Patients showing no biochemical response to first-generation SRL and pasireotide LAR with particularly large tumours with a lower SST₂ receptor expression are prone to achieve tumour shrinkage during pasireotide LAR treatment. Tumour volume decreased from baseline in 33 patients (73.3%) and this shrinkage was significant ($\geq 25\%$) in only 15 cases (33.3%). However, tumour volume increased from baseline was observed in 12 patients; 6 (13%) of which had a significant ($\geq 25\%$) tumour volume increase. This observation was not clinically relevant in most (5/6, 83%) patients, except for one which showed aggressive and treatment-resistant

pituitary tumour behaviour throughout previous treatment modalities. All in all, these data do suggest that pasireotide LAR may not cause a clinically significant increase in tumour size in the majority of acromegaly patients. Previous clinical studies^(7, 8) and our study suggest that pasireotide LAR might exert a greater effect on tumour control, especially in patients whose disease is inadequately controlled on first-generation SRLs.

The observed positive relation between SST₂ receptor and biochemical responsiveness to pasireotide LAR treatment is in line with previous *in vitro* studies⁽⁹⁻¹¹⁾, carried out in primary cultures of GH-secreting tumours, showing that pasireotide exerts its anti-secretory activity mainly by the activation of SST_{2a} receptor⁽¹¹⁻¹³⁾. Although the effects of pasireotide LAR and first-generation SRLs on the reduction of GH levels were superimposable both *in vitro* and *in vivo*, pasireotide LAR therapy was more effective in lowering IGF-I levels^(7, 13). It is plausible that the enhanced efficacy of pasireotide LAR compared with first-generation SRLs is mediated by its stronger reduction of insulin secretion, rendering the GHR in the liver less sensitive to GH action⁽¹⁴⁾.

Our results, however, are in contrast with findings of Iacovazzo *et al.*⁽¹⁵⁾, which suggest that SST₅ receptor expression on GH-secreting tumours drives the responsiveness to pasireotide LAR treatment. The main difference between the study from Iacovazzo *et al.* and our study is related to a different patient selection: the former included only first-generation SRL resistant patients⁽¹⁵⁾, while we included mainly patients who were partially responsive to first-generation SRLs and received combination treatment with pegvisomant.

Aiming to summarize the available preclinical and clinical data on the role of SST receptor expression in driving pasireotide LAR efficacy in GH-secreting tumours, I postulate that the biological effects of pasireotide LAR are mainly driven by SST₂ receptor. However, some evidence suggest that in the presence of low SST₂ receptor expression or in tumours resistant to SST₂ receptor targeting, differently from first-generation SRLs, pasireotide LAR may exert some effects throughout the activation of SST₅ receptor^(13, 15). Although a classification into SST₂-targeted vs SST₅-targeted drugs seems to be too simplistic, I propose that the classification of acromegaly proposed by Cuevas-Ramos *et al.*⁽¹⁶⁾ better captures the complex relationship between SST receptor expression and the efficacy of pasireotide LAR. Pasireotide LAR mainly acts via SST₂ receptor in type 1 patients (biochemical response to first-generation SRLs and high SST₂ receptor expression), while its effects are likely mediated via SST₅ receptor in type 2 and type

3 patients (partial or no biochemical response to first-generation SRLs and low SST₂ receptor expression).

The finding that lower IGF-I levels during pasireotide LAR treatment was associated with a higher T2-signal intensity is contrary to previous studies on first-generation SRLs, reporting greater IGF-I reduction during first-generation SRL treatment in T2-hypointense adenomas⁽¹⁷⁻²⁰⁾. Thus, T2-weighted MRI signal intensity may provide complementary predictive information concerning the responses to both kinds of drugs. However, these results are not unexpected considering that patients with higher T2-signal intensity tumours may have lower IGF-I levels at diagnosis⁽²¹⁾. Consequently, these patients require less IGF-I reduction in order to obtain biochemical control.

We observed a dissociation between achieved IGF-I levels and the presence of significant tumour shrinkage during pasireotide LAR treatment. In other words, hormone secretion and cell growth in the tumour are not synchronous. As discussed in Chapter 4, two mechanisms may account for this dissociation: 1) the different roles of SST receptor subtypes and; 2) the tumour size.

First, the dissociation between anti-secretory and anti-proliferative effects can be based on the different roles of SST receptor subtypes in GH-secreting pituitary tumours. Previous *in vitro* studies^(19, 29, 30) and our data in Chapter 3 indicate that, overall, pasireotide exerts its anti-secretory activity mainly by the activation of SST₂. Patients with significant tumour shrinkage had significantly lower SST₂ receptor expression, as well as a lower SST₂/SST₅ receptor ratio compared to patients without significant tumour shrinkage, which probably accounts for the lack of biochemical control.

Secondly, the tumour size may explain why significant tumour shrinkage is not accompanied by lower IGF-I levels. In our cohort, absolute tumour shrinkage during pasireotide LAR treatment was positively correlated with larger tumours and higher GH levels at baseline. These results confirm and build upon a previous study stating that IGF-I levels remain unaffected above a certain threshold of GH concentrations⁽²²⁾. Knowing that larger tumours secrete more GH⁽²³⁾, it becomes apparent that pasireotide LAR can affect tumour size and thereby GH levels, without affecting IGF-I levels.

A limitation is the lack of generalizability, as most selected patients did not receive surgery and may not reflect the general population of acromegaly patients. Moreover, IGF-I levels measured after three months of pasireotide LAR treatment may be partly influenced by the carryover effect of withdrawal of first-generation SRLs at the start of

the study. Another drawback is that we were unable to assess T2-signal intensity of the diagnostic MRIs due to overall low resolution of MRIs, and therefore were not able to evaluate the T2-signal intensity during first-generation SRL treatment.

2. Pasireotide: mechanism of action and clinical applications

In Chapter 5 and 6 we aimed to assess the clinical response and safety of switching to pasireotide LAR, in patients with acromegaly who were well controlled on a combination of first-generation SRL and pegvisomant up to nine months.

2.1 Hormonal responsiveness

With regard to the hormonal responsiveness to pasireotide LAR (Chapter 5), we found that the efficacy of pasireotide LAR treatment was long-term sustained, as 77% of patients achieved normalized IGF-I levels at nine months. Also we could reconfirm the pegvisomant-sparing effect of pasireotide LAR after 9 months' follow-up with a cumulative pegvisomant dose reduction of 52%, compared with combination therapy with first-generation SRLs. As a consequence, the pegvisomant therapy could be discontinued in 51% of patients. In conclusion, even in acromegaly patients in whom (biochemical) disease activity can't be controlled by first-generation SRLs, pasireotide LAR (as second- or third-line therapy option), either as monotherapy or in combination with pegvisomant, can achieve normalized IGF-I concentrations in most patients.

Our results show that pasireotide LAR treatment reduces IGF-I concentration after two injections, which parallels the reduction in insulin levels, which also occurs after two injections. Although the early-onset of effect of pasireotide LAR is to some extent influenced by the carry-over effect of first-generation SRLs, it may be (partly) mediated by suppressing insulin secretion. This is further supported by the observation that although the effects of pasireotide LAR on GH suppression were superimposable compared with octreotide, pasireotide LAR treatment induced a greater suppression of IGF-I⁽⁷⁾. Another explanation is that unlike octreotide, pasireotide induces less SST₂ receptor internalization which could imply that there is a lower likelihood desensitization to pasireotide treatment.

An important observation in our study was the diverse clinical response to pasireotide LAR treatment ranging from patients that were resistant to very sensitive. This may be

explained by the inclusion of a heterogeneous acromegaly population. This assumption is supported by the observation that patients in the pasireotide LAR monotherapy group had significantly lower GH and IGF-I levels at baseline and used lower pegvisomant doses, compared to patients in the combination therapy group. However, as mentioned in Chapter 3 and 5, we found a strong correlation between the percentage of IGF-I reduction after pasireotide LAR treatment and response to first-generation SRLs. In other words, patients who respond well to first-generation SRL treatment also seemed to respond to pasireotide LAR treatment, which is best illustrated by our observation that the three patients using the highest doses of pegvisomant could not reduce their dose after switching to pasireotide LAR.

2.2 Tumoural responsiveness

In Chapter 4, we report tumoural responses to pasireotide LAR therapy, which were compared with responsiveness to first-generation SRLs. In Chapter 6, we report the novel finding of a potential antitumour activity of pasireotide LAR when we observed a change in mean T2-signal intensity ratio and a 3-fold higher incidence of T2-hyperintense tumours in our cohort, compared with patients receiving first-generation SRLs. Although not biochemically controlled during first-generation SRLs, patients with T2-hyperintense tumours had a significantly larger decrease in IGF-I concentrations after three months of treatment with pasireotide LAR, compared with the total cohort. Substantial decreases in IGF-I concentrations warranted treatment dosage reduction in two of 14 patients, indicating that the observed cystic degeneration and/or tumour cell necrosis in these patients reduced disease activity. Besides, we observed significant ($\geq 25\%$) tumour shrinkage in 5 of the 14 patients with adenomas in which we observed increased T2-signal intensity during nine months of pasireotide LAR treatment. Up to 30 months of pasireotide LAR treatment, an additional decrease in tumour volume was observed in the adenomas of five patients.

Generally speaking, a T2-hyperintense signal indicates cystic degeneration, tumour cell necrosis, or both, which is suggestive of an anti-tumour effect. We believe, however, that it is necessary to differentiate tumour shrinkage from antitumour effects. While pasireotide LAR shows similar inhibitory effects on tumour growth as octreotide in GH-secreting tumour cell cultures⁽²⁴⁾, it additionally showed anti-tumour effects in this subgroup of patients, which have not been observed over the past three decades

of clinical experience with first-generation SRLs. Although first-generation SRLs does not significantly change the mean T2-signal intensity ratio in GH-secreting tumours, Bonneville *and colleagues*⁽²⁰⁾ reported that tumours can shift to T2-hyperintensity during first-generation SRL treatment in up to ten percent of treatment-naïve patients. Hence, an antitumour effect from this class of drugs could be considered. On the contrary, the change in mean T2-signal intensity ratio after pasireotide LAR treatment, and the 3-fold higher incidence of T2-hyperintense adenomas in our cohort indicate that this is a peculiar effect of pasireotide.

We should be aware that in patients with prolactinomas some rare cases of cystic degeneration and/or necrotic changes were observed after administration of dopamine agonists, which is the first time described by Gen *and colleagues*⁽²⁵⁾. In some of these cases new hormonal deficiencies were revealed⁽²⁶⁾. Although no new-onset anterior pituitary deficiencies were observed in our cohort nor the C2305 and C2404 study^(7, 8), we recommend close monitoring of pituitary tumour status during pasireotide LAR treatment as there still may be a risk of developing new hormonal deficiencies as clearly shown by previous cases⁽²⁷⁾.

Among the SST receptor subtypes, only SST₂ and SST₃ might be responsible for the pasireotide LAR-induced cystic degeneration, tumour cell necrosis, or both. In patients with increased T2-signal intense adenomas, pasireotide LAR-induced anti-tumour effects might reduce disease activity by achieving greater IGF-I reduction which is driven by SST₂ receptor (Chapter 4). This poses the question whether SST₂ and possibly SST₃ may be relevant in inducing hormonal as well as anti-tumour effects, while other SST receptors may be relevant in mediating tumour shrinkage during pasireotide LAR treatment.

This is the first report in humans of pituitary cystic degeneration and/or tumour cell necrosis during pasireotide LAR treatment and, therefore, has important implications for medical and surgical treatment options in patients with acromegaly, because first-generation SRLs are not known for their tumour cell necrotic effects. Firstly, the proposed antitumour activity of pasireotide LAR on pituitary tumours in acromegaly might, in contrast to first-generation SRLs, alleviate symptoms, reduce disease activity, and even ‘cure’ patients. Consequently, in case of significant necrosis of the pituitary tumour it might be necessary to reconsider the treatment regimen. Secondly, preoperative treatment with pasireotide LAR might induce (partial) cystic degeneration, tumour cell

necrosis, or both, which could improve surgical outcomes (*e.g.* in medically-naïve patients with larger tumours).

All in all, these data do suggest that pasireotide LAR is superior to first-generation SRLs therapy concerning the tumoural responsiveness (*i.e.* tumour shrinkage and/or anti-tumour effects). However, additional pituitary histology is needed to confirm whether cystic degeneration, tumour cell necrosis, or both leads to increased T2-signal weighted intensity. Moreover, the identification of genetic, molecular, and clinical characteristics associated with the effects of pasireotide LAR will improve clinical practice.

2.3 Safety and tolerability

Pasireotide LAR is well tolerated and has a safety profile comparable to that of first-generation SRLs, except for a greater frequency and degree of hyperglycemia-related adverse events and development of type 2 diabetes^(7, 8, 28). The incidence of type 2 diabetes in our study increased up to 77% (from 69% at three months) at nine months, and nine patients discontinued pasireotide LAR treatment, mainly because of severe hyperglycemia. The risk for developing type 2 diabetes during pasireotide LAR treatment seems inversely related to insulin secretion at baseline.

The patients using pasireotide LAR and pegvisomant combination therapy did not have a lower HbA1c level than patients using pasireotide LAR alone. This observation suggests that the insulin-sensitizing effect of pegvisomant cannot compensate for the insulin and incretin suppressive effect of pasireotide LAR⁽²⁹⁾.

We observed a higher frequency of hyperglycaemia-related adverse events (98%; of which 77% were grade 1 and 2) than in previous studies^(7, 8, 28, 30-33). This can be explained by the broader inclusion criteria, such as higher HbA1c levels and older patients, and the reduced pegvisomant dose during the run-in phase, leading to more insulin resistance due to an increased diabetogenic activity of GH.

To achieve a sound and solid safety profile, early-onset proactive management is required to initially control type 2 diabetes as a long-term risk factor for (cardio-) vascular disease in acromegaly and improve patient compliance and long-term continuation with pasireotide LAR therapy. In accordance with other previous studies, this early-onset proactive management should include assessing baseline fasting plasma glucose and HbA1c levels prior to initiating pasireotide LAR therapy⁽³⁴⁻³⁷⁾. In case of impaired fasting glucose and/or type 2 diabetes at baseline, lifestyle management and/or adequate

antidiabetic treatment should be started or optimized before initiating pasireotide LAR. After initiating pasireotide LAR, proactive glucose monitoring (*i.e.* measure fasting plasma glucose and HbA1c) is especially important in the first three months, afterwards, the intensity of glucose monitoring can be decreased^(35, 36, 38). We favour a less stringent and more liberal strategy in frequency of monitoring our patients⁽³⁸⁾. In our experience, the patients who eventually required insulin treatment were the patients that developed rapid hyperglycaemia after the first injection of pasireotide LAR. Therefore, it is paramount to be aware of early-onset severe hyperglycaemia and promptly initiate insulin treatment. On the other hand, the onset of hyperglycaemia in patients without insulin treatment at baseline had a slower onset of hyperglycaemia that could be managed with solely oral antidiabetic medication after the third injection.

Although we have not performed a cost-effectiveness analysis, it is likely that switching to pasireotide LAR will not result in a long-term reduction of overall treatment costs as the pegvisomant sparing effect of pasireotide LAR would likely be offset by the type 2 diabetes-related medication and healthcare costs.

2.4 Recommendations for the medical management of acromegaly

In figure 1 we present our recommendations for the medical management of acromegaly with a focus on the differences with the current consensus criteria⁽³⁹⁾. We present in this paragraph our recommendations to define the position of pasireotide LAR in the treatment of acromegaly based on our experiences with the pasireotide LAR and pegvisomant combination study and the available basic or clinical studies on pasireotide LAR and acromegaly.

2.4.1 First-line treatment

In accordance with the consensus criteria of Melmed *and colleagues*⁽³⁹⁾, first-generation SRLs monotherapy are considered the first-line medical treatment option for acromegaly (Figure 1).

2.4.2 Second-line and alternative treatments

According to the consensus criteria, pegvisomant substituted for or combined with first-generation SRL therapy is recommended for patients with no significant response (<20% IGF-I reduction) during first-generation SRLs monotherapy⁽³⁹⁾. However, we recommend

that combination therapy with first-generation SRL and pegvisomant is the best choice as second-line option in all non-responders (defined as IGF-I $>1.3 \times \text{ULN}$) by using the following arguments⁽³⁸⁾:

1. The potential advantage of combination therapy is that a lower pegvisomant dosage is needed to normalize IGF-I levels compared with monotherapy of pegvisomant, leading to a reduction in injection frequency for patients⁽⁴⁰⁻⁴²⁾.
2. There are indications that combination therapy might have a favourable effect on QoL compared to first-generation SRLs monotherapy, including the ones who are biochemically controlled⁽⁴³⁾.
3. Although monotherapy with pegvisomant does not reduce tumour size, combination therapy with first-generation SRL may result in tumour size control or even tumour shrinkage in most patients⁽⁴¹⁾.
4. Headaches may be alleviated during combination therapy with pegvisomant and first-generation SRL. It is proposed that nociceptive peptides are inhibited by first-generation SRLs, making it the favourable treatment option for patients with headaches^(44, 45).

However, initiating combination therapy with first-generation SRLs and pegvisomant is not preferred in patients showing poor control of type 2 diabetes during first-generation SRLs monotherapy. In patients receiving first-generation SRLs with worsening of glucose control, previous studies have shown that pegvisomant therapy had a more favourable effect on the glycaemic control^(22, 46, 47). In those cases, pegvisomant monotherapy would be a more suitable option as it may improve glucose metabolism by reducing insulin resistance^(22, 46, 47). This is more or less in accordance with the consensus criteria⁽³⁹⁾, that recommend patients with pre-existing clinically relevant impaired glucose metabolism should be switched from first-generation SRLs to pegvisomant monotherapy. Besides, in patients with no biochemical significant response ($<20\%$ IGF-I reduction) and without significant tumour shrinkage ($<25\%$ tumour volume reduction) during first-generation SRLs monotherapy, pegvisomant monotherapy is recommended.

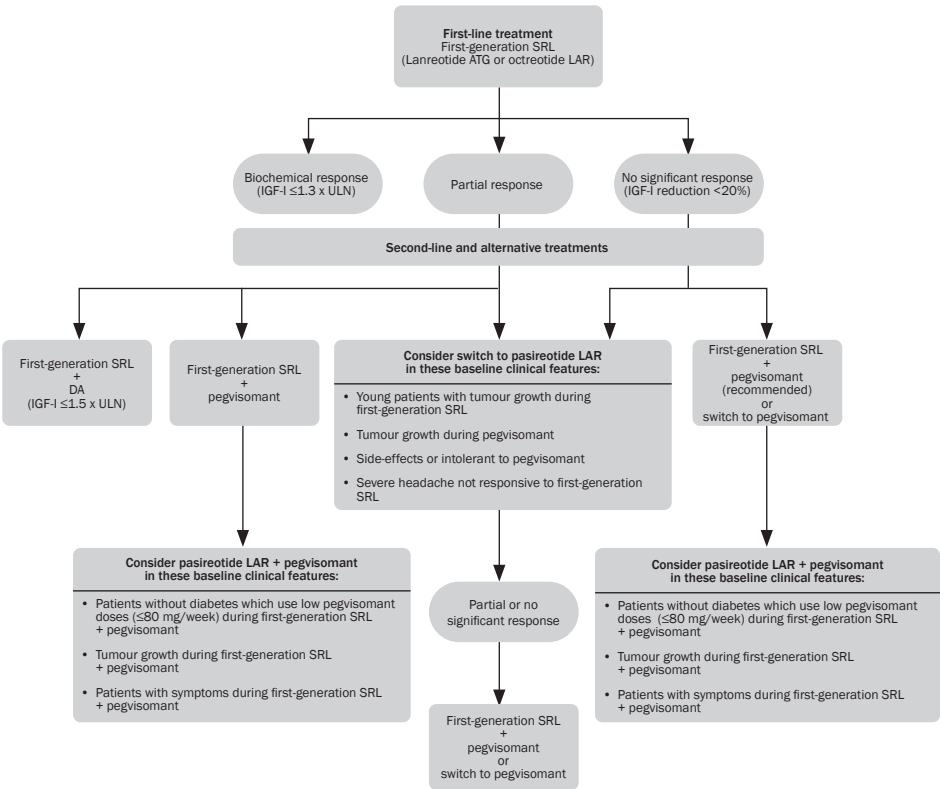
In contrast to the current consensus⁽³⁹⁾, who propose considering co-treatment with cabergoline (if IGF-I $<2.5 \times \text{ULN}$) in patients with inadequate control on first-generation SRL treatment, we recommend co-treatment with cabergoline if IGF-I levels remains

modestly elevated ($\text{IGF-I} \leq 1.5 \times \text{ULN}$), since IGF-I normalization was observed only in those patients⁽⁴⁸⁾.

The current consensus criteria⁽³⁹⁾ advocate pasireotide LAR monotherapy as second-line treatment for patients without biochemical response to first-generation SRLs if a clinically relevant residual tumour that is unsuitable for resection is present. In accordance with the current consensus criteria⁽³⁹⁾, we hypothesize that in particular young patients (age <40 years) with macroadenomas that show tumour growth during first-generation SRL monotherapy or pegvisomant (*i.e.* clinically aggressive tumours⁽⁴⁹⁾), pasireotide LAR monotherapy can be considered as a next treatment step before starting with radiotherapy⁽³⁸⁾. The same strategy can be applied for patients whose disease was previously not controlled by first-generation SRLs with tumour growth (*i.e.* reflecting the presence of more aggressive tumours) during pegvisomant monotherapy⁽³⁸⁾. In addition, we recommend to switch to pasireotide LAR monotherapy as an alternative to pegvisomant monotherapy or combination therapy for patients with the following baseline clinical features⁽³⁸⁾:

1. Patients whose disease was previously not controlled by first-generation SRLs, who experience side-effects, or who are intolerant to pegvisomant monotherapy;
2. Patients with severe headaches not responsive to first-generation SRLs. Headaches may be alleviated during pasireotide LAR treatment.

FIGURE 1. Proposed algorithm for the medical management of acromegaly.



Source: Coopmans EC, How to position pasireotide LAR treatment in acromegaly, *JCEM*, 2019; 104:1978-1988. Image reproduced with permission of the rights holder, *JCEM*. Radiotherapy is not mentioned in this algorithm, but it should be considered for patients with biochemically persistent disease or tumour growth despite surgery or medical therapy. DA, dopamine agonist.

The current consensus criteria⁽³⁹⁾ do not address the current position of pasireotide LAR in combination with pegvisomant in the medical management of acromegaly since it was held before our data was published^(28, 50). Preferred baseline clinical features for the use of pasireotide LAR in combination with pegvisomant would be patients without type 2 diabetes using low pegvisomant doses (≤ 80 mg/week) during combination therapy with first-generation SRLs⁽³⁸⁾. The pegvisomant dosages can be reduced or sometimes even discontinued due to the pegvisomant sparing effect of pasireotide

LAR⁽²⁸⁾. It should be stressed that it is likely that those patients end up with pasireotide LAR monotherapy. Patients biochemically controlled during first-generation SRL and pegvisomant combination therapy, who use first-generation SRLs every three weeks or have symptoms of active acromegaly in the fourth week after first-generation SRL administration, may experience symptomatic improvement after switching to pasireotide LAR and pegvisomant combination therapy⁽³⁸⁾. At last, we postulate that pasireotide LAR treatment may improve tumour size control or even tumour shrinkage. Therefore, patients experiencing tumour growth during first-generation SRL and pegvisomant combination therapy could be switched to pasireotide LAR and pegvisomant combination therapy⁽³⁸⁾.

The challenge in daily practice for every endocrinologist is to decide for each patient with acromegaly whether the potential advantages of biochemical and tumour control outweigh the potential disadvantages of potential (short- and long-term) complications of type 2 diabetes during pasireotide LAR treatment.

3. Emerging treatment option: very-low-carbohydrate ketogenic diet

As physiology has learned us that portal insulin is the driver of GHR expression on the hepatocytes, we applied in the proof-of-concept trial in Chapter 7 an adjuvant eucaloric ketogenic diet to reduce portal insulin and, therefore, make the liver GH-resistant.

By ingeniously combining first-generation SRLs with a eucaloric ketogenic diet, we are able to exert insulin-induced IGF-I normalization in 10 out of the 11 acromegaly patients we studied, without the unwanted increase in GH, and, according to the available guidelines, induce in these acromegaly subjects a biochemical control of their disease activity. In three of six patients who continued the diet (increasing carbohydrates from 35 g to 80 g) until three months, the IGF-I and GH levels remained normal and dose reduction was possible. In two of them, termination of first-generation SRL was even possible. This suggests the key role is played by the diet in both the IGF-I and GH lowering effects.

The high incidence of type 2 diabetes in acromegaly can be cause for concern when introducing a ketogenic diet. Generally speaking, the ketogenic diets are safe. Over the past century, they have been used without severe adverse effects to treat drug-resistant epilepsy in children⁽⁵¹⁾. Common adverse events such as lightheadedness, dizziness, fatigue, difficulty exercising, poor sleep, and constipation tended to pass in a few days to a

few weeks. Also in our study, safety defined as the occurrence of adverse effects ≥ 2 grade based on common terminology criteria of adverse events (CTCAE), posed no issue.

Considering patients with type 2 diabetes, those taking insulin or oral hypoglycemic medications can experience serious hypoglycemia on a ketogenic diet. Thus, this is not a do-it-yourself diet. Patients need training to follow and adhere to the diet and experienced clinicians should be consulted to safely adjust medications when initiating it. Although none of the participants had type 2 diabetes or hypoglycemia-related adverse events, we would recommend tight glycemic control in patients taking antidiabetic medication.

Further, interesting to note are the benefits of a ketogenic diet on disease control in diabetic patients. Although the evidence for ketogenic diets for diabetes management is still preliminary, insulin sensitivity may improve during the diet⁽⁵²⁾ along with glycemic control⁽⁵³⁾. Moreover, it seems to reduce the requirement for antidiabetic medications and improve HbA1c, which is a clinical endpoint for diabetes⁽⁵⁴⁻⁵⁶⁾. We assume, for patients with type 2 diabetes the risks of poor glycemic control from excessive carbohydrate intake far outweigh the possible risks of low-carbohydrate intake. Thus, most patients with diabetes, including those with acromegaly, could benefit from limiting their carbohydrate intake.

Regarding ketosis; it is a natural phenomenon that occurs in humans during fasting and lactation. As none of the subjects in our cohort were diabetes patients, it is completely safe to be in nutritional ketosis. Interestingly, these three recent studies including patients with type 2 diabetes⁽⁵⁴⁻⁵⁶⁾ report normal acid-base physiology and no cases of metabolic acidosis. Nonetheless, we still should be precautionary with this diet in patients with diabetes.

Considering that dehydration is the most common side effect of a ketogenic diet, especially in patients with a deteriorated glucose metabolism and renal vulnerability, such as acromegaly patients, appropriate management and follow-up of dehydration is essential. To address the importance of primary prevention of dehydration in acromegaly patient we advise an adequate fluid intake (≥ 2 liters per day).

Our results could affect the clinical management of acromegaly as a eucaloric ketogenic diet might deploy as an effective adjuvant treatment in some patients before initiating pegvisomant treatment. However, we acknowledge that this is a short-term proof-of-concept study and additional studies are needed to evaluate the long-term safety and efficacy of, as well as adherence to, a eucaloric ketogenic diet.

It should be stressed that our study has several limitations. First of all, the lack of a control group, which made it difficult for comparative evaluation to determine if the standard therapy (*i.e.* first-generation SRL monotherapy) also produced the same outcomes. Although a double-blind randomized study is not possible due to obvious visible differences between the normal and ketogenic diet, a crossover study design would be an eligible option. In a crossover study, the study participants will be switched throughout to two the treatment groups (both test and reference diet) after a washout period. Being the same set of the population the advantage of crossover studies is that patients act as their own controls.

Secondly, we did not measure QoL. We hypothesize that adding a ketogenic diet to first-generation SRL therapy in uncontrolled patients with acromegaly, will improve QoL by reducing IGF-I and GH levels, thereby preventing the switch to the expensive drug pegvisomant and potentially lowering the dose of first-generation SRL in these patients. Lastly, we have not performed a cost-effectiveness analysis, but it is also likely that adding a ketogenic diet to first-generation SRL will lead to a long-term reduction of overall treatment costs. The above-mentioned research questions will be answered utilizing a single-centre matched interventional cohort study, as part of the Efficiency Research Grant of the Erasmus Medical Center.

4. Role of *AIP* variants in pituitary tumours and thyroid carcinomas

In Chapter 8 we performed the first study that analyses the prevalence of *AIP* gene mutations and mutations in genes that have been associated with neuroendocrine tumours in series of tumours from patients presenting with both pituitary adenomas and DTCs.

We showed that genetic alterations were observed in 71.4% (10/14) of DTCs and in 13.3% (2/15) of pituitary adenomas tissues, while there was no overlap between genetic alterations within tissues from the same patient. The absence of somatic *AIP* mutations observed in patients with pituitary adenomas and concomitant DTCs suggest that their contribution to tumoural pathogenesis is probably limited and seems unlikely the genetic cause predisposing to the higher DTC risk observed in these patients.

Among patients with pathological germline *AIP* variants, one *AIP* variant c.853 C>T; p.Q285* was confirmed in the FTC specimen, including evidence of loss of the *AIP* wild-type allele at chromosome 11q13, based on the relatively high allele frequency (83%) of the germline mutation in the tumour DNA. This patient came from an *AIP*-mutated FIPA kindred, however, her pituitary gland was unaffected. To strengthen the hypothesis that *AIP* may be involved in thyroid carcinogenesis and not other driver genes are responsible for the FTC, we examined the most common FTC and PTC driver gene alterations in the FTC specimen. We did not observe *PTEN*, *TERT*, *BRAF V600E*, *RET*, *NRAS*, *HRAS* and *KRAS* mutations nor *RET/PTC* or *PAX8/PPAR γ* chromosomal rearrangements. This opens up a potential role for *AIP* mutation as an initiating event, at least in *AIP* mutation-positive FIPA kindred, thereby echoing a recent finding of an *AIP* mutation carrier by Daly *et al.*⁽⁵⁷⁾ with concomitant FTC. Further studies of *AIP* genetic status among DTCs in FIPA kindred cohorts are warranted to answer this question.

It is noteworthy that although the most common mechanism to lose the wild-type copy of a tumour suppressor gene (e.g. *AIP*) in DTC specimen is a large deletion affecting the wild-type allele, other mechanisms could also play a role, such as another somatic mutation in other parts of the gene, or silencing of the wild-type copy with epigenetic mechanism-promoter methylation or microRNAs which are not covered by NGS. We should emphasize that DTCs are more progressed in transformation since they are malignant when compared with pituitary adenomas. Therefore, it might be interesting to investigate the role of *AIP* mutation in thyroid adenomas (*i.e.* earlier in the transformation) in further studies.

In line with our findings, studying 12 DTC patients with acromegaly, Mian *et al.* reported that 70% of PTC patients with acromegaly were *BRAF* positive⁽⁵⁸⁾. Moreover, *AIP* expression was similar between neoplastic and normal tissue, while the aryl-hydrocarbon receptor (AHR) was expressed more in PTCs carrying *BRAF* mutations than in normal tissue, irrespective of acromegaly status⁽⁵⁸⁾. These data suggest that *BRAF* mutations and AHR overexpression may be associated with DTC risk in acromegaly, at least in patients with concomitant PTC.

A strength of our study lies in the relatively large number of patients in which with targeted NGS the pituitary adenoma and concomitant DTC tumour tissue were systematically investigated. The main limitations of our study lie in the retrospective collection of tumour samples and that we had to exclude several samples due to poor

quality. Another limitation of our study is the lack of clinical data from the patients, including follow-up data (e.g. received radiotherapy). At last, we should be borne in mind that these patients are examined more accurately, which may increase the number of diagnoses of thyroid cancer.

Conclusion

The findings presented in this thesis represent a step forward in our understanding of the position of current treatment options including pasireotide LAR and the emerging treatment option of an adjuvant ketogenic diet in the medical treatment of acromegaly.

We did identify clinical predictors for first-generation SRL treatment response in acromegaly. Baseline IGF-I concentration, bodyweight and the presence of type 2 diabetes can contribute in distinguishing complete biochemical from partial response during first-generation SRL treatment. Non-response during first-generation SRLs occurred more in patients that were younger at diagnosis and tended to harbour larger tumours and underwent surgery less often.

Likewise, we also identified clinical predictors for pasireotide LAR treatment response. The IGF-I lowering effects of pasireotide LAR treatment correlated to SST₂ receptor expression and not to SST₅ receptor. The IGF-I lowering effects correlated with the effect of first-generation SRL treatment, whereas these lower IGF-I levels are associated with a higher T2-signal intensity in the tumour. Surprisingly, biochemical response is not accompanied by tumour shrinkage during pasireotide LAR treatment. Patients showing no biochemical response to first-generation SRL and pasireotide LAR with particularly large tumours with a lower SST₂ receptor expression are prone to achieve tumour shrinkage during pasireotide LAR treatment

The results of the PAPE study demonstrated that pasireotide LAR has a high efficacy, illustrated by a 52% pegvisomant sparing effect, and that in half of the patients pegvisomant therapy could be discontinued. Besides, pasireotide LAR seems to exert a greater effect on tumour control, especially in patients whose disease is inadequately controlled on first-generation SRLs. The increased T2-weighted signal intensity reflects underlying cystic degeneration or necrosis of the tumour and might indicate an antitumour effect of pasireotide LAR. The main conclusion of our recommendations is

that, in general, first-generation SRL and pegvisomant combination treatment remains the second-line medical treatment option of choice, and pasireotide LAR should be reserved as a third-line option. Concerning the long-term treatment of acromegaly with pasireotide LAR, the potential advantages of biochemical and tumour control should be weighed against the disadvantages of potential (short- and long-term) complications of type 2 diabetes.

Our proof-of-concept study showed that a eucaloric very low-carbohydrate ketogenic diet reduced IGF-I concentrations in 11 uncontrolled acromegaly patients, and led to a lower first-generation SRL dose in half of the patients who continued the diet for three months. Our results could affect the clinical management of acromegaly as a ketogenic diet might deploy as an emerging adjuvant treatment in some patients before initiating pegvisomant treatment.

Finally, we examine the role of *AIP* variants in acromegaly patients with concomitant DTCs. The absence of somatic *AIP* mutations observed in patients with pituitary adenomas and concomitant DTCs suggest that their contribution to tumoural pathogenesis is probably limited and seems unlikely the genetic cause predisposing to the higher DTC risk observed in these patients.

Future perspectives on current treatment options

Treatment of acromegaly is complex and most cases require a stepwise, multidisciplinary approach to control the disease, while acromegaly is associated with significant morbidity and mortality if left untreated. Managing and treating acromegaly is inherently expensive because the disorder is chronic, requiring years of management, sophisticated imaging and biochemical testing, and new effective medical therapies are costly. This thesis attempted to further elucidate the correct selection of clinical predictors and therapy for acromegaly and highlights a ketogenic diet as a promising adjuvant treatment option.

Expanded knowledge on imaging, affinity of somatostatin receptors and development of new clinical markers of response and resistance to first-generation SRLs and pasireotide LAR emphasised the importance of a personalised approach rather than following a universal algorithm for therapy. Although the initial therapy choice will largely be driven by biochemical and tumour characteristics, other patient-specific and disease-specific factors should be considered as well to tailor the therapeutic approach. For example, the

presence of type 2 diabetes can contribute in distinguishing complete biochemical from partial response during first-generation SRLs therapy, whereas a higher SST₂ receptor expression and a higher T2-signal intensity in the tumour are associated with a better biochemical response to pasireotide LAR. These markers, however, require further prospective validation and harmonization of scoring systems to determine a precise, personalized approach to therapy⁽⁵⁹⁾.

Interestingly, the available data on the efficacy and safety of pasireotide LAR versus those of the first-generation SRLs strongly suggest that pasireotide LAR is more effective and that the safety issue is manageable. Accomplishing a better biochemical control when IGF-I levels are concerned, and with a good chance of significant tumour reduction and potential cell degeneration, tumour cell necrosis, or both, could result on favouring pasireotide LAR over first-generation SRL therapy. Moreover, pasireotide LAR therapy might have a favourable effect on QoL. However, before we move pasireotide LAR up towards the first-line treatment, we should better define the efficacy and safety of pasireotide LAR in medically or treatment-naïve patients. Studies with large series of cases are required, also for identifying prognostic markers of response to the treatment, as proven for first-generation SRL-resistant acromegaly patients by Iacovazzo *et al.*⁽¹³⁾ and the two studies presented in this thesis (Chapter 3 and 4).

In the last few years, novel familial syndromes and somatic mutations in sporadic GH-secreting pituitary tumours have been described. We utilised mutations in genes that have been associated with neuroendocrine tumours to analyse its role in the development of non-pituitary tumour types in acromegaly, such as DTCs presented in this thesis (Chapter 8). This study opens up a potential role for *AIP* mutation as an initiating event in *AIP* mutation-positive FIPA kindred, and further studies among DTCs in FIPA kindred cohorts are warranted to answer this question. Likely, integrating results of whole genome, whole exome, and transcriptional sequencing will also play a central role in our understanding of the pathophysiology of tumour formation in somatotrophinomas as well as in the testing of novel therapies. Preclinical transgenic animal models incorporating these mutations are suitable models to investigate novel therapies.

Future perspectives on the emerging treatment option: ketogenic diet

In this thesis we highlight a ketogenic diet as a promising adjuvant treatment option. As abovementioned, we acknowledge that this is a short-term proof-of-concept study that did not measure QoL and lacks a control group. Therefore, additional studies are needed to evaluate the long-term safety and efficacy of, as well as adherence to, a eucaloric ketogenic diet. Below you can find future perspectives on the emerging treatment option ketogenic diet.

Bring acromegaly to a halt with a diet

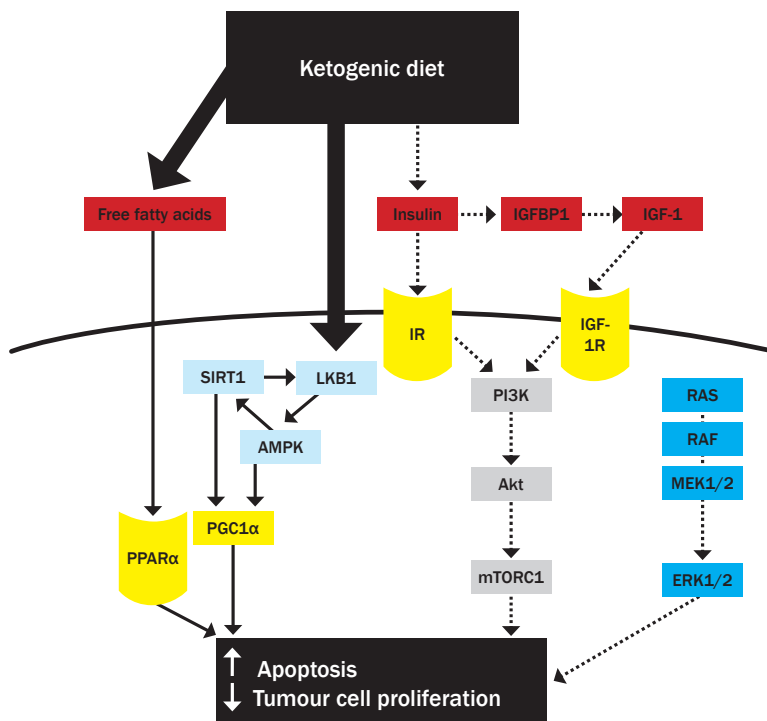
The promising results provide the incentive to further explore the beneficial effects of a ketogenic diet in active acromegaly. To this end, it is of particular interest to investigate the early initiation of a ketogenic diet in acromegaly subjects. These studies can further elucidate whether the diet's effects on insulin and therefore GH sensitivity can slow down, delay or even prevent the development of acromegaly when compared to a normal diet. By following parameters such as IGF-I and GH concentrations, gigantism, organomegaly, changes in bodyweight and composition (*i.e.* increased lean body mass), and the development of type 2 diabetes one can uncover the effect of the intervention. Since acromegaly is a complex disease with an insidious onset and often diagnosed many years after the commencement of symptoms, animal models that mimic the early phase of the disease until full-disease penetrance of acromegaly are required.

Bring concomitant the tumour to a halt

Insulin is well established as a growth-stimulating hormone⁽⁶⁰⁾. Insulin receptor and IGF-I receptor are expressed by most human tumours, including acromegaly⁽⁶¹⁾. High insulin and IGF-I levels provide a plausible mechanism of tumourigenesis through anti-apoptotic signalling and metabolic reprogramming mediated by the phosphatidylinositol-3-kinase (PI3K)–AKT–mammalian target of rapamycin (mTORC1) pathway and the RAS–RAF–MEK1/2–extracellular signal-regulated kinase (ERK)1/2 pathway^(62, 63) (Figure 2). In contrast, by reducing insulin and IGF-I levels, dietary restriction of carbohydrates activates an intracellular network consisting of AMPK, SIRT1, LKB1, PGC-1 α and PPAR α with the potential to counteract tumour cell proliferation⁽⁶³⁾ (Fig. 2). The relevance

of the mTORC1 and ERK1/2 pathways for tumourigenesis is demonstrated by the fact that patients with congenital IGF-I deficiency (*i.e.* Laron syndrome) have extremely low IGF-I and reduced insulin concentrations and usually do not develop cancer^(64, 65). Unfortunately, there is no data about the influence of a ketogenic diet on tumourigenesis in acromegaly and this has not been studied in our proof-of-concept trial.

FIGURE 2. Insulin and IGF-I signalling network and its modulation by a ketogenic diet.



The PI3K-AKT-mTORC1 pathway (grey boxes) and RAS-RAF-MEK1/2-ERK1/2 pathway (blue boxes) are inhibited by a ketogenic diet (dotted arrows). In contrast, the AMPK, SIRT1, LKB1, PGC-1α and PPARα pathway (light blue and yellow) is stimulated by a ketogenic diet (solid arrows). IGF-IR, IGF-I receptor, IR, insulin receptor.

Improve the clinical symptoms

Our proof-of-concept trial data suggest that a ketogenic diet can down-regulate hepatic GHRs in patients with acromegaly, and since the liver is the primary source of circulating IGF-I^(66, 67), it produces less IGF-I⁽⁶⁸⁾. In other words, a ketogenic diet regulates hepatic GH sensitivity, which is well-described in the literature⁽⁶⁹⁻⁷²⁾.

To elucidate molecular mechanisms responsible for ketogenic diet responsiveness, and since the peripheral tissues (*e.g.* skeletal muscle or fat) express SSTs receptors, it may be interesting to elucidate GH action in peripheral tissues as well. In the treatment of acromegaly, it is important to reduce GH action not only in the liver (as described in the previous paragraph) but also in the peripheral tissues⁽⁷³⁾. When activation of GHR expression, as well as engagement of its downstream signalling pathway in peripheral tissues, remains unaltered in response to a ketogenic diet, patients might still suffer acromegaly-related symptoms (*e.g.* headache or fatigue)⁽⁷³⁾. Assessment of GH signalling includes phosphorylated STAT5B, JAK2 and the expression of target genes including IGF-I, SOCS1–3 and CISH, the latter acting as a feedback inhibitor of GH signalling⁽⁷⁴⁾. The interplay between GHR expression in the peripheral tissues (*e.g.* skeletal muscle or subcutaneous fat) and a reduced carbohydrate intake is inconsistent in the literature⁽⁶⁹⁻⁷²⁾. However, recent data from Nakao *et al.* demonstrated that a ketogenic diet exerts an effecting in lowering GH activity in peripheral tissues⁽⁷⁵⁾. If a ketogenic diet acts via a similar mechanism of reducing portal insulin impacts GH signalling in peripheral tissues, this may lead to QoL improvements in patients.

Surprisingly, in our proof-of-concept trial patients reported positive effects on their well-being (not published), that were so favourable that six of the participants wanted to continue the diet. This suggests that a ketogenic diet can reduce GH-activity in the peripheral tissues as well. However, we cannot rule out that patients participating in the study have a more proactive attitude, which helped them to achieve improvements in well-being (*i.e.* selection bias). Of note, the other five participants continued the diet as well but decided to refrain from extra study visits.

Animal models can serve as an important source of *in vivo* information and allow a more detailed analysis of the role of ketogenic diet and the hormonal and tumoural action and alterations in the GHR expression. A good model of acromegaly is the homozygous pituitary-specific *AIP* knockout mice (*Aip*^{Flox/Flox}; *Hesx1*^{Cre/+}; *Rosa*^{FloxSTOPYFP/FloxSTOPYFP})⁽⁷⁶⁾. These animals share many features with patients carrying heterozygous

AIP mutations and developing aggressive pituitary tumours and acromegaly in a familial setting and are known to have a poor response to first-generation SRLs^(77, 78). Another good acromegaly model is the tetracycline-inducible somatotroph-specific *GNAS* knock-in mice (*Gli-Cre^{Tg/+}; Rosa26^{FloxSTOPPrTA/FloxSTOPPrTA}; tetO-Gnas^{Tg/Tg}*)⁽⁷⁹⁾. In contrast to the first mice model, this one share many features with patients with the most common defect in somatotrophinomas (~40%), which are more frequently found in those with less aggressive pituitary tumours that respond well to first-generation SRL^(80, 81).

Significance of the research for the relevant field

Future studies will gain a more comprehensive understanding of the mechanisms by which a carbohydrate restriction influences IGF-I, GH and insulin actions in acromegaly. They will determine if a ketogenic diet may slow down or even prevent the development of acromegaly, show anti-tumoural effects and alter GHR expression in these acromegaly mouse models. These results will have important therapeutic implications for patients with pituitary tumours and/or acromegaly:

Firstly, if the metabolic benefits of IGF-I and GH lowering effect is confirmed in the animal models, this may lead ultimately to an effective new treatment option for acromegaly patients, leading to the normalization of GH and IGF-I levels which can mitigate the increased mortality risk⁽⁸²⁻⁸⁵⁾. It could also play an important role in minimising the permanent phenotypic alterations such as gigantism. Moreover, the diet may reduce secondary complications (*e.g.* organomegaly, type 2 diabetes) associated with acromegaly⁽⁸⁶⁻⁸⁸⁾.

Secondly, studying the effects of insulin, IGF-I and GH on tumourigenesis will benefit patients with pituitary tumours, with the aim to understand the mechanism of tumourigenesis thus paving the way for targeted treatments in these aggressive tumours. Indeed, despite their prevalence, the exact pathophysiological mechanisms that lead to pituitary tumour predisposition and formation are still not fully elucidated. However, the familial form of diseases have always presented opportunities for researchers to elucidate the underlying genetic factors as well as the mechanisms involved in tumourigenesis. Investigating the influence of ketogenic diet on pituitary tumourigenesis and assess the PI3K–AKT–mTORC1, RAS–RAF–MEK1/2–ERK1/2 and the AMPK, SIRT1, PPAR α and PGC-1 α pathways in pituitary tissue, might reveal important new insights on pituitary tumour predisposition and formation.

Thirdly, investigating this novel therapeutic option will give the possibility to improve QoL in patients affected by acromegaly. Symptoms of the disease negatively impact the patient's ability to take active part in society, and the current medical treatment for acromegaly cannot reduce GH-activity in both the liver and the peripheral tissues⁽⁸⁹⁾, thus not impacting the QoL. Of note, although the ketogenic diet can be a challenge, especially for patients with a strong sweet tooth, most patients (90.9%, 10 out of 11 patients) in the proof-of-concept trial found it, within an organised program, surprisingly easy to make the switch.

Finally, the ketogenic diet could represent a cost-effective treatment option and could reduce the cost of expensive drugs. Currently, acromegaly patients have as only treatment option to ameliorate the symptoms, the expensive drug pegvisomant (average annual cost per patient is €60,000) which has no effect on tumour growth. Moreover, pegvisomant is not available in many countries. The results of this study could affect the clinical management of acromegaly as a ketogenic diet might result in an effective and low-cost treatment in patients before initiating pegvisomant as well as it may be used in countries where pegvisomant is not available. Because clinical endocrinology practice moves inexorably toward value-based reimbursements, analyses for treatment cost-effectiveness are required to define optimal control of disease progression for the individual patient. We have not performed a cost-effectiveness analysis, but it is also likely that adding a ketogenic diet to first-generation SRL will lead to a long-term reduction of overall treatment costs. To this end, our research group is planning to set-up a prospective a single-centre matched ketogenic diet interventional cohort study, as part of the Efficiency Research Grant of the Erasmus University Medical Center.

References

1. Petrossians P, Tichomirowa MA, Stevenaert A, Martin D, Daly AF, Beckers A. The Liege Acromegaly Survey (LAS): a new software tool for the study of acromegaly. *Ann Endocrinol (Paris)*. 2012;73(3):190-201.
2. Newman CB, Melmed S, Snyder PJ, Young WF, Boyajy LD, Levy R, et al. Safety and efficacy of long-term octreotide therapy of acromegaly: results of a multicenter trial in 103 patients--a clinical research center study. *J Clin Endocrinol Metab*. 1995;80(9):2768-75.
3. Cuevas-Ramos D, Flaseriu M. Somatostatin receptor ligands and resistance to treatment in pituitary adenomas. *J Mol Endocrinol*. 2014;52(3):R223-40.
4. Gordon MB, Molitch ME, Woodmansee WW, Ioachimescu AG, Gambetti OV, Carver D, et al. Body mass index as one of determinants of biochemical control in acromegaly: 2-year data from the soda registry. *Endocrine Reviews*. 2017;38(3).
5. Fieffe S, Morange I, Petrossians P, Chanson P, Rohmer V, Cortet C, et al. Diabetes in acromegaly, prevalence, risk factors, and evolution: data from the French Acromegaly Registry. *Eur J Endocrinol*. 2011;164(6):877-84.
6. Colao A, Auriemma RS, Lombardi G, Pivonello R. Resistance to somatostatin analogs in acromegaly. *Endocrine Reviews*. 2011;32(2):247-71.
7. Colao A, Bronstein MD, Freda P, Gu F, Shen CC, Gadelha M, et al. Pasireotide versus octreotide in acromegaly: a head-to-head superiority study. *J Clin Endocrinol Metab*. 2014;99(3):791-9.
8. Gadelha MR, Bronstein MD, Brue T, Coculescu M, Flaseriu M, Guitelman M, et al. Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol*. 2014;2(11):875-84.
9. Gatto F, Feelders RA, Franck SE, van Koetsveld PM, Dogan F, Kros JM, et al. In Vitro Head-to-Head Comparison Between Octreotide and Pasireotide in GH-Secreting Pituitary Adenomas. *J Clin Endocrinol Metab*. 2017;102(6):2009-18.
10. Jaquet P, Saveanu A, Gunz G, Fina F, Zamora AJ, Grino M, et al. Human somatostatin receptor subtypes in acromegaly: distinct patterns of messenger ribonucleic acid expression and hormone suppression identify different tumoral phenotypes. *J Clin Endocrinol Metab*. 2000;85(2):781-92.

11. Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, et al. The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. *J Clin Endocrinol Metab.* 2004;89(4):1577-85.
12. Murray RD, Melmed S. A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. *J Clin Endocrinol Metab.* 2008;93(8):2957-68.
13. Gatto F, Feelders RA, Franck SE, van Koetsveld PM, Dogan F, Kros JM, et al. In Vitro Head-to-Head Comparison Between Octreotide and Pasireotide in GH-Secreting Pituitary Adenomas. *J Clin Endocrinol Metab.* 2017;102(6):2009-18.
14. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab.* 2000;85(12):4712-20.
15. Iacovazzo D, Carlsen E, Lugli F, Chiloiro S, Piacentini S, Bianchi A, et al. Factors predicting pasireotide responsiveness in somatotroph pituitary adenomas resistant to first-generation somatostatin analogues: an immunohistochemical study. *Eur J Endocrinol.* 2016;174(2):241-50.
16. Cuevas-Ramos D, Carmichael JD, Cooper O, Bonert VS, Gertych A, Mamelak AN, et al. A structural and functional acromegaly classification. *J Clin Endocrinol Metab.* 2015;100(1):122-31.
17. Potorac I, Petrossians P, Daly AF, Alexopoulou O, Borot S, Sahnoun-Fathallah M, et al. T2-weighted MRI signal predicts hormone and tumor responses to somatostatin analogs in acromegaly. *Endocr Relat Cancer.* 2016;23(11):871-81.
18. Shen M, Zhang Q, Liu W, Wang M, Zhu J, Ma Z, et al. Predictive value of T2 relative signal intensity for response to somatostatin analogs in newly diagnosed acromegaly. *Neuroradiology.* 2016;58(11):1057-65.
19. Heck A, Ringstad G, Fougner SL, Casar-Borota O, Nome T, Ramm-Petersen J, et al. Intensity of pituitary adenoma on T2-weighted magnetic resonance imaging predicts the response to octreotide treatment in newly diagnosed acromegaly. *Clin Endocrinol (Oxf).* 2012;77(1):72-8.
20. Bonneville F, Riviere LD, Petersenn S, Bevan J, Houchard A, Sert C, et al. MRI T2 signal intensity and tumor response in patients with GH-secreting pituitary macroadenoma: PRIMARYS post-hoc analysis. *Eur J Endocrinol.* 2018.
21. Potorac I, Petrossians P, Daly AF, Schillo F, Ben Slama C, Nagi S, et al. Pituitary MRI characteristics in 297 acromegaly patients based on T2-weighted sequences. *Endocr Relat Cancer.* 2015;22(2):169-77.

22. Barkan AL, Burman P, Clemmons DR, Drake WM, Gagel RF, Harris PE, et al. Glucose homeostasis and safety in patients with acromegaly converted from long-acting octreotide to pegvisomant. *J Clin Endocrinol Metab.* 2005;90(10):5684-91.
23. Petrossians P, Daly AF, Natchev E, Maione L, Blijdorp K, Sahnoun-Fathallah M, et al. Acromegaly at diagnosis in 3173 patients from the Liege Acromegaly Survey (LAS) Database. *Endocr Relat Cancer.* 2017;24(10):505-18.
24. Ibanez-Costa A, Rivero-Cortes E, Vazquez-Borrego MC, Gahete MD, Jimenez-Reina L, Venegas-Moreno E, et al. Octreotide and pasireotide (dis)similarly inhibit pituitary tumor cells in vitro. *J Endocrinol.* 2016;231(2):135-45.
25. Gen M, Uozumi T, Ohta M, Ito A, Kajiwara H, Mori S. Necrotic changes in prolactinomas after long term administration of bromocriptine. *J Clin Endocrinol Metab.* 1984;59(3):463-70.
26. Selvan C, Dutta D, Ghosh S, Mukhopadhyay S, Chowdhury S. Growth hormone deficiency, secondary hypothyroidism, and empty sella following treatment of childhood macroprolactinoma. *Indian J Endocrinol Metab.* 2013;17(Suppl 1):S132-4.
27. Coopmans EC, van der Lely AJ, Schneiders JJ, Neggers S. Potential antitumour activity of pasireotide on pituitary tumours in acromegaly. *Lancet Diabetes Endocrinol.* 2019.
28. Muhammad A, van der Lely AJ, Delhanty PJD, Dallenga AHG, Haitsma IK, Janssen J, et al. Efficacy and Safety of Switching to Pasireotide in Patients With Acromegaly Controlled With Pegvisomant and First-Generation Somatostatin Analogues (PAPE Study). *J Clin Endocrinol Metab.* 2018;103(2):586-95.
29. Schmid HA, Brue T, Colao A, Gadelha MR, Shimon I, Kapur K, et al. Effect of pasireotide on glucose- and growth hormone-related biomarkers in patients with inadequately controlled acromegaly. *Endocrine.* 2016;53(1):210-9.
30. Bronstein MD, Fleseriu M, Neggers S, Colao A, Sheppard M, Gu F, et al. Switching patients with acromegaly from octreotide to pasireotide improves biochemical control: crossover extension to a randomized, double-blind, Phase III study. *BMC Endocr Disord.* 2016;16:16.
31. Fleseriu M, Rusch E, Geer EB, Investigators AS. Safety and tolerability of pasireotide long-acting release in acromegaly-results from the acromegaly, open-label, multicenter, safety monitoring program for treating patients who have a need to receive medical therapy (ACCESS) study. *Endocrine.* 2017;55(1):247-55.
32. Tahara S, Murakami M, Kaneko T, Shimatsu A. Efficacy and safety of long-acting pasireotide in Japanese patients with acromegaly or pituitary gigantism: results from a multicenter, open-label, randomized, phase 2 study. *Endocr J.* 2017;64(7):735-47.

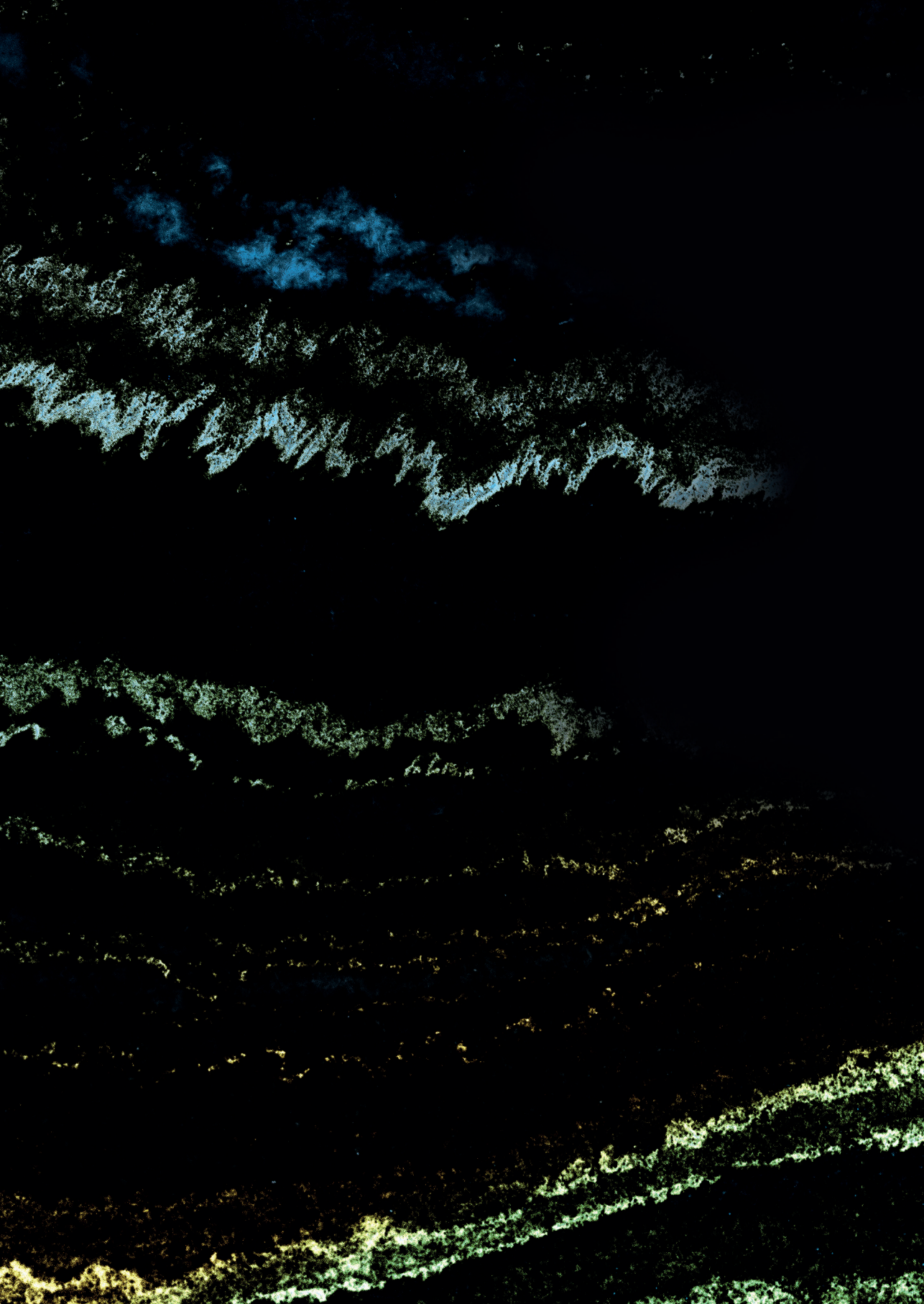
33. Gadelha MR, Bronstein MD, Brue T, De Marinis L, Fleseriu M, Guitelman M, et al. Long-acting pasireotide provides clinical benefit in patients with acromegaly: Results from a 30-month analysis of the phase 3 PAOLA study. *Endocrine Reviews*. [Abstract]. In press Volume 38, Issue 3, Supplement 1, June 2017.
34. Luger A. Hyperglycemia in pasireotide-treated patients with acromegaly and its treatment. *Endocrine*. 2016;54(1):1-2.
35. Samson SL. Management of Hyperglycemia in Patients With Acromegaly Treated With Pasireotide LAR. *Drugs*. 2016;76(13):1235-43.
36. Wildemberg LE, Gadelha MR. Pasireotide for the treatment of acromegaly. *Expert Opin Pharmacother*. 2016;17(4):579-88.
37. Breitschaft A, Hu K, Hermosillo Resendiz K, Darstein C, Golor G. Management of hyperglycemia associated with pasireotide (SOM230): healthy volunteer study. *Diabetes Res Clin Pract*. 2014;103(3):458-65.
38. Coopmans EC, Muhammad A, van der Lely AJ, Janssen J, Neggers S. How to Position Pasireotide LAR Treatment in Acromegaly. *J Clin Endocrinol Metab*. 2019;104(6):1978-88.
39. Melmed S, Bronstein MD, Chanson P, Klibanski A, Casanueva FF, Wass JAH, et al. A Consensus Statement on acromegaly therapeutic outcomes. *Nat Rev Endocrinol*. 2018;14(9):552-61.
40. Neggers SJ, van der Lely AJ. Combination treatment with somatostatin analogues and pegvisomant in acromegaly. *Growth Horm IGF Res*. 2011;21(3):129-33.
41. Neggers SJ, Franck SE, de Rooij FW, Dallenga AH, Poublon RM, Feelders RA, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. *J Clin Endocrinol Metab*. 2014;99(10):3644-52.
42. Trainer PJ, Ezzat S, D'Souza GA, Layton G, Strasburger CJ. A randomized, controlled, multicentre trial comparing pegvisomant alone with combination therapy of pegvisomant and long-acting octreotide in patients with acromegaly. *Clin Endocrinol (Oxf)*. 2009;71(4):549-57.
43. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, et al. Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. *J Clin Endocrinol Metab*. 2008;93(10):3853-9.
44. Levy MJ, Matharu M, Goadsby PJ. Chronic headache and pituitary tumors. *Curr Pain Headache Rep*. 2008;12(1):74-8.

45. Lin TH, Hu K, Flarakos J, Sharr-McMahon M, Mangold JB, He H, et al. Assessment of the absorption, metabolism and excretion of [(1)(4)C]pasireotide in healthy volunteers using accelerator mass spectrometry. *Cancer Chemother Pharmacol.* 2013;72(1):181-8.
46. Ghigo E, Biller BM, Colao A, Kourides IA, Rajicic N, Hutson RK, et al. Comparison of pegvisomant and long-acting octreotide in patients with acromegaly naive to radiation and medical therapy. *J Endocrinol Invest.* 2009;32(11):924-33.
47. Drake WM, Rowles SV, Roberts ME, Fode FK, Besser GM, Monson JP, et al. Insulin sensitivity and glucose tolerance improve in patients with acromegaly converted from depot octreotide to pegvisomant. *Eur J Endocrinol.* 2003;149(6):521-7.
48. Sandret L, Maison P, Chanson P. Place of cabergoline in acromegaly: a meta-analysis. *J Clin Endocrinol Metab.* 2011;96(5):1327-35.
49. Besser GM, Burman P, Daly AF. Predictors and rates of treatment-resistant tumor growth in acromegaly. *Eur J Endocrinol.* 2005;153(2):187-93.
50. Muhammad A, Coopmans EC, Delhanty PJD, Dallenga AHG, Haitma IK, Janssen J, et al. Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study. *Eur J Endocrinol.* 2018;179(5):269-77.
51. Roehl K, Sewak SL. Practice Paper of the Academy of Nutrition and Dietetics: Classic and Modified Ketogenic Diets for Treatment of Epilepsy. *J Acad Nutr Diet.* 2017;117(8):1279-92.
52. Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a Low-Carbohydrate Diet on Appetite, Blood Glucose Levels, and Insulin Resistance in Obese Patients with Type 2 Diabetes. *Ann Intern Med.* 2005;142(6):403-11.
53. Yancy WS, Foy M, Chalecki AM, Vernon MC, Westman EC. A low-carbohydrate, ketogenic diet to treat type 2 diabetes. *Nutr Metab (Lond).* 2005;2(1):34.
54. Lean MEJ, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet.* 2018;391(10120):541-51.
55. Pot GK, Battjes-Fries MCE, Patijn ON, Pijl H, Witkamp RF, de Visser M, et al. Nutrition and lifestyle intervention in type 2 diabetes: pilot study in the Netherlands showing improved glucose control and reduction in glucose lowering medication. *BMJ Nutr Prev Health.* 2019:bmjnph-2018-000012.

56. Hallberg SJ, McKenzie AL, Williams PT, Bhanpuri NH, Peters AL, Campbell WW, et al. Effectiveness and Safety of a Novel Care Model for the Management of Type 2 Diabetes at 1 Year: An Open-Label, Non-Randomized, Controlled Study. *Diabetes Ther.* 2018;9(2):583-612.
57. Daly AF, Rostomyan L, Betea D, Bonneville JF, Villa C, Pellegata NS, et al. AIP-mutated acromegaly resistant to first-generation somatostatin analogs: long-term control with pasireotide LAR in two patients. *Endocr Connect.* 2019;8(4):367-77.
58. Mian C, Ceccato F, Barollo S, Watutantrige-Fernando S, Albiger N, Regazzo D, et al. AHR Over-Expression in Papillary Thyroid Carcinoma: Clinical and Molecular Assessments in a Series of Italian Acromegalic Patients with a Long-Term Follow-Up. *PLOS ONE.* 2014;9(7):e101560.
59. Melmed S. Pituitary Medicine From Discovery to Patient-Focused Outcomes. *J Clin Endocrinol Metab.* 2016;101(3):769-77.
60. Straus DS. Growth-stimulatory actions of insulin in vitro and in vivo. *Endocr Rev.* 1984;5(2):356-69.
61. Belfiore A, Frasca F, Pandini G, Sciacca L, Vigneri R. Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. *Endocr Rev.* 2009;30(6):586-623.
62. Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. *Oncogene.* 2007;26(22):3279-90.
63. Grabacka M, Pierzchalska M, Reiss K. Peroxisome proliferator activated receptor alpha ligands as anticancer drugs targeting mitochondrial metabolism. *Curr Pharm Biotechnol.* 2013;14(3):342-56.
64. Laron Z. The GH-IGF1 axis and longevity. The paradigm of IGF1 deficiency. *Hormones (Athens).* 2008;7(1):24-7.
65. Janecka A, Kolodziej-Rzepa M, Biesaga B. Clinical and Molecular Features of Laron Syndrome, A Genetic Disorder Protecting from Cancer. *In Vivo.* 2016;30(4):375-81.
66. Clemmons DR. Involvement of insulin-like growth factor-I in the control of glucose homeostasis. *Curr Opin Pharmacol.* 2006;6(6):620-5.
67. Ho PJ, Friberg RD, Barkan AL. Regulation of pulsatile growth hormone secretion by fasting in normal subjects and patients with acromegaly. *J Clin Endocrinol Metab.* 1992;75(3):812-9.
68. Coopmans EC, Berk KAC, El-Sayed N, Neggers S, van der Lely AJ. Eucaloric Very-Low-Carbohydrate Ketogenic Diet in Acromegaly Treatment. *N Engl J Med.* 2020;382(22):2161-2.

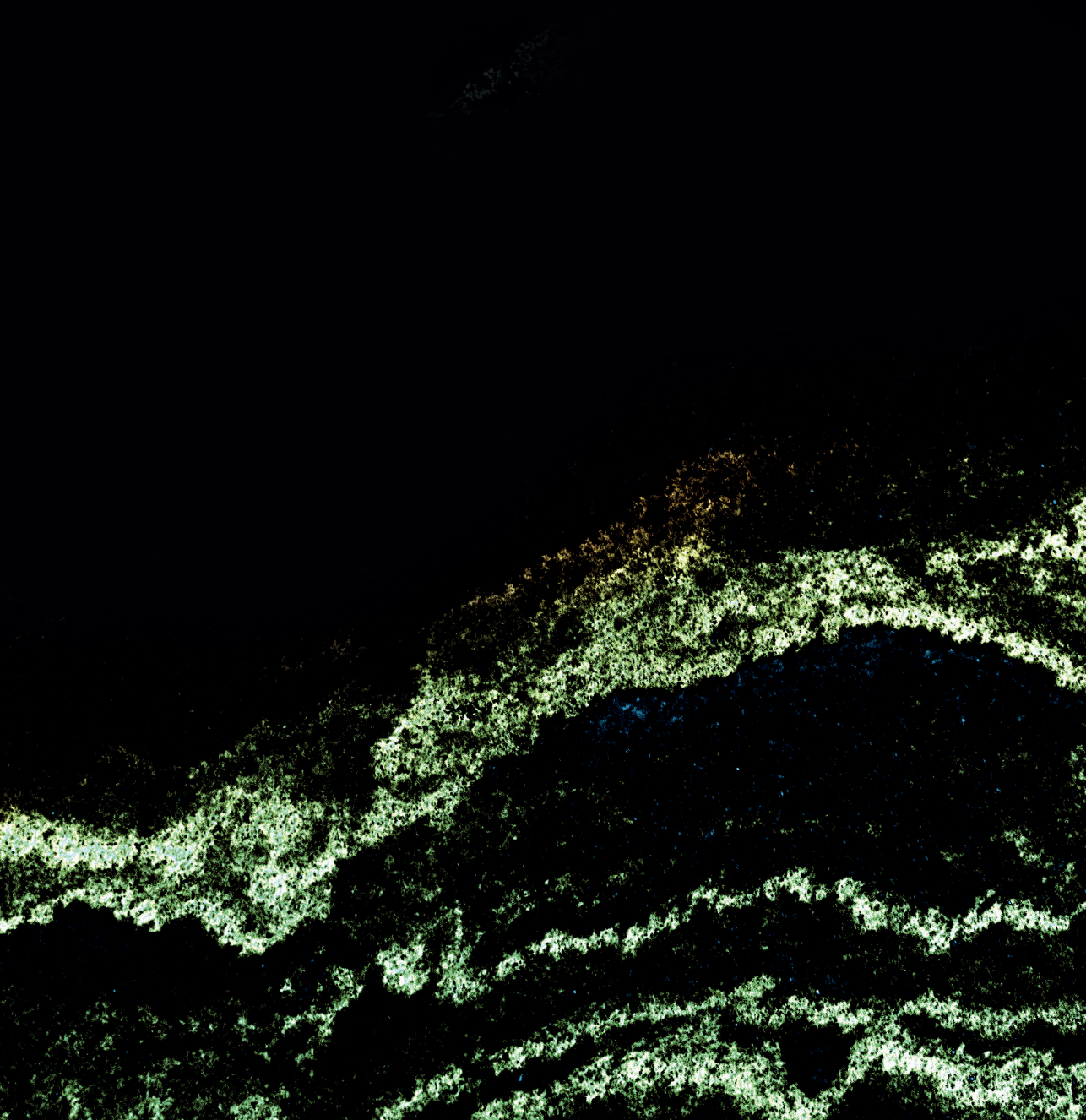
69. Moller L, Dalman L, Norrelund H, Billestrup N, Frystyk J, Moller N, et al. Impact of fasting on growth hormone signaling and action in muscle and fat. *J Clin Endocrinol Metab.* 2009;94(3):965-72.
70. Vendelbo MH, Christensen B, Gronbaek SB, Hogild M, Madsen M, Pedersen SB, et al. GH signaling in human adipose and muscle tissue during 'feast and famine': amplification of exercise stimulation following fasting compared to glucose administration. *Eur J Endocrinol.* 2015;173(3):283-90.
71. Bielohuby M, Sawitzky M, Stoeckl BJM, Stock P, Menhofer D, Ebensing S, et al. Lack of Dietary Carbohydrates Induces Hepatic Growth Hormone (GH) Resistance in Rats. *Endocrinology.* 2011;152(5):1948-60.
72. Olsson B, Bohlooly-Y M, Fitzgerald SM, Frick F, Ljungberg A, Ahrén B, et al. Bovine Growth Hormone Transgenic Mice Are Resistant to Diet-Induced Obesity but Develop Hyperphagia, Dyslipidemia, and Diabetes on a High-Fat Diet. *Endocrinology.* 2005;146(2):920-30.
73. Neggers SJ, Kopchick JJ, Jorgensen JO, van der Lely AJ. Hypothesis: Extra-hepatic acromegaly: a new paradigm? *Eur J Endocrinol.* 2011;164(1):11-6.
74. Dal J, Hoyer KL, Pedersen SB, Magnusson NE, Bjerring P, Frystyk J, et al. Growth Hormone and Insulin Signaling in Acromegaly: Impact of Surgery Versus Somatostatin Analog Treatment. *J Clin Endocrinol Metab.* 2016;101(10):3716-23.
75. Nakao R, Abe T, Yamamoto S, Oishi K. Ketogenic diet induces skeletal muscle atrophy via reducing muscle protein synthesis and possibly activating proteolysis in mice. *Sci Rep.* 2019;9(1):19652.
76. Barry S, Carlsen E, Marques P, Stiles CE, Gadaleta E, Berney DM, et al. Tumor microenvironment defines the invasive phenotype of AIP-mutation-positive pituitary tumors. *Oncogene.* 2019;38(27):5381-95.
77. Marques P, Caimari F, Hernandez-Ramirez LC, Collier D, Iacovazzo D, Ronaldson A, et al. Significant Benefits of AIP Testing and Clinical Screening in Familial Isolated and Young-onset Pituitary Tumors. *J Clin Endocrinol Metab.* 2020;105(6).
78. Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. *J Clin Endocrinol Metab.* 2010;95(11):E373-83.
79. Cheung L, Samper P, Lafont C, Mollard PE, Le Tissier PR, editors. *Endocrine Rev* 34:1122013.

80. Neou M, Villa C, Armignacco R, Jouinot A, Raffin-Sanson ML, Septier A, et al. Pangenomic Classification of Pituitary Neuroendocrine Tumors. *Cancer Cell*. 2020;37(1):123-34 e5.
81. Gadelha MR, Kasuki L, Korbonits M. The genetic background of acromegaly. *Pituitary*. 2017;20(1):10-21.
82. Beauregard C, Truong U, Hardy J, Serri O. Long-term outcome and mortality after transsphenoidal adenomectomy for acromegaly. *Clin Endocrinol (Oxf)*. 2003;58(1):86-91.
83. Holdaway IM, Rajasoorya CR, Gamble GD, Stewart AW. Long-term treatment outcome in acromegaly. *Growth Horm IGF Res*. 2003;13(4):185-92.
84. Holdaway IM, Rajasoorya RC, Gamble GD. Factors influencing mortality in acromegaly. *J Clin Endocrinol Metab*. 2004;89(2):667-74.
85. Swearingen B, Barker FG, 2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, et al. Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. *J Clin Endocrinol Metab*. 1998;83(10):3419-26.
86. Colao A, Ferone D, Marzullo P, Lombardi G. Systemic Complications of Acromegaly: Epidemiology, Pathogenesis, and Management. *Endocrine Reviews*. 2004;25(1):102-52.
87. Melmed S. Medical progress: Acromegaly. *N Engl J Med*. 2006;355(24):2558-73.
88. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP. Mortality in acromegaly: a metaanalysis. *J Clin Endocrinol Metab*. 2008;93(1):61-7.
89. Neggers SJCMM, van Aken MO, de Herder WW, Felders RA, Janssen JAMJL, Badia X, et al. Quality of Life in Acromegalic Patients during Long-Term Somatostatin Analog Treatment with and without Pegvisomant. *J Clin Endocrinol Metab*. 2008;93(10):3853-9.



Part VI

Summary Samenvatting



Summary

In **Chapter 1**, the current medical treatment options for acromegaly, a chronic systemic condition caused by excessive GH secretion from a pituitary tumour, are described. The clinical predictors for the treatment response in acromegaly are discussed in this chapter, focussing on first-generation SRL and pasireotide LAR, either as monotherapy or in combination with pegvisomant. This chapter further focuses on the effect of GH on glucose, lipid and protein metabolism, in particular on the important role of insulin and carbohydrate restriction. Subsequently, in this chapter we describe that although the pathogenesis of GH-secreting pituitary tumours remains elusive, rare cases of familial acromegaly have been reported, and up to 30% of these tumours have been linked to germline mutations in *AIP*. *AIP* gene mutations and mutations in genes that have been associated with neuroendocrine tumours can be utilised to investigate their role in the development of non-pituitary tumour types in acromegaly, such as the high frequency of concomitant DTCs.

In **Chapter 2**, of this thesis baseline IGF-I concentration, bodyweight and the presence of type 2 diabetes can contribute in distinguishing complete biochemical from partial first-generation SRL treatment response in acromegaly. Non-response during first-generation SRLs occurred more in patients that were younger at diagnosis and tended to harbour larger tumours and underwent surgery less often.

Chapter 3. In this cohort, the higher binding affinity to SST₂ receptor expression and not SST₅ receptor is responsible for driving the response (*i.e.* IGF-I lowering effects) of pasireotide LAR treatment. This observation is further supported by the finding that we found in the same patients a positive correlation between the percentage of IGF-I reduction after first-generation SRL treatment and response to pasireotide LAR treatment after both three and nine months' treatment.

Chapter 4. Patients with acromegaly showing no biochemical response to first-generation SRL and pasireotide LAR with particularly large tumours with a lower SST₂ receptor expression are prone to achieve tumour shrinkage during pasireotide LAR treatment. On the contrary, lower IGF-I levels during pasireotide LAR are associated with a higher SST₂ receptor expression and a higher T2-signal intensity in the tumour. As discussed in Chapter 6, a potential antitumour activity of pasireotide LAR emerged when we observed a change in mean T2-signal intensity ratio in GH-secreting tumours

after pasireotide LAR treatment in our cohort. In patients with increased T2-signal intense tumours, pasireotide LAR-induced cystic degeneration, tumour cell necrosis, or both, might reduce disease activity by achieving greater IGF-I reduction in patients with relatively larger adenomas.

Chapter 5. We found that pasireotide LAR treatment normalized IGF-I levels in 77% of acromegaly patients and on average 50% of the pegvisomant dose could be reduced. The frequency of type 2 diabetes increased up to 77% at nine months, and nine patients discontinued pasireotide LAR treatment, mainly because of severe hyperglycemia. The risk for developing type 2 diabetes during pasireotide LAR treatment seems inversely related to insulin secretion at baseline.

Chapter 6. Surprisingly, the T2-weighted MRI signal of the GH-secreting tumour was increased in 14 (30%) of the 47 patients during pasireotide LAR treatment. The increase in T2-signal was particularly substantial (>50%) in eight patients, where the majority of the tumour became T2-hyperintense. Although not biochemically controlled during first-generation SRLs, patients with T2-hyperintense tumours (n=14) had a significantly larger decrease in IGF-I concentrations after three months of treatment with pasireotide LAR, compared with the total cohort. Substantial decreases in IGF-I concentrations warranted treatment dosage reduction in two of 14 patients. Besides, we observed clinically significant ($\geq 25\%$) tumour shrinkage in 5 of the 14 patients with adenomas in which we observed increased T2-signal intensity during nine months of pasireotide LAR treatment. Up to 30 months of pasireotide LAR treatment, an additional decrease in tumour volume was observed in the adenomas of five patients.

Chapter 7. By combining first-generation SRLs with a eucaloric ketogenic diet, we are able to exert insulin-induced IGF-I normalization in 10 out of the 11 acromegaly patients we studied, without the unwanted increase in GH. In three of six patients who continued the diet (increasing carbohydrates from 35 g to 80 g) until three months, the IGF-I and GH levels remained normal and dose reduction was possible. In two of them, termination of first-generation SRL was even possible.

Chapter 8. We showed that genetic alterations were observed in ten (71.4%) of the 14 DTCs and in two (13.3%) of 15 pituitary adenomas tissues, while there was no overlap between genetic alterations within tissues from the same patient. The absence of somatic *AIP* mutations observed in patients with pituitary adenomas and concomitant DTCs suggest that their contribution to tumoural pathogenesis is probably limited and seems

unlikely the genetic cause predisposing to the higher DTC risk observed in acromegaly patients. Among patients with pathological germline *AIP* variants, one *AIP* variant c.853 C>T; p.Q285* was confirmed in the FTC specimen from a FIPA kindred, including evidence of loss of the *AIP* wild-type allele at chromosome 11q13, based on the relatively high allele frequency (83%) of the germline mutation in the tumour DNA. This opens up a potential role for *AIP* mutation as an initiating event, at least in *AIP* mutation-positive FIPA kindred.

In **Chapter 9**, the discussion section of this thesis, previous chapters are discussed, connected and put in the context of other literature. In particular the most recent findings in the research field of the medical treatment of acromegaly, the methodological pitfalls and future perspective on the research field including the role of a ketogenic diet as an emerging treatment option are further elaborated on.

Samenvatting

In **Hoofdstuk 1** van dit proefschrift worden de huidige medische behandelingsopties voor patiënten met acromegalie beschreven. Acromegalie is een chronische systemische aandoening veroorzaakt door overmatige groeihormoon (GH) secretie afkomstig van een hypofyse tumor. Voorspellende factoren voor een goede respons op medicamenteuze therapie bij acromegalie worden besproken in dit hoofdstuk. De nadruk zal liggen op eerste-generatie somatostatine-analogen en pasireotide, hetzij als monotherapie of in combinatie met pegvisomant. Dit hoofdstuk richt zich verder op het effect van GH op het glucose-, lipiden- en eiwitmetabolisme en in het bijzonder op de belangrijke rol van insuline en een koolhydraatbeperkt dieet. Vervolgens beschrijven we in dit hoofdstuk dat, hoewel de pathogenese van GH-producerende hypofysetumoren ongrijpbaar blijft, er zeldzame gevallen van familiale acromegalie zijn gerapporteerd en dat tot 30% van deze tumoren in verband worden gebracht met kiembaanmutaties in het *AIP*-gen. Deze *AIP*-mutatie en andere mutaties geassocieerd met neuro-endocriene tumoren kunnen worden ingezet om de ontwikkeling van andere tumoren bij acromegalie nader te onderzoeken. Zo is het interessant om de rol van een *AIP*-mutatie te onderzoeken bij patiënten die tegelijkertijd een gedifferentieerde schildkliercarcinoom hebben; een tumor die relatief vaak voorkomt bij patiënten met acromegalie.

In **Hoofdstuk 2** van dit proefschrift kunnen baseline IGF-I concentratie, lichaamsgewicht en de aanwezigheid van diabetes type 2, een onderscheid maken tussen volledig en partiële respons op eerste-generatie somatostatine-analogen in patiënten met acromegalie. Therapieresistentie voor eerste-generatie somatostatine-analogen komt vaker voor bij patiënten die jonger waren tijdens de diagnose, een relatief grotere tumor hadden en minder vaak een operatie ondergingen.

Hoofdstuk 3 bediscussieert dat SST₂-receptor en niet SST₅-receptor expressie verantwoordelijk is voor de biochemische respons (d.w.z. IGF-I verlagende effecten) tijdens pasireotide therapie. Deze waarneming wordt verder ondersteund door de bevinding dat we bij dezelfde patiënten een positieve correlatie vinden tussen het percentage IGF-I-reductie na eerste-generatie somatostatine-analogen en de respons op pasireotide therapie na zowel drie als negen maanden.

Hoofdstuk 4 zet uiteen dat patiënten met acromegalie die geen biochemische respons vertonen op zowel eerste-generatie somatostatine-analogen als pasireotide,

met relatief grote tumoren en een lagere SST₂-receptor expressie, vaker tumorafname vertonen tijdens pasireotide therapie. Integendeel, lagere IGF-I waardes tijdens pasireotide therapie worden geassocieerd met een hogere SST₂-receptor expressie en een hogere T2-signaal intensiteit in de tumor. Zoals besproken in Hoofdstuk 6, een potentiële antitumor activiteit van pasireotide openbaart zich toen we een verandering in de T2-signaal intensiteit in GH-producerende tumoren observeerden na pasireotide therapie. Bij patiënten met een verhoogde T2-signaal intensiteit kan pasireotide-geïnduceerde cysteuze degeneratie en/of tumorcel necrose mogelijk de ziekteactiviteit verminderen. Dit zien we ook terug in onze patiëntengroep, waarbij er in deze subgroep een grotere IGF-I reductie wordt bereikt, met name bij diegene met een relatief groot adenoom.

In **Hoofdstuk 5** ontdekken we dat tijdens behandeling met pasireotide in 77% van de acromegalie patiënten de IGF-I waardes normaliseren. Tevens kan in gemiddeld 50% van de patiënten de pegvisomant dosis verlaagd worden. De frequentie van diabetes type 2 nam toe tot 77% na negen maanden behandeling met pasireotide, waarvan negen patiënten stopten vanwege de ontwikkeling van ernstige hyperglycemie. Het risico op het ontwikkelen van diabetes type 2 tijdens behandeling met pasireotide lijkt omgekeerd evenredig te zijn met de insulinesecretie tijdens de start van de studie.

Verrassend genoeg was het T2-gewogen MRI signaal van de GH-producerende tumor verhoogd bij 14 (30%) van de 47 patiënten tijdens pasireotide therapie. Dit beschrijven we in **Hoofdstuk 6**. De toename van de T2-signaal intensiteit is substantieel (>50%) bij acht patiënten, waarin het overgrote deel van de tumor T2-hyperintens werd. Hoewel deze patiënten niet biochemisch gecontroleerd zijn tijdens eerste-generatie somatostatine-analogen, laten diegene met een T2-hyperintense tumor (n=14) een grotere afname in IGF-I concentraties zien na drie maanden therapie met pasireotide in vergelijking met het totale cohort. Deze IGF-I afname rechtvaardigt verlaging van de pasireotide dosering bij twee van de 14 patiënten. Bovendien observeren we in deze subgroep een klinisch significante (≥25%) tumorafname bij vijf van de 14 patiënten na negen maanden pasireotide therapie. Na 30 maanden wordt een extra afname van het tumorvolume waargenomen bij vijf van deze patiënten.

Hoofdstuk 7 zet uiteen dat insuline-geïnduceerde IGF-I normalisatie bereikt kan worden in 10 van de 11 acromegalie patiënten door eerste-generatie somatostatine-analogen te combineren met een eucalorisch ketogeen dieet. Hierbij vindt geen ongewenste toename van de GH concentraties plaats. De IGF-I en GH concentraties

blijven ook genormaliseerd in de zes patiënten die het dieet voortzetten voor drie maanden (inname van aantal koolhydraten wordt verhoogd van 35 gram naar 80 gram per dag). In drie van de zes patiënten was een dosisverlaging van de eerste-generatie somatostatine analogen mogelijk, en in twee daarvan was het zelfs mogelijk om de somatostatine-analogen therapie volledig te stoppen.

In **Hoofdstuk 8** wordt beschreven dat genetische varianten werden waargenomen in tien (71.4%) van de 14 gedifferentieerde schildkliercarcinomen en in twee (13.3%) van de 15 hypofyse adenomen. Er was geen overlap tussen de genetische varianten in beide weefsels van dezelfde patiënt. Aangezien er geen somatische *AIP*-mutaties worden waargenomen bij patiënten met een hypofyse adenoom die gelijktijdig een gedifferentieerde schildkliercarcinoom hebben, suggereert dit dat de bijdrage van een *AIP*-mutatie aan de tumorale pathogenese waarschijnlijk beperkt is. Met andere woorden, *AIP*-mutaties lijken onwaarschijnlijk de genetische oorzaak die predisponeert voor het hogere aantal gedifferentieerde schildkliercarcinomen in patiënten met acromegalie. In patiënten met een pathologische kiembaan *AIP*-variant, werd een van de *AIP*-varianten (c.853C>T; p.Q285*) bevestigd in het folliculaire schildkliercarcinoom weefsel van een familiaal geïsoleerd hypofyseadenoom (FIPA) verwant. Hierbij was ook sprake van een verlies van het wildtype *AIP*-allel op chromosoom 11q13, gebaseerd op de relatief hoge allel frequentie (83%) van de kiembaanmutatie in het tumor DNA. Dit opent een potentiële rol voor een *AIP*-mutatie als initiërende gebeurtenis, althans in *AIP*-mutatie-positieve FIPA-verwanten.

In **Hoofdstuk 9**, de discussie van dit proefschrift, worden de bovengenoemde hoofdstukken besproken, verbonden, en in de context van de huidige literatuur geplaatst. Voornamelijk de meest recente bevindingen in het onderzoeksveld dat zich richt op de medische behandeling van acromegalie, de methodologische valkuilen en toekomst perspectieven voor het onderzoeksveld zoals de rol van een ketogeen dieet als nieuwe behandelingsoptie worden besproken.



List of abbreviations

Erasmus MC PhD Portfolio

Publications not in this thesis

Word of thanks

About the author

List of abbreviations

AcroQoL	Acromegaly quality of life questionnaire
ACTH	Adrenocorticotrophin hormone
ADA	American Diabetes Association
ADH	Antidiuretic Hormone
AE	Adverse event
AIP	Aryl hydrocarbon receptor-interacting protein
AUC	Area under curve
BMI	Body mass index
CI	Confidence interval
CN	Cranial nerve
CTCAE	Common terminology criteria for adverse events
DM	Diabetes mellitus
DSMB	Data safety monitoring board
DPP4	Dipeptidyl peptidase 4
DTC	Differentiated thyroid carcinomas
EMA	European Medicines Agency
FDA	Food and Drug Administration
FFA	Free fatty acids
FFPE	Formalin-fixed, paraffin-embedded
FIPA	Familial isolated pituitary adenoma
FPG	Fasting plasma glucose
FSH	Follicular stimulating hormone
GH	Growth hormone
GHRH	Growth hormone releasing hormone
GHR	Growth hormone receptor
GLP-I	Glucagon-like peptide-I
GNAS	Guanine nucleotide-binding protein α -stimulating polypeptide
Gy	Gray
HbA1c	Glycosylated hemoglobin, type A1C
HE	Haematoxylin eosin
IGF-I	Insulin-like growth factor I

IGFBP1	Insulin-like growth factor-binding protein 1
IHC	Immunohistochemistry
IQR	Interquartile range
IRS	Immunoreactivity score
JAK2	Janus kinase 2 (P 106)
LAN	Lanreotide autogel
LAR	Long-acting release
LAS	Liège acromegaly survey
LH	Luteinizing Hormone
LOCF	Last observation carried forward
MRI	Magnetic resonance imaging
OGTT	Oral glucose tolerance test
PAPE	Pasireotide LAR and pegvisomant study
PASQ	Patient-assessed acromegaly symptom questionnaire
PCR	Polymerase chain reaction
PI3K	Phosphoinositide 3-kinase
PRL	Prolactin
PTC	Papillary thyroid carcinoma
QoL	Quality of life
SAE	Serious adverse event
SDS	Standard deviation
SE	Standard error
SRL	Somatostatin receptor ligand
SRIF	Somatotropin releasing-inhibiting factor
SST	Somatostatin
STAT5B	Signal transducer and activator of transcription 5B
TSH	Thyroid releasing hormone
TSS	Transsphenoidal surgery
ULN	Upper limit of normal
WHO	World health organisation
X-LAG	X-linked acrogigantism

Erasmus MC PhD Portfolio

PhD Portfolio

Name PhD student:	Eva Christine Coopmans
Erasmus MC dept:	Internal Medicine, section Endocrinology, Pituitary Center Rotterdam
Research School:	Netherlands Institute for Health Sciences (NIHES)
PhD period:	March 2017 – March 2020
Promotors:	Prof. dr. A.J. van der Lelij
Co-promotor:	Dr. S.J.C.M.M. Neggers

TRAINING	YEAR(S)
Courses	
Basic Course for Clinical Investigators (BROK)	2017
Biomedical English Writing Course	2017
Patient-Oriented Research (CPO) course: design, conduct and analysis	2017
Biostatistical Methods I: Basic Principles, part A (CCO2a)	2017
Microsoft Excel 2010: Basic & Advanced (Molmed)	2017
Photoshop and Illustrator CS6 workshop (Molmed)	2017
Indesign workshop (Molmed)	2017
Introduction in GraphPad Prism Version 6 (Molmed)	2017
Endnote and PubMed (and other databases) courses	2017
Basic course on R (Molmed)	2018
Research Integrity course	2018
Research skills	
Weekly research meeting Endocrinology Laboratory	2017 – 2020
Research monographs	
1. Article in national patient journal of the Dutch Pituitary Foundation 'Hyponieus' (November 2018) entitled 'New therapy effective for specific group of acromegaly patients'.	2018
2. Article in scientific journal 'Endocrinologie' for researchers and clinicians in the endocrinology field (September 2019, Jaargang 12) entitled 'Pasireotide can induce tumour necrosis in acromegaly patients'.	2019

3. Article in national patient journal of the Dutch Pituitary Foundation 'Hyponieus' (February 2020) entitled 'Can medical therapy with pasireotide LAR kill tumour cells'.	2020
4. Cover story on Amazing Erasmus MC website of the Erasmus Medical Center for the general public (June 2020) entitled 'Treating giant growth with a very strict diet'.	2020
5. Article on 'Voeding & Visie' website for the general public (June 2020) entitled 'Treating giant growth with a strict ketogenic diet'.	2020
6. Article in national patient journal of the Dutch Pituitary Foundation 'Hyponieus' (Augustus 2020) entitled 'Treating giant growth with a very strict diet'.	2020
7. Article in national journal of the Dutch Food and Dietician Journal ('Nederlands Tijdschrift voor Voeding en Dietetiek' (September 2020) entitled 'Treating giant growth with a strict ketogenic diet'.	2020

(Inter)national conferences

Endocrine disorders are prominent clinical features in patients with primary antibody deficiencies

ESE Summer School on Endocrinology (Berlin, Germany) – poster presentation	2017
5 th EYES Meeting (Porto, Portugal) – oral presentation	2017
JNVE conference 2017 (Leiden, The Netherlands) – oral presentation	2017
3 th International Primary Immunodeficiencies Congress 2017 (Dubai, United Arab Emirates) – poster presentation	2017
Internal Medicine Science Days 2018 (Antwerp, Belgium) – poster presentation	2018
Dutch Endocrine Meeting 2018 (Noordwijkerhout, The Netherlands) – oral presentation	2018
De Nationale werkgroep immuundeficiënties (WID) 2019 (Utrecht, The Netherlands) – oral presentation	2019

Efficacy and Safety of switching to Pasireotide in Acromegaly Patients controlled with Pegvisomant and Somatostatin Analogues: PAPE extension study.

Dutch Endocrine Meeting 2018 (Noordwijkerhout, The Netherlands) – poster presentation	2018
100 th Endocrine Society Annual Meeting (ENDO) 2018 (Chicago, USA) – oral presentation	2018
20 th European Congress of Endocrinology (Barcelona, Spain) – poster presentation	2019
6 th EYES Meeting (Poznan, Poland) – oral presentation	2019
T2-Signal Intensity, SSSTR Expression and Somatostatin Analogues Efficacy Predict Response to Pasireotide in Acromegaly	2019
101 st Endocrine Society Annual Meeting (ENDO) 2019 (New Orleans, USA) – poster presentation	2019
21 st European Congress of Endocrinology 2019 (Lyon, France) – oral presentation	2020

Eucaloric ketogenic diet as a new supportive treatment modality for acromegaly

Dutch Endocrine Meeting 2019 (Noordwijkerhout, The Netherlands) – oral presentation	
---	--

Clinical activities

Outpatient clinic for acromegaly patients (Clinical Research Unit)	2017–2019
European Pasireotide LAR Observational Study in Acromegaly (ACRONIS) – co-investigator	2017–2019

Symposia, seminars & workshops

Department of Internal Medicine/Erasmus MC lectures (Rotterdam, The Netherlands)	2017–2020
Erasmus MC PhD Day (Rotterdam, The Netherlands)	2017
Capital Course (Leiden, The Netherlands)	2018

9th Annual European Meeting on the Management of Acromegaly (Zagreb, Croatia)	2019
Early Career Forum before Endocrine Society Annual Meeting (ENDO) 2019 (New Orleans, USA)	2019
Dutch Pituitary Society Patient Day (Utrecht, The Netherlands)	2019
Career workshop ESE (Lyon, France)	2019
Teaching activities	
Teaching skills training 1 st -year medical students, subject: <i>Thyroid</i> Erasmus University Medical Center, Rotterdam, The Netherlands	2017
Teaching skills training 1 st -year medical students, subject: <i>Hypercortisolism</i> Erasmus University Medical Center, Rotterdam, The Netherlands	2018
Invited presentations	
<i>Pituitary disease</i>	
Plenary lecture 3 rd -year pre-medical students. Erasmus University College, Rotterdam, The Netherlands.	2019, 2021
<i>How to Position Pegvisomant and Pasireotide LAR Treatment in Acromegaly</i>	
Invited speaker (Invited by Prof De Marinis) at the 6th Annual Meeting on Pituitary Tumours (PIT-NET), Rome, Italy.	2019
<i>Medical treatment and diet options in acromegaly</i>	
Invited speaker (Invited by Prof M. Ruchata and Prof A. Syrenicz) at the XXII Congress of Polish Society of Endocrinology 2021, Szczecin, Poland.	2020
<i>Pituitary and adrenal cases.</i>	
Invited speaker (Invited by European Society of Endocrinology) at the 27 th ESE Postgraduate Training Course for Clinical Endocrinology, Diabetes and Metabolism, Bucharest, Romania	2020
<i>Eucaloric ketogenic diet as a new supportive treatment modality for acromegaly</i>	
Invites speaker (Invited by Italian Young Endocrinologists) at the 37th SIE congress 2021 in Rome, Italy	2020
<i>Opening EYES symposium and introducing EYES</i>	
Invited speaker (Invited by European Society of Endocrinology) at the European Congress of Endocrinology 2020, online congress.	2020
Supervising Bachelor's theses	
S.W.F. van Meyel, Erasmus University College (± 2 years)	2017–2019
N. el-Sayed, Erasmus University College (± 1.5 years)	2017–2019
Professional societies	
Abstract reviewer for ENDO 2019 Annual Meeting, New Orleans, November 2018 –Review panel member	2019
Abstract reviewer for ENDO 2020 Annual Meeting, San Francisco, November 2019 –Review panel member	2020
Chairing positions	
20 th European Congress of Endocrinology (ECE) – Acromegaly guided poster tour, Barcelona, Spain	2018

101 st Endocrine Society Annual Meeting (ENDO) – Pituitary Disease Markers and Case series oral session, New Orleans, USA.	2019
21 st European Congress of Endocrinology (ECE) – Miscellaneous Pituitary guided poster tour, Lyon, France	2019

Organisation of scientific meetings

ESE Summer School on Endocrinology 2018, Berlin, Germany	2018
Jonge Nederlandse Vereniging voor Endocrinologie (JNVE; Young Dutch Endocrine Society) Congress, Nijmegen, The Netherlands	2018–2020
22nd European Congress of Endocrinology (ECE) – EYES symposium, Prague, Czech Republic	2020

Board membership

Young Investigator Group Organisation team, part of ESE Summer School – started in May 2018	2018-2020
JNVE member of board, part of NVE – started in June 2018	2018-2020
EYES co-chair of board, part of ESE – started in August 2018	2018-2020
Member of the Communities of Practice Working Group, part of Endocrine Society – started in September 2018	2018-2020

PRIZES AND GRANTS

Prizes

Best Clinical Abstract Presentation Award for my oral presentation ' <i>Eucaloric very low-carbohydrate ketogenic diet as a new supportive treatment modality for acromegaly?</i> ', provided by the Dutch Endocrine Meeting during Dutch Endocrine Meeting 2020 Noordwijkerhout (The Netherlands).	2020
Prize: Interview, photoshoot and honourable mention on Dutch Endocrine Society	
Best Poster Case Presentation Award for my poster presentation ' <i>Excellent response to pasireotide therapy in an aggressive and dopamine-resistant prolactinoma?</i> ', provided by the EndoBridge during EndoBridge 2019 Antalya (Turkey).	2019
Prize: Complimentary registration for EndoBridge 2020	
Best Abstract Award in the Adult Pituitary Research Field for my poster presentation on ' <i>T2-signal intensity, SST receptor expression and 1st-generation somatostatin analogues efficacy predict hormone and tumour responses to pasireotide in acromegaly?</i> ', provided by the ENDO Today during the US Endocrine Society 2019 Annual meeting in New Orleans (US).	2019
Prize: Live-interview and honourable mention on ENDO Today website.	

Personal grants

Goodlife Healthcare Travel Grant granted by the Dutch Endocrine Society (NVE) to attend the US Endocrine Society 2019 Annual meeting in New Orleans (US) and give an oral presentation on ' <i>T2-signal intensity, SST receptor expression and somatostatin analogues efficacy predict response to pasireotide in acromegaly?</i> '	2019
Young Investigator Award granted by the Women in Endocrinology (WE) to attend the US Endocrine Society 2018 Annual meeting in Chicago (US), and give an oral presentation on ' <i>Efficacy and safety of switching to pasireotide in acromegaly patients controlled with pegvisomant and somatostatin analogues: PAPE extension study?</i> '	2018
Junior Doctor Travel Grant granted by the International Patient Organisation for Primary Immunodeficiencies (IPOPI) to attend the International Primary Immunodeficiencies Congress (IPIC) 2017 in Dubai (UAE) and give a poster presentation on ' <i>Endocrine disorders are prominent clinical features in patients with primary antibody deficiencies?</i> '	2017

FUNDING

European Joint Programme Rare Diseases Research Mobility Fellowship granted by the European Reference Networks	2021
Early Career Grant granted by the Society for Endocrinology, UK	2020
Prins Bernhard Cultuurfonds granted by the Crone-Haver Droeze Fonds, The Netherlands	2020
FEEL Rolf Gaillard Award and Bursary granted by the Union Européenne des Médecins Spécialistes of Endocrinology	2020
ESE Short-Term Fellowship Grant granted by the European Society of Endocrinology, UK	2020
Bioscientifica Trust Grant granted by The Bioscientifica Trust, UK	2020
Erasmus + Program (EPLUS) granted by the Erasmus University Medical Center, The Netherlands	2020
Jo Kolk financial support granted by Foundation of Woman in Higher Education, The Netherlands	2019
Stichting De Drie Lichten financial support granted by Stichting De Drie Lichten, The Netherlands	2019

Publications not in this thesis

1. **Coopmans EC**, Postma MR, Wolters TLC, van Meyel SWF, Netea-Maier R, Neggers SJCMM, van Beek AP. Predictors for Remission after Transsphenoidal Surgery in Acromegaly: A Dutch Multicenter Study. *J Clin Endocrinol Metab*, 2021. 106(6): p. 1783-1792.
2. Fuchtbauer, L, Olsson, D. S, **Coopmans, E.C**, Bengtsson, B.A, Norrman, L.L, Neggers, Sjcmm, Hellstrom, A, Johannsson, G. Increased number of retinal vessels in acromegaly. *Eur J Endocrinol*, 2020. 182(3): p. 293-302.
3. **Coopmans EC**, El-Sayed N, Frystyk J, Magnusson NE, Jørgensen JOL, van der Lely AJ, Janssen JAMJL, Muhammad A, Neggers SJCMM. Soluble Klotho: a possible predictor of quality of life in acromegaly patients. *Endocrine*, 2020. 69(1): p. 165-174.
4. **Coopmans EC**, van Meyel SWF, Pieterman KJ, van Ipenburg JA, Hofland L, Donga E, Daly AF, Beckers A, Van der Lely AJ, Neggers SJCMM. Excellent response to pasireotide therapy in an aggressive and dopamine-resistant prolactinoma. *Eur J Endocrinol* 2019; 181(2): 21-27.
5. **Coopmans EC**, Chunharojrith P, van der Ent MW, Barendregt BH, Swagemakers SMA, Hollink IH, van der Spek PJ, van der Lely AJ, van Hagen PM, Neggers SJCMM, Dalm VASH. Endocrine disorders are prominent features in patients with primary antibody deficiencies. *Frontiers in Immunology* 2019; 10: 1-9.
6. van der Lely AJ, Kuhn E, Muhammad A, **Coopmans EC**, Neggers SJCMM, Chanson P. Pegvisomant and not somatostatin receptor ligands (SRLs) is first-line medical therapy for acromegaly. *Eur J Endocrinol*, 2020. 182(6): p. D17-D29.

Word of thanks

De hypotheses, studies en interpretaties beschreven in de artikelen die dit proefschrift vormgeven zijn door het werk van een collectief tot stand gekomen. Ik hoop dat deze thesis, gefundeerd op deze gezamenlijke inspanningen een voortschrijdend wetenschappelijk inzicht kan faciliteren. Ik kijk er naar uit om ons werk te verbeteren, te repliceren en verdere stappen te zetten richting het leveren van wetenschappelijke handvatten voor de klinische praktijk.

Ik ben dankbaar voor alle hulp en steun die ik in de afgelopen jaren ontvangen heb. Ik zal mij beperken tot hen die een bijzondere stempel op mijn proefschrift hebben gedrukt.

Zonder twijfel wil ik de **patiënten** als eerste bedanken. Jullie toewijding en enthousiasme om deel te nemen aan wetenschappelijk onderzoek, ondanks jullie beperkte tijd, de reisafstand en het hebben van een chronische ziekte, is inspirerend. Ik heb erg van jullie genoten tijdens jullie deelname aan onze ketogeen dieet studie. Bedankt voor jullie leuke mailtjes, foto's van het dieet (en van wildlife die op de weg naar het ziekenhuis werd gefotografeerd) en bereidheid om jullie ongezoeten mening over het dieet te delen met mij als jullie arts en via social media. Ik vind het heel bijzonder en ben vereerd dat enkelen van jullie (op afstand) bij mijn verdediging aanwezig zijn.

Geachte **dr. Neggers, beste Sebastian**, in september 2013 mocht ik beginnen als onderzoekstudent bij jou en **dr. Dalm** op het onderwerp endocriene dysfuncties in patiënten met een primaire immuundeficiëntie. De samenwerking is mij destijds zo goed bevallen dat ik graag bij jou een promotietraject wilde starten. De afgelopen drie á vier jaar ben je mijn co-promotor en eerste aanspreekpunt geweest. Ik ben je dankbaar voor je vertrouwen en de vrijheden om zelf invulling te geven aan mijn onderzoek. Je hebt de (zware) taak gehad om mij te leren hoe ik mij diplomatieker kan opstellen. Daarnaast stond je altijd open voor nieuwe (en ludieke) ideeën. Je hebt mij gestimuleerd om presentaties op veel (inter-)nationale congressen te geven, wat ik ten zeerste heb gewaardeerd. Via jou ben ik o.a. in contact gekomen met *ESE Young Endocrinologists and Scientists (EYES)*, hier maak ik nog steeds met veel plezier onderdeel van uit. Ik bewonder je creativiteit en je belezenheid. Verder heb ik je leren kennen als een feestbeest met tevens een goed gevoel voor humor, en ik kijk dan ook uit naar onze verdere samenwerking.

Geachte **Prof. Dr. Van der Lelij, beste Aart-Jan**, de afgelopen jaren was je mijn promotor en mentor. Ik heb fijn met je samengewerkt en veel van je geleerd over vasten

en de rol van een ketogeen dieet. Bedankt voor je (altijd) constructieve feedback, je hebt daarmee een belangrijke stempel gedrukt op de artikelen in dit proefschrift. In jouw rol als bestuurder heb je mij kunnen inspireren om ook een bestuursfunctie te gaan bekleden bij *The European Society of Endocrinology*. Ik kijk nu al uit naar het volgende congres waarbij we onder het genot van een goed glas wijn op de dansvloer staan, jij beter danst dan ik, en mensen aan mij komen vragen of jij inderdaad dé AJ bent.

Ik wil graag de leden van mijn promotiecommissie, **prof. dr. Biermasz, prof. dr. Hofland, prof. dr. Vernooij, prof. dr. Bisschop, prof. dr. Clemens, dr. Daly, prof. dr. van Hagen en prof. dr. de Herder** bedanken voor de bereidheid zitting te nemen in de promotiecommissie en voor uw zorgvuldige evaluatie van mijn proefschrift.

Geachte **Drs. Blažević en Drs. Van der Valk, beste Anela en Eline**, mijn paranimfen. De afgelopen jaren heb ik jullie leren kennen als talentvolle collega's en waardevolle vriendinnen. Ik heb genoten van de koffiemomentjes maar ook van de meer serieuze borrels op congressen zoals de DEM, JNVE en feestjes zoals op DTRH, de Parade, IFFR en Eline's bruiloft. Daarnaast mochten we samen internationale congressen bezoeken in Lissabon en Berlijn. Met jullie mocht ik altijd sparren over nieuwe onderzoeksideeën, moeizame analyses en hoe te dealen met de "bazen". Ik waardeer jullie oprechtheid, doorzettingsvermogen en creativiteit. Jullie zijn vrienden voor het leven geworden en hopelijk worden we (opnieuw) weer elkaars directe collega's in de toekomst. Ik ben er trots op dat jullie mij een rebel vinden. Mooi om te horen dat jullie je wel eens de vraag stellen "Wat zou Eva doen" om zo ook een beetje de rebel in jullie los te maken ;).

Geachte **Drs. Visser, beste Jenny**, mijn mentor. Hartelijk dank voor je bereidheid om met mij te sparren over mijn toekomstige carrière, alsmede je hulp bij beursaanvragen. Ook heb je mij uitgenodigd om lid te worden van de *early-career special interest group* van de *Endocrine Society*, waar we samen nog steeds met veel plezier onderdeel van uitmaken. Ten slotte wil ik je ook bedanken voor de gastvrijheid op "jouw lab". Hopelijk zullen we nog een vruchtbare samenwerking hebben op het gebied van *sex differences*, AMH of PCOS. En ik pak graag nog een keer een film op het IFFR met je!

Dear dr. Daly, dear Adrian, many thanks for your carefull reading of almost all my research papers. Your corrections were superb and your input was always of high quality. Also a big thanks to the whole Liege group including **Prof. Dr. Beckers and dr. Petrossians** for the collaboration and opportunity to work with the LAS database.

Geachte **Prof. Dr. Van Hagen en dr. Dalm, beste Martin en Virgil**, bedankt voor de mogelijkheid om met de immunologie samen te mogen werken en onderzoek te doen. Ik heb mij ook een beetje als onderdeel van jullie onderzoeksgroep gevoeld met mijn deelname aan het IPIC congres in Dubai en een praatje in het UMCU voor alle Nederlandse immunologen. In het bijzonder wil ik Virgil bedanken voor zijn hulp als mijn mentor. De koffietjes waarin we kletsten over onderzoek, beurzen aanvragen maar ook privé zaken zoals de grote dartwedstrijden waar je graag heen ging staan mij nog steeds bij. Ik ben vereerd dat Martin deel wilt nemen aan mijn promotiecommissie en hoop dat onze paden nog eens mogen kruisen.

Honourable **Prof. dr. Korbonits, dear Marta**, many thanks for facilitating my first postdoctoral research encounters in London and infecting me with your enthusiasm for genetics in pituitary diseases. Your work and enthusiasm are very inspiring, as is your sincerity and passion. I consider this period as part of the foundation for further postdoctoral research and I look forward to our collaborative research efforts.

Geachte **Prof. dr. Pereira en Prof. dr. Biermasz, beste Alberto en Nienke**, bedankt voor het vertrouwen. Ik kijk erg uit naar mijn postdoctorale onderzoekspositie binnen jullie onderzoeksgroep. Ik hoop in jullie team te mogen leren hoe multidisciplinaire hypofysezorg in zijn werk gaat en hoe we zorgpaden voor patiënten met hypofysetumoren kunnen optimaliseren.

Beste **Nour en Sebastian**, mijn briljante onderzoekstudenten. Wat een geluk dat ik jullie tegen ben gekomen. Wat begon met een *Capstone* onderzoeksproject voor jullie bachelor is uitgemond tot heuse onderzoeksprojecten met 3 publicaties per persoon. Ik heb genoten van jullie leergierigheid en enthousiasme. Daarnaast hebben jullie mij bijgeschoold in het geven van prachtige presentaties met een levendige presentatiestijl. Het verbaasde mij dan ook niet dat jullie allebei de prijs voor de beste presentatie tijdens de JNVE hebben gewonnen. Zo trots als een pauw was ik als jullie met mij mee waren naar internationale congressen in Berlijn, Athene en Lyon. Ik ben trots op jullie en blijf graag jullie mentor.

Geachte **drs. Scheffers, van der Ven, van den Bosch, Jongasma, van Genuchten, Linda, Jelle, Eva, Myrthe, Wouter**, bedankt voor de ultieme Z5 flat *experience*. Niks was te gek op de Z5. Allemaal een eigen kamer (in tegenstelling tot onze profs), kerstboom in de gang versierd met bloedbuizen, eeuwige koffietjes en vrijdagmiddagborrels en met

als kers op de taart de uit de hand gelopen tequila avond, mijn afscheid voordat ik naar London vertrok. Jullie hebben mij geleerd hoe lekker Bobby's Gin is (met name uit mijn gecostumed eigen fles) en ik ga graag op de uitnodiging in om nog eens naar zo'n gin avond te gaan. Ten slotte zijn Linda en ik goede vriendinnen geworden en mag ik voldoende mooie feestjes op uitnodiging van Linda meemaken. Ik vind het tof om te zien hoe goed je het naar je zin hebt in het RotJong bestuur en ik wil graag nog veel mooie RotJong events met je beleven.

Alle collega's van het *Metabolism and Reproduction Lab*, **Patric, Aldo, Hans, Martin, Cobie, Anke, Bas, Selvetta, Guido, Keng, Karina, Ammar, Margreet en Loes**, bedankt voor de leerzame en gezellige tijd tijdens de laboratoriumbesprekingen op maandagochtend en de ENDO in Chicago en New Orleans. Ik heb veel geleerd van jullie feedback tijdens de presentatie van mijn data, maar minstens zo belangrijk is dat ik meer inzicht heb gekregen in het boeiende basaal metabool onderzoek dat jullie verrichten. Loes, jou wil ik speciaal bedanken voor jouw ludieke idee om een hardloop wedstrijd te rennen in New Orleans waarbij we om 08.00 AM finishte, daarna onbeperkt gratis bier mochten drinken, en ik 2^e prijs ontving voor mijn toptijd in mijn leeftijdsklasse (alleen maar omdat ik jou probeerde bij te houden)!

Geachte **Drs. van de Beukel, beste Anneke**, veel dank voor de koffietjes bij Starbucks om vol enthousiasme te sparren over de toekomst van EYES. Ik ben trots om nu in jouw voetsporen te mogen treden en middels als EYES co-chair. Ik hoop dat we elkaar nog vaak zullen treffen rondom endocrinologie aangelegenheden en daarbuiten.

Beste **Trudie Korpershoek-van Voorden**, bedankt voor de prettige samenwerking tijdens de acromegalie poli op vrijdagochtend. Tevens dank voor je interesse en ondersteuning van de ketogeen dieet studie. Hopelijk tot snel, als ik over een paar jaar weer terug ben in het Erasmus Medisch Centrum.

Beste **Karin van der Zwaan**, wat heb je veel voor me geregeld de afgelopen jaren. Dat is zeker een vermelding waard in dit boekje! Samen met Anneke (Hokke) hebben jullie o.a. de *Internal Medicine* dagen georganiseerd waar ik dankbaar aan heb mogen deelnemen.

Dear **EYES board members, dear Ljiljana, Ayse, Lina, Antoan, Luis, Daniele, Juan, Lubov and Walter**, thank you for the international collaboration and all the fun we had! It was a great pleasure to working on great ESE and EYES projects

such as the EYES Annual Meetings, EYES symposium and ESE Postgraduate course. And a big thanks to **Ayse**, my co-chair. I feel truly honored and grateful for the time spent within the ESE and EYES community.

Geachte **JNVE bestuur, Mariëtte, Dirk, Maxime, Mariska, Margreet, Mark, Charlotte, Anouk, Jose, Stan, Jiska en Evie**, bedankt de prettige nationale samenwerking binnen de JNVE. Ik ben trots op de professionalisering die we als team hebben aangebracht in de JNVE. Dit is terug te zien in onze zeer geslaagde JNVE congressen.

Alle overige coauteurs, **Joop Jansen, Tim Korevaar, Adrian Daly, Phillipe Chanson, Thierry Brue, Brigitte Delemer, Václav Hána Jr., Annamaria Colao, Davide Carvalho, Marie-Lise Jaffrain-Rea, Günter K. Stalla, Claudia Fajardo-Montañana, Albert Beckers, Patrick Petrossians, Federico Gatto, Sanne E. Franck, Ammar Muhammad, Nicole Erler, Julia Potorac, Patric Delhanty, Alof Dallenga, Ian Haitsma, Kirsten Berk, Folkert van Kemenade, Marij de Haan en Esther Korpershoek**, hartelijk dank voor jullie bijdrage aan dit proefschrift.

Lieve familie, Mam, Pap, Fraukje, Nadia en Koen, bedankt voor jullie onvoorwaardelijke liefde en steun. Frauk deze thesis draag ik op aan jou. Ik weet zeker dat je over de capaciteiten bezit om een promotie met succes af te ronden en weet dat ik super trots op je ben! Bedankt dat jullie je best deden om geïnteresseerd over te komen als ik weer eindeloze verhalen vertelde over groeihormoon tumoren en mijn patiënten. Lieve familie, we gaan er een feest van maken vanavond!

Geachte drs. Jansen, lieve Nico, we werden verliefd tijdens het begin van de Geneeskunde opleiding. We bleken voorbestemde geestverwanten te zijn. “*Alea iacta est*”, de teerling is geworpen is van ons op toepassing. Mijn uitleg volgt hieronder.

De machteloosheid van het menselijk bestaan is dat het nooit helder is of de keuze die op een bepaald moment gemaakt wordt de beste is. Maar zonder de aangeboren vrijheid en de verantwoordelijkheid die deze met zich meebrengt om keuzes in ons leven te maken zou ons leven zinloos zijn. Jij en ik maakte de keuze om onderzoek te gaan doen, een promotietraject aan te gaan en naar Londen te verhuizen. Dit lot betekende voor ons dat we tijdens het avondeten of de afwas met elkaar van mening wisselde over onze onderzoeken. Tijdens deze sessies werd de nodige kritiek geuit maar werden ook cruciale wijzigingen (met name door jou) aangebracht in de lopende studies. Ook was het jouw lot als *native speaker* om mij een beetje te ondersteunen. Zo las en corrigeerde jij

geregeld mijn artikelen en grants, met als spektakel het artikel wat gepubliceerd werd in de *Lancet Diabetes & Endocrinologie*. Het was mijn lot om slechts een klinische PhD te doen, terwijl het “echte onderzoek” (en denkwerk) door jou in het basale onderzoek plaatsvond. Ik maakte samen met jou de keuze om een postdoctoraal positie in Londen aan te gaan om daar met acromegalie muizen te gaan werken. Jij zou een halfjaar later na mij volgen en daar ook starten als postdoc. Ons avontuur om samen te emigreren naar Londen. Dat ons verblijf in Londen anders verliep dan hoe wij het ons hadden voorgesteld, is daarmee juist bewijs van onze vrijheid. Wij hebben onze verantwoordelijkheid op ons genomen en in het moment in vrijheid de keuze gemaakt die wij op dat moment prioriteit achtten. Een belangrijke vermelding is dat je mij zonder te mopperen weer met auto en aanhangwagen voor twee fietsen kwam ophalen uit hartje Londen.

Jij bent het allerbelangrijkste voor mij in het maken van deze (uitdagende) keuzes. Ik meen dat ik kan ervaren hoe de blik van de Ander (lees: Nico) mij in bepaalde mate gerust kan stellen, omdat dit het bewijs vormt tegen een solipsistisch wereldbeeld dat het subject zelf gecreëerd heeft en waaraan alleen het ik betekenis geeft. De Ander die mij zijn blik schenkt heeft de bekwaamheid om mij als Ander in de wereld te plaatsen, mijn subjectieve perspectief te ontnemen en mij te zien als object waaraan hij eigenschappen kan toekennen en dat hij kan beoordelen. In eerste instantie zal ik denken dat de ogen en evaluatie van de Ander mijn vrijheid zullen ontnemen, maar later realiseerde ik mij dat, hoewel ik onvrij ben ten opzichte van de blik van de Ander, ik die blik juist nodig heb om vrij te zijn ten opzichte van mijzelf, en om bevestigd te worden als een belichaamd wezen dat in de wereld verkeert. Zo kan de Ander mij een spiegel voorhouden en mij als mens ten opzichte van een ander mens laten voelen. Met alleen de eigen blik meen ik dat de mens verloren zou zijn; ik heb de blik van de Ander nodig om mijzelf erkend en herkend te voelen.

Bedankt Nico dat je samen met mij deze keuzes wilt maken en de Ander voor mij wilt zijn.

About the author

Eva Coopmans was born on April 17th 1991 in Zwijndrecht, the Netherlands. She completed high school at the Walburg College Zwijndrecht in 2009 and started medical school at the Erasmus University Medical Center in that same year.

She started her research internship during the first year of her Master in Medicine within the divisions of Endocrinology and Immunology (Erasmus University Medical Center). She conducted in six months a prospective study that has led to a new consensus that assessment of endocrine axes should be incorporated in clinical management of patients with common variable immune deficiency and she published this as first author in 'Frontiers in Immunology'. She received the faculty award for the 'Best Master Thesis higher degree research student' and she was invited as a speaker at the Dutch Internal Medicine Science Days in Antwerp, Belgium.

Her interest in Endocrinology, particularly in pituitary diseases, lead her to continue my research in a PhD project within the group of Prof van der Lely. The thesis explores the therapeutic efficacy of somatostatin analogues and in particular pasireotide in acromegaly patients via analysis of large datasets from international multicentre cohorts and a large-scale phase IV study. She considers the conception of a proof-of-concept study applying a ketogenic diet in acromegaly patients, a potential new treatment option, as her most outstanding personal achievement.

Having completed the clinical research part of her PhD and currently in writing-up status, she joined the group of Prof Korbonits in London to close the knowledge gap between her clinical research experience and translational science aspects which she is also interested in. In London, she has gained preliminary data on body length and IGF-1 levels during somatostatin analogues treatment in *Aip*- and *Gnas*-mutated mice.

Unfortunately, she had to interrupt her stay in London due to the COVID-19 pandemic.

Currently, besides her full-time job as a resident not-in-training at Leiden University Medical Center, she joined the research group of Prof Pereira Arias and Prof Biermasz as a research fellow.

