PITUITARY GIGANTISM

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Pituitary Gigantism

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Sous la direction du
Professeur Albert Beckers
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Université de Liège

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Illustration on cover (by author) inspired by a contemporary publicity poster from the historical records kept in the *Musee des Sciences Naturelles*, Mons, Belgium.

A pencil drawing depicting a historical case of pituitary gigantism, Julius Koch (his stage name was Giant Constantin, or *Le Géant Constantin*), who reached up to 2.59 m in height as a result of an early-onset overgrowth due to pituitary tumor. A paleogenetic study (presented in Chapter 14) of >100 year-old DNA obtained from bone was consistent with a diagnosis of X- linked acrogigantism syndrome, thereby making him the tallest individual with pituitary gigantism in whom a genetic diagnosis of gigantism has been established.

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To my parents Arminé and Hrant

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SUMMARY

Pituitary gigantism is a rare but important form of overgrowth due to GH/IGF-1 excess. The initial aim of the present research was to design and implement a comprehensive cohort study of the etiology, clinical diagnosis and management of this rare disease. This work describes the findings of an international collaborative study involving the largest pituitary gigantism population described to date (208 patients) from 47 centers across the globe to explore the specific characteristics of these patients and the genetic background of pituitary gigantism. Overall, the work undertaken has permitted us to identify the clinical phenotype and treatment outcomes in patients with pituitary gigantism; these features differ significantly from those in adult somatotropinoma patients with acromegaly. Patients with gigantism presented clear a male predominance (95%) and differ in their presentation based on gender, with females presenting significantly earlier than males. Increased somatic growth in pituitary gigantism is associated with an early onset form of GH/IGF-1 hypersecretion due to pituitary tumors that are highly resistant to treatment. These characteristics point to specific molecular mechanisms in pituitary tumor formation. Until recently, pituitary gigantism has been a well-known disease but poorly understood from genetic point of view. Underlying genetic causes have been studied comprehensively and identified in half of the cases in our large international series. While complex multi-organ syndromes (such as McCune-Albright syndrome (MAS), MEN1 and Carney Complex) counted only for rare cases of pituitary gigantism (7% in total), the most frequent genetic etiologies appear to be those leading to disease isolated to the pituitary, such as *AIP* mutations (29%) and X-linked acrogigantism syndrome (X-LAG) (10%). The latter is a new genetic form of infant-onset acrogigantism, occurring sporadically and in familial setting, which was described for the first time during the course of this work. X-LAG remains rare and only about 33 genetically confirmed cases have been published to date.

X-LAG is a dramatically aggressive disorder affecting children from a very young age (usually during the first year), who are predominantly female (70%). Despite the very young age at disease onset, X-LAG patients develop large pituitary lesions (frequently mixed GH and prolactin secreting adenomas and/or hyperplasia) with extremely elevated hormonal levels. This contributes certainly to excessively rapid somatic growth leading to severe overgrowth. The remarkable phenotype of X-LAG syndrome is underlined by an unusual genetic mechanism; it is due to a microduplication

on Xq26.3 including always *GPR101* gene, whereas previously described genetic mechanisms in pituitary tumorigenesis are mainly triggered by a point mutation or deletions in a single gene. Additionally, a novel genetic technology (digital droplet PCR (ddPCR)) revealed that males with X-LAG syndrome can be mosaics for the *GPR101* duplication, and as few as 16% of duplicated cells could lead to severe overgrowth. Many of the tallest giants in history had a clinical history that exactly mirrors this phenotype. The molecular diagnosis of X-LAG due to a duplication in *GPR101* was made using paleogenetic extraction techniques in combination with modern ddPCR on DNA successfully isolated from the century-old remains of the historical case of *The Giant Constantin* (2.59m) who had autopsy findings of a pituitary adenoma. It can be considered as the tallest genetically proven case of gigantism available.

It was also noted that more than 50% of cases remain genetically unexplained. Importantly, these genetic subgroups have statistically significant differences in terms of features at presentation/diagnosis, however all pituitary giants, including the genetically negative group, have aggressive clinical characteristics.

Further studies were focused on the association of genetic events, in particular *AIP* mutations, with the aggressive phenotype of somatotropinomas that are resistant to conventional treatment. The clinical experience in patients with pituitary gigantism that have failed previous therapy with first generation somatostatin analogues, showed the role of other treatment options (pegvisomant, paseriotide) in hormonal and tumoral control in genetically negative and *AIP* mutated cases.

A severe disease burden was highlighted in a comprehensive autopsy and genetic analysis in an adult male patient with a complex clinical profile of MAS including pituitary gigantism. The pathological findings and the presence of *GNAS1* mutation in a mosaic state in different endocrine and non-endocrine tissues, combined with the clinical description of this case in the medical records, illustrated the challenges in treatment and consequences of disease activity.

Crucially, the results derived from our large pituitary gigantism cohort and our further studies in specific genetically predisposed forms (such as X-LAG, *AIP* mutation– or MAS– related cases) pointed out that pituitary gigantism is a severe therapeutic challenge, requiring a multimodal treatment approach. However, one of the major findings of our research shows that early recognition and effective management in terms of sustained hormonal control and pituitary tumor shrinkage are essential for limiting the pathological effects on height and multi-organ disease burden.

RÉSUMÉ

Le gigantisme hypophysaire est une forme rare d'une surcroissance importante due à l'excès de GH et IGF-1. Le but initial de nos travaux était d'organiser une étude complète sur l'étiologie, le diagnostic clinique et la prise en charge de cette maladie rare. Nous décrivons les résultats d'une collaboration internationale impliquant la plus grande population de gigantisme hypophysaire décrite à ce jour (208 patients) de 47 centres à travers le monde pour explorer les caractéristiques spécifiques de ces patients et le contexte génétique du gigantisme hypophysaire. La présentation clinique montre une maladie sévère et invalidante qui affecte généralement la population jeune (enfants, adolescents et jeunes adultes). Dans l'ensemble, ce travail a permis d'identifier le phénotype clinique et les résultats du traitement chez les patients atteints de gigantisme hypophysaire; ces caractéristiques sont différentes de celles bien établies chez les adultes atteints d'acromégalie due à une adénome somatotrope. Les patients atteints de gigantisme présentaient une nette prédominance masculine (95%) et différaient dans leur présentation en fonction du sexe, les femmes se présentant significativement plus tôt que les hommes. Une croissance accrue est associée à une forme précoce d'hypersécrétion de GH / IGF-1 due à des tumeurs hypophysaires très résistantes au traitement. Ces caractéristiques sont les conséquences des mécanismes moléculaires impliqués dans la formation de tumeurs hypophysaires. Jusqu'à très récemment, le gigantisme hypophysaire était une maladie bien connue visuellement, mais mal comprise du point de vue génétique. Les causes génétiques ont été étudiées et révélées dans presque la moitié des cas dans notre grande série internationale. Alors que les syndromes complexes multi-organes (tels que le syndrome de McCune-Albright (MAS), NEM1, et Complex du Carney) ne comptaient que pour de rares cas de gigantisme hypophysaire (7%), les étiologies génétiques les plus fréquentes semblent être celles conduisant à adénomes hypophysaire familiaux isolées (FIPA), comme les mutations *AIP* (29%) et le syndrome du X-linked acrogigantism (X-LAG) (10%). Ce dernier est une nouvelle forme génétique d'acrogigantisme infantile, apparaissant de façon sporadique et familiale, décrite pour la première fois dans ce travail. L'X-LAG reste une maladie rare et seulement environ 33 cas confirmés génétiquement ont été publiés à ce jour. X-LAG est un maladie extrêmement agressive affectant les enfants dès leur plus jeune âge (généralement au cours de la première année), avec une prédominance chez les femmes (70%). Malgré le jeune âge au début de la maladie, les patients X-LAG développent des grandes lésions hypophysaires (ce sont souvent des adénomes mixtes qui sécrètent de la GH et de la prolactine et/ou une hyperplasie) avec des taux hormonaux extrêmement élevés. Ceci contribue certainement à une croissance excessivement rapide conduisant à une taille finale extrême. Le phénotype remarquable de l'X-LAG est dû à un mécanisme génétique inhabituel;

il est dû à une microduplication sur Xq26.3 incluant toujours le gène *GPR101*, alors que les autres mécanismes génétiques bien décrits précédemment dans la tumorigenèse hypophysaire sont déclenchés par une mutation ponctuelle ou des délétions dans un seul gène. De plus, une nouvelle technologie génétique (digital droplet PCR (ddPCR)) a révélé que les mâles atteints du syndrome X-LAG peuvent être des mosaïques pour la duplication *GPR101*, et que 16% seulement des cellules dupliquées pourraient conduire à une croissance extrême. Les plus grands géants de l'histoire avaient une présentation clinique qui reflète exactement ce phénotype. Le diagnostic moléculaire de X-LAG dû à une duplication du *GPR101* a été fait par la technique paléogénétique en combinaison avec le ddPCR moderne sur l'ADN obtenu à partir du squelette centenaire d'un cas historique du *Géant Constantin* (2.59m) et qui a eu un adénome hypophysaire selon les résultats d'autopsie. Compte tenu de la description clinique de ce cas dans les archives historiques, il peut être considéré comme le plus grand cas de gigantisme génétiquement prouvé disponible.

Plus de 50% des cas restent génétiquement inexpliqués. Les groupes de géants avec des causes génétiques différentes et ceux qui ont été génétiquement négatifs, présentent des caractéristiques distinctes au diagnostic, mais tous les géants hypophysaires, y compris le groupe génétiquement négatif, ont un phénotype agressif. Des études ultérieures se sont concentrées sur l'association d'événements génétiques, en particulier de mutations *AIP*, avec le phénotype agressif du somatotropinome résistant au traitement conventionnel. L'expérience clinique chez les patients atteints de gigantisme hypophysaire qui n'ont répondu au traitement antérieur avec des analogues du somatostatine de première génération, a montré le rôle d'autres options thérapeutiques (pegvisomant, paseriotide) dans le contrôle hormonal et tumoral dans les cas génétiquement négatifs et *AIP* mutés.

La morbidité sévère a été mise en évidence lors d'une autopsie complète et d'une analyse génétique chez un patient adulte avec un profil clinique complexe de MAS géant. Les résultats pathologiques et la présence de la mutation *GNAS1* dans un état mosaïque dans différents tissus endocriniens et non endocriniens, combinés avec la description clinique de ce cas dans le dossier médical, ont illustré les défis du traitement et les conséquences de l'activité de la maladie.

Fondamentalement, les résultats de notre grande série de patients atteints du gigantisme hypophysaire et nos études ultérieures dans des formes spécifiques génétiquement prédisposées (comme les cas X-LAG, *AIP* positifs ou MAS) ont montré que le gigantisme hypophysaire est un challenge thérapeutique nécessitant une approche de traitement multimodal. En plus, nous avons montré qu'une reconnaissance précoce et une prise en charge efficace en termes de contrôle hormonal constant et de diminution du volume de tumeur hypophysaire sont essentielles pour limiter les effets pathologiques sur la taille finale et la charge de morbidité multi-organes.

LIST OF ABBREVIATIONS

3PAs	Association of PA and pheochromocytomas/ paraganglioma (3P Association)
17-OHP	17- hydroxyprogesterone
aCGH	Array-based comparative genomic hybridization
ACTH	Adrenocorticotropic hormone
AHR	Aryl hydrocarbon receptor
AIP	Aryl hydrocarbon receptor-interacting protein
ALS	Acid labile subunit
ARNT	Aryl hydrocarbon receptor nuclear translocator
cAMP	Cyclic adenosine monophosphate
ATP	Adenosine triphosphate
BMI	Body mass index
CCND1	Cyclin D1
CDC73	Cell cycle division 73
CDKN	Cyclin-dependent kinase inhibitor
CNC	Carney complex
CNV	Copy number variation
DA	Dopamine agonist
ddPCR	Digital droplet polymerase chain reaction
DHEAS	Dehydroepiandrosterone sulfate
DNA	Deoxyribonucleic acid
GADD	Growth arrest and DNA damage-inducible protein
$G\alpha_i$	Inhibitory Gα protein
GH	Growth hormone
GHBP	GH-binding protein
GH-R	GH-receptor
GHRH	Growth hormone releasing hormone
GHRH-R	Growth hormone releasing hormone receptor
Ghrhr	Murine growth hormone releasing hormone receptor gene
GHS-R	GH-secretagogue receptor
GnRH	Gonadotropin releasing hormone
gsp	Stimulatory G-protein
HSP90	Heat shock protein 90
IGF	Insulin-like growth factor
IGF-1R	IGF-1 receptor
IGFBP	IGF-binding protein
FIPA	Familial isolated pituitary adenoma
FSH	Follicle stimulating hormone
JAK2	Janus kinase 2
LAR	Long acting release
LAS	Liege acromegaly survey
LH	Luteinizing hormone
MAPK	Mitogen activated protein kinase
MAS	McCune-Albright syndrome
MAX	MYC-associated factor X
MEN	Multiple endocrine neoplasia
miRNA	Micro ribonucleic acid
MLPA	Multiple ligation probe amplification
MRI	Magnetic resonance imaging
NFPA	Non-functioning pituitary adenoma
OGTT	Oral glucose tolerance test
p23	Co-chaperon encoded by prostaglandin E synthase 3 gene
PA	Pituitary adenoma
Pit-1	Pituitary-specific transcript factor 1

PKA	Protein kinase A
PKC	Protein kinase C
PPNAD	Primary pigmented nodular adrenocortical disease
PRKACB	Protein kinase A catalytic subunit B
PRKAR1A	Protein kinase A regulatory subunit 1A
PROP1	Prophet of Pit-1
PTTG1	Pituitary Tumor Transforming Gene 1
R	Receptor
SD	Standard deviation
SDH	Succinate dehydrogenase complex flavoprotein
SSA	Somatostatin analogue
SST	Somatostatin receptor
STAT	Signal transducer and activator of transcription
T4	Thyroxine
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
X-LAG	X-linked acrogigantism
XRE/DRE	Xenobiotic- /dioxin-response elements
ZAC1	Zinc finger regulator of apoptosis and cell cycle arrest

TABLE OF CONTENTS

GENERAL INTRODUCTION

Chapter 1: Historical overview

1.1 Cultural aspects

Giants have always fascinated due to their unusual appearance and have produced among the general public feelings of wonder and curiosity, bordering on admiration or fear.

1.1.1 Giants in myths, legends and epics

Creatures with an astounding body size appear in myths and legends across many cultures (Figure 1.1; Appendix - Table s1). The Assyrian cuneiform tablets have allusions to the giant Izdubar- Gilgamesh, "towering above people like cedar above bushes"(1). The word *gigantism* is derived from Ancient Greek Γίγαντες (gígantes) - heroes with extraordinary large body size and superhuman strength who are common personages in Ancient Greece mythology (2). Literally all ancient written accounts that have come down to us - the Bible, Avesta, Veda, Edda, Chinese and Tibetan chronicles, etc., all mention the presence of giants or other characters of extraordinarily large size.

Figure 1.1 Twin brothers Sanasar and Baghdasar, in the Armenian national epic "Daredevils of Sassoun" they are the ancestors of several generations of heroes, all with outstanding physical stature and abilities. Illustration by M. Sosoyan (1984)(3)

In almost all cosmogonies, there is a story of the primordial giant(s), whose bones, flesh and blood constitute the world and everything in the Universe, or explain parts of the landscape and natural phenomena, like the giant Mimas breathing out through the volcano

Vesuvius. In most religions of antiquity (e.g. Greco-Roman polytheism), the pantheon of gods and goddesses includes giant characters, which are frequently the personification of powerful natural forces, such as floods, storms or avalanches and reflect the frailty of people in the face of the unrestrained power of Nature.

Ambivalence about the concept of large size, which can awake feelings of fear as much as admiration, is fundamental in the universal appearance of giants in many cultures. This is mirrored in the popular tradition of depicting as giants both threatening beings as well as protector heroic characters. Symbolic reflection of evil and danger comprises the negative aspect of giants, which are depicted in this setting as fearsome in appearance. Martial glory reflects another familiar trope of giants linked to foundation or defense of a city, and who are supposed to protect its population from enemy invasion, as well as from evil spirits and natural hardships. Heroic narrative of battles between giants can be deeply rooted in local history and reflect many real historical events (Appendix - Table s1).

1.1.2 Giants in visual arts, literature and folklore

Real and imaginary giants are plentifully depicted in literature and visual arts for all epochs. The famous Lemuel Gulliver is the main character of "Gulliver's Travels" by Jonathan Swift, whose name we use in a figurative sense, implying a giant. Folklore and fairytales of many countries contain many impressive giants' stories. People dream about legendary Seven-league boots, admire giant-heroes and their intriguing feats, and all are terrified by cruel giant monsters and ogres. Some of them could be the ancestral memories of real-life large and powerful people who lived long time ago in the same location. A prehistoric skeleton of a man who measured 2.4m in life was unearthed in the St. Michael's Mount in Cornwall, where according to a story from Britain's chronicles (then transformed into a nursery tale "Jack and the Beanstalk"), a terrible giant Cormoran was trapped and defeated by Jack-the-Giant-Killer.

1.1.3 Processional giants

A tradition of giants in processions and carnivals' corteges appeared in Western Europe at the end of the 14th century. One of the first to appear was the figure of St. Christopher (Antwerp, 1398), famous for assisting people to cross a dangerous river due to his size (2.3m) and strength and then found himself in great difficulty to reach the other side when he carried on his shoulders a very heavy child later discovering that this was Christ. His wicker or wood mannequin was a part of the traditional cortege with the clergy, trades and magistrates.

The morphology and behaviors of the processional giants are generally considered in the context of the giants described in medieval texts, in scriptures or classical mythology. Gigantic figures of Goliath and Madame Goliath became a part of processions in many cities throughout the Western Europe, becoming the most noble processional giants. Various new processional giants appeared from local legends, history or representing the typical professions of the region. Local celebrities (such as Jean Bihin de Venders) were known colossi during their lives (Figure 1.2), whereas others were attested with significant degree of "gigantification" of their normal physical appearance to attract the crowds (4). Initially, gigantic figures appeared in street-shows as a means of dramatic expression that promoted their popularization. Further transformation of processional giants as the symbol of celebration and triumph, led to the giants' tradition being preserved.

Figure 1.2 The famous Belgian giant, Jean Antoine Bihin, le Géant de La Reid, depicted in the engraving (left) and a photo of a processional giant in his effigy. Bihin travelled all over Europe and North America starring at different shows and became a celebrity in the 19th century due to his height of 2.43m.
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1.1.4 Artefacts and giants' fossil findings

Besides thousands of legends of giants, there are numerous accounts of cases with large body size and the features related to acromegaly, reported in historical and archeological record. These historical personalities were frequently depicted by artists, and some are well described by chroniclers. Their physical appearance, abilities and deeds were frequently exaggerated as the accounts of them pass into legend.

One of the oldest historical descriptions of acro-gigantism is linked to the figure of Gaius Julius Verus Maximinus "Thrax", the Roman emperor from 235 to 238 AD, who was described as a "human mountain" - a man of extraordinarily great size (over 2.5m) and superhuman strength. A coin of his time shows his profile with perfect aspect of

acromegalic deformities: marked mandibular prognathism, large nose and prominent brow (5-7).

Examination of rare skeletal remains of inordinate size from archaeological excavations revealed some historical cases with signs of skull deformities and other abnormalities suggestive of pituitary disease (8-11). Recent assessment of remains of the Egyptian pharaoh Sa-Nakht, who lived 5,700 years ago, established him probably as the oldest paleontological acro-gigantism case (12).

1.1.5 Giants in royal courts

Dwarfs and giants were frequently adopted by the rich and kept at the palaces for amusement. In the 17th century, the Welsh giant William Evans, who reportedly had a height of 2.29m, became a porter to King Charles I of England and joined the collection of "The Royal Menagerie of Curiosities and Freaks of Nature". He was displayed in a pair with a tiny Jeffrey Hudson, a proportionate dwarf, in order to entertain at court by a contrast in size of these two anatomical anomalies.

Due to their abilities and strength giants also attracted the attention of kings and nobles in order to be used as their personal bodyguards. Another tall man from the Royal collection, the Cornish giant Antony Payne, measuring 2.24m, served as a personal bodyguard of the King Charles I. At the beginning of the 18th century, very tall men were selected throughout the Europe by the King of Prussia, Frederick William I, for his famous army of giants *(Giants from Potsdam)*. Many of them were forcibly recruited in other countries and sent to Friedrich Wilhelm I in order to encourage friendly relations. The notorious Giant Bourgeois from France, who measured approximately 2.27m, tried in every way to avoid being recruited in the Potsdam Army, but then his formidable size and abilities gained him the attention of Russian King Peter the Great, who hired him and brought to Russia as a personal bodyguard (Figure 1.3).

Figure 1.3 The skeleton of Giant Bourgeois, preserved in Peter's the Great Museum of Anthropology and Ethnography (Kunstkamera), Sankt-Petersburg, Russia. On the picture in the left bottom part, his enlarged heart is demonstrated next to the normal-sized human heart. Photos from author's collection.

After his death at age of 42, his body was dissected and studied by Dr. John Arnutia Acarithi, who described deformations of the skull, hypertrophied epiphyses of tubular bones, large internal organs (heart, stomach) and small testis. The extensive description of his body examination bear witness to an early scientific interest to gigantism.

1.1.6 Giants in shows and sports

Known as an attribute of heroes from legends and origin stories, great stature of real individuals was perceived by people as a visual spectacle and led to public fascination. This led many giants, the Irish Cornelius Magrath (2.36m), Charles Byrne (2.31m) and Patrick Cotter O'Brien (2.44m), the Russian Feodor Machnow (2.38m) and the Chinese Chang Yu Sing (2.44m) to be promoted to exhibit themselves for profit in various attraction shows and spectacles in circuses. For these shows, it was always of great importance to exhibit the tallest giants and the data on height measurements were therefore cherished as a trademark.

People with gigantism have struggled also with unwanted attention, as well as prejudice and discrimination due to their outstanding body size. Particularly notorious is the case of the tallest human in history, Robert Pershing Wadlow, the Alton giant (1918 – 1940), who reached 2.72m in height. Medical records and visual evidence make him irrefutably the tallest person ever, who did not stop growing until his death at age of 22 (13, 14).

Because of superior body size, tall individuals have advantages in some competitive sports. Sport federations and committees chronicled a number of giants, like the French professional wrestler and actor, André The Giant (2.24m). A Belgian giant, Fernand Bachelard (Figure 1.4), known as *Le Géant Atlas* (2.35m), performed at wrestling tours, where he was never beaten (15).

Figure 1.4 The Belgian giant, Fernand Bachelard, Le Géant Atlas (2.35m), who ran a famous cafe with his name "Au Géant Atlas" in Bon-Secours, Belgium. Postcard from around 1960 depicting Fernand and his mother in Café au Géant Atlas, reprinted with permission from La Belgique d'Antan.

In the past, few suspected that what was admired was actually a disabling disease. After their death the huge skeletons of giants frequently received the attention of anatomists and ended up in museums.

1.2 History of studies of human growth

The history of growth studies begins in antiquity, in the 6th century BC, when human growth was described by **Solon the Athenian**, who was a Greek statesman, lawmaker and poet. He divided the human life cycle into ten stages, "*hebdomas*", each of which consisted of seven years, where he gave an accurate description of the growth process from infancy to adulthood. Nevertheless, the first anthropometric data only appeared in the 17th and 18th centuries, and it was mainly the systematic measurements of men recruited into European armies (Figure 1.5) (16, 17). The data on height along with date of birth and parents' occupation, were collected in all the Merchant Navy and Royal Navy recruits from 1786 for several decades and allowed then to evaluate the secular trends of the height and the study of social and economic conditions of this specific group of individuals in English society at the end of the 18th and 19th centuries (16, 17).

Figure 1.5 Sketch of the measurement of recruits for the Duke of Sachsen-Weimar's army by Johann Wolfgang von Goethe (1779; Goethe National Museum, Germany) (16).

The first longitudinal study was done by **Philibert Gueneau Montbeillard**, who measured his son from birth to adulthood between 1759 and 1777. These measurements were published as a supplement to the Natural History of Georges Buffon in 1777 (16, 18). The first cross-sectional study was that of the anatomist **Christian Friedrich Jampert** in Germany. He measured a series of children and young people aged from 1 to 25, from the Royal Berlin Orphanage, and published in 1754 the first tables on height measurements arranged by sex and age group (17).

The Belgian statistician **Lambert-Adolf-Jacques Quetelet** (1796-1874) contributed greatly to the study of growth in terms of statistics (19). He was the first to observe the distribution of the height of the conscripts "in the form of a hat", known later as normal distribution (Figure 1.6).

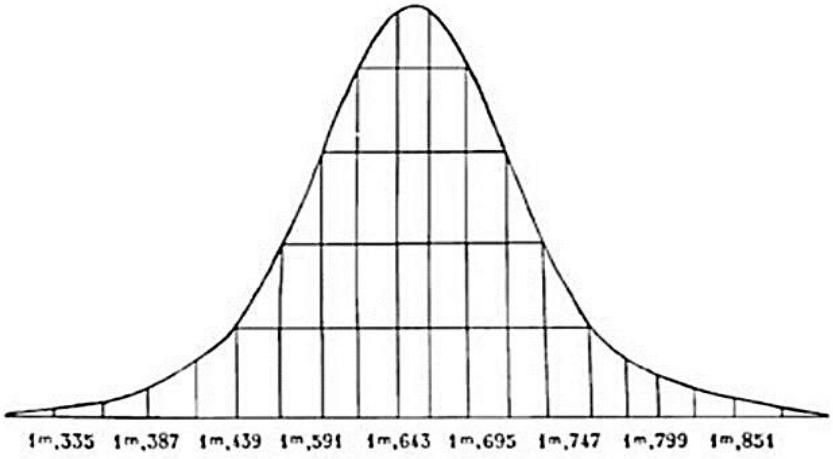


Figure 1.6 The height of Belgians aged from 18 to 20 years. Reprinted from "*Physique sociale ou Essai sur le développement des facultés de l'homme*" *(p.355), by A. Quetelet, 1997 [1869], Brussels: Académie Royale de Belgique. Copyright 1997 by Académie Royale de Belgique. Reprinted with permission (20).*

He also observed the Gaussian error around "the average height", then defined as standard deviation. He also noted the relationship between nutrition and growth from the weight and height data in newborns at the Maternity Hospital in Brussels.

In the United States, a series of studies began on the growth of public and private college students employing modern statistical methods. **Henry Bowditch** applied Galton's percentiles around the mean height from the late 19th century and was the first to publish reference curves in 1885 (16). Finally, the graphic charts with ±1 and ±2 deviation curves around the mean height were suggested for growth monitoring separately in girls and boys by **Brailsford Robertson** (21, 22).

Later, in France, **Michel Sempe** and his collaborators carried out a longitudinal study following the same subjects since birth (mostly during the years 1953 and 1954) until the end of adolescence. This study resulted in the development of tables and charts published in medical journals that were widely used by pediatric practitioners and school health services (23).

Evolution of scientific knowledge on human growth includes the major works of the British pediatric endocrinologist **James Mourilyan Tanner**, who is known primarily for his invention of a scale of measurement of the different stages of sexual development during puberty (the Tanner scale) and modern growth charts (24). He also studied the

impact of genetic and environmental factors on children's growth and the effects of early growth hormone use in significant growth delay (25, 26). His impact in the development of growth studies can be considered as one of the most important in modern auxology (27). Tanner also pointed to the influence of inheritance and environment on normal growth mechanisms, setting the stage for relating anthropometric abnormalities to the larger physiological problems of growth and development. In this context, extraordinary height was no longer interpreted as a signs of mythical health, but, on the contrary, this condition began to be considered as a pathological state, associated with increased morbidity and mortality.

1.3 Recognition of pituitary involvement in gigantism

The path to scientific knowledge about etiology of gigantism included various speculations based on careful clinical observations. Early adequate description of cases of gigantism can be found in the historical records since the 16th century (28, 29). Medical reports provide information of clinically relevant symptoms as well as evidence of an enlarged pituitary sella and tumoral process in the pituitary gland in individuals with gigantism (12, 29, 30). Interpretation of these findings raised several questions mainly concerning the implication of the pituitary in the pathological mechanisms of their diseases, which had long been debated through history.

In 1871, the Austrian anatomist **Carl Langer** described a series of gigantism cases in the "*Human skeletal growth in regard of Gigantism*", where he noted facial changes (large jaw, lips and nostrils) in some cases, and also outlined that only in these cases an enlarged sella turcica was observed (31). Although the focal point of his work supported a link to the pituitary, its causative role in the development of gigantism was still to be elucidated.

In 1884, the Swiss **Christian Fritsche** and **Edwin Klebs** described comprehensively in a separate monograph a case of a tall patient, pointing out clinical signs of acral overgrowth and hypertrophy of internal organs, as well as substantial changes in the pituitary area (32). In the discussion supported by Langer's work, they concluded that the gigantism is constantly accompanied with the hypertrophy of the pituitary, however wrongly considering the pituitary enlargement as a result of the general overgrowth, but not the initial site of the pathological process (33). Furthermore, overgrowth and clinical characteristics were attributed to a disturbance in the normal growth process, but specified that the acromegalic changes occurred as a result of a disease occurring later in life when normal growth has ceased.

Clinical pictures and anatomical findings not dissimilar to this recognized case of the disease, were also reported by other physicians, to which the various names and interpretations were attributed. In 1822, the French dermatologist **Jean-Louis-Marc Alibert** wrote about *géant scrofuleux* (34). In 1864, the Italian neurologist **Andrea Verga** reported dysmorphic facial changes in a woman with what he called *prosopectasia* (Greek: "face enlargement"). It is interesting to mention in this connection, that post-mortem findings revealed a walnut-sized sellar tumor that destroyed the sphenoid bone and compressed the optic chiasma (35). In 1866, the German neurologist **Nicola Friedreich** described similar clinical picture as general *hyperostosis* (36), and later, in 1869, the Italian psychiatrist **Cesare Lombroso** characterized it as *macrosomia* (37). In 1877, the Italian doctor **Vincenzo Brigidi** described similar changes, but considered the disease as a specific skeletal pathology - *rheumatitis deformans* (38).

Historically, the emergence of the term *acromegaly* (Greek: ἄκρον (akron) – "extremity" and μέγα (mega)- "large") as first full description of the disease and its recognition as a new clinical entity are credited to the French neurologist **Pierre Marie**. In an article published in 1886, Marie presented two cases with "a disease characterized by hypertrophy of the hands, legs and face ...". Reviewing clinical cases with previous descriptions of similar morbid presentations and other hypertrophic diseases he delimited this new pathology from other conditions such as "myxoedema, Paget disease (osteitis deformans) and the leontiasis ossea described by Virchow" (39). In contrast to previous views, Marie believed that the disease was not limited solely to skeletal disorders, but manifested itself across the body with a progressive increase in size of the limbs, soft tissues and internal organs. Among the probable causes of this pathology, Marie considered rheumatism, affection of the sympathetic nervous system, or a congenital familial anomaly in the anatomical development of the body, but didn't point out any causative link to the pituitary in this first article (39). Marie made a mention of the pituitary hypertrophy but explained it as a part of the generalized process of visceromegaly (enlargement of organs) and bony deformities observed in acromegaly

In 1887, the German physiologist **Oskar Minkowski**, reporting post-mortem cases of acromegaly first made an important statement about the enlargement of the pituitary consistently associated with and responsible for acromegaly (40). During the next years, the enlarged sella and persistent hypertrophy of the pituitary gland were then definitively demonstrated in all acromegalic cases in available autopsy results reviewed by Marie and his co-workers: the French surgeon **Auguste Broca** (41), the Romanian neurologist **Georges Marinesco** (42) and the Brazilian physician **José Dantas de Souza-Leite** (43).

Furthermore, based on advances in roentgenographic visualization of the bone structures inside the skull, the German neurologist **Hermann Oppenheim** in 1901 demonstrated an enlarged sella on X-ray in a patient with acromegaly (44).

Despite accumulation of valuable clinical descriptions and pathology studies, the question concerning the connection between acromegaly and gigantism, remained debatable. Marie strongly pursued the hypothesis of the entirely different origin of acromegaly and gigantism, considering the latter as an extreme presentation of the normal physiology. At the time of Marie, the pituitary was supposed to have predominantly a suppressive effect on the growth and development of the body, and therefore Marie and de Souza-Leite considered acromegaly as a result of the hypofunction of the pituitary gland. According to his hypothesis, hypertrophy and the subsequent destruction of the pituitary gland discontinued its tonic inhibitory effects on the body, leading to progressive and uncontrolled growth (43).

By putting together the previously published cases with similar symptoms and personal observations in the pituitary area the Italian doctor **Roberto Massalongo** attributed the cause of both acromegaly and gigantism to the same pathological process arising from pituitary hypertrophy and its hyperfunction (45). Finally, the French neurologist **Henry Meige** in collaboration with the French physician and pathologist **Édouard Brissaud** accurately concluded that gigantism and acromegaly have the same pathogenesis, but "gigantism is the acromegaly of the young" (46). Over the next years there were many more reports of acromegaly and gigantism cases described with large pituitary tumors, in particular those accumulated in a monograph by the French physicians **Pierre Emile Launois** and **Pierre Roy** are of great historical and medical interest (47). Further evidence for the pituitary etiology of acromegaly and gigantism has been greatly aided by exploration of the remains of historical gigantism cases preserved in the museums and scientific collections. The Scottish anatomist **Daniel John Cunningham** examined the skeleton the Irish giant Cornelius Magrath from the 18th century (2.26m) preserved in Trinity College, Dublin (Figure 1.7), and described generalized gigantism associated with acromegaly-like phenotype (48).

Figure 1.7 Cornelius Magrath pictured on an engraving by Maag in 1756 (Trinity College, Ireland) (48, 49).

The notorious story of Charles Byrne (called *O'Brien*, 2.34m), another famous Irish giant from the 18th century, the tallest man at his time (Figure 1.8), and the studies of his skeleton at the Royal College of Surgeons of England in London, shed more light to the pathogenic mechanism linked to the pituitary.

After his death at age of 22, Byrne's body came into the possession of the Scottish surgeon **John Hunter**, who proceeded to prepare it for display (50). A long time after, the American surgeon **Harvey Cushing** with the curator of the Hunterian Museum, Arthur Keith, were the first to open the skull of the giant in 1909 and revealed a greatly enlarged and destroyed sella turcica (51, 52).

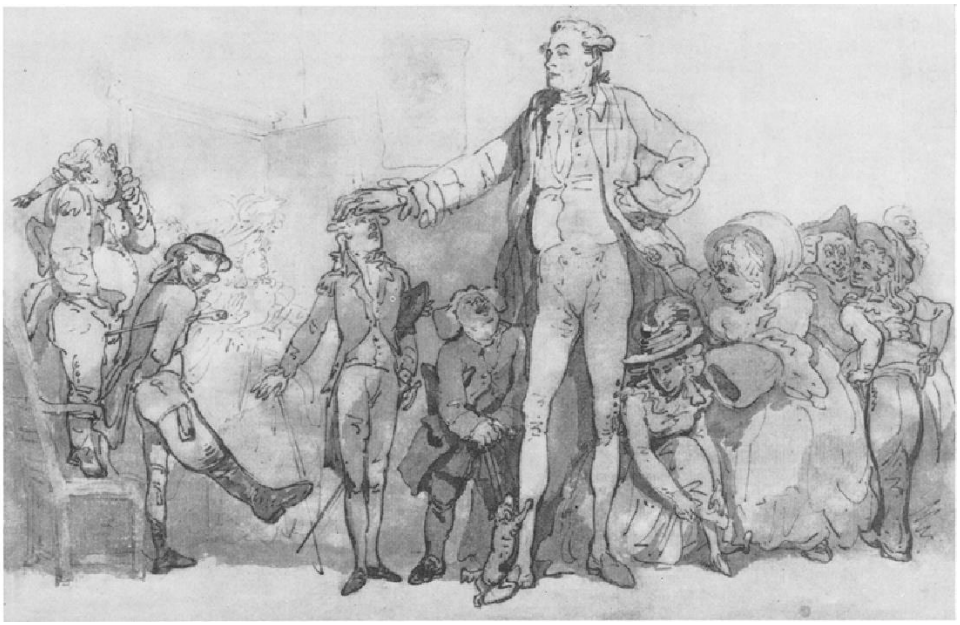


Figure 1.8 A cartoon by Rowlandson showing the Irish Giant with friends (1785, The Hunterian Museum, London)(53)

The contribution of Cushing to pituitary studies is obviously not limited to the exploration of historical cases, but was based on many personal observations. In 1909, he performed a "partial hypophysectomy" in a patient with acromegaly, and pointed to the remission of clinical symptoms of acromegaly after pituitary surgery, supporting the idea that acromegaly might be a manifestation of pituitary hyperfunction (54). In his subsequent works, Cushing also suggested that the pituitary plays a central role in endocrine regulation. Finally, he was the first who postulated a "hormone of growth" in the pituitary and indicated its involvement in growth regulation (55).

Characteristic pathological changes, such as a frequent presence of eosinophilic pituitary cells, were simultaneously discovered in various studies of histopathology in acromegaly. In 1900, the German microbiologist **Carl Benda** was the first to draw attention to an accumulation of eosinophilic cells he described in the pituitary, as a source of its hypersecretion (56). A few years later, the Austrian pathologist **Jacob Erdheim** in the post-mortem study of an adult acromegaly patient, found a pituitary tissue mass composed of eosinophil cells displaced into the sphenoidal sinus (57). In 1927, Cushing and his student the American surgeon **Leo Davidoff** clearly disclosed an eosinophil pituitary adenoma as a cause of pituitary hyperfunction leading to the development of disease (58).

The revelation of the etiological role of the pituitary in growth was supported by animal studies. In 1912, the Austrian physiologist **Bernhard Aschner** demonstrated that body growth in experimental animals (20 dogs) was arrested after hypophysectomy. Based on these results, he concluded that the cause of acromegaly is hyperfunction of the pituitary gland (59). In 1921, the presence of a growth stimulating substance in the pituitary and its hyperfunction in the development of acromegaly were demonstrated by the American anatomist and embryologist **Herbert McLean Evans** in an experiment, where intraperitoneal injections of an extract of the anterior pituitary to rats led to changes seen in acromegaly-gigantism (60). Over the next decades, the development of the theory of pituitary hyperfunction was accompanied by many studies making indirect measurements of the substance which stimulated growth. Thus, the administration to animals of an extract from the anterior pituitary gland or acromegaly subjects allowed the determination of its peripheral actions and metabolic effects, in particular the development of acromegaly symptoms and the hormonal stimulation of longitudinal bone growth (61, 62).

Fundamental advances in understanding of the physiology and pathology of the pituitary gland came when the human growth hormone (GH) was isolated from pituitaries collected at autopsy independently by **Choh Hao Li** and **Harold Papkoff**, in California, and

Maurice Raben, in Massachusetts (63, 64). Further studies devoted to morphofunctional organization of the pituitary somatotroph axis are briefly presented in the Table 1.1.

Table 1.1: Studies identifying pituitary GH action

GH	
1956	Li and Papkoff and Raben were the first to isolate human GH (63, 64).
1961	The elevated GH levels in acromegaly patients were confirmed by radioimmunoassay techniques (65, 66).
IGFs	
1957	Salmon and Daughaday introduced a secondary substance (initially termed "sulfation factor"), which mediates the effects of GH on skeletal tissue, in particular GH-dependent incorporation of sulphate into chondroitin sulphate (67).
1959	The "sulfation factor" was found with elevated levels in active acromegaly, whereas after the remission its level generally came to the normal ranges (68).
1966	Metabolic activity of the "sulfation factor" were discovered, in particular its intrinsic insulin-like effects that could not be inhibited by anti-insulin antibodies, subsequently it was termed "nonsuppressible insulin-like activity" (69).
1972	The term "somatomedin" was proposed as for a key mediator of GH action (70).
1977	Purification and measurements of serum concentration of the basic peptide (somatomedin C) by radioimmunoassay (71).
1978	Somatomedins were renamed to insulin-like growth factors (IGFs), when structural homology with proinsulin was demonstrated by Rinderknecht and Humbel (72).
Hypothalamic regulation	
late 1940s	The concept of the "neurohumoral regulation" of the anterior pituitary by hypothalamus suggested by Geoffrey Harris (73)
1954-1972	"Hypothalamic releasing factors" released into the hypophyseal portal system were identified by Drs. Andrew Schally's and Roger Guillemin's groups
1973	Somatostatin was isolated, characterized, and sequenced (74)
1982	Growth hormone releasing hormone was isolated, characterized and sequenced from a pancreatic tumor from acromegalic patient (75-79)

1.4 Discovery of inherited and syndromic forms of acrogigantism

The early descriptions included rare cases of acromegaly and gigantism in families that underpin the familial occurrence and hereditary forms of the disease (55, 80, 81). The famous biblical Philistine giants, descendants of the Anakim – Goliath, his brothers and

sons, all described with height exceeding 2 m perhaps could referred as the oldest written description of familial gigantism (82, 83). Interestingly, Charles Byrne was depicted in a sketch by John Kay with cousins the Knipe twins from the neighboring village, both suffering from gigantism (84). Byrne's great height was then related to a particular *AIP* mutation that explained the occurrence of his pituitary lesion, as well as unraveled its possible hereditary context (85).

In 1901, **Max Fraenkel** described two cases of the *Alpine giants*, the siblings Baptista (1876-1916) and Paolo Antonio Hugo (1887-1914), with heights of 2.3m and 2.25m, respectively (Figure 1.9). At post-mortem, in the younger brother, who died at age of 27, a large tumor was found in pituitary with supra-, para- and retrosellar growth and compression of the optic chiasma (81).

Figure 1.9 Baptista (2.3m) and Paolo Antonio (2.25m) Hugo with their family are pictured in a photograph from the early 20th century. Picture from the collection of Dr. W. de Herder (86).

A case of a young acrogigantism patient operated by Cushing was documented in his monograph as someone from the family of Kentucky giant (55), who was exhibiting in a traveling circus.

The medical literature continued to provide records of familial gigantism, while technological advances brought knowledge of molecular mechanisms underlying the familial and syndromic forms of acromegaly-gigantism (discussed in the Chapter 5). Inherited forms of acromegaly and gigantism have been the most frequently related to Familial Isolated Pituitary Adenoma (FIPA) (87-92). This hereditary condition exclusively limited to pituitary tumors was first described in Liège in late 1990s by **Albert Beckers**; its detailed description is presented later in this thesis.

Syndromic forms of GH-secreting pituitary adenomas, going back to the first description by Jacob Erdheim of enlarged parathyroid glands at autopsy in an acromegalic patient in 1903, were reported long before the identification of the causative role of particular genes (93). In 1927, Cushing and Davidoff found multiple tumors of parathyroids and pancreatic islet cells in an acromegalic patient (58). In 1954, the American internist **Paul Wermer** pointed to an inherited association of multiple adenomas of endocrine glands (94). Various combinations of endocrine tumors were assembled under the new term "multiple endocrine neoplasia" (MEN), coined later, in 1968, by Steiner et al (95); subsequently MEN1 was used to describe what previously was named Wermer's syndrome.

In 1985, the Irish pathologist **J. Aidan Carney** first defined a new multiorgan syndrome as an association of myxomas, skin hyperpigmentation, and endocrine overactivity, including acromegaly (96). Interestingly, Cushing appears to give a description of clinical symptoms and post-mortem findings corresponding to the diagnostic criteria of Carney complex in an acromegalic patient, from his own observations between 1913 and 1932, which was later confirmed genetically (58, 97, 98).

In historical records Thomas Hasler, the *Tegernsee giant*, from the 19th century, was described as having severe skull and facial deformities along with a height of 2.35m at the age of 25 when he died. His skeleton, which is preserved in the Institute of Pathology at Munich University, was explored more than a century after his death, revealing an enlarged sella turcica, supportive of a pituitary macroadenoma, and fibrous dysplasia of the skull and multiple bones (99, 100). This case appears to be the oldest description of an association of acromegalic gigantism with fibrous dysplasia. This corresponds to a classical picture of a rare multiorgan syndrome first defined by American doctors **Donovan McCune** and **Fuller Albright** as McCune-Albright syndrome in 1937 (101, 102).

Although rare, gigantism cases have been always known in the human history, and the appearance of these individuals always excited general curiosity; whereas their physical

anomalies became the subject of scientific analysis leading to a significant enrichment of medical knowledge. However, gigantism still holds many secrets and continues to inspire research.

Chapter 2: Normal linear growth and attainment of adult height

Somatic growth is a dynamic process that relies on the correct activation and deactivation of powerful biological signals via a highly integrated control system. In each individual, a unique pattern of growth and adult height is determined by multiple genetic factors, but these can be then modified by various external factors (nutrition, socioeconomic situation, psychological well-being, and various medical conditions) (103-106). A secular trend towards increased growth and adult height over generations has been noted since the middle of the 19th century in many countries. This has unequal distribution across the globe, which most likely reflects the variable improvement in quality of the environment and life conditions, especially nutrition, and access to healthcare(107-111).

In children, normal physical growth is established as an important indicator of general health status and well-being (103).

2.1 Normal growth stages

In order to reach final adult height, each individual passes through several key stages, when physical growth occurs at different rates and is governed by different mechanisms of growth regulation: fetal period, infancy (birth to 2 years old), childhood (3 to 11 years old), and puberty/adolescence (12 to 18 years old) (112) (Figure 2.1).

In humans, the *fetal period* is characterized by the highest growth rates (113). Prenatal growth is determined by genetics, maternal factors (uterine growth potential, diabetes, etc.), nutrition and fetal growth factors (placental GH, IGF-2, insulin, cytokines, etc.) (114-116). Evaluation of birth length can provide useful information on prenatal well-being as well as on congenital growth disorders in the newborn infant who is small or large for gestational age.

In the *infancy period*, a deceleration of growth velocity occurs after birth, but linear growth continues at an increased rate; overall, normal-term infants grow 25 cm during the first year, then about half of this during the second year of life. Depending on their birth size, small or large for gestational age children during infancy can demonstrate "catch-up" or "catch-down" growth, respectively (117). Height velocity is predominantly nutrition-dependent, but can vary also due in part to perinatal factors, health condition and thyroid function. Rapid deceleration of growth velocity continues up to the third year when the growth curve becomes smoother.

The *childhood period* is characterized by a prolonged phase of more stable growth until puberty (117). Hormonal regulation of growth in this period becomes more important and

includes the GH/IGF-1 axis and thyroid hormones, whereas nutrition has a less significant influence and intrauterine factors largely cease (118-120). At the age of six to eight years, some transient growth acceleration occurs in all children, which corresponds to the period of adrenarche due to activation of the adrenal cortex and secretion of androgenic steroids.

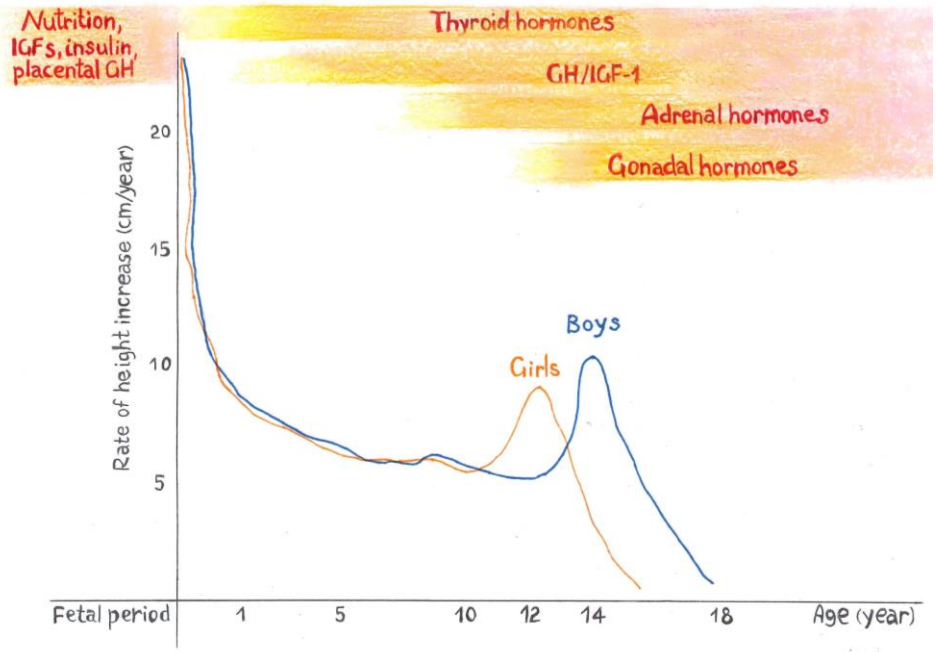


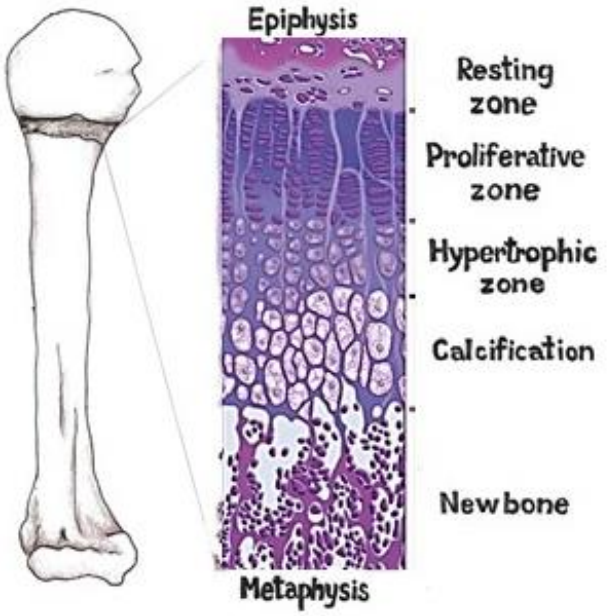
Figure 2.1: Peak height velocity curves for boys and girls. Redrawn from (121).

Growth rates in girls and boys exhibit only small sex differences until the *pubertal period*, when a significant growth spurt occurs due to interplay between the GH/IGF-1 axis and increased levels of gonadal steroids. Due to differences in onset of puberty, in boys, growth acceleration starts later and has greater overall magnitude than in girls (122, 123). The maximal peak growth velocity is reached at mid-puberty (124), and two years later (about 14 years in girls and 16 years in boys), the growth rate falls to 1-2cm/year, and the linear growth generally is nearly finished one year later. This happens due to complete fusion of growth plates induced by estrogens in both sexes. Growth of the spine continues after tubular bones and increases the total height by 2-3cm. There are however individual and ethnic variations in the timing of puberty, pubertal growth acceleration and progression through puberty (123, 124). In general, changes in pubertal growth velocity lead to a predictable height gain of about 25 (±5) cm in females and 30 (±7) cm in males (125, 126). Thus, the gender-related difference in adult height of approximately 13 cm occurs mainly due to the earlier cessation of growth in girls and longer prepubertal growth and more potent growth spurt in boys (127).

2.2 Overview of growth plate physiology

Longitudinal bone growth after birth occurs in immature bones at the epiphyseal plate through the process of endochondral ossification (via prior cartilage formation). The growth plate consists of coherent zones of chondrocytes which are present at different stages and remain active until the end of the puberty (Figure 2.2).

Figure 2.2: Growth plate zones – thin remnant cartilage localized at the ends of tubular bones.



Stem-like cells from the *resting zone* are recruited to the *proliferative zone*, where they undergo numerous mitoses and are arranged in columns parallel to the long axis of the bone. Then they increase their height and become differentiated into *hypertrophic zone* chondrocytes; also these cells produce extracellular matrix. Subsequently, when the matrix calcifies, the cartilage cells undergo degeneration, blood vessels and osteogenic cells invade from the perichondrium, which in turn transforms to the bone-producing periosteum, leading eventually to cartilage remodeling into bone tissue. Every newly formed bone stratum appears directly beneath the epiphyseal plate resulting in the elongation of the long bone diaphysis (128). The end of longitudinal bone growth occurs due to decrease of chondrocyte proliferation with age, which is signified by senescence of the growth plate cells, and epiphysial fusion.

The GH/IGF-1 system appear to be the most important hormonal factor in cartilage formation and remodeling, whereas growth plate fusion occurs at the end of puberty due to estrogens. Furthermore, regulation of the growth plate includes a number of other hormones and growth factors (such as thyroid hormone, insulin, leptin, vitamin D,

fibroblast growth factor 21 and others) and their interplay with components of somatotroph axis and sex steroids.

2.3 Somatotroph axis

The somatotroph axis plays a key role mainly in postnatal growth, but it is also involved in the fetal growth process. Prenatal growth appears to be mainly GH-independent and congenital defects involving GH/ GH-R action affect postnatal growth, whereas IGF-1 deficiency leads to intrauterine and postnatal growth failure (129-133). After birth the entire somatotroph system has a crucial effect on linear growth and mainly targets the cartilage cells of the growth plate until their closure.

2.3.1 Growth hormone

Growth hormone (GH) is encoded by the *GH-1* or *hGH-N* (h = human N = normal) gene located on the long arm of chromosome 17, within a 66-kb cluster among four other related and highly similar genes (placental GH gene: *GH-2*, two chorionic somatomammotropin genes: *CSH-1* and *CSH-2*, and a pseudogene *CSHP1*), which are specifically expressed in placental tissue (134-137).

Pituitary GH is a single chain polypeptide with two disulfide bridges. Most of the biological activity is carried by a 191 amino acid isoform with a molecular weight of 22 kDa (90% of pituitary and circulating GH), and a smaller proportion exists as the 20kD and other minor isoforms (138), as well as aggregates of these forms (homo- or heterodimers and higher oligomers) (139). GH is synthetized and secreted by somatotroph cells which represent about 50% of anterior pituitary cells. They are mainly located in the lateral parts of the pituitary and have multiple secretory granules containing GH and other pituitary hormones (e.g. prolactin). During pituitary organogenesis, somatotroph cells develop at around eight weeks of gestation. Transcription factors Pit-1 (pituitary-specific transcript factor 1) and PROP1 (prophet of Pit-1) are responsible for differentiation of a cell subtype that is a precursor of somatotroph, lactotroph and thyrotroph cell lines. These transcription factors are essential for activation of the *GH-1* gene promotor and are responsible for its high level expression in somatotrophs (140).

GH production by the somatotrophs and its release into the bloodstream are regulated at a higher level by the antagonistic influences of two main hypothalamic neuropeptides: ***GH releasing hormone*** (GHRH) and ***somatostatin*** (Figure 2.3). Both GHRH and somatostatin reach the anterior pituitary via the hypothalamic- hypophyseal portal venous system and bind to specific receptors on somatotroph cells. GHRH is secreted in a pulsatile manner and is stimulated by hypoglycemia, nutrition, stress, physical activity,

sleep, estrogen, androgen and thyroxine (141). The GHRH receptor (GHRH-R) is a seven- transmembrane domain G-protein coupled receptor. Specific interaction of GHRH with extracellular regions of the receptor leads to transmembrane signal transduction and activation of Gs-protein (142). This launches a cascade of intracellular reactions in somatotrophs, predominantly involving cAMP and Ca2+ pathways, and thereby stimulates GH release, GH gene transcription and GH storage in somatotrophs (143). Besides its secretagogue action, GHRH plays an important role in the development of the anterior pituitary and as a mitogenic factor stimulates the proliferation of somatotrophs (144). Somatostatin is secreted continuously and inhibits the release of GH via high affinity receptors (SST) (141). SSTs are seven- transmembrane domain G protein-coupled receptors. The SST family includes five structurally related subtypes (SST1-5). SST2 and SST5 are mainly expressed in the anterior pituitary and exhibit greater inhibitory effect on the secretion of GH than other SST subtypes (145, 146).

GH is released into the general circulation by secretory impulses (147). GH pulse amplitude is maintained by GHRH stimulation, whereas timing of GH pulse generation depends on intermittent withdrawal of somatostatin (148, 149).

Somatotroph cells function under ***negative feedback control*** (Figure 2.3), which involves both GH and IGF-1(150). GH acts at the hypothalamic level with a direct inhibitory impact on GHRH release and its stimulatory effect on SST tone, as well as in the short feedback loop where GH autoregulates its own secretion in the somatotrophs. IGF-1 interacts with GH production in a negative feedback mechanism that effects both at the hypothalamic and pituitary levels and decrease both GH peak amplitude and frequency (151).

GH secretion occurs throughout the day at a relatively low level reaching the highest peak amplitude several hours after the onset of ***sleep.*** In children and young adults, the maximal nocturnal GH release occurs within minutes of the onset of deep sleep. This is possibly due to decreases in somatostatin tone during sleep, although circadian rhythmicity might also modulate GH release (150, 152, 153).

GH release is also stimulated by the gastric peptide ***ghrelin*** (154), which has a probable role in situations of starvation to maintain blood glucose (155). In the pituitary, ghrelin interacts with a specific GH secretagogue receptor (GHS-R, ghrelin receptor) on somatotrophs (156, 157). Ghrelin has additive effects with GHRH in increasing circulating GH (158, 159). This protein has also been found in the arcuate nucleus of the hypothalamus where activation of GHS-R upregulates *GHRH* gene expression. Furthermore, ghrelin may play a role in inhibiting somatostatin (159). Ghrelin stimulates

GH secretion in several conditions such as stress, hypoglycemia and ingestion of protein (high levels of circulating amino acids) (160).

Other factors are also known to enhance GH/IGF-1 secretion, such as exercise, fasting, thyroxine, sex steroids and physiological levels of glucocorticoids. In contrast, free fatty acids, glucose, chronically raised levels glucocorticoids, adiposity, hyperinsulinemia and leptin secreted by adipocytes negatively influence the somatotroph axis. These mechanisms have been reviewed in detail elsewhere (144, 149, 161-170).

The variability of GH secretion changes with ***age***. The frequency of GH pulses is highest in the newborn, passes through a nadir in the prepubertal period and accelerates to culminate at the end of puberty, when GH secretion rates are tripled (171, 172). GH levels then gradually decline in normal aging due to the decrease in the secretory activity of somatotrophs with age with lower GH peak amplitude in the elderly (173).

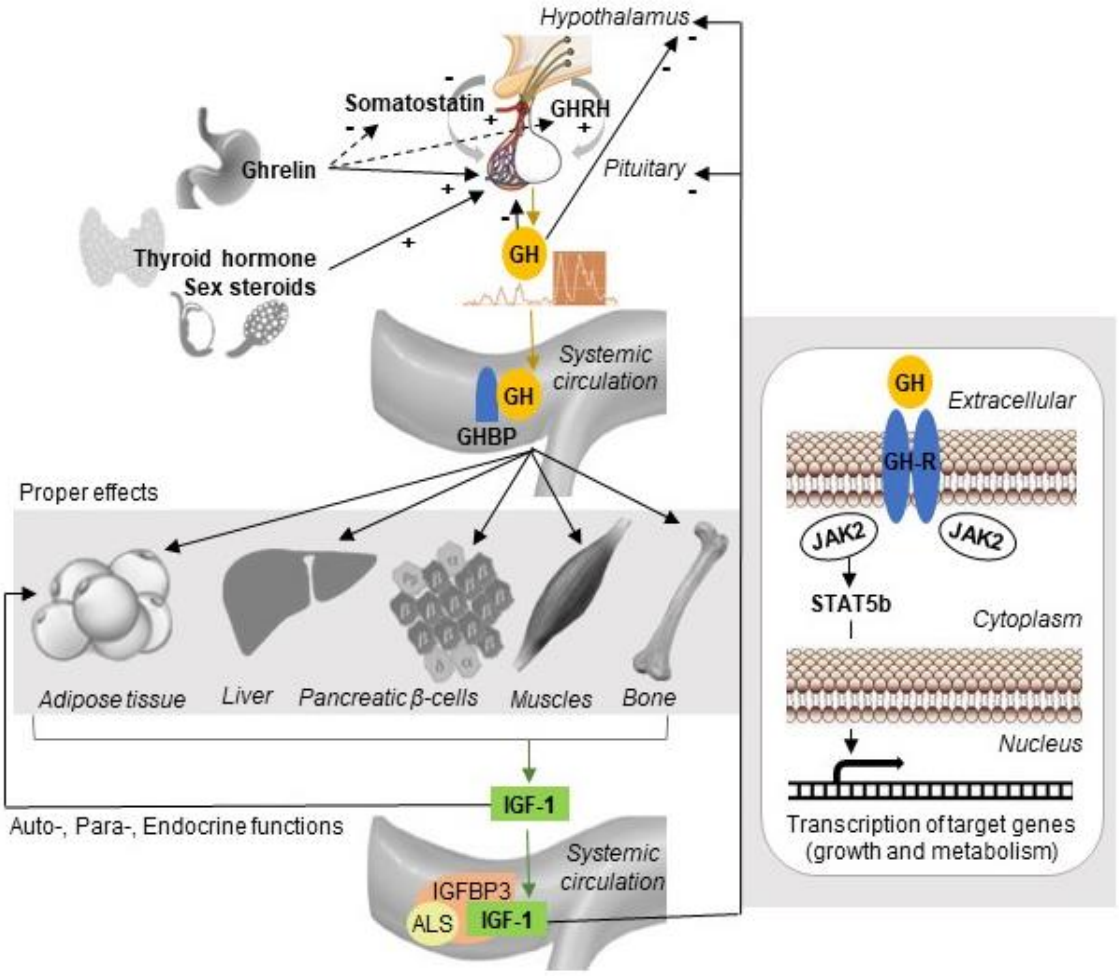


Figure 2.3: Regulation of GH-IGF-1 synthesis, secretion and signaling in humans. GHBP – GH-binding protein; IGFBP3 – IGF-binding protein 3; ALS – acid labile subunit; GHR – GH-receptor; JAK2 – Janus kinase 2; STAT5b – signal transducer and activator of transcription 5b.

In target cells, GH interacts via its specific ***GH-receptor*** (GH-R) (Figure 2.3). GH-R is present virtually in all tissues, with a predominance in the liver and in the cartilage of the epiphyseal growth plate (174, 175). Proteolytic cleavage of the extracellular domain of GH-R forms a high-affinity GH-binding protein (GHBP) and about half of plasma GH circulates in a complex with GHBP, which modulates GH action in target cells (176). GH has two binding sites, each of which interacts with a separate GH-R (Figure 2.3). Thus, in the target tissues, one molecule of GH binds to the extracellular parts of two GH-Rs (177). This provokes an internalization of the receptors with repositioning of their intracellular domains, and initiates the further signal translation via activation of the JAK2–STAT pathway (178). In this signaling cascade the dimerization of GH-R leads to co-localization of two molecules of the Janus associated kinase (JAK2) and the transphosphorylation of one by the other (179). This results in the phosphorylation of the distal part of the GH-R and its binding to cytoplasmic mediators- signal transducers and transcription activators (STAT). STAT is, in turn, phosphorylated and transported to the nucleus, where it activates gene transcription. In particular, there is a STAT5b binding site in the *IGF-1* gene promoter region that activates *IGF-1* gene transcription, and thus mediates growth-promoting actions of GH in humans (180, 181).

In bone tissue, GH generally acts through IGFs, although GH exerts direct IGF- independent effects on the growth plate (182, 183). GH stimulates chondrogenesis in the growth plate by recruitment of stem-like cells to the proliferative zone.

Metabolic effects of GH (including protein synthesis, lipolysis, insulin resistance through induced gluconeogenesis and reduced peripheral glucose utilization, etc.) occur in most tissues independently of the production of IGF-1, but the exact mechanisms are still a matter of debate (184). These additional GH-induced signaling involve the mitogen activated protein kinase (MAPK) pathway, extracellular regulated kinases (ERK), protein kinase C (PKC), negative effects on insulin signaling and other interactions (178, 185, 186), although their role in skeletal growth is uncertain (187).

2.3.2 IGF-1

IGF-1 is the predominant mediator of GH in postnatal growth. IGF-1 has a homologous structure with insulin and IGF-2 (188). IGF-2 is another growth factor which is less important for postnatal period, but is involved mainly in prenatal growth and can be associated with overgrowth in some congenital syndromes (Beckwith-Wiedemann syndrome) (189).

IGF-1 is encoded by the *IGF-1* gene located on the long arm of chromosome 12. Organ-specific gene targeting studies demonstrated that the majority (approximately 75%) of

circulating plasma IGF-1 is liver-derived and is primarily maintained by circulating GH levels (190). The diurnal IGF-1 concentration is stable and reflects GH secretion over a 24-hour period. In turn, IGF-1 directly inhibits GH-R function and is involved in local regulation of GH-induced signaling (191). Control of *IGF-1* gene expression varies according to the different stages of development; concentrations of IGF-1 are low during fetal life and gradually increase from birth reaching their maximum levels in puberty, and decrease progressively in adults along with the age-dependent decline of GH levels (161, 162).

While the liver is the major source of circulating IGF-1, it is also produced in other peripheral tissues (bone, cartilage, erythroid cell precursor, ovary, central nervous system, muscle and kidney) (Figure 2.3). Regulation of tissue IGF-1 is also GH dependent and has a more important role in postnatal local tissue growth control (192, 193); in particular, IGF-1 secretion by muscle, adipose tissue, and bone is under GH regulation and exhibits mainly paracrine/autocrine effects (194-196).

IGF-1 circulates predominantly (almost 100%) in plasma bound with high-affinity ***IGF-binding proteins*** (IGFBPs). They are involved in regulation of the biological activity of IGFs by diverse mechanisms (197). Among six IGFBPs (1-6), IGFBP3 has the highest affinity to IGF-1 and transports more than 75% of it, controlling its half-life and availability in the vascular circulation. Further, it modulates IGF-1's access to tissues and binding to its receptors in target cells (198). The IGFBP3/IGF-1 complex circulates in association with a protein called acid labile subunit (ALS) (198, 199) (Figure 2.3). Both IGFBP3 and ALS are produced in the liver in a GH-dependent fashion (200). ALS has no affinity for IGF-1, or IGFBP3, however it binds to IGFBP3/IGF-1 forming a ternary complex. As a result, the half-life of IGF-1 is significantly extended in the stabilized IGFBP3/IGF-1/ALS complex (up to 12-15 hours) compared to the half-life of free IGF- 1 (10 mins).

IGF-1 mediates its effects mainly by binding to the ***IGF-1 receptor*** (IGF-1R), which is widely distributed in different tissues. It has a tetrameric structure (two α- extracellular and two β- transmembrane subunits) similar to the insulin receptor with tyrosine-kinase activity (201). Autoactivation of IGF-1R is triggered by binding of IGF-1 to its extracellular domain and consecutive conformation of its intracellular domain. The tyrosine kinases of each β-subunit catalyze the phosphorylation of selected tyrosine residues of the other β-subunit and initiate signal transduction (202). The activation of downstream components of the IGF-1 signaling pathway results in stimulation of DNA and protein synthesis, cell growth and inhibition of apoptosis.

Through these mechanisms, IGF-1 is involved in the processes of cell cycle regulation, injury repair, cell differentiation and cell proliferation in different tissues (bone, muscle, ovary and testis, adrenal cortex, thyroid follicular cells, hematopoietic cells, skin and others). In addition, IGF-1 has anabolic effects, which includes enhanced protein synthesis, peripheral glucose uptake and lipolysis (162, 203-206).

In the growth plate, IGF-1 positively influences proliferation, hypertrophy and ossification of the cells, as well as skeletal growth being supported via its diverse metabolic effects (104, 196, 207).

2.4 Sex steroids

Gender-specific GH/IGF-1 concentrations are determined by significant gender differences in plasma sex hormones and their modulating effects on GH secretion and its peripheral action (208, 209). Sex steroids act as strong modulators of GH responsiveness and modify its metabolic effects, whereas their effects on central stimulation of GH secretion are not fully elucidated. Estrogens produce a relative "resistance" to GH in the liver by suppressing GHR signaling, and thus stimulate the secretion of GH indirectly by reducing IGF-1 negative feedback (172). In contrast, androgens enhance GH stimulatory effect on IGF-1 production. These aspects explain why in healthy women GH secretion is 50% greater, but they have lower levels of IGF-1 than healthy males in the same age group (161, 162, 210, 211).

In children, sex steroids have dual effects on normal skeletal growth. They are crucial for inducing a pubertal growth spurt in early puberty and for epiphyseal fusion in late puberty. Concomitantly with the augmentation of sex hormones in puberty, spontaneous daily GH secretion increases by about threefold in girls and doubles in boys compared to the prepubertal stage, inducing pubertal growth acceleration. Pubertal changes in GH and increased growth velocity are primarily induced by estrogen in both sexes, although these happen earlier in girls than in boys, along with gender dimorphism in pubertal timing (122, 172, 212-214).

On the other hand, an increased production of sex steroids, mainly estrogens, accelerates the maturation of chondrocytes and the switch from cartilage template to bone in both sexes. Estrogens are known to advance growth plate senescence (215, 216). The complete ossification of the growth plate occurs at the end of puberty, when the proliferative capacity of chondrocytes is exhausted, thus resulting in epiphyseal fusion and halting of linear growth. Estrogen receptors (ERα and ERβ) are expressed in growth plate cartilage and promote direct effects of estrogen on growth plate including stimulation of

chondrocytogenesis and bone maturation (207, 217-219). The effects of androgens on bone are mainly mediated by their aromatization to estrogens in peripheral tissues, but local conversion of androgens to estrogen also occur in the growth plate (220, 221). Androgens also have direct effects on growth plate via androgen receptors and can thereby contribute to pubertal growth (207, 217). GH-independent stimulation of proliferation in growth plate by sex steroids may be regulated in part via enhanced local IGF-1 expression (222, 223).

2.5 Thyroid

Thyroid function has an important modulating effect on physical growth and development. Thyroid hormones mainly potentiate GHRH production in the hypothalamus, but they also modulate GHRH binding to its receptor on the surface of somatotrophs via regulation of the number GHRH-R and their affinity for GHRH. Thyroid hormones may also have a direct impact on *GH* gene transcription (224). Furthermore, thyroid hormones also have GH-independent growth accelerating effects on chondrocyte proliferation and maturation via thyroid hormone receptors (TRα and TRβ) expressed in growth plate cartilage (118, 225, 226).

Growth is also influenced by many other hormones and growth factors (insulin, glucocorticoids, leptin) via both generally on metabolism, as well as acting locally in growth plate chondrocytes as mediators of the interplay between environmental factors and growth. Their regulatory roles and mechanisms of interaction are described elsewhere (223).

Disruption of the hormonal regulation of the growth plate can lead to important clinical disorders characterized by growth abnormalities. These disease states include pathological overgrowth, which should be distinguished from normal physiological variations of growth.

Chapter 3: Definition and classification of gigantism

3.1 Definition of gigantism

The definition of "how tall is too tall" varies and is unclear in the literature. Identifying which growth abnormality needs additional investigation and medical intervention remains challenging. Extremes of growth can best be defined in comparison with the normal variance of growth of the population.

The average height of the global population is 1.71m for men and 1.59m for women (227). Adult height above 2m in men and 1.9m in women is generally considered excessive regardless of the location. However, the diagnosis of growth abnormalities could be biased if based only on these generalized average criteria. The average population height is specific for the country, race/ethnicity and gender, thus the normal growth rate should be defined following consideration of these factors and on the basis of the geographical origin of the individual and the population.

Another important point is that the extremes of final adult height exceeding +2 SD already have a poor medico-social prognosis. Appropriate medical evaluation of accelerated growth should be performed as early as possible at young age (in childhood and adolescence) before reaching excessively tall final adult height.

In 2006, the World Health Organization (WHO) published international growth curves following an optimized approach to growth, using data from a wide variety of countries (228). According to the WHO standards a Z-score cut-off point of more than +2 SD (which corresponds to the 97,7th percentile) can be used to classify elevated height-for-age as overgrowth in young patients before end of the puberty and closure of epiphyseal growth plates. Usually a child with a height above the 97,7th percentile of the growth curve is considered "too tall" just as a child growing below the 2,3th percentile is considered "too short". The rationale for this is the statistical definition of the central 95% of a distribution as the "normal" range. By definition and from the purely statistical point of view, there are as many children with short stature as with tall stature. Short stature is a common presentation for pediatricians and a structured approach to the assessment and management of the child with short stature is well established (229). In contrast, referrals for assessment of children with excessively rapid growth and tall stature are much less common. In contrast to short stature, increased height is usually considered by society and parents as a physical feature associated with life success (230, 231).

For an adult population, excessively tall stature can be considered using the same cut-off value, i.e.an individual whose final height differs by more than +2 SD scores from the local population standards.

The diagnosis of gigantism can best be defined as current or previous evidence of abnormal, progressive and excessively rapid growth velocity for age (> 97,7th percentile), or the final height greater than +2 SD above mean for relevant population (Figure 3.1). Therefore, both patients who have reached the final height or are still growing should be evaluated.

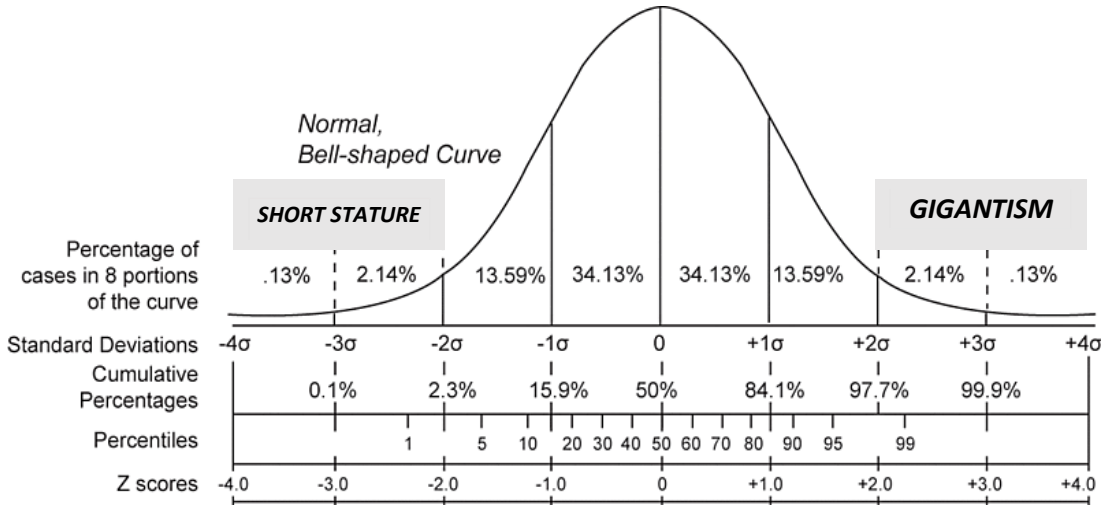


Figure 3.1: Graphic representation of the normal distribution (Bell curve) demonstrating standard deviations, percentiles and Z-scores.

Auxology, the study of growth, is based on dynamic evaluation of stature, including height measurements and growth velocity, which are sensitive indicators for individual growth assessments. Accurately measuring the height of an individual in a standardized manner is crucial in defining any deviation from normal growth. A standard technique of length/height measurement has been suggested (232). A standing height is typically measured from the age of two, whereas in infants and toddlers, a lying length is plotted on a separate growth curve (for 0-24 months). All height measurements are recorded on the growth chart or are evaluated using the growth percentile tables; both include anthropometric data of the reference population who share a genetic background and environmental factors. For this purpose, certain software systems using population-specific anthropometric data (e.g. neonatal records) are available (233).

3.1.1 Prediction of adult height based on parental heights is used to determine individual height targets. Many formulas have been proposed to predict the target height of a child according to the parental heights (234). In parents, in whom diminution of height has set in, a correction for age can be used by adding 1.5cm to height measurement for 45-50

years old, and 3cm after the age of 55. The calculation of mid-parental height (MPH) was first suggested by Tanner and is used as a standard procedure for crude prediction of child's potential adult height (235). The average of the parent's heights should be plotted on a chart at the line corresponding to the height at age 18. However, to consider sex differences in adult height, the population average of this indicator (that is 13cm) should be added to mother's height plotted on a boy's chart, and the reverse should be done with father's height on a girl's chart. The range of target adult height, two SD interval difference from the calculated MPH, is useful for the evaluation of current height. This parameter is also used to exclude a familial tendency to tallness in individuals without underlying genetic disease presenting with excessive linear growth (236).

However, socioeconomic factors could influence the height of parents leading to the incomplete realization of their genetic potential. This should be taken into account, as in such families calculated MPH will not correspond to the expected height in a child who lives in better socioeconomic conditions.

Interestingly, the phenomenon of regression to the mean may occur from one generation to the next. Thus, tall parents tend to have children taller than average, however significantly tall individuals' offspring tend to be less extreme in their tall stature than their parents (237). An adjustment for regression to the mean for extreme parental heights assumes a subtraction from calculated MPH of 1cm for every 5cm that MPH deviates from the population mean (238).

Furthermore, accurate records of heights of grown siblings provide additionally useful information about the familial background in the evaluation of growth abnormalities.

In addition, child's skeletal age or "bone age" is assessed by radiological appearance of indicators of skeletal maturity on X-rays of the hand-wrist. However this approach remains approximative, particularly for extreme variants (239). There are different techniques to assess "bone age"; the most prevalently employed methods are Greulich and Pyle and Tanner-Whitehouse. Both are simple and provide valuable criteria for skeletal growth and maturation (240). Deviation of skeletal age should only be approximately about 10% from the chronological age (241). Tables and formulas for height prediction have been suggested and usually include gender, chronological and skeletal age, and height and growth rates at certain time-points (240). Such adult height prediction, obviously, becomes more accurate with increasing age (242). It is important to note, that all growth predictions using skeletal age are based on reference databases derived from the examination of the children who are healthy and growing normally, however these data are less reliable for final height prediction in children with impaired

growth or in syndromic cases. However in those populations “bone age” assessments are of great importance to distinguish between different growth disorders and to follow growth during treatment (241).

3.2 Clinical evaluation of tall stature

Evaluation of a child with linear growth acceleration and tall stature is based on distinguishing the disease state from normal growth patterns with physiological growth spurts. Diagnosis of overgrowth has been extensively reviewed in the medical literature and different structured approaches to the clinical assessment have been suggested (117, 236, 243-249). A coherent approach to the evaluation of growth abnormalities includes detailed family history, which should consist of information on ethnic origin, search for consanguinity, data on parents’ and siblings’ heights and medical history. Additional information on the pregnancy, results of antenatal ultrasound and measurements at birth are also of special importance. A dietary survey should be performed to characterize the nutrition during the first years of life and to quantify the current caloric intake.

Clinical evaluation should focus on specifying the psychomotor and pubertal development stage, skeletal maturation (bone age), and how these parameters correspond to the chronological age, as well as identifying dysmorphism (long limbs, marfanoid habitus, enlarged hand and feet, facial coarsening, arachnodactyly, true gynecomastia, etc.), malformations and associated signs (e.g. heart murmur, scoliosis, pectus excavatum, hyper elastic skin, organomegaly) and attendant pathologies.

Measurements of height, weight, growth rate, and BMI should be plotted on appropriate reference curves or tables and analyzed according to the age and pubertal development. Precise growth curve analysis provides information on the timing of growth acceleration and growth pattern, which is essential for differentiating sustained abnormal growth from a recent acceleration. Moreover, measurements of cranial perimeter, arm span and lengths of limb segments supply additional information on whether the growth is proportional or not.

3.5 Etiologies of overgrowth and gigantism

There are number of health conditions that should be considered at the initial assessment, as the most appropriate treatment strategy for halting abnormal growth depends closely to its etiology (Figure 3.2).

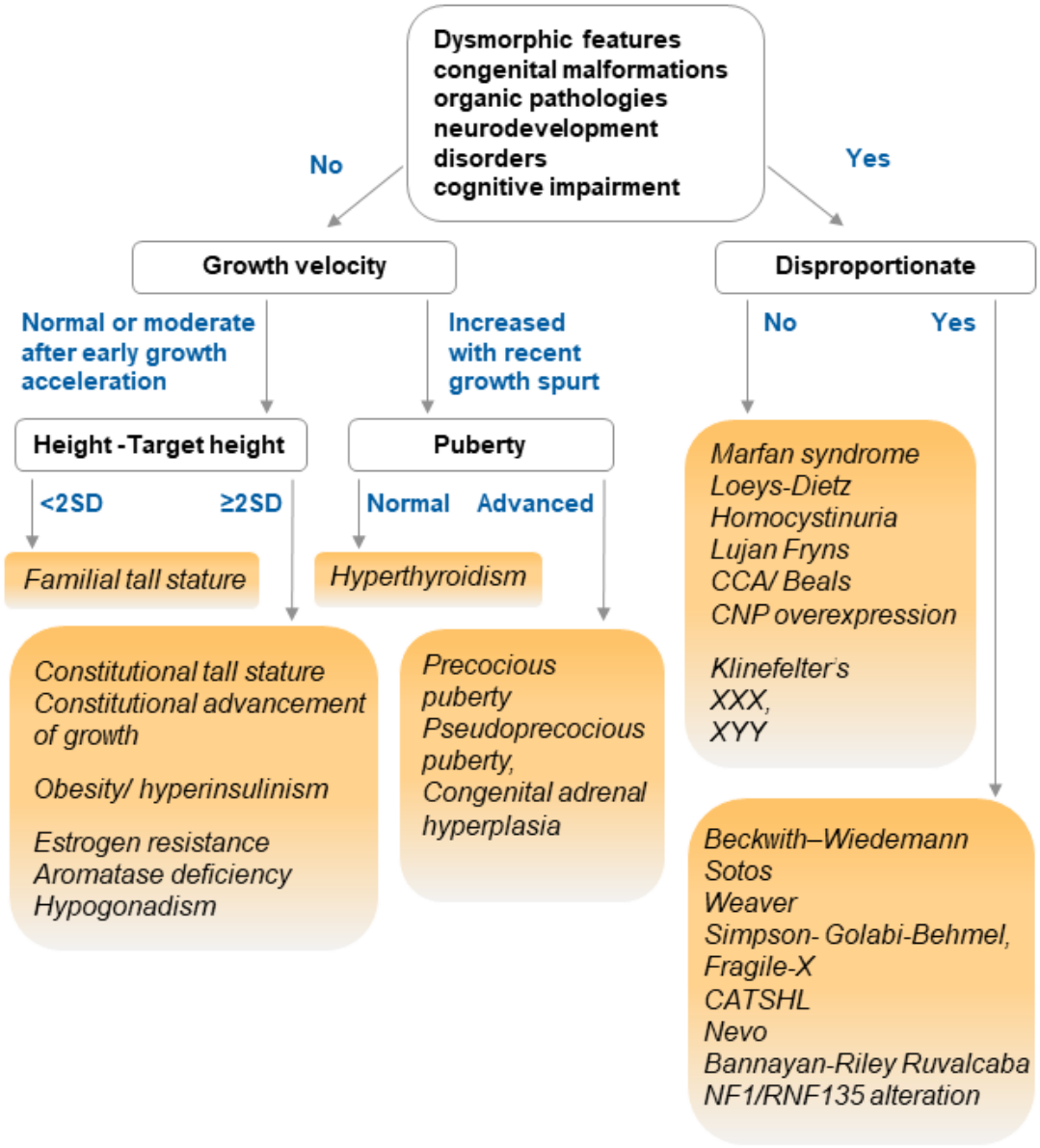


Figure 3.2: Flow-chart of the clinical evaluation of a child with GH- independent tall stature. Congenital syndromes with overgrowth are listed in the Appendix - Table s3.

3.5.1 Constitutional or idiopathic tall stature mainly concerns children from tall families, however it can also occur as a non-familial idiopathic form. It is characterized by an elevated growth rate in the absence of congenital overgrowth syndromes, or hormonal and nutritional disorders (250). Constitutional tall stature can be considered as a variant of normal growth. This is the most common cause of tallness and accounts for more than 80-90% of cases with excessive height (245). It can be defined as familial tall stature if appears in a family with excessive height in one or several family members (parents, grand-parents, siblings). Because of excessive parental height, the calculated target height is above +2 SD. Most of these children have normal anthropometric measurements at

birth and excessively rapid growth usually starts soon after birth. A gradual growth acceleration continues, and the child follows their growth curve toward the target height (250). In these individuals GH, IGF-1 and IGFBP-3 levels are often at the upper limit of the normal range, with more frequent rhythmic secretion of GH (250-252).

Early growth acceleration during the first two to four years of life is also characteristic for *constitutional advancement of growth*. In such cases, a regular high growth rate within the upper channel of the growth curve is observed up to the onset of puberty (250), which usually happens earlier than average (particularly in girls). In contrast to constitutional tall stature, in these individuals advanced puberty contributes to normalization of growth and as a result, final height is achieved within the normal range (253). The exact mechanism of increased stature in children with constitutional advancement of growth is not clear. There is evidence of an increase in regulatory components of somatotroph axis (levels of GH, IGF-1 and IGFBP-3 close to the upper limits of normal ranges, more frequent GH pulses) prior to puberty in these individuals (254).

Thus, the majority of cases with overgrowth will present with non-pathological tall stature, although it is important to note that this remains a diagnosis of exclusion, and is reached in otherwise healthy individuals when the presence of other possible causes are eliminated during the diagnostic work-up (245).

3.5.2 Pathologically rapid growth and tall stature can be classified as GH-dependent and GH-independent, which is the most appropriate in the context of this thesis. GH-independent tall stature includes various primary defects intrinsic to the growth plate, and secondary disorders affecting chondrocyte differentiation in the growth plate during its development mainly due to hormonal dysregulation. From the clinical point of view, it is relevant to distinguish various overgrowth syndromes due to monogenic or chromosomal abnormalities, as well as epigenetic disturbances, as this group of medical conditions usually present specific neurodevelopmental symptoms and congenital morphological anomalies.

3.5.2.1 Overgrowth syndromes are heterogeneous in terms of underlying genetic causes and clinical presentation (255). A particular molecular mechanism can be suspected in the presence of certain specific features or a family history of a similar condition. Clinical characteristics and molecular alterations for known overgrowth syndromes are listed in the Appendix - Table s3 and reviewed in details elsewhere. Regarding statural abnormalities, it is important to note that in congenital overgrowth syndromes, excessive body length can be present in infants from birth due to interference with the antenatal growth process (256, 257). Accelerated linear growth and tall stature are usually observed

during childhood but there is some normalization of the growth curve with age, resulting in a slightly above average adult height.

3.5.2.2 Hormonal dysregulation other than alterations in the GH/IGF-1 axis, occurring at different growth stages, can underlie a particular abnormal growth pattern and excessive height. Assessment of growth velocity and timing of growth acceleration can be helpful to discriminate between different conditions affecting hormonal regulation of linear growth (Table 3.1). Thus, postnatal abrupt increase in growth velocity before puberty is suspicious for endocrine disorders (sex steroid excess, hyperthyroidism and GH excess), while early-onset overgrowth with normal or moderately increased growth velocity is typical for overfeeding in early life and childhood-onset obesity. Prolonged longitudinal growth in adolescence and early adulthood is usually suggestive of impaired sex steroid production or metabolism, and, as a result, a delay in epiphyseal growth plate fusion.

3.5.2.2a Excess thyroid hormone leads to advanced skeletal maturation and growth acceleration. Clinically these children are characterized first by functional signs of hyperthyroidism (tachycardia, palpitations, sweating, asthenia, weight loss), and they usually demonstrate decreased school performance and hyperactivity. Graves' disease is the most common etiology, which occurs usually in adolescents with a goiter and a personal or family medical history of autoimmune disease. Acceleration of linear growth is observed from the onset of hyperthyroidism and can lead to tall stature, however the adult height depends mainly on when sustained control of thyroid hypersecretion is achieved (258).

3.5.2.2b Precocious puberty, either GnRH-dependent or GnRH-independent, presents with a recent increase in growth rate and an advanced bone age obtained on hand-wrist radiographs (259). Excessive secretion of sex steroids arises from alterations in one of the elements of the hypothalamus - pituitary – gonadal and/or adrenal axes. Central precocious puberty is caused by increased hypothalamic LH- releasing hormone pulses, which stimulate gonadotropic hormone secretion and sex steroids produced by peripheral target organs. GnRH -independent early activation of sex hormones secretion in gonads or adrenals is usually caused by hormonally active tumors of these glands or congenital dysfunction of the adrenal cortex. This form of precocious puberty also includes chorionic gonadotropin secreting tumors: chorionic epitheliomas, hepatomas and teratomas.

In children with precocious puberty, pubertal growth acceleration and the appearance of secondary sexual characteristics happens earlier than in their peers. These children may have a growth rate typical for a pubertal “growth spurt” and rapidly become tall for their

age, however, they generally end up with a final adult height lower than average due to early epiphyseal growth plate fusion.

3.5.2.2c Hypogonadism due to congenital or acquired causes induces prolonged linear growth and tall stature in both sexes. Low sex hormone secretion occurs due to compression of hypothalamic-pituitary structures by tumors, congenital malformations, or anomalies of sexual differentiation. This is caused by pathology during gonadal formation and by congenital defects of the biosynthesis of sex steroids or impairment of their cellular metabolism. Hypogonadotropic hypogonadism can be congenital. Clinical evaluation can reveal cryptorchidism and micropenis after birth, as well as associated conditions that can be present raising suspicions of the presence of specific syndromes (e.g. anosmia in Kallmann syndrome) (260).

Children with hypogonadism have the same growth pattern as their peers up to 13-14 years of age. Their further growth is characterized by the absence of a pubertal "growth spurt" and a slightly decreased growth rate. Impaired epiphyseal fusion leads to the growth of the extremities, with a decreased ratio of the upper and lower segments of the body (eunuchoid proportions). As the opposite of precocious puberty, the bone age in children with hypogonadism is delayed starting from the age of 13-14 years; they can also fail to reach peak bone mass and develop osteoporosis due to low sex steroid effects on the bone. These patients develop tall stature only later in life, since the growth plates remain open for a longer time and growth continues into adulthood. The adult height can greatly exceed the average and the calculated target height (> +2 SD) in untreated individuals, while timely initiation of replacement therapy with sex steroids results in puberty onset with normalization of the growth curve, and an adult height corresponding to the target height.

A similar abnormal growth pattern is seen in aromatase deficiency (caused by an inactivating mutation in the cytochrome *CYP19A1* gene) and resistance to estrogens (due to a mutation in the estrogen receptor alfa *ESR1* gene). In these rare conditions absence of a pubertal growth spurt, severely delayed epiphyseal closure and osteoporosis are also caused by impaired estrogenic action on bones (261).

3.5.2.2d Familial glucocorticoid deficiency is a rare inherited condition characterized by ACTH resistance due to defects in its receptor or signaling pathways. Some cases associated with *MC2-R* gene locus (18p11.2) abnormalities exhibit tall stature (262). It has been proposed, that elevated ACTH levels because of deficient negative feedback, may interact via melanocortin receptors in bone and modulate growth (263, 264).

Excessive skeletal growth occurs independently of GH/IGF-1 by an as yet unknown mechanism (262).

3.5.2.2e Overweight and obesity in childhood are accompanied by moderately increased linear growth comparing to their normal-weight peers. Overnutrition in early life leads to progressive weight gain followed by growth acceleration. This may be related to complex neurohormonal interactions involving GH/IGF1/ghrelin/insulin and leptin/GnRH pathways, however the exact growth promoting mechanisms are unclear. Increases in body fat are associated with premature adrenarche and accelerated pubertal maturation resulting in decreased growth velocity with advanced bone age during adolescence and normal or slightly above average adult height (265).

Table 3.1 : Diagnostic tests in overgrowth causing endocrine disorders.

Endocrine causes		***Adult height***	***Diagnostic test***
a. Hyperthyroidism		Rarely above +2 SD, within normal range if adequately treated	TSH, free T4
b. Precocious puberty	GnRH-dependent	Short stature	LH, FSH, GnRH test, sex steroids
	GnRH-independent (pseudoprecocious puberty)	Short stature	ACTH, DHEAS, 17-OHP, estradiol, testosterone, androstenedione
c. Hypogonadism		Tall stature, eunuchoid proportions	LH, FSH, sex steroids
d. Familial glucocorticoid deficiency		Tall stature	ACTH, cortisol
e. Obesity		Normal / slightly above average	BMI, bone age
f. GH hypersecretion		Tall stature, acromegalic changes	GH, IGF-1, GH in OGTT

3.5.2.2f Pituitary GH hypersecretion that occurs while the epiphyseal growth plates are open, lead to excessively rapid growth rate and tall stature, which represent the main subject of the research discussed in this thesis and is presented in greater details below.

Chapter 4: The clinical syndrome of acromegaly-gigantism

4.1 Etiology

Chronic GH excess can clinically manifest with two distinct phenotypes – as gigantism, when disease starts in young individuals before the closure of the epiphyseal growth plates, and as acromegaly in adults (266). These are rare, life-threatening and chronically debilitating conditions. In most cases (>98%) GH hypersecretion is caused by GH-secreting pituitary adenoma (PA) (267). In rare cases ectopic GHRH secretion due to endocranial and extracranial tumors (such as hypothalamic hamartomas, ganglioneuromas, lung carcinoids, islet pancreatic tumors, small-cell lung cancer, adrenal adenoma, medullary thyroid cancer, pheochromocytoma, etc.) can produce pituitary hyperplasia, and/or a somatotroph adenoma (268-272). Even rarer are the cases of acromegaly due to an ectopic PA, with pituitary tissue found in the sphenoid sinus or clivus (273). Individual cases of acromegaly due to GH-producing lymphoma (274) and GH secretion by an ectopic pancreatic islet-cell tumor (275) have also been reported. Exceptional cases of pituitary GH-producing carcinomas, characterized by invasive growth, resistance to treatment and early intra- and/or extracranial (lymph nodes, liver, lungs, spine) metastasis have been reported (276).

4.2 Epidemiology

Pituitary adenomas are benign tumors of the anterior pituitary found in approximately 17% of the general population (14% in autopsy studies and 23% in radiologic studies) (277). The prevalence of clinically relevant PA is approximatively 1 in 1000 in general population, as was demonstrated in an epidemiological study conducted in three districts in Liège (278). Similar results were found by population-based studies in other countries worldwide (1/865-1/1322) (279-284). PA occur typically in adults, although rare cases (5-10%) are diagnosed in younger patients, before the age of 20 (285, 286).

Among all clinically relevant PA, prolactinomas are the most frequent phenotype (about 50%), followed by NFPA (about 30%), whereas GH-secreting lesions account for about 10% (6.5-16.5%), and corticotropinomas and thyrotropinomas are rarer (<6%) (278-284, 287). In the pediatric population, the distribution of PA secretion types is switched, with more frequent ACTH-secreting in prepubertal children, and prolactinomas most likely occurring in adolescents (288, 289), whereas the prevalence of GH-secreting pediatric PA appear to be similar to that in adults (13% in a large surgical series of pediatric PA) (285).

The prevalence of GH-secreting PA is estimated at 1:7000-14,000 inhabitants and its annual incidence varies from 1 cases per 109,000 to 1 per 323,000 population per year (278, 280-284, 287, 290-298). These indicators increase significantly with age, with the prevalence ranging from 1:27,000- 34,500 among children aged 0–17 years and up to 1:5500 among adults aged 65 years and older (295, 296). Since acromegaly is included in the category of orphan diseases, national and international registers and databases appear to be the most appropriate way of obtaining accurate data on the demographics, clinical features and treatment outcomes. According to the Liège Acromegaly Survey (LAS) - the largest international acromegaly dataset, which includes 3173 acromegaly patients, the disease begins and is diagnosed at average age around 40 years of age with a slightly greater frequency in women (54%) (299). Usually acromegaly is diagnosed rather late, from 4 to 10 years after the onset of the disease, although there is a decrease in the duration of symptoms until diagnosis in more recent periods.

Pituitary gigantism is presented in the medical literature mainly as case reports or limited descriptions of small series. Up until 2010 less than 150 documented patients with pituitary gigantism had been reported (300-303). The real prevalence of pituitary gigantism is unknown. Indirect estimates come from the proportion of acromegaly patients with gigantism. The onset of disease precedes the fusion of epiphyseal cartilages and most frequently is reported in adolescents, although it can be diagnosed at any time. Among 3173 acromegaly patients included in the LAS database, pediatric cases account for only 2% (299). In a survey of 2367 surgically treated pituitary tumors, 816 were somatotropinomas, and gigantism was diagnosed in only 1.84% of GH-secreting PA cases (285). In some previous series 5% to 20% of somatotropinoma patients were described as with excessive stature; in a more recent study in a somatotropinoma population approximately 10% presented with gigantism (301, 304, 305).

4.3 Classification of GH-producing pituitary tumors

A classification based on the maximal tumor size distinguishes pituitary microadenomas (<10mm) and macroadenomas (≥10mm), the latter includes also giant adenomas (≥ 40mm).

Most of GH-producing PA originate from the somatotroph pituitary cells and 20% occur as GH and prolactin co-secreting adenomas. The latter lesions are either mixed GH- and prolactin-secreting cell tumors, or are derived from mamosomatotroph or acidophil stem cells (276).

At the ultrastructural level pure GH-producing adenomas are classified as sparsely and densely granulated. Densely-granulated adenomas show perinuclear immunostaining pattern of cytokeratin (CAM5.2) in more than 70% of cells and are frequently associated with somatic *GNAS1* mutations (discussed in the next chapter). They are mainly characterized as less invasive and more sensitive to treatment with SST2-specific somatostatin analogues (SSA). Sparsely-granulated tumors immunostain for cytokeratin accumulated in so-called fibrous bodies and show a more aggressive biological profile (306-308).

4.4 Clinical features, comorbidities and mortality in acromegaly-gigantism

In both young patients and adults, clinical manifestations are related to GH/IGF-1 hypersecretion and local tumor mass effects due to the PA (266) (Figure 4.1).

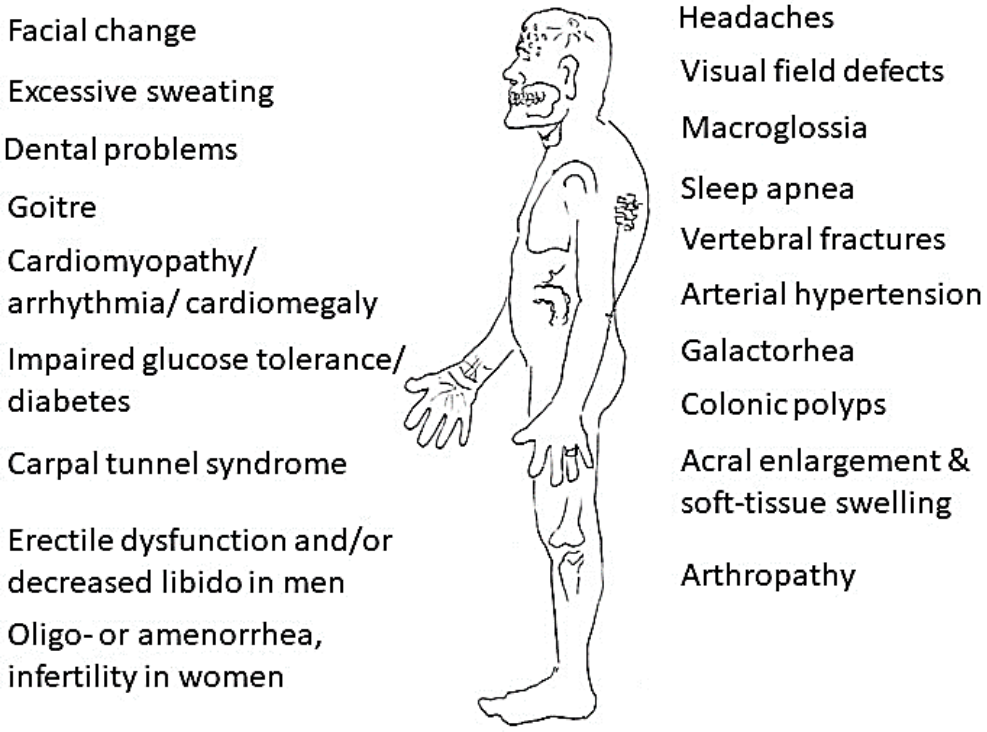


Figure 4.1: Systemic effects of GH/IGF-1 excess and local tumor mass effects

Clinical signs of acromegaly due to chronic GH/IGF-1 excess consist of cartilage, bone and soft tissues overgrowth and include the increase in the size of the hands and feet, facial dysmorphism (enlargement of nose, frontal bones and mandible), thickened skin, hyperhidrosis, seborrhea and enlargement of visceral organs (266, 309). Systemic effects of GH/IGF-1 hypersecretion generally result in complications involving the musculoskeletal system (arthralgias, degenerative osteoarthritis, paresthesia, proximal myopathy, carpal tunnel syndrome), calcium metabolism (hypercalciuria and

hyperphosphatemia, increase in bone turnover; increase in the mineral density in the cortical bone and a decrease in the trabecular bone) (266, 310), the cardiovascular system (hypertension, left ventricular and septal hypertrophy, diastolic dysfunction, coronary heart disease, heart failure or arrhythmias, acromegalic cardiomyopathy with extensive myocardial fibrosis) (311), the respiratory system (snoring, sleep apnea) (266, 312), glucose and lipid metabolism (insulin resistance and glucose intolerance; hypertriglyceridemia, dyslipidemia and atherosclerosis) (313, 314).

Long-term GH/IGF-1 excess may increase the risk of some begin neoplastic lesions (thyroid nodules and adenomatous colonic polyps) and some malignancies (colorectal, thyroid, breast, gastric and urinary tract cancer) (315, 316). Although, overall cancer risk, due to prolonged GH/IGF-1 excess, is only slightly elevated in acromegaly compared with general population, and less pronounced in population-based studies (316, 317).

In children and adolescents with incomplete physiological growth, GH/IGF-1 excess manifests as relatively proportional accelerated growth of the bones of the axial skeleton, soft tissues and visceral organs. Along with development of excessively tall stature, these patients are also characterized by increased body mass for their age. In the absence of adequate treatment and continuous GH/IGF-1 hyperproduction, patients with pituitary gigantism can develop typical symptoms and comorbidities of adult acromegaly.

Local tumor mass effects are associated with headache mainly due to increased intracranial pressure by the tumor, and the endocrine role of GH hypersecretion in the pathogenesis of headache has been also suggested (318, 319). Visual field impairment (includes different combinations of hemianopsia or homonymous bitemporal quadranopsia) is common in macroadenomas with suprasellar expansion and compression of the optic chiasm. Invasive PA invading the cavernous sinuses can compress the cranial nerves (III, IV, and VI) (266). Large PA can compress the normal pituitary gland, resulting in variable hormonal deficiencies, which may also occur after surgical treatment or with radiotherapy. Menstrual abnormalities in women are frequent and sexual dysfunction in men occurs between 20 and 30% of patients. Hyperprolactinemia can be caused by prolactin co-secretion by the PA or by compression of the pituitary stalk.

Life expectancy in uncontrolled acromegaly is reduced by around 10 years and mortality is increased by 72% compared to the general population, due to the presence of important comorbidities (320-326). The major cause of mortality is related to cardiovascular, cerebrovascular and respiratory complications (323, 327, 328), whereas diabetes mellitus and hypertension can also contribute by increasing cardiovascular risk (321, 324-326,

329, 330). In patients with persistent active disease, the standardized mortality rate is approximately 2-3 times higher than in the general population, whereas adequate disease control in treated patients decreases the mortality risk to similar to that of the general population (320-327, 329-331). Some studies reported increased mortality due to high treatment burden, particularly in those undergone radiotherapy (322, 323, 332). Recent studies tend to show a trend in normalization of mortality over time with cancer becoming the leading cause of death in acromegaly, which is similar to that observed in general population (326). These changes in mortality over time occurred with the use of new pharmacological agents, although more data is needed to define the effect of different specific treatments on longevity (333, 334). Historical reports of pituitary gigantism cases from pre-treatment epoch show a conspicuous mortality at a young age due to uncontrolled disease (29). Early mortality remains an important issue in patients with unrecognized disease or due to absence of effective treatment.

4.5 Diagnosis

The clinical and biochemical workup of acromegaly-gigantism is based on the evaluation of clinical signs and symptoms that are suspicious for GH hypersecretion, with laboratory tests to confirm GH/IGF-1 axis disturbances in those presenting with typical clinical features of acromegaly. Increased plasma IGF-1 levels, compared to sex and age- adjusted reference values, are a reliable marker of somototroph axis hyperactivity and are recommended as a first-line test for the biochemical diagnosis of acromegaly (266).

IGF-1 levels can be falsely altered in several physiological or pathological conditions, such as pregnancy, puberty, the use of oral contraceptives, uncontrolled diabetes, severe infections, and kidney or hepatic failure. In patients with elevated IGF-1 concentrations, the diagnosis is confirmed by increased serum GH levels, that are not suppressed to less than 1ng/mL (or by current guidelines, less than 0.4ng/mL using ultrasensitive assays) within 2 hours after oral glucose load (OGTT) (266, 335, 336).

An important part of the clinical diagnosis is pituitary imaging. MRI is recommended as the most informative imaging method for the evaluation of pituitary tumor size and its growth pattern. High resolution MRI techniques allow to visualized small microadenomas (266, 334, 337). An MRI based grading system is suggested for describing cavernous sinus involvement in invasive macroadenomas (338, 339). These 0 to 4 grades represent increasing levels of tumor lateral extent and its relation to the internal carotid artery in coronal view.

Somatotropinomas usually appear as hypointense lesions in T1-weighted MRI sequences and after injection of contrast. MRI signal characteristics on T2- weighted sequences are suggested to predict the behavior of GH-secreting PA. Interestingly, a T2- hypointense signal is associated with less aggressive features (smaller tumors less frequently invading the cavernous sinus) than hyper- and iso-intense PA. Moreover, these T2-hypointense lesions have a better response to SSA treatment (340-346).

Initial evaluation of hormonal function of the adenohypophysis is recommended to exclude mixed hormonal hypersecretions and hypopituitarism, and a visual field test is necessary to evaluate the involvement of the optic chiasma (266, 334).

4.6 Treatment

Treatment options for acromegaly include neurosurgery, medical therapies and radiotherapy. The appropriate management with an optimal sequence of treatment modalities depends on the pituitary tumor characteristics (size, location of the tumor, invasion and compression of optic chiasma) and on the patient's clinical status (presence of comorbidities). Other aspects such as the availability of neurosurgical treatment and adequate medical therapies are relevant. According to the Endocrine society clinical practice guidelines and Expert consensus statement, treatment of acromegaly aims to normalize hormonal levels safely and effectively (a random GH level <1ng/mL and a normal IGF-1 level, post-glucose GH <1ng/mL or <0.4ng/mL when ultrasensitive assay is used), to reduce or control tumor volume, to control disease symptoms and prevent systemic comorbidities, also reducing excess mortality (266, 334).

4.6.1 Neurosurgery

Microscopic or endoscopic transsphenoidal resection of adenoma is the primary treatment choice (266, 334) (Figure 4.2), and it is best performed by an experienced neurosurgeon (347). The disease control rate ranges between 60 and 90% for microadenomas, and between 40 and 50% for non-invasive macroadenomas and <10% for invasive adenomas, when the strictest criteria for cure are used (GH <0.4ng/mL) (333, 348-351). In young somatotropinoma patients disease control occurs in 35-50% (303, 352, 353). Surgical resection can be complicated due to large macroadenomas in young patients as well as the small size and immature skull bones with incomplete pneumatization of the sphenoid sinus. Transcranial surgery is reserved for macroadenomas greater than 4 cm with posterior or parasellar extension. In cases with large invasive PA primary surgery is usually non-curative. As was initially demonstrated in a study performed in Liège, debulking permits a higher control rate with postoperative SSA (354-356).

4.6.2 Medical treatment

Somatostatin receptor analogues (SSA) or ligands are the most frequently used primary and adjuvant medical therapy (Figure 4.2). SSA inhibit the secretion of GH and somatotroph proliferation through their interaction with specific receptors – SSTs (somatostatin receptors). The first-generation SSA (octreotide (357) and lanreotide (358)) that are typically used in acromegaly, bind preferentially to SST type 2, the most common SST that are expressed in GH-producing tumors (359-361). These medical agents are mostly used as secondary treatment when there is a persistent disease after surgery (266, 334). They can also be considered as a primary treatment option, in patients who have contraindications for or refuse neurosurgery. Presurgical SSA treatment has been proposed to improve surgical outcomes in patients (362-369), although results are not uniformly positive (370-373). Hormonal control, in both primary SSA treatment or postoperatively, is seen in about 25-45% of patients overall (266, 334, 374, 375), although higher percentages have been reported (over 50%) (376). Clinically relevant reduction in tumor size (≥20%) can be achieved in more than 50% of patients (377-381).

Some predictive factors for responses to SSA treatment exist. Lower therapeutic response to SSA administration is associated with young age, male sex, higher levels of GH, sparsely granulated adenomas, and cavernous sinus invasion (382, 383). Additionally, as mentioned above, T2 - weighted MRI signal intensity represents a valuable marker for GH-secreting PA characteristics and may be a presurgical predictor of response to SSA treatment in acromegaly. Thus, T2- hypointense somatotropinomas generally have better hormonal responses and tumor shrinkage with primary or adjuvant SSA treatment than T2-iso- or hyperintense PA (340-346).

The response to SSA treatment depends on SST expression patterns. Predictors of a poorer therapeutic response are a low expression or absence of SST2, a low ratio of SST2/SST5, a high expression of truncated SST5 coupled transmembrane domain 4 (*SSTR5TMD4*) (382). A poor response to SSA is also associated with high expression of Ki-67, low AIP expression, decreased *ZAC1* gene expression, low E-cadherin and low Raf Kinase Inhibitory Protein (RKIP) (266, 334, 382, 383).

In acromegaly patients who are inadequately controlled with postsurgical first-generation SSA the treatment algorithm suggests SSA dose escalation or more frequent dosing, a switch to monotherapy with another medical agent available for acromegaly treatment (pasireotide, pegvisomant), or a combination of these drugs; eventually new surgical

intervention and radiotherapy, either stereotactic or conventional, might be needed (Figure 4.2).

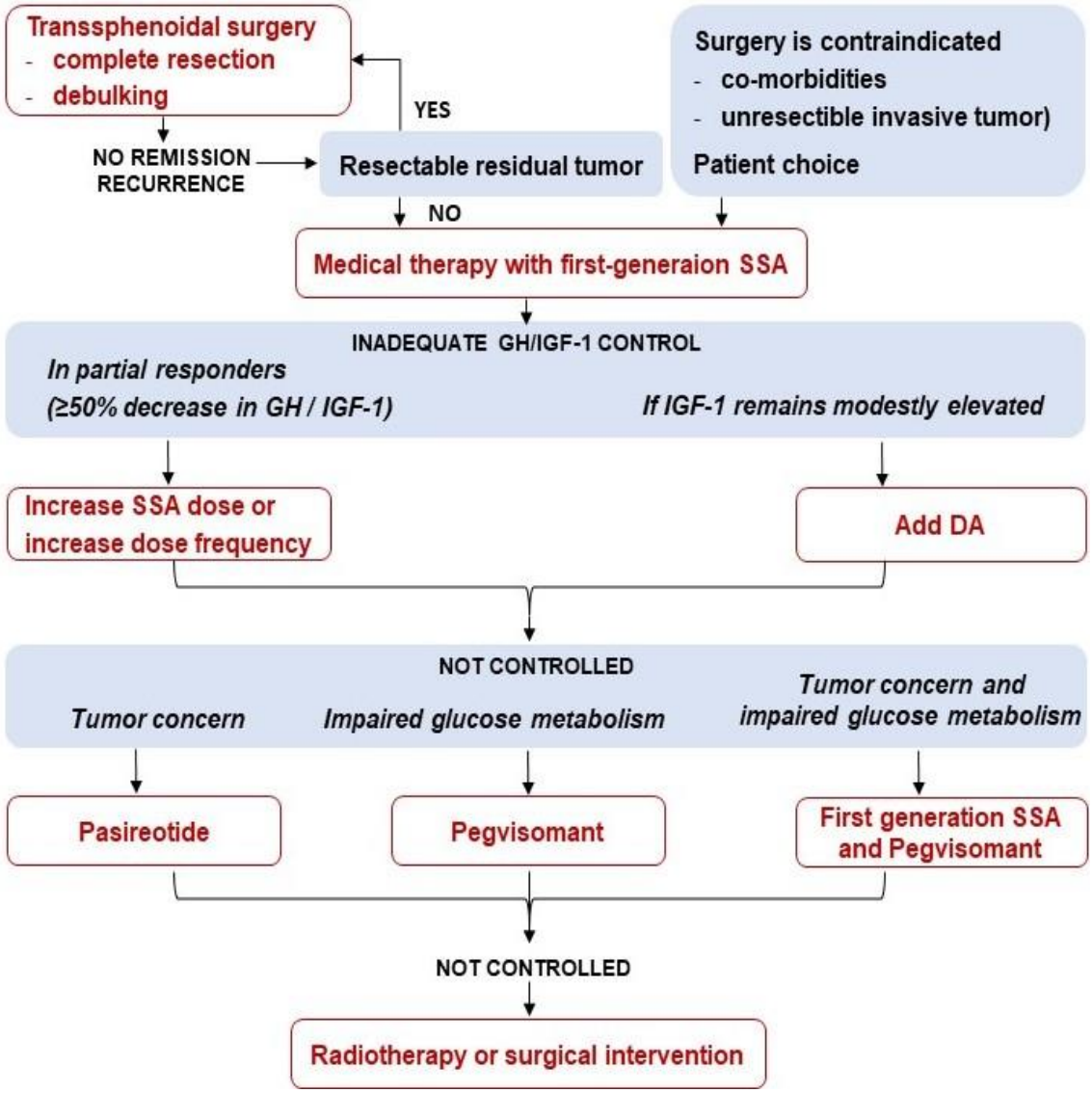


Figure 4.2: Algorithm of therapeutic options' choice. Adapted from "A consensus statement on acromegaly therapeutic outcomes" (334). SSA – somatostatin analogues; DA – dopamine agonists.

The second-generation SSA, pasireotide is a SST ligand which binds to SST types 1, 2 , 3 and 5 (384). Hormonal control can be achieved using this drug in 38-57% of patients (385-387), although the tumor volume control varies between 54-81% (385, 388, 389). About 70% of individuals treated with this drug develop glucose metabolism impairment, which has to be taken into account when considering this treatment modality. Therefore, pasireotide is recommended preferably in those patients without glucose intolerance, and for whom surgery is not an option or has not been curative and who are resistant to first-generation SSA (334, 388-390).

Pegvisomant is a GH receptor antagonist that prevents functional dimerization of the GH receptor. It is highly effective for controlling the hepatic production of IGF-1 which is normalized in 54-97% of acromegaly patients (391-393). The effect on IGF-1 is dose dependent (391), and therapeutic control with the appropriate dose can be obtained in most patients (391, 394-397). It is recommended in patients who are not cured by surgery and/or radiation therapy and in whom IGF-1 concentrations are not normalized with SSA treatment (334, 398). There are historical case reports and small series demonstrating the efficacy of pegvisomant treatment in IGF-1 control in young somatotropinoma patients (303) and in pituitary gigantism (399-402). Given that pegvisomant reduces IGF-1 levels, the negative feedback at the pituitary level is lost, inducing the potential risk of tumor growth (403, 404). Although tumor growth is observed in very rare cases (393), careful tumor follow up is required especially in cases with PA close to the optic chiasm. Because of these issues, the administration of pegvisomant in combination with SSA can be a clinically safe and effective strategy (266, 334, 405).

Dopaminergic agonists (DA) can decrease the secretion of GH and IGF-1 in both mixed GH- and prolactin-producing adenomas and pure GH-secreting PA. It is recommended as an initial adjuvant medical treatment in cases with mildly elevated IGF-1 levels (266, 334) and may be proposed acromegalic patients with mixed GH/prolactin secreting tumors (406). Cabergoline is effective for biochemical control in a proportion of patients as single-agent therapy and particularly as an adjuvant treatment in patients treated with SSA (407). At doses used in endocrinology (median weekly dose of 1mg of cabergoline), cabergoline does not produce clinically significant cardiac valve changes (408-410).

4.6.3 Radiotherapy

External beam radiotherapy is generally considered as a third line treatment option and reserved for patients with persistent disease, who have an active remnant tumor postsurgically and who are intolerant or resistant to pharmacological treatment (Figure 4.2). Biochemical control can be achieved in 20-60% of cases, however, the effect on tumor volume and hypersecretion can take years to become evident (333, 411-414). The impact of radiotherapy is frequently associated with increased risk of adverse events, which, along with the delayed time to disease control, are the main limitations to its more common use in the management of acromegaly (266, 334). In irradiated patients, hypopituitarism develops in more than 50%, and this increases with a longer follow-up period and with higher doses of irradiation (412, 415-417). Other severe side effects, such as optic nerve damage, radionecrosis, secondary brain tumors, vascular injuries and cerebrovascular events can be also observed, however they are rare (418-420). More

modern techniques, such as high single dose radiosurgery, allow the targeting of the tumor, lowering irradiation-related effects on the surrounding tissues (417, 418, 420, 421).

Chapter 5: Genetic pathophysiology of GH-secreting adenomas

5.1 Molecular basis for sporadic GH-secreting PA

Most GH-secreting PA are believed to be sporadic monoclonal tumors that arise from the proliferation of a single mutated cell of the anterior pituitary, that can include various somatic genetic changes (422-424). Advances in genetics have provided increasing evidence of such somatic molecular changes in PA formation (425). These initial genetic events include the activation of oncogenes and the inactivation of tumor suppressor genes. Activating mutations of the *GNAS1* gene, encoding for the stimulatory G-protein (gsp) are the best known of these mechanisms (Figure 5.1). Point mutations affecting codon 201 (usually R201C and R201H) or 227 of the α-subunit of the gsp result in excessive GH-cell proliferation and hormonal hypersecretion. These mutations occur in up to 40% of sporadic acromegaly cases (426, 427), and always located on maternal allele (428). Interestingly, some studies found that tumors with *GNAS1* mutations usually are smaller in size and less invasive, mostly densely granulated somatotroph adenomas and more responsive to medical treatment (429, 430).

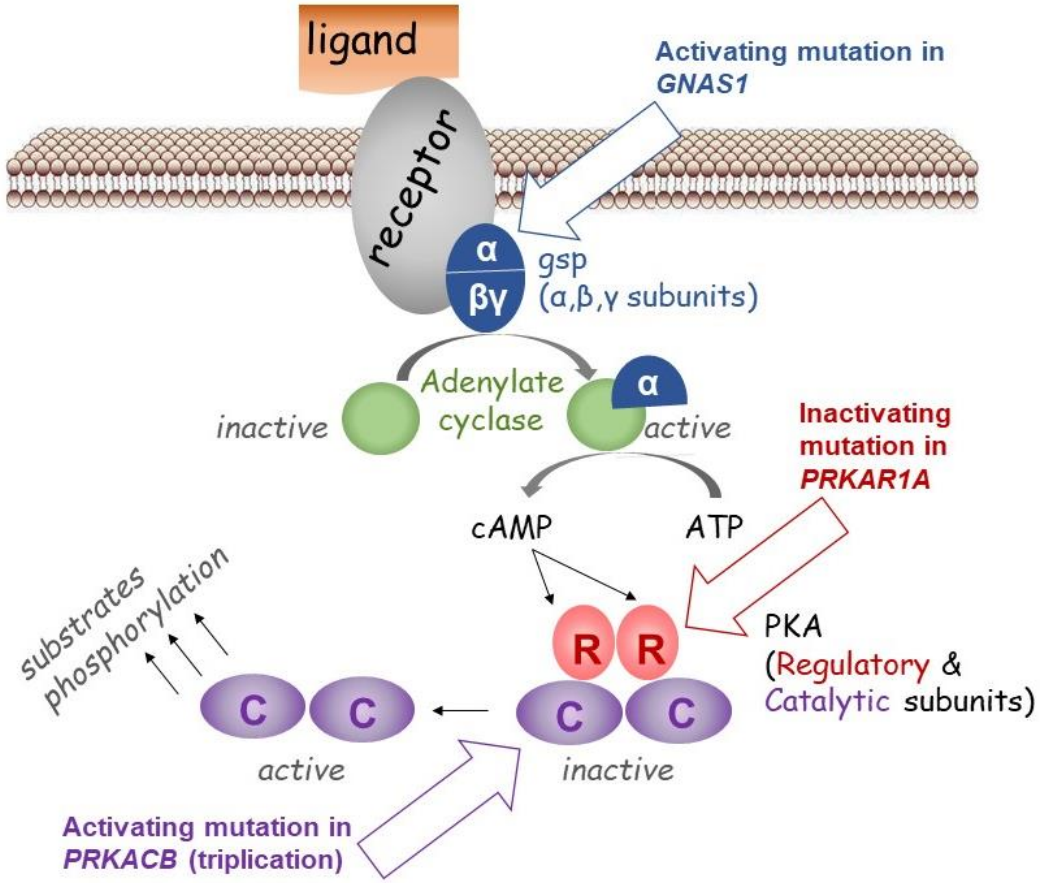


Figure 5.1: Dysregulation of adenylyl cyclase and increase of cAMP (cyclic adenosine monophosphate); α, β, γ – subunits of the gsp (stimulatory G-protein); ATP – adenosine triphosphate; R , C– regulatory and catalytic subunits, respectively, of PKA (protein kinase A).

More recent studies investigating the genomic profile of PA confirmed the exclusive appearance of *GNAS1* mutations in GH-secreting adenomas, whereas no other specific recurrent somatic mutations have been identified in these PA (431-433). Low expression of *GADD45* (growth arrest and DNA damage-inducible protein) and overexpression of *PTTG1* (pituitary tumor transforming gene 1, results in cell cycle disruption, which correlates with PA invasiveness), *STAT3* (signal transducer and activator of transcription 3; increases GH synthesis), *CDKN1A* gene (p21, cyclin-dependent kinase inhibitor, which determines malignant transformation of somatotroph cells) and *CCND1* (Cyclin D1, which regulates the transition from G1- to S-phase) are other molecular changes that have been described in GH-producing tumors (425, 426, 434-437). Advanced approaches in genetic investigations (including multiplex next generation sequencing and whole exome sequencing) have revealed heterogeneity in copy number alterations and genomic instability in GH-secreting PA (431-433). Finally, a single mono- or plurihormonal tumor or multiple lesions can arise against a background of pituitary hyperplasia. Multiple PA each with its own clonal origin can be also observed in rare cases (438, 439).

Besides an initial genetic event, the multistep process of pituitary tumorigenesis includes extracellular factors supporting its growth and expansion (growth factors, cytokines, neuropeptides, hormones), as well as further genetic events responsible for tumor progression and pathological features (invasiveness, apoptosis, resistance to medical agents, recurrence and metastatic dissemination in rare cases) (435, 440).

5.2 Hereditary syndromes with acromegaly-gigantism

Regarding inherited causes, few cases (about 5-10% of all PA) have a genetic predisposition and develop as an isolated pituitary pathology or in the context of multiorgan syndromic endocrine neoplasia (441). Several genes have been identified as being involved in inherited conditions associated with somatotropinomas (442).

5.2.1 FIPA

An inherited condition of non-syndromic PA was identified in a single- center study conducted in Liège at the end of the 1990s and defined as Familial Isolated Pituitary Adenoma (FIPA) (87-90, 92). FIPA is defined as the occurrence of PA in at least two related members of a family in the absence of other syndromic causes (MEN1, Carney complex, …). Various phenotypes of PA, not exclusively somatotropinomas, were noted in families. FIPA was then characterized in an international multicenter study including 64 FIPA families (138 PA patients in total) (90). Since then several hundred FIPA

families have been described by different international research groups (92, 443). FIPA accounts for approximately 2-3% of all PA (90).

Clinical presentation

FIPA kindreds can present as homogeneous families (same tumor phenotype in all affected family members) or heterogeneous families (different PA phenotypes in the family) (90). Although all types of hormonal secretion and NFPA were reported in FIPA, somatotropinomas (pure or mixed GH/prolactin-secreting adenomas) along with prolactinomas are the most frequent PA types and account for 29-47% and 27-40%, respectively (90, 92, 443-445). Despite the varied prevalence in different series, nevertheless, the proportion of GH-secreting PA is much higher in the setting of FIPA than in the general population (10-13%).

Patients with somatotropinomas were described in both homogeneous and heterogeneous FIPA in equal proportions. In particular, homogeneous acromegaly families represent about 20-28% of FIPA and comprise more than half of all homogeneous kindreds (90, 92, 441, 446-448). Clinically, familial GH-secreting PA are characterized by a young age of onset. They are diagnosed about 5 years earlier (10 years earlier in homogeneous somatotropinoma FIPA) and with larger lesions compared with sporadic acromegaly (90).

AIP gene mutations

The genetic basis of FIPA is partly explained by mutations in the aryl hydrocarbon receptor interacting protein (*AIP*) gene, located on chromosome 11q13. *AIP* consists of 6 exons and encodes a 330 amino acid protein. It was discovered to be a PA predisposition gene in FIPA families with GH- and prolactin-secreting PA from Northern Finland and Italy (449). In those studies, *AIP* was identified as a tumor suppressor gene. When an inactivating germinal *AIP* mutation is present, loss of heterozygosity (or a somatic point-mutation) in the second allele is required, according to the Knudson two-hit hypothesis, to cause a pituitary tumor (449).

Conventional knockout mice have been generated to assess the effect of *Aip* on pituitary tumorigenesis. Knockout mice homozygous for *Aip* mutations ($Aip^{-/-}$) die during the embryonic period due to cardiac malformation, whereas heterozygous mice ($Aip^{+/-}$) develop GH/prolactin-secreting PA, which may appear against a background of hyperplasia (450-452). *AIP*-related pituitary tumorigenesis preceded by pituitary cell hyperplasia has been also reported in humans(439).

AIP is a cytoplasmic protein that is wildly distributed in most tissues. In the pituitary it is expressed in large amounts in somatotrophs and lactotrophs (92, 445). Its functional domains are involved in various protein-protein interactions, including with aryl

hydrocarbon receptor (AHR) (453). AHR forms a the complex with AIP in cytoplasm. Interacting with its ligand (e.g. dioxin), AHR is activated and the complex is translocated to the nucleus, where it modifies gene transcription participating in xenobiotic and drug metabolism, cell cycle control and cell-cell communication (454-456) (Figure 5.2).

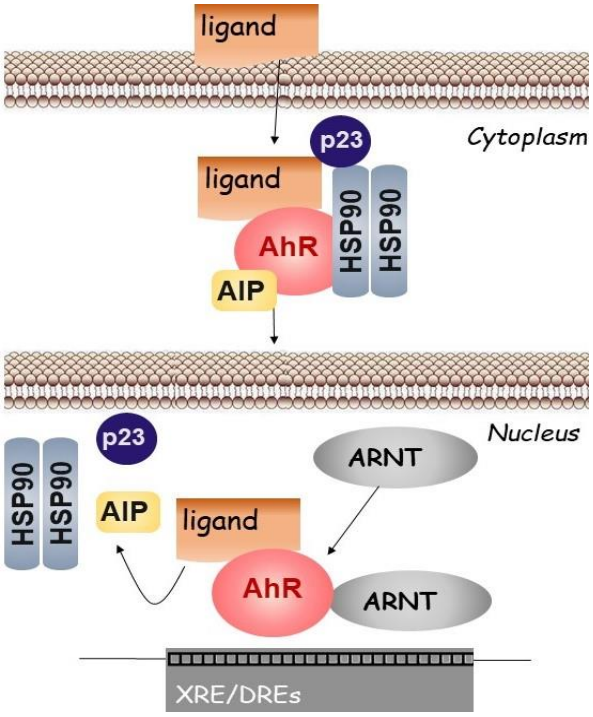


Figure 5.2 : AIP-AHR (aryl hydrocarbon receptor)-ligand interactions.
AHR is associated with AIP, HSP90 (heat shock protein 90) and p23 (co-chaperone).
Ligand binding leads to conformational changes in AHR and its translocation to the nucleus, where it forms a heterodimer with ARNT (AHR nuclear translocator) and binds to the xenobiotic- /dioxin- response elements (XRE/DRE).

AIP has been suggested to be involved in cAMP pathway activation, which is important in somatotroph tumor formation, however, the full range of its interacting partners and their mechanisms of interplay has not been fully elucidated (457). It has been identified as a mediator of somatotroph growth and GH secretion (457-462), as well as a modifier of the effects of SSA in pituitary cells (443). Several miRNAs has been suggested to be involved in the interaction with AIP (463-465). A substantial biological role has been recently assigned for high miR-34a expression in *AIP* mutation-related somatotropinomas. In these tumors, the overexpression of miR-34a transduces cell proliferation via cAMP pathway modulation and impairs the response to treatment with SSA (465).

Most of the molecular changes in the *AIP* gene are inactivating point mutations detectable by direct sequencing; deletions of exons or more extensive deletions including the whole *AIP* gene not visible by direct sequencing have been also reported (92, 443). Such deletions can be detected by Multiple Ligation Probe Amplification (MLPA) as it was previously established for *MEN1* gene (represent about 1–5% of mutations in *MEN1*, most frequently in families) (466). The same technique is employed in germline DNA for detecting large genomic rearrangements in the *AIP* gene (467). To date only rare cases of

such large genomic rearrangements in *AIP* have been reported, mainly in familial settings, and the use of the MLPA technique is usually recommended mainly in FIPA families and giants negative for *AIP* mutations on direct sequencing.

Germline inactivating mutations and deletions of *AIP* are implicated in 15-20% of FIPA, and in 50% of families with homogeneous somatotropinoma (92, 444, 468). *AIP* mutations have incomplete penetrance with reported rates of 15-30% (92, 443, 448, 469-471). Because of incomplete penetrance, *AIP* mutation-related PA can present as a simplex case (only one PA-affected mutation carrier in a family when other carriers remain unaffected). In FIPA kindreds associated with *AIP* mutations, patients develop mainly somatotropinomas (80%) and, more rarely prolactinomas (15%). Although other PA phenotypes can develop in *AIP*-positive FIPA families, at least one case of GH- or prolactin-secreting PA is usually found in such kindreds (92).

Besides FIPA, *AIP* mutations have been identified and studied extensively in different PA patients populations (92, 444, 466, 472-475). This, in turn, permitted the emergence of typical *AIP* mutation-associated PA phenotype. The majority of *AIP* positive cases develop PA before age of 30 (92, 443). Patients with *AIP* mutations frequently present with FIPA. Familial and non-familial *AIP*-related PA are usually large and invasive at presentation.

AIP mutation carriers develop predominantly pure or mixed somatotropinomas (75-80%), followed by prolactinomas (15%) (305, 443). The distinguishing characteristic of *AIP*-mutation associated somatotropinomas is the younger age of onset (20 years earlier) than sporadic acromegaly. Usually they are diagnosed as adolescents and young adults, with median age of 17.5 years. Indeed, comparing to sporadic acromegaly these cases present with more frequent gigantism cases (32% vs 6.5%) (305). *AIP*-related somatotropinomas occur predominantly in males (> 60%), and are larger and more frequently invasive PA, secreting higher GH levels with greater frequency of prolactin co-secretion compared to *AIP*-negative cases. Surgical cure of such aggressive pituitary disease is difficult and the management of patients requires multiple therapeutic modalities. Moreover, both hormonal and tumoral responses to SSA treatment are significantly reduced in *AIP*-positive acromegaly compared to sporadic cases (305, 443, 444, 476). This might happen due to loss of AIP expression in the tumor tissue, which appears to be a prognostic factor of aggressive PA behavior and poor response to SSA even in non-mutated acromegaly (458, 460).

5.2.2 Multiple endocrine neoplasia type 1 (MEN1) and MEN1-like phenocopies

MEN1 is a rare multi-organ syndrome, that affects about one in 10,000 to 100,000 individuals (477, 478). Clinically MEN1 syndrome is characterized by multiple tumors,

primarily in the parathyroid glands, endocrine pancreas, and anterior pituitary. Additional less common endocrine and non-endocrine tumors also occur and the neoplastic process in MEN1 can involve over 25 different tissues (479-481).

PA occur in 30-40% of MEN1 patients, and in about 17% of patients the PA is the first manifestation of MEN1 (477, 482-487). Isolated PA associated with *MEN1* mutations but without other MEN1 manifestations are rare (488, 489). The distribution of PA subtypes is similar to that of sporadic forms and most predominant MEN1-associated PA are prolactinomas (50- 60%), whereas somatotropinomas account for 7-10% of PA.

PA in MEN1 are more frequently macroadenomas (in 85% vs 42% of sporadic PA cases) with local compression symptoms, which develop at a younger age and more frequently demonstrate resistance to medical therapy with dopamine agonists (481, 485, 489). Plurihormonal PA are more frequent in the setting of MEN1 than in sporadic cases, and double PA were also reported in MEN1 (490-492). Rare cases of MEN1-related pituitary carcinomas have also been reported (409, 493, 494).

Loss-of-function mutations in the *MEN1* gene are the most frequent genetic cause underlying MEN1 syndrome and familial forms of isolated MEN1 manifestations (e.g. familial isolated hyperparathyroidism) (478, 495, 496). *MEN1* (located on chromosome 11q13) consists of 10 exons and encodes a 610 amino acid nuclear protein menin. Menin is ubiquitously expressed at all stages of development. It plays a vital role in cell cycle control, transcriptional and oxidative stress regulation, and DNA processing and repair (480, 481, 497). *MEN1* behaves as a tumor suppressor gene and loss of menin leads to tumor formation (480), although a comprehensive understanding of the specific role of *MEN1* in tumorigenesis is lacking.

MEN1 syndrome can occur in familial settings with autosomal dominant inheritance (481, 497). MEN1 disease has a high penetrance, almost all MEN1 patients (>95%) developed at least one clinical manifestation before age of 50 years (487, 489). PA can be potentially the first manifestation of MEN1. Sporadic forms with aggressive PA due to *de novo* mutations should be considered in the young population, as *MEN1* mutations have been reported in young somatotropinoma cases (473-475). Additionally, it should be kept in mind that rare MEN1 cases can develop acromegaly because of ectopic secretion of GHRH or GH from a non-pituitary neuroendocrine tumor (272).

Besides *MEN1* gene abnormalities, a MEN1-like phenotype can develop due to inactivating mutations in *CDKN1B* gene, which codes cyclin-dependent kinase inhibitor 1B (p27Kip1) (498). This condition is defined as MENX in a rat model and as MEN4 syndrome in humans. PA of various secretion types occur in MEN4, however only

rare GH-producing tumors have been described in MEN4 patients (499-502) and the contribution of *CDKN1B* mutations in familial and sporadic somatotropinoma occurrence is very limited (473, 499, 500, 503).

5.2.3 Carney Complex

Carney Complex (CNC) is a multiorgan syndrome occurring as a combination of myxomas, skin lesions and overactivity of endocrine glands - most frequently primary pigmented nodular adrenocortical disease (PPNAD) that lead to Cushing's syndrome (96). Pituitary GH/prolactin hypersecretion is a less frequent endocrine manifestation of CNC (504). Clinically relevant GH-secreting PA and symptomatic acromegaly occurs in a minority of CNC cases (10%). In about 75% of cases, disordered GH/ IGF-1 and prolactin secretion are present (505-508). The majority of these patients exhibit a paradoxical response to functional tests (OGTT and TRH test). Acromegaly in CNC is usually due to multifocal hyperplasia of mammosomatotroph cells with the formation of mixed GH/prolactin-secreting tumors. The onset of pituitary disease in CNC most frequently occurs during the third decade. Therefore only exceptionally rare cases of gigantism has been reported (509).

Most CNC cases (~60%, up to 80% in familial forms) are explained by loss-of-function mutations in protein kinase A regulatory subunit type 1A gene (*PRKAR1A*) (96, 504, 510, 511) (Figure 5.1). There is a very high disease penetrance in *PKRAR1A* mutations carriers (> 95% at age of 50 years) (512-514). Linkage analysis in CNC families revealed a second locus (CNC2) which is mapped to 2p16 (515, 516). A less severe phenotype with a later disease onset was reported in such cases (512). Finally, a patient with CNC-like phenotype presented with acromegaly, pigmented spots and myxomas, has been reported to have a triplication on chromosome 1p31.1 including *PRKACB* gene (517). This gene encodes the B catalytic subunit of PKA and a gain of function mutation leads to a similar molecular dysregulation and clinical phenotype as seen in cases with *PKRAR1A* inactivating mutations (Figure 5.1). Despite the GH hypersecretion and occasional cases of acromegaly described in CNC, all of the evidence indicates that genetic alterations associated with CNC appear to account for only a minority of somatotropinoma patients.

5.2.4 Association of paraganglioma/pheochromocytoma with PA

In 2012, Xekouki et al. described an association of PA and pheochromocytomas/ paraganglioma as a specific endocrine neoplasia syndrome (518), which was termed the 3P association (3PAs) (519). Mutations or large deletions in genes associated with pheochromocytoma/paraganglioma formation and familial isolated pheochromocytomas (e.g. subunits of the succinate dehydrogenase complex flavoprotein *(SDHx)* and MYC-

associated factor X (*MAX*)) have been recently demonstrated to be genetic causes of PA in the setting of 3PAs. Rare early-onset cases with macroadenomas were described in acromegaly patients due to germline mutations in these genes, however their contribution to pituitary gigantism cases has yet to be reported (518-527).

5.2.5. McCune-Albright syndrome (MAS)

MAS is a rare, sporadic syndrome due to mosaicism for post-zygotic activating mutations in the *GNAS1* gene on chromosome 20q13.2. In cells affected by *GNAS1* mutation a constitutive activation of the α subunit of gsp stimulates adenylyl cyclase and increases cAMP levels, which leads to cell dysregulation and tumorigenesis (528-530) (Figure 5.1). The syndrome is clinically characterized by the typical triad consisting of *café-au-lait* skin spots, polyostotic fibrous dysplasia and hyperactivity of endocrine system (precocious puberty, hyperthyroidism, pituitary hyperplasia or tumor with increased secretion of GH/IGF1 and prolactin, hypercortisolism) (530, 531). However, clinical presentation can be more diverse than the classical triad and can vary significantly from one patient to another. This heterogeneity in clinical spectrum is due to the different distribution of cells affected by *GNAS1* mosaicism (multiple endocrine and non-endocrine organs) (532). Germline DNA sequencing is often disappointing due to mosaic *GNAS1* mutations irregularly distributed in tissues, and less than 50% of cases can be identified with mutations even in the presence of the classical MAS triad. No familial cases of MAS have been reported in humans so far, suggesting that germline mutations are incompatible with life (530). However, theoretically in cases with gonadal germline involvement, the transmission to offspring is possible. Such inheritance of disease producing mutation was described in transgenic mice (533).

Pituitary manifestations occur in 20-30% of cases and include somatotroph hypersecretion, as well as hyperplasic and tumoral transformation (531, 534-536). Asymptomatic hypersecretion of GH/IGF1 with failure to suppress GH on the OGTT was reported in the majority of patients. GH hypersecretion is typically associated with moderate hyperprolactinemia (530, 537) and is generally due to diffuse hyperplasia, whereas PA are detected on neuroradiological imaging in about 33-54% (531, 534). Symptomatic acromegaly cases are rare and occur at young age (around age of 20), leading to tall stature in 7% of MAS patients (531, 534, 538-542).

PERSONAL CONTRIBUTION

Chapter 6: Aims and presentation of the research

6.1 Aims of the research

This research was performed with the intention of determining the characteristic disease features in patients with pituitary gigantism, considering that they could differ strikingly from the adult acromegaly phenotype which had been previously well described in the medical literature.

Secondly, the work aimed to define the etiologies of pituitary gigantism, and, therefore, to conduct a study on the genetic causes of GH hypersecretion in a large cohort.

These studies provided new insights into the genetic alterations underlying pituitary gigantism, and hence we aimed to comprehensively explore the clinical and biochemical profile of patients with the new genetic form of infantile acrogigantism *X-linked acrogigantism* (X-LAG) due to microduplication on chromosome Xq26.3, and to investigate the molecular mechanisms and pathophysiology of X-LAG syndrome.

A further objective was to explore clinical presentation and response to conventional treatment options in relation to the genetic background of pituitary gigantism.

6.2 Presentation of the research

6.2.1 Preamble

In recent years, a variety of genetic factors that predispose to somatotroph adenomas or hyperplasia have been identified (543). Mutations in genes such as *GNAS1, PRKAR1A* and *MEN1* are well known to be associated with pituitary pathology in the context of McCune-Albright syndrome, Carney complex and MEN type 1, respectively, and could explain rare cases of acromegaly and gigantism occurring in the settings of these specific genetic disorders. FIPA is a pituitary disease distinct from those syndromic forms. As it was explained in the introduction, the first study was conducted in the late 1990's that arose the emergence of FIPA as a novel disease entity. The initial publication included the observations of unexplained familial occurrence of PA in a Liège-based single-center registry of 1500 PA cases (87, 88). These first results have led to the next step to study FIPA in the larger international cohort (89, 90) and revealed that FIPA represents the most frequent hereditary form of PA and in particular somatotropinomas.

The next step was driven by the discovery of *AIP* gene being a PA predisposition gene in several FIPA kindreds (449), which led then to the comprehensive exploration of the role of *AIP* in FIPA covering its clinical and genetic aspects (444). PA are caused by mutations in the *AIP* gene in 20% of FIPA cases, and these mutations become more frequent (up to 50%) in homogeneous somatotropinoma FIPA kindreds (92, 444).

Over the last 10 years FIPA and *AIP* mutation- associated PA became a subject for a number of genetic and clinical studies. These patients develop pituitary tumors with more aggressive behavior than sporadic PA. These observations gave rise to the extensive investigation of the contribution of *AIP* mutations in the various phenotype of PA, in particular GH-secreting (305). Compared to *AIP* negative acromegaly patients, those with *AIP* mutation-related PA have larger tumors affecting younger patients, more resistant to treatment and with frequent tumor regrowth despite surgery and medical therapy (305). As a consequence of this pituitary disease profile, GH-secreting PA with *AIP* mutations more frequently lead to gigantism than those with *AIP*-negative somatotropinomas (32% vs 6.5%) (305). The results of that study, drew on focus to the aggressive tumor profile occurring in young onset GH/IGF-1 excess and led us to conceive of the idea to study clinical and genetic characteristics of pituitary gigantism. This population of patients had never been the subject of specific scientific research.

6.2.2 Organization of the research and methodology

Previously, only individual cases or small series of patients with pituitary gigantism had been published (300, 302, 303, 402, 536, 540, 541, 544-564). Therefore, we were interested to study comprehensively this particular population in a large cohort that would provide statistically robust grounds for the interpretation of results. In 2011, we organized the first large-scale collaborative international study, which included patients with gigantism and GH/IGF-1 hypersecretion by a visualized pituitary lesion (adenoma/hyperplasia). This was a retrospective cohort study conducted in the Service d'Endocrinologie, C.H.U. de Liège in collaboration with 46 international centers in 18 countries worldwide. The data of the patients (anonymized demographics, medical and familial history, genetics, clinical examination, laboratory investigations, radiology, disease status during the follow up, treatment modalities and response to therapy) were systematically collected in the participating centers and recorded in a case report form designed for the current study. ***Chapter 9*** (Publication I) presents the results of this research revealing in detail the distinct clinical and biochemical profile, evolution and medical complications of the disease, and treatment outcomes in these patients (565).

Considering that inherited forms of somatotropinomas, in particular those associated with *AIP* mutations, have a more aggressive disease course and are less responsive to treatment, genetic analysis was performed in those patients that consented to genetic testing (N=149), in order to rule out abnormalities in known PA predisposition genes (*AIP*, *MEN1*, *PRKAR1A*, *GNAS1*, *CDKN1B*).

Besides those known PA predisposition gene abnormalities, a new genomic change - Xq26.3 microduplication, which was not previously reported to be involved in pituitary tumorigenesis or growth disturbances, was identified. The role of Xq26.3 microduplication in the pathogenesis of infantile gigantism was investigated in a collaboration between the research groups from Liège University and the National Institute of Health (Bethesda, USA), mentored by Albert Beckers and Constantine Stratakis, respectively. This formed the second part of the research on pituitary gigantism, described in ***Chapter 10*** (Publication II). The study of rare genetic phenotypes requires, by definition, an extensive network of collaborating clinicians. Due to our large international databases on pituitary tumors and gigantism, we were able to involve a large number of pediatric patients with early-onset pituitary gigantism. Array-based comparative genomic hybridization (aCGH) – a genome-wide tool, that is used to investigate disease-causing CNV (566), was performed in these patients to study the prospective region on X chromosome for the copy number abnormalities and revealed new cases of accentuate phenotype of early-onset gigantism with similar genomic defects (Xq26.3 microduplications). This permitted us to confirm the localization of the suspected region on X chromosome and to reduce the number of genes in the smallest region of overlap up to 4 duplicated genes in common: *CD40L*, *ARHGEF6*, *RBMX* and *GPR101*. Disease characteristics appeared to be significantly different from those in Xq26.3 microduplication-negative gigantism patients. Thus, a new genetic syndrome of infantile gigantism was isolated and defined as X-linked acrogigantism (X-LAG) (567).

The next step involved an investigation of the expression of candidate genes in pituitary lesions from two patients with Xq26.3 microduplications undergone neurosurgical resection. This revealed *GPR101* gene, encoding an orphan G-protein–coupled receptor, being significantly overexpressed in X-LAG syndrome comparing to sporadic somatotropinomas and normal pituitary. Implication of this gene was then evaluated in sporadic acromegaly, *AIP*- and Xq26.3 microduplication- negative pituitary gigantism and FIPA. These data and initial description of X-LAG were first presented in Publication II to establish the existence of this condition. Subsequently, clinical, hormonal,

neuroradiological and pathological characteristics of X-LAG were studied on an expanded cohort (Publication III in ***Chapter 11***).

Patients with X-LAG syndrome comprised 10% of our international cohort, and represent the second most frequent known genetic cause of pituitary gigantism following the *AIP* gene mutations (found in 29% of cases in our cohort), while more than half of genetically studied cases remain with unknown cause. In Publication I we compared the disease profile in subgroups with *AIP* mutations-associated gigantism, X-LAG, and those with no detected genetic cause. Clinical presentation was affected by genetics of PA, but all pituitary gigantism subgroups demonstrated an aggressive clinical phenotype.

The next part of the research described in ***Chapter 12*** (Publication IV) aimed to explore the pathophysiology of pituitary secretory abnormalities, in particular the role of GHRH in X-LAG syndrome. A series of hormonal profiles was performed in a young female sporadic X-LAG patient, as well as a primary pituitary tumor culture following neurosurgical resection in this patient was obtained and studied *in vitro*.

Publication V in ***Chapter 13*** describes the use of a specific technique, disease-specific ddPCR to compare *GPR101* copy number with that of *ZIC3*, the closest to *GPR101* protein-coding gene unaffected in X-LAG. The ddPCR is a novel technique that permitted to deepen investigations of the genetic and genomic pathophysiology of disorders causing endocrine tumors. Initially, ddPCR methodology was validated by our research group for establishing molecular diagnoses of MAS, described in Publication IX. In X-LAG population, ddPCR allowed us to reveal somatic mosaicism for *GPR101* duplications in sporadic males. Additionally, a new ddPCR- screening tool was used for detecting CNV of *GPR101* gene, consistent with a duplication, in cases clinically suspicious for X-LAG syndrome.

Furthermore, similar ddPCR method was employed in combination with the paleogenetic DNA recovery techniques to investigate the genetic etiology of a severe pituitary gigantism in a historical person – Julius Koch (*The Giant Constantin*), who died more than a century ago. His case was published in the medical literature at that time and the nature of his pituitary gigantism due to a massive PA was confirmed. These historical records and his skeleton were preserved in the Mons Regional Natural Science Museum. According to the data obtained from these materials, Julius Koch had a clinical course that was highly suggestive for X-LAG syndrome. Moreover, a large pituitary lesion had been described as an autopsy finding and was supportive of the clinical diagnosis. As he lived in pre-treatment era, his pituitary disease remained uncontrolled throughout his life, making him the tallest person of his epoch, with a height reaching 2.59m. Cochlear DNA

was retrieved from the petrous part of the temporal bone, which is a highly protected site for DNA recovery (568). Then, a ddPCR reliable for *GPR101* CNV screening, was exploited and showed increased *GPR101* copy numbers consistent with the clinically suspicious X-LAG syndrome (569). The assay and results are detailed in ***Chapter 14*** (Publication VI).

In ***Chapter 15*** (Publication VII) we characterized, clinically and genetically, a local pituitary gigantism cohort in a center specialized in pituitary pathology in order to evaluate its prevalence among somatotropinoma patients and the role of genetic defects (in particular *AIP* mutations) in the response to treatment. Along with important clinical and therapeutic aspects, a large-scale genetic assessment was performed for the first time in this particular somatotropinoma population to study a full spectrum of known PA predisposition genes.

Chapter 16 (Publication VIII) details the specific experience with SSA treatment in two *AIP*-related acromegaly patients, one of whom presented with linear growth acceleration. The findings in this pituitary gigantism patient are remarkable in terms of hormonal and growth control during long term treatment with pasireotide LAR, as well as relentless shrinkage of her large tumor residue to the point where it was barely visible on MRI.

Over more than 25 years the relevance of mutations in *GNAS1* gene to GH secreting pituitary tumors and MAS was well recognized. Pituitary pathology in the context of MAS diffusely involves GH-producing cells, resulting in GH hypersecretion since young age, growth acceleration and acrogigantism. Severe polyostic fibrous dysplasia burdens skeletal overgrowth and acromegalic deformities and rendered the treatment options of pituitary lesion limited. ***Chapter 17*** (Publication IX) describes a dramatic case of gigantism associated with MAS. Anatomopathological and genetic studies were performed at postmortem. In our study, ddPCR methodology was employed for molecular diagnosis. For a long time, *GNAS1* mutations in the context of MAS remained associated with lesions in specific tissues due to mosaicism. In the current case, apart from the typical endocrine and non-endocrine MAS manifestations, *GNAS1* mutations were detected in number of tissues that previously were rarely reported or undescribed in the association with MAS.

Chapter 7: General discussion

Chronic GH/IGF-1 overproduction due to a somatotroph adenoma underlies both pituitary gigantism and adult acromegaly. By definition, abnormal childhood growth patterns and an excessive final height are key features in pituitary gigantism. This happens because the GH/IGF-1 excess develops in growing pediatric or adolescent population before the fusion of the epiphyseal plates, while acromegaly occurs afterwards in adults. Given that pituitary gigantism and acromegaly are due to GH secreting PA, guidelines for diagnostic, treatment and management of adult acromegaly are usually extrapolated to pituitary gigantism. However, importantly, such an approach does not take into account the significantly younger age at exposure to elevated hormonal levels and thus earlier onset of GH-related health issues, which include also growth abnormalities.

Despite the fact that differences between adult acromegaly and gigantism are quite obvious, no specific studies were undertaken in pituitary gigantism until recently. Single case descriptions and small case series have been reported in the medical literature and rare somatotropinoma patients with tall stature have been described in the setting of acromegaly cohort studies. This is due to the fact that pituitary gigantism is a very rare disease. Development of evidence-based recommendations in pituitary gigantism have previously faced various challenges, such as the small number of observations and insufficient statistical power.

In order to overcome these limitations, we conducted a multicenter comprehensive collaboration on pituitary gigantism. Including a large series of patients (n=208) we were able to explore underlying genetic causes and characterize pituitary gigantism as a severe medical condition with a wider assortment of health problems than those usually seen in adult acromegaly.

7.1 Clinical presentation and diagnostic challenges

7.1.1 Gender distribution

Despite their interlinked nature, pituitary gigantism was revealed to have many characteristics that distinguish it from sporadic acromegaly. In the largest international series of >3100 patients, acromegaly was diagnosed with approximatively a 10-year delay and the median age at diagnosis was 45.2 years with a slight female predominance (54.5%) (299) (Table 7.1). In contrast, pituitary gigantism overwhelmingly affects males (78% of patients in our international cohort). There is no clear explanation of male predominance in gigantism, although this could be partly a matter of *AIP* mutations present in almost

1/3 of those gigantism patients in our cohort (565). This is in agreement with previous reports on *AIP* positive somatotropinomas frequently affecting males (92, 305, 474).

Table 7.1: Clinical characteristics of patients with pituitary gigantism (565, 570) compared with adult acromegaly (299).

	Pituitary gigantism (n=208)	**Acromegaly** (n=3173)
Sex	78% male	54.5% female
Age at diagnosis (median)	21 years	45.2 years
Age at first symptoms (median)	14 years	33.5 years
Delay in diagnosis (median)	5.3 years	9 years
Maximum tumor diameter (median)	22mm	15mm
Macroadenoma	84.3%	71.8%
Invasion at diagnosis	54.5%	47.6%
Prolactin co-secretion	34%	10%
Glucose metabolism disorders at diagnosis	14% - glucose intolerance 10% - diabetes mellitus	27.5%
Hypertension at diagnosis	26.5%	28.8%
Sleep apnea at diagnosis	25.6%	25.5%

7.1.2 Disease onset and recognition

An acromegalic appearance and disease manifestations produced by GH excess in adults are undoubtedly unwanted, therefore they usually lead patients to seek medical care. Although the diagnosis of acromegaly in adults can be delayed for years, there is a trend for earlier recognition and diagnostic, probably due to wider availability of MRI and other diagnostic methods and better awareness from the healthcare providers about clinical symptoms and signs associated with GH hypersecretion (299). Consequently, an increase in diagnosis of milder forms of acromegaly with small PA has been noticed in older people in more recent decades, while previously such mild clinical presentations were frequently not detected (299).

Unlike adult patients with dysmorphic changes and symptoms of acromegaly, a rapidly growing child is usually not initially considered unhealthy and timely detection of abnormally rapid growth, as a major clinical sign of GH/IGF-1 disorder in children, can be a very challenging problem. In children, early growth acceleration may be left ignored by parents for several years. This may be due to that, in contrast to short stature, rapid

growth and tallness are usually considered by parents and society as indicators of physical health and a positive trait associated with life success (230, 231). For this reason, at the first referral the height is usually significantly exceeding the relevant growth curve.

We showed that females with pituitary gigantism were younger compared to male patients at diagnosis (15.8 vs 21.5 years). Also females were significantly younger than males at the time when excessively rapid growth was first noticed (11 vs. 13 years). This time difference of disease onset could be partially explained by the female predominance (70%) in X-LAG - a genetic form of severe gigantism with particularly early onset (567, 571).

Sex-related differences in the age at rapid growth onset and at diagnosis end up with an even longer delay in recognition of the disease in males (6.2 vs 2.5 years) compared to females. This could be due to social perceptions regarding tallness in the two sexes. In girls, earlier referrals and investigation can be influenced by the social context in which tall stature in girls has been regarded as less desirable feature in girls than in boys (572). Another medical reason for referrals, for which parents of girls are more sensitized than parents of boys, is related to delayed timing of pubertal events in pediatric somatotropinoma patients. Thus, puberty delay occurs in 29% of our pituitary gigantism cohort due to compression of normal pituitary gland by large adenoma and accompanying hyperprolactinemia. As generally the age of pubertal onset is younger in girls, their parents may be more likely to be alerted earlier because of puberty delay. In males, the physiological onset of puberty occurs later and pubertal delay might remain unrecognized for longer period, potentially leading to later cessation of growth and advanced stature in boys (565).

In those, whose height measurements still fit within 97th percentile, an abnormality in height development could be suspected initially by an early change in normal growth pattern. The majority of gigantism patients have an onset of disease at adolescence, that is, at the age corresponding to the timing of normal pubertal growth acceleration. For this reason, an increase in growth velocity may not cause suspicion in many cases for some time. Besides growth abnormalities, pituitary GH hypersecretion can produce various clinical signs typical of adult acromegaly (such as excessive perspiration, facial coarsening, skin hypertrophy, etc.), which could, however, be mistakenly considered as pubertal changes driven by sex steroid action. Therefore, an abnormal growth spurt is difficult to suspect in most cases, leading to significant delay in the investigation, diagnosis and treatment. Importantly, the Endocrine society clinical practice guideline on acromegaly does not point out diagnostic awareness concerning a subset of young patients

without typical acromegaly features, and a recommendation to rule out acromegaly in rapidly growing pediatric patients is missing.

7.1.3 Final height

The more increased Z-scores for final height were associated with younger age of disease onset and a larger size and more intensive hormonal activity of the pituitary tumor. These features are interconnected, as it has been well established in *Liège Acromegaly Survey* (LAS) database (299), and demonstrate the aggressive nature of somatotropinomas in younger patients developing larger and biologically more active tumors.

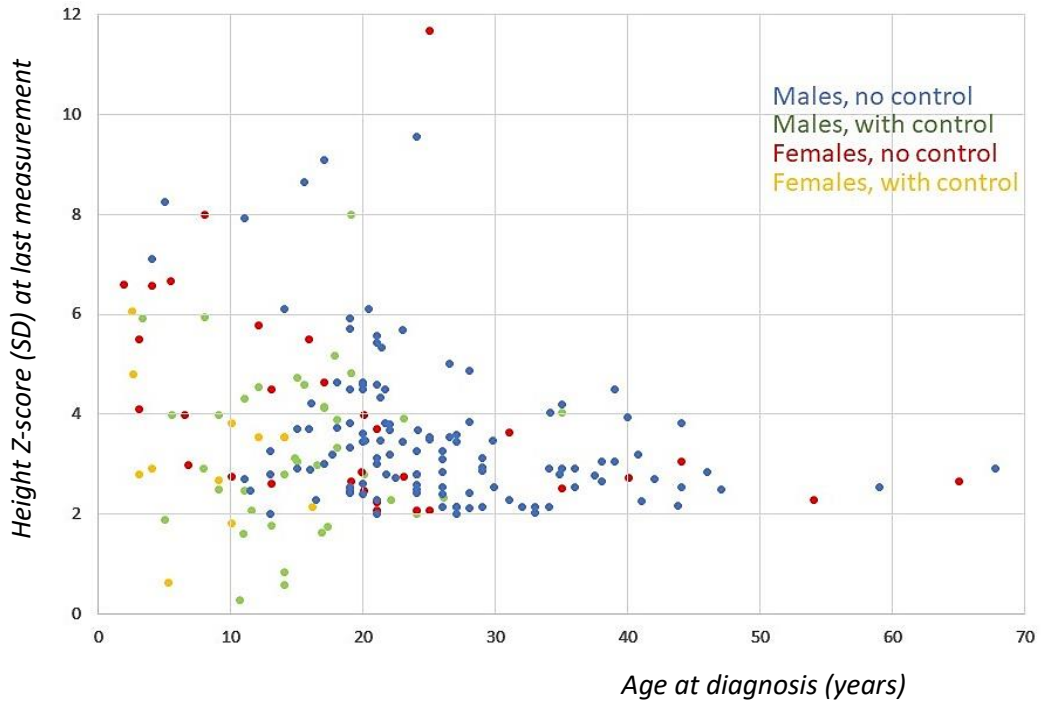


Figure 7.1: Sex and height characteristics of patients with pituitary gigantism (565)

Despite the shorter delay in disease recognition in girls, in terms of final height, both males and females developed similarly excessive stature with the median Z-scores of +3.1 SD and no significant sex-related differences. Both males and females with pituitary gigantism were similarly taller than their parents.

The most important and clinically relevant finding of our study is related to link between excess in the final height and protracted period of the active disease. In general, effective hormonal control was achieved after prolonged period of time in both genders and thus produced statural effects of similar magnitude in males and females in our cohort (Figure 7.1). We established that early disease recognition and hormonal control lead to less accentuated final heigh. This supports the importance of prompt therapeutic control of hormonal hypersecretion after timely diagnosis.

7.1.4 Pituitary adenoma characteristics

Pituitary gigantism patients developed aggressive PA in term of local extension and hormonal hypersecretion in both males and females. The vast majority of gigantism cases develop very large PA (median maximal tumor dimension 22mm), and present with macroadenomas (in 84,3%) and even "giant" adenomas (in 15%) at diagnosis with half of them being already invasive and extrasellar extension in 75%. These pituitary lesions are hormonally very active. They produce highly elevated GH levels, consequently leading to marked IGF-1 hypersecretion, and frequently co-secrete prolactin (in 34%). This pituitary disease profile is clearly a characteristic of young somatotropinomas as it has been evidenced from acromegaly research registries (299, 573) and in somatotropinoma cohort studies, where larger and more aggressive PA occur in younger patients (285, 305, 536, 574). In these previous works, an important role in development of aggressive PA has been allotted to underlying PA genetic background. In particular, *AIP* mutations present frequently with early-onset large GH-secreting PA and, thus, increase the potential to develop GH-induced growth acceleration and gigantism (305). Based on experience in somatotropinoma population studies, one would expect more severe pituitary gigantism cases due to *AIP* mutations than those *AIP* mutation-negative. However, our study established that pituitary gigantism is typified by aggressive PA irrespective of whether the genetic cause is known or not.

In terms of pituitary disease profile and consequences of GH/IGF-1 hypersecretion, the most severe form of somatotroph tumorigenesis is due to X-LAG syndrome. X-LAG develops large pituitary tumors, which produce startlingly high GH and IGF-1 levels since very young age, thus predisposing to its implication in many of the tallest cases in our study and in human history (569, 571). Importantly, X-LAG cases presented by pituitary hyperplasia for long time, in whom hypothalamic GHRH stimulation may play a role in development of pituitary cell-proliferation and hyperactivity (575). Furthermore, proliferation of co-secreting both GH and prolactin pituitary cell-populations that is encountered in X-LAG, becomes a source of prolactin dysregulation in almost all cases. This pattern of morphological changes in the pituitary tissue makes X-LAG significantly different from sporadic forms of somatotroph overactivity.

7.1.4.1 Pituitary apoplexy

Pituitary apoplexy is known to be a rare event, complicating 1.6-7.9% in population of unselected PA (280, 576, 577), and GH-secreting PA and *AIP* mutation-related PA have been reported to be more prone to apoplexy (443, 578). In our pituitary gigantism cohort, pituitary tumor apoplexy occurred in relatively high rate (8%) at substantial young age

(565), suggesting that these young-onset PA might grow rapidly in this particular population. In our study, pituitary apoplexy appears to be rarely associated with abnormalities in *AIP* gene and only one *AIP* mutation was found in a pituitary apoplexy case from FIPA family. This case and the previously reported *AIP*-positive sporadic and FIPA cases with pituitary apoplexy, including familial apoplexy in *AIP*-positive kindreds (92, 305, 439, 443, 448, 471, 579, 580), highlight a potential role for *AIP* in the development of this complication in some *AIP*-mutation positive cases. Although, further investigations need to be done to clarify whether the particularly aggressive *AIP*-related PA profile (young age, large size) promotes apoplexy development or a specific molecular process linked to genetic changes in the somatotrophs is responsible for this complication.

Furthermore, in our study, another familial pituitary gigantism case presented with pituitary apoplexy as the first clinically significant manifestation but was found with no known genetic causes; this suggests that the question of whether there are other genetic factors predisposing to pituitary apoplexy remains unclear.

7.1.5 Clinical symptoms and signs of acromegaly

Large macroadenomas and exposure to highly elevated hormonal levels contribute to a heavy burden of signs and symptoms of local tumor- mass effects and GH/IGF-1 excess. Acromegaly cases with uncontrolled GH/IGF-1 have increased mortality (320, 321, 324-326). Co-morbidities, such as cardiovascular disorders, respiratory disease and cancer, occurring in acromegaly contribute to reduced life expectancy in these patients (328). In adult acromegaly, an older age at diagnosis and a prolonged latency period before the diagnosis support development of co-morbidities, such as cardiovascular disease (299).

Despite their younger age at onset and diagnosis, gigantism patients presented with typical acromegaly features (565) (Figure 7.2). Although the GH/IGF-1 excess-related symptoms depend on the hormonal excess duration and occur more frequently in relatively older patients, but even the young-age group (≤19 years old at diagnosis), already have a high disease burden at the time of first referral. This includes carbohydrates metabolism disturbances, sleep apnea, joint disorders, arterial hypertension and heart disease that are known to be a common problem in adult acromegaly (299, 312, 328, 581, 582).

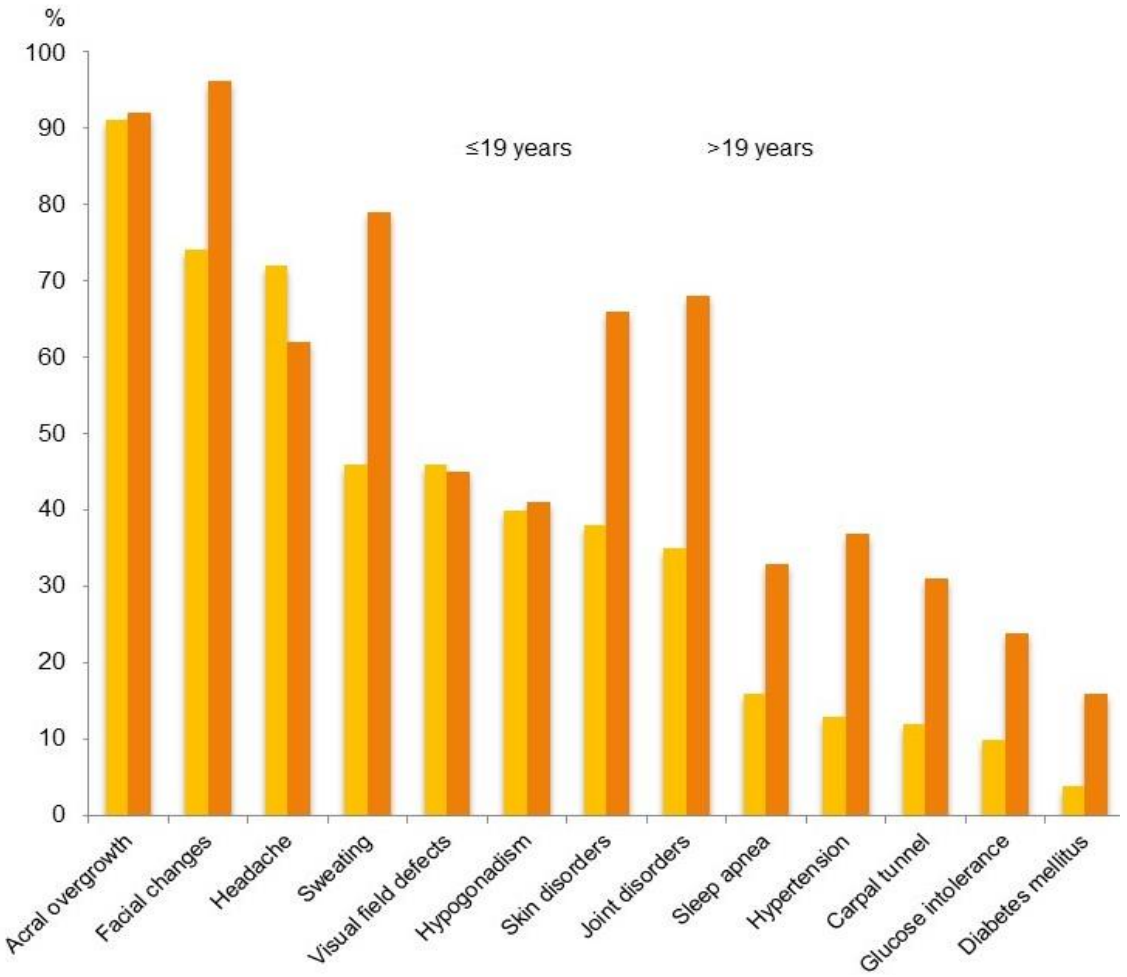


Figure 7.2: Clinical presentation at diagnosis (565)

7.1.5.1 Disease evolution

Patients with long-term uncontrolled disease, can develop a complex spectrum of signs of systemic effects of GH/IGF-1 hypersecretion. Consequently, chronic GH excess in uncontrolled patients results in earlier decompensation and increased disease-related mortality (583). Cardiovascular disease, which represents an important cause of morbidity and mortality in acromegaly accounting for 80% of complications and 44- 50% of deaths in different series (320, 321, 324-326), is frequently reported in the pituitary gigantism group (36.5% at baseline and reported in 38.3% on follow-up). These mainly include left ventricular hypertrophy (21%) and diastolic dysfunction (10%) (565, 584). Importantly, the acromegaly-related co-morbidities, such as arterial hypertension, insulin resistance and disorders of glucose and lipid metabolism, which are known as cardiovascular risk factors in adult acromegaly (328), have been already present at baseline in children and adolescents with pituitary gigantism. High rates of these cardiovascular risk factors since young age might predispose in part to the frequent occurrence of heart disease in the gigantism population (565).

It is worth noting another disabling morbidity due to GH/IGF-1 hypersecretion namely joint disorders. These were seen in a substantial proportion of patients in our cohort at baseline and rose with increasing age. Joint disease in acromegaly is associated with development of arthropathy, which is produced first as partially reversible changes in the cartilage and periarticular ligaments, then if persisting over time results in degenerative joint disease (581). Our results showed that this leads to a high disease burden during follow up in patients with pituitary gigantism (565).

The clinical impact of persistently elevated hormonal levels from a young age on the development of morbidities is dramatic, as these co-morbidities were not greatly improved on treatment, and new symptoms also occur during follow up (565). Therefore, early and sustained control of somatotroph axis activity becomes even more important in these patients for avoiding severe co-morbidities related to GH/IGF-1 effects on end-organs in addition to reducing final height (565, 584).

Some well-described historical cases of pituitary gigantism, in whom disease control was not possible due to absence of effective treatments, usually developed severe forms of tall stature complicated by multiorgan disease and died at a remarkably young age. The enhanced understanding of the pathological mechanisms of GH-secreting PA and the development of effective treatments to deal with GH/IGF-1 excess, have led to less frequent cases of severe gigantism in the Western world, whereas untreated cases develop pronounced gigantism symptoms and systemic complications. The most dramatic course can be seen in X-LAG as a result of particularly aggressive pituitary disease progression since very young age (569, 585). However, atypical of that profound overgrowth and multiple co-morbidities would be expected only in untreated pituitary gigantism cases, they remain an important consequence of uncontrolled GH/IGH-1 excess also in individuals, who have access to multiple treatment modalities, due to resistant-to-treatment pituitary disease frequently seen in pituitary gigantism (565).

7.2 Treatment strategies and follow-up

To date, no intervention studies evaluating treatment strategies have been published for pituitary gigantism and the management of these patients remains challenging. Clinical guidelines and treatment recommendations established for adult acromegaly provide the definition of the main goals of treatment for this disease, however height issues are not included in the current guidelines (266, 334). In pituitary gigantism, chronic tumoral GH/IGF-1 hypersecretion requires long-term control of GH and/or IGF-1 levels, pituitary tumor shrinkage/growth control, decrease of GH/IGF-1 hypersecretion symptoms and

local tumor mass effects. Halting the excessive linear growth should be underlined as an important additional treatment objective in young patients. Similarly, treatment outcome evaluation software programs that assess and monitor disease activity, such as SAGIT and ACRODAT, are focused on hormonal and tumor profile, signs and symptoms of acromegaly and systemic co-morbidities, but does not include data on height and linear growth pattern, that represents an objective indicator of disease activity in pituitary gigantism (586, 587).

One of the main characteristics - the young age of gigantism patients, raises issues regarding the use of somatotropinoma treatment algorithms derived from adults, as available medical treatments are not comprehensively studied and labelled for pediatric age group. Therapeutic effects of most of these medication on somatotroph axis are expected from doses that are appropriate for use in adults and the absence of pediatric guidelines for these drugs creates challenges in the treatment of children and adolescents with gigantism. Most medications indicated for acromegaly treatment must be used off-label in pediatric population, which assumes the individual determination of appropriate dosages for safely disease control.

7.2.1 Pubertal growth spurt - why time matters

The pathophysiological mechanism at the heart of pituitary gigantism – the GH-secreting adenoma, is considered as a potentially curable disease, whereas the excessive stature, which develops quickly as a consequence of its hormonal activity, is an irreversible morphological change. This raises an important issue of the time-frame for effective limitation of height gain. We found that earlier hormonal control was associated with lower final height (565). Early disease recognition and the early use of the most appropriate and effective treatment are required in order to achieve sustained hormonal control as soon as possible.

Another important particularity in pituitary gigantism, that should be considered to achieve the treatment goals, is related to the presence of physiological aspects (e.g. normal pubertal events) and pathological factors (e.g. hypogonadism, frequent genetic abnormalities). The physiology of normal growth process involves a growth spurt due to increased levels of gonadal steroids in puberty, which, should be considered as an important limiting factor for timely disease control in the youngest prepubertal cases of pituitary gigantism. The onset and duration of the pubertal growth spurt are critical parameters for determining the growth rate during puberty and eventually influence the final height. Therefore, the normalization of GH/IGF-1 hypersecretion and the rapid growth at the rate of < +2 SD is the best to be achieved before the onset of puberty.

Despite the large variation in age at onset and progression through puberty, the magnitude of the pubertal growth spurt remains less variable between individuals (123). Owning to the mean growth velocity at the onset of puberty and the peak velocity in girls and boys, a fixed increment in height can be expected as 25 (±5) cm in females and 30 (±7) cm in males, during puberty (126).

The height gain in puberty is promoted together by the somatotroph and gonadotroph axes. Tumoral GH hypersecretion in pituitary gigantism first occurring around the age of puberty or remaining uncontrolled at the time of pubertal onset, and superimposed on pubertal activation of the gonadal axis, can exacerbate the physiologically established pubertal growth spurt, producing a more elevated final height. For this reason, in children with pituitary GH hypersecretion, who enter the puberty with normal gonadal status, halting the GH-induced excessive growth is an urgent and challenging problem.

Furthermore, adequate sex steroid production is necessary to provide for physiological closure of epiphyseal growth cartilage and to decrease the excessive height-related concerns.

7.2.2 Hypogonadism and pubertal delay aggravating the height prognosis

Late onset of pubertal growth spurt in normally maturing children without GH-excess increases their final height, because epiphyseal maturation and fusion are delayed due to the absence of steroid action on the growth plates. (588, 589). Similarly, in pituitary gigantism, the deficiency of gonadal function in growing patients can prolong the period of GH-induced excess growth and exacerbate the height problem. Indeed, in our study those pituitary gigantism patients with normal gonadal function had lower final heights than patients with hypogonadism or pubertal delay (565). Recognition of hypogonadism is crucial. There are several factors in pituitary gigantism that can influence the functional gonadal state. Impairment of normal gonadotroph function may result from the gonadotroph cells being compressed and damaged by a large GH-secreting macroadenoma, frequently encountered in pituitary gigantism. A further decline in gonadal function can be caused by hyperprolactinemia, which was present in one third of cases in our cohort, due to either pituitary stalk compression by tumor mass, or prolactin co-secretion by the pituitary lesion; the latter is common in cases with genetic predisposition. Furthermore, hypogonadotropic hypogonadism can be acquired as a consequence of the heavy treatment burden in an effort to promptly control GH/IGF-1 hypersecretion (95% had undergone one or multiple neurosurgeries and 53% had received pituitary radiotherapy).

7.2.3 Treatment challenges

In gigantism, PA are not only large and highly secretory at diagnosis, but also are, in general, resistant to treatment. In acromegaly, neurosurgical resection of the pituitary tumor is recommended as first-line treatment (334, 590) with the transsphenoidal approach as the gold standard for PA resection (591, 592). Many pituitary gigantism cases require additional therapeutic options for hormonal control post-operatively: repeated surgeries and radiotherapy with adjuvant medical treatment (565). Moreover, in one third of pituitary gigantism cases in our series, aggressive pituitary disease led to the application of three or more treatment modalities. However, even the use of various treatments and a frequent multimodal approach did not ensure cure, and in the long-term only 39% of treated patients achieved GH/IGF-1 control.

Which initial treatment (medical or neurosurgical) is more beneficial for sustained hormonal control and whether preoperative use of medical therapy ameliorates surgical outcomes in pituitary gigantism is unclear based on the available observations regarding treatment strategies in pituitary gigantism population. We believed that even as a rare condition, pituitary gigantism requires a highly individual approach and expert evaluation in reference centers supported with multidisciplinary surgical and medical teams.

7.2.3.1 Neurosurgery

Surgical treatment in pituitary gigantism can be curative, but the outcomes in young somatotropinoma patients tend to be worse than in adults (285, 565, 593, 594).

A unique aspect of cranial anatomy particular for young age makes minimally invasive neurosurgery challenging in pediatric population. First, the relatively smaller endoscopic endonasal corridor in children makes visualization difficult and requires specific instrumentation. Secondly, the variable degree of sphenoid sinus development can become a substantial barrier to employing the transsphenoidal approach to access the sella region. The process of pneumatization of sphenoid sinus begins generally around age of 3 years, but usually does not reach maturity until approximately age of 10 - 14 years (595-597). Pituitary gigantism patients often have an incompletely pneumatized sphenoid sinus. Applying the transsphenoidal surgical approach in these patients can require drilling of the sphenoid bone to access the PA. Furthermore, the bone tends to be predominantly marrow-rich, and can bleed severely during the drilling manipulations. This can potentially increase operative time (596, 598). However, widespread adoption of intraoperative image guidance for landmark orientation and for identifying the important parasellar structures (e.g. carotids) appears to be helpful to safely perform endoscopic skull base surgery in the pediatric population, including the youngest of

patients, and to ameliorate intraoperative and surgical outcomes independently of the sphenoid pneumatization pattern (598-601).

The majority of PA in pituitary gigantism are macroadenomas with extrasellar extension and invasion, which are the local factors making the transsphenoidal approach more difficult and reducing the chance of curative initial neurosurgery. In our cohort only 26% of operated patients were controlled after first surgery, which is lower than the rate of remissions after surgery reported in adult acromegaly with macroadenomas (40-50%) (333, 348-351). When only partial tumor resection is performed, similarly to adult acromegaly in general, the surgical debulking effect should be beneficial for control with postoperative SSA therapy (354, 602).

Furthermore, the anterior pituitary is affected by diffuse hyperplasia in some severe gigantism cases with an underlying genetic background, such as MAS and X-LAG (531, 571, 603). The remnant hyperplasic tissue can recurrently secrete excessive hormonal levels with the potential for life-long active acromegaly and occasionally only radical resection of entire anterior pituitary tissue could be curative in these individuals.

7.2.3.2 Medical treatment

Primary pharmacotherapy may provide rapid and safe reduction of excess GH/IGF-1 levels in acromegaly (266), but mentioned above, the absence of treatment protocols for children may hinder its use. Moreover, given to that young somatotropinomas are frequently treatment-resistant, a sustained hormonal control is difficult to be obtained in long-term with medical treatment as a single therapeutic modality. Thus, primary medical treatment demonstrated poor results in our pituitary gigantism cohort, with primary control using medical therapy only in 7%. Secondary medical treatment after surgical resection with or without radiotherapy showed better response rates (34%). However, first generation SSA alone were not uniformly effective and required their combination with pegvisomant and dopamine agonists in substantial number of patients. Studies in acromegaly on the efficacy of monotherapy with long acting SSA have shown higher control rates with normalization of hormonal levels in about half of treated patients (604). Some molecular changes involved in pituitary tumor formation can lead to resistance to medical therapy. In particular, the phenotype of resistance to SSA associated with *AIP* mutations has been well established (305). *AIP* mutated acromegaly patients have a significantly lower hormonal response and a decreased rate of tumor shrinkage on treatment with first-generation, SST2-specific SSA (octreotide and lanreotide). While in previous studies relatively poor response to SST2-specific therapeutic agents appears to be linked to the loss of AIP in the tumor tissue (458, 460, 605) and occurs via $G\alpha_i$ or

ZAC1 signaling, a key factors in determination of SST2-mediated hormonal and proliferative responses to SSA (457, 459, 606), more recent data evidenced that the role of AIP staining in predicting responses to medical treatment in acromegaly might be limited (607). In *AIP* mutation carriers, the level of AIP expression in tumoral tissue depends on the type of alteration in the *AIP* gene: from preserved expression rates of AIP in patients with missense mutations and up to the entire absence or very low levels of AIP immunostaining in patients with truncated *AIP* mutations (458, 608). Also, truncated mutations in *AIP* are suggested to be frequently associated with more severe PA phenotype than non-truncated mutations in one series (443).

Thus, the aggressive behavior of somatotropinomas usually complicates the management of *AIP* mutation-related pituitary gigantism. Given to that these PA have less chance to be cured by initial neurosurgical intervention as they are frequently large and invasive at presentation, their resistance to octreotide and lanreotide represents a sustained problem in terms of timely and effective control of GH-related overgrowth (565).

Pasireotide, a new generation SSA with affinity for multiple SSTs, is indicated for the treatment of somatotropinomas (361). However, the experience of pasireotide treatment in patients with pituitary gigantism is very limited. Its high affinity for SST2, SST3 and SST5 could permit pasireotide to improve control in somatotropinomas that are resistant to first-generation SSA. Owing to frequent resistance to octreotide/lanreotide in these patients, consideration of pasireotide in the pituitary gigantism treatment strategy could be of clinical interest. In our international cohort of pituitary gigantism, only one patient, with *AIP* mutation-related macroadenoma, received pasireotide for two months as presurgical medical treatment, with uncertain outcome due to the short period of follow up. More recently, we reported long-term effects of pasireotide LAR therapy in two *AIP*-mutation positive somatotropinoma patients resistant to first-generation SSA, one of whom had GH-induced overgrowth (609). We showed that continued long-term pasireotide LAR treatment lead to hormonal control and remarkably potent tumor regression. These treatment effects were lost when switched back to octreotide, probably because the pituitary tumor was positive for SST5 but showed loss of SST2 and AIP expression. Preclinical studies suggested that biological effects of pasireotide in corticotroph lesions occur via SST5-targeted pathways, whereas in somatotropinomas the anti-secretory and anti-tumoral effects of pasireotide are predominantly mediated by SST2 (610). In this context of cell-specific activity, our data indicates that pasireotide might also exert SST5-targeted actions in somatotropinomas with impaired *AIP* function, similarly to that observed in corticotrophs (609). Although the implicated molecular

mechanisms remain to be elucidated. These results are in line with previous studies suggesting that its effects are mediated via SST5 in some somatotropinoma cohorts with low SST2 expression, in particular in those with *AIP* mutations (611, 612). Given to that *AIP* mutations underlie for about one third of pituitary gigantism cases, we believe that treatment with pasireotide might be a beneficial treatment option in some of cases. However, carbohydrate metabolism disorders are frequently revealed in pituitary gigantism at the time of diagnosis with even more elevated occurrence during follow-up, which can be an important limitation for the potential use of pasireotide. Remarkably, our case study demonstrated that the worsening of existing diabetes on chronic pasireotide treatment can be reversible after treatment removal. Thus, in one of the described patients, the impaired glucose metabolism required intensification of multimodal anti-diabetic therapy, but then it returned to baseline levels when pasireotide was stopped after >10 years of treatment (609). This observation pointed out the potential for pasireotide therapy in acromegaly patients with considering its metabolic effects.

7.2.3.2a Pegvisomant

The GH receptor antagonist pegvisomant is generally recommended in patients who are not cured by surgery and/or radiation therapy and in whom IGF-1 concentrations are not normalized with SSA treatment (334, 398). It is highly effective in blocking the biological action of GH and reducing excessive levels of IGF-1 in adult acromegaly, with control rates of 63% after 5 years use in large cohort trials (392, 393) and in virtually all patients if it is used in adequate dose in combination with SSA.

Data in the pediatric somatotropinoma population are limited (303). Disease control with pegvisomant was achieved in individual case reports and small series of pituitary gigantism, where it was generally used after the failure of other treatment modalities (399-402, 476, 613, 614). Our experience supports the idea that the administration of pegvisomant is effective to control the disease symptoms and excessive height gain by decreasing IGF-1 levels in pituitary gigantism cases. This is particularly important in gigantism with a genetic predisposition (X-LAG syndrome and *AIP* mutation-related), which is frequently difficult to treat by surgery and SSA (571, 615). However, pegvisomant could have several limitations in pituitary gigantism. Treatment with this medication does not reduce GH production and the volume of PA; in contrast, it can potentially induce tumor growth, although such cases are very rare (393). Tumor enlargement during therapy has been reported in young patients with pituitary gigantism (303, 402). For this reason, pegvisomant is more often considered in combination therapy with SSA or cabergoline, which increase the additive efficacy and additionally decrease

GH levels and provide tumoral control (391, 616-618). In our cohort of pituitary gigantism, combining pegvisomant with SSA and DA, as primary treatment or after pituitary surgery and radiotherapy, controlled 53.5% of cases (565). We believe that in the setting of pituitary gigantism, when the prompt effective therapy is crucial to control height, pegvisomant has a major advantage as it rapidly produces normalization of IGF-1 levels and clinical improvement (476, 571, 615). As we observed in particularly aggressive cases of pituitary gigantism, in a study of series from tertiary referral center, the combination therapy including pegvisomant, produced effective and safe normalization of IGF-1 levels as well as amelioration of clinical symptoms and final height prognosis (615).

7.2.3.2b Dopamine agonists

In some older publications dopamine agonists were administrated with adjunctive radiotherapy (549, 619), as well as combination therapy with other pharmacological agents has been used successfully to control the pituitary disease in some children with gigantism (620). The use of dopamine agonists in our cohort of pituitary gigantism corresponds to this previous experience. In most cases, they were administrated in combination with other medical therapies and the treatment course varied among centers due to different available therapeutic modalities (565). The efficacy of dopamine agonists in reducing serum GH levels was variable and they were also prescribed to normalize the concomitant hyperprolactinemia. The latter is a frequent finding in patients with pituitary gigantism, which could serve to augment the occurrence of gonadal dysregulation and thus prolong the delay in growth plate fusion. Adjuvant dopamine agonist treatment could be useful to normalize the prolactin levels, thus ameliorate its impairing effects on gonadal function particularly important in young patients (565, 571, 615).

7.2.3.3 Radiotherapy

Radiotherapy is an option in multi-modal therapy of somatotropinomas for long-term tumoral and hormonal control. However, its therapeutic effect slowly develops during several years and cannot provide rapid control of height gain. Usually radiotherapy is used after primary or repeated neurosurgeries fail to control tumor size and when adjuvant medical therapy is not sufficient to guarantee hormonal control. In our study, disease control in long-term follow-up (after a median of 14 years) was achieved in 43% of patients who received secondary radiotherapy, and two patients, in whom surgery was contraindicated or refused, were treated with primary radiotherapy with opposed outcomes (565). Another important point against radiotherapy in pituitary gigantism is

linked to increased occurrence of hypopituitarism and risk of neurocognitive dysfunction in irradiated children (417, 621).

7.2.4 Disease evolution and long-term control

One of the aims of effective treatment include the need to reduce the effects of hormonal hypersecretion on end-organ function and alleviate tumor compression of local structures. This appears to be difficult to attain in pituitary gigantism patients with increasing need of multiple treatment modalities (402, 565, 614, 615). In our study, low rate of sustained hormonal control was achieved, expectedly leading to the poor symptom evolution over the long-term. At last follow-up, improvement of some manifestations and worsening of other problems was noted, whereas the majority of symptoms remained unchanged (Figure 7.3).

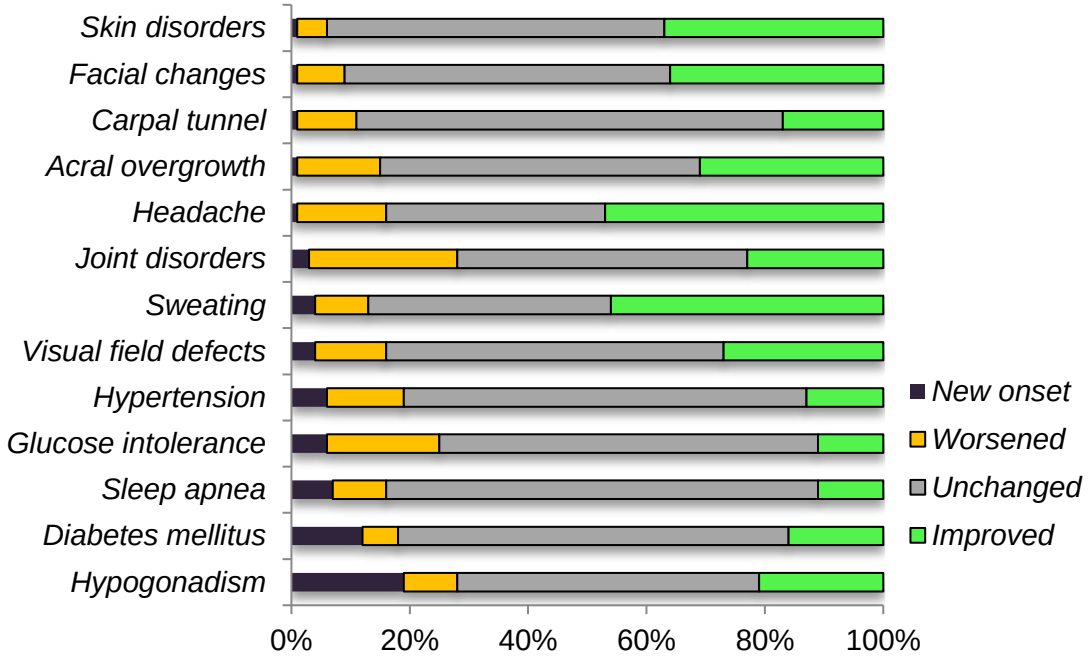


Figure 7.3: Evolution of signs and symptoms at last follow-up (565, 570)

Moreover, new symptoms were frequent and increased disease burden (in particular joint disorders, glucose metabolism impairment, sleep apnea and arterial hypertension). These typical for adult acromegaly symptoms (328, 581) appeared in much younger patients with pituitary gigantism and were mainly influenced by persistent GH/IGF-1 hypersecretion. As it was outlined above, cardiovascular disease in these relatively young population is not infrequent and was reported with even higher prevalence on follow-up (38%), including functional and structural changes of the heart (565). Evolution of these complications was influenced by delay in hormonal control and high rates of GH-related cardiovascular risk factors since young age (impaired glucose metabolism, arterial

hypertension) (Figure 7.2) (565, 584). These findings of our study emphasize how important is to prevent with all efforts the extended lack of hormonal control in pituitary gigantism.

7.2.4.1 Hypopituitarism

The reverse side of treatment of PA is hypopituitarism that is frequently acquired as a result of neurosurgery and radiotherapy. This is a particularly undesirable consequence in young patients already having a high risk of hypopituitarism due to the large size of their pituitary tumors (565). Permanent hypopituitarism is known to increase morbidity and worsen life-expectancy in acromegaly, and occurs more frequently in younger patients and in those with larger pituitary tumors (622). In pituitary gigantism, one quarter of patients already have at least one pituitary axis deficiency at presentation due to large macroadenomas (565, 614, 615). Subsequently, implementation of multiple neurosurgical and radiotherapeutic treatments increased the prevalence of cases with hypopituitarism in our cohort up to 64% during follow-up. Gonadal axis deficit was the most frequently seen in this population (62%) and probably contributed to prolonged growth and the taller final adult height, as discussed above. On another hand, hypopituitarism as a consequence of radical neurosurgical resection of the pituitary tumor or radiotherapy in cured patients, can include dysfunction of remaining somatotrophs resulting in GH deficiency, growth deceleration and insufficient final height. The prevalence of GH axis deficit at the last follow-up accounts for 10% of evaluated cases in our gigantism cohort. In children, this requires adequate GH replacement and control of growth pattern over time (567, 593). Finally, GH substitution therapy could be considered for acquired GH deficiency also in cured adult patients with the final height above +2SD (623). Pituitary deficiency involved adrenal and thyroid axis as well in 47% and 41%, respectively, thereby contributing to increasing morbidity.

7.2.5 Specific treatments for height control

There are several specific treatments for height control in constitutional tall stature. Although there is no evidence for application of these specific therapeutic options to arrest continuing skeletal growth in individuals with GH-induced overgrowth.

7.2.5.1 Orthopedic surgery

Surgical bilateral knee growth cartilage epiphysiodesis is used to limit final adult height, but it has mainly been studied in individuals with constitutional tall stature. There are few studies on this topic (126, 624-627). These studies suggest an effective reduction of final height from predicted height of 5cm, and adverse effects are rare if performed by experienced surgeons. In patients with pituitary gigantism, the clinical expediency of

growth limiting orthopedic intervention is unknown. In patients, who lack sustained hormonal control, persistent GH/IGF-1 hypersecretion or hormonal recurrence after treatment can increase risk of complications, including asymmetrical leg growth and other skeletal deformities. Therefore, particular emphasis here should be placed on the ensuring stable GH/IGF-1 control prior to these measures, as an unwarranted interference with skeletal growth appear to be unsafe when hormonal levels are not maintained at normal range (628).

7.2.5.2 Pharmacological treatment with sex steroids

Linear growth can be halted by exogeneous administration of sex steroids in patients with an excessive calculated growth potential. However, to date sex steroids are no longer widely recommended for growth deceleration due to their short and long-term consequences. In girls with constitutional tall stature, the use of estrogenic treatment at the beginning of puberty can potentially produce complications, including a decrease in fertility and an increased risk of estrogen-dependent cancer (629-631). Treatment with estrogen in males is not widely used, and is limited to exceptional cases with LH receptor defects. Moreover, reversible gynecomastia can occur on estrogen treatment. In males, testosterone is usually used for aromatization to estrogen to cause epiphyseal maturation, but is frequently accompanied by complications, such as acne and aggressive behavior, and it remains currently unclear if testosterone treatment increases cancer risk (for example for prostate cancer) in these men (632, 633). Therefore, the use of sex steroids to cause premature epiphyseal cartilage fusion and growth arrest in children with constitutional tall stature is currently highly controversial (634, 635). In both sexes, the effect of sex steroids on growth varies in treated individuals and the final height reduction depends on the bone age at which treatment is initiated (636).

As discussed above, hypogonadism with pubertal arrest in pituitary gigantism can delay epiphyseal maturation and fusion providing longer period for growth. In these cases, even when GH/IGF-1 is inhibited, there can be a potential for further statural growth, and the estimated final height can still significantly exceed +2 SD. Successful limiting of growth by sex hormone administration has been reported in pituitary gigantism with hypogonadism (476). Although, the question whether sex steroid treatment can have a general role in acceleration of epiphyseal fusion and reduction in growth velocity in pituitary gigantism, remains open. We believe that the indications for sex steroids use in pituitary gigantism are best considered in patients with obvious hypogonadism.

7.3 Genetics of pituitary gigantism

Scientific and technological advances in the recent 15-20 years have led to better understanding of genetic predisposition to GH-secreting PA (286, 543, 637). Genetic abnormalities in known PA genes can affect the secretory and proliferative function of somatotroph cells conferring specific characteristics to these lesions. Thus, inherited or syndromic forms of somatotropinomas are well distinguished from sporadic acromegaly by being more aggressive difficult-to-treat PA occurring at younger age. As discussed above, this clinical presentation is typical for pituitary gigantism and hence genetic forms have been previously reported in some somatotropinoma patients with tall stature (305, 570). In our study, genetic causes of pituitary gigantism were extensively investigated in a large cohort. Genetic mutations or pathological copy number variation underlie half of pituitary gigantism cases (565).

7.3.1 FIPA and role of AIP mutations in pituitary gigantism

FIPA is the most frequent inherited form of somatotropinomas with abnormalities in *AIP* gene revealed in about 20% of FIPA and in 50% of homogeneous FIPA kindreds with somatotropinomas. Familial cases were not infrequently found in our pituitary gigantism cohort (22.3%) (565). Unsurprisingly, most of gigantism patients from FIPA kindreds were diagnosed with *AIP* mutations (305, 443, 565, 570). Familial occurrence can be underestimated and *AIP*-associated gigantism cases can be improperly considered as sporadic, when family history is incomplete and leaves out distant relatives with PA. Moreover, *AIP* mutation- related gigantism can appear as a simplex case, when the pituitary phenotype is produced in a single patient in a kindred, whereas other asymptomatic carriers remain unrevealed. This happens due to incomplete penetrance, which has been reported with variable rates (15-30%) (92, 469-471).

7.3.1.1 Clinical characteristics of AIP mutation positive gigantism

AIP mutation-related gigantism patients, both - in the settings of FIPA or sporadic, differ from their *AIP*-negative counterparts and X-LAG syndrome by their pronounced male predominance (95%). This is consistent with the evidence that all secretion types of *AIP*-related PA more frequently occur in males (50-60%) (92, 305, 443).

Non-syndromic somatotropinomas due to germline mutations in *AIP* gene, affect usually adolescents (with the median age at disease onset of 17.5 years) and have generally large and invasive pituitary lesions which are resistant to treatment (85, 92, 305, 444, 474). Given the aggressive phenotype of *AIP* mutation-associated pituitary tumors frequently occurring during the physiological growth period, cases with pituitary gigantism were reported in a larger proportion (32%) of *AIP*-positive acromegaly as compared to *AIP*-

negative acromegaly (6.5%) (305). We found *AIP* mutations in nearly one third of gigantism cases, with an even higher occurrence in patients from FIPA families (42%) (565). Thus, germline *AIP* mutations represent the most frequent genetic cause of GH- secreting PA in gigantism patients. Another research group reported that 56 of 120 (46.7%) patients with pituitary gigantism had *AIP* mutations, however, in most of patients in this study, mutations in other genes were ruled out beforehand and patients with other than *AIP* gene abnormalities were not included in the total group (443). A higher prevalence of *AIP* related somatotropinomas, including those causing gigantism, can be observed due to increased mutation carrier frequency that is highly concentrated in a local population of a discrete geographical region (85, 638, 639). Such founder mutations can be transmitted in large kindreds. Founder *AIP* mutations (R304X, etc.) have been reported in some groups of PA patients from Finland, Italy and Ireland (85, 92, 449, 638, 640). The *AIP* mutation, p.F269_H275dup, was found in apparently sporadic gigantism patients and in several apparently unrelated FIPA kindreds from different countries (linked by a history of steady population immigration or geographical proximity) (445, 641) with high occurrence of gigantism, and the evidence suggested a common ancestor for the affected cases. According to the established prevalence of *AIP* mutations in different PA populations, several groups at-risk for *AIP* mutations have been identified. These include FIPA patients, especially those from homogeneous somatotropinoma kindreds, pediatric PA and young patients with large macroadenomas, whereas in unselected PA patients *AIP* mutations are rare (0-4%) (92). Our study demonstrated that *AIP* mutations are the most prevalent cause of pituitary gigantism, in whom the *AIP*-related pituitary disease leads to a distinct clinical phenotype. Thereby, these observations make the pituitary gigantism patients a target population for the analysis of the *AIP* status for correct treatment choices and family screening. We believe that the genetic screening for *AIP* mutations is useful for clinical decision and treatment planning in pituitary gigantism (642).

Given the role of *AIP* mutations in the etiopathology of early-onset aggressive PA, clinical screening is justified from a young age in all mutation carriers, including those asymptomatic, for early identification and treatment of *AIP*-related PA in order to avoid development of large tumors with compression of local structures, as well as clinical manifestations of hormonal abnormalities, including tall stature. However, asymptomatic microadenomas are frequently revealed in *AIP* mutation carriers (that might mirror the frequent occurrence of small pituitary incidentalomas in the general population) and that rarely progress with time (643). On another hand, evidence from some *AIP* mutation positive cases provides an insight into the natural history of *AIP*-related PA with their

development into larger adenomas with a more severe phenotype (609). We reported a case of a pediatric *AIP* mutation-positive microprolactinoma treated initially with cabergoline. However, this pituitary tumor had early progression and grew rapidly into an invasive macroadenoma with GH hypersecretion, the onset of clinical signs of GH/IGF-1 excess and accelerated linear growth (609). This has led to a question being raised whether there is an interaction of various factors and what is the particular role of this gene in the sequence of genetic events. We believe that, tumoral transformation in the pituitary cell-lines might be initiated by *AIP* mutation, whereas tumor progression and aggressive phenotype might be potentially influenced by subsequent additional genetic alterations in the tumor tissue or by the local environment factors. Although a different scenario of tumor development could also be assumed, which might imply the emergence of the PA driven initially by mutations in genes other than *AIP* and then worsen after their complementary interaction with molecular pathways involving *AIP*.

Some specific features, as pituitary hyperplasia, may establish the pituitary disease development. Thus, pituitary tumorigenesis preceded by pituitary cell hyperplasia has been reported in *Aip* knockout mice and in humans with germline *AIP* mutations (439, 450-452). *AIP* is a tumor suppressor gene and inactivation of the wild-type allele is required to develop the *AIP*-related phenotype. In a previous study, loss of heterozygosity as a second hit at the *AIP* locus appeared to be a later event than hyperplasia in the PA of two siblings (439). This is indicative that *AIP* mutation positive PA may appear on the background of hyperplasia.

7.3.1.2 Large genomic rearrangements of AIP

All except two *AIP*-positive patients in our gigantism cohort had inactivating point mutations in *AIP* gene detected by direct sequencing. Large intragenic deletions in *AIP* were revealed with the use of the MLPA technique in pituitary gigantism cases from two FIPA kindreds negative for *AIP* mutations on direct sequencing (565). This is consistent with the previous experience on detection of large genomic rearrangement in *AIP* in various PA populations (92, 443). Therefore, the use of the MLPA technique in pituitary gigantism is potentially useful for *AIP* testing.

7.3.2 X-LAG syndrome

One of the most important findings of our research was the discovery of a new genetic syndrome – X-LAG, characterized by GH and prolactin hypersecretion due to pituitary hyperplasia or adenoma. Since the initial publication in 2014 including the description of the first X-LAG cohort, this remains very rare condition, with about 33 cases reported to date worldwide (567, 571, 575, 585, 644-647).

Patients with X-LAG syndrome showed a remarkably consistent phenotype that distinguishes X-LAG from other cases and allows us to have an idea about the clinical prognosis.

7.3.2.1 Clinical presentation and growth pattern in X-LAG

Despite having a congenital disorder, at birth these children usually do not stand out in terms of development. Usually they are born from uncomplicated pregnancies and are normally sized at birth, cases with low or increased birth heights and weights have been reported (571, 646). The onset of an abnormal pattern of rapidly increasing growth occurs during infancy (usually the first year of life), which is a distinguishing clinical characteristic of X-LAG. Most patients also had increased weight and head circumference, not always in parallel with height. In affected children anthropometric measurements and their chronological age and age-related development are discordant. By the time of diagnosis, which is generally made at the median age of three years (almost always before age of 5), these children already had an elevated Z-score for height (about +4 SD) (567, 571). Besides severe overgrowth, these young patients presented at diagnosis with signs typical for adult acromegaly: coarsened facial features, enlarged nasal bridge, prominent jaw with widely spaced teeth, large hands and feet, and increased perspiration. Increased appetite and hunger were reported in one-third of X-LAG cases, but was not seen in other forms of pituitary gigantism. Moreover, in some X-LAG cases, acanthosis nigricans has been noticed suggesting that insulin resistance might be present in X-LAG (571).

The rapid increase in body size in X-LAG is GH-dependent and associated with GH-secreting pituitary lesions. These tumors have features that are different from the sporadic somatotroph adenomas in terms of histological structure and secretory activity. They are biologically very active with greatly elevated levels of GH, and with prolactin co-secretion in almost all cases (567, 571). Mixed GH- and prolactin-secreting PA, as well as mammosomatotroph cases, are predominant in X-LAG (571). Such findings have been described also in other genetic forms of acromegaly (92), whereas plurihormonal secretion is rare in sporadic PA cases, in which concomitant hyperprolactinemia is mainly caused by pituitary stalk compression by large tumor.

7.3.2.2 Pituitary lesions in X-LAG and the role of GHRH in tumorigenesis

Despite the very young age of disease onset, most of children with X-LAG develop large pituitary macroadenomas, while others have pituitary hyperplasia. Furthermore, adenoma formation within the hyperplasic tissue was detected at pathology examination of operated samples, in rare cases with multiple microadenomas against the background of

pituitary cell hyperplasia (571). Thus, the process behind tumorigenesis in X-LAG might involve an evolution from normal tissue through hyperplasia to adenoma formation. This spectrum of pathological findings in X-LAG appears to mirror closely those seen in GHRH transgenic mice (648, 649) influenced by elevated circulating GHRH levels. In humans, hyperstimulation of anterior pituitary cells by highly elevated circulating GHRH levels in rare cases of GHRH-secreting NETs produce acromegaly due to pituitary hyperplasia, rather than somatotroph adenoma, with GH hypersecretion (270). In Publication IV we reported clinical and *in vitro* tumor studies in an X-LAG case, in which the implication of GHRH dysregulation in the pathophysiology of X-LAG has been explored. Importantly, peripheral GHRH levels were consistently elevated but below those suggestive of an ectopic GHRH source. Adenomatous and hyperplasic tissues were strongly positive for GHRH-R as compared to normal pituitary tissue. In contrast, GHRH staining was low in the pituitary lesion samples, arguing against a source of GHRH at the pituitary level (571). Previously published X-LAG cases were also reported with elevated levels of GHRH (553). Elevated plasma GHRH levels at diagnosis accompanied by high levels of GHRH-R in the hyperplastic and adenomatous pituitary tissue, are suggestive for a causative role of hypothalamic dysregulation (575). Another argument for the GHRH-driven process underlying X-LAG is the inhibition of GH and prolactin secretion *in vitro* by a GHRH receptor antagonist (575). Finally, recent experiments in mouse model, transgenic for *GPR101* duplication, provided new insights into signaling pathways in X-LAG (650). In this model, a transgenic *Ghrhr*^{Gpr101} construct linked *Gpr101* to *Ghrhr* via its promoter, thus ensuring Gpr101 overexpression occurrence exclusively in the pituitary cells. As a result, this enhanced greatly GH and prolactin secretion in the pituitary, leading to somatic overgrowth and metabolic disturbances similar to acromegaly. Importantly, in the current animal model GPR101 overexpression in the pituitary level didn't provoke pituitary hyperplasia or tumor formation and hormonal hypersecretion occurs from the unchanged pituitary gland, unlike what was observed in X-LAG syndrome in humans. X-LAG phenotype include PA or hyperplasia formation, which might occur due to interference of multiple pathways involving GPR101 overexpression in both pituitary and hypothalamic levels. Thus, due to GPR101 overexpression presented only in pituitary cells, current transgenic model produced an incomplete phenotype of X-LAG without hypothalamus-mediated morphological changes in the pituitary tissue, which is, in fact, a feature distinguishing it from X-LAG presentation in humans (650). The entirety of these data indicates that specific pathological mechanism of pituitary tumorigenesis in X-LAG may be maintained by

hypothalamic influence via increased GHRH levels that eventually promotes mammosomatotroph cells proliferation and hyperactivity.

7.3.2.3 Treatment challenges in X-LAG

Referring to treatment of the pituitary disease in X-LAG, we pointed out that the unusual tumor biology leads to exceptional challenges in management of these lesions. SSA and DA are insufficient to bring hormonal levels and growth under control. Treatment with DA can, however, be effective for normalization of prolactin levels. Most of the patients showed very poor responses to SSA treatment either primary or postoperative. Interestingly, this was not caused by low SST in pituitary tumors from affected patients (571). The currently available SSA bind preferentially to SST2, however some X-LAG patients are not responsive to therapy despite preserved expression of this receptor subtype. Downregulation of important post-receptor signaling pathways could underlie this phenomenon (460). The impairment of some pathways linked to SSA effects in somatotropinomas with poor SSA response is associated with loss of AIP (459, 460, 606, 651). But in pituitary tumors from X-LAG patients, AIP staining was preserved, indicating that other signaling elements and pathways involved in SSA resistance in X-LAG yet to be identified. Given the frequent diffuse hyperplasia in X-LAG, radical neurosurgical resection is of particular importance to insure the disease control. However, as it was explained above, this approach contributes to high rates of hypopituitarism. The large size of PA and unusual tumor biology lead to increased recurrence rates; thereby reoperations and radiotherapy are frequently required as SSA were shown being not effective treatment option. The GH antagonist pegvisomant can be used successfully as long-term adjuvant therapy. The role of new-generation SSA pasireotide in treatment of X-LAG patients has not been established. According to the immunostaining results, SST5 expression was preserved in tumors from X-LAG patients. Given to this data, treatment with multireceptor-targeted SSA potentially could represent a way to control the marked hormonal hypersecretion and overgrowth in some X-LAG patients.

7.3.2.4 X-LAG Genetics

The molecular findings in X-LAG were equally surprising and novel as the clinical characteristics of the patients. The role of CNV has been established as being responsible for human genomic disorders (652). CNV and genomic rearrangements have not been previously known to be associated with pituitary tumorigenesis. Our studies demonstrate for the first time that pituitary gigantism can occur as a genomic trait due to Xq26.3 microduplication that encompasses the gene *GPR101*.

GPR101, the gene which is invariably involved in microduplications and is responsible for X-LAG (567, 571, 575, 585, 644-647). It is an orphan G-protein coupled receptor that partly resembles adrenergic and serotonin receptors. No endogenous ligand of GPR101 has been identified, and its physiological function, signaling pathways and the mechanisms of dysregulation of *GPR101* are yet unknown. The data from X-LAG patients and *in vitro* studies provide strong evidence for the involvement of GPR101 in the somatotroph axis. Expression of GPR101 is age and tissue specific. In particular, it is strongly expressed in the arcuate nucleus in the hypothalamus (653), suggesting an additional argument that hypothalamic involvement could play a role in the pathogenesis of X-LAG, as discussed above.

Implication of *GPR101* duplication in pituitary tumorigenesis in X-LAG syndrome arose a question whether abnormalities in this gene could be involved in sporadic acromegaly and other PA or congenital pituitary deficiency (567, 654-656). Although, rare mutations in *GPR101* have been detected in those populations, so far, all the available evidence strongly suggests that the most consistent clinical phenotype attributed to *GPR101* abnormalities occur due to its duplication leading to X-LAG syndrome.

7.3.2.4a Mosaicism in X-LAG

Implementation of aCGH was important to initially reveal the role of pathological CNV in pituitary tumorigenesis and X-LAG. Employment of another technique, ddPCR, permitted us to reveal *GPR101* gene duplications in the mosaic state (644). Interestingly, this was found to be the underlying cause of X-LAG phenotype exclusively in sporadic male patients, whereas females and males from X-LAG families had constitutive duplications (585, 644, 646). Remarkably, duplications involving as low as 16% of cells is capable of inducing X-LAG with dramatic gigantism, explaining some of the tallest recorded cases. This novel finding elucidates a new mechanism - mosaicism for a duplication, in pituitary tumorigenesis. In addition, one of the ddPCR techniques that we employed was useful as a screening tool, identifying a new case of X-LAG among 64 patients with acromegaly and gigantism. Thus, it may have implications for diagnosis of X-LAG syndrome.

7.3.2.4b FIPA due to X-LAG

Actually, familial form of X-LAG represents a homogeneous FIPA with acrogigantism in all affected members. To date, three X-LAG kindreds have been described with identical (unique for each family) Xq26.3 duplication transmission from affected mothers to affected sons (567, 645). The penetrance of the pituitary disease occurring due to

Xq26.3 microduplication is supposed to be 100% and no cases of unaffected *GPR101* CNV carriers were seen sporadically or among parents and siblings of X-LAG patients.

7.3.2.5 X-LAG phenotype in historical cases of gigantism

A scientific literature review identified at least 15 case reports, which were phenotypically suggestive as candidates for X-LAG (567). Some of those were later confirmed genetically (645, 646), confirming the consistent pattern of clinical presentation of X-LAG, even if identified retrospectively. We also explored early childhood-onset gigantism cases reaching further back into the historical data and found that the clinical phenotype of X-LAG is consistent with some historical cases of gigantism (571). Thus, like our patients' population, these cases came from families without growth disorders that would exclude familial tall stature and other GH-unrelated causes of overgrowth in the family. According to available records, normally proportioned at birth, they started growing abnormally before the age of three. Many historical cases of gigantism were described with large pituitary lesions and a dramatic clinical course. They presented marked overgrowth and heights far in excess of normal (approximately + 4 SD).

Figure 7.4: Historical images of the tallest recorded cases of pituitary gigantism with early childhood-onset corresponding to the established distinctive clinical presentation of X-LAG. Two grey silhouettes illustrate the average human height (1.75m) (571, 657).

Julius Koch from 19th century with a height reaching 2.59m was one of the tallest humans in history, whose remains were available for exploration. We analyzed the clinical and anatomopathological description of this case in the preserved medical records that were consistent with the X-LAG phenotype (569). Findings from our study concluded that X-LAG syndrome likely explains many of the tallest people, like Julius Koch, and made his case the tallest genetically established gigantism case on record.

It is important to note, that in the tallest people in history, like Robert Wadlow and Julius Koch, the pituitary disease occurred in the era before effective treatments were developed.

Thus, in those individuals with markedly active pituitary disease from infancy, as is typical for X-LAG syndrome, the lack of an adequate treatment produced the tallest individuals in human history (571, 657).

These descriptions provide valuable information about natural history of the pituitary tumors and GH excess-driven pathological changes in pituitary gigantism. Aggressive natural progression of X-LAG-associated pituitary tumor can be captured and described in contemporary patients when it is left without treatment during a considerable period, with spectacular increase in height being seen (585). Such cases illustrate clearly the dangers inherent in this condition and emphasizes the importance of early recognition, diagnosis and effective treatment.

7.3.3 Genetically negative pituitary gigantism cases

In half of patients (54%), who were included in our genetic analysis, no molecular abnormality has been identified in PA predisposition genes (565). After distinguishing the X-LAG and the *AIP*-related disease as a separate genetic form of gigantism, the genetically negative cohort still represents an interesting phenotype of large, aggressive, biologically very active somatotropinomas, with poor therapeutic response (565, 570). In the pituitary gigantism population, *AIP* mutations make a notable contribution to the severe phenotype of pituitary disease (443, 565) which is in line with the established profile of *AIP* mutation-positive acromegaly (305). Meanwhile, we have demonstrated here that all pituitary gigantism cases are conferred with features of aggressive pituitary disease regardless their genetic status, in contradistinction to cases of sporadic *AIP*-negative somatotropinomas with less aggressive clinical features compared to *AIP* mutation-related acromegaly patients (305).

In particular, compared to *AIP* mutation-related gigantism, pituitary disease in the genetically-negative group appeared to be more severe in terms of higher hormonal levels and greater resistance to SSA treatment, whereas these *AIP*-negative cases are older at first presentation (fewer cases were diagnosed before age of 19) and have a longer disease latency. Thus, against this background we believe that this might be very heterogeneous group, which contains cases exhibiting aggressive pituitary disease and somatotropinomas with a milder course, including as yet unidentified causative genes (565, 646).

7.3.4 Syndromic cases

Apart from familial isolated forms, PA can occur as a part of multiorgan syndromes. However, inherited molecular abnormalities in syndromic setting are rarely associated with acromegaly. For instance, clinical MEN1 was identified in 6,6% among a large

cohort of patients with acromegaly (658). Furthermore, MEN1 patients with acromegaly showed low likelihood of positive genetic testing for mutations in genes associated with this syndrome (658, 659).

In our international series, pituitary gigantism in the context of complex syndromes, accounts in total for 7% and includes cases with MEN1 (1%), CNC (1%) and MAS (5%) (565).

7.3.4.1 Pituitary gigantism in the setting of MAS

MAS-related GH hypersecretion produces one of the most severe clinical presentation of pituitary gigantism, which is usually difficult to treat. In our international cohort of GH-related gigantism, MAS comprised 5% of genetically studied cases and is the third frequent cause of pituitary gigantism (565). While somatic *GNAS1* defects in somatotropinomas contribute to rather "mild" PA phenotype, germline mutations in this gene are known as being incompatible with life. In MAS, *GNAS1* mutations occur in the mosaic state and hence demonstrate a spectrum of abnormalities in a variety of affected tissues (528, 530). MAS was previously established as classical presentation as precocious puberty, fibrous dysplasia and *café-au-laits* skin spots (101, 102). The multiorgan affection can also include endocrine gland hyperactivity creating a varied clinical picture (530, 603, 660, 661). GH hypersecretion due to pituitary tumor or diffuse cell hyperplasia occurs in about 10-20% of MAS cases, while some series report its prevalence up to 30%, and about 7% ended up with a final height > 2m (531, 534, 542). Precocious puberty is a classical problem in MAS leading to premature fusion of epiphyseal plates, thus preventing from the development of excessive stature. On another hand, there are dramatically severe cases of MAS with acrogigantism and serious craniofacial deformities. In publication IX, we reported a MAS case with a complex clinical presentation, underlining the significance of the interplay between disease effects across the dysregulated systems (603). Craniofacial fibrous dysplasia was significantly influenced by the effects of long-term GH excess on affected bones; in turn therapeutic efforts failed to counteract hormonal hypersecretion. The diffuse character of pituitary changes and the influence of fibrous dysplasia and skull deformities on MRI make the visualization of pituitary abnormalities in MAS challenging. In our case, a cystic lesion in the sellar region with a normal sized pituitary became visible after two decades of persistent biochemical acromegaly. The somatotropinoma was surrounded by diffuse hyperplasia, however this was not seen on multiple MRI and was only revealed at autopsy. Additive effects of symptoms and difficult disease management in this case demonstrate how a serious clinical picture of gigantism develops in MAS cases from

young age. Onset of pituitary pathology in MAS usually occurs as in our case around the age of 25 (531), although rare cases of MAS have been reported being associated with very early-onset of GH-related overgrowth (538-541). Regarding the differential diagnosis, although GH hypersecretion and overgrowth can occur in MAS from very young age, pathological growth acceleration is typically diagnosed after or along with associated pathology, in particular polyostic fibrous dysplasia and *café-au-lait* macules. These extra-pituitary manifestations of MAS are helpful to distinguish from non-syndromic infantile and childhood-onset gigantism. On another hand, the diagnosis of GH hypersecretion can be delayed due to resemblance of changes in skull bones affected with polyostic fibrous dysplasia and typical for acromegaly facial features (531, 662). However, early recognition of GH hypersecretion and genetic confirmation of MAS is particularly important in young patients, in whom the combination of different pathologies arising from the mosaic distribution of mutated cells, can increase the disease burden (663).

7.3.4.1a Additive challenges of polyostic fibrous dysplasia and GH hypersecretion in MAS

Bone involvement in pathological processes induced by polyostic fibrous dysplasia can be exacerbated by GH hypersecretion, whereas scoliosis and joint disease occurring in tall individuals can be worsened by co-existing skeletal deformities. Earlier intervention for hormonal control (at least before 18 years old) is vital to decrease the progression of fibrous dysplasia and prevent the worsening of craniofacial deformities (531, 542, 664). Finally, GH hypersecretion in MAS is challenging to treat due to underlying diffuse pituitary hyperplasia and since craniofacial fibrous dysplasia complicates the management of the pituitary disease in MAS patients by limiting applicable treatment modalities. Cranial bone deformities makes surgical access difficult (665). In this context, the presence of diffuse pituitary hyperplasia as the most common cause of hypersecretion in MAS, makes radical neurosurgery unlikely, if not impossible. Another determinant of neurosurgical complexity, extensive dystrophic calcification in PA has been described in a patient with gigantism in the context of MAS, that rendered a gross total resection more difficult (666). Moreover, the use of radiotherapy is limited due to the increased risk of malignant transformation of cranial fibrous dysplasia after irradiation (537, 663, 665, 667). Response to SSA treatment is usually partial, and pegvisomant alone or in combination could be effective in such cases (565, 668-670). Cranial nerves pathology is an extraskeletal manifestation generally reported in MAS due to bone deformities (663).There is some evidence that the harmful role of GH hypersecretion on craniofacial fibrous dysplasia effects in MAS (i.e. cranial neuropathy, anatomical structures'

dislocation) can be decreased with early intervention (in childhood/adolescence) to control GH/IGF-1 levels (537). In our case, GH/IGF-1 levels remained uncontrolled since childhood as neurosurgery was impossible due to profound facial and cranial deformity and the absence of visible pituitary lesion (SSA treatment was not available) (603). Dramatic worsening of craniofacial deformity was caused by persistent hormonal excess and resulted in severe visual and hearing impairment. This case emphasizes the particular importance of early control of somatotroph axis hyperfunction in MAS to prevent progression of bone pathology and other disease associated conditions.

7.3.4.1b MAS genetics

Clinical diversity in associations of MAS manifestations produced by mosaic state of different cell types, and, besides classical MAS features, can include uncommon symptoms. On another hand, the mosaic state underlines challenges in genetic diagnosis in patients with a clinical suspicion of MAS due to low-abundance causative genetic alteration, an activating mutation in *GNAS1* gene, which frequently remains undetected by direct sequencing in blood and tissue samples.

We comprehensively studied a MAS case with severe acrogigantism associated with classical MAS-related fibrous dysplasia and skin defects, as well as with rarer and unusual MAS-affected organs (hyperparathyroidism, hyperplasia of pancreas and thymus) (603). To study our case genetically, we employed a specific technical approach with use of ddPCR, which is a highly sensitive technique to increase the detection rate of low frequency genetic variants (671, 672). Implication of ddPCR permitted us to obtain genetic diagnosis of MAS by identifying *GNAS1* mutation in DNA obtained from the post-mortem samples of different tissues in various rate (603) and, thus demonstrate that even low level of mosaicism can be detected in affected tissues.

7.3.4.2 Other monogenic syndromes associated with PA

None of the pituitary gigantism patients in our cohort was identified with other rare conditions due to *CDKN1B*, *SDHx* and *MAX* mutations. Although mutations in these genes can be associated with PA in MEN4 or 3PAs, somatotropinomas are infrequent (499, 500, 518, 522, 526). A case of pituitary gigantism has been reported in a patient with an alteration in *CDKN1B* gene (673) and pituitary gigantism related to *SDHx* and *MAX* mutations has not yet been reported. Thus, our findings along with the previous reports showed that young-onset somatotropinomas leading to gigantism are exceptionally rare in monogenic multiorgan disease.

7.3.5 Genetic screening in pituitary gigantism

Risk criteria for harboring germline mutations have been established based on published retrospective screening studies in different PA populations. Several recommendations have been made regarding what genetic test should be performed in pituitary GH hypersecretion and who should be referred for testing. Guidelines only exist for screening in multiorgan tumor syndromes, depending on the clinical criteria for the disease in individual or affected family members. Based on published recommendations and our experience, a decision strategy for optimal choice of genetic tests is proposed in Figure 7.5. Apart from individuals with associated syndromic pathologies and familial cases, patients with other clinical features, such as pediatric / adolescent onset, large and invasive pituitary tumors resistant to medical treatment, are suggested to be initially considered for genetic testing.

7.3.5.1 Screening strategy in syndromic presentation

Personal or family history suggestive of multiple endocrine tumor syndromes and associated pathologies of non-endocrine tissues, should focus genetic testing on specific search for mutations in appropriate genes implicated in a particular syndromic phenotype (543). For instance, clinically relevant GH hypersecretion can occur in the settings of MAS, and as a rule these cases demonstrate clinical signs or symptoms suggestive for MAS (*café-au-lait* spots, and/or fibrous dysplasia, and/or a previous history of precocious puberty) since an early age, thus guiding the differential diagnosis and further investigation forward the syndromic form.

According to our findings and previous case reports from the scientific literature, it should be kept in mind that rare cases of pituitary gigantism can be associated with syndromic conditions such as MAS, MEN1 and CNC (565), while there is little evidence to consider other syndromic forms of GH-secreting PA (MEN4 and 3PAs) to be an important cause of pituitary gigantism (473).

While patients with extra-pituitary manifestations or family history of endocrine tumor syndromes should be initially directed for specific for that pathology genes screening, there is considerable uncertainty whether these genetic etiologies should be tested in gigantism cases with isolated to pituitary disease. In general, in patients with MEN1 or Carney complex PA usually develop at adult age and rarely appear as a first manifestation in young individuals, however rare cases of pediatric-onset GH-secreting PA with increased height velocity and tall stature have been reported in these settings (473, 509, 536, 565). Confirmation of the genetic forms is important for being aware that a carrier

may acquire with time certain disease manifestations, emphasizing the importance of early genetic diagnosis for adequate surveillance since young age. As it was outlined above regarding somatotropinoma management, other pathological manifestations of syndromic disease in affected person can have additive to elevated GH/IGF-1 levels morbidity (e.g. craniofacial fibrous dysplasia and hormonal effects in MAS can be mutually exacerbating) or require particular therapeutic approach in these individuals.

7.3.5.2 Screening strategy in familial presentation

Familial forms of PA are uncommon with most frequent presentation as FIPA kindreds making up approximatively 2% of all PA (90, 92). The occurrence of FIPA is 10-times higher in pituitary gigantism population (22.3%) (565). The majority of FIPA gigantism cases had *AIP* mutations, and X-LAG syndrome was found to be an underling genetic cause in three homogeneous acrogigantism FIPA kindreds described so far (567, 645, 674, 675). While all detected FIPA cases with Xq26.3 microduplication presented with only acrogigantism, individuals with *AIP* mutation-related gigantism can be from either homogeneous or heterogeneous FIPA with various pituitary secretory tumor types in the affected family members. Moreover, all carriers of Xq26.3 microduplication described so far, developed early-onset clinical manifestations of pituitary GH hypersecretion, whereas familial *AIP* mutations show incomplete penetrance in such kindreds.

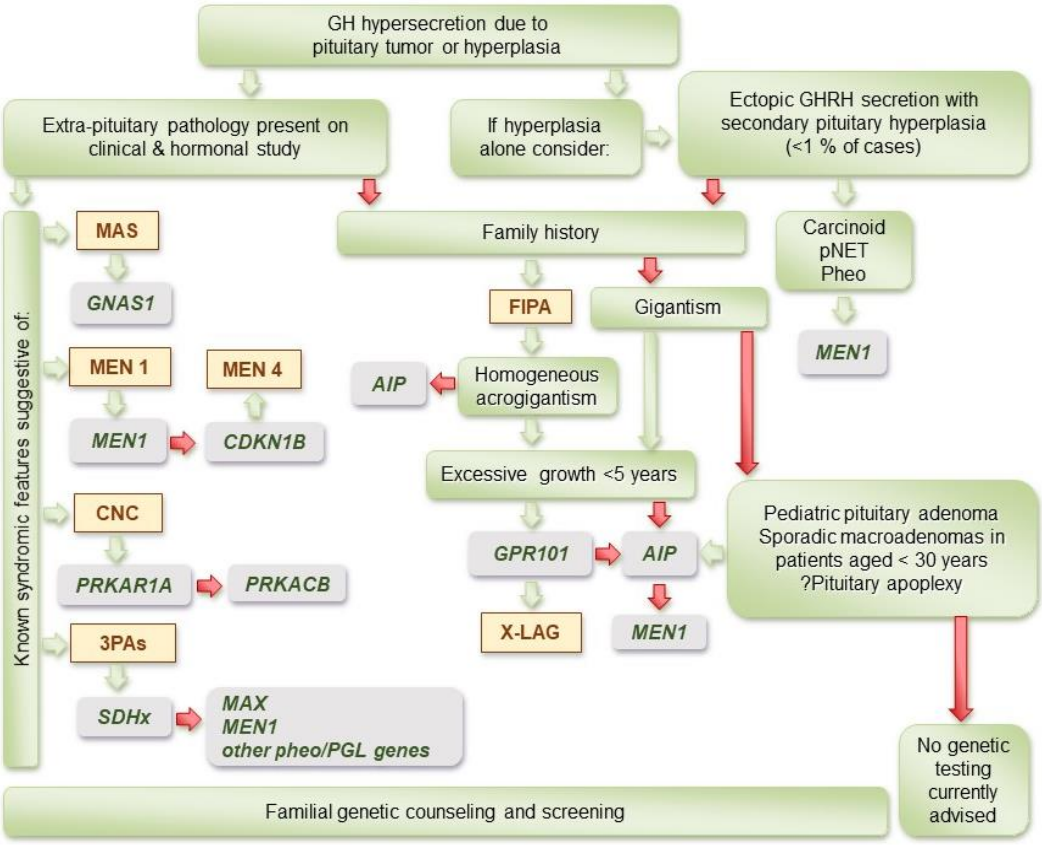


Figure 7.5: Genetic screening algorithm in GH-secreting PA. Adapted from (543)

7.3.5.3 Genetically distinct groups of isolated pituitary gigantism

In our international pituitary gigantism cohort two main groups with identified genetic background presented with pituitary isolated disease related either to *AIP* mutations or X- LAG syndrome (29% and 10%, respectively) and had distinct phenotypes. In these genetic forms of isolated pituitary gigantism, clinically the most explicit characteristic is related to different age at disease onset, which therefore becomes a key feature for the choice of genetic testing algorithm. Indeed, the pituitary gigantism population meets the criteria for high risk of *AIP* mutations and should be certainly considered for screening (607, 637, 676). However, those with *AIP* mutations are predominantly adolescents and young adults, whereas all X-LAG patients have a very consistent onset of the pituitary disease in early infancy. We suggest CGH as an initial genetic investigation in pituitary gigantism with pathologically rapid growth starting during infancy, in order to check for *GPR101* CNV in the context of X-LAG syndrome. In our pituitary gigantism population, the youngest cases were uniformly linked to X-LAG syndrome, however for very young-age pediatric somatotropinomas other genetic causes should be also kept in mind, as rare cases of isolated pituitary gigantism occurring since very young age have been also reported in the literature due to other molecular abnormalities. *AIP* mutation-related cases have been reported with the beginning of symptoms as young as 4 years (305), while PA can occur in very young children in the setting of complex multiorgan syndrome (473-475, 531, 673). Accelerated linear growth due to GH secreting PA has been diagnosed in a MEN1 mutation carrier at age as young as 5 years (677, 678) and *MEN1* mutations were detected in 8% of a young sporadic PA population including cases with GH-secreting tumors (473, 475). Furthermore one case of pediatric-onset somatotropinoma with a germline variant in the promoter region of the *CDKN1B* gene has been reported (673).

Chapter 8: Conclusions and perspectives

8.1 General conclusions

The present thesis comprehensively describes the clinical, therapeutic and genetic features of pituitary gigantism – a pathology previously only rarely reported in the medical literature. The principal contributions of the present research can be listed as follow:

Clinical and therapeutic aspects

We have designed and conducted the first large-scale international multicenter study in this complex rare disease and, thus, have been able to collect comprehensive data in patients with strict criteria of GH-induced skeletal overgrowth exceeding +2 SD above local normal range.

In this study, we have demonstrated the clinical aspects of the young somatotropinoma population, which contrast markedly with adult acromegaly. Pituitary gigantism has strong male predominance and large and aggressive GH-secreting pituitary macroadenomas frequently accompanied by prolactin co-secretion. Females in our cohort had earlier symptom onset and were diagnosed with pituitary disease earlier than males.

We have shown that early diagnosis, as well as early treatment initiation, and improved management reduces final height and decreases the substantial disease burden attributable to chronic excessive levels of GH. Multimodal therapeutic approaches are needed to optimize early and effective disease control. Combined medical therapy (long-acting SSA and pegvisomant) as primary or secondary (postsurgical and/or after irradiation) treatment can be effective in hormonal and growth control.

Resistant to treatment phenotypes were established and provide new elements in our understanding of the biology of PA.

Genetic aspects

Our studies revealed that pituitary gigantism can be due to an inherited pituitary disease like multiple tumor syndromes, FIPA, or isolated pituitary lesions. Genetic etiologies have been identified in about 50% of cases.

We have revealed that the most common genetic causes were related to *AIP* mutations/deletions and X-LAG syndrome and account for about 30% and 10%, respectively, of pituitary gigantism.

In our series, more than half of the pituitary gigantism cases were not produced by an abnormality in any established PA predisposition genes. The phenotype could vary in different genetic forms and pituitary gigantism patients with as-yet unidentifiable genetic

cause. Thus, *AIP* mutations and *GPR101* duplications were described as genetic causes underlying particularly challenging pituitary gigantism cases. However, all patients had aggressive disease features, and genetically negative pituitary tumors often showed substantially aggressive behavior in terms of higher levels of GH and IGF-1 and delayed disease control due to resistance to different treatment options, requiring more frequently multimodal therapy.

The complementary genetic studies in cases of pituitary gigantism brought the identification of a new form of severe acrogigantism, which was termed X-linked acrogigantism or X-LAG. This syndrome is associated with a phenotype of overgrowth due to mixed GH- and prolactin-secreting PA / hyperplasia with the onset in the early infancy (usually the first year of life) and may explain the etiology of the youngest and the most severe cases of gigantism, including rare FIPA families.

The detailed clinical and therapeutic characterization of X-LAG was elaborated, which permitted us to conclude that X-LAG syndrome is a severe disease that is hard to manage due to the high levels of GH hypersecretion, presence of diffuse mammosomatotroph hyperplasia and poor SSA responses despite the moderate to high levels of SST2 expression. Effective control of pituitary tumors and abnormal statural growth requires radical neurosurgery and multimodal therapy including pegvisomant. The evidence obtained in these studies underlines that these cases of dramatic overgrowth need to receive effective therapy with all possible means to arrest GH-induced growth before puberty. Since its first description, X-LAG remains a rare disease: currently about 33 cases have been reported by our and other research groups.

With regard to the genetics, an Xq26.3 microduplication, which invariably includes *GPR101*, was identified in cases with clinical presentation of X-LAG. Further evidence confirmed that increased expression of *GPR101* in the pituitary appears to be a causative molecular mechanism of X-LAG syndrome. Clinical presentation of very early-onset isolated GH-induced gigantism is almost always associated with a duplication of *GPR101*. These results on Xq26.3 rearrangements revealed a new tumorigenic mechanism implicated at the pituitary level. *GPR101* is associated with growth regulation via a new pathway.

The clinical observation and postsurgical *in vitro* tumor studies in an X-LAG case provided new evidence that hypothalamic dysregulation is possibly associated with X- LAG syndrome, through hypothalamic GHRH contributing to GH and prolactin hypersecretion by development of pituitary hyperplasia or tumor formation. The study

revealed that a GHRH antagonist appears to inhibit GH and prolactin secretion, suggesting new therapeutic perspectives in X-LAG.

The existence of somatic mosaicism for the *GPR101* duplication was revealed in sporadic males with X-LAG, while females and familial males (from FIPA kindreds) have apparently constitutional duplications. This evidence revealed that diverse genetic mechanisms can take place in X-LAG syndrome.

The use of high-definition aCGH and personalized junction-specific ddPCR techniques, permitted to demonstrate that each X-LAG microduplication has unique breakpoints, and that an identical Xq26.3 microduplication, transmitted from mother to son, can underlie familial acrogigantism in some *AIP* mutation negative FIPA. Additionally, an approach using ddPCR screening based on CNV at *GPR101* was suggested as an informative tool in those cases with similar to X-LAG presentation.

By reviewing medical literature and historical records, some earlier cases of infant-onset pituitary gigantism were found to closely resemble those of X-LAG. It was also noted that many of the tallest patients in history had an early childhood-onset gigantism phenotype similar to that of X-LAG syndrome. This supported the hypothesis that *GPR101* duplication may explain these historical cases of gigantism. Our research group had an opportunity to study genetically skeletal remains of a historical case of pituitary gigantism with early onset of growth acceleration, increased appetite, lifelong growth and a final height of 2.59m. The genetic analysis identified an increased *GPR101* copy number suggesting this case as being associated with X-LAG syndrome.

This historical case study also highlighted some valuable medical aspects of X-LAG concerning the natural history of pituitary tumors and dramatic evolution of untreated disease leading to extreme gigantism, thus stressing the importance of optimization of the early and effective management of this severe and difficult to treat condition today.

Finally, the last study of this thesis was based on a comprehensive clinical, pathologic, and genetic evaluation of an adult male patient with MAS and helped to expand the understanding of this syndromic form of pituitary gigantism. The data obtained was particularly valuable to underline the diverse tissue-specific involvement of *GNAS1* somatic mutations. Clinical and genetic factors in MAS makes pituitary disease control challenging, producing thus one of the most dramatic forms of gigantism.

Based on the experience obtained in the total of studies described here, a decision strategy was developed for clinical evaluation and genetic testing in pituitary gigantism. The latter includs the main PA predisposition genes: *AIP*, *GPR101*, *MEN1*, *PRKAR1A*, *PRKACB*, *CDKN1B*, *SDHx*, *MAX1* and *GNAS1*. Initial investigation of patients with pituitary

gigantism comprises proper clinical evaluation and family history, which then leads to an appropriate screening algorithm for possible genetic etiologies.

Appropriate genetic counselling is recommended in family members of individuals with identified causative mutations. Family tree visualization and orderly screening for the mutation found in the proband is helpful to distinguished the non-carriers and carriers for further targeted clinical evaluation and follow-up.

The studies presented in this thesis underline that multicenter international collaborations provide medically important information on rare diseases, and methodological advances in genetics help to expand the understanding of their molecular mechanisms. Our studies in gigantism provided valuable information on pituitary predisposition genes, that can be of great utility for clinicians. Beyond this, we believe that the results will stimulate scientific and medical interest on this rare and dramatic disease, increasing general awareness of the severe symptomatology and the need for early recognition and therapeutic control.

8.2 Perspectives and future directions of research

Recent scientific advances led to the unravelling a number of genetic mechanisms in pituitary gigantism. However, the etiology of many gigantism cases remains obscure. Patients that are negative for all genetic causes known so far, present an intriguing population with an aggressive disease course, which is a priority for further studies. New genetic/genomic methodologies and tools are increasingly being developed and used to investigate disease causing molecular mechanisms.

Phenotypical characteristics of the majority of gigantism cases include a sustained resistance to widely available medical therapies (first generation SSA). The reasons underlying this poor somatostatin analog efficacy requires molecular study of receptor subtypes and signaling pathway activation. Another aim will be to assess the potency of novel multireceptor-targeted SSA (pasireotide) in pituitary gigantism cases resistant to first generation SSA.

An upcoming study will be focused on different clinical aspects, consequences and co-morbidities of hormonal hypersecretion in patients with pituitary gigantism. As a follow-up study, we will examine cardiovascular disease, effects on the skeleton and bone, pituitary MRI and histological/immunohistochemical characteristics, and their evolution during treatment in pituitary gigantism.

Timely diagnosis and adequate therapeutic control in acromegaly is known to improve the quality of life and to maintain work capacity. A future step will be to develop a new

health-related quality of life questionnaire for patients with pituitary gigantism. Besides pituitary disease-specific assessments based on available Acromegaly Quality of Life Questionnaire (ACROQoL), it could be expanded to include height/growth-related aspects impacting in patients' lives and items that are important in young patients.

In adult acromegaly, multimodal treatment in an experienced center and appropriate management of co-morbidities can contribute to a reduction in mortality by 2-5 times. In our cohort, seven patients died from various causes, all at a relatively young age. However, the disease impact on life span in pituitary gigantism patients was not previously studied. It will be of interest to evaluate the influence of young age of onset, uncontrolled pituitary disease, co-morbidities, use of multiple therapeutic modalities and overgrowth–related factors on the life expectancy in pituitary gigantism cases.

Our results in X-LAG provide strong evidence for the involvement of *GPR101* in the somatotrope axis and offer an opportunity to study new pathways involved in pituitary secretion and the role of this new key player in the physiology of growth, particularly in early childhood. We are pursuing further studies to unravel these molecular mechanisms. An important focus of further research will be to understand the main pattern of expression of the *GPR101* protein and to identify the primary ligand (if any) of this receptor. This will shed light on the true role of this gene in the pituitary and its implication in growth regulation. Further clarification of the role of *GPR101* will also imply studies in animal models that hopefully will provide new insight into physiology of *GPR101*.

We are interested to understand the nature of a connection, if there is any, between *GPR101* and energy balance/appetite regulation. Animal data suggest that inactivation in the region of interest plays an important role in obesity. As we have shown that the duplication in *GPR101* in X-LAG is associated with somatic overgrowth accompanied with height and weight gain and increased hunger, further studies including implication of dysregulation in *GPR101* in populations with overweight and eating behavior disturbances, might be of interest.

Further work will involve the identification of more X-LAG cases and development of clinical and pathological patient databases in order to better understand the disease characteristics related to this genomic disorder. We will continue studying the skeletal remains from ancient subjects with features reminiscent of X-LAG syndrome. Genetic

testing in newly diagnosed and historical cases will extend number of such genomic alterations in a larger number of individuals with this rare disease.

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PERSONAL PUBLICATIONS

CHAPTER 9: Publication I

Clinical and Genetic Characterization of Pituitary Gigantism: An International Collaborative Study in 208 Patients

Rostomyan* L., A. F. Daly*, P. Petrossians, E. Nachev, A. R. Lila, A. L. Lecoq, B. Lecumberri, G. Trivellin, R. Salvatori, A. G. Moraitis, I. Holdaway, D. J. Kranenburg-van Klaveren, M. Chiara Zatelli, N. Palacios, C. Nozieres, M. Zacharin, T. Ebeling, M. Ojaniemi, L. Rozhinskaya, E. Verrua, M. L. Jaffrain-Rea, S. Filipponi, D. Gusakova, V. Pronin, J. Bertherat, Z. Belaya, I. Ilovayskaya, M. Sahnoun-Fathallah, C. Sievers, G. K. Stalla, E. Castermans, J. H. Caberg, E. Sorkina, R. S. Auriemma, S. Mittal, M. Kareva, P. A. Lysy, P. Emy, E. De Menis, C. S. Choong, G. Mantovani, V. Bours, W. De Herder, T. Brue, A. Barlier, S. J. Neggers, S. Zacharieva, P. Chanson, N. S. Shah, C. A. Stratakis, L. A. Naves, and A. Beckers.

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CHAPTER 10: Publication II

Gigantism and Acromegaly Due to Xq26 Microduplications and *GPR101* Mutation

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CHAPTER 11: Publication III

X-Linked Acrogigantism Syndrome: Clinical Profile and Therapeutic Responses

Beckers A.*, M. B. Lodish*, G. Trivellin, L. Rostomyan, M. Lee, F. R. Faucz, B. Yuan, C. S. Choong, J. H. Caberg, E. Verrua, L. A. Naves, T. D. Cheetham, J. Young, P. A. Lysy, P. Petrossians, A. Cotterill, N. S. Shah, D. Metzger, E. Castermans, M. R. Ambrosio, C. Villa, N. Strebkova, N. Mazerkina, S. Gaillard, G. B. Barra, L. A. Casulari, S. J. Neggers, R. Salvatori, M. L. Jaffrain-Rea, M. Zacharin, B. L. Santamaria, S. Zacharieva, E. M. Lim, G. Mantovani, M. C. Zatelli, M. T. Collins, J. F. Bonneville, M. Quezado, P. Chittiboina, E. H. Oldfield, V. Bours, P. Liu, W. de Herder W, N. Pellegata, J. R. Lupski, A. F. Daly, and C. A. Stratakis.

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CHAPTER 12: Publication IV

GHRH Excess and Blockade in X-LAG Syndrome

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CHAPTER 13: Publication V

Somatic Mosaicism Underlies X-Linked Acrogigantism Syndrome in Sporadic Male Subjects

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CHAPTER 14: Publication VI

Paleogenetic Study of Ancient DNA Suggestive of X-Linked Acrogigantism

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CHAPTER 15: Publication VII

Combined Treatment with Octreotide LAR and Pegvisomant in Patients with Pituitary Gigantism: Clinical Evaluation and Genetic Screening

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CHAPTER 16: Publication VIII

Hormonal and tumoral control of acromegaly with pasireotide LAR in patients with *AIP* mutations and resistance to first generation somatostatin analogs

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CHAPTER 17: Publication IX

McCune-Albright Syndrome: A Detailed Pathological and Genetic Analysis of Disease Effects in an Adult Patient

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SUPPORTING PUBLICATIONS

Supporting Publication 1 (S1)

Screening for Genetic Causes of Growth Hormone Hypersecretion

Rostomyan L. and A. Beckers.

Growth Horm IGF Res 30-31 (Oct - Dec 2016): 52-57.

Supporting Publication 2 (S2)

Pituitary Gigantism: Causes and Clinical Characteristics

Rostomyan L., A. F. Daly, and A. Beckers.

Ann Endocrinol (Paris) 76, no. 6 (Dec 2015): 643-9.

Supporting Publication 3 (S3)

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Supporting Publication 4 (S4)

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X-LAG: How Did They Grow So Tall?

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Ann Endocrinol (Paris) 78, no. 2 (Jun 2017): 131-36.

Supporting Publication 7 (S7)

Characterization of GPR101 Transcript Structure and Expression Patterns

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J Mol Endocrinol 57, no. 2 (Aug 2016): 97-111.

Supporting Publication 8 (S8)

GPR101 Mutations Are Not a Frequent Cause of Congenital Isolated Growth Hormone Deficiency

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APPENDIX

Appendix 1

Table s1 for Chapter 1: Giants in legends and foundation myths from different traditions

The Bible describes giants as groups of people that lived together (the Nefilim, Rephaite, Emim, Anakim) and individuals of great stature in several places before and after the Flood. Perhaps the most famous biblical representatives of prodigious body size have been Og, the king of Basham, who was linked to the Rephaite and slept on a 4m long iron bed, and the Philistine giants, descendants of the Anakim – Goliath, defeated by the young David, his brothers and sons, all with height exceeding 2m (82, 83). Goliath is also mentioned in **the Koran** as Galut.
Greek mythology tells of three races – titans, giants and cyclops. The Titans were not only predecessors, but also the progenitors of the Olympian gods: one of them, Kronos, was the father of Zeus, the supreme ruler of Olympus. However, this did not prevent the war for power between the gods and the titans to flare up, in which the latter were defeated. The titans were replaced by their stepbrothers — giants, mortals, but no less powerful. The war with them – gigantomachy – also ended in victory for the Olympians. Atlas was one of the titans who fought against Zeus. When the titans lost, Zeus punished Atlas, forcing him to keep the sky above the Earth forever.
Norse mythology is rich with stories about giants, attested to in the older poetic Edda and the prose Edda. The giant Ymir was the first creature, who then created the world from his flesh. *Aces* were gods who appeared later and partially took their origin from the giants. Among them Thor was the most popular god. Similarly to the Greek mythology, everything ended in war, from which the gods emerged victorious. But a couple of giants still survived and gave birth to a new generation, harboring fierce hatred against the gods. Hrungnir, who had a stone head and stone heart, was the strongest *jötunn*, which is an apparent synonym to giant. The giant lost his battle against Thor, but his dead body felt on his enemy, crushing Thor's throat with his knee. No one could even lift the leg of the giant. Then the infant son of Thor and the giantess Jarnsaxa grew up in three days and freed Thor (679).
In **British myths**, giant creatures were led by Gogmagog whose name means the land, and protected Albion against the invaders.
In **Irish mythology**, the Fomorians were supernatural creatures representing demonic, dark forces of chaos, who were sometimes described as giants, with whom the ancient inhabitants of Ireland constantly had to fight. Fionn mac Cumhaill, who was a hero with supernatural abilities from the Fenian Cycle of Gaelic mythology, was sometimes portrayed as a giant as he was closely associated with the story about The Giant's Causeway and many geographical parts nearby. The legend says that when he was challenged to fight a monstrous giant from Scotland, he drove a number of big stones to the water and thus built a bridge to cross the sea. His enemy then fled in horror, destroying the bridge along the way, while Fionn threw a rock at the fleeing giant that landed as an islet near the coast.

Bogatyrs (akin to knight-errant), the main heroes of many *bylinas* (**East Slavic** medieval epic poems), are characterized by large body proportions, immense strength, courage and bravery. In Russian bylinas and folklore stories some bogatyrs were brothers and the protectors of their homeland. A mythical giant Svyatogor surpasses other bogatyrs and represents an enormous force.

Ilya Muromets is the most famous bogatyr of the bylinas and was also mentioned in German epic poems of the 13th century as a mighty knight of a noble family – Russian Ilya. He had outstanding physical and spiritual strength and became a hero in many stories where he battled with enemies and beasts protecting people and the Homeland. Interestingly, Ilya is the only bogatyr canonized as a Saint of the Russian Orthodox Church, because this character is linked to a real historical person, who was a warrior, and then become a monk, named Ilya Pechersky. His relics are kept in the Anthony Caves of the Kiev-Pechersk Monastery and were studied several times. A well-preserved mummy belongs to an adult man who lived in the 11th-12th century and was rather tall for his time and had signs of various injuries and bone deformities, which correspond to the features and adventures in some of the legends about bogatyr Ilya.

The Nart sagas, the epic of peoples of the **North Caucasus** (including the Ossetians, Abkhaz-Adyg peoples, Vainakhs, Balkarians and Karachais, also Svans and others), are based on legends about the origin and adventures of knight-heroes "*narts*", who were very brave warriors. They were created by the supreme god with a particular goal – to establish order on the earth, to defeat evil and dragons. However, they were fighting not only with monsters, but also with other nations and with each other.

Iovan Iorgovan is a character in a cycle of legends about giants in **Romanian mythology**, a hero who kills a serpent-monster, rescuing a beautiful girl. On the other hand, he was also an anti-hero, an aggressor, who turns all around into stone.

The central plot in the ancient **Armenian mythology** is the resistance of proto-Armenians or Armenians to foreign adversity, represented by the struggle between mythical giants. Giant heroes were transformed into eponyms of all Armenians, the founders of the country and statehood, while the evil giant-demons symbolized ethnic nations of the enemy countries. Armenians call themselves *Hay* and their country *Hayastan*, which originate from the name of Hayk, a hero-archer, the handsome giant with remarkable strength, who valued independence most of all. He led his people from the plain of Sennaar to the cold but free mountains of Armenia, escaping from the tyranny of Bel, another giant from Babylon. When Bel's innumerous army invaded Armenia, Hayk with his small-numbered but brave soldiers gave battle and killed the enemy with a well-aimed arrow shot.

The Armenian national epic described four generations of heroes from the same family, the founders of Sassoun, all with outstanding physical stature and abilities, and their struggle against the tyrant Msra Melik, who had come to conquer Sassoun.

In Armenian legends, the mountains, an important part of the local landscape, usually have an anthropomorphic origin. They were giant brothers, who met every morning to greet each other, being tightly girded with their belts. But with time, they became lazy, did not want to wake up early and greeted each other without tightening the belts. For this the brothers were punished by gods, who turned them into mountains, their belts into green valleys, and tears into fresh springs.

In **Mesopotamia**, according to the Sumero-Akkadian epic of creation "Enuma Elish", Tiamat, a goddess of salt sea or primordial ocean-chaos, from which everything took its origin (including the gods), mixed her waters with Abzu, the god of fresh water, thereby giving rise to the world. New gods battled with her, and Marduk, the storm-god, killed her and created heaven and the earth from her divided body.
Kumbhakarna is a giant demon from the **Hindu** epic Ramayana. Kumbhakarna asked Brahma for a long sleep by mistake instead of a blessing. As a result, the giant slept six months per year, and when he woke up, he ate everyone around. When Kumbhakarn's help was needed for a battle against the troops of Prince Rama, only a clatter made by a thousand elephants could wake him.
In the **Aztec mythology**, the Quinametzin are characterized by exceptional stature and strength. They were punished by the gods for rebellion and sins they had committed. There were 4 generations of giants, among those giants-founders of the pyramid of Cholula and the City of Teotihuacan. The deity-creators Tezcatlipoca and Quetzalcoatl defeated a giant crocodile Cipactli creating the heaven and the earth from her body. After the last destruction of the Earth, the planet was in desolation and the sky fell on Earth. Gods brought four giants (Cuauhtemoc, Izcoalt, Izcaqlli and Tenexuche) to raise the sky. Tezcatlipoca and Quetzalcoatl then turned into trees to help the giants to support the sky.
Kua Fu, a giant in ancient **Chinese mythology** wanted to catch and capture the sun. In one of myths described in Shan Hai Jin (Book of Mountains and Seas), he drained all of the waters dry and died of thirst, then after his death his body was transformed into the Grove of Fertility, a huge evergreen garden of peach trees. Xingtian is a divine giant in ancient Chinese mythology known for his struggle with the heavenly emperor Huangdi. Xingtian was defeated and decapitated. Xingtian's head was buried by near Changyang Shan Mountain, but the headless Xingtian made another face on his torso and continued the fight, thereby symbolizing the indomitable spirit that never gives up and retains the will to resist.
The Jentilak, known also as "*the jentil"*, are a race of giants in **Basque mythology** who represented the pre-Christian Basque people. They have superhuman strength and usually throw big rocks at their enemies. They were believed to build the megaliths, dolmens and cromlechs – stone structures around the Basque Country.
In Finnish creation myths, Ilmatar is the primordial giant goddess, who creates the reliefs of the Earth and was the mother of Väinämöinen, a demigod knight-hero. The national epic of **Karelia and Finland**, the Kalevala, also tells the story of the ancient dead giant Antero Vipunen whose body has long been overgrown with trees. He possessed very important magical spells. Väinämöinen descends into the giant's womb and tries to bring him back to life, he tortured him with sharp stakes in order to get the missing words in the magic spell that he knew.

Appendix 2

Table s3 for Chapter 3: Clinical characteristics and etiologies of overgrowth syndromes (255, 680-702).

Congenital syndromes with overgrowth	**Growth pattern and major clinical features**	**Genetic mechanisms/ inheritance**
• *Monogenic*		
Marfan	*Disproportionate tall stature, arachnodactyly, scoliosis, hyperextensible joints, lens ectopia and other ocular problems, risk of aortic root dilatation and valve prolapse*	*Mutations of the FBN1 at 15q21.1* *Autosomal dominant*
Fragile X	*Childhood and preadolescent proportionate height and/or weight overgrowth, adult height is close to the normal or lower than normal, dysmorphic facial features, increased head circumference, cognitive impairment, behavioral problems, macroorchidism and hyperextensibility of the joints*	*Transcriptional silencing due to hypermethylated CGG repeat expansions in FMR1 gene at Xq27.3* *Microdeletions and intragenic mutations in FMR1*
Beckwith-Wiedemann	*Neonatal macrosomia, proportionate postnatal overgrowth with organomegaly* *Final height is usually within the normal range or upper limit.* *Hyperinsulinemic hypoglycemia, abdominal wall defects, macroglossia, midface hypoplasia, anterior linear earlobe creases and helical ear pits, increased risk of embryonal tumors*	*Mutations in CDKN1C* *Dysregulation of imprinted genes at 11p15.5 (IGF2, H19, KCNQ1, CDKN1C, KCNQ1OT1)* *Could be presented as somatic mosaicism*
NF1 (Neurofibromatosis type 1)	*Proportionate tall stature in about 50%, neurofibromas, in severe cases – cognitive deficiency, increased risk of malignancy*	*Mutation in NF1 at 17q11.2* *Autosomal dominant* *In 5% more severe phenotype due to microdel 17q11.2 including RNF135*
Homocystinuria	*Similar to Marfan syndrome phenotypic features, osteoporosis and scoliosis, mental retardation and psychiatric disorders, and predisposition to thromboembolic events*	*Mutations in CBS at 21q22.3* *Autosomal recessive*

Sotos	*Generalized pre- and postnatal overgrowth with advanced bone age and dental maturation; adult height is not affected* *'Triangular-shaped' facies, Neonatal hypotonia and feeding problems, macrocephaly, intellectual disability, elevated risk of malignancy*	*Haploinsufficiency in NSD1 at 5q35.2–35.3 – 60-90%* *CNV on chromosomes 10, 14, 15 and X* *Autosomal dominant*
Weaver	*Proportionate pre- or postnatal overgrowth, accelerated osseous maturation, unusual craniofacial appearance with a hoarse and low-pitched cry, hypertonia, and camptodactyly*	*Some cases with NSD1 mutations* *RNF135*
CATSHL (Camptodactyly-tall stature-scoliosis-hearing loss)	*Large for gestational age, proportionate tall stature, camptodactyly, sensorineural hearing loss, development delay, microcephaly, scoliosis and/or pectus excavatum*	*Missense mutation in FGFR3 at 4p16.3, autosomal dominant*
Overgrowth-Macrocephaly-Facial Dysmorphism due to RNF135 alterations	*Postnatal overgrowth, macrocephaly, dysmorphic facial characteristics, advanced bone age, developmental delay, hearing problems and eye abnormalities*	*Mutations in RNF135* *Autosomal dominant*
Epiphyseal chondrodysplasia, Miura type (CNP overexpression)	*Large for gestational age, postnatal statural overgrowth (>97th percentile), Adult height is markedly increased (> 4 or more SDS), mild dysmorphic features, marfanoid habitus, scoliosis, very long halluces and metaphyseal-epiphyseal dysplasia*	*Activating mutation in NPR2* *Translocation involving 2q37.1 near the NPPC gene* *Autosomal dominant*
Perlman	*Increased length at birth with decrease in growth rate to normal/below-normal, typical facial features, alongside macrosomia, nephromegaly, hypotonia, and cryptorchidism, hyperplasia of the islets of Langerhans, developmental delay, increased risk of Wilms tumors*	*Mutations in DIS3L2 at 2q37* *Autosomal recessive*
Sclerosteosis	*Tall stature/ adult height at the upper limit, mandibular overgrowth since childhood, conductive hearing loss, facial palsy, variable syndactyly and hyperostosis and sclerosis of tubular bones, frequent fractures*	*Inactivating variant in SOST gene at 17q21.31* *Autosomal recessive*

Primrose (Intellectual disability-cataracts-calcified pinnae-myopathy)	*Macrocephaly, hypotonia, intellectual disability, autism and other behavioral concerns, unusual facial features, sparse body hair, tall stature, with diabetes, deafness, progressive muscle wasting and ectopic calcifications*	*Missense mutations in ZBTB20 at 3q13.31*
Loeys-Dietz (Marfan type II)	*Disproportionate skeletal and cardiovascular Marfanoid pathologies, no ocular involvement*	*Mutations of the TGFBR1 and TGFBR2 Haploinsufficiency in TGFB2 gene Autosomal dominant*
CCA (congenital contractural arachnodactyly) /Beals	*Disproportionate Marfanoid tall stature, congenital contractures (knees, elbows, fingers), arachnodactyly, kyphoscoliosis, muscular hypoplasia, crumpled ears, aortic root dilatation*	*Mutations in FBN2 at 5q23.3 Autosomal dominant*
Lujan -Fryns	*Marfanoid habitus after puberty, adult height at normal upper limit, typical craniofacial dysmorphism, long and narrow face, crowded teeth, macrocephaly, hyperextensibility of digits and dysgenesis of corpus callosum, mental retardation and behavioral problems*	*Mutation in MED12 or UPF3B, both on X chromosome*
Nevo (Ehlers–Danlos syndrome type VIA)	*Increased perinatal length with some degree of proportionate tall stature in infancy and mid-childhood, talipes calcaneovalgus, kyphoscoliosis, generalized hypotonia, edematous palms and soles, and spindle shaped fingers*	*Mutation in PLOD1 gene at 1p36.22 Autosomal recessive*
Simpson-Golabi-Behmel	*Pre- and postnatal proportionate overgrowth, with organomegaly. Final adult height is usually >97th centile. Characteristic dysmorphic features, supernumerary nipples, hand anomalies, speech delay, cardiac anomalies, risk of embryonal cancers*	*Mutation in GPC3 gene at Xq26.2*
PTEN-hamartoma (Bannayan-Riley Ruvalcaba)	*Proportionate postnatal overgrowth, growth deceleration during childhood, adult height is normal, macrocephaly, hamartomas and lipomas, and penile macules, intellectual disability, hypotonia, joint hypermobility and elevated risk of cancer*	*Haploinsufficiency of PTEN gene at 10q23.31 Autosomal dominant*

Congenital syndromes with overgrowth	Growth pattern and major clinical features	Genetic mechanisms/ inheritance
• *Chromosomal aneuploidy*		
Klinefelter	*Tall stature from around 2 years of age, eunuchoid body proportions, clinodactyly, hypertelorism, elbow dysplasia, a high arched palate and hypotonia, androgen deficiency and pubertal delay, gynecomastia, variable cognitive/behavioral problems with difficulties in language, problem solving and planning, risk of germ cell tumors, breast cancer and osteoporosis*	*47, XXY* *less commonly,* *48, XXXY;* *48,XXYY; 49,XXXXY or* *46,XY/47, XXY mosaicism*
Trisomy X	*Normally sized at birth, normal growth rate until age of 4, tall stature during childhood and adolescence, adult height upper limit or mildly above 2SD, mild ocular hypertelorism, epicanthal folds, pes planus and 5th finger clinodactyly, variable intellectual disability and developmental delay*	*47, XXX*
47, XYY	*Normally sized at birth, tall adult stature, mild dysmorphic features, variable behavioral problems and cognitive impairment*	*47, XYY*
Pallister Killian	*Increased birth length and weight, postnatal deceleration of growth rate and overweight, characteristic dysmorphic facial features, pigmentary streaks on the skin, intellectual disability and seizures*	*Mosaicism of a supernumerary 12p isochromosome*

Congenital syndromes with overgrowth	**Growth pattern and major clinical features**	**Genetic mechanisms/ inheritance**
• *Genomic microrearrangements*		
Gorlin / nevoid basal cell carcinoma syndrome (PTCH1 9q22.32-33 microdel)	*Macrosomia, macrocephaly, keratocystic odontogenic tumours, small pits in the skin of the palms of the hands and soles of the feet, increased risk of skin cancer, brain tumors, cardiac and ovarian fibromas*	*Haploinsufficiency of PTCH1 due to 9q22.32-33 microdeletion Autosomal dominant*
Angelman syndrome with tall stature (due to paternal uniparental disomy)	*Tall stature only if paternal chromosome deletion, mental retardation, microcephaly, absence of speech, ataxia*	*Paternally derived deletion of 15q11-13*
Trisomy IGF-1R	*Large for gestational age, tall adult stature, dysmorphic features learning disorders and sometimes congenital malformations*	*Duplication and triplication 15q26.1*
Microdeletion 3q13.31	*Increased postnatal growth hypotonia, macrocephaly, intellectual disability, disturbed behavior and unusual facial features*	*Microdeletion 3q13.31*
Phelan- McDermid	*Hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech, autistic behavior, and minor dysmorphic features*	*Microdeletion 22q13.3*
19p13.13 deletion syndrome	*Tall stature, macrocephaly, mildly dysmorphic facial features, variable intellectual disability, developmental delay, hypotonia, ataxia, seizures, abnormalities of brain structure, strabismus, underdevelopment of the optic nerves*	*Microdeletion 19p13.13*

Congenital syndromes with overgrowth	Growth pattern and major clinical features	Genetic mechanisms/ inheritance
• *Other genetic disorders with tall stature*		
Marshall–Smith syndrome	*Tall stature and advanced bone age at birth, facial dysmorphism, short and conical phalanges, recurrent respiratory infections, failure to thrive*	*Unknown*
Association of SEC23A & MAN1B1 mutations	*Tall stature, developmental delay, obesity, macrocephaly, mild dysmorphic features, hypertelorism, maloccluded teeth, intellectual disability, and flat feet*	*Homozygous in both SEC23A and MAN1B1 genes*

Appendix 3

Supplemental materials for Chapter 9 (Publication I)

Appendix 4:

Supplemental materials for Chapter 10 (Publication II)

Appendix 5:

Supplemental materials for Chapter 11 (Publication III)

Appendix 6:

Supplemental materials for Chapter 13 (Publication V)

Appendix 7:

Supplemental materials for Chapter 14 (Publication VI)

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PERSONAL PROFILE

Dr. Liliya Rostomyan

Researcher at the Department of Endocrinology (Prof. A. Beckers), University of Liège, Liège, Belgium

Specialty Areas: pituitary adenomas; acromegaly-gigantism; growth disorders; multiple endocrine neoplasia syndromes, genetic studies in endocrine tumors

Chronology:

MD: Moscow Medical Academy, Moscow, Russia, 1999-2005;

Endocrinology Residency: Federal State Research Center for Endocrinology, Moscow, Russia, 2005-2009;

Specialty Fellowship Training in Neuroendocrinology: Federal State Research Center for Endocrinology, Moscow, Russia, 2009-2011;

PhD in endocrinology: Federal State Research Center for Endocrinology, Moscow, Russia 2011;

PhD in endocrinology: University of Liège, Liège, Belgium, 2011-2021;

R&D at the Department of endocrinology (Prof. A. Beckers), CHU de Liège, Liège, Belgium, since April 2018-present.

Biosketch

Dr. Rostomyan qualified in Medicine with highest honors award and golden medal from Moscow Medical Academy, Moscow, Russia and undertook her Residency in Endocrinology, Fellowship in Neuroendocrinology and a doctorate in Endocrinology at the Federal State Research Center for Endocrinology, Moscow, Russia. Since residency Dr. Rostomyan has studied the characteristics of endocrine tumors, in particular pituitary adenomas, in the context of genetic syndromes. Drawing on this experience, Dr. Rostomyan joined the research group of Prof. Albert Beckers in Liège, Belgium in 2011, where she focused on exploring the molecular and genetic basis of pituitary tumorigenesis, factors impacting aggressive behavior and treatment outcomes of patients with pituitary tumors.

As part of her work at the Beckers group Dr. Rostomyan is involved in international collaboration and networking in large number of countries worldwide. She is responsible for the professional connections with centers in Russia and countries across the Commonwealth of Independent States and their participation in international collaborations on neuroendocrine diseases conducted in Liège. Particularly, the first large-scale study of the clinical and genetic characteristics of patients with pituitary gigantism was designed and implemented in 2011, that became the core of her PhD project.

Dr. Rostomyan is an enthusiastic supporter of local and international scientific events (educational courses, roundtable discussions, workshops and conferences) both as a participant and an invited speaker.

Dr. Rostomyan is an active member of Belgian Endocrine Society, EYES, ESE, ENEA, The Endocrine Society, Women in Endocrinology and has received a number of travel grants, young researcher and outstanding abstract awards, prizes and honours from Belgian and international endocrinology societies, including “Belgian Endocrine Society Lecture Award 2019” prize awarded to Dr. Rostomyan for the work entitled : “Pituitary gigantism: clinical characteristics and new insights into the genetics of GH-secreting pituitary adenomas”.

She has authored so far, 25 peer-reviewed articles in international scientific journals (7 as first author), 7 book chapters, and has presented >50 abstracts as first author in different national and international endocrinology meetings.

List of personal publications

Books and book chapters:

1. Potorac, I., L. Rostomyan, J. F. Bonneville, P. Petrossians and A. Beckers. "**Pituitary Update.**" University of Liege. Merelbeke: IPSEN, 2014.
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6. Rostomyan L., A. Beckers and P. Petrossians **"History of the identification of gigantism and acromegaly"** In Gigantism and Acromegaly, edited by C. Stratakis: Elsevier Academic Press, 2021, P 1-16
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Articles:

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** Ces auteurs ont contribué de façon équivalente à la publication*

Artistic representation of acromegaly-gigantism by Dr. Liliya Rostomyan

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