Thesis for the degree of Doctor of Philosophy (Medicine)

Prediction Models and Pharmacogenomics in Adult Growth Hormone Deficiency

By

Edna de Jesus Litenski Barbosa

Department of Endocrinology, Institute of Medicine,
Sahlgrenska Academy
University of Gothenburg

Göteborg, Sweden 2012

Prediction Models and Pharmacogenomics in Adult Growth Hormone Deficiency

Edna de Jesus Litenski Barbosa

ISBN 978-91-628-8546-5

http://hdl.handle.net/2077/29711

Edna J. L. Barbosa
Institute of Medicine, Department of Endocrinology
Gröna Straket 8
Sahlgrenska University Hospital,
SE-413 45, Gothenburg, Sweden

edna.barbosa@medic.gu.se
ednajlbarbosa@gmail.com

Phone: +55 41 9102 0353; Fax: +55 41 3523 9184

Cover photo: aerial view of the Sahlgrenska University Hospital by Göran Olofsson, taken on 21 June, 2006, added to Gothenburg University Library on 13 December, 2007.

Printed by Kompendiet, Gothenburg, Sweden, 2012

To my family

"Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less."

Marie Curie

Abstract

Barbosa, EJL 2012. Prediction Models and Pharmacogenomics in Adult Growth Hormone Deficiency, Department of Internal Medicine, Sahlgrenska Academy at the University of Gothenburg, Sweden.
ISBN 978-91-628-8546-5

The overall aim of this thesis was to study clinical and genetic factors that influence response to growth hormone replacement therapy (GHRT) in GH deficient (GHD) adults. The patients were part of a cohort of adults with hypopituitarism and severe GHD who were studied before and after 12 months of GHRT. The dose regimen was individualized in order to attain normal IGF-I levels. Logistic regression (LR) analysis was used to identify good and poor responders to GHRT. The candidate gene approach was used to study single nucleotide polymorphisms (SNPs) in the GH receptor (GHR) gene, in genes related to GH signaling pathways, lipid metabolism and renal tubular function. Changes in IGF-I levels, body composition (BC), lipid profile and extracellular water (ECW) were analyzed as the GHRT outcomes. We identified gender and insulin levels at baseline as predictors for changes in IGF-I levels, and gender, height and lean body mass (LBM) at baseline as predictors for changes in BC. The accuracy of the equations obtained by LR to predict whether a patient will be a GR or PR was 70% for IGF-I and 80% for BC responses. The d3 allele polymorphism in the *GHR* gene did not influence IGF-I levels and BF at baseline and their changes after GHRT. At baseline, distinct SNPs of the cholesteryl ester transfer protein (*CETP*) gene were associated with higher total cholesterol (TC), HDL-C and LDL-C, those of the apolipoprotein E (*APOE*) gene with lower TC and LDL-C, *APOB* gene with higher serum HDL-C, and those of the peroxisome proliferator-activated receptor gamma (*PPARG*) gene with lower LDL-C and the *APOE/C1/C4/C2* cluster with lower tryglicerides (TG). After GHRT, greater reductions in TC and LDL-C were associated with SNPs in the *APOB* and *PPARG*, explaining 5% of the variation. SNPs in the signal transducer and activator of transcription 5B (*STAT5B),* in the phosphoinositide-3-kinase, catalytic, beta polypeptide (*PIK3CB)* and in the sodium/potassium/chloride transporter member 1 (*SLC12A1)* genes were associated with differences in ECW in GHD patients. We conclude that gender, body height, LBM and insulin levels were the best predictors of IGF-I and BC responses to GHRT in GHD adults. The presence of the d3-GHR allele did not influence responses to GHRT, but we found that some SNPs in genes related to lipid metabolism, GH signaling pathways and water balance impact the baseline characteristics of GHD and their response to GHRT.

KEYWORDS: growth hormone deficiency, hypopituitarism, growth hormone replacement therapy, prediction models, candidate gene approach, polymorphisms, body composition, growth hormone receptor, lipid metabolism, extracellular water, pharmacogenomics

Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. **Barbosa EJL, Koranyi J, Filipsson H, Bengtsson B-Å, Boguszewski C, Johannsson G.** Models to predict changes in serum IGF-I and body composition in response to GH replacement therapy in GH-deficient adults. European Journal of Endocrinology (2010) 162: 869-878.

II. **Barbosa EJL, Palming J, Glad CAM, Filipsson H, Koranyi J, Bengtsson B-Å, Carlsson LMS, Boguszewski CL, Johannsson G.** Influence of the exon3-deleted/full-length growth hormone (GH) receptor polymorphism on the response to GH replacement therapy in adults with severe GH deficiency. J Clin Endocrinol Metab (2009) 94: 639-641.

III. **Barbosa EJL, Glad CAM, Nilsson AG, Nyström HF, Götherström G, Svensson P-A, Vinotti I, Bengtsson B-A, Nilsson S, Boguszewski CL, Johannsson G.** Genotypes associated with lipid metabolism contribute to differences in serum lipid profile of growth hormone deficient (GHD) adults before and after GH replacement therapy. Eur J Endocrinol (2012) 167: 353-362.

IV. **Barbosa EJL, Glad CAM, Nilsson AG, Bosaeus N, Nyström HF, , Svensson P-A, Bengtsson B-A, Nilsson S, Bosaeus I, Boguszewski CL, Johannsson G.** Genotypes associated with growth hormone (GH) signaling pathway and renal function contribute to differences in the extracellular water volume of GH deficient adults. Manuscript.

Table of Contents

Summary in English

Introduction: There is considerable individual variation in the clinical response to growth hormone (GH) replacement therapy (GHRT). Useful predictors of treatment response are lacking, particularly in GH deficient (GHD) adults.
Aims: The overall aim of this thesis was to identify clinical and genetic factors that influence the response to GHRT in GHD adults.
Hypothesis: The more specific research questions were: can we develop prediction models for serum IGF-I and body composition (BC) responses to GHRT in GHD adults? Does the GH receptor polymorphism (d3-GHR) influence the response to GHRT in terms of IGF-I levels and body fat (BF) in GHD adults? Do single nucleotide polymorphisms (SNPs) in genes related to lipid metabolism influence serum lipid concentrations in GHD adults and their changes with GHRT? Can SNPs in genes related to renal sodium and water balance and SNPs in genes related to the GH signaling pathway predict extracellular water (ECW) volumes in GHD adults and their changes with GHRT?
Methods: The patients in this thesis were part of a longitudinal cohort of adults with hypopituitarism and severe GHD who received GHRT in a titrated dose regimen to attain normal IGF-I levels. Adult GHD patients were studied before and after 12 months of GHRT. To develop prediction models for GHRT response, 167 patients (103 men; median age 49.8 yrs) were studied. Serum IGF-I levels and BC using dual-energy x-ray absorptiometry (DXA) were assessed. The GHD patients were classified as "Good Responders" (GR) or "Poor Responders" (PR) when the response to GHRT was greater than the 60th or lower than the 40th percentile, respectively. Logistic regression (LR) was used to identify GR or PR. The candidate gene approach was used in the genetic studies and genomic DNA was extracted from whole blood. The influence of the d3 allele of the GHR gene on the IGF-I and BF responses to GHRT was studied in 124 GHD adults (79 men; median age 50.1 yrs). BF was assessed by the 4-compartment model, and multiplex PCR with fragment detection by gel electrophoresis was used for determining the genotype in the GHR exon 3 locus. The impact of 9 genes related to lipid metabolism on lipid response to GHRT was studied in 318 GHD adults (184 men; median age 51 yrs). Twenty SNPs were selected because they had already been shown to have a significant association with serum lipid concentrations and cardiovascular conditions in other populations. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were assessed in serum samples. The association of 5 genes related to GH signaling pathways and 9 genes in renal tubular function on ECW volume was investigated in 311 GHD adults (181 men; median 51 yrs). These genes were selected because of their well-established role in the GH signaling pathways or their influence on the renal tubular function and renal sodium and water balance regulation. A total of 41 SNPs were genotyped using TaqMan or the Sequenom platform. ECW measurements were estimated using bioelectrical impedance analysis (BIA) at a frequency of 50kHz (BIA-50kHz).
Results: For IGF-I response, the clinical predictors were gender and insulin levels at baseline. Men were 5.6 times more likely to be GR than women. Mean baseline insulin levels were similar in GR and PR, but patients with higher insulin levels were more likely to be GR. For BC changes [body fat (BF) and lean body mass (LBM)], the best predictors were gender, height and LBM at baseline. Men, taller patients and those with lower LBM were more likely to be GR. The accuracy of the equations obtained by LR to predict whether a patient will be a GR or PR was 70% for IGF-I and 80% for BC responses.
In the analysis of the GHR polymorphism, 58% of the GHD patients had two wild-type alleles (fl/fl-GHR; Group 1), while 42% had at least one d3-GHR allele (Group 2), comprising 32% with one d3-GHR allele (d3/fl-GHR) and 10% with two GHR-alleles (d3/d3-GHR). No significant difference was found between patients from Group 1 and Group 2 in terms of IGF-I and BF changes after 12 months of GHRT. Our investigation of SNPs related to serum lipid concentrations in GHD adults showed that serum TC concentrations were associated with the cholesteryl ester transfer protein (*CETP)* gene SNPs rs708272 and rs1800775 and with apolipoprotein E *(APOE)* gene SNP rs7412. Moreover, serum HDL-C concentrations were associated with *CETP* SNPs rs708272, rs1800775 and rs3764261 and the apolipoprotein B *(APOB)* gene SNP rs693. Serum LDL-C concentrations were associated with *APOE* SNP rs7412, peroxisome proliferator-activated receptor gamma (*PPARG)* gene SNP rs10865710 and *CETP* SNP rs1800775. Triglyceride (TG) concentrations were associated with *APOE/C1/C4/C2* SNP rs35136575. After treatment, the *APOB* SNP rs676210 GG genotype was associated with larger

decreases in TC and LDL-C, and the *PPARG* SNP rs10865710 CC genotype with greater decreases in TC. These two SNPs explained 5% of the lipid profile variation in response to GHRT. All associations remained significant when adjusted for age, sex and BMI. Three SNPs were associated with ECW volume in GHD patients: the signal transducer and activator of transcription 5B (*STAT5B) SNP* rs6503691; the phosphoinositide-3-kinase, catalytic, beta polypeptide (*PIK3CB) SNP* rs361072; and the solute carrier family 12 (sodium/potassium/chloride transporters), member 1 (*SLC12A1) SNP* rs2291340. After 12 months of GHRT, no SNP was found to have influenced changes in ECW in response to GHRT.

Conclusions: mathematical models to predict GH responsiveness in GHD adults were developed using gender and serum insulin levels as the major clinical predictors for IGF-I response, and gender, body height and baseline LBM for BC response. The presence of the d3-GHR allele did not influence the IGF-I and BF responses to GHRT in GHD adults. Multiple SNPs in genes related to lipid metabolism contributed to individual differences in the serum lipid concentrations of GHD adults. Moreover, polymorphisms in the *APOB* and *PPARG* had a small but significant influence on the changes in the serum lipid profile after GHRT. The *STAT5B, PIK3CB* and *SLC12A1* polymorphisms contributed to the interindividual variability in the ECW volume in untreated GHD adults.

Summary in Swedish/Sammanfattning på svenska

Bakgrund: Det finns en stor individuell variation i kliniskt svar på ersättningbehandling med tillväxthormon (GH). Användbara prediktorer för behandlingssvar saknas, särskilt hos vuxna med brist på GH.
Syfte: Det övergripande syftet med denna avhandling var att identifiera kliniska och genetiska faktorer som påverkar svar på GH ersättningsbehandling hos vuxna individer med GH-brist.
Hypotes: De mer specifika forskningsfrågorna var: kan prediktionsmodeller för förändring i serum IGF-I och kroppssammansättning som följd av GH ersättningsbehandling hos vuxna individer med GH-brist utvecklas? Påverkar en polymorfism i GH receptorn (d3-GHR) svaret på GH ersättningsbehandling vad gäller IGF-I nivåer och kroppsfett hos vuxna individer med GH-brist? Påverkar enkla basparssubstitutioner, s.k. SNPs, i gener relaterade till lipidmetabolism serum lipidnivåer hos vuxna individer med GH-brist och förändring i lipidnivåer under GH ersättningsbehandling? Kan SNPs i gener relaterade till njurens natrium- och vattenbalans eller GH signaleringskaskad prediktera volym av extracellulärvatten (ECW) samt förändring av ECW volym under GH ersättningsbehandling hos vuxna individer med GH-brist?
Metoder: Patienterna i denna studie ingick i en longitudinell studie på patienter med hypofyssvikt och grav GH-brist som fått GH ersättningsbehandling i en titrerad dosregim i syftet att erhålla normaliserade IGF-I värden. Vuxna individer med GH-brist studerades före och efter 12 månader med GH ersättningsbehandling. För att utveckla prediktionsmodeller för GH ersättningsbehandlingssvar studerades 167 patienter (79 män; median ålder 49,8 år). Serum IGF-I nivåer och kroppssammansättning mätt med dual-energy x-ray absorptiometry (DEXA) undersöktes. Patienter med GH-brist klassificerades som ”good responders” (GR) eller ”poor responders” (PR) när responsen på GH ersättningsbehandling var över den 60e eller under den 40e percentilen, respektive. Logistisk regression (LR) användes för att identifiera GR och PR. En kandidatgensapproach användes i de genetiska studierna och genomiskt DNA isolerades från helblod. Påverkan av d3-GHR på IGF-I och kroppsfett under/efter GH ersättningsbehandling studerades hos 124 vuxna individer med GH-brist (79 män; median ålder 50,1 år). Kroppsfett bestämdes med en s.k. 4-compartment modell och multiplex PCR med fragmentdetektion via gelelektrofores användes för att bestämma genotyp för d3-GHR. Effekten av nio gener involverade i lipidmetabolism på lipidrespons under GH ersättningsbehandling studerades hos 318 vuxna individer med GH-brist (184 män; median ålder 51 år). 20 SNPs valdes ut baserat på att de tidigare visats ha en signifikant koppling till serum lipidkoncentrationer och/eller kardiovaskulär funktion i andra populationer. Totalkolesterol (TC), low-density lipoprotein kolesterol (LDL-C), high-density lipoprotein kolesterol (HDL-C) samt triglycerider (TG) mättes i serumprover. Påverkan av fem gener inom GH signaleringskaskad och nio gener med effekt på njurfunktion på ECW volym undersöktes hos 311 vuxna individer med GH-brist (181 män; median ålder 51 år). Dessa gener valdes ut med anledning av deras väletablerade roll inom GH-signalering eller dess påverkan på njurfunktion och reglering av vattenbalans. Totalt genotypades 41 SNPs med TaqMan eller Sequenom plattformarna. ECW mätningar utfördes med s.k. bioimpedisk impedansanalys (BIA) vid en frekvens av 50 kHz (BIA-50kHz).
Resultat: Prediktorer för IGF-I respons var kön samt insulinnivåer vid behandlingsstart. Män var 5,6 gånger troligare att vara GR än kvinnor. Medel insulinnivåer vid start var likvärdiga hos GR och PR, men patienter med högre insulinnivåer var troligare GR. För förändring i kroppssammansättning [kroppsfett (BF) och muskelmassa (LBM)] var de bästa prediktorerna kön, ålder samt LBM vid behandlingsstart. Män, längre individer och patienter med lägre LBM var troligare en GR. Säkerheten för framtagna ekvationer att prediktera huruvida en patient är en GR eller PR var 70% för IGF-I och 80% för kroppssammansättning. Analysen av GHR polymorfismen visade att 58% av de vuxna individerna med GH-brist bar på två vildtypsalleler (fl/fl-GHR; Grupp 1), medan 42% bar på åtminstone en d3-allel (Grupp 2; där 32% hade en d3-allel (d3/fl-GHR) och 10% hade två d3-alleler (d3/d3-GHR)). Inga signifikanta skillnader uppmättes mellan Grupp 1 och Grupp 2 vad gäller förändring av IGF-I och kroppsfett efter 12 månader med GH ersättningsbehandling. Vår undersökning av tolv SNPs relaterade till lipidmetabolism hos vuxna individer med GH-brist visade att serum TC koncentrationer var kopplade till cholesteryl ester transfer protein (*CETP)* SNPs rs708272 och rs1800775 samt till apolipoprotein E *(APOE)* SNP rs7412. Serum HDL-C koncentrationer var kopplade till *CETP* SNPs rs708272, rs1800775 och rs3764261 samt till

apolipoprotein B *(APOB)* SNP rs693, serum LDL-C koncentrationer var kopplade till *APOE* SNP rs7412, peroxisome proliferator-activated receptor gamma (*PPARG)* SNP rs10865710 samt till *CETP* SNP rs1800775. Serum TG koncentrationer var kopplade till *APOE/C1/C4/C2* SNP rs35136575. Efter behandling var *APOB* SNP rs676210 GG genotyp kopplad till en större sänkning av TC och LDL-C, och *PPARG* SNP rs10865710 CC genotyp var kopplad till en större sänkning av TC. Dessa två SNPs förklarade 5% av variationen i lipidprofil under GH ersättningsbehandling. Alla associationer kvarstod som signifikanta efter justeringar för ålder, kön samt BMI. Tre SNPs var kopplade till ECW volym hos individer med GH-brist: signal transducer and activator of transcription 5B (*STAT5B) SNP* rs6503691, phosphoinositide-3-kinase, catalytic, beta polypeptide (*PIK3CB) SNP* rs361072 samt solute carrier family 12 (sodium/potassium/chloride transporters), member 1 (*SLC12A1) SNP* rs2291340. Ingen SNP påverkade förändring i ECW volym efter 12 månader med GH ersättningsbehandling.

Slutsatser: Specifika matematiska modeller för att prediktera svar på GH behandling hos vuxna individer med GH-brist utvecklades, där kön och serum insulin nivåer var de viktigaste kliniska prediktorerna för IGF-I respons och kön, längd och LBM vid behandlingsstart var de viktigaste prediktorerna för kroppsammansättningsrespons. d3-GHR påverkade inte förändring av IGF-I nivåer och kroppsfett under GH ersättningsbehandling hos vuxna individer med GH-brist. Flertalet SNPs i gener relaterade till lipidmetabolism bidrog till individuella skillnader i serum lipid koncentrationer hos vuxna individer med GH-brist. Polymorfismer i *APOB* och *PPARG* generna hade en liten men signifikant påverkan på lipidprofil efter GH ersättningsbehandling. Polymorfismer i *STAT5B, PIK3CB* och *SLC12A1* generna bidrog till den interindividuella variabiliteten i ECW volym hos obehandlade vuxna med GH-brist.

Abbreviations

AGT	angiotensinogen
APOB	apolipoprotein B
APOC	apolipoprotien C
APOE	apolipoprotein E
BC	body composition
BF	body fat
BIA	bioelectrical impedance analysis
BMI	body mass index
BMC	bone mineral content
BMD	bone mineral density
BP	blood pressure
BW	body weight
CAD	coronary artery disease
CASR	calcium-sensing receptor
CETP	cholesteryl ester transfer protein
CV	coefficient of variation
CVD	cardiovascular disease
DBP	diastolic blood pressure
DEXA	dual-energy X-ray absorptiometry
DNA	deoxyribonucleic acid
DXA	dual-energy X-ray absorptiometry
ECW	extracellular water
FDA	Food and Drug administration
GH	growth hormone
GHBP	growth hormone binding protein
GHD	growth hormone deficiency
GHRH	growth hormone releasing hormone
GHRT	growth hormone replacement therapy
GR	good responder
GWAS	genome-wide association studies
HDL	high-density lipoprotein
HWE	Hardy –Weinberg equilibrium
IGF-I	insulin-like growth factor-1
ITT	insulin tolerance test
JAK2	Janus Kinase 2
LBM	lean body mass
LDL-C	low-density lipoprotein cholesterol
LDLR	low-density lipoprotein receptor
LPL	lipoprotein lipase
LR	logistic regression
MAF	minor allele frequency
OR	odds ratio
PCR	polymerase chain reaction
PPARG	peroxisome proliferator-activated receptor gamma
PR	poor responder
RIA	radioimmunoassay
ROMK	potassium inwardly-rectifying channel, subfamily J, member 1
SNP	single-nucleotide polymorphism
SBP	systolic blood pressure
SDS	standard deviation score
STAT	signal transducer and activator of transcription
TBW	total body water
TC	total cholesterol
VLDL	very low-density lipoprotein
WC	waist circumference
WHR	waist hip ratio
WNK1	lysine deficient protein kinase 1 gene

Introduction

For over twenty-five years, GHRT has been used to treat GHD in adults. However, the determination of the most adequate dose for individual patients has proved difficult and response to the treatment varies greatly from one GHD adult to another. Many possible reasons have been suggested to account for why individual response to GHRT varies so much. The GH has a wide range of biological actions in the body and it affects several targets. In particular, the GH acts on the liver, adipose tissue, muscle, bone, kidney and brain, and these tissues in turn have factors which interfere with the response. This situation is complicated further still by the fact that some of the GH's actions are directly or indirectly mediated by the endocrine and paracrine actions of IGF-I (1, 2).

The response to GHRT is influenced by clinical parameters and might be impacted by genetic factors. Clinical factors, such as age, gender and BMI, account for a small part of the variability in the GH response (3). In children, there have been attempts to develop prediction models for growth response to GHRT (4). In adults, however, such mathematical models have not been tested to distinguish good and poor responders to GHRT.

In recent years, genetic factors and their influence on responsiveness to drug therapies have attracted great deal of interest and opened a myriad of possibilities to enable more individualized treatments. The potential use of pharmacogenomic approaches could lead to more effective and safer therapies, tailored to the individual needs of each patient.

There have been many different ways of testing the interactions between genetics, human biology and pharmacology. A single-nucleotide polymorphism (SNP) is a DNA sequence variation that occurs when a single nucleotide — A, T, C or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. A single SNP may cause a Mendelian disorder, while in most diseases SNPs do not function individually, but work in coordination with other SNPs to produce a certain condition. Thus, an investigation of SNPs could reveal unexpected physiological and pathological aspects of human diseases. This can be done through genome-wide association studies (GWAS), which scan the entire genome for common genetic variations, or by candidate gene analysis, involving the study of pre-specified genes of interest, e.g., genes coding for membrane transporters that can affect drug bioavailability, plasma concentration and/or delivery to the target site. In this study, we have adopted the candidate gene approach to explore the potential role of various SNPs in the baseline characteristics of patients with hypopituitarism and severe GHD and their impact on the responsiveness to 12 months of GHRT.

Historical background of GH replacement therapy in adults

The earliest attempts at isolating human GH from the pituitary gland were made in the 1950s by Li and Papokoff in California (5). Raben, in 1958, reported on the treatment of a pituitary dwarf with pituitary GH (6). In the 1960s, the therapeutic use of pituitary GH, extracted from human cadavers, was widely accepted for treating children with severe growth retardation. From 1963 to 1985, thousands of GHD children in different parts of the world received pituitary GH. However, this treatment was abruptly terminated when it was discovered that a number of patients developed Creuzfeldt-Jacob disease, a degenerative neurological prion-mediated disorder (7-8). The link between pituitary GH and this disease was quickly recognized by regulatory agencies. However, at the same time, a new development with great clinical potential took place: recombinant DNA-derived human GH.

The GH gene was cloned for the first time in 1979, and in 1981 the first recombinant human GH was synthetically produced (9). The process was improved over the next few years and received FDA approval in 1985. Prior to this, GH therapy was available only to the most severely affected GHD children. With the development and standardization of the recombinant techniques, the availability of treatment became a reality both to a wider range of short children with many different disorders and adults with hypopituitarism. Hence, in the 1990s, the new clinical entity of severe GHD in adults began to be defined and characterized by increased body fat, decreased muscle mass, reduced

extracellular fluid volume, reduced bone density and altered lipid profile, reduced exercise capacity and impaired quality of life (10-13).
One of the first challenges in this new field of adult GH treatment was to define the appropriate dose of GH. In the early days, the dose was based on the patient's weight or body surface area, using the same strategy employed in children. However, despite the effectiveness of the treatment, it often resulted in a number of side effects related to fluid retention and insulin resistance due to high doses and individual patient sensitivity to the hormone (14-18). As a consequence, initial GH doses were progressively reduced with subsequent up-titration doses based on individual response to changes in IGF-I, body composition, quality of life and metabolic profile, which is the current recommendation for treatment of adults with GHD. Individualized dose titration had the same beneficial effects, but with fewer side effects and lower GH maintenance doses (19-20).
Holmes and Shalet (21) made one of the first attempts to identify the factors that influence GH sensitivity. They observed that older and more obese patients with adult-onset GHD were more sensitive to GH. Furthermore, other studies showed that the effects of GH on body composition, lipid profile and markers of bone turnover were more pronounced in men (22-24). It was also observed that women receiving oral estrogen replacement therapy were more resistant to GH in the serum IGF-I response (25) and less prone to develop peripheral edema and other fluid-related side effects of GHRT (26).

GH deficiency in adults

GHD adults exhibit abnormal body composition, characterized by a significant increase in fat mass, especially visceral fat, and a decrease in lean body mass (11, 27-32), total body water (TBW) and extracellular fluid volume (12, 33, 34). GHD patients have approximately 7% more fat mass in comparison to the predicted values adjusted for age, sex and height (35, 16). They often have diminished strength and exercise capacity (36-37). Their bone mineral density (BMD) is reduced, especially in young adults with GHD, and bone fractures are more prevalent (31, 38). Adults with GHD also have an altered serum lipid profile and are more prone to cardiovascular abnormalities (33, 39-46). These patients might that their quality of life (QoL) is poor and that they suffer from social isolation and decreased energy and vitality (41, 46-48). GHD may also contribute to the reduced life expectancy secondary to increased cerebro- and cardiovascular disease that has been reported in several epidemiological studies of patients with hypopituitarism (11, 13, 18, 42-45).

Outcomes of GH replacement therapy

In contrast with GH therapy in children, there are many endpoints to be evaluated in an adult GHD patient treated with GHRT, including changes in BC, lipid and metabolic profile, bone health and quality of life. Serum IGF-I concentrations are used to monitor the GHRT in adults and they are especially helpful for detecting over-replacement. The serum IGF-I response to the administration of GH mainly reflects the hepatic effect of GH, as over 70% of the circulating IGF-I is produced in the liver (1).
Reduced fat mass after GHRT has mostly been described in the visceral fat through anthropometric measurements and imaging (18, 16, 49). A recent study concerning the effect of GH replacement on different fat compartments analyzed by whole-body magnetic resonance imaging found that GHRT may affect both subcutaneous and visceral fat mass compartments (50-51).
GHD patients have lower levels of total body water (TBW) as a result of reduced volumes of ECW (34). The GHRT increases ECW as a result of the antinatriuretic effects of GH and IGF-I (16, 18, 52-55). In most patients, ECW volume is normalized by the same GH dose employed to obtain a normal serum IGF-I level (56). Alterations in ECW might be a more useful end-point for GHRT monitoring because these changes are more consistent during therapy (57).
GH regulates lipoprotein metabolism by enhancing clearance of LDL by activating the expression of hepatic LDL receptors (58-59). Most studies that have evaluated the effects of GHRT on the lipid

profile of GHD adults have shown a reduction in serum levels of total cholesterol (TC), LDL cholesterol and apolipoprotein B, and an increase in HDL cholesterol concentrations (59) with little or no change in triglycerides (59).
GHD in adults is associated with insulin resistance. In the short term, GHRT may worsen insulin sensitivity due to anti-insulin effects of GH (60, 61). However, in the long-term, GH-induced lipolysis overcomes its diabetogenic effect and no significant changes have been observed in serum glucose and insulin level and in the index of insulin sensitivity, such as HOMA-IR (62-64).
QoL has been studied by global and disease-derived questionnaires to measure the response to treatment. Most of the patients who score badly at baseline find that their QoL has improved after treatment. On the other hand, no changes have been reported by patients with good QoL at baseline. Thus, the worse the patient is, the more these scores improve. Once the beneficial psychological effects have been felt, they are sustained in the long term (66-67).
A progressive increase in bone mineral content (BMC) and bone mineral density (BMD) in GHD patients can only be detected after at least 18 months of GHRT. The lumbar spine increases by around 9%, with men responding more than women (63, 67). BMC and bone area are increased as a result of the increase in the amount of bone, and this leads to a less marked effect on BMD (68). This reduced effect is due to increased endosteal and periosteal bone formation in cortical bone with a less marked effect on trabecular bone. This is evident in the histomorphometric data of men who had childhood onset disease and received GH treatment for 5 years (69). In most patients, BMD normalizes (63, 51), and this is likely to be result in a lower risk of bone fractures.
The effect on muscle strength and muscle function is normally not detectable until approximately 18 months of treatment (24). After two to five years of GHRT, muscle strength can be normalized in a significant proportion of patients (70).

Clinical predictors of individual responsiveness to GH replacement therapy

As mentioned above, individual responsiveness to GHRT is highly variable, both in children and adults, depending on a number of documented factors. Mathematical models to predict growth response in children are mainly based on multivariate analysis of a patient's characteristics and treatment modalities, and they have been reported on for children with GHD, girls with Turner Syndrome and short children born small for gestational age (4, 71-76). These algorithms can explain, with a low margin of error, a high degree of the observed variability of the response during the first and subsequent prepubertal treatment years (4) In children, the target for the prediction models are growth velocity and final adult height, while in GHD adults the targets are multiple and may vary among patients. Patients may exhibit a good response in one or two outcomes, but not in all. Clinical factors such as baseline BMI, serum GHBP levels, age and gender, were found to influence the GH response in adults, although they account for only a part of the variability. The response to GH in BC has been shown to be poorly correlated with the dose of GH, whereas BMI, age, gender and GHBP levels are weak prediction factors (3, 19, 76-77). On the other hand, IGF-I response has been found to be associated with GH dose (78), gender (23) and route of oestrogen replacement (26, 79-83). Patients with a higher BMI are more likely to have a lower reduction in body fat (BF), while patients with low baseline GHBP levels are more likely to have the most marked increase in lean body mass. In terms to gender, GHD men experience a more pronounced increase in serum IGF-I concentrations and TBW than GHD women (3). But even after adjustments for these clinical factors, individual response to GHRT remains highly variable.
Compliance is always a question of concern in all patients with apparently suboptimal response to GHRT. Management is currently based on daily sc injections that patients often find cumbersome and therefore may affect adherence to treatment. Misperceptions concerning the consequences of missed GH doses, discomfort with injections, dissatisfaction with treatment results and inadequate contact with health-care providers (along with other factors) have been strongly associated with approximately 70% of noncompliance (84, 85). To promote continuous GH use, routine education should emphasize

therapeutic end points and their relationship to compliance with GH therapy in an effort to convince and empower patients with GH deficiency to use self-care strategies to achieve their treatment goals.

Genetic predictors of individual responsiveness to GH replacement therapy

Genetic background could potentially have an impact on GH responsiveness to GHRT. For instance, a polymorphism in the GH receptor (GHR) gene leading to the deletion of exon 3 (d3-GHR) has been linked to the growth response to GH therapy in short children. Dos Santos et al (86) studied the effect of this polymorphism in a large sample of short children born small for gestational age (SGA) and children with idiopathic short stature (ISS), who have normal birth size but grow at a decreased rate. During the first 2 years of GHRT, growth response was greater in children bearing at least one allele encoding the d3-GHR isoform. Positive associations have been published on children with GHD (87-88), ISS and SGA (86, 89), whereas others have found no link between the GHR genotype and the efficacy of GHRT on GHD (90-91) and non-GHD children (92-94).

Aims of the thesis

The main overall objective of this thesis was to study clinical and genetic factors that could influence response to GHRT in GHD adults. The more specific primary aims were:

Paper I To identify good and poor responders to GHRT and to develop mathematical models using clinical factors to predict response to GHRT.

Paper II To assess the influence of the exon 3-deleted (d3-GHR) and full-length (fl-GHR) GH receptor isoforms on the response in serum IGF-I levels and BF to GHRT.

Paper III To evaluate the influence of SNPs in genes related to lipid metabolism on the response in the serum lipid profile to GHRT.

Paper IV To examine the influence of SNPs in genes related to the GH signaling pathways and renal tubular function on the response in ECW to GHRT.

Subjects and Methods

Patients

The patients in this study were part of a large prospective longitudinal cohort of adults with hypopituitarism and severe GHD treated at the Sahlgrenska University Hospital, Gothenburg, Sweden from as early as 1993 until 2009 (Table 1)**.** The number of patients included in each study varied due to exclusion criteria and the willingness of individuals to participate in genetic testing. An insulin tolerance test (ITT) was used to confirm the diagnosis of GHD in over 75% of the patients. Eligibility was determined by a maximum peak GH response ≤ 3.0 μg/l during the ITT (blood glucose nadir ≤ 2.2 mmol/l). Patients with contraindications for the ITT were subjected to other tests, such as GHRH-arginine, GHRH-pyridostigmine or glucagon in order to confirm severe GHD. In the GHRH-arginine test, the appropriate cutoff points for diagnosing GHD were 11.5 μg/l for those with a BMI less than 25 kg/m^2, 8.0 μg/l for a BMI of 25–30 kg/m^2, and 4.2 μg/l for those with a BMI greater than 30 kg/m^2 (95). A cutoff of 10 μg/l for the GHRH-pyridostigmine and between 2.5 and 3 μg/l for glucagon stimulation tests seems to have the appropriate specificity and sensitivity for the diagnosis of GHD (96-98).
The most common cause of GHD was non-functional pituitary adenoma and the majority of patients had adult-onset GHD (AO-GHD). None of the AO-GHD had previously received GH therapy. All adults with childhood-onset GHD (CO-GHD) had previously received GH therapy but it had been terminated a considerable amount of time before they were retested prior to GHRT in adulthood, most of them having gone without treatment for at least four years. The remaining few had not received treatment for at least 6 months. Furthermore, most patients had multiple pituitary hormonal deficiencies. When required, patients received adequate and stable replacement therapy with glucocorticoids, thyroid hormone (levothyroxine), gonadal steroids and desmopressin for at least 6 months before beginning GHRT.

Considerations on patient selection

The patients in this study are mostly patients with hypothalamic pituitary disease and hypopituitarism. They are all tested and offered GHRT if a deficiency is found. This reduces patient selection bias, but may have an impact when drawing comparisons with other studies, as other centres in Sweden and those in other countries have different criteria for testing patients and providing them with treatment.

Ethics

All the studies included in this thesis were approved by the Ethics Committee of the University of Gothenburg, Sweden and conducted in accordance with the Declaration of Helsinki.
Written informed consent was obtained from all patients after they had received oral and written information regarding the study.

Table 1. Characteristics of the adults with GH deficiency included in *Papers I-IV*.

Variable	*Paper I*	*Paper II*	*Paper III*	*Paper IV*
No. of patients, (Men/women)	167 (61.7/38.3)	124 (63.7/36.3)	318 (57.9/42.1)	311 (58.2/41.8)
Age, years	49.8 (19-76)	50.1 (18-76)	51 (17-77)	51 (17-77)
No. of patients with childhood-onset GHD	19 (11.4)	16 (12.9)	31(9.7)	32 (10.3)
Known duration of hypopituitarism, years	2 (0-42)	2 (0-42)	2 (0-47)	2 (0-47)
Causes of pituitary deficiency				
Non-secreting pituitary adenoma	75 (44.9)	56 (45.2)	129 (40.6)	131 (42.1)
Prolactinoma	15 (9)	13 (10.5)	24 (7.5)	27 (8.7)
Craniopharyngioma	17 (10.2)	11 (8.9)	24 (7.5)	24 (7.7)
Idiopathic	19 (11.4)	12 (9.7)	28 (8.8)	27 (8.7)
Other	41 (24.6)	32 (25.8)	113 (35.5)	102 (32.8)
Hormone replacement therapy				
Glucocorticoid	95 (56.9)	69 (55.6)	163 (51.3)	156 (50.2)
Levothyroxine	135 (80.8)	102 (82.3)	240 (75.5)	231 (74.3)
Sex steroids	121 (72.5)	91(73.3)	198 (62.2)	191 (61.5)
Isolated GHD	21 (12.6)	12 (9.7)	37 (11.6)	35 (11.3)

Data are presented as median (range or percentage).

GH replacement therapy

The patients in all four studies received recombinant human GH, administered s.c. every evening. The initial median dose was 0.17 mg/day, varying from 0.1 to 1.3. The dose was titrated according to age- and gender-adjusted serum IGF-I concentrations after 1 and 4 weeks of GHRT and every 3 months thereafter to maintain IGF-I levels between the median and the upper limit of the normal reference range. The individual serum IGF-I levels were transformed into standard deviation scores (SDS) according to age- and sex-adjusted reference values. The reference population had previously been described in detail (104) and randomly selected from the same geographical area (Western Sweden) as the patients in the present study. The following formulae were used to calculate the predicted IGF-I values (105): [292.7 - 2.1 x age] for men, and [375.7 - 3.7 x age] for women. The calculations of the IGF-I SDS were carried out using two other formulae: [observed IGF-I - predicted IGF-I/48] for men, and [observed IGF-I - predicted IGF-I/54.7] for women.

Study design

The design was an open uncontrolled open labeled longitudinal study. Clinical and biochemical evaluation was performed at baseline, 1 and 3 months and then every 3 months during the first year and thereafter every 6 months. Measures of BC were taken at baseline and after 6 and 12 months.

Paper I: To determine whether a patient was a good or poor responder to GHRT, the absolute changes in BC and IGF-I responses after 12 months of treatment were calculated. Changes in IGF-I levels (μg/l) from baseline were adjusted according to the cumulative GH dose (cGH, mg) that each patient received during the whole period of treatment, using the following formula to calculate IGF-I response (μg/per mg of GH): [ΔIGF-I/cGH]. Patients were categorized as good responders (GR) or poor responders (PR) to GHRT using this ratio. Patients with IGF-I response above the 60th percentile were categorized as GR and those with a response below the 40th percentile as PR. For BC, patients were classified as GR when LBM increased and BF decreased above the 60th percentile, while in the PR, changes in LBM and BF were below the 40th percentile.

Paper II: Based on genotype, patients were divided into Group 1 (those with two wild-type alleles, fl/fl-GHR) and Group 2 (those with at least one d3-GHR allele). At baseline, the genotype frequency was studied, in addition to its relation with clinical and laboratorial characteristics. After 12 months of GHRT, the genotype was linked to the following outcome variables and their change following therapy: 1) serum IGF-I concentrations; 2) IGF-I SDS; 3) BF; and 4) GH dose.

Paper III: Patients were divided into two groups: 1) those with two major alleles of genes related to lipid metabolism and 2) those carrying at least one minor allele. Genotypes were linked to variations in lipid profile before and after 12 months of GHRT in GHD adults.

Paper IV: Patients were divided into two groups: 1) those patients with two major alleles of genes related to GH signaling pathways and renal tubular function and 2) those carrying at least one minor allele. Genotypes were linked to variations in ECW before and after 12 months of GHRT in GHD adults.

Considerations on study design

In **Paper II**, we studied the polymorphism of GHR because GH actions on different tissues are mediated by interaction with its receptor.

In **Paper III**, the candidate genes were selected based on their known physiological influence on lipid metabolism and in the cardiovascular status in other populations (Table 2).

In **Paper IV**, the candidate genes were selected based on their known physiological role in the GH signaling pathways and their influence on renal tubular function and regulation of the renal sodium and water balance in other populations (Table 3).

Table 2. Summary of the genes and single nucleotide polymorphisms analyzed in *Paper III* and their importance in previous non-GHD studies.

Gene	dbSNP ID	MAF (%)	Minor Allele	Previous findings (reference)
PPARg3	rs10865710	20.8	G	Associated with increased height and plasma LDL-C in a French population (99). Also associated with colorectal cancer risk in Chinese population (100).
CETP	rs1800775	45	C	Associated with HDL-C response to GH replacement and modified by glucocorticoid treatment in GHD adults (101).
	rs708272	47.8	T	Associated with higher HDL levels and lower risk of MI in healthy women (103).
	rs3764261	36.7	T	Associated with HDL-C in type II Diabetes and aging population (103).
PCSK9	rs11206510	15	C	Associated with LDL-C and CAD (103).
LPL	rs1801177	1.7	A	N-allele associated with MI in type 2 diabetes (104). Higher remnant lipoproteins and lower a2 HDL particle levels in patients with type diabetes and MI (105).
	rs12678919	14.2	G	Associated with HDL-C (103).
	rs6993414	13.2	G	Associated with TG conc (103).
APOB	rs676210	19.2	A	Located in a domain involved in structural changes of apolipoprotein B during conversion of VLDL to LDL-C in general population (106).
	rs1042031	20.8	A	Located in a region known to regulate binding to the receptor (106)
	rs679899	46.6	A	Located in a crucial region for lipidation (106).
APOE E2	rs429358 and rs7412	3.9	T/T	ApoE associated with cardiovascular disease in postmenopausal women (107). Genotyping can be performed using SNPs rs429358 and rs7412. APOE E2 genotype = rs429358 (T) + rs7412 (T), APOE E3 = rs429358 (C) + rs7412 (T), APOE E4 = rs429358 (C) + rs7412 (C).
APOE E3		76.7	C/T	
APOE E4		19.4	C/C	
MR (NR3C2)	rs5522	11.7	G	Association with BMI and LDL-C levels in general population (108-109).
LDLR	rs1433099	30	A	Associations with baseline LDL-C and TG levels and CHD and CVD in aging population (110).
	rs2738466	20.7	G	
APOE-APOC cluster	rs35136575	25	G	Influences plasma Apo E (111) in Caucasian, African and Mexican Americans and LDL-C levels in type II diabetes and aging populations (103).
	rs4420638	18.3	G	
APOB	rs562338	22.5	T	SNP associated with LDL-C concentrations (103).

Apolipoprotein B gene, *APOB*; LDL receptor, *LDLR*; lipoprotein lipase, *LPL;* cholesteryl ester transfer protein, *CETP*; apolipoprotein E, *APOE*; apolipoprotein E/C1/C4/C2 gene cluster, *APOE*/C1/C4/C2; peroxisome proliferator-activated receptor gamma, *PPARG;* proprotein convertase subtilisin kexin type 9 *PCSK9;* and nuclear receptor subfamily 3, group C, member 2, *NR3C2*. MAF: minor allele frequency.

Table 3. Summary of the genes and single nucleotide polymorphisms analyzed in *Paper IV* and their importance in previous non-GHD studies.

Gene	dbSNP ID	MAF (%)	Minor Allele	Previous findings (reference)
GH-signaling pathway				
GHR	rs6873545	25.8	C	tagSNP for the GHR exon 3 deleted/full-length polymorphism in GHD adults (112).
PI3KCB	rs361072	42.5	G	Associated with HOMA IR dependent on BMI, the C-allele creates a GATA binding site capable of increasing transcription of PI3KCB in obese children (113). Also associated with IGF-I levels and longevity in long-lived people (114).
JAK2	rs7849191	50	C	Associated with higher central fat, % central fat and waist circumference in white female twin subject (115).
	rs3780378	47.5	T	Associated with higher serum apoA, TC, LDL-C and lower TG (115).
STAT5b	rs6503691	10.8	C	tagSNP, this SNP together with STAT3 SNP haplotype associated with breast cancer in German population (116).
SOCS2	rs11107116	20	T	Associated with peak height velocity during puberty in the northern Finland birth cohort (117).
Renal tubular function				
AGT	rs699	38.3	C	Associated with renal dysfunction and CVD in diabetic women (118).
STK39	rs6749447	27.5	G	Associated with BP in Amish and non-Amish populations (119).
	rs3754777	13.3	A	
WNK1	rs880054	44.0	G	Associations with mean 24-hour SBP and DBP in the general population (120).
	rs765250	34.7	G	
	rs1159744	25.8	C	Contributed to variations of BP response to thiazide in adults with essential hypertension (121).
SLC12A1	rs2291340	17.5	C	Associated with BP (122) in Japanese population; gene modulated by GH (123).
SLC12A3	rs11643718	15.8	A	Associated with primary hypertension (124), renal albumin loss, and diabetic nephropaty in young women (124).
SCNN1A	rs2228576	25.8	A	In vitro affected ENaC's surface expression and has been associated with hypertension in patients with type II diabetes with and without end-stage renal disease (125).
SCNN1G	rs5723	27.6	G	Predispose to hypertension susceptible to diuretic therapy in Chinese hamster ovary cells (126).
	rs5729	26.8	A	
	rs13331086	28.3	T	Associated with systolic blood pressure (SBP) in healthy Caucasians (127).
KCNJ1 (ROMK)	rs2846679	16	A	Associated with mean 24-hour SBP in the general Australian white population (128).
	rs2186832	20	C	Associated with mean 24-hour DBP (128, 129).
	rs675759	16.1	G	Associated with BP and left ventricular mass (128, 129).
CASR	rs1965357	15.0	C	Associated with urinary calcium excretion in African Americans (130).

Genes related to GH-signaling pathways: growth hormone receptor gene, *GHR*; Janus kinase 2, *JAK2;* signal transducer and activator of transcription 5B, *STAT5B;* phosphoinositide-3-kinase, catalytic, beta polypeptide, *PIK3CB*, suppressor of cytokine signaling 2, *SOCS2*;
Genes related to renal tubular function: angiotensinogen, *AGT;* sodium channel, non-voltage-gated 1 alpha subunit *SCNN1A*; sodium channel, non-voltage-gated 1 gamma subunit, *SCNN1G*; solute carrier family 12 (sodium/potassium/chloride transporters), member 1 *SLC12A1/NKCC2*; solute carrier family 12 (sodium/chloride transporters), member 3 (thiazide-sensitive sodium-chloride cotransporter) *SLC12A3*; potassium inwardly-rectifying channel, subfamily J, member 1, *KCNJ1(ROMK*); WNK lysine deficient protein kinase 1, *WNK1*; calcium-sensing receptor, *CASR*; serine threonine kinase 39 *STK39*.

Anthropometric methods

In all four papers, body weight (BW), body height, waist circumference (WC) and hip girth were measured in the morning. The BMI was calculated as BW in kilograms divided by height in meters squared (kg). Systolic blood pressure (SBP) and diastolic BP (DBP) were measured using the sphygmomanometric cuff method.

Biochemical methods

All laboratorial measurements were taken out at the Department of Clinical Chemistry at the Sahlgrenska University Hospital. Serum IGF-I levels were determined in serum samples collected following an overnight fast, using a hydrochloric acid-ethanol extraction RIA with authentic serum IGF-I for labelling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). From June 2004 to September 2006, these levels were determined using an automated chemiluminescent immunoassay (Advantage) from Nichols. Thereafter, serum IGF-I was determined using an automated chemiluminescent assay system (IMMULITE 2500®, Diagnostic Products Corp., Los Angeles, CA, USA). All assays had a detection limit $\leq$ 20 µg/l and an inter-assay CV $\leq$ 8.6%. The individual serum IGF-I levels were transformed into SD scores according to age– and sex–adjusted, population based reference values (131-132).
Serum insulin was measured using immunometric method with chemiluminescence technology (ADVIA Centaur; Bayer Diagnostics).
TC and triglyceride (TG) concentrations were determined using enzymatic methods (Roche Molecular Biochemicals) and expressed as mmol/l. HDL-C levels were determined after the precipitation of apoB-containing lipoproteins with $MgCl_2$ and heparin (133). LDL-C was calculated using Friedewald's formula adjusted to SI units (134).

Body composition

Total BF and LBM were assessed at baseline and after 6 and 12 months of GHRT using dual-energy X-ray absorptiometry (DXA) **(**Lunar DPX-L, Lunar Corporation, Madison, WI, USA). The precision errors (1 S.D.) of the scanner were 1.7% for BF and 0.7% for LBM, which were determined by double examinations of ten healthy subjects (135).
In Paper II, a four-compartment model (4-C) was used to study body composition (136). This model was based on the simultaneous determination of total body potassium, total body water and body weight. Fat-free extracellular solids, body cell mass, extracellular water and BF were calculated from these measurements. Total body potassium was assessed in a whole-body counter with a CV of 2.2%. Total body water was determined by the isotope dilution of titrated water with a CV of 3.2%.
ECW measurements were estimated using bioelectrical impedance analysis (BIA) at a frequency of 50 kHz (BIA-50kHz) (137). In a subset of patients, ECW was measured using sodium bromide dilution, which is the gold-standard method for this measurement.

Genetic methods

Genomic DNA was isolated from whole blood using the FlexiGene DNA kit (QIAGEN, Hilden, Germany). To determine the genotype in the GHR exon 3 locus, a multiplex polymerase chain reaction (PCR) with fragment detection by gel electrophoresis was performed using the primers G1, G2, and G3 (GenBank accession no. AF155912) as previously described (138). A second PCR using only the primers G1 and G3 was conducted whenever a perfect d3/d3 genotype or a d3/d3 with weak 935-bp amplicon (suspected d3/fl genotype) was detected (139).

In *Papers II, III and IV,* genomic DNA was isolated from whole blood using the FlexiGene DNA kit (QIAGEN, Hilden, Germany).

Tables 2 and 3 summarize the SNPs analyzed in *Papers III* and *IV* and their importance in previous non-GHD studies. *APOE* gene SNP rs429358, *SLC12A3* gene SNP rs11643718, *STK39* gene SNP rs3754777 and *GHR* gene SNP rs6873545 were genotyped using TaqMan SNP genotyping and the remaining SNPs with the Sequenom platform. The Sequenom genotyping was performed at the Mutation Analysis facility at Karolinska University Hospital using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Sequenom Inc., San Diego, CA, USA). iPLEX assays were designed using SpectroDESIGNER software (Sequenom Inc.).

Statistical methods

Paper I

Bivariate analyses (Pearson, chi square and independent samples test) were conducted to test the significance of the association between each predictor and the outcome variables. Multivariate logistic regression analyses were used to identify significant predictors of the GH response in each outcome variable (140). Many variables were tested, of which the most important were gender, age, BMI, total BF (kg), LBM (kg), insulin levels, peak GH during stimulation test, IGF-I levels, glucocorticoid replacement dose and GH starting dose. The results were presented as odds ratios (OR) with 95% confidence intervals. Two methods were used to measure accuracy: the Hosmer-Lemeshow goodness-of-fit test, which showed that the prediction models for IGF-I and BC responses were well fitted ($P > 0.05$); and the classification tables to show how well the models identified the GR and PR by assessing sensitivity, specificity and predictive value. Using the specified cut-off value of 0.5, the model categorized a subject as a GR if the estimated probability was 0.5 or more and a PR if the estimated probability was less than 0.5. All the values presented were two-tailed, and values of < 0.05 were considered indicative of statistical significance.

Paper II

Differences between genotype groups (Group 1: fl/fl-GHR vs. Group 2: d3/d3+d3/fl) were assessed using the Mann-Whitney *U* Test, chi square or Fisher's Exact Test, and the correlations were calculated in accordance with Pearson or Spearman, when appropriate. To determine the influence of the d3/fl-GHR genotype on the response of IGF-I and BF to GHRT, further mixed models (141) with repeat measurements in the same individual were used.

Paper III

The genetic association of individual SNPs to the lipid concentrations at baseline as well as to their changes after 12 months was analyzed with a two-sample *t*-test and ANCOVA, adjusting for significant covariates of sex, age and baseline BMI. While the lipids were slightly right skewed and therefore usually log transformed, this transformation was not performed since the ANCOVA had a better fit with untransformed data, enabling a more straightforward interpretation. Correction for multiple testing was done by permutation for the 88 tests related to changes in lipid concentrations.

Paper IV

The genetic association of individual SNPs to the ECW volumes at baseline as well as to their changes after 12 months was analyzed with ANCOVA, adjusting for significant covariates. The covariates that were analyzed included age, sex, height, weight, BMI, testosterone replacement (142), glucocorticoid, thyroxine and the dosage of the latter two, use of anti-hypertensive drugs, prior treatment for Cushing's disease and acromegaly. $P < 0.05$ was assumed to represent a significant difference. Correction for multiple testing was done using the Bonferroni method.

Methodological considerations

Considerations on body composition

There is no universal consensus as to the best way to measure body composition. DXA was used to measure BC in *Paper I* since this was the method that had been most widely used on patients throughout their treatment. Some researchers have voiced concern over the possibility that DXA could be affected by fluid changes in the lean soft tissue. This means that there are slight predictive errors in the calculation of lean soft tissue using DXA, and these can arise in the wake of fluid distribution changes (143-144). DXA might underestimate BF in patients with a large waist circumference because its main weakness is that the travel distance of the rays through the body is unknown. As the tissue is considered homogeneous, the result is not as accurate in areas such as the abdomen, which have several tissues.
Even though the sodium bromide is the gold standard for measuring ECW, it is invasive, time consuming and expensive, whereas the BIA is non-invasive, rapid and lower in cost (145). While BIA methods may not be considered as reliable as the standard, especially for patients such as GHD adults, who do not have normal ECW/TBW ratios, in our study a comparison was made between the standard data and the BIA data of the 67 patients whose bromide data were available and both methods proved to be equally reliable.

Considerations on genetic methods

Two methods were used for the genotyping of the SNPs in *Papers III and IV*: TaqMan and Sequenom. The TaqMan genotyping method is excellent for analysing single SNPs. In that context it is fast, reliable and straightforward. Assays can be ordered pre-designed, and despite the expense, it is now commonly accessible to most research groups focusing on genetics. In this specific situation however, when analysing more than 20 SNPs for a single project, TaqMan SNP genotyping is not the best option, particularly due to reagent costs, DNA template use and time consumption (since SNPs are analysed separately). Therefore, the Sequenom platform is a better choice because it allows simultaneous analysis of over 20 SNPs, thereby making it more cost effective, faster and requiring less starting material (DNA). The equipment for this method is highly sensitive and expensive, but core facilities such as the mutation analysis facility at the Karolinska University Hospital make it accessible to the public. However, it is worth mentioning that due to the nature of the Sequenom genotyping, where the primers are designed to fit into a pool and allow for the SNPs to be genotyped based on the mass of the extended PCR product, sometimes some primer design fails and the corresponding SNP has to be genotyped using another method or in a separate pool, which is expensive. When the SNPs were not suitable for Sequenom, they were run separately using TaqMan SNP genotyping.
In *Paper IV*, for the genotyping of the GHR exon 3 deleted/full-length (d3/fl) polymorphism we decided to use the tagSNP rs6873545, known as a marker thereof (146). The main reason for this is that the tagSNP allows for genotyping using TaqMan SNP genotyping, which is more suitable when working with larger cohorts. It is also less time-consuming and requires a smaller amount of DNA than the conventional multiplex PCR with fragment detection by gel electrophoresis (138). A previous study involving the same population showed that use of the tagSNP is as accurate as the original genotyping method (112).

Considerations of statistical methods

In *Paper III*, two-sample *t* tests and ANCOVA were used to analyze the genetic association of individual SNPs with lipid concentrations at baseline and changes after 12 months. A log transformation is better in the case of the *t* test, but for the ANCOVA untransformed data are preferred. For this reason, the untransformed data were maintained as this type of data is more easily

interpreted and does not heighten the risk of false positives in the *t* test as the normal approximation of the means would serve in any case with a sample of the size under study.

It is also important to point out that it was deemed more statistically reliable to present the homozygotes and heterozygotes in two genetic groups rather than three due to the small number of homozygotes for the minor allele in ten of the twenty SNPs. This is acceptable because the dominant, additive and recessive genetic models all had the same results.

Correction for multiple testing was conducted in both papers, with permutation analysis being used in *Paper III* and the Bonferroni method being used in *Paper IV*. In neither case did any SNP remain significant following adjustment. However, it should be taken into account that these methods are considered somewhat harsh and findings cannot be summarily invalidated due to a loss of significance after adjustment in a candidate gene approach.

Main Results

Prediction models for GHRT response in adult GHD patients (Paper I)

Definition of GR and PR to GHRT

The 60th and 40th percentile cut-off values were used to define GR and PR, respectively. After 12 months of GHRT, the mean serum IGF-I response was 1.2 μg/l per mg of GH. Fifty-two men and 15 women, with a median age of 49.1 years, were classified as GR in terms of serum IGF-I response. GR had a serum IGF-I response > 1.33, while that of PR was < 0.98. The PRs were 26 men and 41 women, with a median age of 51.2 years. The mean change in LBM was 2.4 kg, and the cut-off value for GR was > 2.79 kg, and for PR was < 1.71 kg. For BF, the mean change was a decrease of 2.3 kg, with a reduction > 2.74 kg characterizing GR and a reduction < 1.34 kg or increase characterizing PR. For BC, taking into account both LBM and BF, 35 patients (31 men and 4 women, median age 50) were classified as GR.

Prediction model for serum IGF-I response

At baseline, the height, BW and LBM of GR were higher than those of the PR. At the end of the 12-month treatment, GR were receiving lower daily GH and cumulative GH doses than PR. However, these differences were caused by the increased prevalence of men among the GR. The logistic regression (LR) model found gender and baseline serum insulin levels to be predictors of IGF-I response (Table 4). Gender was a highly significant factor, as men were 5.6 times more likely to be GR than women. Moreover, it was more likely for patients with higher insulin levels to be GR than patients with lower insulin levels, despite the fact that there was no difference between the GR and PR in terms of mean insulin levels. The logistic regression resulted in the equation shown below, which can be used to estimate serum IGF-I response to 12 months of GHRT. The accuracy of the model to predict whether a patient will be a GR or PR was approximately 70%. Using the specified cut-off value of 0.5, the sensitivity of the prediction model, i.e. the correctly predicted percentage of patients in the GR category, was 77.6%. The specificity of the prediction, i.e. the correctly predicted percentage of patients in the PR category, was 59.7%. The result of the LR was the following aforementioned equation, which can be used to estimate the probability of being GR in serum IGF-I response to 12 months of GHRT.

$$P(GR\_IGF-I)=\frac{1}{1+(e)^{-(-1.60+1.73.Gender+0.06.insulinlevels)}}$$

Where P (GR_IGF-I) is the predicted probability of being a GR in IGF-I.
Gender: men = 1 and women = 0; Serum insulin levels at baseline (mU/L); "*e"* is 2.718.
GR if estimated probability P (GR_IGF-I) is ≥ 0.5, and PR if P (GR_IGF-I) < 0.5.

Table 4. Significant baseline predictors for serum IGF-I, LBM and BF responses (changes) to 12-month GH replacement therapy using logistic regression analyses.

Predictors	Regression coefficient	Odds Ratio (95% CI)	*P* value	Goodness-of-fit test
IGF-I response				
Constant (β)	– 1.60			0.674
Gender	1.73	5.62 (2.59, 12.18)	< 0.0001	
Basal Insulin (mU/L)	0.06	1.06 (1.00, 1.12)	0.049	
LBM response				
Constant (β)	5.98			0.726
Gender	2.46	11.67 (3.94, 34.55)	< 0.0001	
Basal BMI (kg/m^2)	– 0.44	0.65 (0.52, 0.80)	< 0.0001	
Basal BF (kg)	0.15	1.17 (1.05, 1.29)	0.003	
BF response				
Constant (β)	– 6.08			0.738
Height (cm)	0.04	1.04 (1.00, 1.07)	0.05	
Body composition response (LBM + BF)				
Constant (β)	– 28.08			0.702
Gender	2.37	10.72 (1.36, 84.18)	0.024	
Height (cm)	0.21	1.23 (1.09, 1.40)	0.001	
Basal LBM (kg)	– 0.20	0.82 (0.73, 0.92)	0.001	

An odds ratio >1 indicates an increased probability of being GR. CI, confidence interval. The Goodness–of–fit test (Hosmer & Lemeshow) showed that the prediction models for IGF-Iand Body composition (LBM + BF) responses were well fitted ($P > 0.05$).

Prediction model for BC response

Gender was also a highly significant factor that impacted changes in BC. Out of 16 women, 4 were GR and 12 were PR, while out of 49 men, 31 were GR and 18 were PR. At baseline, the GR were taller, their BMI was lower and their insulin levels were lower than that of PR. Furthermore, at the end of the study, the GR were taking a higher cumulative dose of GH than the PR. Nevertheless, the daily GH dose after 12 months was similar. Logistic regression showed that gender, body height and LBM at baseline contributed significantly to the variations in BC (Table 4). Men, taller patients and those with lower LBM were much more likely to be GR. The logistic regression resulted in the equation shown below, which can be used to predict whether a patient will be a GR to GHRT in terms of BC. The model is approximately 80% accurate. Using the specified cut-off value of 0.5, the sensitivity of the prediction was 88.6% and the specificity was 70%. The result of the LR was the following equation, which can be used to estimate BF response to 12 months of GHRT.

$$P(GR\_BC) = \frac{1}{1 + (e)^{-(-28.08 + 0.21.Height - 0.20.basalLBM + 2.37.Gender)}}$$

Where P (GR_BC) is the predicted probability of being a GR in BC;
Gender: men = 1 and women = 0; height (cm); LBM at baseline (kg); "*e"* is 2.718.
GR if estimated probability P (GR_BC) ≥ 0.5, and PR if P (GR_BC) < 0.5.

Genetic studies in adult GHD patients before and after 12 months of GHRT

Influence of the exon 3-deleted/full-length GH receptor polymorphism (*Paper II*)

Approximately 57% of the originally assigned d3/d3 homozygotes proved to be d3/fl heterozygotes after the second PCR was conducted, demonstrating that this second PCR was indeed necessary.
Of the 124 GHD patients, 72 (58%) had two wild-type alleles (fl/fl-GHR; Group 1) and 52 (42%) had at least one d3-GHR allele (Group 2). In this group, 40 (32%) patients had one d3-GHR allele (d3/fl-GHR) and 12 (10%) had two GHR-alleles (d3/d3-GHR). Prior to treatment, there were no significant differences in the clinical characteristics of the two groups. Men and women were analyzed separately, and no gender difference was found in the prevalence of GHR genotypes. After 12 months, the men in Group 1 were on a lower daily dose of GH than those in Group 2 (median dose 0.3 (range 0.1, 0.7) vs. 0.4 (0.3, 0.8), respectively, $p = 0.03$), but with no difference in GH cumulative dose. No significant difference was found between the two groups in terms of changes in IGF-I levels and body fat after 12 months, indicating no influence of GHR polymorphisms in the variability of GHRT response.

Influence of SNPs in genes related to lipid metabolism on serum lipid profile (*Paper III*)

Genotyping for *Paper III and IV* was conducted simultaneously. Every genotyping assay had a success rate > 94.9%. Furthermore, the re-genotyping of 27% of the study samples resulted in 99.9% concordance following the Sequenom run. No Mendelian errors were detected in the 14 HapMap families and concordance analyses with the HapMap data showed concordance rates of 100% for almost all of the analysed SNPs available in HapMap, although the concordance rate of those that did not fully comply was at least 90%. Minor allele frequencies (MAF), genotype distributions and concordance with the Hardy-Weinberg equilibrium (HWE) of the 20 SNPs are shown in Table 5.

TABLE 5. Summary of the allele frequencies, genotype distributions and Hardy-Weinberg equilibrium (HWE) of single nucleotide polymorphisms (SNPs) in genes related to lipid metabolism

Gene	SNP	Major (M)	Minor (m)	MAF (m)	MM	Mm	mm	missing	HWE*p*	[1]d
APOB	rs693	C	T	0.465	92	149	70	7	0.808	0.35
APOB	rs562338	C	T	0.147	219	91	0	8	0.010	0.35
APOB	rs676210	G	A	0.214	198	101	17	2	0.688	0.33
APOB	rs679899	G	A	0.477	86	152	72	8	0.954	0.36
APOB	rs1042031	G	A	0.204	201	95	16	6	0.562	0.33
LDLR	rs1433099	G	A	0.267	174	114	27	3	0.415	0.32
LDLR	rs2738466	A	G	0.240	180	114	18	6	1.000	0.32
LPL	rs1801177	G	A	0.018	299	11	0	8	0.951	0.86
LPL	rs6993414	A	G	0.113	251	62	5	0	0.875	0.39
LPL	rs12678919	A	G	0.096	255	52	4	7	0.772	0.41
CETP	rs708272[2]	G	A	0.401	119	142	56	1	0.488	0.33
CETP	rs1800775[2]	C	A	0.441	105	143	68	2	0.342	0.34
CETP	rs3764261	G	T	0.307	160	121	37	0	0.173	0.32
APOE	rs7412[3]	C	T	0.068	270	36	3	9	0.369	0.48
APOE	rs429358[3]	T	C	0.169	213	99	4	2	0.129	0.34
APOE/C[4]	rs4420638	A	G	0.199	200	106	10	2	0.666	0.33
APOE/C[4]	rs35136575	C	G	0.260	175	110	26	7	0.353	0.32
PPARG	rs10865710	C	G	0.235	177	122	12	7	0.269	0.32
PCSK9	rs11206510	T	C	0.165	219	83	10	6	0.827	0.35
NR3C2	rs5522	A	G	0.120	244	70	3	1	0.710	0.37

MAF, minor allele frequence. **MM**, homozygote for the common (major) allele (two major alleles). **Mm**, heterozygote (one major allele, and one minor allele). **mm**, homozygote for the rare (minor) allele (two minor alleles). [1]**d,** the standardized difference detectable with 80% power at the 5% significance level for each SNP. [2]The former abbreviations for *CETP* SNP rs708272 is *Taq*IB polymorphism and for *CETP* SNP rs1800775 is -629A/C. [3]The polymorphisms, rs429358 and rs7412, in the *APOE* gene together define the APOE ε2, ε3 and ε4 alleles. [4]*APOE/C* is an abbreviation for *APOE/C1/C4/C2* gene cluster.

Genotype and baseline lipid profile

Table 6 summarizes the SNPs significantly associated with serum lipid concentrations at baseline. All associations between SNPs and baseline lipid concentrations remained significant when adjusted for sex, age and BMI and the use of glucocorticoid and levothyroxine replacement.

TABLE 6. Single nucleotide polymorphisms (SNPs) found to be associated with lipid concentrations at baseline in GHD adults carriers of two major alleles (MM) vs. carriers of at least one minor allele (Mm and mm)

SNPs	**Study parameters**	**MM (n)**	**Mm & mm (n)**	***P* §**	***P* adj***
CETP (rs708272)		GG (119)	GA and AA (198)		
	Basal TC	5.48±1.12	5.86±1.28	0.009	0.006
	Basal HDL-C	1.19±0.40	1.31±0.41	0.01	0.005
CETP (rs1800775)		CC (105)	CA and AA (211)		
	Basal TC	5.45±1.13	5.84±1.26	0.008	0.005
	Basal HDL-C	1.19±0.40	1.31±0.41	0.01	0.01
	Basal LDL-C	3.51±1.07	3.79±1.14	0.04	0.03
CETP (rs3764261)		GG (160)	GT and TT (158)		
	Basal HDL-C	1.18±0.38	1.35±0.43	0.0004	0.0004
APOB (rs693)		CC (92)	CT and TT (219)		
	Basal HDL-C	1.18±0.35	1.30±0.43	0.03	0.02
APOE (rs7412)		CC (270)	CT and TT (39)		
	Basal TC	5.82±1.22	5.00±1.02	0.0001	0.0002
	Basal LDL-C	3.78±1.12	3.08±0.88	0.0003	0.0004
PPARG (rs10865710)		CC (177)	CG and GG (134)		
	Basal LDL-C	3.81±1.13	3.51±1.06	0.02	0.02
APOE/C (rs35136575)		CC (175)	CG and GG (136)		
	Basal TG	1.88±1.29	1.58±0.79	0.02	0.007

Lipid concentrations are presented as mean ± SD. TC, total cholesterol (mmol/l). LDL-C, low-density lipoprotein cholesterol (mmol/l). TG, triglycerides (mmol/l). HDL, high-density lipoprotein cholesterol (mmol/l). *APOE/C, APOE/C1/C4/C2* gene cluster. §*P*-value for an independent *t*-test between the genotype groups. ***P*-value indicating association in ANCOVA.

Genotype and GH-induced changes on serum lipid concentrations

Table 7 summarizes the SNPs that showed a statistically significant association with changes in plasma lipid concentrations in response to GHRT. All associations remained significant when adjusted for age, sex and BMI and the use of glucocorticoid and levothyroxine replacement. Additional analyses excluding patients with lipid-lowering drugs (n = 28), patients with previous Cushing's disease (n = 20) and acromegaly (n = 12) did not alter these results.

TABLE 7. Single nucleotide polymorphisms (SNPs) found to be associated with changes in plasma lipid concentrations in response to 1-year GH therapy in GHD adults carriers of two major alleles (MM) vs. carriers of at least one minor allele (Mm and mm)

SNPs	Response	MM	Mm & mm	[1]*P*	[2]*D*	[3]P_{adj}	[4]P_{corr}
APOB rs676210		GG	GA & AA				
	ΔTC	-0.34 ± 0.89	-0.06 ± 0.72	0.003	0.36	0.004	0.24
	ΔLDL-C	-0.40 ± 0.75	-0.19 ± 0.66	0.016	0.19	0.024	0.78
PPARG rs10865710		CC	CG &GG				
	ΔTC	-0.33 ± 0.87	-0.01 ± 0.81	0.02	0.32	0.010	0.49

Changes in lipid concentrations are presented as mean ± SD. TC, total cholesterol (mmol/l). LDL-C, low-density lipoprotein cholesterol (mmol/l). HDL, high-density lipoprotein cholesterol (mmol/l). [1]*P*-value for an independent *t*-test between the genotype groups.
[2]Estimated difference between genotype groups in ANCOVA.
[3]*P*-value indicating association in ANCOVA.
[4]*P*-value for ANCOVA corrected for 88 tests by permutation.

After GHRT, there was a greater reduction in TC concentrations in homozygous G-allele carriers of the *APOB* SNP rs676210 and homozygous C-allele carriers of *PPARG* SNP rs10865710. The reduction in LDL-C concentrations was greater in homozygous G-allele carriers of the *APOB* SNP rs676210 (Figure 1 A, B, C). Together, SNPs rs676210 in the *APOB* gene and rs10865710 in the *PPARG* gene explained 5% of the variance in the TC concentrations after 1 year of GHRT. These associations were not significant when corrected for multiple testing.

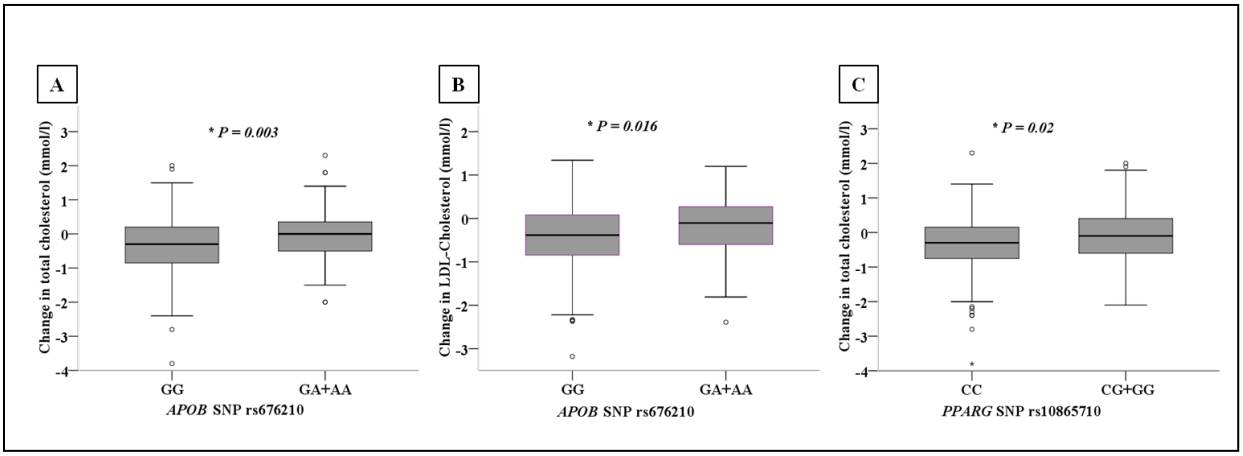

Figure 1. Individual changes in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in response to GH replacement according to SNP rs676210 in the *APOB* gene (A, *P=0.003 and B, *P=0.016) and changes in TC according to SNP rs10865710 in the *PPARG* gene (C, *P=0.02).

Influence of SNPs in genes related to the GH signaling pathways and renal tubular function on ECW (*Paper IV*)

Comparison between ECW measurements using bioelectrical impedance analysis and sodium bromide dilution methods

We compared the ECW volumes of 64 GHD adults calculated using BIA-50 kHz with the ECW volumes obtained using sodium bromide dilution, which is the gold-standard method for this measurement. The BIA-50kHz method proved to be as accurate as the sodium bromide dilution in this case. The correlation coefficient was 0.9 ($p < 0.0001$) (unpublished data).

Genotype and variation of ECW volume in adults with GH deficiency

The minor allele frequencies (MAF), genotype distributions and concordance with the Hardy-Weinberg equilibrium (HWE) of 6 SNPs in genes related to the GH signaling pathways and 15 in genes related to renal tubular function in 311 patients are shown in Table 8.

Table 8. Minor allele frequencies, genotype distributions and concordance with Hardy-Weinberg equilibrium of the 21 SNPs

Gene	SNP	Major(A)	Minor(B)	MAF(B)	AA	AB	BB	missing	HWEp	d
GHR	rs6873545	T	C	0.24	180	107	20	4	0.75	0.33
JAK2	rs3780378	T	C	0.48	73	169	61	8	0.12	0.38
JAK2	rs7849191	C	T	0.37	122	140	42	7	0.98	0.33
STAT5B	rs6503691	C	T	0.12	241	63	7	0	0.51	0.38
PIK3CB	rs361072	A	G	0.40	107	156	47	1	0.72	0.34
SOCS2	rs11107116	G	T	0.27	167	118	23	3	0.94	0.32
AGT	rs699	A	G	0.44	101	144	64	2	0.63	0.34
SCNN1A	rs2228576	C	T	0.37	120	144	41	6	0.98	0.33
SCNN1G	rs5723	C	G	0.21	193	104	12	2	0.91	0.33
SCNN1G	rs5729	T	A	0.21	193	105	13	0	0.96	0.33
SCNN1G	rs13331086	T	G	0.22	185	105	13	8	0.93	0.33
SLC12A1	rs2291340	T	C	0.19	205	93	13	0	0.84	0.34
SLC12A3	rs11643718	G	A	0.09	256	51	2	2	0.95	0.42
KCNJ1	rs675759	G	C	0.17	220	79	12	0	0.36	0.35
KCNJ1	rs2186832	C	G	0.19	207	86	17	1	0.15	0.34
STK39	rs3754777	C	T	0.15	221	82	5	3	0.70	0.36
STK39	rs6749447	T	G	0.24	168	124	12	7	0.17	0.32
WNK1	rs880054	T	C	0.38	124	137	48	2	0.61	0.33
WNK1	rs765250	T	C	0.24	179	112	20	0	0.91	0.32
WNK1	rs1159744	G	C	0.18	205	86	13	7	0.59	0.34
CASR	rs1965357	T	C	0.12	235	65	5	6	0.98	0.38

MAF, minor allele frequency. **MM**, homozygote for the common (major) allele (two major alleles). **Mm**, heterozygote (one major allele, and one minor allele). **mm**, homozygote for the rare (minor) allele (two minor alleles). **HWE*p*,** *p*-value for Hardy-Weinberg equilibrium. **d,** the standardized difference detectable with each SNP needed to have 80% power at the 5% significance level.

Table 9 summarizes the SNPs that were significantly associated with ECW volumes at baseline. After adjustment for the significant covariates, sex and BMI, *PIK3CB* SNP rs361072, *STAT5B* SNP rs6503691 and *SLC12A1* SNP rs2291340 were associated with ECW volume at baseline.
The ECW volume of the minor allele carriers of the SNPs in *PIK3CB* and *STAT5B* genes was 0.6 liters higher than that of the major allele homozygotes.
The ECW volume of the major allele homozygotes of SNP rs2291340 in the *SLC12A1* gene was 0.5 liters higher than that of the major allele carriers.
No association remained significant when corrected for multiple testing (Bonferroni correction).
After 12 months of GHRT, the significant covariates were sex, BMI and GH dose. No single SNP influenced the ECW changes observed after GHRT.

TABLE 9. Single nucleotide polymorphisms (SNPs) found to be associated with extracellular water (ECW) volumes at baseline in 311 growth hormone deficient (GHD) adults carriers of two major alleles (MM) vs. carriers of at least one minor allele (Mm and mm)

Gene	**SNPs**	**MM (n)**	**Mm & mm (n)**	***Adjusted estimate***	***Adjusted *P*-value**
PIK3CB	rs361072	AA (107)	AG and GG (203)		
		17.27 ± 4.06	18.00 ± 4.31	0.604	0.02
STAT5B	rs6503691	CC (241)	CT and TT (70)		
		17.64 ± 4.21	18.07 ± 4.30	0.610	0.04
SLC12A1	rs2291340	TT (205)	TC and CC (106)		
		18.01 ± 4.42	17.22 ± 3.80	-0.546	0.039

Mean values and SD of ECW volumes expressed in liters. The adjusted estimate is the estimated difference between genotype groups in ANCOVA, after adjustment for sex and BMI.
*****P*-value indicating association in ANCOVA, after adjustment for sex and BMI.

Discussion

We studied a large cohort of well-characterized adults with severe GHD who were treated with GH for 12 months at a single center. Mathematical models to predict GH responsiveness to GHRT in GHD adults were developed using gender and serum insulin levels as the major clinical predictors for good response in serum IGF-I, and gender, body height, and baseline LBM, for good response in BC. The presence of the d3-GHR allele did not influence the IGF-I and BF responses to GHRT in GHD adults. Multiple SNPs in genes related to lipid metabolism were associated with individual differences in the serum lipid concentrations of untreated GHD adults. Moreover, polymorphisms in the *APOB* and *PPARG* had a slight, but significant, influence on the changes in serum lipid profile after GHRT. The *STAT5B, PIK3CB* and *SLC12A1* polymorphisms were associated with the interindividual variability in the ECW volume of untreated GHD adults.

The concept that genetic variation contributes to variability in disease phenotypes and drug responses is widely accepted. The response to GHRT is clearly multifactorial. In fact, the present study is a quantitative genetic study of a trait (the response to GHRT). Therefore, the analysis should take into account the risk of false negativity. There are a number of features in adult patients with hypopituitarism and severe GHD before and after GHRT that could increase the risk of false negativity. First, adult GHD is somewhat heterogeneous in terms of etiology, age of onset, other anterior pituitary hormone deficits and severity. Second, the improvement achieved in the adult GHD abnormalities under GHRT is partly influenced by GH dose, injection frequency and the duration of therapy. The plasma pattern of GH, including pulse frequency and basal and total integrated levels, is also influenced by several factors, such as gender, age and adiposity (147-148). Therefore, the pattern of GH delivery and exposure may have an impact on the biological effects of the treatment. The importance of the mode of GH administration is supported by a number of animal studies demonstrating that the pattern of GH exposure has different effects on growth and metabolism (148). Third, current management is based on daily sc injections before bedtime that do not reproduce the physiological pattern of GH secretion and, furthermore, is often felt by the patient to be cumbersome, and may therefore affect adherence to treatment. Taken together, all these factors only add to the difficulties involved in finding associations with specific genotypes and contribute to any false negativity. On the other hand, false positive findings are more likely to occur with small sample sizes, and phenotypic variations tend to be overestimated in quantitative trait studies (149). However, large-scale studies in well established conditions and with homogeneity of phenotypes are not easily accomplished in patients with hypopituitarism. Despite these problems, the strength of our study lies in the fact that the investigation was performed in a well-characterized cohort of adult GHD patients who were monitored in a single medical center with a uniform therapeutic approach using GH dose titration and the likelihood that those with poor compliance would be excluded from the study group. We assumed in all our models that the patients had homogenous drug exposure and that we should search for tissue related factors that might explain the variability in therapeutic response.

Our research project was initiated with the aim of developing mathematical models to predict GR and PR to GHRT based on clinical factors responsible for individual variability in response to therapy. For clinical purposes, prediction models can be helpful by selecting patients who benefit most from any given therapy. GHRT is a long-term, fairly expensive treatment, with daily sc injections. The approach adopted for the treatment of GHD adults varies from country to country for a number of reasons. There are also many different outcomes to be examined in GHD adults and, for an individual patient, the decision to initiate GHRT should take into account a wide spectrum of variables (150).

Although no single parameter can clearly distinguish GR from PR and any definition to this end is arbitrary, we decided to adopt IGF-I and BF as the investigational endpoints because they were considered the best biomarkers to reflect a direct GH effect. In our patients, response to GHRT followed a Gaussian curve. We did not use the median response as the cut-off to distinguish GR and PR because the results around the median were very similar. The best mathematical models were those

with cut-offs at the 60th and 40th percentiles, leaving a good size sample with significant statistical power.

Using IGF-I and BC as the final endpoints, we confirmed the findings of previous studies in the literature that demonstrate the importance of gender in the response to GHRT (23, 18, 82, 83, 151). Both *in vivo* and *in vitro* models have shown that hepatic gene expression after GH administration is sexually dimorphic (152). There is a sex-related difference in the metabolic responses to pulsatile GH exposure. Men have a higher lipolytic rate in response to the pulsatile GH injection than women, which is confirmed by a more significant reduction in intra-abdominal fat in GHD men in response to GHRT (23, 153, 154). In addition, male patients exhibit the most marked increase in LBM in response to GHRT (151, 155). The mechanism underlying the greater GH responsiveness in men is the synergistic effect of testosterone on GH actions, enhancing the lipolytic action of GH (156) and the effect of GH on serum IGF-I (157). We also found serum insulin levels at baseline to be a predictor for IGF-I response. It was more likely for patients with higher insulin levels to be GR than patients with lower insulin levels. One possible explanation for this finding is the effect of insulin up-regulating the surface availability of the GHR in human liver cells (158). In our study, the logistic regression model revealed that men and taller GHD patients with lower LBM at baseline were more likely to be GR in BC changes. Although height and LBM contributed significantly to this prediction model, it is possible that gender had an influence on the selection of these variables as predictors since the proportion of men was twice as high as the proportion of women in the GR group (159-161)
The predicted accuracy of the mathematical models we developed to distinguish between GR and PR to GHRT based on gender, insulin levels, height and lean body mass (LBM) at baseline was 70% for IGF-I and 80% for BC. Further prospective, multicenter studies, involving a large number of GHD adult patients, would be desirable in order to confirm the utility of our prediction models.

In addition to clinical parameters, our research project continued to search for genetic factors that might also impact the response to GHRT. The d3 allele of the GHR gene was the first candidate to be studied due to its key role in GH actions. The d3GHR polymorphism has been linked to increased receptor activity as a result of enhanced signal transduction (86) and has been shown to influence growth velocity in GHD children (87-88) as well as in other groups of short children (86, 89, 162, 163). Van der Klaauw et al. (164) conducted a similar study using serum IGF-I response and lipid profile as end-points in a Dutch cohort of 99 adult GHD patients. They found a higher increase in serum IGF-I levels in patients bearing at least one d3-GHR allele after 12 months of GHRT, although this effect was not maintained after 5 years. In contrast, we did not find any association between the GHR genotypes and the IGF-I response. Of three additional studies conducted in different European countries, two did not succeed in demonstrating an association between GHR polymorphisms and IGF-I response (165-166) and one showed a significant relationship of the d3/d3 on IGF-I response to GHRT (167). Other end-points have been investigated, such as body composition, lipid profile, quality of life and GH dose (165-168) (Table 10). Adetunji *et al.* (165) and Giavoli *et al.* (166) also included BF as an end-point, showing that the d3-GHR allele did not contribute to the variability of response to GHRT, which is in agreement with our results. In our study, male carriers of the d3-GHR allele required a higher daily dose of GH than men with fl/fl to attain the same IGF-I level. Adetunji et al (165) found that the polymorphism had no influence on the GH dose, although Meyer et al. (168) found that d3-GHR carriers require approximately 25% less GH than fl carriers.

Table 10. Overview of publications on the pharmacogenetic effects of GH in relation to d3GHR genotype on the different clinical endpoints in response to GH treatment in adults with growth hormone deficiency (GHD)

	van der Klaauw et al., 2008	**Barbosa et al., 2009**	**Adetunji et al., 2009**	**Meyer et al., 2009**	**Giavoli et al., 2010**	**Moyes et al., 2010**
No. of patients	99 (1yr)/ 53 (5yrs)	124	131	133	100 (1yr)/ 50 (5yrs)	194
Gender Males (%)	43 (43.4%)	79 (63.7%)	71 (54%)	66 (49.6%)	62 (62%)	81 (41.8%)
Mean age (yrs)	51	50	50	45.4	46	
Follow-up after GHRT	1yr/5yrs	1yr	1yr	1yr	1yr/5yrs	6 mo/1 yr
fl/fl	55 (56%)	72 (58%)	72 (55%)		48 (48%)	101 (52%)
fl/d3	38 (38%)	40 (32%)	51 (39%)		45 (45%)	75 (38.7%)
d3/d3	6 (6%)	12 (10%)	8 (6%)		7 (7%)	18 (9.3%)
Significant effect of d3GHR polymorphism on the End Points						
IGF1 levels	↑ IGF1 (1yr)	No effect	No effect	No effect	No effect	↑Δ IGF1(1 yr), homozygous d3
Lipid profile	↓TC, ↓LDL-C, ↑ HDL-C (1yr)	NA	NA		↑ HDL-C (1yr), but ↓TC, ↓LDL-C (5yrs)	No effect
Anthropometric parameters	No effect	NA	NA		NA	NA
Bone mineral density	No effect	NA	NA		NA	NA
Body fat (BF)	NA	No effect	No effect		No effect	NA
Quality of life	NA	NA	No effect		NA	No effect
GH dose	NA	NA	No effect	Lower GH dose	NA	NA
BMI	NA	NA	NA		No effect	NA
Glucose homeostasis	NA	NA	NA		↑fasting glucose (1yr/ 5yrs)	NA
WHR	NA	NA	NA		NA	No effect

NA: not applicable.

In our second genetic study, we used the candidate gene approach to investigate the influence of 20 SNPs located in 9 genes on the serum lipid profile of GHD adults and their changes after GHRT. These SNPs were selected based on their role in lipid metabolism and their previous associations with serum lipids in other large cohorts of individuals (99, 103-104, 106-108, 110-111).

GHD adults exhibit an abnormal lipid profile characterized by increased TC and LDL-C, increased apolipoprotein B and, according to some reports, increased TG and reduced HDL-C concentrations (60). The GH/IGF-I interacts with the LDL receptor and hepatic enzymes involved in lipoprotein metabolism. In hypophysectomized rats and animal models, the cholesterol metabolism in the liver is reduced, as well as the LDL mRNA and LDL receptor number. The GH has been shown to have an important role in the regulation of hepatic LDL receptor expression and plasma lipoprotein levels (58, 59). The GH increases lipolysis in adipose tissue and stimulates the synthesis and secretion of VLDL triglycerides by the liver (169, 170, 171). It also stimulates the pos-transcriptional editing of apo B mRNA in rat liver (172), a regulatory action that will enrich the TG-rich VLDL with the truncated form of apoB, apoB48, and hence drive these lipoproteins into a more rapid catabolic pathway (173). GHRT in GHD adults has been associated with reduced TC and LDL-C. However, as observed with other end-points of GHRT, there is considerable individual variability in the treatment response . A meta-analysis of placebo-controlled studies showed that GHRT was associated with a mean reduction of -0.3 ± 0.3 mmol/L in TC and -0.5 ± 0.3 mmol/L in LDL-C (60). Conversely, HDL-C concentrations have been reported to have increased (60), remain unchanged (174) or decrease (175) following GHRT.

We found that GHD adult carriers of G allele of the *PPARG* gene had lower serum LDL-C levels. In a French population-based study, carriers of the G allele were taller and had increased plasma LDL-C concentrations (99), which were explained by the modulation of the *PPARG3* gene transcription by the STAT5B pathway. The *PPARG* gene regulates adipocyte differentiation and insulin sensitization and influences lipid metabolism by its activation through the STAT5B pathway. An *in vitro* study (99) demonstrated that the GH/STAT5B pathway could activate the promoter of the *PPARG*3 variant in 3T3-L1 cells, whereas the *PPARG* SNP rs10865710 prevented this activation by abolishing the binding of STAT5B to this promoter. These findings might explain that in a state of GHD, such as in our patients, G-allele carriers presented lower levels of LDL-C.

After GHRT, our G-allele carrier patients showed a lower reduction in TC and LDL-C levels in comparison with homozygous C carriers. Natural or synthetic PPARG ligands have been shown to inhibit STAT activity in a PPARG-dependent manner (176-177). It is possible that the polymorphism in the PPARG gene modulates STAT signaling and potentially interferes with GH effects.

In our study, homozygous G carriers of SNP rs676210 in the *APOB* gene were found to have the greatest reductions in TC and LDL-C concentrations in response to GHRT. Although the reasons for this finding are not completely understood, we speculated that this polymorphism may promote structural changes in apolipoprotein B, affecting the conversion of VLDL to LDL (178 - 180). This alteration would result in a reduction in the number of VLDL particles and/or affect VLDL surface properties. Another possibility is increased LDL clearance by the LDL-receptor (106) in patients with this genotype

Seven SNPs located in five genes, the *CETP*, *APOE*, *APOB*, *APOE/C1/C4/C2* cluster and *PPARG*, turned out to have an effect on lipid concentrations in GHD adults following a similar pattern as that described in other reports (103, 106, 111, 181-183,). In our study, all the carriers of the minor alleles of the three SNPs in the *CETP* gene (rs1800775, rs708272 and rs3764261) had higher serum HDL-C concentrations, in agreement with studies in other populations (102, 105, 181-182). Moreover, higher TC levels were associated with minor alleles of SNPs rs1800775 and rs708272 and higher LDL-C levels with SNP rs1800775. The *CETP* gene, encoding the cholesteryl ester transfer protein, enables the transfer of cholesteryl ester in plasma from HDL towards TG-rich lipoproteins (116). In general, the genetic deficiency of the CETP protein is associated with very high HDL-C levels and SNP rs1800775 in the *CETP* gene has been shown to regulate CETP transcriptional activity *in vitro* (184). SNP rs708272 and SNP rs1800775 have also been associated with decreased TG concentrations and a negligible impact on LDL-C and apolipoprotein B (185), while SNP rs3764261 was also found to be associated with LDL-C levels (182). In a population of GHD adults, Dullart et al. (101) observed that SNP 1800775 was associated with HDL-C response to GHRT only in patients treated with glucocorticoids, while in our study this gene had no impact on lipid changes after therapy.

The apolipoprotein E (*APOE*) gene is composed of three alleles (ε2, ε3 and ε4) that are responsible for the synthesis of apolipoprotein E (apoE) (107). The apoE protein affects the metabolism of TC and TG by binding to receptors in the liver, mediating the clearance of chylomicron remnants and VLDL from the circulation. Allelic variation in the *APOE* has consistently been associated with plasma concentrations of TC, LDL-C and apolipoprotein B (107). We observed that GHD adults carrying the T allele of *APOE* SNP rs7412 have lower TC and LDL-C levels at baseline, which is in agreement with a previous study in a population of patients with dementia (186).
The *APOE/C1/C4/C2* gene cluster SNP rs35136575 influences hepatic control region 2, which regulates the hepatic expression and transcription of all four apolipoprotein genes in the cluster. The present study found that homozygous carriers of the C-allele had higher serum TG concentrations at baseline than G-allele carriers. This is in agreement with a previous study that demonstrates an association between this SNP and TG concentrations in the general population (111).

We have also examined the association of polymorphisms in genes related to the GH signaling pathway and renal tubular function with the ECW volume before and after GHRT in GHD adults. First, the methodology for measuring ECW was validated by comparing the ECW volumes calculated by BIA-50 kHz with those measured by the sodium bromide dilution, which is considered the gold-standard. Our results demonstrated that the estimates by the BIA-50kHz method correlated very well with the estimates made by sodium bromide dilution, allowing the use of the former in our study population.
Out of the 21 SNPs included in our candidate gene approach, three were found to be associated with the ECW volume in untreated GHD patients and none with ECW changes after GHRT. The ECW volume measured in the carriers of the minor allele of SNP rs361072 in the *PIK3CB* gene and of SNP rs6503691 in the STAT5B gene was 0.6 liters higher than that of major allele homozygotes. The protein encoded by the *STAT5B* gene is a member of the STAT family of transcription factors. This protein mediates the signal transduction triggered by various cell ligands, including GH. SNP rs6503691 is located within the region of 17q21 that includes the entire STAT5A gene, the 5´ end of STAT5B and 3´end of the STAT3 genes. It might create variations in the activity of the STAT 5b protein, which would influence the variation of the ECW volumes in a GHD state. There have been no other studies relating this polymorphism with ECW, but an association has been shown between this SNP and serum cholesterol levels in children with GHD before and after GHRT (187).
The *PIK3CB* gene encodes an isoform of phosphoinositide 3-kinase (PI3K) that is important in the GH signaling pathway. We studied rs361072 in the *PIK3CB* gene because it is very common in people of European ancestry, is located within the *p110β* gene promoter (10) and has been associated with plasma IGF-I levels. Healthy people carrying TT and TC genotypes have lower free IGF-I plasma levels in comparison CC genotypes (114). It is not known how this SNP interferes with the control mechanisms of ECW volume, but one possibility is that genetic variation in the *PIK3CB* gene would create variable activity of PI3-kinase heterodimers, influencing ECW volumes.
Homozygotes of the major allele of SNP rs2291340 in the *SLC12A1* gene showed 0.5 liters higher ECW volume than that of the minor allele carriers. This gene plays a key role in concentrating urine and accounts for most NaCl resorption. Na-K-Cl cotransporter activity is affected by a large variety of hormonal stimuli, including GH (33, 123). GH acutely increases renal electrolyte and water reabsorption by modulating the Na-K-Cl co-transporter in distal tubular segments. The renal effects of chronic GH exposure seem to be the result of an initial phase of sodium and water retention, the balance of which is subsequently restored. We selected SNP rs2291340 because of its contribution to blood pressure variation previously reported in the literature (19).

Conclusions

1) Mathematical models were developed for GHD adults to enable the prediction of good and poor responders to GHRT based on gender and baseline serum insulin levels for changes in serum IGF-I levels, and gender, body height and baseline LBM for changes in BC.

2) The presence of the d3-GHR allele in GHD adults did not influence their clinical and laboratorial findings and had no impact on serum IGF-I and total BF changes after GHRT.

3) Serum lipid concentrations in GHD adults were affected by polymorphisms in the *CETP*, *APOE*, *APOB*, *APOE/C1/C4/C2* cluster and *PPARG* genes, while polymorphisms in the *APOB* and *PPARG* genes were associated with changes in TC and LDL-C levels during GHRT.

4) ECW volume in GHD adults was affected by polymorphisms in two genes related to the GH signaling pathways (*PI3KCB* and *STAT5B*) and in one gene related to renal sodium and water balance (*SLC12A1*).

Future Aspects

Prediction models

The model as it now stands is already very useful. It facilitates the definition of a good or poor responder, allowing physicians to set the adequate dose for each patient more rapidly and efficiently, enabling more effective treatment in a shorter time. As patients can normalize their condition more quickly on a lower dose of GH, the cost of the treatment is lowered. As cost is an important factor, this will allow more patients to receive treatment. A suggestion for future studies might be to produce separate prediction models for men and women. This would especially require a much larger number of women, as they tend to respond more poorly to treatment. It would also be interesting to increase the number of predictors in order to improve the result of treatment, making it more cost feasible, safer and more effective. It is also necessary for the model to be tested on other cohorts and populations in a variety of centres around the world.

Genetic studies

Now that genome wide searches and the mapping of haplotypes have become a reality, a number of possibilities for future research have been revealed. A genome wide search can help to identify other candidate genes and pertinent SNPs and see how their influence could be applied to future prediction models for response to growth hormone treatment. The findings of numerous studies can be applied to a database with those of other populations and this would be of great help for the purposes of validation, with findings being confirmed or refuted. Databases can be linked in order to make available larger homogeneous and heterogeneous samples.

Acknowledgements

Many people have helped me to get this thesis into print during the six years that I worked on this project. I would especially like to acknowledge:

Gudmundur Johannsson, my supervisor, for giving me a place on his team, assigning people to help me in a new environment and having the confidence to give me a line of research. Without his patience and invaluable help, this thesis would not be possible.

Cesar Luiz Boguszewski, my co-supervisor, for introducing me to the Sahlgrenska University Hospital and acting as my go-between in the early days and helping me with my research and articles throughout the project. The value of his comments, suggestions and insight cannot be overstated.

I would especially like to thank Camilla Glad for all her work on the genetic part of the study and for providing laboratory space for this purpose, taking the time to read my manuscripts and making invaluable suggestions. I am grateful for her friendship, quick replies to my messages and for proofreading the thesis book.
The nurses and auxiliary medical staff at the Research Centre for Endocrinology and Metabolism (CEM), especially Lena Wirén, Anna-Lena Jönsson, Ingrid Hansson, Sigrid Lindstrand, Annika Reibring, Anna Olsson, Jenny Tiberg and Annika Alklind for introducing me to the working-life and routines of the CEM. Together with all the other staff (Ulla-Britt Liebera and Kajsa Torstensson, Sophie Halden), they helped to create a friendly and welcoming ambience for me as a bewildered newcomer and were always a joy to be with whether at work or in moments of relaxation.

Ingrid Hannson, Vibeke Malmros and Niklas Bosaeus at Sahlgrenska University Hospital for helping me with the medical records and files, collecting and checking information, especially concerning the work on extra cellular water. Thank you for all your kindness and patience.

Professor Olle Isaksson, my first contact at the Sahlgrenska University Hospital, for pointing me in the right direction and suggesting that clinical research was the best choice for me.

My co-authors, Josef Koranyi, Helena Filipsson Nyström, Bengt-Åke Bengtsson, Jenny Palming, Camilla A. M. Glad, Lena M. S. Carlsson, Anna G. Nilsson, Galina Götherström, Per-Arne Svensson, Isabela Vinotti, Staffan Nilsson, Niklas Bosaeus and Ingvar Bosaeus for their valuable help, support, suggestions and contributions to this project. An extra special thanks Helena for taking the time to read my manuscripts and making helpful suggestions.

Staffan Nilsson, for his excellent work on the statistics for the two most recent studies in this thesis, providing greater insight into the data.

Anna Nilsson for taking the time and trouble to find specific extra information and data at the CEM.

The Mutation Analysis Facility at Karolinska University Hospital for their work on the Sequenom part of this project, especially Linda Berglind and Kristina Duvefelt, without whose specialist knowledge the work on Papers III and IV would not be possible in such a short time.

All the professors, researchers and staff of the SEMPR Research Centre of the Federal University of Paraná, Curitiba, Brazil, for their help, friendship and a good working atmosphere throughout this project.

Lise-Lott Norrman, Celina Franco and Ragnhildur Bergthorsdottir for their friendship during my stay in Sweden.

Robert Stewart, my English teacher, for all his help over the past four years.

My mother, Maria J. Lima, for all her help when things began to get on top of me and being there for me whenever I needed her. All my love and thanks.

My family, Ney, Lillian and Matheus, for all their understanding and support over the past six years. This project would never be possible without their patience, and words cannot express how grateful I am to you all.

The Institute of Medicine, Department of Internal Medicine, Sahlgrenska Academy of the University of Gothenburg for their general support and especially the Department of Endocrinology at Sahlgrenska University Hospital for recognizing the potential of this project and without whose support it would never have become a reality.

This project was supported by the Swedish federal government under the LUA/ALF agreement, Swedish Research Council (K2008-65p-20758-01-4), NovoNordisk and Pfizer.

References

1. Sjogren K, Liu JL, Blad K, Skrtic S, Vidal O, et al. (1999) Liver-derived insulin-like growth factor I (IGF-I) is the principal source of IGF-I in blood but is not required for postnatal body growth in mice. Proc Natl Acad Sci U S A 96: 7088-7092.
2. Gibney J, Johannsson G (2004) Long-term monitoring of insulin-like growth factor I in adult growth hormone deficiency: a critical appraisal. Horm Res 62 Suppl 1: 66-72.
3. Johannsson G, Bjarnason R, Bramnert M, Carlsson LM, Degerblad M, et al. (1996) The individual responsiveness to growth hormone (GH) treatment in GH-deficient adults is dependent on the level of GH-binding protein, body mass index, age, and gender. J Clin Endocrinol Metab 81: 1575-1581.
4 Ranke MB, Lindberg A (2010) Observed and predicted growth responses in prepubertal children with growth disorders: guidance of growth hormone treatment by empirical variables. J Clin Endocrinol Metab 95: 1229-1237.
5 Li CH, Papkoff H (1956) Preparation and properties of growth hormone from human and monkey pituitary glands. Science 124: 1293-1294.Stingl JC, Brockmöller J (2011) Why, when, and how should pharmacogenetics be applied in clinical studies?: Current and future approaches to study designs. Clinical Pharmacology & Therapeutics 89 (2): 198-209.
6 Raben MS (1958) Treatment of a pituitary dwarf with human growth hormone. J Clin Endocrinol Metab 18: 901-903.
7 Koch TK, Berg BO, De Armond SJ, Gravina RF (1985) Creutzfeldt-Jakob disease in a young adult with idiopathic hypopituitarism. Possible relation to the administration of cadaveric human growth hormone. N Engl J Med 313: 731-733.
8 Mills JL, Schonberger LB, Wysowski DK, Brown P, Durako SJ, et al. (2004) Long-term mortality in the United States cohort of pituitary-derived growth hormone recipients. J Pediatr 144: 430-436.
9 Cronin MJ (1997) Pioneering recombinant growth hormone manufacturing: pounds produced per mile of height. J Pediatr 131: S5-7.
10 Cuneo RC, Salomon F, McGauley GA, Sonksen PH (1992) The growth hormone deficiency syndrome in adults. Clin Endocrinol (Oxf) 37: 387-397.
11 Rosen T, Bengtsson BA (1990) Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336: 285-288.
12 de Boer H, Blok GJ, Van der Veen EA (1995) Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16: 63-86.
13. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, et al. (2001) Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 357: 425-431.
14. Salomon F, Cuneo RC, Hesp R, Sonksen PH (1989) The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. N Engl J Med 321: 1797-1803.
15. Jorgensen JO, Pedersen SA, Thuesen L, Jorgensen J, Ingemann-Hansen T, et al. (1989) Beneficial effects of growth hormone treatment in GH-deficient adults. Lancet 1: 1221-1225.
16. Binnerts A, Swart GR, Wilson JH, Hoogerbrugge N, Pols HA, et al. (1992) The effect of growth hormone administration in growth hormone deficient adults on bone, protein, carbohydrate and lipid homeostasis, as well as on body composition. Clin Endocrinol (Oxf) 37: 79-87.
17. Whitehead HM, Boreham C, McIlrath EM, Sheridan B, Kennedy L, et al. (1992) Growth hormone treatment of adults with growth hormone deficiency: results of a 13-month placebo controlled cross-over study. Clin Endocrinol (Oxf) 36: 45-52.
18. Bengtsson BA, Koppeschaar HP, Abs R, Bennmarker H, Hernberg-Stahl E, et al. (1999) Growth hormone replacement therapy is not associated with any increase in mortality. KIMS Study Group. J Clin Endocrinol Metab 84: 4291-4292.

19 Johannsson G, Rosen T, Bengtsson BA (1997) Individualized dose titration of growth hormone (GH) during GH replacement in hypopituitary adults. Clin Endocrinol (Oxf) 47: 571-581.
20 Drake WM, Coyte D, Camacho-Hubner C, Jivanji NM, Kaltsas G, et al. (1998) Optimizing growth hormone replacement therapy by dose titration in hypopituitary adults. J Clin Endocrinol Metab 83: 3913-3919.
21 Holmes SJ, Shalet SM (1995) Which adults develop side-effects of growth hormone replacement? Clin Endocrinol (Oxf) 43: 143-149.
22 Johannsson G, Oscarsson J, Rosen T, Wiklund O, Olsson G, et al. (1995) Effects of 1 year of growth hormone therapy on serum lipoprotein levels in growth hormone-deficient adults. Influence of gender and Apo(a) and ApoE phenotypes. Arterioscler Thromb Vasc Biol 15: 2142-2150.
23. Burman P, Johansson AG, Siegbahn A, Vessby B, Karlsson FA (1997) Growth hormone (GH)-deficient men are more responsive to GH replacement therapy than women. J Clin Endocrinol Metab 82: 550-555.
24 Johannsson G, Grimby G, Sunnerhagen KS, Bengtsson BA (1997) Two years of growth hormone (GH) treatment increase isometric and isokinetic muscle strength in GH-deficient adults. J Clin Endocrinol Metab 82: 2877-2884.
25 Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, et al. (2001) Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. Am J Physiol Endocrinol Metab 281: E1191-1196.
26 Janssen YJ, Helmerhorst F, Frolich M, Roelfsema F (2000) A switch from oral (2 mg/day) to transdermal (50 microg/day) 17beta-estradiol therapy increases serum insulin-like growth factor-I levels in recombinant human growth hormone (GH)-substituted women with GH deficiency. J Clin Endocrinol Metab 85: 464-467.
27 Hoffman DM, O'Sullivan AJ, Freund J, Ho KK (1995) Adults with growth hormone deficiency have abnormal body composition but normal energy metabolism. J Clin Endocrinol Metab 80: 72-77.
28 Snel YE, Brummer RJ, Doerga ME, Zelissen PM, Bakker CJ, et al. (1995) Adipose tissue assessed by magnetic resonance imaging in growth hormone-deficient adults: the effect of growth hormone replacement and a comparison with control subjects. Am J Clin Nutr 61: 1290-1294.
29 de Boer H, Blok GJ, Voerman HJ, Phillips M, Schouten JA (1994) Serum lipid levels in growth hormone-deficient men. Metabolism 43: 199-203.
30 Binnerts A, Deurenberg P, Swart GR, Wilson JH, Lamberts SW (1992) Body composition in growth hormone-deficient adults. Am J Clin Nutr 55: 918-923.
31 Beshyah SA, Freemantle C, Thomas E, Rutherford O, Page B, et al. (1995) Abnormal body composition and reduced bone mass in growth hormone deficient hypopituitary adults. Clin Endocrinol (Oxf) 42: 179-189.
32 Carroll PV, Christ ER, Bengtsson BA, Carlsson L, Christiansen JS, et al. (1998) Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. Growth Hormone Research Society Scientific Committee. J Clin Endocrinol Metab 83: 382-395.
33 Jorgensen JO, Thuesen L, Muller J, Ovesen P, Skakkebaek NE, et al. (1994) Three years of growth hormone treatment in growth hormone-deficient adults: near normalization of body composition and physical performance. Eur J Endocrinol 130: 224-228.
34 Rosen T, Bosaeus I, Tolli J, Lindstedt G, Bengtsson BA (1993) Increased body fat mass and decreased extracellular fluid volume in adults with growth hormone deficiency. Clin Endocrinol (Oxf) 38: 63-71.
35 Snel YE, Doerga ME, Brummer RJ, Zelissen PM, Zonderland ML, et al. (1995) Resting metabolic rate, body composition and related hormonal parameters in growth hormone-deficient adults before and after growth hormone replacement therapy. Eur J Endocrinol 133: 445-450.
36 Cuneo RC, Salomon F, Wiles CM, Sonksen PH (1990) Skeletal muscle performance in adults with growth hormone deficiency. Horm Res 33 Suppl 4: 55-60.

37 Rosen T, Eden S, Larson G, Wilhelmsen L, Bengtsson BA (1993) Cardiovascular risk factors in adult patients with growth hormone deficiency. Acta Endocrinol (Copenh) 129: 195-200.
38 Rosen T, Wilhelmsen L, Landin-Wilhelmsen K, Lappas G, Bengtsson BA (1997) Increased fracture frequency in adult patients with hypopituitarism and GH deficiency. Eur J Endocrinol 137: 240-245.
39 Stabler B, Turner JR, Girdler SS, Light KC, Underwood LE (1992) Reactivity to stress and psychological adjustment in adults with pituitary insufficiency. Clin Endocrinol (Oxf) 36: 467-473.
40 Rosen T, Wiren L, Wilhelmsen L, Wiklund I, Bengtsson BA (1994) Decreased psychological well-being in adult patients with growth hormone deficiency. Clin Endocrinol (Oxf) 40: 111-116.
41 De Boer H, Blok GJ, Voerman HJ, De Vries PM, van der Veen EA (1992) Body composition in adult growth hormone-deficient men, assessed by anthropometry and bioimpedance analysis. J Clin Endocrinol Metab 75: 833-837.
42 Bates AS, Bullivant B, Sheppard MC, Stewart PM (1999) Life expectancy following surgery for pituitary tumours. Clin Endocrinol (Oxf) 50: 315-319.
43 Bates AS, Van't Hoff W, Jones PJ, Clayton RN (1996) The effect of hypopituitarism on life expectancy. J Clin Endocrinol Metab 81: 1169-1172.
44 Bulow B, Hagmar L, Mikoczy Z, Nordstrom CH, Erfurth EM (1997) Increased cerebrovascular mortality in patients with hypopituitarism. Clin Endocrinol (Oxf) 46: 75-81.
45 Besson A, Salemi S, Gallati S, Jenal A, Horn R, et al. (2003) Reduced longevity in untreated patients with isolated growth hormone deficiency. J Clin Endocrinol Metab 88: 3664-3667.
46 Bjork S, Jonsson B, Westphal O, Levin JE (1989) Quality of life of adults with growth hormone deficiency: a controlled study. Acta Paediatr Scand Suppl 356: 55-59; discussion 60, 73-54.
47 Burman P, Broman JE, Hetta J, Wiklund I, Erfurth EM, et al. (1995) Quality of life in adults with growth hormone (GH) deficiency: response to treatment with recombinant human GH in a placebo-controlled 21-month trial. J Clin Endocrinol Metab 80: 3585-3590.
48 McGauley GA, Cuneo RC, Salomon F, Sonksen PH (1990) Psychological well-being before and after growth hormone treatment in adults with growth hormone deficiency. Horm Res 33 Suppl 4: 52-54.
49 Snel YE, Doerga ME, Brummer RM, Zelissen PM, Koppeschaar HP (1995) Magnetic resonance imaging-assessed adipose tissue and serum lipid and insulin concentrations in growth hormone-deficient adults. Effect of growth hormone replacement. Arterioscler Thromb Vasc Biol 15: 1543-1548.
50 Egger A, Buehler T, Boesch C, Diem P, Stettler C, et al. (2011) The effect of GH replacement therapy on different fat compartments: a whole-body magnetic resonance imaging study. Eur J Endocrinol 164: 23-29.
51 Hazem A, Elamin MB, Bancos I, Malaga G, Prutsky G, et al. (2012) Body composition and quality of life in adults treated with GH therapy: a systematic review and meta-analysis. Eur J Endocrinol 166: 13-20.
52 Moller J, Fisker S, Rosenfalck AM, Frandsen E, Jorgensen JO, et al. (1999) Long-term effects of growth hormone (GH) on body fluid distribution in GH deficient adults: a four months double blind placebo controlled trial. Eur J Endocrinol 140: 11-16.
53 Johannsson G, Sverrisdottir YB, Ellegard L, Lundberg PA, Herlitz H (2002) GH increases extracellular volume by stimulating sodium reabsorption in the distal nephron and preventing pressure natriuresis. J Clin Endocrinol Metab 87: 1743-1749.
54 Herlitz H, Jonsson O, Bengtsson BA (1994) Effect of recombinant human growth hormone on cellular sodium metabolism. Clin Sci (Lond) 86: 233-237.
55 Dimke H, Flyvbjerg A, Frische S (2007) Acute and chronic effects of growth hormone on renal regulation of electrolyte and water homeostasis. Growth Horm IGF Res 17: 353-368.
56 .de Boer H, Blok GJ, Popp-Snijders C, Stuurman L, Baxter RC, et al. (1996) Monitoring of growth hormone replacement therapy in adults, based on measurement of serum markers. J Clin Endocrinol Metab 81: 1371-1377.

57 de Boer H, Blok GJ, Voerman B, de Vries P, Popp-Snijders C, et al. (1995) The optimal growth hormone replacement dose in adults, derived from bioimpedance analysis. J Clin Endocrinol Metab 80: 2069-2076.
58 Rudling M, Norstedt G, Olivecrona H, Reihner E, Gustafsson JA, et al. (1992) Importance of growth hormone for the induction of hepatic low density lipoprotein receptors. Proc Natl Acad Sci U S A 89: 6983-6987.
59 Rudling M, Olivecrona H, Eggertsen G, Angelin B (1996) Regulation of rat hepatic low density lipoprotein receptors. In vivo stimulation by growth hormone is not mediated by insulin-like growth factor I. J Clin Invest 97: 292-299.
60 Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, et al. (2004) Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89: 2192-2199.
61 Johansson JO, Fowelin J, Landin K, Lager I, Bengtsson BA (1995) Growth hormone-deficient adults are insulin-resistant. Metabolism 44: 1126-1129.
62 Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO (2002) Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. J Clin Endocrinol Metab 87: 2121-2127.
63 Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, et al. (2001) A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86: 4657-4665.
64 Monson JP, Bengtsson BA, Abs R, Feldt-Rasmussen U, Wuster C (2000) Can growth hormone therapy cause diabetes? KIMS Strategic Committee. Lancet 355: 1728-1729.
65 Murray RD, Skillicorn CJ, Howell SJ, Lissett CA, Rahim A, et al. (1999) Influences on quality of life in GH deficient adults and their effect on response to treatment. Clin Endocrinol (Oxf) 51: 565-573.
66 Wiren L, Bengtsson BA, Johannsson G (1998) Beneficial effects of long-term GH replacement therapy on quality of life in adults with GH deficiency. Clin Endocrinol (Oxf) 48: 613-620.
67 Bex M, Abs R, Maiter D, Beckers A, Lamberigts G, et al. (2002) The effects of growth hormone replacement therapy on bone metabolism in adult-onset growth hormone deficiency: a 2-year open randomized controlled multicenter trial. J Bone Miner Res 17: 1081-1094.
68 Johannsson G, Rosen T, Bosaeus I, Sjostrom L, Bengtsson BA (1996) Two years of growth hormone (GH) treatment increases bone mineral content and density in hypopituitary patients with adult-onset GH deficiency. J Clin Endocrinol Metab 81: 2865-2873.
69 Bravenboer N, Holzmann PJ, ter Maaten JC, Stuurman LM, Roos JC, et al. (2005) Effect of long-term growth hormone treatment on bone mass and bone metabolism in growth hormone-deficient men. J Bone Miner Res 20: 1778-1784.
70 Svensson J, Sunnerhagen KS, Johannsson G (2003) Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. J Clin Endocrinol Metab 88: 2061-2069.
71. Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, et al. (1999) Derivation and validation of a mathematical model for predicting the response to exogenous recombinant human growth hormone (GH) in prepubertal children with idiopathic GH deficiency. KIGS International Board. Kabi Pharmacia International Growth Study. J Clin Endocrinol Metab 84: 1174-1183.
72. Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, et al. (2000) Prediction of long-term response to recombinant human growth hormone in Turner syndrome: development and validation of mathematical models. KIGS International Board. Kabi International Growth Study. J Clin Endocrinol Metab 85: 4212-4218.
73. Ranke MB, Lindberg A, Cowell CT, Wikland KA, Reiter EO, et al. (2003) Prediction of response to growth hormone treatment in short children born small for gestational age: analysis of data from KIGS (Pharmacia International Growth Database). J Clin Endocrinol Metab 88: 125-131.

74. Ranke MB, Lindberg A, Martin DD, Bakker B, Wilton P, et al. (2003) The mathematical model for total pubertal growth in idiopathic growth hormone (GH) deficiency suggests a moderate role of GH dose. J Clin Endocrinol Metab 88: 4748-4753.
75. Dahlgren J, Kristrom B, Niklasson A, Nierop AF, Rosberg S, et al. (2007) Models predicting the growth response to growth hormone treatment in short children independent of GH status, birth size and gestational age. BMC Med Inform Decis Mak 7: 40.71 70.
76. Johansson AG (1999) Gender difference in growth hormone response in adults. J Endocrinol Invest 22: 58-60.
77. Verhelst J, Abs R, Vandeweghe M, Mockel J, Legros JJ, et al. (1997) Two years of replacement therapy in adults with growth hormone deficiency. Clin Endocrinol (Oxf) 47: 485-494.
78. Bengtsson BA, Abs R, Bennmarker H, Monson JP, Feldt-Rasmussen U, et al. (1999) The effects of treatment and the individual responsiveness to growth hormone (GH) replacement therapy in 665 GH-deficient adults. KIMS Study Group and the KIMS International Board. J Clin Endocrinol Metab 84: 3929-3935.
79. Ceda GP, Calbiani B, Maggio M, Perelli-Ercolini S, Benedetti N, et al. (1999) Two-year treatment with rhGH of one patient with chronic congestive heart failure and growth hormone deficiency. J Endocrinol Invest 22: 51-52..
80. Cook DM, Ludlam WH, Cook MB (1999) Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. J Clin Endocrinol Metab 84: 3956-3960.
81. Kam GY, Leung KC, Baxter RC, Ho KK (2000) Estrogens exert route- and dose-dependent effects on insulin-like growth factor (IGF)-binding protein-3 and the acid-labile subunit of the IGF ternary complex. J Clin Endocrinol Metab 85: 1918-1922.
82. Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG (2000) Gender difference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adults: role of sex hormone replacement. J Clin Endocrinol Metab 85: 1121-1125.
83. Span JP, Pieters GF, Sweep FG, Hermus AR, Smals AG (2001) Gender differences in rhGH-induced changes in body composition in GH-deficient adults. J Clin Endocrinol Metab 86: 4161-4165.
84. Rosenfeld RG, Bakker B (2008) Compliance and persistence in pediatric and adult patients receiving growth hormone therapy. Endocr Pract 14: 143-154.
85. Cutfield WS, Derraik JG, Gunn AJ, Reid K, Delany T, et al. (2011) Non-compliance with growth hormone treatment in children is common and impairs linear growth. PLoS One 6: e16223.
86. Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, et al. (2004) A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36: 720-724.
87. Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, et al. (2006) Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 91: 1076-1080.
88. Raz B, Janner M, Petkovic V, Lochmatter D, Eble A, et al. (2008) Influence of growth hormone (GH) receptor deletion of exon 3 and full-length isoforms on GH response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 93: 974-980
89. Tauber M, Ester W, Auriol F, Molinas C, Fauvel J, et al. (2007) GH responsiveness in a large multinational cohort of SGA children with short stature (NESTEGG) is related to the exon 3 GHR polymorphism. Clin Endocrinol (Oxf) 67: 457-461.
90. Pilotta A, Mella P, Filisetti M, Felappi B, Prandi E, et al. (2006) Common polymorphisms of the growth hormone (GH) receptor do not correlate with the growth response to exogenous recombinant human GH in GH-deficient children. J Clin Endocrinol Metab 91: 1178-1180.
91. Blum WF, Machinis K, Shavrikova EP, Keller A, Stobbe H, et al. (2006) The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. J Clin Endocrinol Metab 91: 4171-4174.

92. Carrascosa A, Esteban C, Espadero R, Fernandez-Cancio M, Andaluz P, et al. (2006) The d3/fl-growth hormone (GH) receptor polymorphism does not influence the effect of GH treatment (66 microg/kg per day) or the spontaneous growth in short non-GH-deficient small-for-gestational-age children: results from a two-year controlled prospective study in 170 Spanish patients. J Clin Endocrinol Metab 91: 3281-3286.
93. Carrascosa A, Audi L, Esteban C, Fernandez-Cancio M, Andaluz P, et al. (2008) Growth hormone (GH) dose, but not exon 3-deleted/full-length GH receptor polymorphism genotypes, influences growth response to two-year GH Therapy in Short Small-for-Gestational-Age Children. J Clin Endocrinol Metab 93: 147-153.
94. Carrascosa A, Audi L, Fernandez-Cancio M, Esteban C, Andaluz P, et al. (2008) The exon 3-deleted/full-length growth hormone receptor polymorphism did not influence growth response to growth hormone therapy over two years in prepubertal short children born at term with adequate weight and length for gestational age. J Clin Endocrinol Metab 93: 764-770.
95. Corneli G, Di Somma C, Prodam F, Bellone J, Bellone S, et al. (2007) Cut-off limits of the GH response to GHRH plus arginine test and IGF-I levels for the diagnosis of GH deficiency in late adolescents and young adults. Eur J Endocrinol 157: 701-708.
96. Longobardi S, Merola B, Pivonello R, Di Rella F, Di Somma C, et al. (1996) Reevaluation of growth hormone (GH) secretion in 69 adults diagnosed as GH-deficient patients during childhood. J Clin Endocrinol Metab 81: 1244-1247.
97. Berg C, Meinel T, Lahner H, Yuece A, Mann K, et al. (2010) Diagnostic utility of the glucagon stimulation test in comparison to the insulin tolerance test in patients following pituitary surgery. Eur J Endocrinol 162: 477-482.
98. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Vance ML (2011) Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96: 1587-1609.
99. Meirhaeghe A, Fajas L, Gouilleux F, Cottel D, Helbecque N, et al. (2003) A functional polymorphism in a STAT5B site of the human PPAR gamma 3 gene promoter affects height and lipid metabolism in a French population. Arterioscler Thromb Vasc Biol 23: 289-294.
100. Koh WP, Yuan JM, Van Den Berg D, Ingles SA, Yu MC (2006) Peroxisome proliferator-activated receptor (PPAR) gamma gene polymorphisms and colorectal cancer risk among Chinese in Singapore. Carcinogenesis 27: 1797-1802..
101. Dullaart RP, van den Berg G, van der Knaap AM, Dijck-Brouwer J, Dallinga-Thie GM, et al. (2010) HDL cholesterol response to GH replacement is associated with common cholesteryl ester transfer protein gene variation (-629C>A) and modified by glucocorticoid treatment. Eur J Endocrinol 162: 227-234.
102. Ridker PM, Pare G, Parker AN, Zee RY, Miletich JP, et al. (2009) Polymorphism in the CETP gene region, HDL cholesterol, and risk of future myocardial infarction: Genomewide analysis among 18 245 initially healthy women from the Women's Genome Health Study. Circ Cardiovasc Genet 2: 26-33.
103. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, et al. (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40: 161-169.
104. Izar MC, Helfenstein T, Ihara SS, Relvas WG, Santos AO, et al. (2009) Association of lipoprotein lipase D9N polymorphism with myocardial infarction in type 2 diabetes: the genetics, outcomes, and lipids in type 2 diabetes (GOLD) study. Atherosclerosis 204: 165-170.
105. Lamon-Fava S, Asztalos BF, Howard TD, Reboussin DM, Horvath KV, et al. (2010) Association of polymorphisms in genes involved in lipoprotein metabolism with plasma concentrations of remnant lipoproteins and HDL subpopulations before and after hormone therapy in postmenopausal women. Clin Endocrinol (Oxf) 72: 169-175.
106. Benn M, Stene MC, Nordestgaard BG, Jensen GB, Steffensen R, et al. (2008) Common and rare alleles in apolipoprotein B contribute to plasma levels of low-density lipoprotein cholesterol in the general population. J Clin Endocrinol Metab 93: 1038-1045.
107. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, et al. (2002) Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. Am J Epidemiol 155: 487-495.

108. Derijk RH, van Leeuwen N, Klok MD, Zitman FG (2008) Corticosteroid receptor-gene variants: modulators of the stress-response and implications for mental health. Eur J Pharmacol 585: 492-501.
109. Fernandes-Rosa FL, Bueno AC, de Souza RM, de Castro M, dos Santos JE, et al. (2010) Mineralocorticoid receptor p.I180V polymorphism: association with body mass index and LDL-cholesterol levels. J Endocrinol Invest 33: 472-477.
110. Polisecki E, Muallem H, Maeda N, Peter I, Robertson M, et al. (2008) Genetic variation at the LDL receptor and HMG-CoA reductase gene loci, lipid levels, statin response, and cardiovascular disease incidence in PROSPER. Atherosclerosis 200: 109-114
111. Klos K, Shimmin L, Ballantyne C, Boerwinkle E, Clark A, et al. (2008) APOE/C1/C4/C2 hepatic control region polymorphism influences plasma apoE and LDL cholesterol levels. Hum Mol Genet 17: 2039-2046.
112. Glad CA, Johannsson G, Carlsson LM, Svensson PA (2010) Rapid and high throughput genotyping of the growth hormone receptor exon 3 deleted/full-length polymorphism using a tagSNP. Growth Horm IGF Res 20: 270-273.
113. Le Stunff C, Dechartres A, Mariot V, Lotton C, Trainor C, et al. (2008) Association analysis indicates that a variant GATA-binding site in the PIK3CB promoter is a Cis-acting expression quantitative trait locus for this gene and attenuates insulin resistance in obese children. Diabetes 57: 494-502.
114. Bonafe M, Barbieri M, Marchegiani F, Olivieri F, Ragno E, et al. (2003) Polymorphic variants of insulin-like growth factor I (IGF-I) receptor and phosphoinositide 3-kinase genes affect IGF-I plasma levels and human longevity: cues for an evolutionarily conserved mechanism of life span control. J Clin Endocrinol Metab 88: 3299-3304.
115. Ge D, Gooljar SB, Kyriakou T, Collins LJ, Swaminathan R, et al. (2008) Association of common JAK2 variants with body fat, insulin sensitivity and lipid profile. Obesity (Silver Spring) 16: 492-496.
116. Vaclavicek A, Bermejo JL, Schmutzler RK, Sutter C, Wappenschmidt B, et al. (2007) Polymorphisms in the Janus kinase 2 (JAK)/signal transducer and activator of transcription (STAT) genes: putative association of the STAT gene region with familial breast cancer. Endocr Relat Cancer 14: 267-277.
117. Sovio U, Bennett AJ, Millwood IY, Molitor J, O'Reilly PF, et al. (2009) Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. PLoS Genet 5: e1000409.
118. Lin J, Hu FB, Qi L, Curhan GC (2009) Genetic polymorphisms of angiotensin-1 type 1 receptor and angiotensinogen and risk of renal dysfunction and coronary heart disease in type 2 diabetes mellitus. BMC Nephrol 10:9.
119. Wang Y, O'Connell JR, McArdle PF, et al. (2009) From the Cover:Whole-genome association study identifies STK39 as a hypertension susceptibility gene.Proc Natl Acad Sci USA 106: 226-31.
120. Tobin MD, Raleigh SM, Newhouse S, Braund P, Bodycote C, et al. (2005) Association of WNK1 gene polymorphisms and haplotypes with ambulatory blood pressure in the general population. Circulation 112: 3423-3429.
121. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E (2005) WNK1 kinase polymorphism and blood pressure response to a thiazide diuretic. Hypertension 46: 758-765.
122. Iwai N, Tago N, Yasui N, Kokubo Y, Inamoto N, et al. (2004) Genetic analysis of 22 candidate genes for hypertension in the Japanese population. J Hypertens 22: 1119-1126.
123. Dimke H, Flyvbjerg A, Frische S (2007) Acute and chronic effects of growth hormone on renal regulation of electrolyte and water homeostasis. Growth Horm IGF Res 17: 353-368.
124. Matsuo A, Katsuya T, Ishikawa K, Sugimoto K, Iwashima Y, et al. (2004) G2736A polymorphism of thiazide-sensitive Na-Cl cotransporter gene predisposes to hypertension in young women. J Hypertens 22: 2123-2127.
125. Kim JH, Shin HD, Park BL, et al. (2006) SLC12A3 (solute carrier family 12 member [sodium/chloride] 3) polymorphisms are associated with end-stage renal disease in diabetic nephropathy. Diabetes 55: 843-848.

126. Tong Q, Menon AG, Stockand JD (2006) Functional polymorphisms in the alpha-subunit of the human epithelial Na+ channel increase activity. Am J Physiol Renal Physiol 290: F821-827.
127. Vormfelde SV, Sehrt D, Toliat MR, Schirmer M, Meineke I, et al. (2007) Genetic variation in the renal sodium transporters NKCC2, NCC, and ENaC in relation to the effects of loop diuretic drugs. Clin Pharmacol Ther 82: 300-309.
128. Busst CJ, Scurrah KJ, Ellis JA, Harrap SB (2007) Selective genotyping reveals association between the epithelial sodium channel gamma-subunit and systolic blood pressure. Hypertension 50: 672-678.
129. Tobin MD, Tomaszewski M, Braund PS, Hajat C, Raleigh SM, et al. (2008) Common variants in genes underlying monogenic hypertension and hypotension and blood pressure in the general population. Hypertension 51: 1658-1664.
130. Jung J, Foroud, TM, Eckert GJ, et al. (2009) Association of the calcium-sensing receptor gene with blood pressure and urinary calcium in African-Americans. J Clin Endocrinol Metab 94:1042-8.
131. Landin-Wilhelmsen K, Wilhelmsen L, Lappas G, Rosen T, Lindstedt G, et al. (1994) Serum insulin-like growth factor I in a random population sample of men and women: relation to age, sex, smoking habits, coffee consumption and physical activity, blood pressure and concentrations of plasma lipids, fibrinogen, parathyroid hormone and osteocalcin. Clin Endocrinol (Oxf) 41: 351-357.
132. Svensson J, Johannsson G, Bengtsson BA (1997) Insulin-like growth factor-I in growth hormone-deficient adults: relationship to population-based normal values, body composition and insulin tolerance test. Clin Endocrinol (Oxf) 46: 579-586.
133. Wiklund O, Fager G, olofsson SO, Wilhelmsson C, Bondjers G (1980) Serum apolipoprotein levels in relation to acute myocardial infarction and its risk factors--determination of apolipoprotein D. Atherosclerosis 37: 631-636.
134. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502.
135. Bosaeus I, Johannsson G, Rosen T, Hallgren P, Tolli J, et al. (1996) Comparison of methods to estimate body fat in growth hormone deficient adults. Clin Endocrinol (Oxf) 44: 395-402.
136. Bruce A, Andersson M, Arvidsson B, Isaksson B (1980) Body composition. Prediction of normal body potassium, body water and body fat in adults on the basis of body height, body weight and age. Scand J Clin Lab Invest 40: 461-473.
137. Jaffrin MY, Morel H (2009) Extracellular volume measurements using bioimpedance spectroscopy-Hanai method and wrist-ankle resistance at 50 kHz. Med Biol Eng Comput 47: 77-84.
138. Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, et al. (2000) Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. J Biol Chem 275: 18664-18669.
139. Audi L, Esteban C, Carrascosa A, Espadero R, Perez-Arroyo A, et al. (2006) Exon 3-deleted/full-length growth hormone receptor polymorphism genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents (n = 247) and in an adult control population (n = 289) show increased fl/fl in short SGA. J Clin Endocrinol Metab 91: 5038-5043.
140. Hosmer DW & Lemeshow S. (2000) Applied Logistic Regression. 2nd ed. New York, USA: John Wiley & Sons.
141. Brown H, Prescott R (2006) Applied mixed models in medicine. 2nd ed. New York, USA: John Wiley & Sons Ltd.
142. Johannsson G, Gibney J, Wolthers T, Leung KC, Ho KK (2005) Independent and combined effects of testosterone and growth hormone on extracellular water in hypopituitary men. J Clin Endocrinol Metab 90: 3989-3994.
143. Pietrobelli A, Wang Z, Heymsfield SB (1998) Techniques used in measuring human body composition. Curr Opin Clin Nutr Metab Care 1: 439-448.

144. Filipsson H (2009) Aspects of diagnosis and treatment of hypopituitarism in adult life. Department of Internal Medicine, Sahlgrenska Academy at University of Gothenburg, Sweden. ISBN 978-91-628-7694-4.
145. Silva AM, Heymsfield SB, Gallagher D, Albu J, Pi-Sunyer XF, et al. (2008) Evaluation of between-methods agreement of extracellular water measurements in adults and children. Am J Clin Nutr 88: 315-323.
146. Lettre G, Lange C, Hirschhorn JN (2007) Genetic model testing and statistical power in population-based association studies of quantitative traits. Genet Epidemiol 31: 358-362.
147. Giustina A, Veldhuis JD (1998) Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19: 717-797.
148. Johannsson G (2011) Long-acting growth hormone for replacement therapy. J Clin Endocrinol Metab 96: 1668-1670.
149. Xu S (2003) Theoretical basis of the Beavis effect. Genetics 165: 2259-2268.
150. Svensson J, Finer N, Bouloux P, Bevan J, Jonsson B, et al. (2007) Growth hormone (GH) replacement therapy in GH deficient adults: predictors of one-year metabolic and clinical response. Growth Horm IGF Res 17: 67-76.
151. Johannsson G, Rosen T, Lindstedt G, et al. (1996) Effects of 2 years of growth hormone treatment on body composition and cardiovascular risk factors in adults with growth hormone deficiency. Endocrinol Metab 3(Suppl A): 3-12.
152. Thangavel C, Shapiro BH (2007) A molecular basis for the sexually dimorphic response to growth hormone. Endocrinology 148: 2894-2903.
153. Franco C, Koranyi J, Brandberg J, Lonn L, Bengtsson BK, et al. (2009) The reduction in visceral fat mass in response to growth hormone is more marked in men than in oestrogen-deficient women. Growth Horm IGF Res 19: 112-120.
154. Surya S, Horowitz JF, Goldenberg N, Sakharova A, Harber M, et al. (2009) The pattern of growth hormone delivery to peripheral tissues determines insulin-like growth factor-1 and lipolytic responses in obese subjects. J Clin Endocrinol Metab 94: 2828-2834.
155. Ezzat S, Fear S, Gaillard RC, Gayle C, Marcovitz S, et al. (2003) Circulating IGF-I levels in monitoring and predicting efficacy during long-term GH treatment of GH-deficient adults. Eur J Endocrinol 149: 499-509.
156. Yang S, Xu X, Björntorp P, Edén S (1995) Additive effects of growth hormone and testosterone on lipolysis in adipocytes of hypophysectomized rats J Endocrinol 147: 147-152.
157. Ho KK, Gibney J, Johannsson G, Wolthers T (2006) Regulating of growth hormone sensitivity by sex steroids: implications for therapy. Front Horm Res 35: 115-128.
158. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK (2000) Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. J Clin Endocrinol Metab 85: 4712-4720.
159. Deurenberg P, Weststrate JA, Seidell JC (1991) Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. Br J Nutr 65: 105-114.
160. Deurenberg P, van der Kooy K, Leenen R, Weststrate JA, Seidell JC (1991) Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross-validation study. Int J Obes 15: 17-25.
161. Bruce A, Andersson M, Arvidsson B, Isaksson B (1980) Body composition. Prediction of normal body potassium, body water and body fat in adults on the basis of body height, body weight and age. Scand J Clin Lab Invest 40: 461-473.
162. Binder G, Baur F, Schweizer R, Ranke MB (2006) The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational-age children. J Clin Endocrinol Metab 91: 659-664.
163. Binder G, Trebar B, Baur F, Schweizer R, Ranke MB (2008) Homozygosity of the d3-growth hormone receptor polymorphism is associated with a high total effect of GH on growth and a low BMI in girls with Turner syndrome. Clin Endocrinol (Oxf) 68: 567-572.
164. van der Klaauw AA, van der Straaten T, Baak-Pablo R, Biermasz NR, Guchelaar HJ, et al. (2008) Influence of the d3-growth hormone (GH) receptor isoform on short-term and long-term treatment response to GH replacement in GH-deficient adults. J Clin Endocrinol Metab 93: 2828-2834.

165. Adetunji OR, MacFarlane IA, Javadpour M, Alfirevic A, Pirmohamed M, et al. (2009) The d3/fl-GH receptor gene polymorphism does not influence quality of life and body composition in GH-deficient adults receiving GH replacement therapy. Eur J Endocrinol 161: 541-546.
166. Giavoli C, Ferrante E, Profka E, Olgiati L, Bergamaschi S, et al. (2010) Influence of the d3GH receptor polymorphism on the metabolic and biochemical phenotype of GH-deficient adults at baseline and during short- and long-term recombinant human GH replacement therapy. Eur J Endocrinol 163: 361-368.
167. Moyes VJ, Walker DM, Owusu-Antwi S, Maher KT, Metherell L, et al. (2010) d3-GHR genotype does not explain heterogeneity in GH responsiveness in hypopituitary adults. Clin Endocrinol (Oxf) 72: 807-813.
168. Meyer S, Schaefer S, Stolk L, Arp P, Uitterlinden AG, et al. (2009) Association of the exon 3 deleted/full-length GHR polymorphism with recombinant growth hormone dose in growth hormone-deficient adults. Pharmacogenomics 10: 1599-1608.
169. Angelin B, Rudling M (1994) Growth hormone and hepatic lipoprotein metabolism. Curr Opin Lipidol 5: 160-165.
170. Elam MB, Simkevich CP, Solomon SS, Wilcox HG, Heimberg M (1988) Stimulation of in vitro triglyceride synthesis in the rat hepatocyte by growth hormone treatment in vivo. Endocrinology 122: 1397-1402.
171. Elam MB, Wilcox HG, Solomon SS, Heimberg M (1992) In vivo growth hormone treatment stimulates secretion of very low density lipoprotein by the isolated perfused rat liver. Endocrinology 131: 2717-2722.
172. Sjoberg A, Oscarsson J, Bostrom K, Innerarity TL, Eden S, et al. (1992) Effects of growth hormone on apolipoprotein-B (apoB) messenger ribonucleic acid editing, and apoB 48 and apoB 100 synthesis and secretion in the rat liver. Endocrinology 130: 3356-3364..
173 Sparks CE, Marsh JB (1981) Metabolic heterogeneity of apolipoprotein B in the rat. J Lipid Res 22: 519-527.
174. Garry P, Collins P, Devlin JG (1996) An open 36-month study of lipid changes with growth hormone in adults: lipid changes following replacement of growth hormone in adult acquired growth hormone deficiency. Eur J Endocrinol 134: 61-66.
175. Leese GP, Wallymahmed M, VanHeyningen C, Tames F, Wieringa G, et al. (1998) HDL-cholesterol reductions associated with adult growth hormone replacement. Clin Endocrinol (Oxf) 49: 673-677.
176. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 391: 79-82.
177. Ricote M, Huang J, Fajas L, Li A, Welch J, et al. (1998) Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci U S A 95: 7614-7619.
178. Sunyaev S, Ramensky V, Koch I, Lathe W, 3rd, Kondrashov AS, et al. (2001) Prediction of deleterious human alleles. Hum Mol Genet 10: 591-597.
179. Ng PC, Henikoff S (2003) SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 31: 3812-3814.
180. Wojczynski MK, Gao G, Borecki I, Hopkins PN, Parnell L, et al. (2010) Apolipoprotein B genetic variants modify the response to fenofibrate: a GOLDN study. J Lipid Res 51: 3316-3323.
181. Sarzynski MA, Jacobson P, Rankinen T, Carlsson B, Sjostrom L, et al. (2011) Association of GWAS-based candidate genes with HDL-cholesterol levels before and after bariatric surgery in the Swedish obese subjects study. J Clin Endocrinol Metab 96: E953-957.
182. Talmud PJ, Drenos F, Shah S, Shah T, Palmen J, et al. (2009) Gene-centric association signals for lipids and apolipoproteins identified via the HumanCVD BeadChip. Am J Hum Genet 85: 628-642.
183. Bennet AM, Reynolds CA, Gatz M, Blennow K, Pedersen NL, et al. (2010) Pleiotropy in the presence of allelic heterogeneity: alternative genetic models for the influence of APOE on serum LDL, CSF amyloid-beta42, and dementia. J Alzheimers Dis 22: 129-134.

184. Dachet C, Poirier O, Cambien F, Chapman J, Rouis M (2000) New functional promoter polymorphism, CETP/-629, in cholesteryl ester transfer protein (CETP) gene related to CETP mass and high density lipoprotein cholesterol levels: role of Sp1/Sp3 in transcriptional regulation. Arterioscler Thromb Vasc Biol 20: 507-515.
185. Thompson A, Di Angelantonio E, Sarwar N, Ergou S, Saleheen D, Dullaart RPF, Keavney B, Ye Z, Danesh J (2008) Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. JAMA 299: 2777-2788.
186. Bennet AM, Reynolds CA, Gatz M, Blennow K, Pedersen NL, et al. (2010) Pleiotropy in the presence of allelic heterogeneity: alternative genetic models for the influence of APOE on serum LDL, CSF amyloid-beta42, and dementia. J Alzheimers Dis 22: 129-134.
187 Makimura M, Ihara K, Kojima-Ishii K, Nozaki T, Ohkubo K, et al. (2011) The signal transducer and activator of transcription 5B gene polymorphism contributes to the cholesterol metabolism in Japanese children with growth hormone deficiency. Clin Endocrinol (Oxf) 74: 611-617.
188. Haas M, Forbush B, 3rd (1998) The Na-K-Cl cotransporters. J Bioenerg Biomembr 30: 161-172.