GROWTH HORMONE GENES AND PROSTATE CANCER RISK

by

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Background: Growth hormone (*GH*) SNPs are associated with breast cancer and colon cancer. The author investigated the association of prostate cancer with genetic polymorphisms in *GH* SNPs in the Ancillary MrOS study. **Methods:** Included in the current investigation were 128 men with prostate cancer and 743 healthy men, 65 years of age or older. SNPs were tested in Growth Hormone 1 (*GH1*, n=4), Growth Hormone Receptors (*GHR*, n=15), Growth Hormone-Releasing Hormone (*GHRH*, n=4), Growth Hormone-Releasing Hormone Receptors (*GHRHR*, n=10), Ghrelin (*GHRL*, n=8), and Growth Hormone Secretagogue Receptor (*GHSR*, n=9) genes for an association with prostate cancer risk. SNPs were selected based on HapMap Phase 1 and based on functional variation. The SNPs were genotyped using Illumina Assay and were included if the minor allele frequency was 1% or greater. Logistic regression analysis was used to examine associations, adjusted for age, weight, BMI, truncal % fat, total % fat, and diabetes. Similarly, tests of trends and tests of dominant/recessive effect were performed. **Results:** After adjusting for potential confounding factors, two *GH1* SNPs, one *GHR* SNP, one *GHRH* SNP, two *GHRHR* SNPs, one *GHRL* SNP, and one *GHSR* SNP showed significant associations with prostate cancer risk. **Public Health Significance:** If the relationships observed in this study are confirmed, it would justify the investigation of approaches that would reduce the activity of GH in those at high risk for prostate cancer. **Conclusions:** The results of

the current study suggest that *GH* SNPs are associated with prostate cancer risk. This provides support for replication of these findings in other studies.

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PREFACE

I want to thank my committee for their encouragement, support, and feedback on my thesis. Their sound advice and careful guidance of this project is deeply appreciated. Much gratitude is due to my mentors, Dr. Bunker and Dr. Weissfeld, who guided me through the process from the formative stages to the final draft.

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This thesis is dedicated to the memory of my grandmother, Anna Swann Sisk. Her unconditional love, guidance, and support helped me to succeed and instilled in me the confidence that I am capable of doing anything I put my mind to.

1.0 INTRODUCTION

Prostate cancer is one of the most common cancers of males in developed countries.[1] Incidence rates of prostate cancer increased in the United States[2], Canada[3], England and other parts of Western Europe[4] during the early 1990s due to improved screening tests based on prostate specific antigens (PSA).[5] Although there has been a decline in prostate cancer incidence since 1994[6], it remains a significant public health concern. The National Cancer Institute estimated 218,890 men will be diagnosed with and 27,050 men in the U.S. will die of cancer of the prostate in 2007.[7] The latest SEER statistics stated that in 2003, there were 1,937,807 men alive with a history of prostate cancer, and one in six men will be diagnosed with prostate cancer at some point in their life.[8] In addition, it has been estimated that approximately $8 billion is spent on prostate cancer treatment each year in the U.S.[9] The impact of prostate cancer on quality of life, economic expenditure, and survival is immense and further research is needed to understand the etiology of and risk factors for prostate cancer.

Known risk factors of prostate cancer include age, race/ethnicity, and family history. Age is a strong risk factor for prostate cancer and the likelihood of developing prostate cancer increases with advancing age. Incidence and mortality rates increase after the age of 50 [10], and the probability of being diagnosed with prostate cancer before the age of 40 is 1 in 10,149, 1 in 28 for men between the ages of 50 and 60, and 1 in 7 for men aged 60 and older.[11] Race/ethnicity is another significant risk factor. African American men have the highest risk of

developing prostate cancer; it tends to be diagnosed at younger ages and the cancer grows faster compared to men of other racial/ethnic backgrounds.[12] Next, it is most common in Caucasian men and is followed by Hispanic and Native American men; men of Asian ancestry are at the lowest risk of developing prostate cancer. Family history of prostate cancer also increases one's risk of developing this disease, especially if a father or brother had prostate cancer. Several epidemiologic / twin studies have shown that hereditary prostate cancer is characterized by early onset and autosomal dominant inheritance.[13]

Several susceptibility genes for prostate cancer have been identified, such as ELAC2, PCAP, RNASEL and HPC1 polymorphisms.[14-17] These polymorphisms are not common and the genetic influence on prostate cancer is relatively small; rare, highly penetrant polymorphisms in these genes probably account for less than 10% of susceptibility in prostate cancer cases.[18, 19]

It is likely that more common polymorphisms with a relatively weak effect have a larger overall impact on prostate cancer risk because these variants may occur at higher frequencies. The combined effect of risk factors – such as race, age, and family history – and genetic factors influence the etiology of prostate cancer, making it a multifactorial disease.[8] The heterogeneity of prostate cancer (i.e. slow- versus fast-growing) makes it difficult to identify genetic factors associated with incident cases. Further research is needed to identify multiple genetic factors associated with the etiology of prostate cancer.

1.1 GENETICS OF GROWTH HORMONE GENES

1.1.1 Growth Hormone 1 (*GH1*)

Genes associated with growth hormones are of significant interest in cancer research and recent data suggests higher levels of growth hormone are associated with increased risk of breast cancer[20], neuroendocrine tumors[21], and colorectal cancer[22]. *GH1* is transcribed in the somatotrophic cells of the pituitary and GH1 is responsible for the release of GH into circulation [see figure 1]. The *GH1* gene is positioned on chromosome 17q23 and is located in a pituitary-specific transcription factor binding region, PIT-1. Transcription of *GH1* depends on proximal promoter regions that house two binding sites for *PIT-1/GH1*. In addition, the proximal region of the GH1 gene promoter exhibits a high level of sequence variation with 16 single nucleotide polymorphisms (SNPs) within a 535 base-pair region[23], and the SNPs significantly influence the expression of GH1.[24]

However, SNPs located in the promoter region do not fully explain transcription of *GH1*; a transgenic study of human (h)*GH* expression revealed that the promoter region is not enough to initiate gene expression of (h)GH in vivo.[25] *GH1* expression is also under the influence of a locus control region (LCR) which is upstream of the *GH1* between 14.5 kb and 32 kb.[26] This LCR includes several DNase I hypersensitive sites, which are required for the activation of the GH1 gene. Two DNase I hypersensitive sites (I and II) have binding sites for the pituitary transcription factor *PIT-1* and they are responsible for the expression of the somatotrope expression of *GH1*.[27] Building on this, another study found that the LCR increases the activity of the proximal promoter of a *GH1* haplotype and the effect of a particular proximal promoter

haplotype is differentially affected by differing LCR haplotypes.[24] The interaction between the different LCR and *GH1* haplotypes seem to determine the extent to which *GH1* is expressed.

1.1.2 Growth Hormone Receptors (*GHR*)

The *GHR* gene is positioned on chromosome 5p12 and it is present on in most cells. The actions of *GH* are mediated by the binding gh1 to the transmembrane receptor of GHR, which is present on the surface of most cells. Binding of *GH1* results in receptor dimerization and activation, and a short isoform of the receptor circulates as a binding protein (*GHBP*); the main function of *GHBP* is to act as a physiological buffer that stabilizes GH in plasma.[28] Following dimerization and activation, this leads to internalization and down-regulation of *GHR* and is processed by one of two pathways: 1) a small rapid, nondegradative pathway or 2) a slower lysosomal degradative pathway.[29] A polymorphism of *GHR* has been identified and it has an effect on *GHBP*, thereby influencing *GH* in the plasma. *hGHRtr* contains a 26-bp deletion, leading to the creation of a stop codon at position 280, which truncates approximately 97% of the intracellular domain of *hGHR*.[30] After comparing *GHR* to *GHRtr*, *GHRtr* was shown to have a significantly increased ability to generate soluble GHBP.[31] This association has been found in animal studies (i.e. rat) and in studies utilizing human liver cells.[32] This suggests that the association between increased production of *GH* and *GHR* is mediated by *GHBP* and *GHBP* is differentially expressed based on *GHR* polymorphisms.

1.1.3 Growth Hormone-Releasing Hormone (*GHRH*), GHRH-Receptors (*GHRHR*) and Somatostatin

The *GHRH* gene is on chromosome 20q11 and it is responsible for *GHRH*, a peptide hormone secreted from the hypothalamus. *GHRH* stimulates the proliferation of pituitary somatotrophs and induces secretion of *GH* from these cells. This is accomplished by the binding of *GHRH* to a seven-membrane domain receptor of the somatotropes, namely the *GHRH* receptor (*GHRHR*).[33] In addition to *GHRH*, somatostatin is another hypothalamic hormone responsible for the regulation of GH. These two hormones have opposing functions, with *GHRH* stimulating the release of *GH1* by activating cyclic Amp (cAMP) accumulation and somatostatin inhibiting cAMP accumulation.[34]

The *GHRH*-receptor (*GHRHR*) is on chromosome 7p15 and is present in pituitary cells.[35] Ghrh binds to its receptor on the surface of the pituitary somatotroph cells, the coupling of G protein stimulates adenylate cyclase to generate cAMP (cAMP).[36] cAMP then stimulates the transcription of the *PIT1* gene, which stimulates the transcription of *GH1* and *GHRHR*.[36, 37] Antagonists of *GHRHR* also operate to influence transcription of *GH1*; antagonists bind to and prevent *GHRH* from binding to *GHRHR*.[38] Therefore, multiple mechanisms are involved in the expression of *GH* of the pituitary.

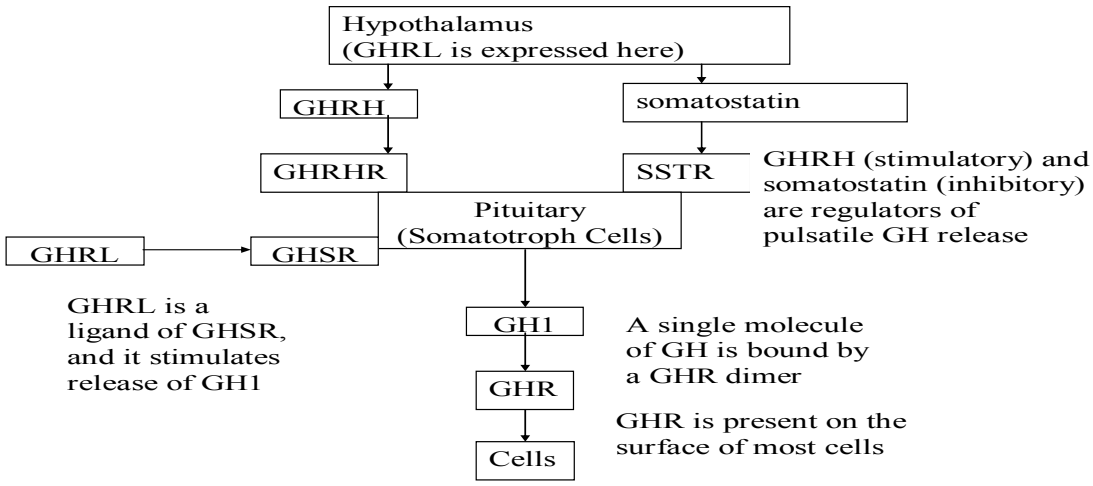

Figure 1. GHRL: ghrelin precursor; GHRH: growth hormone-releasing hormone; GHRHR: growth hormone-releasing hormone receptor; SSTR: somatostatin receptor; GHSR: growth hormone secretagogue receptor; GH1: growth hormone 1; and GHR: growth hormone receptor.

1.1.4 Ghrelin precursor (*GHRL*) and Growth Hormone Secretagogue Receptor (*GHSR*)

The *GHRL* gene is positioned on chromosome 3p25 and the *GHSR* gene is located on chromosome 3q26. The majority of research in this field has identified *GHRH* and somatostatin as the primary regulators of growth hormone secretion; however, recent research reveals a third mechanism, *GHRL* and *GHSR*, involved in *GH* secretion [see Figure 2]. *GHRL* is produced in the stomach and is an endogenous ligand for *GHSR* [39], which is expressed in the pituitary. *GHRL* circulates in human blood at considerable plasma concentrations (120 pmol/g).[40] In addition, *in vitro* and *in vivo* studies of pituitary cells reveal that *GHRL* acts directly on the pituitary by binding with *GHRL* to release *GH*.[41, 42] Several studies also reveal that *GHRL* stimulates *GH* release in a dose-dependent manner.[33, 43] Identification of pathways that regulate

GHRL release in the stomach is not well understood, and further research is needed to elucidate this mechanism.

1.2 THE IMPACT OF GROWTH HORMONE GENES ON CANCER

1.2.1 Breast Cancer

Growth hormones (*GH*) are increasingly implicated in the development of breast cancer; however, little is known about the role of *GH* in cancer and the mechanisms regulating *GH* production. Several studies show an association between several *GH1* SNPs of the proximal promoter region and decreased risk of breast cancer[44, 45], while another study found no association.[46] In addition, several *GH1* haplotypes have also shown a significant association with decreased risk of breast cancer.[47, 48] This is of great interest since *GH* mRNA and *GH* protein have been found at different levels in epithelial cells from normal and malignant breast tissue.[49] There was greater expression of GH mRNA and greater cellular proliferation in cancer cells than normal cells.

Tissue composition and breast density have significant associations with breast cancer [50, 51], and recent findings suggest that *GH1* polymorphisms are associated with breast density.[52] Mulhall et al. found that *GH1*-75A homozygotes had a significantly greater percent density, larger dense area, and a smaller area of non-dense tissue[52], suggesting the role of *GH1* on cellular proliferation.

One study found that a *GHR* haplotype is associated with a significantly decreased risk of breast cancer.[47] *GHR* mRNA has been expressed in both epithelial and stromal components of

breast tissue, and *GHR* mRNA expression has been significantly higher in cancer tissue.[53] In addition, levels of *GHR* have been reported to have an inverse correlation with tumor grade[54], and an upregulation of GHR has been found in cancerous breast tissue compared to adjacent normal breast tissue.[47] This provides evidence of *GHR* expression in breast cancer and indicates a potential role of *GHR* signaling in breast cancer.

GHRH mRNA and protein have been found in both normal and malignant breast tissue.[55] One study found that *GHRH* was detected at a significantly higher ratio in lobular than in ductal carcinomas.[56] *GHRHR* mRNA has also been found in breast cancer.[57] One study found that *GHRHR* C-261T is associated with a decreased risk of breast cancer[48], while such an association was absent in another study.[46]

1.2.2 Colorectal Cancer

Recent findings suggest growth hormone may have a role in the development of colorectal cancer. One study shows that expression of the *IGF-I* gene is regulated primarily by growth hormone[58], and other studies have shown that *IGF* polymorphisms are associated with colorectal cancer.[59, 60] Therefore, it is possible that *GH* exerts its influence on colorectal cancer through the *IGF* pathway. One study showed an inverse association with risk of colorectal cancer and a gene-dosage effect in a *GH1* polymorphism.[61] The adjusted means for the level of plasma IGF-I and the *IGF-I/IGFBP*-3 ratio were lower for the *GH1* A/A genotype than for the T/T genotype (this SNP was not in the present study).[61] In addition, GH serum levels are higher in colorectal cancer cases[62] and *GH* receptors are expressed in advanced colorectal tumors and at lower levels in normal tissues.[63]

1.2.3 Pituitary Carcinomas

GHSR has been identified in the pituitary and because of this, it is possible that GHRL binds to *GHSR* in the pituitary and stimulates secretion of GH. *GHRL* and *GHSR* expression was relatively low in corticotroph tumors and was relatively high in somatotroph tumors compared with normal tissue.[41] Other studies found no association between *GHRL* and *GHSR* and non-functioning pituitary tumors, yet there was a significant association between *GHRL* and *GHSR* with somatotroph tumors.[21, 64-66] Furthermore, several studies showed a negative correlation between *GHSR* mRNA and *GHRL* mRNA expression in pituitary tumors[21, 66]; since the *GHSR / GHRL* is a mechanism that controls secretion of GH from the pituitary, these findings suggest that this pathway may be involved in the pathogenesis of pituitary tumors.

1.2.4 Prostate Cancer

There is a limited amount of research conducted on growth hormones and risk of prostate cancer. Both breast cancer and prostate cancer are affected by hormones; because recent findings suggest that *GH* genes may influence development of breast cancer[44, 45, 47, 48] one might also suggest that GH genes are involved in the pathogenesis of prostate cancer. One study found increased expression of a *GHR* mRNA in prostate cancer tissue compared to normal tissue.[67] Another study found an intense nuclear GHRHR immunoreactivity and cytoplasmic *GHRHR* mRNA expression in the secretory cells of prostate cancer.[68] In addition, *GHRH* and *GHRHR* mRNA co-expression in prostate cancer cell lines is associated with cellular proliferation,[69] consistent with studies on other cancer types.[47, 61] Last, *GHRL* mRNA has been shown to be involved in

cellular proliferation in prostate cancer cells.[70] These studies suggest that growth hormone genes are associated with cellular proliferation and increased risk of prostate cancer.

1.2.5 Growth Hormones and Cancer

Research suggests that *GH1* polymorphisms increase proliferation of colonic epithelial cancer cells[61] and breast cancer cells[47]; in addition, it has been shown that *GHRH* agonists inhibit growth in breast cancer[71] and colorectal cancer.[72] Other studies have shown an association between growth hormone polymorphisms and increased cellular proliferation in prostate cancer cases.[69, 70] Research on prostate cancer tissue and growth hormone expression is limited to one on *GHR*[67], one on *GHRH*[69], two on *GHRHR*[68, 69] and one on *GHRL / GHSR* [70]. There were not any studies identified that focused on growth hormone polymorphisms. Therefore, further research is needed to understand the impact of growth hormone polymorphisms on prostate cancer risk.

1.3 GROWTH HORMONES, BODY COMPOSITION, DIABETES AND PROSTATE CANCER

Research suggests that anthropometric measures are associated with both increased prostate cancer risk and growth hormones. Several studies have shown taller people to be at increased risk of prostate cancer.[73, 74] In addition, BMI and obesity are associated with decreased risk of prostate cancer;[74] however, this relationship is not clear. Obesity in males is associated with lower levels of testosterone[75], and higher levels of testosterone are associated with increased risk

of prostate cancer.[76] Diabetes is also associated with both obesity and decreased levels of testosterone.[77] Recent studies have reported that impaired insulin action is associated with increased fat mass, especially abdominal fat.[78] Compared to subjects of normal weight, growth hormone levels have been shown to be negatively correlated with age, BMI, and waist circumference in a group of obese subjects.[79] It could be hypothesized that multiple mechanisms (i.e. anthropometric measures, growth hormone levels) are involved in the development of prostate cancer.

The prostate gland is essentially undeveloped until puberty, when an interaction between sex and growth hormones induces its development.[80] Therefore, it is biologically plausible that growth hormones and GH polymorphisms impact cellular proliferation that could lead to prostate cancer. The present study utilized MrOS data collected for an ancillary study of candidate gene polymorphisms in pathways which may be related to osteoporosis. Known functional variants were included, and haplotype tagging SNPs were chosen to capture the majority of the variability in each candidate gene. Here we are reporting on the growth hormone pathway in which we screened four GH1 SNPs, fifteen GHR SNPs, four GHRH SNPs, ten GHRHR SNPs, 8 GHRL SNPs, and 9 GHSR SNPs in men diagnosed with prostate cancer and healthy controls. I tested the hypothesis that growth hormone variants and haplotypes are associated with increased risk of prostate cancer independent of body composition measures. In addition, I investigated the gene-dose effect and the effect of recessive and dominant genes on prostate cancer risk.

2.0 METHODS

2.1 STUDY POPULATION

The longitudinal, community-based design of the MrOS study has been published elsewhere.[81] The original sample of MrOS included 5995 men 65 years of age or older recruited between March 2000 and April 2002 from six communities in the United States: Birmingham, AL; Minneapolis, MN; Pittsburgh, PA; Palo Alto, CA; Portland, OR; and San Diego, CA. The eligible participants of the MrOS cohort were able to walk without the assistance of another person, have not had bilateral hip replacement, able to give self-reported consent, attend the clinic visit, complete at least the anthropometric, DEXA, and vertebral X-ray procedures, and were able to answer a self-administered questionnaire over medical history, physical activity, diet, and lifestyle and demographic characteristics. There were no other exclusion criteria.

An ancillary study of candidate gene polymorphisms and bone-related variables among participants from the Pittsburgh and Minneapolis sites was conducted at the University of Pittsburgh.[82] A total 2010 participants were recruited at the two study sites. Of those participants, 124 were excluded for being treated for osteoporosis, taking antiandrogen therapy or oral corticosteroids. The number of minorities at these two sites was small; therefore, 92 minority race participants were not genotyped. Due to budgetary constraints and sample size considerations, a random sample of the remaining 1794 participants at these sites was chosen to

arrive at the final sample of 871. This sample included 74 men reporting prostate cancer at baseline, and 54 incident prostate cancer cases, resulting in 128 prostate cancer cases and 743 healthy controls (see Figure 3). Eligible participants had a mean age of 71.9 years (age range: 65-94).

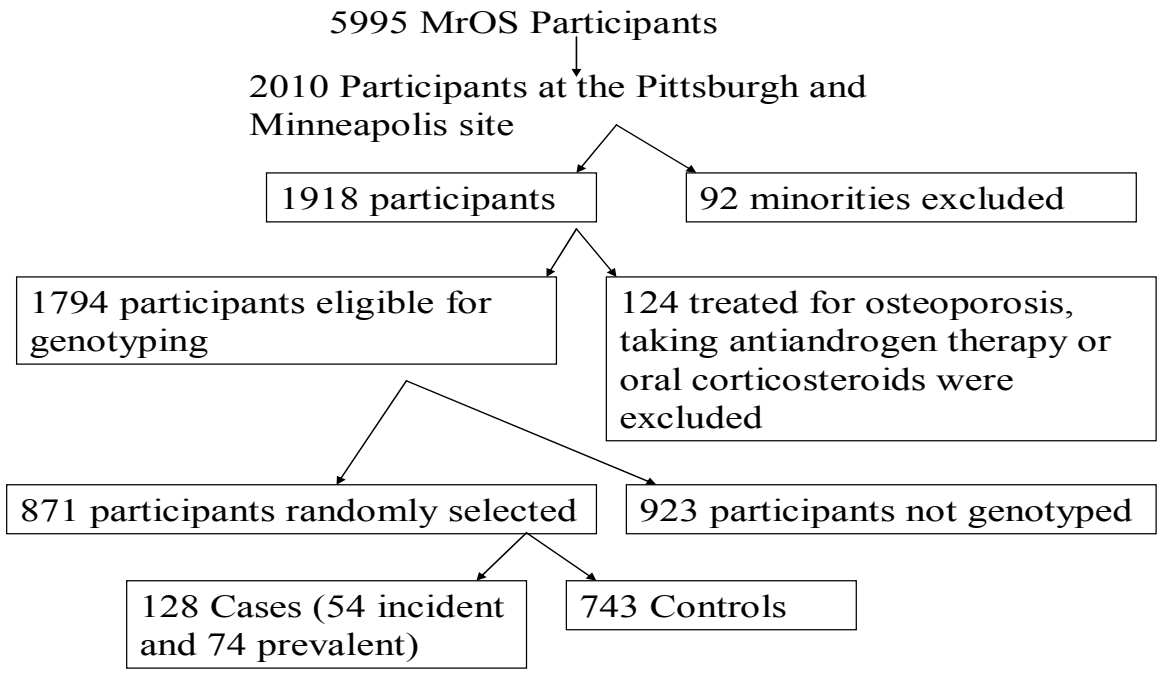

Figure 2. Study Flow Chart

2.2 MEASURES

2.2.1 Baseline Characteristics.

The primary MrOS study collected numerous characteristics of personal and medical history. A structured interview collected information on age, diabetes diagnosis, medications and medical

history, and prostate disease. Current body weight, height, and body composition measures were taken at baseline by an examiner using standard equipment. Weight was determined using the balance beam scale. The Harpenden stadiometer was used to measure height. Two different height measurements were collected; two additional measurements were taken if the difference between measurement 1 and measurement 2 was ≥ 4 mm. DEXA scanners were utilized to determine body composition, total fat, and truncal fat.

2.2.2 Prostate Cancer and Prostate Symptoms

Prevalent prostate cancer cases were determined at baseline in the self-administered questionnaires. Yearly follow-ups consisted of a questionnaire to obtain information about incident prostate biopsies and incident prostate cancer. Permission was granted for study personnel to review medical records for incident cases concerning PSA levels, Gleason scores, tumor stage and grade and type of treatment. The information about the diagnosis was centrally reviewed and adjudicated.

2.3 SNP SELECTION

SNPs were selected from 374 physiologically defined candidate genes by generating a reference SNP panel from the International HapMap database (Phase I) in the gene region (10kb downstream and 30kb upstream). Tag SNPs were selected if they had minor allele frequency (MAF) ≥5% using a pairwise correlation method ($r^2 \geq 0.80$).[83] In addition, functional variation was also targeted if there was: 1) non-synonymous coding SNPs with a reported MAF >1%; or

2) SNPs abolished or created new transcription factor binding sites or altered an exon splice enhancer site (MAF > 2%).

2.4 GENOTYPING PROCEDURES

DNA from frozen whole blood specimens was extracted using Qiagen's Flexigene protocol. High quality, genomic DNA samples were selected for genotyping on the Illumina Golden Gate Assay platform at the University of Pittsburgh's Genomics and Proteomics Core Facility. 37 patient samples were genotyped in duplicate and 4 internal controls were included per plate to ensure reproducibility. A 100% reproducibility rate among the internal controls and a 99.9% reproducibility rate among replicate patient samples was observed.

Loci which had a minor allele frequency less than 1%, did not conform to Hardy-Weinberg equilibrium, or had a low call rate were not included in analysis. Hardy-Weinberg was assessed for the total sample, and was also assessed separately for cases and controls. ,026 SNPs in 368 gene regions meeting these stringent quality control criteria were included in the final analysis. Individual samples with a low call rate or that were highly correlated with another (indicating relatedness) were excluded from the analysis. In total, 871 unique participant samples were used for the final analysis.

2.5 STATISTICAL ANALYSIS

Differences in demographic characteristics, selected variables, and distribution of genotypes across polymorphisms of GH1, GHR, GHRH, GHRHR, GHRL and GHSR between cases and controls were assessed using the $\chi 2$ test (for categorical variables) and student *t*-test (for continuous variables). The association between GH1, GHR, GHRH, GHRHR, GHRL and GHSR polymorphisms and prostate cancer risk were estimated by determining the ORs and 95% CIs from multivariate logistic regression analyses. Multivariate models assessed the impact of dominant gene effect and dose effect on prostate cancer risk. Multivariate models were further adjusted for other potentially confounding factors, such as age, weight, height, BMI, trunk % fat, total % fat, and diabetes. SAS/Genetics and Haploview were used to test for Hardy-Weinberg Equilibrium (HWE), Linkage Disequilibrium (LD), and tests of trends. All SNPs were tested for conformation with Hardy-Weinberg expectations in both the Pittsburgh site and the Minneapolis site. Allele and genotype association was calculated using χ^2 and trend tests. Linkage disequilibrium (LD) was calculated using SAS/Genetics and Haploview. Both Lewontin's D and r^2 values were used to assess LD. Haplotype analysis was also completed using SAS/Genetics. HAPLOVIEW was used to double-check haplotype frequencies. Global tests of association were performed on haplotype analysis; individual haplotype analysis were preformed if the global test is significant. A p-value of < 0.05 indicated significant results for all analyses. All the statistical analyses were performed with SPSS, SAS, or Haploview software.

3.0 RESULTS

3.1 BASELINE CHARACTERISTICS

Table 1 shows relevant characteristics of the subjects by case-control status. Cases with prostate cancer and controls were similar in weight, height, BMI, trunk % fat, total % fat, and diabetes. Combined and prevalent cases were older than control subjects. The study sample (n=871) had a higher BMI at baseline compared to white subjects not selected for genotyping from the Pittsburgh and Minneapolis sites (n=1047; p=0.01; data not shown).

Table 1. Baseline Characteristics

	Total Sample (n=871)	Controls (n=743)	Combined Cases (n=128)	Prevalent Cases (n=74)	Incident Cases (n=54)
Age, mean years (SD)	73.6 (5.8)	73.3 (5.7)	75.3 (5.5)*	76.0 (5.2)**	74.2 (5.9)
Weight, mean kg (SD)	85.32 (14.1)	85.5 (14.1)	84.4 (14.1)	84.4 (13.2)	84.3 (15.3)
Height, mean cm (SD)	173.5 (6.7)	173.6 (6.8)	173.3 (6.5)	174.2 (6.9)	172.2 (5.7)
BMI, mean kg/m^2 (SD)	28.3 (4.1)	28.3 (4.2)	28.0 (3.7)	27.7 (3.3)	28.3 (4.1)
Trunk % Fat, mean (SD)	29.3 (6.0)	29.3 (6.1)	29.6 (5.3)	29.8 (4.7)	29.4 (6.2)
Total % Fat, mean (SD)	26.9 (5.3)	26.9 (5.4)	26.9 (4.8)	27.0 (4.6)	26.8 (5.3)
Diabetes, N (%)	110 (12.6)	97 (13.1)	13 (10.2)	7 (9.5)	6 (11.1)

*p-value = 0.001; **p-value < 0.001 (ANOVA p-values)

3.2 ASSOCIATIONS BETWEEN GH SNPS AND BASELINE CHARACTERISTICS

SNPs were included in the present analysis if there were significant associations with cases (e.g., combined, prevalent, or incident) and controls (See Table 6 in Appendix B). Nine of the 50 GH SNPs were significant in one or more of the categories listed above and entered subsequent analysis. Various SNPs are associated with baseline characteristics (See Table 7 in Appendix C). Age is associated with GHRH SNP 1 (p=0.04) in the total study sample and with GH1 SNP 1 (p=0.03) and GHRH SNP 1 (p=0.01) in controls (data not shown). Weight is associated with GHRHR SNP 9 (p=0.04) in the total sample, with GHRHR SNP 10 (p=0.02) in cases (data not shown), and with GHRHR SNP 9 (p=0.03) in controls (data not shown). Height is associated with GHRHR SNP 9 (p=0.005) and GHRL SNP 7 (p=0.008) in the total sample and with GHRHR SNP 9 (p=0.005) and GHRL SNP 7 (p=0.01) in controls (data not shown). BMI is associated with GHRHR SNP 10 (p=0.03) in cases (data not shown). Trunk % fat is associated with GHRHR SNP 10 (p=0.04) in controls (data not shown). Total % fat is associated with GHR SNP 9 (p=0.03) in controls (data not shown). Diabetes is associated with GHRL SNP 7 (p=0.008) in the total sample and with GHRH SNP 9 (p=0.005) in cases (data not shown).

3.3 HARDY-WEINBERG EQUILIBRIUM

Hardy-Weinberg Equilibrium (HWE) was assessed for allele frequencies for all SNPs in the study for the total sample, by case-control status and by site (Pittsburgh vs. Minneapolis). The SNPs that were included in the final analysis are in HWE in the total sample; GHRL SNP 7 is not in HWE for controls and GH1 SNPs 1 and 2 are not in HWE for cases (See Table 8 in

Appendix D). GHRHR SNP 10 (χ^2 9.89; p=0.002) was not consistent with HWE for the Minneapolis site (data not shown).

Table 2. Allelic frequencies of cases and controls

Gene, N (%)	Total Sample (n=871) N (%)	Controls (n=743) N (%)	Combined Cases (n=128) N (%)	Prevalent Cases (n=74) N (%)	Incident Cases (n=54) N (%)
GH1 SNP 1 (rs2854184)					
1	1131 (0.65)	944 (0.64)	187 (0.73)	108 (0.73)	79 (0.73)
2	603 (0.35)	534 (0.36)	69 (0.27)	40 (0.27)	29 (0.27)
χ^2 p-value			***p=0.004***	***p=0.03***	p=0.05
GH1 SNP 2 (rs2070776)					
1	1128 (0.65)	947 (0.64)	181 (0.71)	97 (0.65)	76 (0.70)
2	614 (0.35)	539 (0.36)	75 (0.29)	51 (0.34)	32 (0.30)
χ^2 p-value			***p=0.03***	p=0.08	p=0.16
GHR SNP 2 (rs10473282)					
1	1160 (0.66)	1004 (0.67)	156 (0.61)	97 (0.65)	59 (0.55)
2	582 (0.34)	482 (0.32)	100 (0.39)	51 (0.34)	49 (0.45)
χ^2 p-value			p=0.91	p=0.62	p=0.005
GHR SNP 9 (rs12233949)					
1	1254 (0.72)	1069 (0.72)	185 (0.72)	107 (0.72)	78 (0.72)
2	488 (0.28)	417 (0.28)	71 (0.28)	41 (0.28)	30 (0.28)
χ^2 p-value			p=0.91	p=0.93	p=0.95
GHRH SNP 1 (rs2050093)					
1	1411 (0.81)	1214 (0.82)	197 (0.77)	117 (0.79)	80 (0.74)
2	329 (0.19)	270 (0.18)	59 (0.23)	31 (0.21)	28 (0.26)
χ^2 p-value			p=0.07	p=0.41	***p=0.04***
GHRHR SNP 9 (rs4988498)					
1	1647 (0.95)	1413 (0.95)	234 (0.93)	132 (0.90)	102 (0.96)
2	85 (0.05)	67 (0.05)	18 (0.07)	14 (0.10)	4 (0.04)
χ^2 p-value			p=0.07	***p=0.007***	p=0.72
GHRHR SNP 10 (rs740336)					
1	1704 (0.98)	1459 (0.98)	245 (0.96)	143 (0.97)	102 (0.94)
2	36 (0.02)	25 (0.02)	11 (0.04)	5 (0.03)	6 (0.06)
χ^2 p-value			***p=0.007***	p=0.14	***p=0.004***
GHRL SNP 7 (rs35668)					
1	1049 (0.60)	878 (0.59)	171 (0.67)	105 (0.71)	66 (0.61)
2	691 (0.40)	606 (0.41)	85 (0.33)	43 (0.29)	42 (0.39)
χ^2 p-value			***p=0.02***	***p=0.005***	p=0.69
GHSR SNP 4 (rs2922126)					
1	1167 (0.67)	1001 (0.67)	166 (0.65)	107 (0.72)	59 (0.55)
2	573 (0.33)	483 (0.33)	90 (0.35)	41 (0.23)	49 (0.45)
χ^2 p-value			p=0.41	p=0.23	***p=0.006***

Notes: χ^2 p-values for cases vs. controls; **1**: the dominant allele, and **2**: the recessive allele; **GH1**: growth hormone 1 SNPs; **GHR**: growth hormone receptors SNPs; **GHRH**: growth hormone-releasing hormone SNPs; **GHRHR**: GHRH-receptor SNPs; **GHRL**: ghrelin precursor SNPs; **GHSR**: growth hormone secretagogue receptor SNPs.

3.4 ASSOCIATIONS OF GH SNPS AND PROSTATE CANCER

Table 6 in Appendix A contains descriptive analysis and tests of trends for 9 GH SNPs that entered analysis. Tests of allelic association and prostate cancer risk for the GH SNPs are shown in Table 2. The associations between GH SNPs and prostate cancer risk are shown in Table 3. Test of dominant effect and recessive effect are shown in Tables 4 and 5, respectively. All logistics regression models were adjusted for age, weight, height, BMI, trunk % fat, total % fat and diabetes.

3.4.1 GH1 SNP1

Chi-square statistics show that GH1 SNP 1 is associated with combined prostate cancer cases compared to controls. Combined cases had a significantly higher frequency of the common homozygote compared to controls with frequencies of 57% and 45%, respectively. In addition, tests of trends (dose effect) were significant for combined and prevalent cases compared to controls, with p-values of 0.009 and 0.04, respectively. The test of trend for incident cases compared to controls was marginally significant (p=0.08). Tests of association between alleles and case-control status reveal that combined cases and prevalent cases had a statistically significant greater frequency (both are 73%) of the dominant allele compared to controls (64%). The test of allelic association was borderline significant for incident cases (p=0.05), in which 73% of cases had the dominant allele compared to 64% controls.

Homozygote carriers of the rare GH1 SNP 1 were associated with decreased prostate cancer risk in the combined case-control group (OR, 0.50; p=0.027). Tests for dominant effect revealed significant associations. Combined heterozygote and rare homozygote carriers of GH1

SNP 1 were associated with decreased risk of prostate cancer in the combined case-control group (OR, 0.62; p=0.02) and in the prevalent case-control group (OR, 0.60; p=0.04).

3.4.2 GH1 SNP 2

Chi-squared statistics for GH1 SNP 2 showed a significant association between combined and prevalent cases compared to controls. The frequency of the common homozygote for combined cases, prevalent cases and controls was 54.7%, 55.4%, and 41.2%, respectively. The test of trend for combined cases compared to controls was significant (p=0.03). The frequency of the dominant allele was statistically different in combined cases (71%) and controls (64%; p=0.03).

Heterozygote carriers of GH1 SNP 2 were associated with decreased prostate cancer risk (OR, 0.49; p=0001) in the combined case-control group. Heterozygote carriers of GH1 SNP 2 were associated with decreased risk of prostate cancer in the prevalent case-control (OR, 0.46; p=0.006). Tests for dominant effect of GH1 SNP 2 revealed significant associations for risk of prostate cancer. Combined heterozygote and rare homozygote carriers of GH1 SNP 2 were associated with decreased risk of prostate cancer in the combined case-control group (OR, 0.54; p=0.002) in the prevalent case-control group (OR, 0.52; p=0.01), and in the incident case-control group (OR, 0.55; p=0.04).

3.4.3 GHR SNP 2

GHR SNP 2 was associated with prostate cancer in incident cases compared to controls. GHR SNP 2 common homozygotes have a frequency of 31.5% and 48.9% for incident cases and controls, respectively. Heterozygotes have a frequency of 46.3% and 37.4% for incident cases

and controls, and rare homozygotes have a frequency of 22.2% and 13.7% for incident cases and controls, respectively. The test of trend for incident cases compared to controls was significant (p=0.01). The test for allelic association was significant for cases and controls, with a higher frequency of incident cases with the dominant allele than controls (p=0.005).

Heterozygote carriers of GHR SNP 2 were associated with increased prostate cancer risk in the incident case-control group (OR, 2.05; p=0.03). Rare homozygote carriers of GHR SNP 2 were associated with increased risk of prostate cancer in the incident case-control group (OR, 2.72; p=0.01). In the test for dominant effect, combined heterozygote and rare homozygote carriers of GHR SNP 2 were associated with increased risk of prostate cancer in the incident case-control group (OR, 2.22; p=0.01).

3.4.4 GHR SNP 9

Chi-squared statistics for GHR SNP 9 showed a significant association between prevalent cases and controls. The frequency of the heterozygote SNP is 50.0% and 38.1% for prevalent cases and controls, respectively. However, logistic regression analysis did not show a significant relationship between GHR SNP 9 and prostate cancer risk.

3.4.5 GHRH SNP 1

Chi-squared statistics for GHRH SNP1 had significant associations for combined and incident cases compared to controls. The frequency of the rare homozygote is 7.8%, 9.3%, and 3.1% for combined cases, incident cases, and controls. Tests of trends were marginally significant for combined cases (p=0.07) and incident cases (p=0.05) compared to controls. There is a

statistically higher frequency of the recessive allele for incident cases (26%) compared to controls (18%; p=0.04). Rare homozygote carriers of GHRH SNP 1 were associated with increased prostate cancer risk in the combined case-control group (OR, 2.34; p=0.04).

3.4.6 GHRHR SNP 9

GHRHR SNP 9 rare homozygotes were collapsed into the category of heterozygotes due to small sample size (n=2). The frequency of heterozygote carriers of GHRHR SNP 9 was significantly higher in prevalent cases (16.4%) compared to controls (9.1%); and there was a significant gene-dose effect (p_{trend} =0.007) for prevalent cases compared to controls. The frequency of the recessive allele was statistically different in prevalent cases (10%) compared to controls (0.05; p=0.007). Carriers of GHRHR SNP 9 were associated with increased risk of prostate cancer in the prevalent case-control group (OR, 2.12; p=0.033).

3.4.7 GHRHR SNP 10

GHRHR SNP 10 rare homozygotes were collapsed into the category of heterozygotes due to a small sample size (n=1). Heterozygotes had a frequency of 7.8%, 9.3%, and 3.4% for combined cases, incident cases, and controls, respectively, and these associations were statistically significant. The frequency of the recessive allele was statistically different in combined cases (4%; p=0.007) and incident cases (6%; p=0.004) compared to controls (2%). Carriers of GHRHR SNP 10 were associated with increased prostate cancer risk in the combined case-control group (OR, 2.81; p=0.011) and in the incident case-control group (OR, 3.69; p=0.014).

3.4.8 GHRL SNP 7

Chi-squared statistics for GHRL SNP 7 showed a significant association between cases compared to controls. The frequency of the common homozygote for combined cases, prevalent cases and controls was 57.8%, 60.8%, and 45.3%, respectively. The test of trend for prevalent cases compared to controls was significant (p=0.02). The frequency of the dominant allele was significantly higher in combined cases (67%; p=0.02) and prevalent cases (71%; p=0.005) compared to controls (59%). Heterozygote carriers of GHRL SNP 7 were associated with decreased risk of prostate cancer in the combined case-control group (OR, 0.50; p=0.008) and in the incident case-control group (OR, 0.44; p=0.04). A similar association was marginally significant in the prevalent case-control group (OR, 0.54; p=0.05). In the test for dominant effect, combined heterozygote and rare homozygote carriers were associated with decreased prostate cancer risk in the combined case-control group (OR, 0.62; p=0.01) and in the prevalent case-control group (OR, 0.56; p= 0.02).

3.4.9 GHSR SNP 4

Chi-squared statistics showed a significant association between GHSR SNP 4 and case-control status. The frequency of the rare homozygote is 22.2% and 10.1% for incident cases and controls, respectively. The test of trend (gene-dose effect) for incident cases compared to controls was significant (p=0.006). The frequency of the recessive allele was statistically different for incident cases (45%; p=0.006) compared to controls (33%).

Heterozygote carriers of GHSR SNP 4 were associated with decreased risk of prostate cancer in the prevalent case-control group (OR, 0.53; p=0.02). In addition, the test for dominant

effect showed that combined heterozygote and rare homozygote GHSR SNP 4 carriers have a decreased risk in the prevalent case-control group (OR, 0.57; p=0.03). Furthermore, rare homozygote carriers of GHSR SNP 4 were associated with increased prostate cancer risk in the incident case-control group (OR, 3.00; p=0.006). The test for recessive effect showed that homogygote rare carriers of GHSR SNP 4 were associated with decreased prostate cancer risk in the incident case-control group (OR, 2.48; p=0.01).

Table 3. Logistic regression models.

	Total Sample Frequency (n=871)	Cases and Controls	Prevalent Cases and Control	Incident Cases and Controls
SNP		**OR (p-value)**	**OR (p-value)**	**OR (p-value)**
GH1 SNP 1 (rs2854184)				
0	411	1.00	1.00	1.00
1	309	0.68 (0.08)	0.63 (0.09)	0.77 (0.41)
2	147	**0.50 (0.027)**	0.56 (0.13)	0.42 (0.08)
GH1 SNP 2 (rs2070776)				
0	376	1.00	1.00	1.00
1	376	**0.49 (0.001)**	**0.46 (0.006)**	0.54 (0.05)
2	119	0.69 (0.22)	0.73 (0.40)	0.61 (0.29)
GHR SNP 2 (rs10473282)				
0	416	1.00	1.00	1.00
1	328	1.28 (0.26)	0.91 (0.73)	***2.05 (0.03)***
2	127	1.71 (0.05)	1.24 (0.53)	***2.72 (0.01)***
GHR SNP 9 (rs12233949)				
0	457	1.00	1.00	1.00
1	340	1.25 (0.27)	1.44 (0.15)	1.00 (0.99)
2	74	0.68 (0.34)	0.33 (0.14)	1.03 (0.95)
GHRH SNP 1 (rs2050093)				
0	574	1.00	1.00	1.00
1	263	1.19 (0.42)	1.09 (0.75)	1.34 (0.34)
2	33	***2.34 (0.04)***	2.23 (0.13)	2.61 (0.09)
GHRHR SNP 9 (rs4988498)*				
0	783	1.00	1.00	1.00
1	81	1.29 (0.43)	1.73 (0.14)	0.79 (0.66)
2	2	-	-	-
GHRHR SNP 9 (rs4988498)*				
0	783	1.00	1.00	1.00
1	83	1.49 (0.19)	***2.12 (0.033)***	0.79 (0.66)
GHRHR SNP 10 (rs740336)*				
0	835	1.00	1.00	1.00
1	34	***2.53 (0.026)***	2.34 (0.11)	2.90 (0.06)
2	1	-	-	-
GHRHR SNP 10 (rs740336)*				
0	835	1.00	1.00	1.00
1	35	***2.81 (0.011)***	2.34 (0.11)	***3.69 (0.014)***
GHRL SNP 7 (rs35668)				
0	410	1.00	1.00	1.00
1	231	0.76 (0.24)	0.57 (0.08)	1.08 (0.80)
2	229	***0.50 (0.008)***	0.54 (0.05)	***0.44 (0.04)***
GHSR SNP 4 (rs2922126)				
0	392	1.00	1.00	1.00
1	383	0.80 (0.29)	***0.53 (0.02)***	1.41 (0.29)
2	95	1.39 (0.26)	0.77 (0.54)	***3.00 (0.006)***

Notes: OR: odds ratio; 0: the common homozygote; 1: the heterozygote; and 2: the homozygous rare. GH1: growth hormone; GHR: growth hormone receptor; GHRH: growth hormone-releasing hormone; GHRHR: GHRH receptor; GHRL: ghrelin; GHSR: growth hormone secretagogue receptor. Models adjusted for: age, weight, height, BMI, trunk % fat, total % fat, and diabetes.

Table 4. Test for dominant effect of GH SNPs

	Total Sample Frequency (n=871)	Cases and Controls	Prevalent Cases and Control	Incident Cases and Controls
SNP		**OR (p-value)**	**OR (p-value)**	**OR (p-value)**
GH1 SNP 1 (rs2854184)				
0	411	1.00	1.00	1.00
1	456	***0.62 (0.02)***	***0.60 (0.04)***	0.66 (0.14)
GH1 SNP 2 (rs2070776)				
0	376	1.00	1.00	1.00
1	495	***0.54 (0.002)***	***0.52 (0.01)***	***0.55 (0.04)***
GHR SNP 2 (rs10473282)				
0	416	1.00	1.00	1.00
1	455	1.39 (0.09)	0.99 (0.97)	***2.22 (0.01)***
GHR SNP 9 (rs12233949)				
0	457	1.00	1.00	1.00
1	414	1.14 (0.50)	1.24 (0.39)	1.01 (0.98)
GHRH SNP 1 (rs2050093)				
0	574	1.00	1.00	1.00
1	296	1.31 (0.18)	1.21 (0.46)	1.48 (0.18)
GHRHR SNP 9 (rs4988498)				
0	783	1.00	1.00	1.00
1	83	1.49 (0.19)	***2.12 (0.03)***	0.79 (0.66)
GHRHR SNP 10 (rs740336)				
0	835	1.00	1.00	1.00
1	35	***2.81 (0.011)***	2.34 (0.11)	***3.69 (0.014)***
GHRL SNP 7 (rs35668)				
0	410	1.00	1.00	1.00
1	460	***0.62 (0.01)***	***0.56 (0.02)***	0.74 (0.28)
GHSR SNP 4 (rs2922126)				
0	392	1.00	1.00	1.00
1	478	0.90 (0.61)	***0.57 (0.03)***	1.70 (0.08)

Notes: **OR**: odds ratio; **1**: the heterozygote and the homozygous rare, **0**: the common homozygote; **GH1**: growth hormone 1 SNPs; **GHR**: growth hormone receptors SNPs; **GHRH**: growth hormone-releasing hormone SNPs; **GHRHR**: GHRH-receptor SNPs; **GHRL**: ghrelin precursor SNPs; **GHSR**: growth hormone secretagogue receptor SNPs. All Models were adjusted for the following: age, weight, height, BMI, trunk % fat, total % fat, and diabetes.

Table 5. Test for recessive effect of GH SNPs

	Total Sample Frequency (n=871)	Cases and Controls	Prevalent Cases and Control	Incident Cases and Controls
SNP		**OR (p-value)**	**OR (p-value)**	**OR (p-value)**
GH1 SNP 1 (rs2854184)				
0	720	1.00	1.00	1.00
1	147	0.59 (0.07)	0.68 (0.30)	0.47 (0.12)
GH1 SNP 2 (2070776)				
0	752	1.00	1.00	1.00
1	119	0.94 (0.82)	0.1.02 (0.96)	0.80 (0.62)
GHR SNP 2 (rs10473282)				
0	744	1.00	1.00	1.00
1	127	1.52 (0.09)	1.29 (0.44)	1.87 (0.07)
GHR SNP 9 (rs12233949)				
0	797	1.00	1.00	1.00
1	74	0.60 (0.22)	0.28 (0.08)	1.03 (0.95)
GHRH SNP 1 (rs2050093)				
0	837	1.00	1.00	1.00
1	33	2.18 (0.06)	2.17 (0.14)	2.32 (0.14)
GHRHR SNP 9 (rs4988498)*				
0	864	1.00	1.00	1.00
1	2	-	-	-
GHRHR SNP 10 (rs740336)*				
0	869	1.00	1.00	1.00
1	1	-	-	-
GHRL SNP 7 (rs35668)				
0	639	1.00	1.00	1.00
1	231	0.94 (0.77)	0.68 (0.23)	1.38 (0.29)
GHSR SNP 4 (rs2922126)				
0	775	1.00	1.00	1.00
1	95	1.55 (0.11)	1.03 (0.95)	***2.48 (0.01)***

Notes: **OR**: odds ratio; **0**: the common homozygote and the heterozygote, **1**: the homozygous rare; **GH1**: growth hormone 1 SNPs; **GHR**: growth hormone receptors SNPs; **GHRH**: growth hormone-releasing hormone SNPs; **GHRHR**: GHRH-receptor SNPs; **GHRL**: ghrelin precursor SNPs; **GHSR**: growth hormone secretagogue receptor SNPs. All Models were adjusted for the following: age, weight, height, BMI, trunk % fat, total % fat, and diabetes.
* Recessive models could not be performed due to small frequency of homozygous rare.

3.5 HAPLOTYPE ANALYSIS

Using SNP data, haplotype frequencies for prostate cancer cases and controls were calculated (Figures 4-6). Global tests of significance did not show any associations between GH haplotypes and prostate cancer risk in this study; therefore, individual haplotype block analysis was not performed.

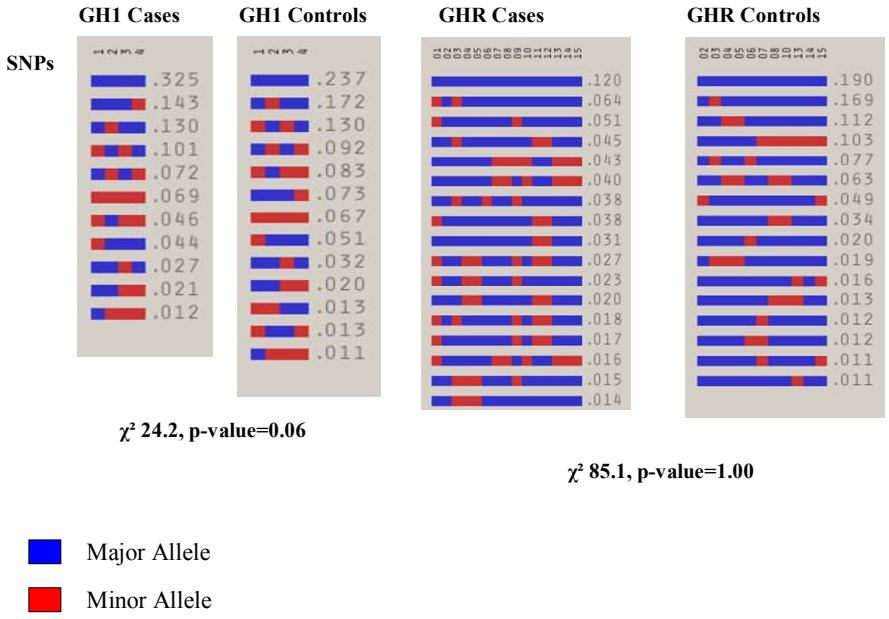

Figure 3. GH1 and GHR haplotype frequency estimates for prostate cancer cases and controls

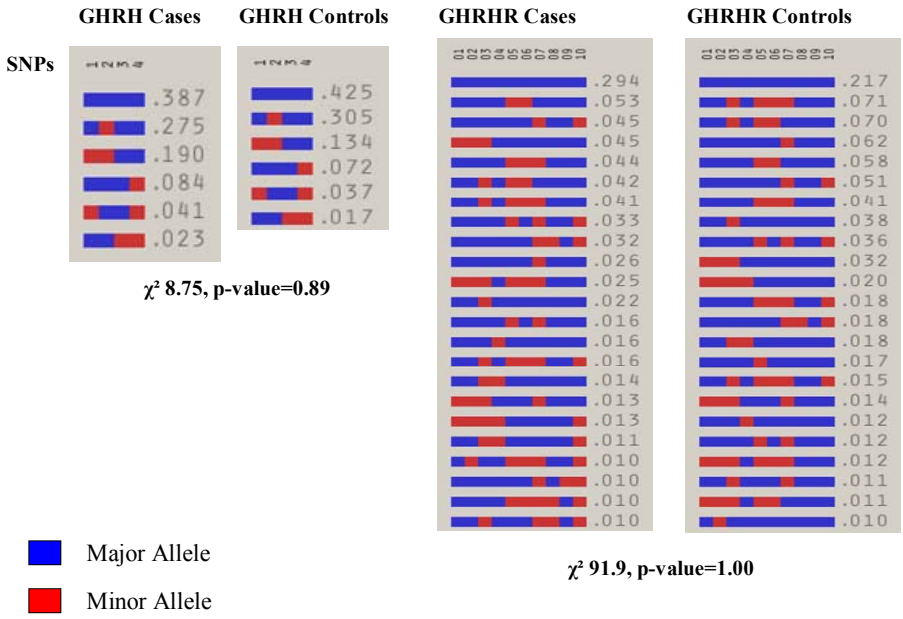

Figure 4. GHRH and GHRHR haplotype frequency estimates for prostate cancer cases and controls

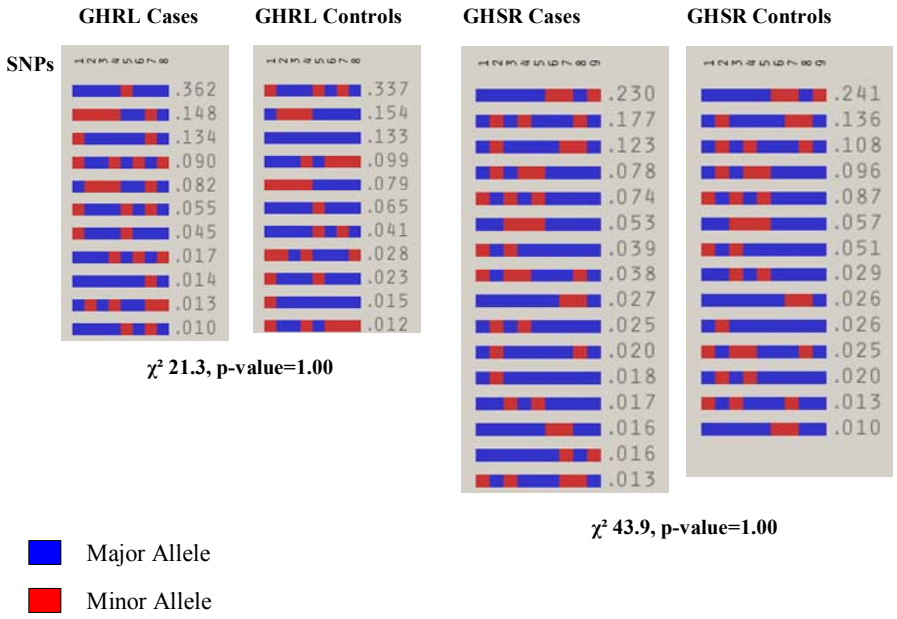

Figure 5. GHRL and GHSR haplotype frequency estimates for prostate cancer cases and controls

3.6 FUNCTIONAL VARIATION

Inclusion of SNPs targeted for functional variation. *GHRHR* SNP 10 was the only SNP in the present analysis to be included based on functional variation; it showed variation with missense mutations and alternative splicing (Appendix F Table 12). *GHRHR* SNP 9 had similar results. In addition, the NCBI SNP database also revealed functional variation in 25 of the 50 *GH* SNPs.

4.0 DISCUSSION

Although expression of *GHR, GHRH, GHRHR*, and *GHRL* has been previously demonstrated in different studies[66-70] investigating its role in prostate cancer tissue compared to normal tissue, this is the first study to report the association of polymorphisms in genomic growth hormone genes (*GH1*, *GHR*, *GHRH*, *GHRHR*, *GHRL* and *GHSR*) and prostate cancer risk.

The results reveal that 8 *GH* SNPs (out of the 50 SNPs genotyped) are associated with prostate cancer risk. *GH1* SNP 1, *GH1* SNP 2, and *GHRL* SNP 7 showed a decreased frequency of the recessive allele in cases compared to control, and the frequency of the heterozygote genotype also had a decreased frequency in cases compared to controls. *GH1* SNP 2 and *GHRL* SNP 7 showed a significantly decreased risk of prostate cancer for the heterozygote carriers, and *GH1* SNP 1 showed a significantly decreased risk of prostate cancer for the rare homozygote carriers. Of these SNPs, *GH1* SNP 2 and *GHRL* 7 had the strongest association with prostate cancer. It should be noted that *GH1* SNP 1 and *GH1* SNP 2 were in HWE for controls, but not for cases.

As described above, *GHR* SNP 2 was associated with increased risk of prostate cancer. A recent study found an association between a *GHR* haplotype and increased prostate cancer risk.[91] McKay et al. investigated the 92 *GHR* SNPs in the haplotype analysis, and the haplotype block that was significantly related to prostate cancer risk contained *GHR* SNP 2. These findings were based on a larger sample size (cases, n =2,863; controls, n=1,737) and this strengthens the

results from the present study. Haplotype analysis was completed in the present study, but the findings were not significant.

GHR SNP 2, *GHRH* SNP 1, *GHRHR* SNP 9, and *GHRHR* SNP 10 showed an increased frequency of the recessive allele in cases compared to controls. The frequency of heterozygote and rare homozygote carriers of *GHR* SNP 2, *GHRHR* SNP 9, and *GHRHR* SNP 10 were higher in cases compared to controls. The frequency of rare homozygote carriers of *GHRH* SNP 1 was higher in cases compared to controls. In addition, *GHR* SNP 2, *GHRHR* SNP 9, and *GHRHR* SNP 10 showed an increased risk of prostate cancer for heterozygote carriers, and *GHR* SNP 2 and *GHRH* SNP 1 showed an increased risk of prostate cancer for rare homozygote carriers.

Although these 8 *GH* SNPs were associated with prostate cancer risk, there were some differences in association between the different case groups (e.g., combined, prevalent, and incident) and controls. *GH1* SNP 1, for example, was associated with prostate cancer risk in the combined case-control group, whereas *GH1* SNP 2 was associated with prostate cancer risk in combined and prevalent case-control groups. However, these are two independent groups of cases, and it provides evidence for replication of these findings in other study populations.

GHSR SNP 4 showed mixed results between the different groups. Heterozygote carriers of *GHSR* SNP 4 showed a significant decrease in prostate cancer risk in the prevalent case-control group, whereas the opposite effect was found in the incident case-control group, which showed a non-significant increase in prostate cancer risk. Likewise, rare homozygote carriers showed an increased risk of prostate cancer in the incident case-control group, whereas the prevalent case-control group showed a non-significant decrease in risk. There are several possibilities for these mixed results. It is possible that these findings are false positive and a larger sample size with more cases would provide greater insight on these specific findings. In

addition, this gene may be associated with more aggressive cases resulting in a survival bias. Last, there are differences in ascertainment of case status in prevalent versus incident groups. Prevalent cases reported a history of prostate cancer at the baseline self-report questionnaire, and incident cases reported prostate cancer incidence at yearly follow-ups, with corroborating data from medical records.

In addition, there were associations of baseline characteristics with *GH* SNPs (See Table 7 in Appendix C). However, these SNPs (with the exception of *GHR* SNP9) remained significantly associated with prostate cancer risk after adjustment for the baseline characteristics. The results suggest that *GH* genotypes influence risk of prostate cancer independently of an effect of age, diabetes and body composition.

The ancillary study utilized tagging for inclusion of SNPs; in addition, SNPs were included if they were shown to have possible functional effects. According to the NCBI Single Nucleotide Polymorphism database, *GHR* SNP 9, *GHRH* SNP 1, and *GHRL* 7 contain polymorphisms in the intron. Several studies show evidence that introns are involved in development of cancer.[84-86] These SNPs were also significantly associated with prostate cancer in cases (combined, prevalent, or incident) compared to controls. Malkinson[87] hypothesized that introns of genes whose products influence tumor development can also affect cancer incidence and regulatory mechanisms that control growth and differentiation steps may be controlled by their intronic structure. Mutations in introns may influence development of a neoplasm because introns can affect the various steps involved in normal expression of the gene even though the intron is not in the final protein.[88]

GHRHR SNP 9 and *GHRHR* SNP 10 were found to have possible functional effects as missense SNPs and alternative splicing. In addition, *GHRHR* SNP 9 and SNP 10 would be in

different regions depending on which splicing of the gene is present. Both SNPs are located in the promoter / regulatory region upstream, with splice variants located in the coding region. The splice variants were found to have the possible functional effect of missense (non-conservative) that causes the domain to be abolished. One study[69] investigated gene expression and prostate cancer tissue; they found that a splice variant of *GHRHR* differs from the full-length receptor in a small part of the extracellular portion of the receptor protein which could affect the strength of the binding of *GHRH* to its receptor. In addition, another study found that cells transfected with *GHRHR* splice variant proliferate faster than the full-length receptor.[89]

There are limitations in this study which should be discussed in the context of the findings. First, there is a relatively small sample size which places limitations on the statistical power to detect effects due to genotype. As another issue, a case-control association study with multiple SNPs or haplotypes in multiple genes represents a statistical multiple comparisons problem. However, 19% of the 50 *GH* SNPs investigated were significantly associated with cases compared to controls suggesting the GH pathway is important. Even though there are methods for addressing multiple comparisons in genetic epidemiology studies, no standard approach has been universally adopted.[90] Second, several SNPs within the growth hormone genes were not included. There was a total of 8 *GH* SNPs (1 *GH1*, 1 *GHRH*, 2 *GHRHR*, and 4 *GHRL*) that failed Illumina and were not included in analysis; these SNPs may be associated with prostate cancer risk. Third, this study sample may be biased. More advanced cancer cases were likely to be excluded since they were taking bone-modulating medications. In addition to this, those included are more likely to have greater mobility and physical function, since men were excluded if they required assistance walking. This could result in a healthier sample population and may not be generalizable to the source population. Fourth, due to budgetary

constraints and sample size calculations, a random sample was taken only from the Pittsburgh and Minneapolis sites. However, those included were comparable to those excluded on all baseline measures except BMI.

A strength of this study includes the random sample from the larger sample of community-dwelling men. The MrOS study investigated risk factors for osteoporosis and fractures in older men; the present study, then, is not affected by selection bias since participants were not selected on the basis case-control status for prostate cancer. In addition, this is the first study to report on these *GH* SNPs and prostate cancer risk. The SNPs in this study were not investigated in the studies looking at the relationship between breast cancer,[47, 49, 53-56] pituitary tumors,[64, 66] and neuroendocrine tumors.[41, 42]

5.0 SUMMARY

In summary, this study investigated the effect of *GH1*, *GHR*, *GHRH*, *GHRHR*, *GHRL* and *GHSR* SNPs on the risk of prostate cancer. The results suggest that eight of these SNPS are associated with risk of prostate cancer. Sample size, power, and issues pertaining to multiple comparisons support the replication of these findings in larger studies. However, this is of public health significance; if the relationships observed in this study are confirmed, it would justify the investigation of approaches that would reduce the activity of GH in those at high risk of prostate cancer.

APPENDIX A

LIST OF ABBREVIATIONS

GH1	Growth Hormone 1
GHR	Growth Hormone Receptor
GHRH	Growth Hormone-Releasing Hormone
GHRHR	Growth Hormone-Releasing Hormone Receptor
GHRL	Ghrelin
GHSR	Growth Hormone Secretagogue Receptor
MAF	Minor Allele Frequency
OR	Odds Ratio
PC	Prostate Cancer
SNP	Single Nucleotide Polymorphism

APPENDIX B

SNPS ENTERING ANALYSIS

Table 6. Association of SNPs with case-control status

Gene, N (%)	Controls (n=743)	Combined Cases (n=128)	Prevalent Cases (n=74)	Incident Cases (n=54)
GH1 SNP 1 (rs2854184)				
0	338 (45.7)	73 (57.0) *	43 (58.1)	30 (55.6)
1	268 (36.3)	41 (32.0)	22 (29.7)	19 (35.2)
2	133 (18.0)	14 (10.9)	9 (12.2)	5 (9.3)
Test of Trend		***p=0.009***	***p=0.04***	p=0.08
GH1 SNP 2 (rs2070776)				
0	306 (41.2)	70 (54.7) **	41 (55.4) *	29 (53.7)
1	335 (45.1)	41 (32.0)	23 (31.1)	18 (33.3)
2	102 (13.7)	17 (13.3)	10 (13.5)	7 (13.0)
Test of Trend		***p=0.03***	p=0.09	p=0.17
GHR SNP 2 (rs10473282)				
0	363 (48.9)	53 (41.4)	36 (48.6)	17 (31.5) *
1	278 (37.40)	50 (39.1)	25 (33.8)	25 (46.3)
2	102 (13.7)	25 (19.5)	13 (17.6)	12 (22.2)
Test of Trend		p=0.05	p=0.64	***p=0.01***
GHR SNP 9 (rs12233949)				
0	393 (52.9)	64 (50.0)	35 (47.3) *	29 (53.7)
1	283 (38.1)	57 (44.5)	37 (50.0)	20 (37.0)
2	67 (9.0)	7 (5.5)	2 (2.7)	5 (9.3)
Test of Trend		p=0.91	p=0.93	p=0.95
GHRH SNP 1 (rs2050093)				
0	495 (66.7)	79 (61.7) *	48 (64.9)	31 (57.4) *
1	224 (30.2)	39 (30.5)	21 (28.4)	18 (33.3)
2	23 (3.1)	10 (7.8)	5 (6.8)	5 (9.3)
Test of Trend		p=0.07	p=0.41	p=0.05
GHRHR SNP 9 (rs4988498)				
0	673 (90.9)	110 (87.3) ***	61 (83.6) ***	49 (92.5)
1	67 (9.1)	14 (11.1)	10 (13.7)	4 (7.5)
2	0 (0)	2 (1.6)	2 (2.7)	0 (0)

Table 6 (Continued)

Test of Trend		p=0.07	***p=0.007***	p=0.71
GHRHR SNP 9 (rs4988498)				
0	673 (90.9)	110 (87.3)	61 (83.6) *	49 (92.5)
1	67 (9.1)	16 (12.7)	12 (16.4)	4 (7.5)
GHRHR SNP 10 (rs740336)				
0	717 (96.6)	118 (92.2) ***	69 (93.2)	49 (90.7) ***
1	25 (3.4)	9 (7.0)	5 (6.8)	4 (7.4)
2	0 (0)	1 (0.8)	0 (0)	1 (1.9)
Test of Trend		***p=0.008***	p=0.14	***p=0.006***
GHRHR SNP 10 (rs740336)				
0	717 (96.6)	118 (92.2) **	69 (93.2)	49 (90.7) *
1	25 (3.4)	10 (7.8)	5 (6.8)	5 (9.3)
GHRL SNP 7 (rs35668)				
0	336 (45.3)	74 (57.8) **	45 (60.8) *	29 (53.7)
1	206 (27.8)	23 (18.0)	15 (20.3)	8 (14.8)
2	200 (27.0)	31 (24.2)	14 (18.9)	17 (31.5)
Test of Trend		p=0.05	***p=0.02***	p=0.74
GHSR SNP 4 (rs2922126)				
0	334 (45.0)	58 (45.3)	41 (55.4)	17 (31.5) **
1	333 (44.9)	50 (39.1)	25 (33.8)	25 (46.3)
2	75 (10.1)	20 (15.6)	8 (10.8)	12 (22.2)
Test of Trend		p=0.41	p=0.23	***p=0.006***

*$0.05 > p \geq 0.025$; **$0.025 > p \geq 0.01$; ***$p < 0.01$. Notes: χ^2 p-values for cases vs. controls; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare; **GH1**: growth hormone 1 SNPs; **GHR**: growth hormone receptors SNPs; **GHRH**: growth hormone-releasing hormone SNPs; **GHRHR**: GHRH-receptor SNPs; **GHRL**: ghrelin precursor SNPs; **GHSR**: growth hormone secretagogue receptor SNPs.

APPENDIX C

Table 7. Association of GH SNPs and Baseline Characteristics

Gene, N (%)	Total Sample, N (%) (n=871)	Age, mean years (SD)	Weight, mean lb (SD)	Height, mean cm (SD)	BMI, mean kg/m^2 (SD)	Trunk % Fat, mean (SD)	Total % Fat, mean (SD)	Diabetes, N (%), (n=110)
GH1 SNP 1 (rs2854184)								
0	411 (47.4)	73.9 (5.7)	188.4 (32.0)	173.9 (6.8)	28.3 (4.3)	29.3 (6.4)	26.9 (5.7)	45 (40.9)
1	309 (35.6)	73.7 (5.8)	186.4 (29.9)	173.4 (6.4)	28.1 (4.0)	29.1 (5.9)	26.8 (5.2)	48 (43.6)
2	147 (17.0)	72.7 (5.6)	188.8 (29.1)	173.3 (7.5)	28.5 (3.6)	29.7 (5.4)	27.2 (4.9)	17 (15.5)
GH1 SNP 2 (rs2070776)								
0	376 (43.2)	73.9 (5.7)	188.4 (32.0)	173.9 (6.8)	28.3 (4.3)	29.3 (6.4)	26.9 (5.7)	45 (40.9)
1	376 (43.2)	73.7 (5.8)	186.4 (29.9)	173.4 (6.4)	28.1 (4.0)	29.1 (5.9)	26.8 (5.2)	48 (43.6)
2	119 (13.7)	72.7 (5.6)	188.8 (29.1)	173.3 (7.5)	28.5 (3.6)	29.7 (5.4)	27.2 (4.9)	17 (15.5)
GHR SNP 2 (rs10473282)								
0	416 (47.8)	73.6 (6.0)	187.5 (29.4)	173.2 (6.7)	28.4 (4.1)	29.1 (6.0)	26.7 (5.2)	41 (37.3)
1	328 (37.7)	5.6 (5.6)	186.0 (31.3)	173.6 (6.7)	28.0 (4.1)	29.0 (6.0)	26.7 (5.4)	48 (43.6)
2	127 (14.6)	73.8 (5.5)	192.6 (30.7)	174.3 (7.3)	28.7 (3.7)	30.6 (6.1)	28.0 (5.2)	21 (19.1)
GHR SNP 9 (rs12233949)								
0	457 (52.5)	73.5 (5.6)	186.1 (30.3)	173.4 (6.8)	28.1 (4.0)	29.1 (6.0)	26.7 (5.3)	57 (51.8)
1	340 (39.0)	73.9 (5.7)	188.0 (30.7)	173.8 (6.9)	28.2 (4.1)	29.2 (6.0)	26.9 (5.3)	42 (38.2)
2	74 (8.5)	72.9 (6.2)	194.3 (30.7)	173.3 (6.0)	29.3 (3.9)	30.7 (6.0)	28.3 (5.5)	11 (10.0)
GHRH SNP 1 (rs2050093)								
0	574 (66.0)	74.0 (5.8) *	188.7 (30.9)	173.5 (6.8)	28.4 (4.2)	29.5 (6.1)	27.1 (5.3)	68 (61.8)
1	263 (30.2)	72.6 (5.4)	185.0 (29.7)	173.8 (6.6)	27.8 (3.8)	28.8 (5.8)	26.4 (5.3)	36 (32.7)
2	33 (3.8)	74.7 (5.5)	187.3 (30.5)	172.4 (7.2)	28.5 (3.4)	30.1 (6.3)	27.5 (5.7)	6 (5.5)
GHRHR SNP 9 (rs4988498)								
0	783 (90.4)	73.6 (5.7)	188.3 (30.9) *	173.8 (6.8) **	28.3 (4.1)	29.3 (6.1)	26.9 (5.4)	102 (93.6)
1	81 (9.4)	73.7 (6.0)	180.5 (26.4)	171.7 (6.5)	27.8 (3.8)	29.1 (5.2)	26.6 (4.7)	6 (5.5)
2	2 (0.2)	75.5 (0.71)	191.1 (7.3)	169.3 (5.6)	30.3 (0.84)	30.6 (1.03)	28.9 (0.23)	1 (0.9)

Table 7 (Continued)

GHRHR SNP 10 (rs740336)								
0	835 (96.0)	73.6 (5.7)	187.7 (30.6)	173.5 (6.7)	28.3 (4.0)	29.4 (6.0)	26.9 (5.3)	106 (96.4)
1	34 (3.9)	73.3 (5.7)	181.9 (29.8)	174.0 (7.5)	27.4 (5.5)	26.8 (6.7)	24.9 (5.7)	3 (2.7)
2 ***	1 (0.1)							1 (0.9)
GHRL SNP 7 (rs35668)								
0	410 (47.1)	73.7 (5.8)	186.6 (31.8)	172.8 (6.9) **	28.3 (4.1)	29.5 (5.7)	26.9 (4.9)	24 (22.0) **
1	231 (26.6)	73.9 (5.9)	186.9 (31.6)	173.4 (6.7)	28.1 (4.1)	29.4 (6.0)	26.9 (5.5)	42 (38.5)
2	229 (26.3)	73.1 (5.5)	190.6 (29.7)	174.5 (6.6)	28.4 (3.8)	29.1 (6.4)	26.8 (5.5)	43 (39.4)
GHSR SNP 4 (rs2922126)								
0	392 (45.1)	73.9 (5.7)	188.3 (30.9)	173.8 (6.9)	28.3 (4.11)	29.4 (5.9)	26.9 (5.2)	55 (50.0)
1	383 (44.0)	73.1 (5.8)	187.7 (31.2)	173.4 (6.6)	28.3 (4.1)	29.3 (6.2)	26.9 (5.5)	43 (39.1)
2	95 (10.9)	74.2 (5.6)	186.1 (32.2)	173.2 (7.0)	28.1 (3.9)	29.2 (6.0)	26.6 (5.0)	12 (10.9)

*$0.05 < p \geq 0.025$; **$p < 0.008$. Notes: ANOVA p-values for SNPs vs. baseline measures; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare; **GH1**: growth hormone 1 SNPs; **GHR**: growth hormone receptors SNPs; **GHRH**: growth hormone-releasing hormone SNPs; **GHRHR**: GHRH-receptor SNPs; **GHRL**: ghrelin precursor SNPs; **GHSR**: growth hormone secretagogue receptor SNPs.

APPENDIX D

Table 8. Hardy-Weinberg Equilibrium

	Cases and Controls (n=871)	Controls (n=743)	Cases (n=128)
Hardy-Weinberg Equilibrium	**χ^2 (p-value)**	**χ^2 (p-value)**	**χ^2 (p-value)**
GH1 SNP 1 (rs2854184)	0.27 (0.60)	2.13 (0.14)	***4.80 (0.03)***
GH1 SNP 2 (rs2070776)	2.57 (0.11)	0.45 (0.50)	***6.58 (0.01)***
GH1 SNP 3 (rs2070720)	0.20 (0.65)	1.49 (0.22)	3.03 (0.08)
GH1 SNP 4 (rs2058194)	0.01 (0.90)	0.002 (0.97)	0.14 (0.70)
GHR SNP 1 (rs3764451)	1.25 (0.26)	2.25 (0.13)	0.65 (0.42)
GHR SNP 2 (rs10473282)	0.07 (0.79)	0.92 (0.34)	2.47 (0.11)
GHR SNP 3 (rs1876790)	***5.79 (0.02)***	3.91 (0.05)	2.21 (0.14)
GHR SNP 4 (rs2036745)	***4.53 (0.03)***	2.79 (0.09)	2.21 (0.14)
GHR SNP 5 (rs11744988)	0.70 (0.40)	0.76 (0.38)	0.13 (0.71)
GHR SNP 6 (rs4866931)	1.73 (0.19)	1.04 (0.31)	0.94 (0.33)
GHR SNP 7 (rs4129472)	0.59 (0.44)	0.04 (0.84)	2.10 (0.15)
GHR SNP 8 (rs7736209)	0.06 (0.81)	0.35 (0.55)	0.67 (0.41)
GHR SNP 9 (rs12233949)	0.90 (0.34)	2.38 (0.12)	1.57 (0.21)
GHR SNP 10 (rs7709790)	0.23 (0.63)	0.32 (0.57)	0.02 (0.89)
GHR SNP 11 (rs7721081)	0.58 (0.45)	1.82 (0.18)	1.27 (0.26)
GHR SNP 12 (rs6179)	0.48 (0.49)	1.11 (0.29)	0.53 (0.46)
GHR SNP 13 (rs4242119)	0.57 (0.45)	1.41 (0.23)	0.79 (0.37)
GHR SNP 14 (rs6180)	0.02 (0.88)	0.13 (0.72)	0.25 (0.62)
GHR SNP 15 (rs719756)	0.06 (0.80)	0.22 (0.63)	0.25 (0.62)
GHRH SNP 1 (rs2050093)	0.17 (0.67)	0.15 (0.70)	2.54 (0.11)
GHRH SNP 2 (rs1073768)	0.02 (0.88)	0.05 (0.82)	0.89 (0.34)
GHRH SNP 3 (rs4988492)	0.34 (0.56)	0.27 (0.60)	0.07 (0.79)
GHRH SNP 4 (rs6032470)	0.003 (0.98)	0.07 (0.78)	0.33 (0.57)
GHRHR SNP 1 (rs7458593)	0.09 (0.75)	0.008 (0.93)	0.33 (0.57)
GHRHR SNP 2 (rs4723034)	0.15 (0.70)	0.003 (0.95)	1.27 (0.26)
GHRHR SNP 3 (rs7384927)	0.30 (0.58)	0.07 (0.79)	0.77 (0.38)
GHRHR SNP 4 (rs6954044)	0.76 (0.38)	0.24 (0.62)	1.13 (0.29)
GHRHR SNP 5 (rs2302019)	0.29 (0.58)	0.01 (0.91)	1.89 (0.17)

Table 8 (Continued)

GHRHR SNP 6 (rs2267721)	0.96 (0.33)	0.44 (0.51)	1.17 (0.28)
GHRHR SNP 7 (rs11771444)	0.31 (0.57)	0.66 (0.42)	0.09 (0.75)
GHRHR SNP 8 (rs2267723)	1.36 (0.24)	0.50 (0.48)	2.06 (0.15)
GHRHR SNP 9 (rs4988498)	0.004 (0.95)	1.66 (0.20)	3.32 (0.06)
GHRHR SNP 10 (rs740336)	1.10 (0.29)	0.22 (0.64)	2.69 (0.10)
GHRL SNP 1 (rs35682)	0.78 (0.38)	1.21 (0.27)	0.13 (0.71)
GHRL SNP 2 (rs10490815)	1.41 (0.23)	3.10 (0.08)	1.31 (0.25)
GHRL SNP 3 (rs10490816)	0.44 (0.51)	0.93 (0.33)	0.34 (0.56)
GHRL SNP 4 (rs1629816)	2.03 (0.15)	2.35 (0.12)	0.0001 (0.99)
GHRL SNP 5 (rs696221)	0.45 (0.50)	0.43 (0.51)	0.03 (0.86)
GHRL SNP 6 (rs697231)	***4.68 (0.03)***	***4.68 (0.03)***	0.18 (0.67)
GHRL SNP 7 (rs35668)	2.87 (0.09)	***6.59 (0.01)***	3.31 (0.07)
GHRL SNP 8 (rs703915)	0.03 (0.87)	0.06 (0.80)	0.04 (0.84)
GHSR SNP 1 (rs558572)	0.003 (0.96)	0.005 (0.94)	0.0008 (0.97)
GHSR SNP 2 (rs1403637)	1.59 (0.21)	1.39 (0.23)	0.17 (0.68)
GHSR SNP 3 (rs4144707)	0.25 (0.61)	0.04 (0.84)	0.94 (0.33)
GHSR SNP 4 (rs2922126)	0.13 (0.72)	0.43 (0.51)	0.22 (0.64)
GHSR SNP 5 (rs9819506)	0.05 (0.82)	0.02 (0.88)	0.01 (0.90)
GHSR SNP 6 (rs863441)	0.20 (0.65)	0.19 (0.66)	0.01 (0.90)
GHSR SNP 7 (rs9881073)	0.68 (0.41)	1.35 (0.24)	0.43 (0.51)
GHSR SNP 8 (rs11713751)	3.77 (0.05)	1.95 (0.16)	2.01 (0.16)
GHSR SNP 9 (rs12638147)	0.54 (0.46)	0.70 (0.40)	0.01 (0.91)

APPENDIX E

LINKAGE DISEQUILIBRIUM

E.1 GH1 SNPS

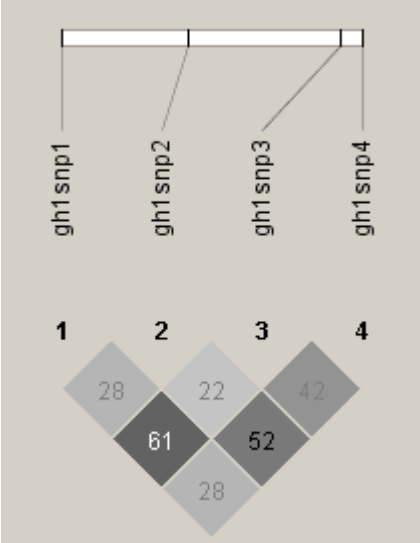

Figure 6 Linkage Disequilibrium of GH1 SNPs

LD structure across the GH1 gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GH1 gene. The color code shows r^2 value of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination (white) or higher correlation (darker). LD was calculated for the entire sample.

E.2 GHR SNPS

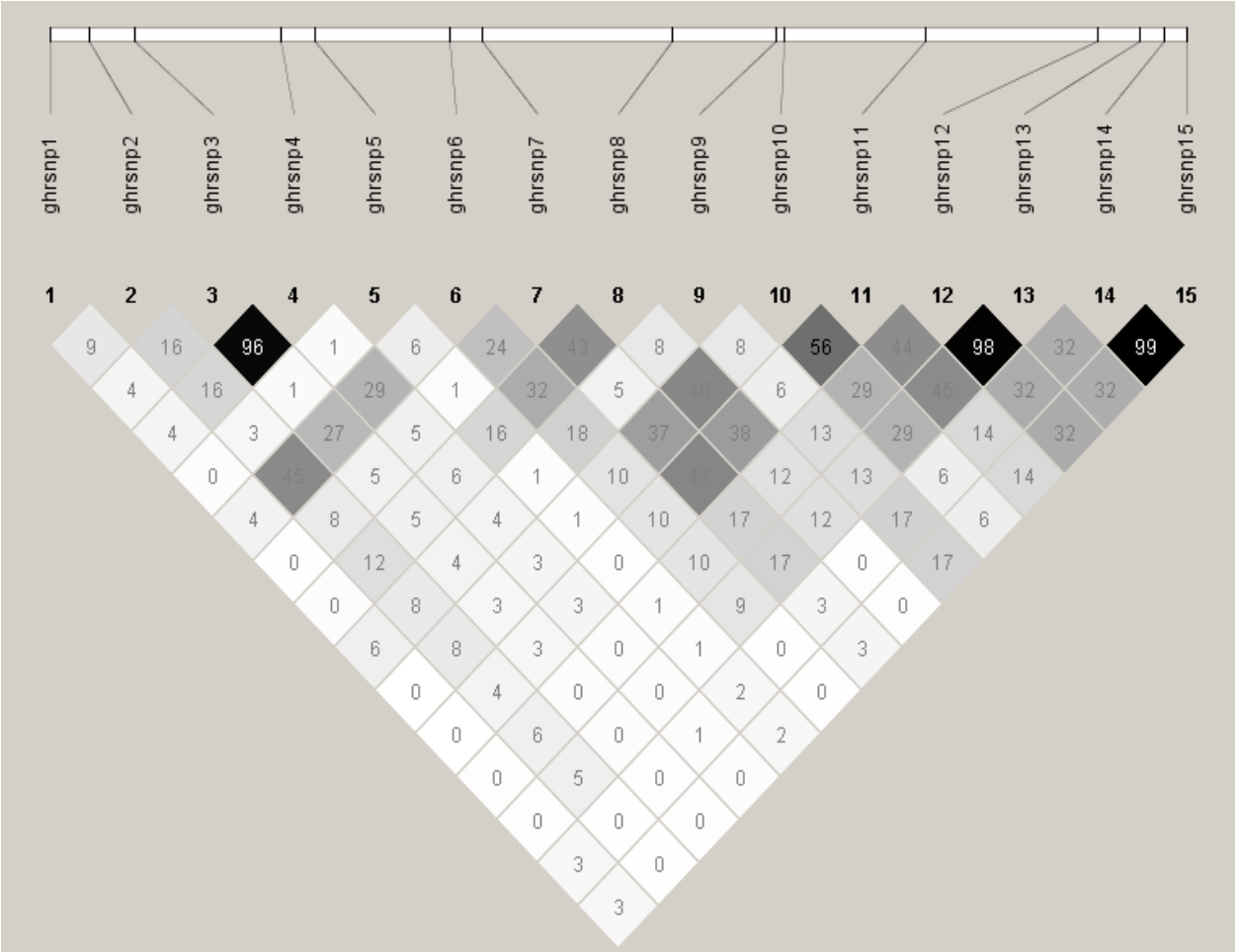

Figure 7. Linkage Disequilibrium of GHR SNPs

LD structure across the GHR gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GH1 gene. The color code shows r^2 value of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination (white) or higher correlation (darker). LD was calculated for the entire sample.

E.3 GHRH SNPS

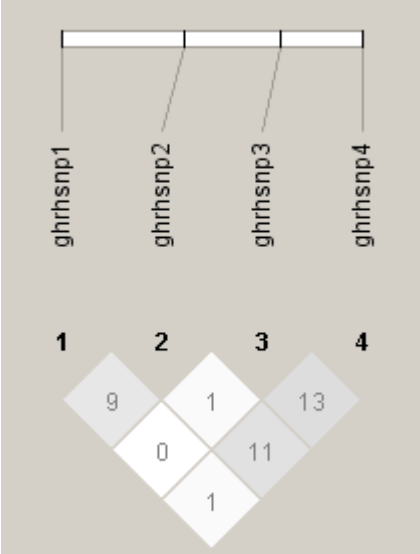

Figure 8. Linkage Disequilibrium of GHRH SNPs

LD structure across the GHRH gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GH1 gene. The color code shows r^2 value of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination (white) or higher correlation (darker). LD was calculated for the entire sample.

E.4 GHRHR SNPS

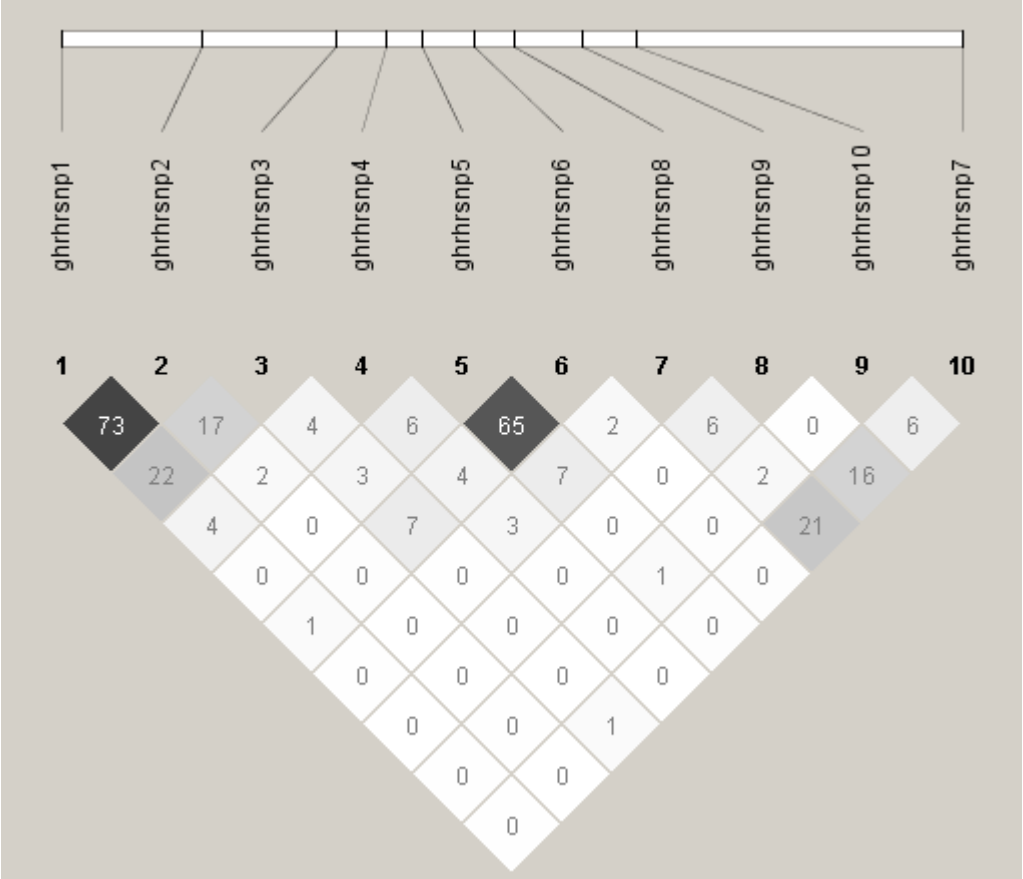

Figure 9. Linkage Disequilibrium of GHRHR SNPs

LD structure across the GHRHR gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GH1 gene. The color code shows r^2 value of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination (white) or higher correlation (darker). LD was calculated for the entire sample.

E.5 GHRL SNPS

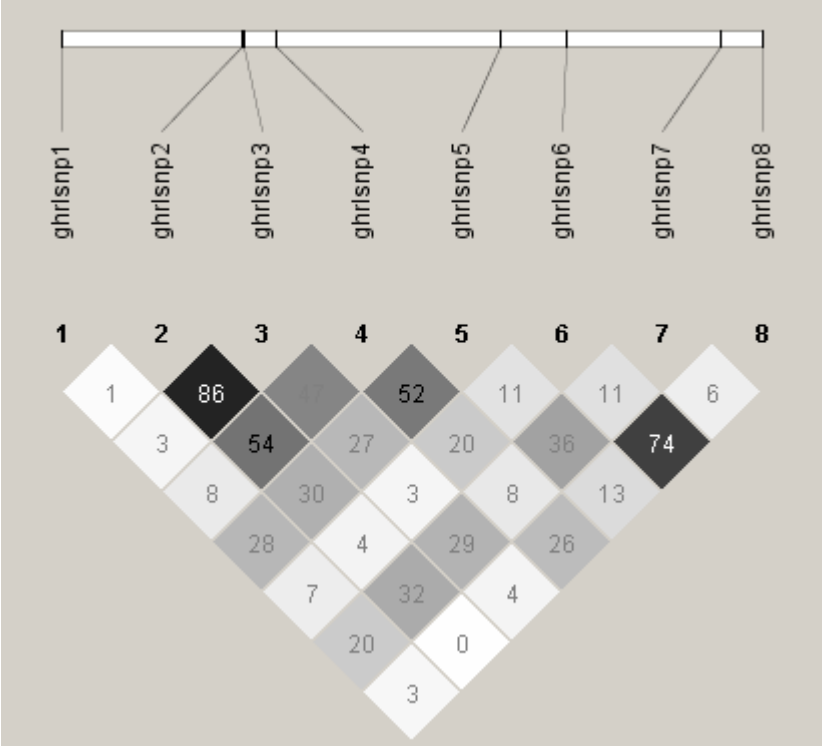

Figure 10. Linkage Disequlibrium of GHRL SNPs

LD structure across the GHRL gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GH1 gene. The color code shows r^2 value of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination (white) or higher correlation (darker). LD was calculated for the entire sample.

E.6 GHSR SNPS

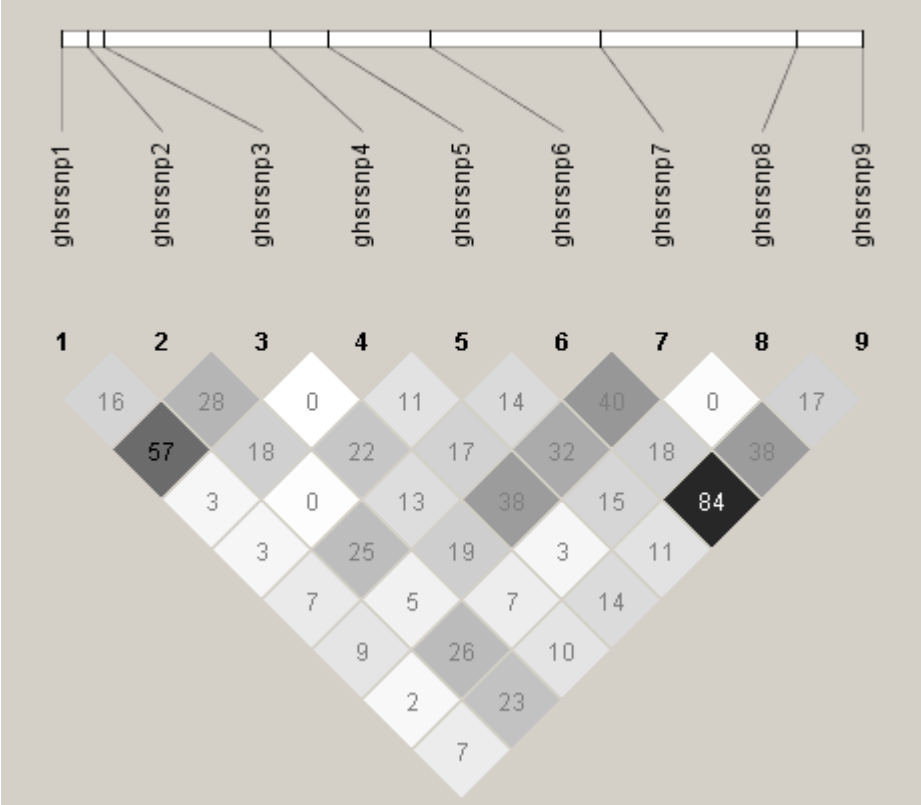

Figure 11. Linkage Disequilibrium of GHSR SNPs

LD structure across the GHSR gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GH1 gene. The color code shows r^2 value of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination (white) or higher correlation (darker). LD was calculated for the entire sample.

APPENDIX F

FREQUENCY, REGION AND FUNCTION OF SNPS

Table 9. GH1 SNPs Frequency, Region and Function

Gene, N (%)	Controls (n=743)	Cases (n=128)	Region / Function
GH1 SNP 1 (rs2854184)			
0	338 (45.7)	73 (57.0)	
1	268 (36.3)	41 (32.0)	
2	133 (18.0)	14 (10.9)	
p-value		*0.035*	
GH1 SNP 2 (rs2070776)			
0	335 (45.1)	70 (54.7)	Intron / synonymous
1	306 (41.2)	41 (32.0)	
2	102 (13.7)	17 (13.3)	
p-value		*0.011*	
GH1 SNP 3 (rs2070720)			
0	333 (44.9)	67 (52.3)	synonymous
1	317 (42.7)	50 (39.1)	
2	92 (12.4)	11 (8.6)	
p-value		0.11	
GH1 SNP 4 (rs2058194)			
0	371 (49.9)	61 (47.7)	nonsynonymous
1	206 (27.7)	41 (32.0)	
2	166 (22.3)	26 (20.3)	
p-value		0.60	

NOTES: p-value (χ^2) of cases vs. controls; **GH1**: growth hormone 1 SNPs; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare.

Table 10. GHR SNPs Frequency, Region and Function

GHR SNPs			
Gene, N (%)	Controls (n=743)	Cases (n=128)	Region / Function
GHR SNP 1 (rs3764451)			
0	557 (75.0)	97 (75.8)	
1	167 (22.5)	30 (23.4)	
2	19 (2.6)	1 (0.8)	
p-value		0.46	
GHR SNP 2 (rs10473282)			
0	363 (48.9)	53 (41.4)	
1	278 (37.4)	50 (39.1)	
2	102 (13.7)	25 (19.5)	
p-value		0.14	
GHR SNP 3 (rs1876790)			
0	475 (63.9)	81 (63.3)	
1	227 (30.6)	38 (29.7)	
2	41 (5.5)	9 (7.0)	
p-value		0.79	
GHR SNP 4 (rs2036745)			
0	475 (64.0)	81 (63.3)	Intron
1	228 (30.7)	38 (29.7)	
2	39 (5.3)	9 (7.0)	
p-value		0.71	
GHR SNP 5 (rs11744988)			
0	658 (88.6)	120 (93.8)	intron
1	81 (10.9)	8 (6.3)	
2	4 (0.5)	0 (0)	
p-value		0.19	
GHR SNP 6 (rs4866931)			
0	355 (47.8)	58 (45.3)	Intron
1	222 (29.9)	41 (32.0)	
2	165 (22.2)	29 (22.7)	
p-value		0.85	
GHR SNP 7 (rs4129472)			
0	509 (68.5)	87 (68.0)	Intron
1	211 (28.4)	34 (26.6)	
2	23 (3.1)	7 (5.5)	
p-value		0.38	
GHR SNP 8 (rs7736209)			
0	435 (58.6)	78 (61.4)	Intron
1	270 (36.4)	41 (32.3)	
2	37 (5.0)	8 (6.3)	
p-value		0.60	
GHR SNP 9 (rs12233949)			
0	393 (52.9)	64 (50.0)	intron
1	283 (38.1)	57 (44.5)	
2	67 (9.0)	7 (5.5)	
p-value		0.23	
GHR SNP 10 (rs7709790)			
0	487 (65.5)	88 (68.8)	Intron
1	232 (31.2)	36 (28.1)	

Table 10 (Continued)

2	24 (3.2)	4 (3.1)	
p-value		0.77	
GHR SNP 11 (rs7721081)			
0	539 (72.5)	92 (71.9)	Intron
1	193 (26.0)	31 (24.2)	
2	11 (1.5)	5 (3.9)	
p-value		0.16	
GHR SNP 12 (rs6179)			
0	391 (52.7)	69 (54.3)	Synonymous
1	303 (40.8)	47 (37.0)	
2	48 (6.5)	11 (8.7)	
p-value		0.54	
GHR SNP 13 (rs4242119)			
0	394 (53.1)	71 (55.5)	Intron
1	302 (40.7)	46 (35.9)	
2	46 (6.2)	11 (8.6)	
p-value		0.43	
GHR SNP 14 (rs6180)			
0	375 (50.5)	61 (47.7)	Nonsynonymous
1	207 (27.9)	37 (28.9)	
2	161 (21.7)	30 (23.4)	
p-value		0.83	
GHR SNP 15 (rs719756)			
0	376 (50.7)	61 (47.7)	
1	206 (27.8)	37 (28.9)	
2	160 (21.6)	30 (23.4)	
p-value		0.81	

NOTES: p-value (χ^2) of cases vs. controls; **GHR**: growth hormone receptor SNPs; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare.

Table 11. GHRH SNPs Frequency, Region and Function

Gene, N (%)	Controls (n=743)	Cases (n=128)	Region / Function
GHRH SNP 1 (rs2050093)			
0	495 (66.7)	79 (61.7)	Intron
1	224 (30.2)	39 (30.5)	
2	23 (3.1)	10 (7.8)	
p-value		*0.033*	
GHRH SNP 2 (rs1073768)			
0	362 (48.9)	69 (53.9)	
1	232 (31.4)	34 (26.6)	
2	146 (19.7)	25 (19.5)	
p-value		0.50	
GHRH SNP 3 (rs4988492)			
0	714 (96.2)	122 (95.3)	Nonsynonymous
1	28 (3.8)	6 (4.7)	
2	0 (0)	0 (0)	
p-value		0.62	
GHRH SNP 4 (rs6032470)			
0	565 (76.1)	92 (71.9)	
1	164 (22.1)	34 (26.6)	
2	13 (1.8)	2 (1.6)	
p-value		0.54	

NOTES: p-value (χ^2) of cases vs. controls; **GHRH**: growth hormone-releasing hormone SNPs; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare.

Table 12. GHRHR SNPs Frequency, Region and Function

GHRHR SNPs			
Gene, N (%)	**Controls (n=743)**	**Cases (n=128)**	**Region / Function**
GHRHR SNP 1 (rs7458593)			
0	564 (75.9)	92 (71.9)	
1	167 (22.5)	34 (26.6)	
2	12 (1.6)	2 (1.6)	
p-value		0.59	
GHRHR SNP 2 (rs4723034)			
0	469 (68.3)	72 (64.9)	
1	197 (28.7)	37 (33.3)	
2	21 (3.1)	2 (1.8)	
p-value		0.49	
GHRHR SNP 3 (rs7384927)			
0	355 (47.8)	62 (48.8)	
1	280 (37.7)	52 (40.9)	
2	108 (14.5)	13 (10.2)	
p-value		0.41	

Table 12 (Continued)

GHRHR SNP 4 (rs6954044)			
0	624 (84.1)	106 (82.8)	Locus
1	114 (15.4)	22 (17.2)	
2	4 (0.5)	0 (0)	
p-value		0.62	
GHRHR SNP 5 (rs2302019) *			
0	366 (49.3)	67 (52.3)	Upstream / with no known function
1	237 (31.9)	47 (36.7)	
2	139 (18.7)	14 (10.9)	
p-value		0.09	
GHRHR SNP 6 (rs2267721) *			
0	341 (46.0)	59 (46.1)	Intronic / with no known function
1	318 (42.9)	60 (46.9)	
2	82 (11.1)	9 (7.0)	
p-value		0.35	
GHRHR SNP 7 (rs11771444)			
0	440 (59.4)	69 (53.9)	
1	267 (36.0)	49 (38.3)	
2	34 (4.6)	10 (7.8)	
p-value		0.23	
GHRHR SNP 8 (rs2267723)			
0	378 (50.9)	70 (54.7)	Intron
1	216 (29.1)	40 (31.3)	
2	149 (20.1)	18 (14.1)	
p-value		0.28	
GHRHR SNP 9 (rs4988498) *			
0	673 (90.9)	110 (87.3)	Missense (nonconservative) / splicing regulation
1	67 (9.1)	14 (11.1)	(domain abolished)
2	0 (0)	2 (1.6)	5 utr promoter – regulatory region / coding
p-value		*0.002*	
GHRHR SNP 10 (rs740336) *			
0	717 (96.6)	118 (92.2)	Coding / missense (nonconsevative) / splicing
1	25 (3.4)	9 (7.0)	regulation (domain abolished); sense-synonymous /
2	0 (0)	1 (0.8)	splicing regulation (domain abolished); promoter -
p-value		*0.008*	regulatory region / 5 upstream; intronic / intronic

NOTES: p-value (χ^2) of cases vs. controls; **GHRHR**: growth hormone-releasing hormone receptor SNPs; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare.
*These genes are functional in our dataset.

Table 13. GHRL SNPs Frequency, Region and Function

GHRL SNPs			
Gene, N (%)	Controls (n=743)	Cases (n=128)	Region / Function
GHRL SNP 1 (rs35682)			
0	356 (48.0)	66 (51.6)	Intron
1	192 (25.9)	33 (25.8)	
2	194 (26.1)	29 (22.7)	
p-value		0.67	
GHRL SNP 2 (rs10490815)			
0	402 (54.1)	65 (50.8)	
1	276 (37.1)	56 (43.8)	
2	65 (8.7)	7 (5.5)	
		0.23	
GHRL SNP 3 (rs10490816)			
0	432 (58.2)	70 (54.7)	
1	262 (35.3)	51 (39.8)	
2	48 (6.5)	7 (5.5)	
p-value		0.59	
GHRL SNP 4 (rs1629816)			
0	316 (43.9))	57 (46.0)	
1	295 (41.0)	51 (41.1)	
2	108 (15.0)	16 (12.9)	
p-value		0.81	
GHRL SNP 5 (rs696221)*			
0	361 (48.7)	63 (49.2)	Downstream / 3utr / with no known function
1	211 (28.4)	34 (26.6)	
2	170 (22.9)	31 (24.2)	
p-value		0.89	
GHRL SNP 6 (rs697231)			
0	592 (79.8)	102 (79.7)	Intron
1	135 (18.2)	24 (18.8)	
2	15 (2.0)	2 (1.6)	
p-value		0.93	
GHRL SNP 7 (rs35668)			
0	336 (45.3)	74 (57.8)	Intron
1	206 (27.8)	23 (18.0)	
2	200 (27.0)	31 (24.2)	
p-value		*0.019*	
GHRL SNP 8 (rs703915)			
0	545 (73.5)	96 (75.0)	Intron
1	180 (24.3)	30 (23.4)	
2	16 (2.2)	2 (1.6)	
p-value		0.88	

NOTES: p-value (χ^2) of cases vs. controls; **GHRL**: ghrelin precursor SNPs; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare. *****These genes are functional in our dataset.

Table 14. GHSR SNPs Frequency, Region and Function

GHSR SNPs			
Gene, N (%)	Controls (n=743)	Cases (n=128)	Region / Function
GHSR SNP 1 (rs558572)			
0	493 (66.4)	87 (68.0)	
1	224 (30.2)	37 (28.9)	
2	25 (3.4)	4 (3.1)	
p-value		0.94	
GHSR SNP 2 (rs1403637)			
0	346 (46.6)	61 (47.7)	
1	259 (34.9)	40 (31.3)	
2	138 (18.6)	27 (21.1)	
p-value		0.67	
GHSR SNP 3 (rs4144707)			
0	377 (50.7)	69 (54.3)	
1	306 (41.2)	52 (40.9)	
2	60 (8.1)	6 (4.7)	
p-value		0.39	
GHSR SNP 4 (rs2922126)			
0	334 (45.0)	58 (45.3)	
1	333 (44.9)	50 (39.1)	
2	75 (10.1)	20 (15.6)	
p-value		0.14	
GHSR SNP 5 (rs9819506)			
0	369 (49.7)	73 (57.0)	
1	308 (41.5)	47 (36.7)	
2	66 (8.9)	8 (6.3)	
p-value		0.26	
GHSR SNP 6 (rs863441)			
0	407 (54.9)	73 (57.0)	
1	282 (38.0)	47 (36.7)	
2	53 (7.1)	8 (6.3)	
p-value		0.87	
GHSR SNP 7 (rs9881073)			
0	354 (47.6)	67 (52.3)	
1	220 (29.6)	37 (28.9)	
2	169 (22.7)	24 (18.8)	
p-value		0.52	
GHSR SNP 8 (rs11713751)			
0	325 (44.5)	47 (37.0)	
1	312 (42.7)	54 (42.5)	
2	94 (12.9)	26 (20.5)	
p-value		0.05	
GHSR SNP 9 (rs12638147)			
0	413 (55.6)	71 (55.5)	
1	276 (37.1)	49 (38.3)	
2	54 (7.3)	8 (6.3)	
p-value		0.91	

NOTES: p-value (χ^2) of cases vs. controls; **GHSR**: growth hormone secretagogue receptor SNPs; **0**: the common homozygote, **1**: the heterozygote, and **2**: the homozygous rare.

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