Research Paper

Association of growth hormone receptor gene variant with longevity in men is due to amelioration of increased mortality risk from hypertension

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Keywords: hypertension, growth hormone receptor, longevity, mortality
Received: March 19, 2021 **Accepted:** May 20, 2021 **Published:** June 1, 2021

ABSTRACT

The single nucleotide polymorphism (SNP) *rs4130113* of the growth hormone receptor gene (*GHR*) is associated with longevity. Here we explored whether longevity-associated genotypes protect against mortality in all individuals, or only in individuals with aging-related diseases. *Rs4130113* genotypes were tested for association with mortality in 3,557 elderly American men of Japanese ancestry. At baseline (1991–1993), 1,000 had diabetes, 730 had coronary heart disease (CHD), 1,901 had hypertension, 485 had cancer, and 919 lacked these diseases. The men were followed from baseline until Dec 31, 2019 or death (mean 10.8 ± 6.5 SD years, range 0.01–28.8 years; 99.0% deceased by that date). In a heterozygote disadvantage model, longevity-associated genotypes were associated with significantly lower mortality risk in individuals having hypertension (covariate-adjusted hazard ratio [HR] 0.83 [95% CI: 0.76–0.93, p = 4.3 x10^{-4}]. But in individuals with diabetes, CHD, and cancer there was no genotypic difference in lifespan. As expected, normotensive men outlived men with hypertension (p = 0.036). There was no effect, however, of genotypic difference on lifespan in normotensive men (p = 0.11). We found that SNP *rs4130113* potentially influenced the binding of transcription factors E2A, MYF, NRSF, TAL1, and TCF12 so as to alter *GHR* expression. We propose that in individuals with hypertension, longevity-associated genetic variation in *GHR* enhances cell resilience mechanisms to help protect against cellular stress caused by hypertension. As a result, hypertension-affected men who possess the longevity-associated genetic variant of *GHR* live as long as normotensive men.

INTRODUCTION

Growth hormone (GH) and its receptor (GHR) are not only important for regulating growth, they have many other important biological functions including response to nutrients, regulation of metabolism and controlling physiological processes related to the hepatobiliary, cardiovascular, renal, gastrointestinal, and reproductive systems [1, 2]. Growth hormone signaling is an important regulator of aging. GH deficiency leads to slower growth, delayed maturation, reduced body size, and can result in attenuation of the rate of aging,

increased health-span, and increased longevity [2]. Key to this are evolutionarily conserved pathways of insulin/insulin-like growth factors and mechanistic target of rapamycin, where there are trade-offs between anabolic processes/growth and lifespan. Accordingly, the GH deficient Ames dwarf mouse is long lived [3], whereas GH transgenic mice have shortened lifespans [2]. Disruption of the GHR in *Ghr*$^{-/-}$ mice leads to 55% and 38% longer lifespan in males and females, respectively [4]. Lifespan extension accompanying targeted deletion of both the GH releasing hormone gene and *Ghr* reduces lean body mass, bone mineral density, and increases adiposity [5]. GH signaling also increases the risk of cancer [2].

We have reported a significant negative association between height and longevity in our large cohort of American men of Japanese ancestry [6]. More recently, in a case-control study of 13 single nucleotide polymorphisms (SNPs) of *GHR* in this cohort (Supplementary Methods, Supplementary Study cohort, and Supplementary Table 1), SNP *rs4130113* was associated with greater lifespan of nonagenarian men aged ≥ 95 years [7] (Supplementary Table 2). Bonferroni corrected *p* value was 0.015 for *rs4130113* in a major allele (*A*) vs minor allele (*G*) carrier recessive model. Besides the longevity findings in *Ghr* null mice, we studied *GHR* because in mouse liver *GHR* was differentially expressed (downregulated 2.1-fold), in response to the well-established longevity effector, caloric restriction [8].

In the present longitudinal study, we tested the hypothesis that genetic variation in *GHR* affects lifespan at least in part by protection against the detrimental effects of one or more aging-related diseases, namely diabetes, hypertension, coronary heart disease, and/or cancer. We identify putative functional differences attributable to our longevity variant and describe how these changes may influence the phenotype of disease resilience.

RESULTS

Characteristics of subjects

Shown in Table 1 are baseline (1991–1993) characteristics of men in the study, adjusting for age, according to each genotype of *GHR* SNP *rs4130113*, and prevalence of medical conditions. Analyses found no evidence of population stratification in the dataset (data not shown). By December 31, 2019, 3521 out of 3557 (99.0%) subjects had died during the overall 29 years of follow-up (mean 10.8 ± 6.5 SD years – range 0.01–28.8 years). At baseline, among the 3557 participants, 28.5% had been diagnosed with diabetes, 53.4% with hypertension, 20.5% with CHD, and 13.6% with cancer. Mean age at death was 88.6 ± 6.1 years for men with at least one disease, and 89.5 ± 6.0 years for those with none ($p < 0.0001$). In hypertensive subjects, prevalence of diabetes, hypertension, CHD and cancer did not differ significantly between each genotype.

GHR genotype and survival in subjects with, and without, the various diseases

Table 2 shows the results for 3 genetic models, i.e., *AA* vs *AG*/*GG*, *AG* vs *AA*/*GG*, and *GG* vs *AA*/*AG* (where *G* is the minor allele), using Cox proportional hazard models by disease status for diabetes, hypertension, CHD and cancer.

The heterozygote disadvantage model showed a significant genotypic association with lifespan difference for just one of the 4 diseases, namely hypertension ($p = 0.00027$ for model 1 and $p = 0.0034$ for model 2; Table 2). After Bonferroni correction for the 32 comparisons in the multivariate analyses, the *p* values remained significant ($p = 0.0086$ and $p = 0.011$, respectively). Compared with the heterozygote (*AG*), being homozygous for either allele combined (*AA* or *GG*) showed significant protection against mortality in hypertensive subjects. The protection was similar for *AA* and *GG* ($p = 0.92$). However, in normotensive subjects, lifespan was significantly longer irrespective of *GHR* genotype (Kaplan-Meier Log-rank $\chi^2 = 24.2$, $p = 8.9 \times 10^{-7}$). Survival curves for hypertensive subjects and normotensive subjects according to whether their genotype was *AG* or *AA*/*GG* are shown in Figure 1. These curves were determined using a Cox proportional hazard model adding an interaction term of *GHR* with hypertension. Hazard ratios for homozygotes vs heterozygotes on mortality by hypertension status are shown in Table 3. In men with hypertension who had the longevity-associated genotype, mortality risk was reduced to normal (Figure 2). No genetic effects of *GHR* genotype on mortality were apparent in subjects with diabetes, CHD and cancer after Bonferroni correction (Table 2). For completeness, we also show the effect of *GHR* SNP *rs4130113* on mortality in the whole cohort irrespective of disease status (Supplementary Table 3). As can be seen, only the heterozygote disadvantage model showed an association of *rs4130113* with longevity.

In a major allele recessive model, carriers of the longevity-associated minor allele (*G*) of *rs4130113* showed a weaker association with lifespan difference in hypertensives compared with major allele homozygotes ($p = 0.015$ for model 1, and $p = 0.059$ for model 2) (Table 2), which were rendered nonsignificant by Bonferroni correction.

Table 1. Characteristics of all subjects at baseline by *GHR rs4130113* genotype.

Characteristics	*AA*	*AG*	*GG*	*p*
n	1256	1692	609	
Age at examination, y	77.9 ± 4.6	77.6 ± 4.6	77.6 ± 4.7	0.14
Birth year	1913.5 ± 4.6	1913.9 ± 4.6	1913.8 ± 4.7	0.10
Anthropometric and physiological				
Height, cm	161.6 ± 5.8	161.7 ± 5.6	162.1 ± 5.7	0.15
Weight, kg	61.7 ± 9.2	61 ± 9	61.9 ± 8.8	0.049
Waist to hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.44
BMI, kg/m^2	23.6 ± 3.2	23.3 ± 3.1	23.5 ± 2.9	0.078
Triceps skinfold thickness, mm	10.4 ± 4.1	9.9 ± 4.0	10.1 ± 3.8	0.013
Subscapular skinfold thickness, mm	16.6 ± 6.2	16.0 ± 6.0	15.8 ± 6	0.0053
Best forced expiratory volume, L	2.0 ± 0.5	2.1 ± 0.4	2.1 ± 0.5	0.11
Grip strength, kg	30.3 ± 5.9	30.2 ± 6.1	30.1 ± 6.5	0.90
Blood pressure, systolic, mmHg	149.9 ± 23.2	149.2 ± 23.6	148.7 ± 23.5	0.57
Blood pressure, diastolic, mmHg	80.1 ± 11.2	80 ± 11.5	79.5 ± 10.9	0.55
Cognitive (CASI) score	82.6 ± 14.5	82.6 ± 14.5	83.1 ± 14.8	0.76
Hematological and biochemical				
Total cholesterol, mg/dL	190.8 ± 33.1	189.1 ± 33.2	189.6 ± 31.2	0.37
HDL cholesterol, mg/dL	51.2 ± 13.6	51.1 ± 13.3	49.9 ± 12.8	0.10
Triglycerides, mg/dL	150.8 ± 95.4	147.3 ± 94.4	150.9 ± 89.1	0.53
Fasting plasma glucose, mg/dL	112.4 ± 26.6	113.3 ± 30.3	113.5 ± 32.5	0.67
Fasting plasma insulin, mIU/dL	16.0 ± 13.5	15.4 ± 13.7	15.0 ± 11.2	0.31
Plasma fibrinogen, mg/dL	306.7 ± 65.3	306.6 ± 62.1	307.4 ± 65.6	0.96
White blood cell count, 10^3/μL	6.2 ± 1.7	6.3 ± 2.5	6.2 ± 1.7	0.12
Health habits				
Current smoker, %	8.0	6.2	6.5	0.17
Past smoker, %	56.8	55.0	53.6	0.41
Smoking, pack-years	27.6 ± 35.4	25.4 ± 33.0	25.1 ± 34.4	0.20
Alcohol consumption, ounces/month	18.1 ± 40.1	19.6 ± 41.8	18.2 ± 37.8	0.58
Physical activity index, metabolic work/day	31 ± 4.6	30.8 ± 4.5	30.8 ± 4.6	0.38
Difficulty in walking 0.8 km, %	17.9	19.4	17.1	0.33
On diabetes medication, %	10.7	11.2	12.3	0.58
Diseases				
Hypertension (160/95), %	53.5	53.5	53.4	1.00
Coronary heart disease, %	19.8	20.3	22.5	0.40
Stroke history, %	4.2	3.9	5.9	0.11
Cancer, %	15.1	13.3	10.7	0.032
Diabetes, %	26.9	28.8	31.1	0.16
Depressive symptoms, %	9.1	10.2	10.3	0.61
Emphysema, %	2.6	2.8	3.5	0.55
Bypass history, %	7.2	6.7	8.5	0.32
Angioplasty, %	5.3	8.0	6.1	0.013
Ankle-brachial index < 0.9%, %	12.6	12.1	13.3	0.74
Sociodemographic				
Education, years	10.4 ± 3	10.6 ± 3.2	10.5 ± 3.1	0.16
Married, %	83.0	83.5	83.1	0.93

Genotype frequencies indicated Hardy-Weinberg equilibrium. The demographic data shown were age-adjusted.

Table 2. Hazard ratios (HR) of heterozygotes vs. homozygotes of *GHR* SNP *rs4130113* and other models with total mortality in men with diabetes, hypertension, CHD and cancer.

			With the disease		*Without the disease*	
Disorder (n with/w'out)	***Cox model***	***Genetic model†***	***HR¥ (95% CI)***	***p***	***HR (95% CI)***	***p***
Diabetes	1*	*AG vs. AA/GG*	1.04 (0.92-1.18)	0.54	1.07 (0.99-1.16)	0.090
(1000, 2508)	2**	*AG vs. AA/GG*	1.06 (0.92-1.21)	0.43	1.07 (0.98-1.16)	0.14
Hypertension	1	*AG vs. AA/GG*	1.18 (1.08-1.30)	0.00027	0.95 (0.86-1.05)	0.32
(1901, 1656)	2	*AG vs. AA/GG*	1.20 (1.09-1.33)	0.00034	0.92 (0.82-1.02)	0.11
CHD	1	*AG vs. AA/GG*	1.05 (0.91-1.22)	0.48	1.08 (1.00-1.16)	0.050
(730, 2827)	2	*AG vs. AA/GG*	1.03 (0.88-1.21)	0.71	1.08 (0.99-1.17)	0.077
Cancer	1	*AG vs. AA/GG*	0.98 (0.82-1.18)	0.85	1.09 (1.01-1.17)	0.022
(485, 3072)	2	*AG vs. AA/GG*	0.95 (0.78-1.17)	0.64	1.08 (1.00-1.17)	0.042
Diabetes	1	*AG/GG vs. AA*	1.04 (0.91-1.19)	0.54	1.02 (0.94-1.10)	0.70
(1000, 2508)	2	*AG/GG vs. AA*	1.05 (0.91-1.21)	0.52	1.00 (0.91-1.09)	0.92
Hypertension	1	*AG/GG vs. AA*	1.12 (1.02-1.24)	0.015	0.93 (0.84-1.03)	0.17
(1901, 1656)	2	*AG/GG vs. AA*	1.11 (1.00-1.23)	0.059	0.90 (0.81-1.01)	0.071
CHD	1	*AG/GG vs. AA*	1.07 (0.92-1.25)	0.37	1.02 (0.94-1.10)	0.63
(730, 2827)	2	*AG/GG vs. AA*	1.02 (0.86-1.21)	0.80	1.01 (0.93-1.10)	0.84
Cancer	1	*AG/GG vs. AA*	0.93 (0.77-1.11)	0.42	1.06 (0.98-1.14)	0.15
(485, 3072)	2	*AG/GG vs. AA*	0.88 (0.71-1.09)	0.24	1.04 (0.96-1.13)	0.37
Diabetes	1	*AA/AG vs. GG*	1.00 (0.85-1.18)	0.97	1.10 (0.99-1.22)	0.085
(1000, 2508)	2	*AA/AG vs. GG*	1.02 (0.86-1.21)	0.82	1.13 (1.01-1.27)	0.040
Hypertension	1	*AA/AG vs. GG*	1.10 (0.98-1.24)	0.11	1.03 (0.90-1.17)	0.68
(1901, 1656)	2	*AA/AG vs. GG*	1.16 (1.02-1.33)	0.028	1.01 (0.88-1.17)	0.86
CHD	1	*AA/AG vs. GG*	0.98 (0.82-1.18)	0.85	1.10 (1.00-1.22)	0.053
(730, 2827)	2	*AA/AG vs. GG*	1.02 (0.83-1.25)	0.87	1.12 (1.00-1.25)	0.041
Cancer	1	*AA/AG vs. GG*	1.12 (0.86-1.45)	0.41	1.06 (0.96-1.16)	0.24
(485, 3072)	2	*AA/AG vs. GG*	1.14 (0.85-1.53)	0.37	1.08 (0.98-1.20)	0.14
Diabetes	1	Additive	1.02 (0.93-1.11)	0.69	0.98 (0.93-1.04)	0.51
(1000, 2508)	2	Additive	1.01 (0.92-1.11)	0.76	0.96 (0.91-1.02)	0.24
Hypertension	1	Additive	1.02 (0.96-1.09)	0.44	0.96 (0.89-1.03)	0.24
(1901, 1656)	2	Additive	1.00 (0.94-1.07)	0.94	0.95 (0.88-1.03)	0.19
CHD	1	Additive	1.04 (0.94-1.15)	0.48	0.98 (0.93-1.03)	0.47
(730, 2827)	2	Additive	1.00 (0.90-1.12)	0.93	0.97 (0.92-1.03)	0.33
Cancer	1	Additive	0.94 (0.82-1.07)	0.32	1.01 (0.96-1.06)	0.75
(485, 3072)	2	Additive	0.91 (0.78-1.05)	0.19	0.99 (0.94-1.05)	0.84

Cox models: *Model 1: Age-adjusted; **Model 2: Covariate-adjusted, where covariates adjusted in Cox model were: age, BMI, glucose, smoking (pack-years), alcohol intake (ounces/month), physical activity index, depression, and stroke.

†Genetic models: Top: heterozygotes (*AG*) vs major allele homozygotes (*AA*) + minor allele homozygotes (*GG*). Middle: Major allele (*A*) vs minor allele (*G*) carrier recessive model. Bottom: Additive model.

¥HR, hazard ratio (95% confidence interval).

After Bonferroni correction for multiple testing for covariate adjusted models (in total 32 models), only the effect of *AG vs. AA/GG* with hypertension was significant, $p_B = 0.011$.

Functional annotations

In an attempt to determine how *GHR* may influence disease resistance and help to identify biological pathways we examined the following: (1) *GHR* tissue expression, (2) transcription factors (TFs) that might be modified by our sentinel SNP (*rs4130113*), (3) SNPs in linkage disequilibrium (LD) that might modify transcription factor binding, (4) the expression patterns of these TFs, and (5) the location of any *cis*-regulatory elements that are physically linked with our sentinel SNP. Supplementary Figure 1 shows that *GHR* is expressed most notably at high levels in adipose tissue, breast, liver, and muscle. We screened SNPs in and around *rs4130113* for functional annotations using the HaploReg database. Our longevity SNP was predicted to influence the binding of transcription factors E2A, MYF, NRSF, TAL1, and TCF12 (Supplementary Figure 2). The effect of the major (i.e., more common) allele is predicted to reduce binding of E2A, MYF, NRSF and TCF12, and increase binding of TAL1. Biological effects, biological pathways, and tissue expression of these transcription factors are shown in Supplementary Table 4, and include expression in muscle, neuronal cells, hematopoietic tissues and skin. While no other SNPs were in strong LD, three putative functional features were in moderate LD with *rs4130113* and were examined in more detail in Supplementary Figure 3 and Supplementary Table 5. A super-enhancer that is active in adipose tissue occupies a large portion of *GHR*. A 3,812 bp downstream promoter, *LOC107963949*, is specific for the most abundant version of the GHR protein (v1) found in liver. Variant *rs10941580* is an intronic expression QTL (ieQTL), which is a variant associated with differences in mRNA expression levels, and may also be a pre-mRNA splicing-related QTL (sQTL) that, by an influence on splicing, would affect protein isoform profiles in adipose tissue, muscle, nerve, thyroid, and breast tissues), as shown in Supplementary Table 6. This variant is also identified as causing "non-sense–mediated transcript decay" (Supplementary Table 4). As well, the SNP has been identified as an eQTL that influences expression and exon usage in the above

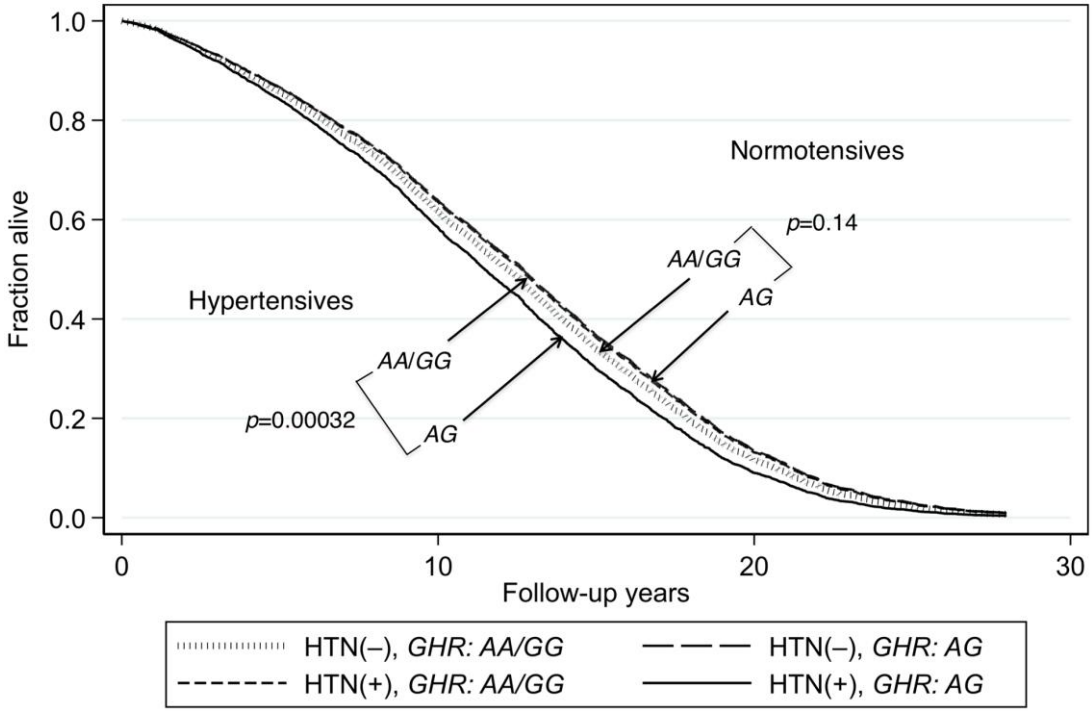


Figure 1. Survival curves spanning the period from baseline (1991–1993) to Dec 31, 2019 for subjects with and without hypertension according to genotypes of *GHR* SNP *rs4130113*. The survival probabilities were estimated from the Cox proportional hazard model: h(t) = h(t0) * exp(β1*Age + β2*BMI + β3*glucose + β4*hypertension + β5**GHR_AG* + β6* (hypertension**GHR_ AG*)), by fixing age at 75 years, BMI at the mean, 23.5 kg/m^2, and glucose at the mean, 113 mg/dL (where β6 is the effect of the interaction of hypertension with *GHR* genotype on mortality, for *AG* vs *AA*/*GG*, i.e., a heterozygote disadvantage model, giving *p*(β6) = 0.0004). Survival curves of *AG* vs. *AA*/*GG* for hypertensive subjects and subjects without hypertension (*p* = 0.0003 and *p* = 0.14, respectively). In men with hypertension who had the longevity-associated genotype *AA* and those with the *GG* genotype, the mortality risk was reduced to a level not significantly different from subjects without hypertension (hypertensive *AA*/*GG* vs. normotensive *AA*/*GG*: *p* = 0.20; hypertensive *AA*/*GG* vs normotensive *AG*: *p* = 0.78).

Table 3. Hazard ratios (HR) of homozygotes (*AA*, *GG*) vs. heterozygotes (*AG*) of *GHR* SNP, *rs4130113*, with total mortality in men by hypertension status.

		Hypertensive (n = 1901)		*Normotensive (n = 1656)*	
Cox model	***Genetic model†***	***HR¥ (95% CI)***	***p***	***HR (95% CI)***	***p***
1*	*AA vs AG*	0.85 (0.77-0.94)	0.0012	1.08 (0.97-1.20)	0.19
	GG vs AG	0.84 (0.74-0.96)	0.0079	1.00 (0.88-1.15)	0.996
2**	*AA vs AG*	0.87 (0.77-0.97)	0.011	1.11 (0.99-1.25)	0.0830
	GG vs AG	0.81 (0.71-0.94)	0.0041	1.02 (0.88-1.19)	0.7702

Cox models: *Model 1: Age-adjusted; **Model 2: Covariate-adjusted, where covariates adjusted in Cox model were: age, BMI, glucose, smoking (pack-years), alcohol intake (oz/mo), physical activity index, depression, cancer, and stroke, CHD, and diabetes.

†Genetic model: Heterozygote disadvantage.

¥HR, hazard ratio; CI, confidence interval.

tissues (see GTEx database). Since these variants are in a non-coding region of the genome, they are presumed to affect transcription and/or mRNA splicing/exon usage, whether directly or indirectly as chromatin modifying units (i.e., *cis*-regulatory elements). The tissue spectrum of *GHR* expression involves high levels in adipose tissue, breast, liver, and muscle, as shown in Supplementary Figure 1. According to the GTEx database, *GHR* has 13 different transcripts that result in five major isoforms. The majority of these are expressed in liver, muscle, breast, and adipose tissue (Supplementary Figure 4). Screening of *GHR* for eQTLs using the GTEx portal identified 4,568 entries in addition to *rs10941580* (not shown) and 18,128 sQTLs in addition to *rs10941580* (not shown). Data supporting the involvement of *rs10941580* with exon usage and the tissues involved are shown in Supplementary Table 6.

In an attempt to determine how the *GHR* longevity SNP might influence biological pathways and to try to define the role that *rs4130113* plays in resilience, we mapped the above potential regulatory sites using the WashU Genome Browser. Supplementary Figure 5 shows the location of *GHR* and its various transcripts (purple herring bones) and the location of the super-enhancer (bar) along with location of the three features: *rs4130113*, LOC107963949, and *rs10941580*. Shown in the Figure are data from ChIP-seq experiments, as follows: (a) DNAse I sensitive sites in muscle, (b) H3Kme3 histone marks in liver, (c), histone H3K27ac marks in muscle, (d) histone H3K27ac in liver, (e) histone H3K4me1 marks in liver, (f) H3K36me3 marks in liver, (g) histone marks in the HepG2 cell line (all of these using chromHMM, which is software for learning and characterizing chromatin states that can integrate multiple chromatin datasets such as ChIP-seq data of various histone modifications to discover *de novo* the major reoccurring combinatorial and spatial patterns of chromatin marks), (h) the location of HNF4A binding, which has been shown to induce the "downstream promoter", (i) locations of RNA polymerase II (RNAPII) binding, and (j) CTCF binding sites and chromatin loop domains. H3K4me3 and DNAse I hypersensitivity are associated with sites of open chromatin, which are associated with activation of transcription of nearby genes [9]. H3K27ac is associated with activation in promoters/enhancers, H3K4me1 with activation in enhancers, and H3K36me3 with activation in gene bodies. The *GHR* promoter, *rs4130113* and "downstream promoter" overlap with sites of H3K4me3, H3K27ac, and DNAse I hypersensitivity, as well as CTCF binding sites, the latter being able to serve as either 3-dimensional insulators or for the grouping of functional features together in *cis*-acting topological domains. Together these features are predicted to form a *cis*-regulatory unit consisting of the super-enhancer, *rs4130113*, and open chromatin sites. This is supported by the locations of CTCF binding sites that generally form insulator domains [10, 11].

DISCUSSION

The present study has found that the longevity-associated *AA* genotype (frequency 35.3%), but also the *GG* genotype (frequency 17.1%), of *GHR* SNP *rs4130113* is associated with protection against risk of mortality in hypertensive elderly American men of Japanese ancestry. As a result, those individuals lived longer, whereas individuals with the *AG* genotype (frequency 47.6%) died sooner. Moreover, the survival curve for hypertensive *AA*/*GG* subjects did not differ significantly from the survival curve for normotensive subjects with the *AA*/*GG* genotype. This indicated that possession of the *GHR* longevity-associated genotype can mitigate the adverse effects on lifespan of having hypertension.

Long-lived *Ghr*$^{-/-}$ mice have elevated subcutaneous fat mass, APOE and insulin sensitivity of cardiac and skeletal muscle, but lower body weight, plasma cholesterol, IGF-I, plasma insulin, glucose tolerance, and cancer [12]. In our subjects, elevated BMI was associated with lower mortality in old age (HR = 0.96; 95% 0.95-0.97, $p < 0.0001$). Lifespan of mice with liver- and fat-specific *Ghr* knockout was not affected, and only a modest lifespan extension in males was seen in muscle-specific *Ghr* knockout mice [13]. Moreover, *Ghr* knockout in almost fully-grown mice can still extend lifespan, indicating the importance of GH-related mechanisms in adulthood [14]. GH secretion is lower and more tightly controlled in offspring of long-lived families when compared with their partners [15]. Subtle differences in GH-related effects are apparent between mice and humans, possibly arising from the fast vs. slow pace of life in each [2]. A notable example is the GHR exon 3 deletion variant that is associated with GH sensitivity, greater height, lower serum IGF-1, and longevity in men [16]. Individuals homozygous for a *GHR* exon 3 deletion (*d3*/*d3*) were reported to exhibit increased lifespan [16]. We did not, however, find an association of *d3*/*d3* with longevity in our cohort. In the covariate-adjusted model, HR was 0.98 in the baseline sample. In the hypertensive subjects, HR was 1.02 (95% CI 0.84–1.24; $p = 0.84$). After adjusting for *d3*/*d3* in the covariate model, the HR for the effect of *GHR* heterozygotes on mortality in hypertensive subjects was unchanged, 1.20 (1.09–1.33; $p = 0.00034$).

Heterozygote disadvantage is when a heterozygote has a lower overall fitness than either homozygote, and can be a potent driver of population genetic divergence [17]. Why then would heterozygotes with hypertension be at a disadvantage? The effect of transcription factor binding, whether positive or negative, would be greater in homozygotes (*AA* and *GG*) than heterozygotes (*AG*). The repertoire of transcription factors would in turn be influenced by external factors, including a prevailing pathophysiological condition, in this case hypertension. To elucidate the mechanism explaining heterozygote disadvantage, future research should aim to ascertain the effect of each individual transcription factor on each genotype in the hypertensive vs normotensive state. As background, the GHR is either a monomer or homodimer. *GHR* is encoded by at least 10 exons, with exons 2–7 encoding the extracellular domain, exon 8 the transmembrane domain, and exons 9–10 the intracellular domain [18]. The predominant isoform, version v1, is shown as ENST00000230882.8 in Supplementary Figure 4. The exon 3 deletion variant, GHGRd3, is shown as ENST00000357703.6, and is the result of a deletion of the exon rather than being caused

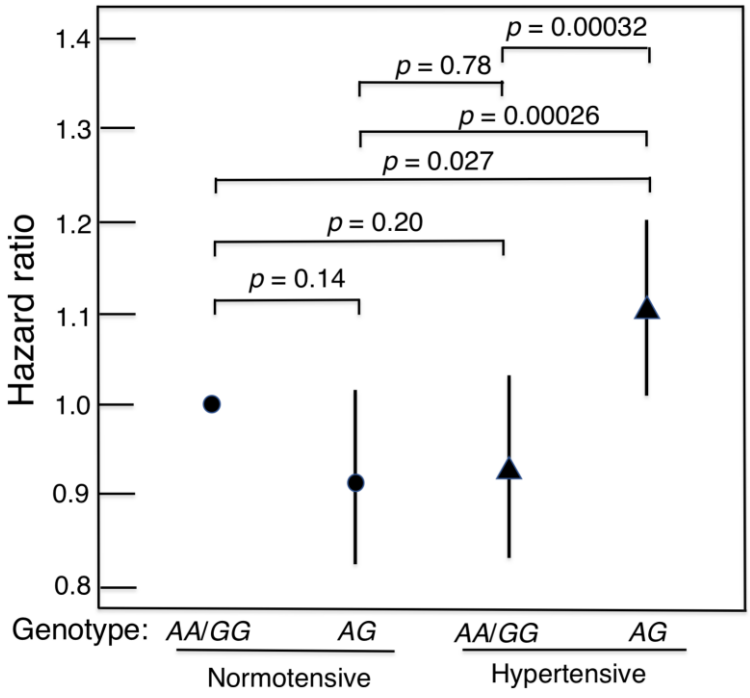


Figure 2. Mortality risk (hazard ratio), adjusted for age, BMI and glucose, for hypertensive subjects and normotensive subjects according to genotype of *GHR* SNP *rs4130113* in heterozygote disadvantage model, *AG* vs. *AA*/*GG*. It can be seen that in men with hypertension who had a longevity-associated genotype, mortality risk was reduced to normal in that it did not differ significantly from the survival curves of normotensive men.

by a splicing event. As can be seen in Supplementary Figure 4 there are multiple (as many as 13) transcripts caused by alternate splicing events, resulting in at least five protein isoforms that are differentially expressed, the majority of which are expressed largely in liver, muscle, and adipose tissue. Two of these alternatively spliced transcripts, at exon 9, GHR-(1–279) and GHR-(1–277), were identified in human liver [19, 20] and function as dominant negative inhibitors of the full-length receptor. GHR-(1–279) lacks the first 26 bp of exon 9 of the full-length receptor (GHRfl), whereas for GHR-(1–277) this exon is deleted in its entirety [20]. Both alternatively spliced isoforms result in a frame shift and a premature stop codon, leading to mRNAs with intact extracellular and transmembrane domains, but lacking more than 90% of the intracellular domain. While these receptor variants have no signaling capacity, they can inhibit GH action mediated by GHRfl in a dominant negative manner [20, 21]. Patients heterozygous for genetic variants or mutations that generate splicing-related deletion of exon 9 are GH-insensitive [21, 22], providing evidence for a pathophysiological role for these truncated receptors. We propose that the heterogeneity of protein isoforms helps to explain the disadvantage that heterozygotes with hypertension have for mortality. A search of the GTEx portal for other functional variants in *GHR* found that there are 18,128 neighboring SNPs associated with exon usage and 4,568 eQTL variants associated with expression levels. There is an ieQTL, *rs10941580*, that influences expression in adipose tissue, muscle, nerve, breast, and thyroid. An ieQTL is a *cis*-regulatory element that is predicted to influence the expression levels of a nearby gene [23].

Alternate splicing has been shown to change with age [24]. We believe that variants that influence transcript splicing are important factors in the heterozygote disadvantage model that we have found to be responsible for *GHR* resilience to aging-related morbidity risk.

In Japanese and other populations, hypertension increases risk of death from CHD [25], cerebrovascular accident (stroke) [25, 26], and dementia [27], each of which has genetic components [28–31]. Other *GHR* SNPs – *rs6182*, *rs6180*, *rs6184* (minor allele frequencies 0.137, 0.387, 0.077) that are non-synonymous (amino acid changes Cys440Phe, Leu544Ile and Pro579Thr, respectively) – have been found to be associated with hypertension and elevated blood pressure in Japanese men [32]. These variants are located in exon 5 of isoform 1 and are missing in the isoform 12 precursor, leading to isoform 5. A UK study found an association of the longevity-associated [16] *GHR* exon 3 deletion variant with hypertension amongst stroke patients [33].

In conclusion, men without hypertension lived the longest, while, in the group with hypertension, those homozygous for either the major (common) or minor (less common) allele of *GHR* SNP *rs4130113* lived longer than those who were heterozygous for this SNP. The overall association of genetic variation in *GHR* with mortality risk was contributed entirely by genotype-dependent amelioration of the increased mortality risk from hypertension.

MATERIALS AND METHODS

Study participants

See Supplementary Methods and Supplementary Study Cohort.

Genotyping

Genotyping methods were as described previously [7] (Supplementary Methods).

Variant search

Variants surrounding *rs4130113* were screened on the RegulomeDB site, which includes known and predicted regulatory elements in the intergenic regions, as well as regions of DNAase hypersensitivity, binding sites for transcription factors, and promoter regions. Sources of these data included public datasets from GEO, the ENCODE project, and published literature [34]. Chromosome 5 locations used the GRCh37.p13 genome build (http://www.gencodegenes.org/releases/19.html).

We also screened the variants using HaploReg, which is a tool for exploring annotations of the noncoding genome at variants on haplotype blocks, such as candidate regulatory SNPs at disease-associated loci [35]. The sentinel SNP *rs4130113* was not in strong LD with any other variants (Supplementary Figure 1). We also searched for transcription factor binding sites predicted to be significantly modified by this SNP.

Statistical analyses

General linear models were used to compare age-adjusted indirect measurements between groups, and logistic models was used to compare the age-adjusted direct measurements. Cox proportional models were used to assess the association of *GHR* for 3 genetic models – namely, *AA* vs *AG*/*GG*, *AG* vs *AA*/*GG*, and *GG* vs *AA*/*AG* – on mortality stratified by disease status, such as by diabetes, by hypertension, by CHD, and by cancer. The effects of the genotype on mortality were corrected for multiple tests using by the Bonferroni method. The significant genetic model was selected and

used in the analyses. The Cox proportional hazard assumption was tested for each Cox model. The effect of interaction of disease with *GHR* genotype on mortality was tested in the Cox model. All statistical analyses were performed using the Statistical Analysis System version 9.4 [36]. Figures were generated using STATA 12 Graphics [37].

AUTHOR CONTRIBUTIONS

B.J.M. and T.A.D. conceived the idea presented, designed the study, steered the analyses and wrote the manuscript. R.C. performed statistical analyses of the data. K.H.M. and B.J.W. managed clinical aspects and collected the data. All authors discussed the results and contributed to the final manuscript.

ACKNOWLEDGMENTS

The authors thank all study participants and their families for their cooperation and the Hawaii State Department of Health for its help. The authors wish to acknowledge Dr. Alvin T. Onaka, Brian Horiuchi, and Caryn Tottori of the Hawaii State Department of Health for providing death certificate data on cause of death for the KHHP participants, Ms. Ayako Elliott and Ms. Eva Ardo for assistance with genotyping, and Ms. Hiromi Nakada and Ms. Ka-on Fong for monitoring the vital status of KHHP participants.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING

Research reported in this publication was supported by the Kuakini Medical Center, the US National Institutes of Health (contract N01-AG-4-2149, Grants 5 U01 AG019349-05, 5R01AG027060 [Kuakini Hawaii Lifespan Study], 5R01AG038707 [Kuakini Hawaii Healthspan Study], 1P20GM125526-01A1 [Kuakini Center of Biomedical Research Excellence for Clinical and Translational Research on Aging]), and contract N01-HC-05102 from the National Heart, Lung, and Blood Institute.

REFERENCES

1. Bartke A, Westbrook R. Metabolic characteristics of long-lived mice. Front Genet. 2012; 3:288. https://doi.org/10.3389/fgene.2012.00288 PMID:23248643

2. Bartke A. Growth hormone and aging. Rev Endocr Metab Disord. 2021; 22:71–80. https://doi.org/10.1007/s11154-020-09593-2 PMID:33001358

3. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. Nature. 1996; 384:33. https://doi.org/10.1038/384033a0 PMID:8900272

4. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology. 2000; 141:2608–13. https://doi.org/10.1210/endo.141.7.7586 PMID:10875265

5. Icyuz M, Zhang F, Fitch MP, Joyner MR, Challa AK, Sun LY. Physiological and metabolic characteristics of novel double-mutant female mice with targeted disruption of both growth hormone-releasing hormone and growth hormone receptor. Aging Cell. 2021; 20:e13339. https://doi.org/10.1111/acel.13339 PMID:33755309

6. He Q, Morris BJ, Grove JS, Petrovitch H, Ross W, Masaki KH, Rodriguez B, Chen R, Donlon TA, Willcox DC, Willcox BJ. Shorter men live longer: association of height with longevity and *FOXO*3 genotype in American men of Japanese ancestry. PLoS One. 2014; 9:e94385. https://doi.org/10.1371/journal.pone.0094385 PMID:24804734

7. Donlon TA, Morris BJ, Chen R, Masaki KH, Allsopp RC, Willcox DC, Tiirikainen M, Willcox BJ. Analysis of polymorphisms in 59 potential candidate genes for association with human longevity. J Gerontol A Biol Sci Med Sci. 2018; 73:1459–64. https://doi.org/10.1093/gerona/glx247 PMID:29300832

8. Estep PW 3rd, Warner JB, Bulyk ML. Short-term calorie restriction in male mice feminizes gene expression and alters key regulators of conserved aging regulatory pathways. PLoS One. 2009; 4:e5242. https://doi.org/10.1371/journal.pone.0005242 PMID:19370158

9. Morris BJ, Willcox BJ, Donlon TA. Genetic and epigenetic regulation of human aging and longevity. Biochim Biophys Acta Mol Basis Dis. 2019; 1865:1718–44. https://doi.org/10.1016/j.bbadis.2018.08.039 PMID:31109447

10. Ali T, Renkawitz R, Bartkuhn M. Insulators and domains of gene expression. Curr Opin Genet Dev. 2016; 37:17–26. https://doi.org/10.1016/j.gde.2015.11.009 PMID:26802288

11. Donlon TA, Morris BJ. In silico analysis of human renin gene-gene interactions and neighborhood topologically associated domains suggests breakdown

of insulators contribute to ageing-associated diseases. Biogerontology. 2019; 20:857–69. https://doi.org/10.1007/s10522-019-09834-1 PMID:31520345

12. Young JA, List EO, Kopchick JJ. Deconstructing the growth hormone receptor (GHR): Physical and metabolic phenotypes of tissue-specific GHR gene-disrupted mice. Prog Mol Biol Transl Sci. 2016; 138:27–39. https://doi.org/10.1016/bs.pmbts.2015.10.014 PMID:26940385

13. List EO, Berryman DE, Ikeno Y, Hubbard GB, Funk K, Comisford R, Young JA, Stout MB, Tchkonia T, Masternak MM, Bartke A, Kirkland JL, Miller RA, Kopchick JJ. Removal of growth hormone receptor (GHR) in muscle of male mice replicates some of the health benefits seen in global GHR-/- mice. Aging (Albany NY). 2015; 7:500–12. https://doi.org/10.18632/aging.100766 PMID:26233957

14. Junnila RK, Duran-Ortiz S, Suer O, Sustarsic EG, Berryman DE, List EO, Kopchick JJ. Disruption of the GH receptor gene in adult mice increases maximal lifespan in females. Endocrinology. 2016; 157:4502–13. https://doi.org/10.1210/en.2016-1649 PMID:27732088

15. van der Spoel E, Jansen SW, Akintola AA, Ballieux BE, Cobbaert CM, Slagboom PE, Blauw GJ, Westendorp RG, Pijl H, Roelfsema F, van Heemst D. Growth hormone secretion is diminished and tightly controlled in humans enriched for familial longevity. Aging Cell. 2016; 15:1126–31. https://doi.org/10.1111/acel.12519 PMID:27605408

16. Ben-Avraham D, Govindaraju DR, Budagov T, Fradin D, Durda P, Liu B, Ott S, Gutman D, Sharvit L, Kaplan R, Bougnères P, Reiner A, Shuldiner AR, et al. The GH receptor exon 3 deletion is a marker of male-specific exceptional longevity associated with increased GH sensitivity and taller stature. Sci Adv. 2017; 3:e1602025. https://doi.org/10.1126/sciadv.1602025 PMID:28630896

17. Láruson ÁJ, Reed FA. Stability of underdominant genetic polymorphisms in population networks. J Theor Biol. 2016; 390:156–63. https://doi.org/10.1016/j.jtbi.2015.11.023 PMID:26656110

18. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature. 1987; 330:537–43. https://doi.org/10.1038/330537a0 PMID:2825030

19. Dastot F, Sobrier ML, Duquesnoy P, Duriez B, Goossens M, Amselem S. Alternatively spliced forms in the cytoplasmic domain of the human growth hormone (GH) receptor regulate its ability to generate a soluble GH-binding protein. Proc Natl Acad Sci USA. 1996; 93:10723–28. https://doi.org/10.1073/pnas.93.20.10723 PMID:8855247

20. Ross RJ, Esposito N, Shen XY, Von Laue S, Chew SL, Dobson PR, Postel-Vinay MC, Finidori J. A short isoform of the human growth hormone receptor functions as a dominant negative inhibitor of the full-length receptor and generates large amounts of binding protein. Mol Endocrinol. 1997; 11:265–73. https://doi.org/10.1210/mend.11.3.9901 PMID:9058373

21. Ayling RM, Ross R, Towner P, Von Laue S, Finidori J, Moutoussamy S, Buchanan CR, Clayton PE, Norman MR. A dominant-negative mutation of the growth hormone receptor causes familial short stature. Nat Genet. 1997; 16:13–14. https://doi.org/10.1038/ng0597-13 PMID:9140387

22. Iida K, Takahashi Y, Kaji H, Nose O, Okimura Y, Abe H, Chihara K. Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. J Clin Endocrinol Metab. 1998; 83:531–37. https://doi.org/10.1210/jcem.83.2.4601 PMID:9467570

23. Fish AE, Capra JA, Bush WS. Are interactions between cis-regulatory variants evidence for biological epistasis or statistical artifacts? Am J Hum Genet. 2016; 99:817–30. https://doi.org/10.1016/j.ajhg.2016.07.022 PMID:27640306

24. Bhadra M, Howell P, Dutta S, Heintz C, Mair WB. Alternative splicing in aging and longevity. Hum Genet. 2020; 139:357–69. https://doi.org/10.1007/s00439-019-02094-6 PMID:31834493

25. Satoh M, Ohkubo T, Asayama K, Murakami Y, Sugiyama D, Yamada M, Saitoh S, Sakata K, Irie F, Sairenchi T, Ishikawa S, Kiyama M, Ohnishi H, et al, and EPOCH-JAPAN Research Group. Lifetime risk of stroke and coronary heart disease deaths according to blood pressure level: EPOCH-JAPAN (Evidence for Cardiovascular Prevention From Observational Cohorts in Japan). Hypertension. 2019; 73:52–59. https://doi.org/10.1161/HYPERTENSIONAHA.118.11635 PMID:30763510

26. Georgakis MK, Gill D, Malik R, Protogerou AD, Webb AJ, Dichgans M. Genetically predicted blood pressure across the lifespan: Differential effects of mean and pulse pressure on stroke risk. Hypertension. 2020; 76:953–61. https://doi.org/10.1161/HYPERTENSIONAHA.120.15136 PMID:32623925

27. Ninomiya T, Ohara T, Hirakawa Y, Yoshida D, Doi Y, Hata J, Kanba S, Iwaki T, Kiyohara Y. Midlife and late-life blood pressure and dementia in Japanese elderly: the Hisayama study. Hypertension. 2011; 58:22–28. https://doi.org/10.1161/HYPERTENSIONAHA.110.163055 PMID:21555680

28. Miyaki K, Htun NC, Song Y, Ikeda S, Muramatsu M, Shimbo T. The combined impact of 12 common variants on hypertension in Japanese men, considering GWAS results. J Hum Hypertens. 2012; 26:430–36. https://doi.org/10.1038/jhh.2011.50 PMID:21633377

29. Lu X, Peloso GM, Liu DJ, Wu Y, Zhang H, Zhou W, Li J, Tang CS, Dorajoo R, Li H, Long J, Guo X, Xu M, et al., and GLGC Consortium. Exome chip meta-analysis identifies novel loci and East Asian-specific coding variants that contribute to lipid levels and coronary artery disease. Nat Genet. 2017; 49:1722–30. https://doi.org/10.1038/ng.3978 PMID:29083407

30. Ehret GB, Ferreira T, Chasman DI, Jackson AU, Schmidt EM, Johnson T, Thorleifsson G, Luan J, Donnelly LA, Kanoni S, Petersen AK, Pihur V, Strawbridge RJ, et al. The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. Nat Genet. 2016; 48:1171–84. https://doi.org/10.1038/ng.3667 PMID:27618452

31. Weinstein G, Beiser AS, Au R, Decarli C, Wolf PA, Seshadri S. Association of parental stroke with brain injury and cognitive measures in offspring: the Framingham Heart Study. Stroke. 2013; 44:812–15. https://doi.org/10.1161/STROKEAHA.112.680520 PMID:23362080

32. Kokubo Y, Tomoike H, Tanaka C, Banno M, Okuda T, Inamoto N, Kamide K, Kawano Y, Miyata T. Association of sixty-one non-synonymous polymorphisms in forty-one hypertension candidate genes with blood pressure variation and hypertension. Hypertens Res. 2006; 29:611–19. https://doi.org/10.1291/hypres.29.611 PMID:17137217

33. Horan M, Newsway V, Ya, Lewis MD, Easter TE, Rees DA, Mahto A, Millar DS, Procter AM, Scanlon MF, Wilkinson IB, Hall IP, Wheatley A, et al. Genetic variation at the growth hormone (*GH1*) and growth hormone receptor (*GHR*) loci as a risk factor for hypertension and stroke. Hum Genet. 2006; 119:527–40. https://doi.org/10.1007/s00439-006-0166-5 PMID:16572267

34. Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM, Snyder M. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 2012; 22:1790–97. https://doi.org/10.1101/gr.137323.112 PMID:22955989

35. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 2012; 40:D930–34. https://doi.org/10.1093/nar/gkr917 PMID:22064851

36. Statistical Analysis System (SAS) version 9.4. SAS Institute, Cary, NC, USA. 2020. https://libguides.library.kent.edu/statconsulting/SAS

37. StataCorp. Stata Statistical Software: Release 12. College Station, TX: StataCorp LP, 2011.

SUPPLEMENTARY MATERIALS

Supplementary Methods

Donlon TA, Chen R, Masaki KH, Willcox DC, Allsopp RC, Willcox BJ, Morris BJ. Association of growth hormone receptor gene variant with longevity in men is due to amelioration of increased mortality risk from hypertension.

Study cohort

The present case-control study was conducted as part of the Kuakini Hawaii Lifespan Study and the Kuakini Hawaii Healthspan Study, an embedded cohort study of healthy aging drawn from the original population of the Kuakini Honolulu Heart Program (KHHP) and Honolulu-Asia Aging Study (HAAS) [1, 2]. As such the current study is a case-control sub-study of the KHHP population-based, prospective study of cardiovascular disease among 8,006 Japanese American men that began in 1965. The KHHP participants were recruited during 1965–1968 from 9,877 men aged 45–68 years who had valid contact information from World War II Selective Service records, were born from 1900–1919, and who were living on the island of Oahu in 1965 [3]. Study participants had parents who were both from Japan, mostly the western, central and southern regions [3, 4]. Although 88% of participants were born in Hawaii, there is a theoretical possibility of confounding of case vs. control status for allele frequencies due to geographic origin. Therefore, for certain analyses, cases and controls were stratified by parental prefecture of origin using conditional logistic regression models. Analyses showed no evidence of population stratification in the dataset (data not shown). Subjects were recruited at the same time and place (Oahu) and case and control status only became evident after death or attainment of ≥95 years of age, meaning there was no apparent reason why genetic background should be different. The KHHP cohort has been described elsewhere [4] and is quite robust for phenotype-genotype associations since the data collection are exceptionally accurate, involving cross validation utilizing an expert Morbidity and Mortality Committee. The Hawaii Japanese population is from a limited geographic area in Japan, with little outbreeding and, based on the authors' unpublished data, exhibits a smaller degree of genetic diversity than the overall population of Japan.

All participants in the current study were drawn from records of study participants updated to February 2012. Archived phenotypic data and blood samples from Examination 4 of the KHHP (1991–1993), which coincided with the commencement of the HAAS, were used as the baseline for our case-control study. The HAAS was begun as an expansion of the KHHP for the study of neurodegenerative diseases, cognitive function, and other aging phenotypes in elderly persons. Participants included 3,741 men aged 71 to 93 at Examination 4 (mean age 77.9 ± 4.7 SD years), approximately half the number of the original KHHP [5], representing 80% of survivors of the original cohort.

Our prior genetics studies have used a quasi-nested case-control design and subjects drawn from the KHHP/HAAS population were as described previously [6]. Subjects were followed with regular examinations and blood work until the present, or death. "Cases" (longevity phenotype) were defined as individuals who had survived to at least 95 years of age. As of February 2012, these were in the upper 1% of the 2010 U.S. birth cohort-specific survival [7]. Of the 440 cases, 317 of the men had died by February 2012 (mean age at death 97.0± 2.1SD years; range 95–106 years) and 123 individuals were still alive (mean age 96.8±1.8 SD years; range 95–107 years). Controls were 374 men of average lifespan randomly selected from KHHP/HAAS cohort as individuals who had died up to the age of 81 years. Mean age at death, 78.1±1.8 SD years (range 72–81 years), accorded with the 3.5 year longer life expectancy of Japanese American men living in Hawaii [8] than the average 1910 U.S. birth cohort-specific survival for middle-aged US men [7].

Procedures performed were in accord with institutional guidelines and were approved by the Institutional Review Board of Kuakini Medical Center. Written informed consent was obtained from all study participants or from family representatives, if participants could not provide consent.

An extensive number of parameters were measured, including hypertension status, total plasma cholesterol level, diabetes, body mass index, cigarette smoking status, alcohol intake, physical activity, and various other parameters. Hypertension was defined as a systolic or diastolic blood pressure of ≥ 160 and ≥ 90 mm Hg, respectively, or use of antihypertensive medication. To be considered normotensive, systolic and diastolic blood pressures needed to be < 140 and < 90 mm Hg, respectively. Men who were neither normotensive nor hypertensive were classified as having borderline hypertension. Study participants were also classified as having diabetes on the basis of a medical history (physician diagnosed or based on the reported use of insulin or the receipt of oral diabetes medication). Assessment of overall metabolic output

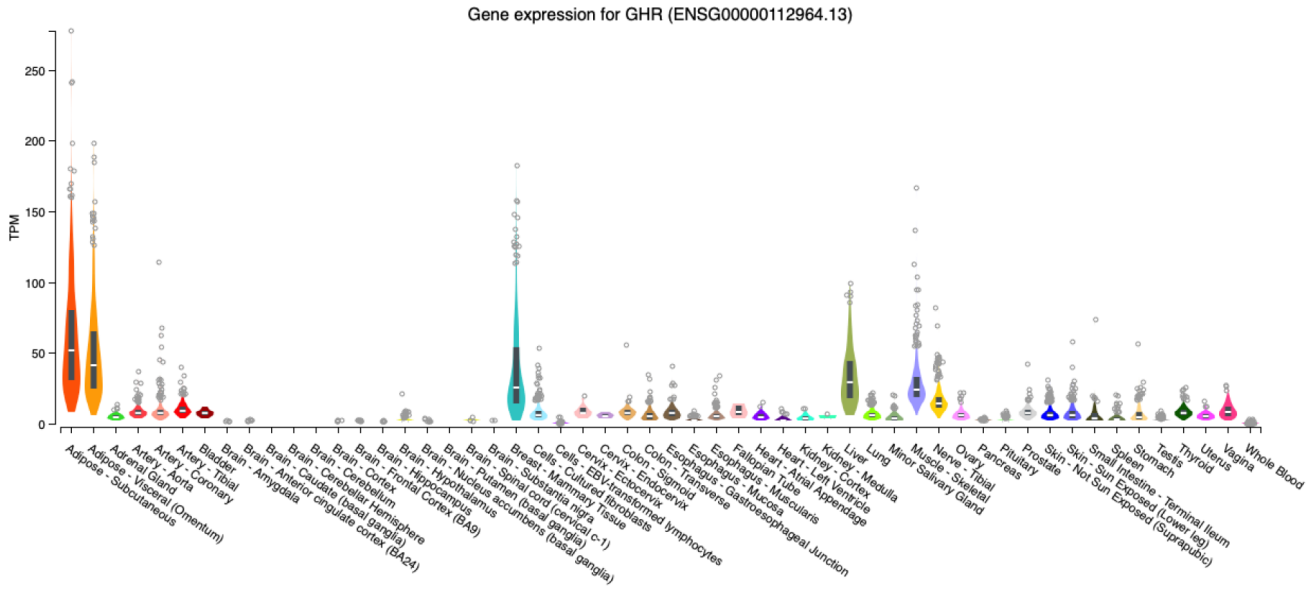
during a typical 24-hour period was based on the use of a physical activity index. The physical activity index was derived by summing the average number of hours per day spent in five different activity levels (basal, sedentary, slight, moderate, and heavy) after each was multiplied by a weighting factor that corresponded to the level of exertion needed to undertake the activity. High levels of the physical activity index indicate active lifestyles, and low levels indicate inactive lifestyles [9, 10].

Genotyping

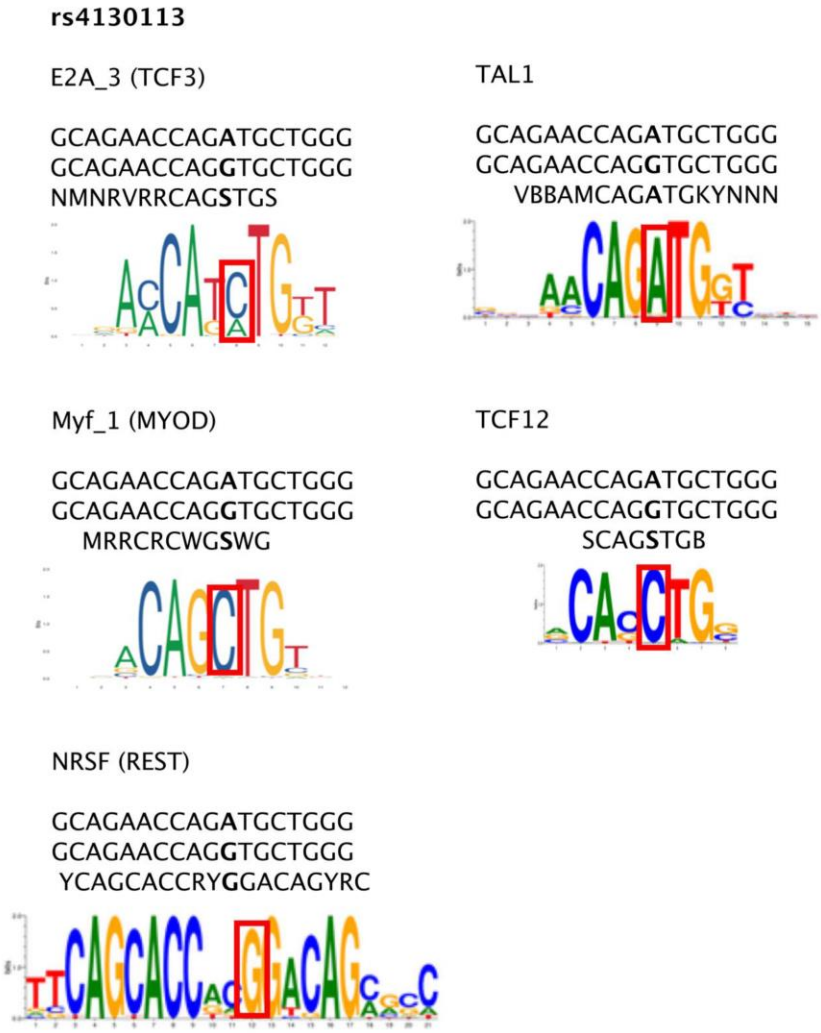
Our original case-control studies (814 subjects) and subjects in the current longitudinal study were performed using total leukocyte DNA isolated using the PureGene system (Gentra Systems, Inc.) and quantified using PicoGreen staining (Molecular Probes, Eugene, OR). Tagging SNPs (tSNPs) were genotyped at the University of Hawaii Cancer Center on the Illumina GoldenGate platform (high-throughput SNP genotyping on universal bead arrays). Genotyping of DNA was performed on the same platform. The longevity study included 2,900 subjects that were genotyped using TaqMan® reagents (purchased from Applied Biosystems, Thermo Fisher Scientific) for PCR amplification under standard conditions with AmpliTaq Gold® DNA polymerase (Perkin-Elmer Corp.). PCR products were detected by TaqMan® assay, using a 6-FAM-labeled FRET probe for one allele and a VIC-labelled probe for the other allele, with minor groove binding (MGB) quenchers to enhance assay signal. PCR products were measured using a QuantStudio 12K Flex system. Genotype data were managed through an integrated database sample management-data processing system of proven accuracy. All positive controls on each genotyping plate were evaluated for consistency. SNP call rates exceeded 98%.

We implemented a series of quality control checks based on the Illumina metrics. For inclusion of data for a SNP its call rate had to exceed 0.95 and the Hardy-Weinberg equilibrium p value needed to be > 0.01.

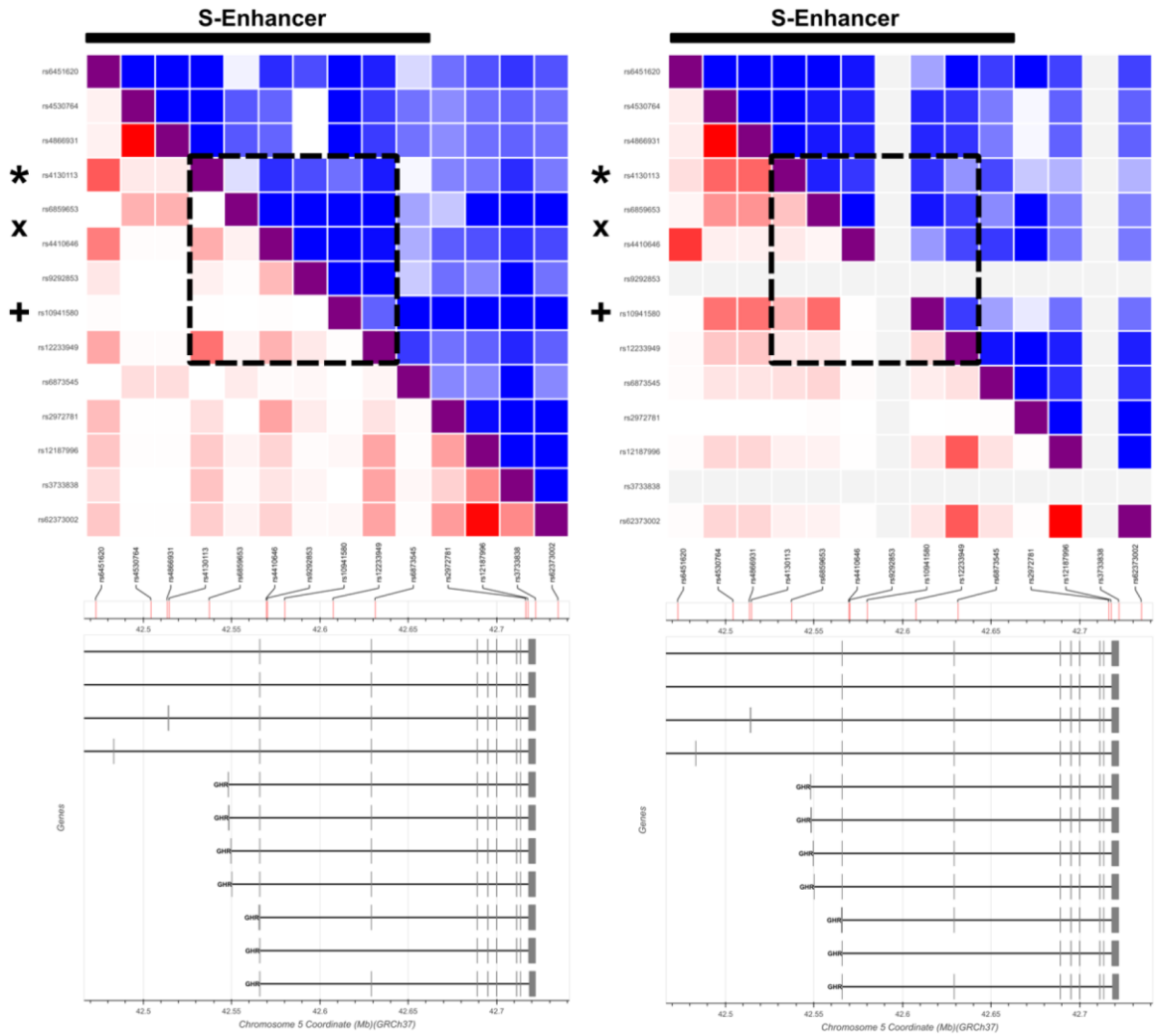
Supplementary Figures



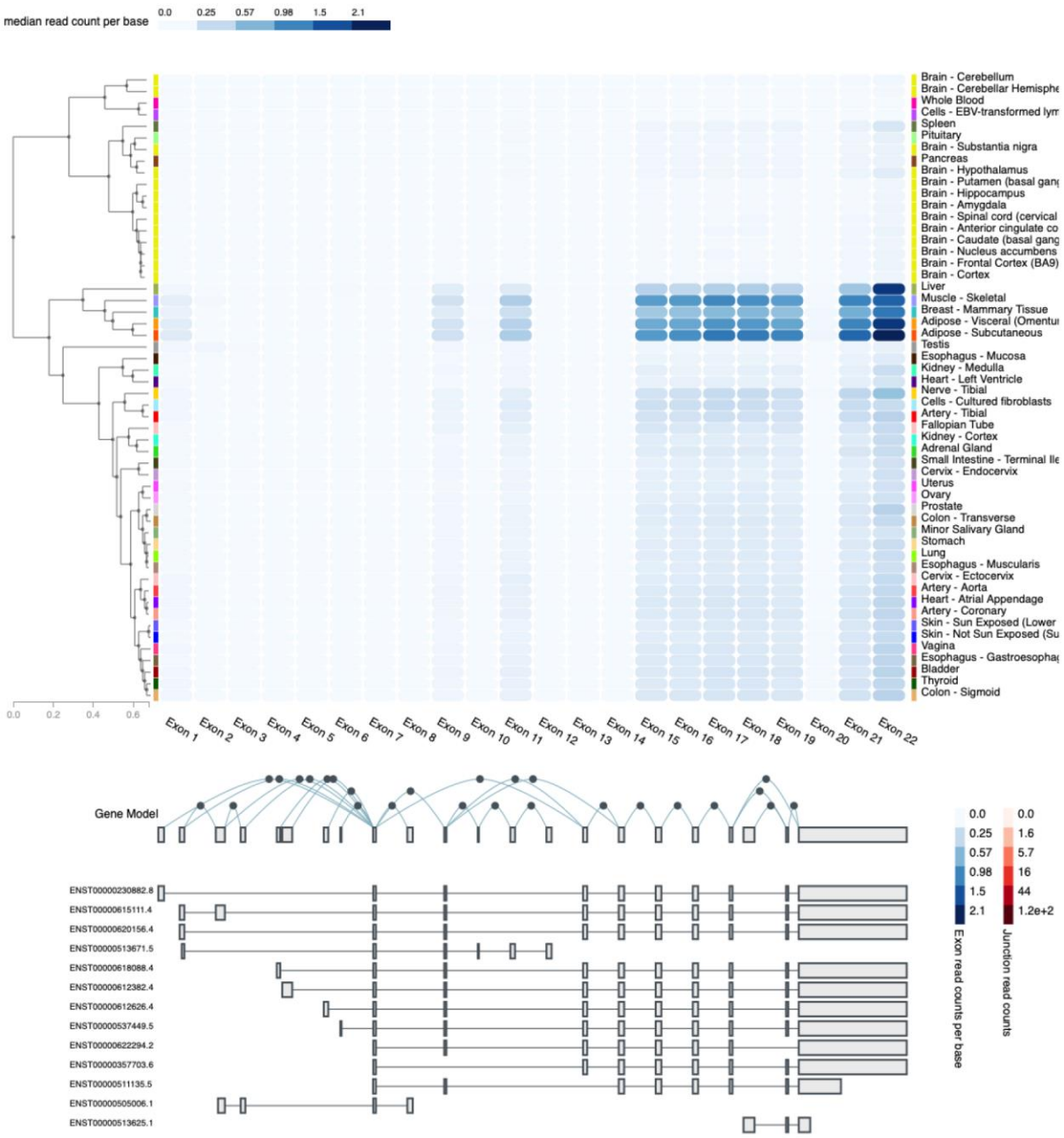
Supplementary Figure 1. Tissue expression of *GHR* from GTEx. GHR is expressed in adipose tissue, breast, liver, and muscle.



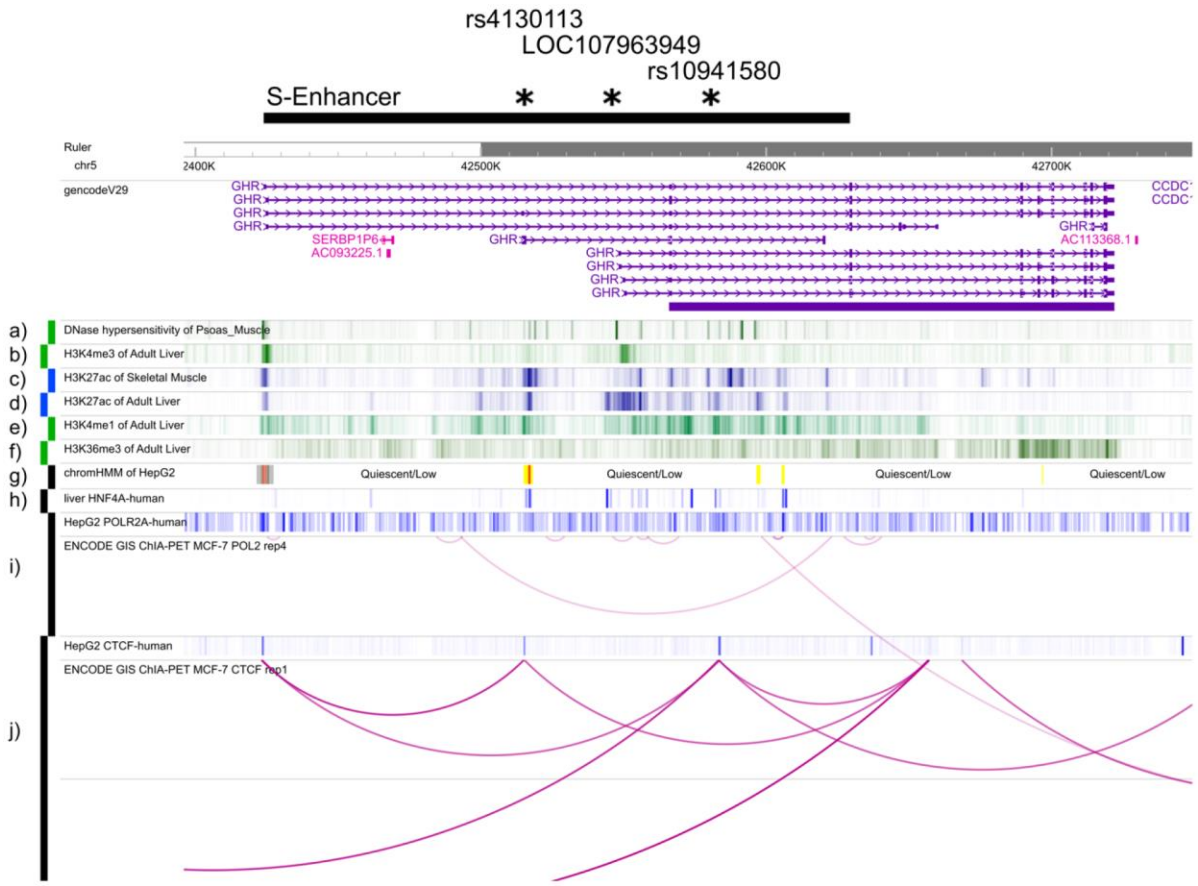
Supplementary Figure 2. Transcription factor binding sites significantly modified by the *GHR* SNP *rs4130113.* The major (*A*) allele of *rs4130113* is predicted to decrease binding of E2A, decrease binding of MYF, decrease binding of NRSF, increase binding of TAL1, and decrease binding of TCF12. Red rectangles denote the variant SNP nucleotide in the transcription factor canonical sequence. The nucleotide sequences above each colored diagram are: 1st line is major allele (*A*); 2nd line is minor allele (*G*); 3rd line is canonical recognition site. Nucleotide ambiguity codes are: B, not A; V, not T; M, C or A; K, T or G; W, A or T; S, C or G).



Supplementary Figure 3. Linkage disequilibrium (LD) matrix of SNPs used in the longevity study. The figure above shows the location of the longevity-associated SNP *rs4130113* ("*") the location of the "downstream promoter" (LOC107963949, "x") and the ieQTL/sQTL/NMD SNP *rs10941580* ("+"). All three are in moderate LD as defined by the dotted square. An ieQTL is a *cis*-regulatory element that is predicted to influence the expression levels of a nearby gene [11]. sQTLs (splicing QTLs) are quantitative trait loci that regulate alternative splicing of pre-mRNAs. Non-sense mediated transcript decay (NMD) transcript variant is a variant in a transcript that is the target of NMD (SO:0001621). NMD = non-sense mediated transcript decay. The downstream promoter represents regulatory module B of the growth hormone receptor gene. It encompasses the downstream promoters for alternate 5' end transcript variants V1, V4, V7, and V8. This sequence includes hepatocyte nuclear factor 4 alpha recognition sites and GAGA sites, which recognize sequence specific transcription factors that positively and negatively regulate gene expression. GHRv1 is the major form and is liver specific [12, 13]. The Japanese (JPT) LD map is shown on the left and the Caucasian (CEU) on the right for comparisons.



Supplementary Figure 4. Exon usage and tissue expression of *GHR*. GHR is encoded by at least 10 exons, with exons 2–7 encoding the extracellular domain, exon 8 the transmembrane domain, and exons 9–10 the intracellular domain [14]. The full-length version, v1, is shown as ENST00000230882.8 in the figure above. The exon 3 deletion variant, GHGRd3, is shown as ENST00000357703.6 above and is the result of a deletion of the exon, rather than being caused by a splicing event. As can be seen in the above figure there are multiple (at least 13) transcript variants caused by alternate splicing events, resulting in at least five protein isoforms that are differentially expressed, the majority of which are expressed largely in liver, muscle, and adipose tissue. Two of these alternatively spliced transcripts, at exon 9, GHR-(1–279) and GHR-(1–277), were identified in human liver [15, 16]. GHR-(1–279) lacks the first 26 bp of exon 9 of the full-length receptor (GHRfl), whereas for GHR-(1–277) this exon is deleted in its entirety [16]. Both alternative splicing events result in a frame shift and a premature stop codon, resulting in mRNAs with intact extracellular and transmembrane domains but lacking more than 90% of the intracellular domain. While these receptor variants have no signaling capacity, they can inhibit GH action mediated by GHRfl in a dominant negative manner [16, 17]. Patients heterozygous for variants/mutations that result in the splicing out of exon 9 are GH insensitive [16, 18], providing evidence for a pathophysiological role for these truncated receptors. A search for other functional variants in *GHR* found that there are 18,128 neighboring SNPs associated with exon usage and 4,568 eQTL variants associated with expression levels (GTEx portal). There is an ieQTL, *rs10941580*, that is expressed in adipose tissue, muscle, nerve, breast, and thyroid. An ieQTL is a *cis*-regulatory element that is predicted to influence the expression level of a nearby gene [11].



Supplementary Figure 5. The locations of *GHR* with its various transcripts (purple herring bones) and the locations of the super-enhancer (bar) along with the three features: *rs4130113*, LOC107963949, and *rs10941580* (*). Shown in "a" through "j" are data from ChIP-seq experiments, as follows: (**a**) DNAse I sensitive sites in muscle, (**b**) H3Kme3 histone marks in liver, (**c**) histone H3K27ac marks in muscle, (**d**) histone H3K27ac in liver, (**e**) histone H3K4me1 marks in liver, (**f**) H3K36me3 marks in liver, (**g**) chromHMM marks in the HepG2 cell line, (**h**) the location of HNF4A binding, which has been shown to induce the "downstream promoter", (**i**) locations of RNA polymerase II binding (RNAPII), and (**j**) CTCF binding sites and chromatin loop domains. H3K4me3 and DNAse I hypersensitivity are associated with sites of open chromatin which are associated with activation of transcription of nearby genes [8]. H3K27ac is associated with activation in promoters/enhancers, H3K4me1 activation in enhancers, and H3K36me3 activation in gene bodies. ChromHMM is software for learning and characterizing chromatin states that can integrate multiple chromatin datasets such as ChIP-seq data of various histone modifications to discover *de novo* the major reoccurring combinatorial and spatial patterns of marks (legend next to a) through j) on left-hand side of diagram). The *GHR* promoter, *rs4130113* and "downstream promoter" overlap with sites of H3K4me3, H3K27ac, and DNAse I hypersensitivity, as well as CTCF binding sites, the latter being able to serve as either 3-dimensional insulators or the grouping of functional features together in *cis*-acting topological domains. Together these features are predicted to form a *cis*-regulatory unit consisting of the super-enhancer, *rs4130113* and open chromatin sites. This is supported by the locations of CTCF binding sites that generally form insulator domains [9, 10].

Supplementary Tables

Supplementary Table 1. The SNPs genotyped in *GHR* and the minor allele frequency of each in control American men of Japanese ancestry in the Kuakini Honolulu Heart Program, and of Japanese subjects in the dbSNP database.

SNP	HHP	dbSNP
*rs4130113**	0.40	0.48
rs9292853	0.20	0.22
rs12187996	0.39	0.34
rs62373002	0.39	0.30
rs6873545	0.14	0.13
rs4866931	0.04	0.10
rs4410646	0.41	0.42
rs4530764	0.04	0.08
rs2972781	0.41	0.48
rs12233949	0.33	0.31
rs3733838	0.18	0.18
rs6451620	0.43	0.45
rs6859653	0.06	0.11

*Denotes the SNP with longevity using a heterozygote disadvantage model.

Supplementary Table 2. Each *GHR* SNP tested in the case-control study, and Bonferroni corrected *p* values for association with longevity.

Gene	SNP ID	*p*-value
GHR	*rs4130113*	0.015*
GHR	*rs9292853*	0.052
GHR	*rs12187996*	0.076
GHR	*rs62373002*	0.076
GHR	*rs6873545*	0.11
GHR	*rs4866931*	0.22
GHR	*rs4410646*	0.28
GHR	*rs4530764*	0.29
GHR	*rs2972781*	0.45
GHR	*rs12233949*	0.456
GHR	*rs3733838*	0.54
GHR	*rs6451620*	0.72
GHR	*rs6859653*	0.996

Probability (*p*) is based on heterozygote disadvantage model.
*The *p* values were obtained after Bonferroni correction for multiple testing.

Supplementary Table 3. The effect of *GHR* SNP *rs4130113* on mortality in the whole cohort for different genetic models by two Cox models.

Cox model	Genetic model	Relative risk	*p*
1	*AG vs. AA/GG*	1.07 (1.00-1.14)	0.042
2	*AG vs. AA/GG*	1.06 (0.99-1.14)	0.10
1	*AA vs. AG/GG*	0.97 (0.90-1.04)	0.38
2	*AA vs. AG/GG*	0.99 (0.92-1.07)	0.74
1	*GG vs. AA/AG*	0.93 (0.85-1.02)	0.12
2	*GG vs. AA/AG*	0.92 (0.83-1.01)	0.086

Model 1: adjusted for age.

Model 2: adjusted for age, BMI, glucose, smoking (pack years), PAI, alcohol intake, depression, stroke, CHD, diabetes, cancer and hypertension. The *p* values shown were obtained after correction for multiple testing by the Bonferroni method.

Supplementary Table 4. Modifications to transcription factor binding by *rs4130113.*

SNP	Transcription factor	Effect of major allele	Biological pathway(s)	Tissue
rs4130113	E2A_3 (TCF3)	reduce	lymphopoiesis	All
	MYF_1 (MYOD)	reduce	muscle cell differentiation	Skeletal muscle
	NRSF (REST)	reduce	oncogene or a tumor suppressor	Undifferentiated neuronal progenitor cells. Low levels in many tissues
	TAL1	increase	erythroid differentiation	Hematopoietic
	TCF12	reduce	lineage-specific differentiation	Skeletal muscle, hematopoietic, skin

Supplementary Table 5. Genetic features in *GHR*.

Feature	Location (hg19)	Characteristic
GHR	42,423,877–42,721,980	Gene, variant 1
Super enhancer	42,421,467–42,633,445	Super enhancer, adipose tissue
rs4130113	42,514,651	Longevity SNP, this study
LOC107963949	42,546,421–42,550,233	Downstream promoter*
rs10941580	42,580,021	ieQTL and sQTL

The table shows the location of the gene *GHR*, a super-enhancer described in adipose tissue, the longevity SNP *rs4130113* used in the present study, a downstream promoter, and an open chromatin feature in the gene, *GHR*. SNP *rs10941580* is predicted to be both an ieQTL and an sQTL for *GHR*. An NMD transcript variant is a variant in a transcript that is the target of non-sense mediated transcript decay (NMD; SO:0001621). An ieQTL is a *cis*-regulatory element that is predicted to influence the expression levels of a nearby gene [11]. sQTLs (splicing QTLs) are quantitative trait loci that regulate alternative splicing of pre-mRNA [13].

*The downstream promoter represents regulatory module B of the growth hormone receptor gene. It encompasses the downstream promoters for alternate 5' end transcript variants V1, V4, V7 and V8. This sequence includes hepatocyte nuclear factor 4α r [12] recognition sites and GAGA sites, which recognize sequence-specific transcription factors that positively and negatively regulate gene expression. GHRv1 is the major form and is liver specific.

Supplementary Table 6. Influence of *rs1094150* on exon usage in *GHR*.

SNP	Intron ID	*p*	NES	Tissue
rs10941580	clu_37892	3.70E–11	0.45	Adipose tissue – visceral (Omentum)
	clu_34535	7.30E–11	0.37	Muscle – skeletal
	clu_39029	7.40E–09	0.36	Adipose tissue – subcutaneous
	clu_40148	1.00E–07	–0.38	Nerve –tibial
	clu_41545	0.0000049	–0.33	Thyroid
	clu_39450	0.000018	0.33	Breast – mammary tissue

The Table shows the predicted exon usage in the tissues described. NES refers to the normalized effect size. All data are from GTEx [19].

Supplementary References

1. Kagan A, Harris BR, Winkelstein W Jr, Johnson KG, Kato H, Syme SL, Rhoads GG, Gay ML, Nichaman MZ, Hamilton HB, Tillotson J. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: demographic, physical, dietary and biochemical characteristics. J Chronic Dis. 1974; 27:345–64. https://doi.org/10.1016/0021-9681(74)90014-9 PMID:4436426

2. Yano K, Reed DM, McGee DL. Ten-year incidence of coronary heart disease in the Honolulu Heart Program. Relationship to biologic and lifestyle characteristics. Am J Epidemiol. 1984; 119:653–66. https://doi.org/10.1093/oxfordjournals.aje.a113787 PMID:6720665

3. Kagan A,. The Honolulu Heart Program: An Epidemiological Study of Coronary Heart Disease and Stroke. Amsterdam, The Netherlands: Harwood Academic Publishers. 1996.

4. Worth RM, Kagan A. Ascertainment of men of Japanese ancestry in Hawaii through World War II Selective Service registration. J Chronic Dis. 1970; 23:389–97. https://doi.org/10.1016/0021-9681(70)90022-6 PMID:5492969

5. White L, Petrovitch H, Ross GW, Masaki KH, Abbott RD, Teng EL, Rodriguez BL, Blanchette PL, Havlik RJ, Wergowske G, Chiu D, Foley DJ, Murdaugh C, Curb JD. Prevalence of dementia in older Japanese-American men in Hawaii: The Honolulu-Asia Aging Study. JAMA. 1996; 276:955–60. PMID:8805729

6. Willcox BJ, Donlon TA, He Q, Chen R, Grove JS, Yano K, Masaki KH, Willcox DC, Rodriguez B, Curb JD. FOXO3A genotype is strongly associated with human longevity. Proc Natl Acad Sci USA. 2008; 105:13987–92. https://doi.org/10.1073/pnas.0801030105 PMID:18765803

7. Arias E. United States life tables, 2008. Natl Vital Stat Rep. 2012; 61:1–63. PMID:24974590

8. Nordyke EC, Lee R, Gardner RW. A profile of Hawaii's elderly population. Papers East West Popul Inst. 1984; 91:13–14.

9. Donahue RP, Abbott RD, Reed DM, Yano K. Physical activity and coronary heart disease in middle-aged and elderly men: the Honolulu Heart Program. Am J Public Health. 1988; 78:683–85. https://doi.org/10.2105/ajph.78.6.683 PMID:3369600

10. Abbott RD, Rodriguez BL, Burchfiel CM, Curb JD. Physical activity in older middle-aged men and reduced risk of stroke: the Honolulu Heart Program. Am J Epidemiol. 1994; 139:881–93. https://doi.org/10.1093/oxfordjournals.aje.a117094 PMID:8166138

11. Fish AE, Capra JA, Bush WS. Are Interactions between cis-regulatory variants evidence for biological epistasis or statistical artifacts? Am J Hum Genet. 2016; 99:817–30. https://doi.org/10.1016/j.ajhg.2016.07.022 PMID:27640306

12. Goodyer CG, Rhani Z, Zheng H. Expression of the hepatic specific V1 messenger ribonucleic acid of the human growth hormone receptor gene is regulated by hepatic nuclear factor (HNF)-4α2 and HNF-4α8. Mol Endocrinol. 2008; 22:485–500. https://doi.org/10.1210/me.2007-0387 PMID:17991764

13. Adriaens ME, Bezzina CR. Genomic approaches for the elucidation of genes and gene networks underlying cardiovascular traits. Biophys Rev. 2018; 10:1053–60. https://doi.org/10.1007/s12551-018-0435-2 PMID:29934864

14. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature. 1987; 330:537–43. https://doi.org/10.1038/330537a0 PMID:2825030

15. Dastot F, Sobrier ML, Duquesnoy P, Duriez B, Goossens M, Amselem S. Alternatively spliced forms in the cytoplasmic domain of the human growth hormone (GH) receptor regulate its ability to generate a soluble GH-binding protein. Proc Natl Acad Sci USA. 1996; 93:10723–28. https://doi.org/10.1073/pnas.93.20.10723 PMID:8855247

16. Ross RJ, Esposito N, Shen XY, Von Laue S, Chew SL, Dobson PR, Postel-Vinay MC, Finidori J. A short isoform of the human growth hormone receptor functions as a dominant negative inhibitor of the full-length receptor and generates large amounts of binding protein. Mol Endocrinol. 1997; 11:265–73. https://doi.org/10.1210/mend.11.3.9901 PMID:9058373

17. Ayling RM, Ross R, Towner P, Von Laue S, Finidori J, Moutoussamy S, Buchanan CR, Clayton PE, Norman MR. A dominant-negative mutation of the growth

hormone receptor causes familial short stature. Nat Genet. 1997; 16:13–14. https://doi.org/10.1038/ng0597-13 PMID:9140387

18. Iida K, Takahashi Y, Kaji H, Nose O, Okimura Y, Abe H, Chihara K. Growth hormone (GH) insensitivity syndrome with high serum GH-binding protein levels caused by a heterozygous splice site mutation of the GH receptor gene producing a lack of intracellular domain. J Clin Endocrinol Metab. 1998; 83:531–37. https://doi.org/10.1210/jcem.83.2.4601 PMID:9467570

19. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. Nat Genet. 2013; 45:580–85. https://doi.org/10.1038/ng.2653 PMID:23715323