RESEARCH NOTE

Association of a single-nucleotide polymorphism (rs6180) in *GHR* gene with plural tissue weight

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Introduction

The growth hormone receptor (GHR) belongs to the cytokine receptor superfamily and mediates majority of growth hormone. Only nonsynonymous single-nucleotide polymorphism (SNP) rs6180 (p.Ile544Leu; c.1630 A>C) in exon 3 of the *GHR* gene is highly polymorphic. In the present study, we focussed on analysing the potential effect of a variation in the *GHR* gene and investigated an effect of SNP rs6180 on human organ weight using 187 autopsy samples. The cardiac weight (g), left ventricular weight (g), cardiac hypertrophy index, body surface area (m^2), right pulmonary weight (g), left renal weight (g), and right renal weight (g) of the AA genotype were significantly higher than those of the CC genotype. This study is the first to investigate the association of an SNP (rs6180) in *GHR* gene and data routinely measured at autopsy, such as organ weight. Some nonsynonymous variations in the *GHR* gene were identified in the cytoplasmic domain of the GHR protein (Van Dyke *et al.* 2009; Filopanti *et al.* 2012).

Three coding SNPs have been reported (rs6182: p.Cys440Phe, rs6180: p.Ile544Leu, rs6184: p.Pro579Thr) in *GHR*. Among them only rs6180 (p.Ile544Leu; c.1630 A>C) in exon 3 is highly polymorphic; ancestral allele of rs6180 is A allele. (Filopanti *et al.* 2012). Although, to date, effect of rs6180 on protein function is unknown, Takada *et al*. (2003) speculated that rs6180 may result in alterations in downstream signal transduction.

Information on externally visible traits such as gender, eye and hair colour, and height provided by DNA-based investigations would be valuable marker for physical traits (Kayser and Schneider 2009). We have investigated the association of various SNPs with human height (Takeshita *et al.* 2011; Fujihara *et al.* 2012). The specimens used in our previous study were obtained at autopsy and data other than height, such as organ weight, can be applied for association study. The facts that GHR is related to GH allowed us to expect possible associations of the SNPs in the *GHR* gene with physical traits (height, organ weight and so on). Therefore, in the present study, we focussed on analysing the potential effect of polymorphic SNP rs6180 in the *GHR* gene on human organ weight using autopsy samples to explore the marker for physical traits comprehensively.

Materials and methods

Study population

Blood samples ($n = 187$) from Japanese subjects autopsied in Shimane Prefecture were collected. The height, weight, organ weight and cardiac parameters in the autopsy characteristics from the study according to gender are shown in table 1. Genomic DNA was extracted from blood samples collected at autopsy using a QIAamp DNA Blood Mini Kit (Qiagen, Venlo, The Netherlands). The study including usage of genomic DNA derived from autopsy cases was reviewed and approved by the Human Ethics Committee of Shimane University School of Medicine.

SNP typing

In this study, one SNP (rs6180, c.1630 A>C) was analysed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis (Takeshita *et al.* 2011; Fujihara *et al.* 2012). Since the substitution sites corresponding to this SNP neither suppressed nor created any

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Table 1. Height, weight, organ weight and cardiac parameters in the autopsy characteristics from the study according to gender[a].

Variables	Female ($n = 68$)	Male ($n = 119$)	P value[b]
Age (years)	68 (26–94)	60 (18–93)	3.58×10^{-2}
Height (cm)	152 (131–171)	165 (141–181)	3.91×10^{-8}
Weight (kg)	48.8 (25.2–78)	57.4 (32–100)	8.39×10^{-3}
Cardiac weight (g)	335 (150–480)	385 (230–810)	9.85×10^{-3}
Left ventricular weight (g)	138 (66–194)	195.5 (122.6–395.2)	0
Cardiac hypertrophy index	2.20 (0.94–3.16)	2.35 (1.45–4.74)	7.38×10^{-2}
Body surface area (m^2)	1.44 (0.97–1.90)	1.63 (1.16–2.11)	3.39×10^{-10}
Left ventricular mass index	92.9 (49.0–147.0)	118.2 (77.6–188.9)	1.64×10^{-11}
Left pulmonary weight (g)	380 (175–662)	490 (150–1160)	2.25×10^{-5}
Right pulmonary weight (g)	451 (210–740)	590 (165–1280)	7.43×10^{-6}
Hepatic weight (g)	1085 (575–2430)	1310 (210–4500)	1.45×10^{-4}
Pancreatic weight (g)	69 (10–395)	75 (24.5–320)	3.25×10^{-1}
Left renal weight (g)	121.3 (65–252)	155 (70–680)	1.66×10^{-6}
Right renal weight (g)	120 (60–235)	140 (25–310)	1.87×10^{-4}
Cerebral weight (g)	1220 (1000–1475)	1380 (875–1700)	9.96×10^{-10}

[a]Values are presented as median (minimum–maximum).
[b]P values were evaluated by Mann–Whitney U test.

known restriction enzyme recognition sites, we used a mismatched PCR-amplification method for genotyping. Incorporation of a deliberate mismatch near the 3′-terminus of a PCR primer allowed the creation of each enzyme recognition site. Primers for the specific amplification of the DNA fragments encompassing a substitution site corresponding to the SNPs were newly designed on the basis of the nucleotide sequence of the human growth hormone receptor gene rs6180-F (5′-ACTTCTGTGAGGCAGATGCCAAAAAGGGC-3′); rs6180-R (5′-TCCCAGCAGCAGTGGTAAGGCTTTCTGT-3′). The underlined residue indicates the mismatched nucleotide to create *Hae*III cutting site when the substitution site is C. Amplification was performed in a 25 μL reaction mixture using ~2 ng of DNA. The reaction mixture contained a buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl), 1.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM of dNTPs, and 1.25 U of *Taq* polymerase (AmpliTaq Gold; Applied Biosystems, Foster City, USA). PCR was performed with a protocol consisting of initial denaturation at 94°C for 3 min followed by 30 cycles with denaturation at 96°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min followed by a final extension at 72°C for 5 min. Two μL of the PCR product obtained using each pair of primers was digested with the *Hae*III enzyme (New England Biolabs, Ipswich, USA) at 37°C for 2 h in a final reaction mixture volume of 15 μL according to the manufacturer's instruction to determine the genotype of each SNP.

The amplified product from the A allele is resistant to *Hae*III digestion, whereas C allele was completely digested with *Hae*III to yield 104-bp and 28-bp fragments. The digests were then subjected to microchip electrophoresis on the Shimadzu MultiNA using a DNA 500 kit (Shimadzu, Kyoto, Japan) (figure 1). Nucleotide sequences of the representative subjects were confirmed by direct sequencing of the PCR products in which a substitution site corresponding to each SNP was included; the dideoxy chain-terminating

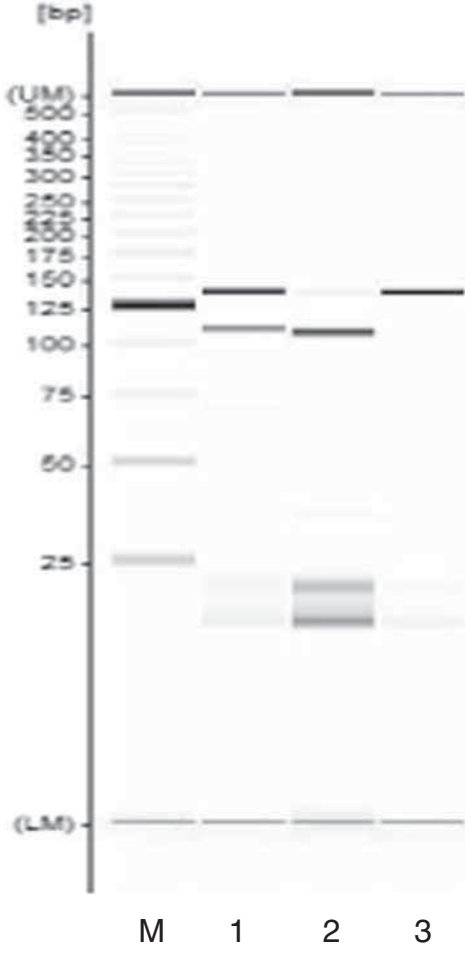

Figure 1. Electrophoretic patterns for genotyping of SNP rs6180 by using the PCR-RFLP method. lane M, marker; lane 1, AC genotype; lane 2, CC genotype; lane 3, AA genotype. The 132-bp fragment was amplified with primers rs6180-F and rs6180-R, and the amplified products were subject to *Hae*III digestion.

Table 2. Genotype distribution and allele frequencies for SNP rs6180.

Genotype	*n* (%) Female ($n = 68$)	Male ($n = 119$)	Total ($n = 187$)	Allele freqeucny (%)		HWE (*P* value)
AA	10 (14.7)	25 (21.1)	35 (36.9)	A	0.409	
CA	27 (39.7)	56 (47.0)	83 (44.4)			10.3 (0.0058)
CC	31 (45.6)	38 (31.9)	69 (18.7)	C	0.591	

Table 3. Comparison of data among different genotypes of *GHR* gene (rs6180) in autopsied subjects[a].

	AA	AC	CC	*P* value[b]
Height (cm)				
Total	160 (146–181)	163 (136–177)	160 (131–173)	0.51
Female	155 (146–163)	151 (136–171)	154 (131–168)	0.61
Male	165 (150–181)	164 (146–177)	166 (140–181)	0.88
Cardiac weight (g)				
Total	395 (235–540)	350 (210–810)	353 (150–645)	0.03
Female	355 (235–475)	332 (210–480)	330 (150–420)	0.15
Male	408 (235–540)	375 (230–810)	372 (240–645)	0.15
Left ventricular weight (g)				
Total	193 (899–268)	177 (89–395)	160 (66–318)	0.02
Female	139 (99–192)	140 (89–194)	136 (66–177)	0.40
Male	209 (125–268)	192 (123–395)	189 (127–318)	0.09
Cardiac hypertrophy index				
Total	2.4 (1.5–3.4)	2.3 (1.3–4.7)	2.2 (1.0–3.8)	0.05
Female	2.2 (1.5–3.0)	2.4 (1.3–3.2)	2.2 (1.0–2.9)	0.006
Male	2.5 (1.5–3.4)	2.3 (1.5–4.7)	2.3 (1.5–3.8)	0.17
Body surface area (m^2)				
Total	1.6 (1.2–2.0)	1.6 (1.2–2.1)	1.5 (1.1–2.0)	0.04
Female	1.5 (1.2–1.8)	1.4 (1.1–1.9)	1.5 (1.0–1.8)	0.52
Male	1.7 (1.5–2.0)	1.6 (1.3–2.1)	1.6 (1.2–2.0)	0.27
Left ventricular mass index				
Total	122 (65–162)	110 (63–187)	105 (49–174)	0.11
Female	89 (65–134)	99 (63–147)	91 (49–130)	0.77
Male	124 (78–162)	115 (88–187)	115 (84–174)	0.26
Left pulmonary weight (g)				
Total	455 (255–1160)	435 (150–1050)	430 (175–1090)	0.11
Female	403 (268–650)	415 (215–740)	450 (210–715)	0.40
Male	460 (255–1160)	483 (150–1050)	498 (250–1090)	0.09
Right pulmonary weight (g)				
Total	550 (330–1280)	515 (165–1130)	490 (210–1095)	0.02
Female	480 (350–660)	415 (215–740)	450 (210–715)	0.70
Male	610 (330–1280)	610 (165–1130)	513 (245–1095)	0.11
Hepatic weight (g)				
Total	1265 (825–2295)	1220 (210–4500)	1230 (510–3070)	0.23
Female	1202 (825–1415)	1050 (665–1800)	1055 (575–2430)	0.84
Male	1330 (830–2295)	1325 (210–4500)	1277 (510–3070)	0.30
Pancreatic weight (g)				
Total	78 (30–265)	70 (10–395)	69 (13–225)	0.25
Female	69 (40–170)	70 (10–395)	66 (13–225)	0.34
Male	80 (30–265)	73 (30–320)	70 (25–225)	0.47
Left renal weight (g)				
Total	160 (95–250)	140 (65–680)	132 (80–390)	0.04
Female	144 (95–195)	120 (65–185)	120 (80–252)	0.50
Male	170 (99–250)	150 (70–680)	143 (95–390)	0.24
Right renal weight (g)				
Total	142 (50–230)	130 (60–310)	120 (25–235)	0.04
Female	127 (90–225)	120 (60–200)	120 (80–252)	0.66
Male	145 (50–230)	138 (65–310)	125 (25–229)	0.11
Cerebral weight (g)				
Total	1330 (1125–1600)	1312 (1085–1650)	1285 (875–1700)	0.33
Female	1225 (1125–1310)	1220 (1070–1440)	1220 (1000–1475)	0.73
Male	1430 (1165–1600)	1345 (1065–1650)	1383 (875–1700)	0.53

[a] Values are presented as median (minimum–maximum).
[b] *P* values different genotypes by univariate regression analysis.

method with the BigDye®Terminator Cycle Sequencing Kit was employed using a Genetic Analyzer 310 (Applied Biosystems) according to the manufacture's instruction.

Statistical analysis

Chi-square (χ^2) analysis was performed to evaluate the Hardy–Weinberg equilibrium (HWE). Mann–Whitney U test was performed to show the gender difference. Univariate (single) regression analysis was used to compare adult height, weight, organ weight and cardiac parameters among different genotypes. These statistics were conducted using the program STATCEL2 (OMS Publishing Inc, Saitama, Japan). Differences at $P<0.05$ were considered to be statistically significant.

Results and discussion

This study is the first to investigate the association of an SNP rs6180 (p.Ile544Leu; c.1630 A>C) in the *GHR* gene and data routinely measured at autopsy, such as organ weight. The allele and genotype distributions of the rs6180 polymorphism are presented in table 2. Frequencies of alleles A and C of the rs6180 SNP (c.1630 A>C) were 40.9% and 59.1%, respectively. Genotype frequencies of the rs6180 SNP was in good agreement with the HWE at the loci. The allele frequencies of A and C alleles for the Asian (Han Chinese), Asian (Japanese in Tokyo), European, and sub-Saharan African are reported to be 38.4% and 61.6%, 41.9% and 58.1%, 53.0 % and 47.0%, and 55.8% and 41.2%, respectively in NCBI database. C allele is predominant in Asian populations but A allele is predominant in European and African populations. The allele frequencies of this study are similar to those from NCBI database of other Asian.

Previously, we showed that a SNP in the *HMGA2* gene is associated with human height and that SNPs in the LIM homeobox 3-quiescin Q6 sulphhydryl oxidase 2 (*LHX3-QSOX2*) gene and *IGF1* gene are not related to human height indicating that the height-related gene is different among populations (Fujihara *et al.* 2012). In the present study, rs6180 is not significantly associated with height. As shown in table 3, rs6180 was significantly related to the cardiac weight (g), left ventricular weight (g), cardiac hypertrophy index, body surface area (BSA (m^2)), right pulmonary weight (g), left renal weight (g), and right renal weight (g). These associations were not observed when statistical analysis was performed for female and male separately except for female cardiac hypertrophy index (table 3). This may be due to difference in sample size of female and males. Further accumulative data is needed to clarify the association.

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