susceptibility genes underlying aspirin hypersensitivity, we further genotyped the two significant SNPs in 282 ATA patients for comparison. In the AIA–ATA association study, the INDO-SNP2 also showed a statistically significant association with AIA ($P = 0.038$) (Table 4), whereas an association of the IL1R2-SNP10 with AIA was marginal ($P = 0.073$) with the same direction of genetic effect of the associated allele on AIA susceptibility (Table 5). In contrast, no significant differences in allele frequencies at the two SNPs were observed between CTR and ATA groups (statistical data not shown). These SNP-based association results indicate that the two SNPs in *INDO* and *IL1R2* are associated with the risk of aspirin hypersensitivity rather than an asthmatic reaction in Japanese population.

Figure 3 shows D'- and r^2-based LD block structures in the genomic regions around *INDO* and *IL1R2*, respectively. We observed a strong LD ($|D'| = 0.98$) between INDO-SNP1 and -SNP2 in the *INDO* region. A highly structured LD pattern, a major LD block structure ($|D'| > 0.7$) covered by IL1R2-SNP6 to -SNP11, was observed in *IL1R2* (Fig. 3). Next, we conducted a haplotype-based association study within the respective LD blocks (Tables 6 and 7). We found that one haplotype of *INDO*, m/m (double minor haplotype) at INDO-SNP1 and -SNP2, was underrepresented in AIA with statistical significance after multiple test correction with Bonferroni's correction (Table 6; $\chi^2 = 6.74$, df = 1, corrected $P = 0.038$), indicating a protective effect of the m/m haplotype of *INDO*. One haplotype of *IL1R2*, M/M/M (triple major haplotype) at IL1R2-SNP6, -SNP10, and -SNP11, showed a highly significant difference between AIA and CTR (Table 7; $\chi^2 = 8.94$, df = 1, corrected $P = 0.011$), indicating that the M/M/M haplotype represented a risk for AIA.

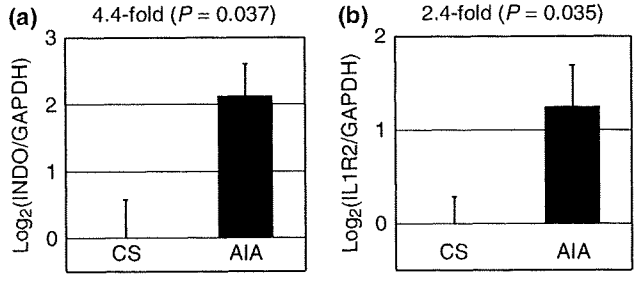

Fig. 2. Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis validates expression differences for two genes, *INDO* and *IL1R2*, in nasal polyps from aspirin-intolerant asthma (AIA) and chronic sinusitis (CS) patients. Relative amounts of the respective genes in nasal polyps (AIA, $n = 10$; CS, $n = 4$) were measured by real-time RT-RCR using TaqMan Gene Expression Assays. *Y*-axes indicate the $\log_2$ relative expression levels, normalized to the amount of *GAPDH* and relative to the averaged expression levels in CS groups. The expression levels of *INDO* and *IL1R2* were significantly higher in AIA than CS nasal polyps by the Welch *t*-test.

INDO encodes indoleamine 2, 3-dioxygenase, which is a rate-limiting enzyme of tryptophan catabolism and is expressed in various cell types such as fibroblasts, macrophages, and dendritic cells [26]. INDO activity is induced by interferons (IFNs) and further enhanced by inflammatory cytokines such as IL-1 [27] but suppressed by anti-inflammatory cytokines such as TGF-β and IL-4 [28, 29]. INDO-induced tryptophan degradation in macrophages results in inhibition of T cell proliferation [30], suggesting that INDO plays an important role in the regulation of T cell-mediated immune responses. Aspirin inhibits INDO activity in stimulated peripheral blood mononuclear cells indirectly, via its inhibitory effect on the production of IFN-γ [31]. Therefore, the functional disturbance of INDO activity due to the INDO-SNP2 might play a role in the pathogenesis of aspirin sensitivity or AIA. A possibility remains that an unknown SNP in tight LD with the INDO-SNP2 or on the m/m haplotype could serve as a *bona fide* causality, which could prevent AIA induction by keeping INDO activity normal. Further studies are needed to resolve the functional significance of the INDO-SNP2 and the m/m haplotype in the genetic aetiology of AIA.

Interleukin 1 receptor type II (IL1R2) acts as a soluble decoy receptor that inhibits IL-1 signalling [32]. The inhibition of IL-1 binding to the receptor in human monocytes results in a reduction of COX-2 activity but not COX-1 activity [33]. As an imbalance in arachidonate

Table 4. Allelic association of *INDO* SNPs with AIA in Japanese population

					MAF		AIA vs. CTR				MAF	AIA vs. ATA	
SNP no.	Position*	Localization	dbSNP ID	Alleles (M/m)†	AIA $n = 219$	CTR $n = 374$	χ^2	Odds ratio (95% CI)	P	Corrected P‡	ATA $n = 282$	χ^2	P
INDO-SNP1	−1953	5′-upstream	rs3808606	T/C	0.414	0.472	3.65	0.79 (0.62–1.01)	0.056	0.17	ND		
INDO-SNP2	6202	intron4	rs7820268	C/T	0.101	0.163	8.47	0.58 (0.40–0.84)	0.0036	0.011	0.145	4.29	0.038
INDO-SNP3	13994	intron9	rs3739319	A/G	0.474	0.436	1.63	1.17 (0.92–1.49)	0.20	0.60	ND		

*Numbers indicate the nucleotide position from the first nucleotide of exon 1.

†M and m denote major and minor alleles, respectively, at each SNP site.

‡Corrected *P* values were obtained using Bonferroni's correction.

MAF, minor allele frequency; AIA, aspirin intolerant asthma; CTR, non-asthmatic control; ATA, aspirin tolerant asthma; ND, not determined; SNP, single nucleotide polymorphism.

Table 5. Allelic association of *IL1R2* SNPs with AIA in Japanese population

SNP No.	Position*	Localization	dbSNP ID	Alleles (M/m)†	MAF AIA $n=219$	MAF CTR $n=374$	AIA vs. CTR χ^2	Odds ratio	P	MAF Corrected P‡	AIA vs. ATA ATA $n=282$	χ^2	P
IL1R2-SNP1	−6913	5′-upstream	rs4851519	C/T	0.272	0.219	4.27	1.34 (1.01-1.76)	0.039	0.43	ND		
IL1R2-SNP2	−4381	5′-upstream	rs35789178	T/G	0.368	0.358	0.12	1.05 (0.81-1.34)	0.73	1	ND		
IL1R2-SNP3	−3657	5′-upstream	rs12467316	A/C	0.299	0.376	7.21	0.71 (0.55-0.91)	0.0072	0.080	ND		
IL1R2-SNP4	−3145	5′-upstream	rs12468239	C/T	0.090	0.093	0.02	0.97 (0.64-1.47)	0.89	1	ND		
IL1R2-SNP5	9147	intron1	rs11691240	C/T	0.439	0.479	1.73	0.85 (0.67-1.08)	0.19	1	ND		
IL1R2-SNP6	14513	intron1	rs3755482	A/G	0.273	0.340	5.58	0.73 (0.56-0.95)	0.018	0.20	ND		
IL1R2-SNP7	15413	intron1	rs719250	G/A	0.381	0.318	4.73	1.32 (1.02-1.69)	0.030	0.33	ND		
IL1R2-SNP8	21335	intron3	rs2110562	C/T	0.179	0.193	0.32	0.91 (0.67-1.25)	0.57	1	ND		
IL1R2-SNP9	40304	3′-downstream	rs4851531	T/C	0.462	0.408	3.16	1.25 (0.98-1.59)	0.075	0.83	ND		
IL1R2-SNP10	42202	3′-downstream	rs11688145	C/A	0.244	0.330	9.23	0.66 (0.50-0.86)	0.0024	0.026	0.296	3.22	0.073
IL1R2-SNP11	54346	3′-downstream	rs7588933	A/G	0.186	0.206	0.70	0.88 (0.65-1.19)	0.40	1	ND		

*Numbers indicate the nucleotide position from the first nucleotide of exon 1.

†M and m denote major and minor alleles, respectively, at each SNP site.

‡Corrected P values were obtained using Bonferroni's correction.

MAF, minor allele frequency; AIA, aspirin intolerant asthma; CTR, non-asthmatic control; ATA, aspirin tolerant asthma; ND, not determined

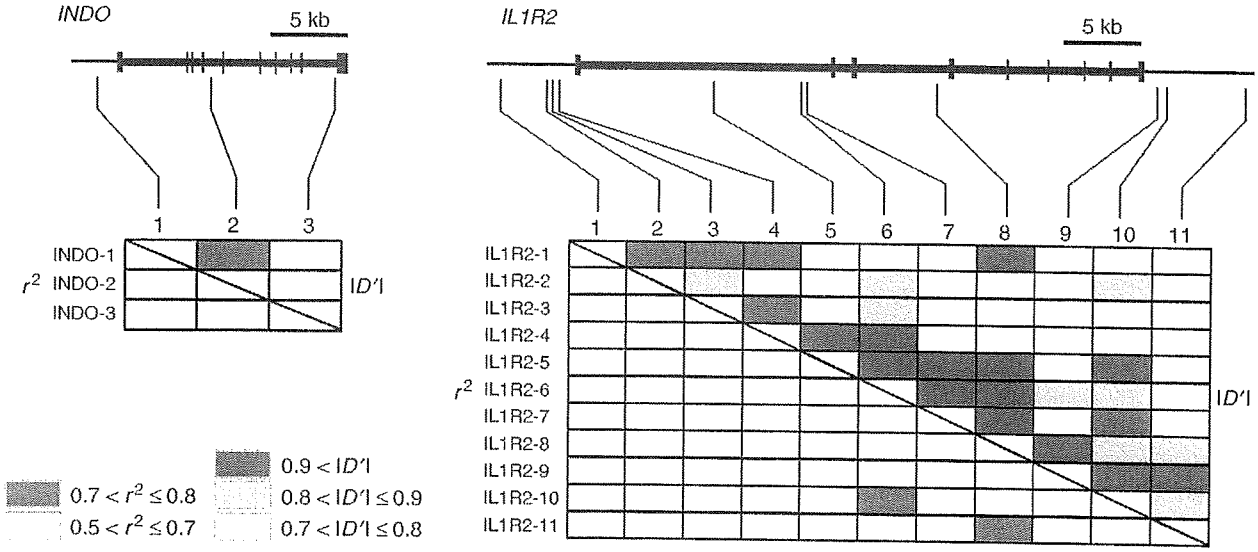

Fig. 3. Linkage disequilibrium pattern of *INDO* and *IL1R2*. The gene structures of *INDO* (left) and *IL1R2* (right), together with positions of the 14 single nucleotide polymorphisms (SNPs) examined, are shown. Pairwise LD coefficients, D' and r^2, were determined and expressed as a block structure. In the schematic block, red boxes indicate a pairwise LD of $|D'|>0.9$, pink $0.9\geqslant|D'|>0.8$, and orange $0.8\geqslant|D'|>0.7$; blue boxes indicate a pairwise LD of $0.8\geqslant r^2>0.7$ and light blue $0.7\geqslant r^2>0.5$. Blank boxes represent $|D'|\leqslant 0.7$ or $r^2\leqslant 0.5$.

metabolism is the usual pathogenesis proposed for AIA, the elevated level of IL1R2 that inhibits the inflammatory effects of IL-1α in the respiratory tract might well be involved in the pathogenesis of AIA and the formation of nasal polyps. Although IL1R2-SNP10 showed the strongest allelic association with AIA (Table 5), the functional impact of an SNP locating 3′-downstream of the gene is currently unclear despite its up-regulated expression in the AIA polyp (Table 3). There is also the possibility that an unidentified functional SNP in LD with the IL1R2-SNP10 could be a causality. Further genetic fine mapping in *IL1R2* will be required to fully understand which genetic variant contributes to the risk of AIA. The functional impacts of the SNP and haplotype also require further investigation.

In conclusion, DNA microarray technology was used to monitor global gene expression patterns specific to AIA nasal polyp tissues to clarify the pathophysiology of AIA. From the gene expression profile, candidate genes underlying AIA were selected and subjected to an association study. We identified SNPs in *INDO* and *IL1R2* that may represent genetic susceptibility to AIA. This genetic study

Table 6. Haplotype-based association of *INDO* with AIA

	Haplotype frequency					
Haplotype (INDO-SNP1/2)*	Total	AIA	CTR	χ^2	*P*	Corrected $P^\dagger$
M/M (T/C)	0.552	0.591	0.531	3.75	0.053	0.21
m/M (C/C)	0.306	0.304	0.307	0.01	0.94	1
m/m (C/T)	0.141	0.105	0.161	6.74	0.0094	0.038
			Global comparison	8.05 (df = 3)	0.045	0.18

*M and m denote major and minor alleles, respectively, at each SNP site. The corresponding nucleotides at the respective sites are shown in parentheses.
†Corrected *P* values were obtained using Bonferroni's correction.
AIA, aspirin intolerant asthma; CTR, non-asthmatic control; SNP, single nucleotide polymorphism.

Table 7. Haplotype-based association of *IL1R2* with AIA

	Haplotype frequency					
Haplotype (IL1R2-SNP6/SNP10/SNP11)*	Total	AIA	CTR	χ^2	*P*	Corrected $P^\dagger$
M/M/M (A/C/A)	0.494	0.552	0.458	8.94	0.0028	0.011
m/m/M (G/A/A)	0.277	0.243	0.299	4.03	0.045	0.18
M/M/m (A/C/G)	0.182	0.170	0.189	0.59	0.44	1
			Global comparison	8.72 (df = 4)	0.069	0.28

*M and m denote major and minor alleles, respectively, at each SNP site. The corresponding nucleotides at the respective sites are shown in parentheses.
†Corrected *P* values were obtained using Bonferroni's correction.
AIA, aspirin intolerant asthma; CTR, non-asthmatic control; SNP, single nucleotide polymorphism.

represents only first-stage evidence of the association because only Japanese individuals were included, and so further replication in independent case–control samples is required to confirm the role of *INDO* and *IL1R2* genotypes in the genetic risk for AIA. A pathophysiological link between the two gene products is unclear and further investigation is evidently needed. In addition, further studies including functional analyses of the SNPs with respect to how genetic variants are responsible for the risk of AIA are also required for a full understanding of the pathogenesis of AIA.

Acknowledgements

We thank tissue and DNA donors and supporting medical staff for making this study possible. This work was supported in part by a Grant-in-Aid for scientific research from the Japanese Ministry of Education, Science, Sports, and Culture. We are grateful to Yoshiko Sakamoto, Hiromi Kamura, and Kozue Otaka for their technical assistance. All of authors have no declared conflicts of interest.

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Association of the growth hormone receptor gene polymorphisms with mandibular height in a Korean population

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ARTICLE INFO

Article history:
Accepted 6 March 2009

Keywords:
Growth hormone receptor gene
Mandible
Korean
Ethnicity

ABSTRACT

Growth hormone receptor gene (*GHR*) is one of the likely candidates for determining morphological traits, because GH is a key regulator of bone growth. The genetic association of *GHR* in exon 10 with mandibular ramus height has been found in different populations, Japanese and Chinese. On the other hand, two common isoforms of *GHR*, one full-length (*fl-GHR*) and the other lacking the extracellular domain encoded by exon 3 (*d3-GHR*), are associated with differences in responsiveness to GH. The purpose of this study involving 159 Korean subjects was to study the associations between a *GHR* polymorphism (*d3/fl-GHR*) that results in genomic deletion of exon 3 and craniofacial morphology, and to study the associations between *GHR* genotypes in exon 10 and craniofacial morphology. Moreover, the allelic frequencies in a multi-ethnic population (24 Han Chinese, 24 African-Americans, 24 European-Americans, and 24 Hispanics) in a *GHR* polymorphism (*d3/fl-GHR*) were compared in this study. The five craniofacial linear measurements (cranial base length, maxillary length, overall mandibular length, mandibular corpus length, and mandibular ramus height) obtained from lateral cephalograms were examined as craniofacial morphology. We found that the *d3/fl-GHR* polymorphism had no association for any measurements, and a statistically significant association ($P = 0.024$) between the *GHR* polymorphisms P561T and C422F in exon 10 and mandibular ramus height. Neither SNPs besides P561T and C422F polymorphisms in exon 10 nor the measurements besides mandibular ramus height have statistically significances. Both derived alleles at P561T and C422F SNPs were highly associated with only one haplotype, haplotype-4 in Korean population. As quantitative haplotype association, the results showed a significant difference in mandibular ramus height between individuals having one haplotype-4 and others without haplotype-4 ($P = 0.028$). Moreover, we found that the *d3/fl-GHR* polymorphism showed diverse frequency in different population. Regarding *GHR* genotypes in exon 10, the present study mostly

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0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2009.03.002

reflected the results obtained for a Japanese population, although our current study does not replicate the correlation between the I526L polymorphism of *GHR* and mandibular ramus height as was reported in a previous study of Han Chinese. The results of the present study suggest that the *GHR* exon 10 SNPs, not *d3/fl-GHR*, contribute to changes in the mandibular ramus height of Koreans.

1. Introduction

Craniofacial morphology is a polygenic, quantitative trait that is determined by genetic and environmental factors.[1] Studies on similarities in craniofacial morphology between close relatives have clarified that the genetic factors play an important role in the determination of craniofacial morphology.[2,3] Furthermore, a comparison of monozygous and dizygous twins has revealed a clear genetic influence on craniofacial morphology.[4] Identifying the genetic susceptibility for specific craniofacial phenotypes would enable more effective diagnosis and treatment of cranial malformations such as mandibular prognathism.[5]

Recent advances in clinical genetics have increased the fund of knowledge on genetic susceptibilities for craniofacial phenotypes.[5–7] It has recently been reported that single nucleotide polymorphisms (SNPs) of the growth hormone receptor (*GHR*) gene are associated with mandibular height in Japanese and Chinese populations.[8,9] It has been reported that the *GHR* polymorphism P561T in exon 10 is associated with mandibular height in the Japanese population.[8] Zhou et al. found that the *GHR* polymorphism I526L in exon 10 is associated with mandibular height in the Chinese population,[9] and they speculated that the discordance between Chinese and Japanese populations is due to either a lack of power in their experiments or a real difference between these populations. Considerable similarities have been observed in the linkage disequilibrium (LD) pattern between the East Asian populations (Korean, Japanese, and Han Chinese). The patterns of haplotype structure and the haplotype frequencies in the Korean population are very similar to those in the Japanese and Han Chinese populations. In particular, the LD patterns and haplotype frequencies of the Korean population show high degrees of similarity with those of the Japanese population.[10]

A polymorphism of *GHR* (*d3/fl-GHR*) that results in genomic deletion of exon 3 is associated with increased responsiveness to growth hormone; i.e., children carrying at least one *d3-GHR* allele show 1.7–2 times greater response to growth hormone than do *fl-GHR/fl-GHR* homozygotes.[11] This common polymorphism of human *GHR* that results in genomic deletion of exon 3 has recently been associated with the degree of height increase in response to GH therapy in French short children who were born small for gestational age or with idiopathic short stature[11], as well as in German Turner syndrome patients[12] and Brazilian GH-deficient children[13]. It may be a clue to understanding the ethnic difference of the association between *GHR* and mandibular ramus height to determine the allelic frequencies in a multiethnic population.

The purpose of this study is to characterise further the roles of the *d3/fl-GHR* SNP of *GHR* and five SNPs in exon 10 of *GHR* in 159 Korean subjects with regard to craniofacial morphology, and to define the allelic frequencies of *d3/fl-GHR* in a multiethnic population, which consisted of Han Chinese, African-Americans, European-Americans, and Hispanics.

2. Material and methods

2.1. *Subjects*

Genomic DNA samples and lateral cephalograms were obtained for the group of 159 Korean subjects, which comprised 100 men (age range, 20–49 years; mean age, 25.24 years) and 59 women (age range, 18–58 years; mean age, 24.39 years). The subjects were patients at dental hospitals or volunteers from the Pusan area. All the individuals were unrelated. Subjects who had congenital disorders, such as cleft palate or general physical disease, were excluded from the study. None of the subjects had received orthodontic or orthopaedic treatment. The 159 Korean subjects were comprised of 87 Class I, 44 Class II, and 28 Class III subjects.

In addition, DNA samples from 24 Han Chinese, 24 African-Americans, 24 European-Americans, and 24 Hispanics without craniofacial measurement data were obtained from the Coriell Cell Repository (Camden, NJ, USA), and used only as reference populations for the allelic frequencies of the exon 3-deleted/full-length (*d3/fl-GHR*) polymorphism.[14]

The protocol used in this study was approved by the Ethical Committee of Pusan National University, and all patients gave their written informed consent to participate in the study before DNA samples were taken.

2.2. *Genotyping and sequencing*

For DNA sample collection, the inside of the mouth was scraped with 10 strokes of a brush (MasterAmp™ Buccal Swab DNA Extraction Kit; AR Brown Co. Ltd., Tokyo, Japan). Four samples were collected from each subject. Genomic DNA was obtained from these samples.

We screened the *GHR* coding region for the five known SNPs of exon 10, [9] and for a polymorphic deletion of exon 3.[14] The analysed SNPs were C422F (dbSNP ID; rs6182), S473S (rs6176), P477T (rs6183), I526L (rs6180), P561T (rs6184), and *d3/fl-GHR*. The dbSNP numbers are taken from the dbSNP database at the NCBI (http://www.ncbi.nlm.nih.gov/SNP/).

Polymerase chain reaction (PCR) was performed according to a standard protocol. To determine the *GHR* exon 10

Table 1 – The relationship between six SNPs in *GHR* and six linear measurements of body height and craniofacial morphology in 159 Korean subjects.

	n	Body height (cm)			N-S (mm)			A′-PTM′ (mm)			Gn-Co (mm)			Pog′-Go (mm)			Co-Go (mm)		
		Mean	S.D.	*P*-value	Mean	S.D.	*P*-value	Mean	S.D.	*P*-value	Mean	S.D.	*P*-value	Mean	S.D.	*P*-value	Mean	S.D.	*P*-value
d3/fl-GHR																			
fl/fl	92	171.2	7.2	0.24	75.3	3.8	0.92	52.0	3.2	0.79	139.6	8.3	0.21	84.8	5.5	0.12	72.0	7.6	0.59
fl/d3	24	169.7	6.3		75.6	3.8		52.2	3.4		137.8	6.9		82.8	5.2		70.7	6.3	
d3/d3	9	173.7	6.9		75.4	4.4		51.3	2.5		142.7	10.4		84.5	4.5		72.5	6.6	
C422F/P561T																			
GG/CC	124	170.6	7.1	0.49	75.2	3.8	0.70	52.1	3.3	0.32	138.7	8.0	0.38	83.9	5.5	0.77	71.9	7.1	0.02*
GT/CA	24	169.6	6.7		75.2	3.7		51.3	2.9		137.7	8.6		84.2	5.3		68.5	5.5	
S473S																			
CC	145																		
CT	3																		
P477T																			
CC	145																		
CA	3																		
I526L																			
AA	62	171.5	7.2	0.19	75.8	3.7	0.24	52.6	3.2	0.24	139.1	8.7	0.71	84.3	5.7	0.82	72.0	7.6	0.68
AC	61	170.0	7.2		74.8	3.3		51.7	3.2		137.9	7.8		83.5	5.3		71.2	6.5	
CC	24	169.0	6.3		75.1	4.5		51.7	3.7		139.2	7.9		84.2	5.8		71.1	7.4	

* $P < 0.05$.

genotypes, PCR amplification was performed in the Gene Amp 9700 (Applied Biosystems, Tokyo, Japan) with a 10-μL PCR reaction that contained 30 ng of genomic DNA, 200 μM of each dNTP, 0.25 U of Ex Taq (Takara, Otsu, Japan), and 0.1 μM of each primer. The PCR involved initial denaturation at 95 °C for 1 min, followed by 40 cycles of 94 °C for 30 s and 72 °C for 1 min. After removal of the remaining primers and dNTP using ExoSAP-IT (GE Healthcare Life Science, USA), the products were subjected to BigDye v1.1 sequencing in the ABI PRISM 3700 DNA Analyzer (Applied Biosystems). Polymorphisms were identified using the Sequencer program (Gene Code Co., Ann Arbor, MI, USA). Each polymorphism was confirmed by sequencing the nucleotides from both strands.

For the genotyping of *d3/fl-GHR* in exon 3, PCR was performed using the G1, G2, and G3 primers (GenBank accession no. AF155912) and the published method,[14] with minor modifications. Briefly, we amplified the wild-type allele (*fl-GHR*) using primer pairs G1 and G2, and the deleted allele (*d3-GHR*) using the primer pairs G1 and G3 in separate 50-μl PCR reactions. Amplified PCR products were electrophoretically analysed on a 1% agarose gel and stained with ethidium bromide.

2.3. *Craniofacial measurements*

The lateral cephalograms were traced and measured by a single examiner (EH. K) using the Power Cephalo software (ReazaNet Co., Tokyo, Japan). To determine the errors associated with digitising and measuring, 30 radiographs were selected randomly. The examiner repeated the digitising and measuring 2 weeks later. A paired t test was applied to the first and second measurements, and no error was found. Cranial base length (nasion-sella; N-S), maxillary length (A'-PTM'), overall mandibular length (gnathion-condylion; Gn-Co), mandibular corpus length (pogonion'-gonion; Pog'-Go), and mandibular ramus height (condylion-gonion; Co-Go) were measured.[8] ANB angle have been measured as this angle was used for testing normal distribution. Body height was also measured for all Korean subjects.

The Kolmogorov–Smirnov test was performed to assess the body height and distribution pattern of craniofacial morphology (ANB angle, a cephalometric measurement of the anterior–posterior relationship of the maxilla to the mandible), and the mean, median, standard deviation, skewness, and kurtosis of each measurement were calculated.

2.4. *Haplotyping, LD analysis, and statistical analysis*

Tests for Hardy–Weinberg equilibrium, and the allele and genotype frequencies were all performed. The associations between six GHR SNPs and craniofacial measurements were analysed using the Mann–Whitney test. Quantitative haplotype analysis was tested for each Haplotype with One-factor ANOVA or the Mann–Whitney test. All the analyses were performed using the SPSS 10.0 (SPSS Inc., Chicago, IL, USA). A P-value < 0.05 was considered significant.

The LD for all possible two-way combinations of SNPs was tested using the D′ and r^2 values.[15,16] Haplotype block analysis was conducted with the Gabriel and the Four Gamete methods.[17,18] SNP haplotypes and their frequencies were estimated by the maximum likelihood method with an expectation-maximisation algorithm.[19] The SNPAlyze 5.1 standard software (DYNACOM, JAPAN) was used to conduct LD and haplotype block.

3. Results

Table 1 shows the frequencies of the six genotypes of *GHR* and the relationships between these genotypes and six linear measurements of body height and craniofacial morphology in 159 Korean subjects. For the distribution pattern and the results of the Kolmogorov–Smirnov test, the heights and ANB angles of the 159 subjects were found to reflect the normal distribution (data not shown). Thus, the subjects of the present study were presumed to represent a normally distributed Korean population in terms of craniofacial morphology and height. The Mann–Whitney U-test was used to identify potential differences in measurements. Heterozygosity for S473S and P477T (genotypes CT and CA, respectively) was found in only three subjects. Therefore, statistical analysis was not performed for S473S and P477T. The genotype-specific association analysis revealed that only Co-Go (mandibular ramus height) was significantly correlated with the P561T (induced by C-to-A transversion) and C422F (induced by G-to-T transversion) variants ($P = 0.024$).

The strength of the LD for each SNP pair was measured using the D′ and r^2 values (Fig. 1). The D′ coefficient was almost equal to 1 for almost all the pairs of SNPs in the Korean population, which indicates that the gene is located in a single LD block. The r^2 parameter, which measures the correlation between alleles, showed that one of the SNP pairs was tightly

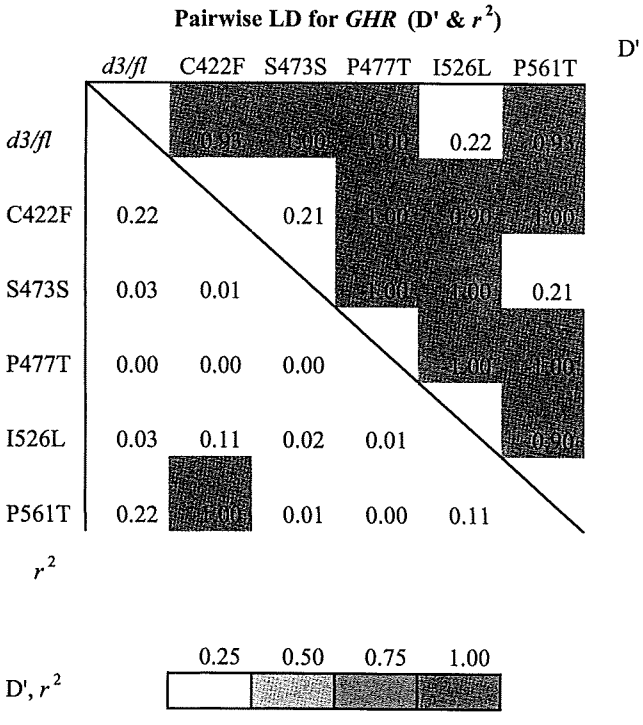

Fig. 1 – Plot of the D′ values of the *GHR* polymorphisms in 159 Korean subjects. LD, Linkage disequilibrium.

Table 2 – Estimated haplotype frequencies of six SNPs.

	Haplotype						Estimated common haplotype frequency
	d3/fl	C422F	S473S	P477T	I526L	P561T	
Haplotype-1	*fl*	G	C	C	A	C	0.4785
Haplotype-2	*fl*	G	C	C	C	C	0.2417
Haplotype-3	*d3*	G	C	C	A	C	0.1214
Haplotype-4	*d3*	T	C	C	C	A	0.0738
Haplotype-5	*d3*	G	C	C	C	C	0.0533

Haplotypes constructed on the basis of genotypic data for six SNPs spanning the LD block covering GHR. Frequency of each haplotype within the sample of 159 Korean individuals (nine haplotypes), listed in decreasing order. The three major haplotypes accounted for 85% of the haplotypes in the sample.

correlated. LD analysis revealed that two SNPs, C422F and P561T, were in complete LD.

Haplotype analysis, which was constructed with six SNPs, showed that three major haplotypes accounted for 85% of the estimated haplotypes in the Korean population. All the other haplotypes had frequencies of less than 10% (Table 2).

As shown in Table 2, both derived alleles at P561T and C422F SNPs were highly associated with only one haplotype, haplotype-4 in Korean population. As quantitative haplotype association, the results showed a significant difference in mandibular ramus height between individuals having one haplotype-4 and others without haplotype-4 ($P = 0.028$) (Table 3).

We detected the *d3/fl-GHR* variant in all five populations (Korean, Han Chinese, African-American, European-American, and Hispanic). The *d3/fl-GHR* genotype frequencies varied among the populations (Table 4), with high frequencies *d3-GHR* allele in African-Americans (47.9%) and European-Americans (31.3%). The remaining populations had *d3/fl-GHR* genotype frequencies of 10–20%.

Table 3 – Haplotype association study of mandibular ramus height (CD-GO).

Copy number	*n*	Mean	S.D.	P-value
Haplotype-1				
0	44	71.4	5.8	0.99
1	52	71.5	7.4	
2	29	71.7	8.1	
Haplotype-2				
0	66	71.4	7.5	0.99
1	45	71.6	6.5	
2	14	71.6	7.1	
Haplotype-3				
0	92	71.1	7.3	0.35
1	33	72.5	6.1	
Haplotype-4				
0	104	72.1	7.0	0.03*
1	21	68.4	6.8	
Haplotype-5				
0	119	71.4	7.1	0.54
1	6	72.6	5.3	

* $P < 0.05$.

4. Discussion

Several studies have succeeded in elucidating susceptibility locus-related non-syndromic craniofacial morphology because craniofacial morphology is strongly influenced by genetic background.[5–9] We demonstrate an association between the P561T and C422F SNPs (not located in exon 3) of the *GHR* locus and mandibular ramus height in a Korean population. In terms of haplotye, we found a significant difference in mandibular ramus height between individuals having one haplotype-4 (*d3*-T-C-C-C-A) and others without haplotype-4 in the Korean population.

Comparisons of the facial morphologies of Korean children with idiopathic short stature and growth hormone deficiency have revealed common characteristics.[20,21] Disproportionate growth of the cranial base structures and jaws results in facial retrognathia, which entails a proportionately smaller posterior than anterior facial height in persons of short stature with growth hormone deficiency.[21] Children who are in receipt of long-term GH replacement therapy show exaggerated growth of the craniofacial skeleton, especially with respect to the height of the mandibular ramus.[22,23] In a comparison of children with Turner syndrome who received recombinant human growth hormone treatment and a large cross-sectional control group, Rongen-Westerlaken et al.[24] have found a statistically significant increase in ramus growth that is associated with mandibular ramus height but not with mandibular body length, maxillary length or anterior cranial base length. Moreover, in *GHR* knockout mice, mandibular ramus height is significantly reduced,[25] and disproportional skeletal growth is reflected by decreased femur: crown-rump and femur:tibia ratios.[26] Therefore, *GHR* is suggested to have a site-, area- or region-specific effects.[27] On the other hand, growth hormone insensitivity syndrome of genetic origin has been linked to many different mutations of the *GHR*, and is associated with a wide range of severities of clinical and biochemical phenotypes. Mandibular growth is also influenced by multiple factors,[28] among which heterozygous *GHR* mutations appear to play a more or less important role, depending on the kind of mutation and on the overall genetic make-up of the individual. Although there is continuing interest in the functional importance of the P56IT and C422F variants, their precise roles remain unknown. The availability of an environmental factor (i.e., orthopaedic treatment) has made it possible to initiate therapeutic trials on children with short ramus height.[27]

Table 4 – Allele distribution of *d3/fl-GHR*.

		Korean (n = 159) (%)	Han Chinese (n = 24) (%)	African American (n = 24) (%)	European Americans (n = 24) (%)	Hispanic (n = 24) (%)
d3/fl-GHR	*fl*	83.2	84.1	52.1	68.8	81.8
	d3	16.8	15.9	47.9	31.3	18.2

The results of these trials, particularly with respect to the differential effects of such therapy on subjects with or without P561T/C422F will be of great interest.

Dos Santos et al.[11] have recently reported an association between the *d3-GHR* genotype and increased responsiveness to high-dosage recombinant human growth hormone therapy in short children without GH deficiency. In the present study, the prevalence of the *d3-GHR* allele was found to be 15–50%. The prevalence of the *d3-GHR* allele in persons of European origin has been previously reported as 25–32%, with a homozygosity frequency of 9–14%.[11,12,14] It has been speculated that this supposedly very flexible region of the GHR protein plays a role in the conformational changes that occur during transactivation of the GHR dimer by GH.[11] Nonetheless, in the present study, there was no association between *d3/fl-GHR* polymorphism and craniofacial measurements in a Korean population.

On an average, the allele frequencies for populations from different continents differ by 16–19%, and for populations within a continent, such as Koreans and Japanese, they differ by 5–10%.[29] In that report, it was described that these differences may be sufficiently large, even among the closely related Korean, Japanese, and Chinese populations, to cause substructural problems for case–control genetic studies of complex traits. Despite, we analysed five SNPs in exon 10 of the *GHR* gene in Korean subjects, and found an association between the P561T and C422F polymorphisms of *GHR* and mandibular ramus height, reflecting the results obtained for a Japanese population.[8] Although a previous study of 95 Han Chinese reported a correlation between the I526L polymorphism of *GHR* and mandibular ramus height, our current study with 159 unselected Korean subjects does not replicate this finding. The reason of the discordance between Chinese and Japanese/Korean populations remains still unclear. A haplotype-based study based on HapMap data is required to assess a difference among Asian populations. The result of the present study also raises the possibilities for a real difference between these populations.

Information on ethnic differences in allele frequency of disease-associated variants is important for better understanding of the pathologic mechanisms of polymorphisms.[30] We could not obtain craniofacial measurements in other ethnic groups analysed. However, we found that the *d3/fl-GHR* polymorphism showed diverse frequency in different population. Craniofacial morphology has ethnic differences.[31–34] It might imply the need for independent studies of craniofacial morphology for *GHR* in each ethnic group, and our work emphasises the importance of close matching of ethnic groups, especially when craniofacial morphology is examined.

Based on the present data, we conclude that there is a significant association between the P561T and C422F polymorphisms of *GHR* (which are in LD) and mandibular ramus height in a Korean population.

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ORIGINAL ARTICLE

Further evidence for an association between mandibular height and the growth hormone receptor gene in a Japanese population

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Introduction: Craniofacial morphology has a significant genetic component. It has recently been reported that single nucleotide polymorphisms (SNPs) in the growth hormone receptor gene (GHR) are associated with mandibular height. To confirm these findings, we genotyped SNPs in healthy Japanese subjects, about 1.7 times the number analyzed in previous reports. **Methods:** By using 5 SNPs in exon 10 of the GHR, we studied the relationships between genotypes and craniofacial linear measurements. The allelic frequencies in a multiethnic population (Han Chinese, African American, European American, and Hispanic) were also observed. **Results:** There was a significant association between SNPs and mandibular ramus height ($P = 0.029$), confirming our previous report of an association between polymorphism P561T and mandibular ramus height. Moreover, the SNP, C422F, was in linkage disequilibrium with P561T. Subjects with genotype CC of polymorphism P561T and genotype GG of polymorphism C422F had significantly greater mandibular ramus height than those with genotypes CA and GT. Four of the 5 SNPs were found almost exclusively in Asians, with the frequencies in other populations extremely small. **Conclusions:** Our results indicate that the GHR polymorphisms P561T and C422F are associated with mandibular ramus height in Japanese population and suggest that the SNPs of the GHR associated with the Japanese are likely to be different in other ethnic groups. This might partly explain the differing craniofacial morphology among different ethnicities. (Am J Orthod Dentofacial Orthop 2009;136:536-41)

Craniofacial morphology has a strong genetic component, but it is also influenced by environmental factors, making it complex to study. On the other hand, growth hormones play a major role in the growth and development of the craniofacial complex by directly and indirectly modulating the size and the angular relationships of the craniofacial structures,[1] and growth hormone receptors are present in the mandibular condyle with the molecular genetic analysis.[2]

Godowski et al[3] reported that the growth hormone receptor gene (GHR) (*600946) has 9 exons that encode the receptor and several additional exons in the 5-prime untranslated region. The coding exons span at least 87 kilobase of chromosome 5. Exon 2 encodes the signal peptide, exons 3 through 7 the extracellular domain, exon 8 the transmembrane domain, and exon 9 and part of exon 10 the intracellular domain. Reports have shown a relationship between the GHR and idiopathic short stature and Laron syndrome (growth hormone insensitivity syndrome), marked by a characteristic facial appearance. Kaji et al[4] identified compound heterozygous GHR mutations in exon 10, associated with severe growth retardation, Laron syndrome, and an undetectable serum growth hormone-binding protein. In a 53-year-old woman and her 57-year-old brother with growth hormone insensitivity syndrome, Milward et al[5] identified a homozygous 22 base-pair deletion in exon 10 of the GHR. Tiulpakov et al[6] described a GHR mutation comprising a guanine deletion at position 1776 in exon 10 in a patient with Laron

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The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.
Supported by the High-Tech Research Center Project for Private Universities and a matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology), 2005-2009.
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Submitted, May 2007; revised and accepted, October 2007.
0889-5406/$36.00

doi:10.1016/j.ajodo.2007.10.054

syndrome. Interestingly, patients with growth hormone receptor deficiency showed significantly decreased vertical facial growth.[7]

We reported first about an association between a single nucleotide polymorphism (SNP) in exon 10 of GHR and mandibular ramus height in Japanese subjects.[8] It has recently been reported that the SNP in exon 10 of GHR is also associated with mandibular height in Chinese people,[9] and GHR is considered a possible genetic marker for mandibular ramus height.[10]

GHR polymorphism I526L is associated with mandibular height in Chinese people, and there might be an ethnic difference in the association of GHR with mandibular ramus height. In patients with Laron syndrome[11,12] or idiopathic short stature,[13-19] the association of GHR is different depending on ethnicity. One hundred Japanese subjects were used in our previous study about the association between the SNP in the GHR and mandibular ramus height; this association was replicated with 95 Han Chinese. However a larger-scale study with distinct ethnicities is required to obtain a conclusive result.[20-23]

Therefore, in this study, we examined the association between 5 SNPs in exon 10 of GHR and craniofacial morphology in 167 normal Japanese subjects, and, to further characterize the importance of the GHR locus, we examined the allelic frequencies of these SNPs in the GHR in a multi-ethnic population of Han Chinese, African Americans, European Americans, and Hispanics.

MATERIAL AND METHODS

Genomic DNA and lateral cephalograms were obtained from 167 Japanese subjects, including 50 men (ages, 20-49 years; average age, 35 years) and 117 women (ages, 18-58 years; average age, 29 years). The men were the same set that we studied previously.[8] The subjects were patients at dental hospitals and volunteers from the Tokyo metropolitan area. They were unrelated. Subjects with congenital disorders such as cleft palate or general physical disease were excluded from the study. None had received orthodontic or orthopedic treatment.

Additionally, DNA samples from 24 Han Chinese, 24 African Americans, 24 European Americans, and 24 Hispanics with no craniofacial measurements were obtained from the Coriell Cell Repository (Camden, NJ) and used only as reference groups for allelic frequencies of the 5 SNPs in exon 10 of the GHR.

The protocol used in this study was approved by the Ethical Committee of Showa University, and all patients gave written informed consent to participate in the study before blood samples were taken.

To collect a sample for DNA analysis, the inside of the mouth was scraped with 10 strokes of a brush (MasterAmp Buccal Swab DNA Extraction Kit, AR Brown, Tokyo, Japan). Four samples were collected from each subject, and genomic DNA was obtained from these samples.

We screened the coding region of the GHR for published polymorphisms and included 5 SNPs: C422F (dbSNP ID; rs6182), S473S (rs6176), P477T (rs6183), I526L (rs6180), and P561T (rs6184).[9] The dbSNP number came from the dbSNP database of National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/).

Polymerase chain reaction amplifications were performed according to a standard protocol. To determine genotypes at exon 10 of the GHR, polymerase chain reaction amplification was performed in a 10-μL polymerase chain reaction volume containing 30 ng of genomic DNA, 200 μmol/L of each dNTP, 0.25 units EX Taq (Takara, Otsu, Japan), and 0.1 μmol/L of each primer on the Gene Amp 9700 (Applied Biosystems, Tokyo, Japan). An initial denaturation was performed at 95°C for 1 minute, 40 cycles at 94°C for 30 seconds, and 72°C for 1 minute. After removal of the remaining primers and dNTPs by using ExoSAP-IT (USB Corp, Cleveland, Ohio), the products were subjected to sequencing on the ABI PRISM 3700 DNA Analyser (BigDye, version 1.1, Applied Biosystems).

Polymorphisms were identified with the sequencer program (Gene Code Co, Ann Arbor, Mich). Each polymorphism was confirmed by sequencing nucleotides from both strands.

For the craniofacial measurements, the lateral cephalograms were traced and measured by 1 examiner (T.Y.) using the computer software Power Cephalo (ReazaNet, Tokyo, Japan), as previously described.[8] Briefly, we measured cranial base length (nasion-sella), maxillary length, overall mandibular length (gnathion-condylion), mandibular corpus length (pogonion-gonion), and mandibular ramus height (condylion-gonion). Body height was also measured in all subjects.

The Kolmogorov-Smirnov test was used to assess body height and the distribution pattern of craniofacial morphology (ANB angle), and the mean, median, standard deviation, skewness, and kurtosis of each measurement were calculated.

Statistical analysis

The associations between 5 SNPs in exon 10 of the GHR and craniofacial measurements were analyzed by using the Mann-Whitney test with SPSS software (version 10.0, SPSS, Chicago, Ill). Tests for Hardy-Weinberg equilibrium, and the allele and genotype frequencies were all done with this software.

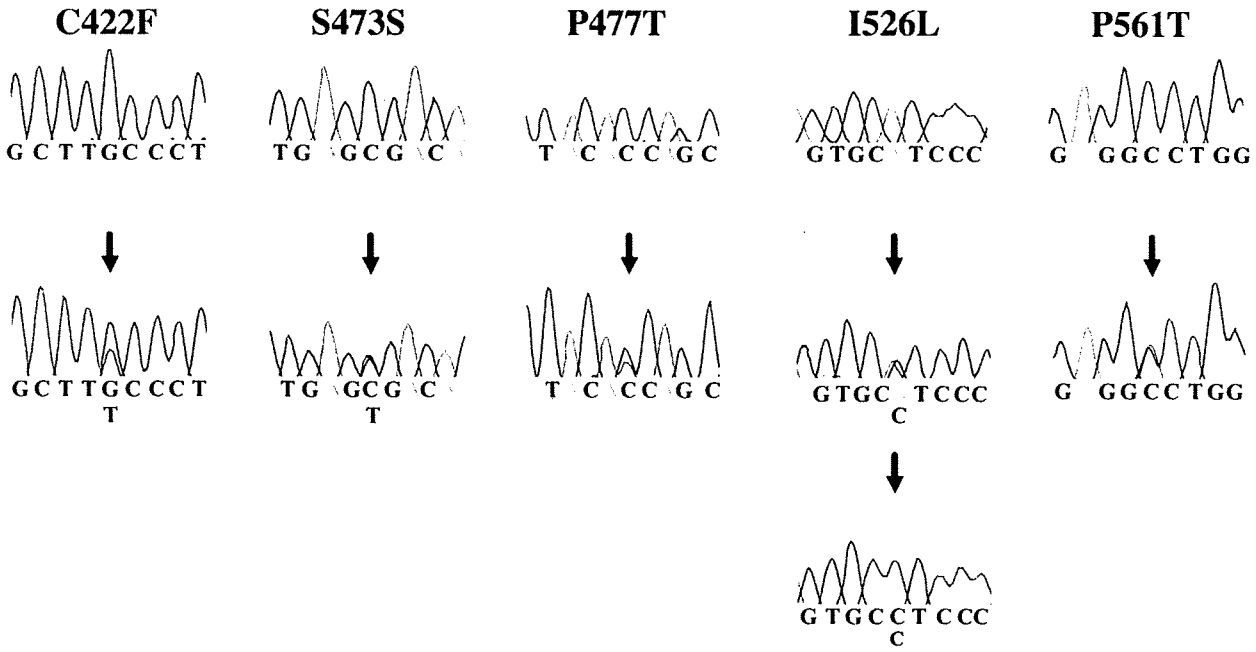

Fig. DNA sequence chromatograms of 5 SNPs in exon 10 of the GHR.

Table I. Allele distribution of 5 SNPs in exon 10 of the GHR

		Japanese (n = 167)	*Han Chinese (n = 24)*	*African American (n = 24)*	*European American (n = 24)*	*Hispanic (n = 24)*
C422F	G	94.1%	79.4%	100.0%	100.0%	100.0%
	T	5.9%	20.6%	0.0%	0.0%	0.0%
S473S	C	96.3%	97.3%	100.0%	97.5%	100.0%
	T	3.7%	2.6%	0.0%	2.5%	0.0%
P477T	C	98.7%	100.0%	100.0%	100.0%	100.0%
	A	1.3%	0.0%	0.0%	0.0%	0.0%
I526L	A	46.7%	38.2%	64.3%	58.3%	62.4%
	C	53.3%	61.8%	35.6%	41.6%	37.5%
P561T	C	94.7%	80.0%	100.0%	100.0%	100.0%
	A	5.2%	19.9%	0.0%	0.0%	0.0%

Haplotypes were inferred, and haplotype frequencies were estimated, by using the expectation-maximization method of haplotype inference in the Arlequin computer program.[24]

RESULTS

For the distribution pattern and the results of the Kolmogorov-Smirnov test, the height and the ANB angle of the 167 subjects reflected normal distribution (data not shown). Thus, these subjects were presumed to represent a normally distributed Japanese population for craniofacial morphology and height.

We detected 5 SNPs in the GHR in all 5 groups: Japanese, Han Chinese, African American, European American, and Hispanic (Fig). The frequencies varied among them, as shown in Table I.

Genotype-specific associations were examined with the Mann-Whitney test and showed that only mandibular ramus height was significantly correlated with the P561T and C422F variants ($P < 0.05$, Table II).

Haplotype analysis, constructed with 5 SNPs, showed 2 major haplotypes accounting for 85% of estimated haplotypes in the Japanese group. All other haplotypes had frequencies less than 10% (Table III).

The strength of linkage disequilibrium for each SNP pair was measured by using D′ and r2 values. The D′ coefficient was equal to or close to 1 for all pairs of SNPs in the Japanese population, indicating that the gene is in single linkage disequilibrium block (Table IV). The r2

Table II. Relationship between 5 SNPs in the GHR and 6 linear measurements of body height and craniofacial morphology in 167 Japanese subjects

			Body height (cm)			*N-S (mm)*			*A'-PTM' (mm)*			*Gn-Co (mm)*			*Pog'-Go (mm)*			*Co-Go (mm)*		
		n	*Mean*	*SD*	P	*Mean*	*SD*	P	*Mean*	*SD*	P	*Mean*	*SD*	P	*Mean*	*SD*	P	*Mean*	*SD*	P
C422F	GG	135	161.6	7.9	0.16	69.7	3.4	0.66	50.0	4.8	0.95	122.9	9.3	0.63	79.5	5.6	0.78	61.6	6.5	0.02*
	GT	16	164.6	10.2		69.3	4.4		49.9	3.1		121.7	8.5		79.9	7.2		57.9	6.1	
S473S	CC	137	161.9	8.4	0.95	69.6	3.5	0.32	49.9	4.8	0.71	122.9	9.2	0.89	79.9	5.9	0.31	61.5	6.5	0.54
	CT	11	161.1	6.1		70.5	2.8		49.8	2.1		123.0	9.7		78.5	6.4		60.9	5.6	
P477T	CC	146	161.6	8.3	0.47	69.6	3.5	0.58	49.9	4.7	0.54	122.7	9.1	0.15	79.8	6.0	0.23	61.3	6.4	0.17
	CA	4	163.8	9.1		69.5	4.8		51.3	3.5		130.5	11.4		83.3	5.7		65.6	4.5	
I526L	AA	77	162.7	8.8	0.47	69.5	3.5	0.56	50.1	3.2	0.06	124.2	9.7	0.19	80.1	5.8	0.82	62.4	6.7	0.13
	AC	44	161.0	7.9		70.2	4.0		50.7	3.1		121.6	8.7		79.6	5.5		61.1	6.8	
	CC	32	161.4	6.9		69.4	2.6		48.1	7.9		121.4	7.7		79.5	6.8		59.7	5.4	
P561T	CC	135	161.6	7.9	0.16	69.7	3.4	0.66	50.0	4.8	0.95	122.9	9.3	0.63	79.5	5.6	0.78	61.6	6.5	0.02*
	CA	16	164.6	10.2		69.3	4.4		49.9	3.1		121.7	8.5		79.9	7.2		57.9	6.1	

N-S, Cranial base length; *A'-PTM'*, maxillary length; *Gn-Co*, overall mandibular length; *Pog'-Go*, mandibular corpus length; *Co-Go*, mandibular ramus height.
*$P < 0.05$.

parameter that measures the correlation between alleles showed that 1 SNP pair was tightly correlated (Table IV). Linkage disequilibrium analysis showed that 2 SNPs, C422F and P561T, were in complete linkage disequilibrium.

DISCUSSION

The quantitative genetic aspects of mandibular morphology were explored in reference to an association with the GHR polymorphisms in Japanese people, with an examination of the differences in the allelic frequencies between ethnicities. Mandibular growth greatly depends on cartilage growth and is a multifactorial phenomenon in which genetic disposition, nutrition, homeostasis, hormones, and growth factors interact.[25] The GHR plays an important role in cartilage growth, which directly affects other features of growth and development.[26] In this study, we showed a relationship between the P56IT and C422F variants at the GHR locus and mandibular ramus height in Japanese subjects.

We analyzed 5 SNPs of exon 10 of the GHR in Japanese subjects and found an association between exon 10 of the GHR polymorphisms, P561T and C422F, and mandibular ramus height. Even though a previous study of 95 Han Chinese reported a correlation between the I526L polymorphism of theGHR and mandibular ramus height, our study with 167 Japanese subjects did not replicate this observation. The reason for this discrepancy remains unclear, but we found widely discordant allele frequencies in exon 10 of the GHR between some ethnic groups. However, we could not obtain craniofacial measurements in other ethnic groups. The association of the GHR is different depending on ethnicity

Table III. Haplotypes constructed on the basis of genotypic data for 5 SNPs spanning the linkage disequilibrium block covering exon 10 of the GHR

Haplotype	*C422F*	*S473S*	*P477T*	*I526L*	*P561T*	*Estimated common haplotype frequency*
1	W	W	W	W	W	0.5016
2	W	W	W	v	W	0.3548
3	v	W	W	v	v	0.0693
4	W	v	W	v	W	0.0462

Haplotypes could be subdivided into 4 major haplotypes. Two major haplotypes were present in the Japanese population.
W, Major allele of each variation; *v*, minor allele of each variation.

in other cases such as Laron syndrome[11,12] and idiopathic short stature.[14-19] These differences might imply the need for independent studies of craniofacial morphology for the GHR in each ethnic group. The mandibles of Japanese subjects appear to be slightly smaller those of European Americans[27] or Caucasians.[28-30] Our work emphasizes the importance of close matching of ethnic groups, especially when craniofacial morphology is examined.

It has been hypothesized that an SNP outside exon 10 could also affect the receptor function to influence mandibular ramus height. Specifically, a polymorphism in the human GHR (d3/fl-GHR) resulting in genomic deletion of exon 3 was reported.[31,32] This common polymorphism of the GHR is associated with increased responsiveness to growth hormone: children carrying at least 1 d3-GHR allele show a 1.7 to 2 times greater response to growth hormone than children with the fl-GHR/fl-GHR homozygote allele.[33] Further epidemiologic studies in Japanese and other populations are

Table IV. Allelic correlations among exon 10 SNPs in the GHR

	C422F	*S473S*	*P477T*	*I526L*	*P561T*
D′					
C422F					
S473S	−1.000				
P477T	−1.000	−1.000			
I526L	1.000	0.672	−1.000		
P561T	1.000	−1.000	−1.000	1.000	
r2					
C422F					
S473S	0.004				
P477T	0.001	0.001			
I526L	0.082	0.028	0.009		
P561T	1.000	0.004	0.001	0.078	

Pairwise linkage disequilibrium between GHR SNPs was measured with 2 coefficients: D′ and r2 in Japanese people. The D′ parameter is close to or equal to 1, indicating that few historical recombinations have occurred in the locus. In contrast, the r2 coefficient, which measures the correlation between alleles, varies broadly. The linkage disequilibrium analysis showed that P561 T and C422F were completely linked with each other ($|D'| = 1.000$, $r2 = 1.000$).

required by genotyping other coding SNPs of the GHR including the exon 3 deletion.

Although various environmental factors have been found to contribute to the morphogenesis of the mandible, genetic factors play a substantial role.[34] However, few reports have examined the correlation between craniofacial morphology and genotype, and our results successfully reproduced the correlation between genotype and mandibular ramus height.[8,9,35,36] Sasaki et al[10] reported on a Japanese patient with ectodermal dysplasia and proposed that the P561T variant could be a genetic marker for mandibular growth. The clinical implication for growing patients is the potential advantage to predict mandibular ramus height growth by using DNA from a simple cheek swab. The sample can be collected without extensive training, potentially facilitating genetic studies in dentistry. It would then be possible to predict the orthopedic reaction before starting treatment, although ethnic differences should be considered. This genetic factor might be considered along with other factors associated with mandibular growth in treatment planning for influencing mandibular height, such as Herbst appliances,[37] functional appliances,[38] headgear,[39] and facemask therapy.[40]

We reported previously a genome-wide linkage analysis with 90 Asian sibling pairs with mandibular prognathism and mapped 3 chromosomal loci, including 1p36, 6q25, and 19p13.2.[36] The loci identified for mandibular prognathism are different from the GHR locus on chromosome 5. In our study, we found no SNPs with a relationship with mandibular corpus length or overall mandibular length, and neither relationship was found in the Chinese.[9] The gonial angle is strongly affected by the masseter muscle, and overall mandibular length is affected by the gonial angle.[41,42] The replication of these results with an independent data set should facilitate better understanding of the development of mandibular prognathism.

CONCLUSIONS

We confirmed an association between polymorphisms P561T and C422F that are in linkage disequilibrium, and mandibular ramus height using a larger sample size than our previous report.[8] Subjects with genotype CC of polymorphism P561T and genotype GG of polymorphism C422F had significantly greater mandibular height than those with genotypes CA and GT. Han Chinese, African Americans, European Americans, and Hispanics have different frequencies in exon 10 of the GHR compared with the Japanese. This knowledge provides insight into the molecular pathways associated with growth and development of the mandible, and might be useful for orthodontic diagnosis and orthopedic treatment of the mandible.

We thank Kozue Otaka for her expert technical assistance.

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Journal of Human Genetics (2009) 54, 543–546

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SHORT COMMUNICATION

Genome-wide association database developed in the Japanese Integrated Database Project

Asako Koike[1], Nao Nishida[2], Ituro Inoue[3], Shoji Tsuji[4] and Katsushi Tokunaga[2]

The establishment of high-throughput single-nucleotide polymorphism (SNP)-typing technologies has enabled astonishing progress to be made in genome-wide association studies (GWAS), and various novel genetic factors associated with complex diseases have been discovered. Our organization has created a public repository database (DB) to achieve a continuous and intensive management of GWAS data and to facilitate data sharing among researchers. In the GWAS DB, information on study design, quality control protocols, allele frequencies, genotype frequencies and statistical genetic analysis results are stored as publicly available data and can be accessed freely, whereas individual genotyping data and raw data are stored as restricted data and can only be accessed with authorization. All data are presented by a graphic viewer, which is designed to be user friendly for researchers who are not familiar with GWAS to accelerate disease-related studies. Furthermore, the DB allows users to compare various study results obtained by different institutions and on different platforms. The same data are also managed as a distributed annotation system to call up useful data from other DBs and to superimpose them on the GWAS data for help in interpretation. The DB is accessible at https://gwas.lifesciencedb.jp/.
Journal of Human Genetics (2009) **54**, 543–546; doi:10.1038/jhg.2009.68; published online 24 July 2009

Keywords: database; genome-wide association; SNP

INTRODUCTION

The accomplishment of sequencing of the entire human genome[1,2] and the HapMap project,[3] coupled with the development of cost-effective high-throughput dense single-nucleotide polymorphism (SNP)-typing techniques, have enabled a genome-wide exploration of various complex disease-associated variants. Currently, the high-throughput SNP-typing methods are expected to cover about 80% of the human genome in linkage disequilibrium.[4] A number of large-scale genome-wide cohort studies and case–control studies, such as seven common disease GWAS by the Wellcome Trust Case Control Consortium (WTCCC, 2007), have been planned, and some of them are underway. So far, more than 100 loci of disease-related/causing candidates for about 40 common diseases and traits have been identified,[5] and some loci have led to new insights into pathophysiology and etiological pathways. Because GWAS yields large amounts of raw data and analysis results, the management of GWAS data has become a matter of serious concern. Furthermore, more and more grant-funding agencies, journal editors and research communities are beginning to require the disclosure of GWAS data. Disclosure and data sharing of GWAS data will primarily lead to the following three possibilities: (1) meta-analysis using data sets produced in multiple studies to find novel disease-related SNP candidates; (2) re-use of GWAS data combined with other experimental data, including pathway data and expression data, to deepen the exploration of each disease; and (3) development of methods to analyze and compute genetic statistics. In the case of meta-analysis in particular, the use of raw data is indispensable for quality control and for consideration of population structures. Some studies have successfully found additional disease-related SNP candidates on the basis of meta-analysis.[6,7]

The National Center for Biotechnology Information launched the database (DB) of Genotype and Phenotype in the fall of 2006 as a centralized GWAS system to archive and distribute GWAS data. Currently, results funded by the Genetic Association Information Network and voluntarily submitted data have been accumulated. The European Genotype Archive was created in the spring of 2008 as a repository system for phenotype–genotype relationships, and results primarily from WTCCC have been accumulated and redistributed. To achieve a continuous and intensive management of GWAS data and data sharing among researchers, we established a new DB that is publicly available. This DB is expected to have an essential role in providing easily accessible GWAS data to researchers in various biomedical fields. Some disease-related SNPs are assumed to be buried because of their insufficient *P*-values caused by an insufficient number of case–control samples. It is possible that these SNPs will be revealed by combining the GWAS analysis results with other data possessed by users.

In this paper, we introduce the GWAS DB.

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Received 3 June 2009; accepted 27 June 2009; published online 24 July 2009

MATERIALS AND METHODS

Database structure

The DB system consists of an internal GWAS DB and a public GWAS DB. For a maximum of 1 year, or until the acceptance of publication, submitted data are stored in the internal GWAS DB and can be accessed only by the research team that submitted the data for greater convenience in data sharing among research team members living in various locations. Currently, the DB systems are implemented using mysql version 5.0 (http://dev.mysql.com/downloads/mysql/5.0.html), and some of the statistical analysis results are also accumulated in a distributed annotation system (DAS) server. A schematic drawing of the GWAS DB is shown in Figure 1.

In this DB, three types of data access, namely, (1) public access, (2) authorized access accompanied by a data use application, and (3) authorized access accompanied by a data use application and its review by a data access committee, are possible. Principally, frequency data of genotypes and alleles and statistical analysis results can be accessed freely. However, automatic access and frequent access are restricted to prevent the release of frequency data of genome-wide genotypes and alleles, as such a large volume of genotype/allele data leads to the specification of whether the given genome is contained in the case or in the control group, as reported previously.[8] These genome-wide frequency data can be obtained by submitting a data use application to the data access committee. For the use of genotype or raw data, an application that

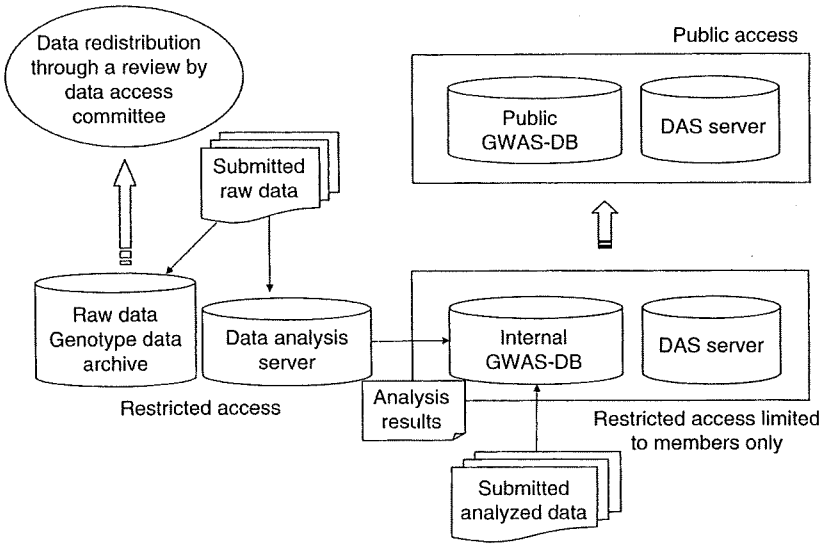

Figure 1 Schematic drawing of genome-wide association study (GWAS) database (DB) systems.

Table 1 Summary of database contents

Contents	*Data sources*
Statistics	
Frequencies of genotypes, alleles and haplotypes	
Statistical genetic analysis	
P-values and odds ratios on genotypic model and allelic model	
P-values and odds ratios on trend model, additive model and recessive model	
Permutation test results	
Bonferroni's corrections and false discovery rate for multiple testing using	
Akaike information criterion	
Hardy–Weinberg equilibrium test	
Haplotype-based χ^2-test	
Epistasis	
Linkage disequilibrium parameters (r^2, D', Lod)	
Other data	
mRNA, amino-acid sequence of each gene	NCBI (http://www.ncbi.nlm.nih.gov/)
mRNA, genome-mapped position	UCSC Hg. 18 (http://hgdownload.cse.ucsc.edu/)
SNP position and SNP kind (cSNP, sSNP, rSNP and so on)	NCBI (http://www.ncbi.nlm.nih.gov/)
OMIM	NCBI (http://www.ncbi.nlm.nih.gov/)
Copy number variation	DGV (http://projects.tcag.ca/variation/)
Gene function	Gene ontology (http://www.geneontology.org/)
Microsatellite polymorphism	UCSC (http://hgdownload.cse.ucsc.edu/)
Manually curated disease-related mutation information	

describes the research purpose and lists the research team members must be submitted to the data access committee. The data access committee deliberates on whether the applicant's research purpose meets the content of the consent form. Only applicants approved by the review committee can use individual genotype data and raw data in accordance with the data handling security rules required by the data access committee and following data use restrictions on the basis of informed consent.

Individual data and raw data are accumulated in the server in a secured computer environment that is different from the public DB server. Only authorized persons can access this server.

Data submission

In principal, both analysis results and unanalyzed data can be submitted. When data have already been analyzed, the analyzed data are accumulated in this DB, along with a detailed description of the analysis protocols. When data have not been analyzed yet, they are analyzed in our site, and the results are accumulated in this DB. When raw data are redistributable under certain conditions, they are also submitted with the contents of the consent form. All data must be submitted with documents explaining the design of the study, as well as ethical consideration.

Data cleaning for quality control

When data are submitted as individual data without analysis results, they are analyzed as follows: (1) SNPs with a call rate <95% and samples with a call rate <95% are removed. (2) SNPs, the Hardy–Weinberg equilibrium test result of which in a control group is less than 0.001 or the minor allele frequency of which is less than 0.05, are removed. (3) The principle component analysis (PCA) of these case–control data, along with HapMap data, is carried out using EIGENSTRAT[9] or other programs so that sample outliers and samples with a possible ethnic mixture or a different ethnicity are removed on the basis of the PCA result. Sample outliers in the plot of heterozygosity versus call rate are also removed. The quantile–quantile plot based on the allelic model is calculated and checked. When only genotype frequency data are submitted, PCA and heterozygosity checks are skipped,

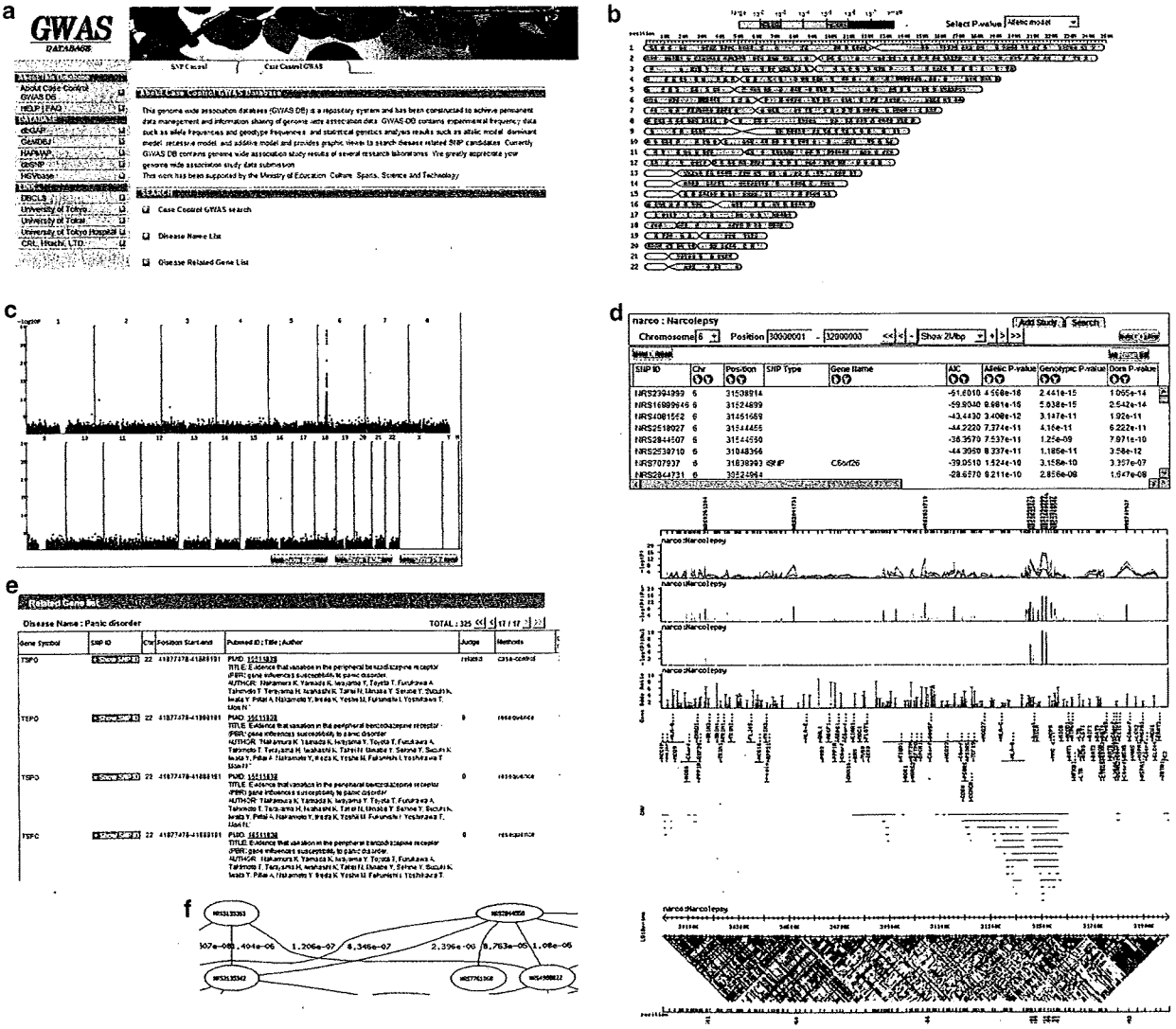

Figure 2 Snapshots of the genome-wide association study (GWAS) database. **(a)** Top page, **(b)** bird's-eye view, **(c)** Manhattan plot, **(d)** region table and graph, **(e)** disease-related gene/single-nucleotide polymorphism (SNP) lists (public data) and **(f)** SNP network based on epistasis.