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ORIGINAL ARTICLE

A functional SNP upstream of the beta-2 adrenergic receptor gene (*ADRB2*) is associated with obesity in Oceanic populations

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OBJECTIVE: Obesity is a growing health concern in the Oceanic populations. To investigate the genetic factors associated with adult obesity in the Oceanic populations, the association of single nucleotide polymorphisms (SNPs) of the beta-2 adrenergic receptor (*ADRB2*) gene with obesity was examined in 694 adults living in Tonga and Solomon Islands.

RESULTS: A screening for variation in 16 Oceanic subjects detected 17 SNPs in the entire region of *ADRB2*, of which nine SNPs including two non-synonymous ones, rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu), were further genotyped for all subjects. The rs34623097-A allele, at a SNP located upstream of *ADRB2*, showed the strongest association with risk for obesity in a logistic regression analysis adjusted for age, sex, and population ($P = 5.6 \times 10^{-4}$, odds ratio [OR] = 2.5, 95% confidence interval [CI] = 1.5–4.2). The 27Glu was also significantly associated with obesity in the single-point association analysis ($P = 0.013$, OR = 2.0, 95%CI = 1.2–3.4); however, this association was no longer significant after adjustment for rs34623097 since these SNPs were in linkage disequilibrium with each other. A copy of the obesity-risk allele, rs34623097-A, led to a 1.6 kg/m^2 increase in body mass index (BMI; defined as weight in kilograms divided by height in meters squared) ($P = 0.0019$). A luciferase reporter assay indicated that rs34623097-A reduced the transcriptional activity of the luciferase reporter gene by approximately 10% compared with rs34623097-G. An electrophoretic mobility shift assay demonstrated that rs34623097 modulated the binding affinity with nuclear factors. An evolutionary analysis implies that a G > A mutation at rs34623097 occurred in the Neandertal genome and then the rs34623097-A allele flowed into the ancestors of present-day humans.

CONCLUSION: The present results suggest that rs34623097-A, which would lead to lower expression of *ADRB2*, contributes to the onset of obesity in the Oceanic populations.

INTRODUCTION

The overall prevalence of adult obesity is increasing in Oceanic populations. Although the change from a traditional to a modern lifestyle largely contributed to the rapid increase in the prevalence of obesity, environmental factors cannot fully explain individual differences in the predisposition to obesity in Oceanic populations as well as other populations. Along with environmental factors, genetic factors also play a key role in the development of obesity.[1]

Genome-wide association studies have shown that common variants in the *FTO* gene are significantly associated with body mass index (BMI) or obesity in European populations,[2–4] and a number of studies have confirmed the association of *FTO* polymorphisms with obesity in European populations. However, our previous study did not replicate the association of *FTO* polymorphism with BMI in Oceanic populations, even though the possibility of false-negative results due to the small sample size

(320 subjects) was not excluded.[5] A recent study with a larger sample size (465 American Samoans and 624 Samoans) also revealed no significant association after corrections for multiple testing.[6] These results suggest differences in genetic factors between Oceanic and European populations that predispose them to obesity.

Total energy consumption in excess of total energy expenditure leads to obesity. It has been suggested that polymorphisms of genes involved in the regulation of catecholamine function are associated with obesity, since catecholamines play a key role in energy expenditure and lipolysis. The beta-2 adrenergic receptor (ADRB2), a member of the G protein coupled receptors, mediates the catecholamine-induced activation of adenylate cyclase through the action of G proteins. Considering the biological function of ADRB2, polymorphisms of the *ADRB2* gene (OMIM 109690) are plausible candidates that are associated with obesity.

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Previous association studies have especially focused on two common nonsynonymous SNPs of the *ADRB2* gene, Arg16Gly (rs1042713) and Gln27Glu (rs1042714). In some studies, Arg16Glu and/or Gln27Glu were associated with obesity and/or BMI,[7–9] but these associations were not found in other studies.[10,11] A meta-analysis for Arg16Gly and Gln27Glu showed that the 27Glu allele was a significant risk factor for obesity in Asians, Pacific Islanders, and Native Americans.[12] However, in the original study of the Tongan population (ie, Pacific Islanders) referred by the meta-analysis, 27Glu tended to be associated with obesity, but the association was not statistically significant,[13] implying questionable association in Oceanic populations. It is also the fact that few Oceanic populations have been investigated. Therefore, in this study, we examined the association of *ADRB2* polymorphisms with obesity in Oceanic populations and performed functional analyses of the SNP associated with risk for obesity.

SUBJECTS AND METHODS

Subjects

A total of 694 adult subjects (18 years old or older) were recruited from five Oceanic populations: (1) Austronesian (AN)-speaking Polynesians in Ha'ano and Fakakakai villages of Ha'apai Island, Tonga ($n = 32$); (2) AN-speaking Polynesians in Nuku'alofa, Tonga ($n = 139$); (3) AN-speaking Melanesians in Munda, Solomon Islands ($n = 170$); (4) AN-speaking Melanesians in Kusaghe (referred to as Paradise in our previous papers), Solomon Islands ($n = 202$); and (5) AN-speaking Micronesians in Rawaki, Solomon Islands ($n = 151$). Rawaki village is located on New Georgia Island, Solomon Islands, but Rawaki villagers are AN-speaking Micronesians who migrated from the Gilbert Islands, Kiribati, about 40 years ago. Thus, in this paper, Rawaki villagers are regarded as Micronesians.

To investigate the allele frequencies of rs34623097 and rs1042714 (Gln27Glu) in other Oceanic populations, 32 non-Austronesian (NAN)-speaking Melanesians (Gidra) living in Western Province, Papua New Guinea, and 32 AN-speaking Melanesians in Balopa, Manus Province, Papua New Guinea, were also analyzed. In addition, the allele frequencies of rs34623097 and rs1042714 (Gln27Glu) were also investigated in 894 Mongolian individuals. The following genomic DNA samples used in the International HapMap Project[14,15] were obtained from the Coriell Cell Repository and subjected to rs34623097 genotyping: 57 subjects from YRI (Yoruba in Ibadan, Nigeria), 58 CEU (CEPH Utah residents with ancestry from northern and western Europe), 43 JPT (Japanese in Tokyo, Japan), and 45 CHB (Han Chinese in Beijing, China). The features of the Oceanic populations have been previously described in detail.[16,17] The locations of the Oceanic populations studied are shown in Supplementary Material, Supplementary Figure S1.

Blood samples were collected after obtaining informed consent from each participant. This study was approved by the Health Ethics Committee, Ministry of Health, Solomon Islands; the Medical Research Advisory Committee of Papua New Guinea; National Health Ethics & Research Committee of Tonga; the Ethics Committee of the Health Science University of Mongolia; the Ethics Committee of Jichi Medical University; and the Research Ethics Committee of the Graduate School of Comprehensive Human Sciences, University of Tsukuba.

Anthropometric measurement

Anthropomorphic phenotypes were directly measured in field settings. Measurements were taken of subjects dressed in light clothing. Body height was measured to the nearest 1 mm by using a field anthrometer (GPM, Zurich, Switzerland), and weight was recorded to the nearest 0.1 kg by using a portable digital scale (Tanita model BC-518, Tokyo, Japan). Subjects with BMI $\geqslant 27$ kg/m^2 were classified as obese regardless of the population, ethnicity, and sex in this study. The reason why obesity was not defined as BMI $\geqslant 30$ kg/m^2 is because there were no obese male subjects in Kusaghe if defined so.

Screening for variation of *ADRB2*

Genomic DNA was extracted from peripheral blood by using a QIAamp Blood Kit (Qiagen, Hilden, Germany). Variations in the ~ 3.8 kb region containing the entire *ADRB2* gene were screened for 16 subjects: two from

Nuku'alofa, two from Ha'apai, four from Kusaghe, four from Munda, and four from Rawaki. For the putative regulatory region, the genomic region ~ 2 kb upstream of the initiator codon ATG was analyzed. Primers were designed within the overlapping region on the basis of the genomic sequence obtained from the National Center for Biotechnology Information database (GRCh37/hg19) (Supplementary Material, Supplementary Figure S2a). Primer sequences are listed in Supplementary Material, Supplementary Figures S2b and S2c. PCR amplification was performed with the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) by using a FastStart Taq DNA Polymerase kit (Roche, Molecular Biochemicals, Mannheim, Germany). PCR products were sequenced with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Genotyping

From 17 SNPs detected by the screening, eight tag SNPs were selected as candidates to be genotyped. Of eight candidate SNPs, seven SNPs, excluding rs2400706, were successfully genotyped. Here, rs2400706 could not be genotyped, since the TaqMan probe for rs2400706 was not designed. Two SNPs, rs1042711 and rs1042714, were additionally selected, since the significant associations of these SNPs with obesity had been previously reported.[7–9,18] Accordingly, a total of nine SNPs were genotyped (Table 2 and Figure 1). The remaining seven SNPs were in LD ($r^2 \geqslant 0.8$) with at least one of nine SNPs genotyped (Supplementary Material, Supplementary Figure S3). Two SNPs, rs1042713 (Arg16Gly) and rs1042714 (Gln27Glu), were genotyped by using the DigiTag2 assay.[19] The other seven SNPs were genotyped using the TaqMan SNP genotyping assays. The assays were run on an ABI 7300 machine (Applied Biosystems, Foster City, CA, USA).

Cell culture

HEK293T (human embryonic kidney cell line) cells were cultured in D-MEM (Wako, Osaka, Japan) supplemented with 10% heat-inactivated FBS in 5% CO_2 at 37 °C.

Luciferase reporter assay

The DNA samples from subjects with homozygotes of rs34623097-A and -G were amplified with a forward primer (5′-TTTTTTGGATCCAGCTC CAAGTTTGGTGAATCTGGAAC-3′) and a reverse primer (5′-TTTTTTGC TAGCTGGGTGACAGAACAAGACTCCATCTC-3′). Accordingly, two different PCR products with 619 bp, including the rs34623097-A and rs34623097-G alleles, were separately obtained. The PCR products had no sequence difference at any sites other than rs34623097. The PCR products for each allele were gel purified by using NucleoSpin Extract II (Macherey Nagel, Düren, Germany) and then cut with BamHI and NheI restriction enzymes. These products were ligated into the BglII and NheI sites in the promoter region located 5′ to the Renilla luciferase gene in the psiCHECK2 vector (Promega, Madison, WI, USA) by using T4 DNA ligase (Thermo Fisher Scientific, Waltham, MA, USA). Accordingly, SV40 early enhancer/promoter in the psiCHECK2 vector was completely replaced by the PCR products. The inserts containing rs34623097-A or rs34623097-G were confirmed by PCR-direct sequencing. Plasmid with rs34623097-A or rs34623097-G was transfected into HEK293T cells using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) and incubated for 24 h. The luciferase activities were determined by using the Dual-Glo Luciferase Assay system (Promega, Madison, WI, USA) with a luminometer. In this system, transcriptional activity was measured by Renilla luciferase activity and was normalized by Firefly luciferase activity in each assay (ie, in the same well). The experiment was replicated five times, with quintuplicate assays performed within each replicate.

Electrophoretic mobility shift assay

Nuclear extracts were extracted from the cultured HEK293T cells by using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo scientific, Rockford, IL, USA) according to the manufacturer's instruction. The oligonucleotide probes (5′-TAATTTCACTTTAGCAATAAAGTCACATGC-3′ for rs34623097-A, 5′-TAATTTCACTTTAGCA̲GTAAAGTC̲ACATGC-3′ for rs346230 97-G, and both complementary sequences) were labeled with a Biotin 3′ End DNA Labeling Kit (Thermo scientific, Rockford, IL, USA). EMSA was performed using a Light Shift Chemiluminescent EMSA Kit (Thermo scientific, Rockford, IL, USA) according to the manufacturer's instructions. Briefly, the reaction mixtures (20 μl) containing 6 μg of nuclear extract,
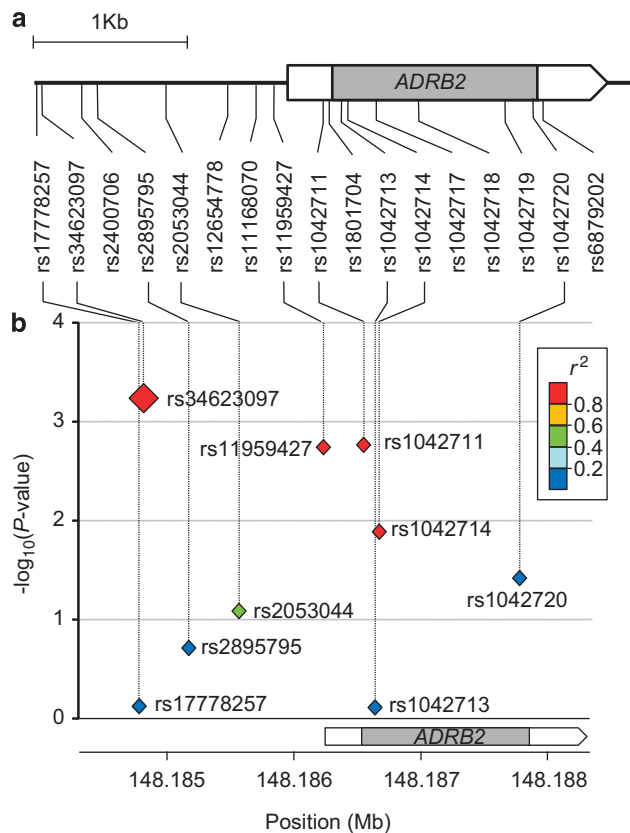
Figure 1. Significant association of rs34623097-A with obesity. (**a**) Location of SNPs. A screening for variation detected 17 SNPs in the entire region of *ADRB2* gene in 16 subjects. (**b**) Association plot of nine SNPs. The association of each *ADRB2* SNP with obesity was examined by multiple regression analysis adjusted for age, sex, and population. Each SNP is plotted as a diamond along the chromosomal position, and r^2 between the SNP and rs34623097 is colored as a scale from low (blue) to high (red).

1 × binding buffer, 50 ng/μl of poly (dI-dC), 5 mM $MgCl_2$, 0.05% NP-40 and 2.5% glycerol were pre-incubated on ice for 10 min and then incubated with 1 nM of the biotin-labeled probe for 20 min at room temperature The reaction mixtures containing no nuclear extract were also incubated with the labeled probes as negative control samples. Each sample was loaded onto the 5% polyacrylamide gel (ATTO, Tokyo, Japan) in 0.5 × TBE. Samples were electrophoresed until the bromophenol blue dye had migrated approximately 3/4 down the length of the gel. The gel and nylon membrane were sandwiched in a clean electrophoretic transfer unit, and the DNA was transferred at 380 mA (∼100 V) for 30 min. Then DNA and membrane were crosslinked by using a UV-light cross-linker instrument. Biotin-labeled DNA was detected by chemiluminescence provided by the LightShift Chemiluminescent EMSA Kit (Thermo scientific, Rockford, IL, USA). For competition EMSA, to compete specifically with labeled oligonucleotide probes, a 200-fold excess of unlabeled oligonucleotide probes for rs34623097-A or rs34623097-G were added to reaction mixtures containing 6 μg of nuclear extract.

Statistical analyses

Deviation of genotype frequencies from Hardy-Weinberg equilibrium was examined by chi-square test. Haplotype frequency and pairwise LD parameters, r^2 and D', were estimated by using Haploview software.[20] In association analyses, only subjects who had been 18 years old or older at the time of anthropometric survey were analyzed. A logistic regression analysis that was adjusted for age, sex, and population was performed to assess the association of each *ADRB2* SNP of *ADRB2* with obesity (BMI ≥ 27 kg/m²). To assess the independent effect of each SNP on obesity, a logistic regression analysis with rs34623097, rs11959427, rs1042711, rs1042714, and rs1042720 as independent variables adjusted for age, sex, and population was also performed. A multiple regression analysis

adjusted for age, sex, and population was performed to assess the association of rs34623097 with BMI. In both regression analyses, the number of copies of a derived allele at each *ADRB2* SNP was used as an independent variable (ie, homozygotes of an ancestral allele, those of a derived allele, and heterozygotes were coded as 0, 2, and 1, respectively). The association of rs34623097 genotypes with BMI was further assessed by analysis of covariance (ANCOVA) adjusted for age, sex, and population. The genotypes of 626 SNPs with minor allele frequency of more than 0 in JPT and CHB were retrieved from the HapMap database,[14,15] and then the r^2 values between rs34623097 and each of 626 SNPs around the *ADRB2* were calculated.

In the luciferase assay, to adjust for the variation of experimental conditions among five replications (ie, transfections) each with quintuplicate assays, the difference in transcriptional activity between rs34623097-A and rs34623097-G was assessed by ANCOVA adjusted for replication.

A SNP upstream of *ADRB2*, rs34623097, was analyzed for a putative transcription factor-binding site by using the web-based software Transcription Factor Binding Sites Search (TFSEARCH; http://www.cbrc.jp/research/db/TFSEARCH.html) (version 1.3),[21] with a threshold score of 90.0.

The genotype data of rs34623097 and rs1042714 for CHB, JPT, CHS, FIN, GBR, IBS, TSI, YRI, LWK, PUR, MXL, and CLM were obtained from the 1000 Genomes Project database (http://www.1000genomes.org).[22] The genotype data of SNPs around rs34623097 in CHB (Han Chinese in Beijing, China), JPT (Japanese in Tokyo, Japan), CHS (Southern Han Chinese), and FIN (Finnish in Finland) populations were also retried from the 1000 Genomes Project database. The Neandertal genome sequences (Vi33.16, Vi33.25, and Vi33.26) at the corresponding sites were obtained from the UCSC Genome Browser (http://genome.ucsc.edu/).[23]

In this study, the significance level was set at 0.05 (ie, P-values of less than 0.05 were regarded as statistically significant), unless otherwise stated. The Bonferroni correction was used when appropriate.

RESULTS

Association test

To assess the association between *ADRB2* polymorphisms and obesity, 694 adults between 18 and 65 years of age were recruited from five Oceanic populations (Table 1 and Supplementary Material, Supplementary Figure S1). Although the mean BMI varied among the five populations, subjects with BMI ≥ 27 kg/m² were classified as obese regardless of the population, ethnicity, and sex. In this study, obesity was not defined as BMI ≥ 30 kg/m², since no male subjects were obese in Kusaghe if defined so. The prevalence of obesity was the highest in the Polynesians (87.8% for Nuku'alofa and 78.1% for Ha'apai), followed by the Micronesians (48.3% for Ravaki) and the Melanesians (42.9% for Munda and 13.4% for Kusaghe) (Table 1).

A screening for variation in the entire region of *ADRB2* detected 17 SNPs in the Oceanic subjects (Figure 1a and Supplementary Material, Supplementary Figure S3a). Seven SNPs were selected from the 17 SNPs based on the linkage disequilibrium (LD) between SNPs (Supplementary Material, Supplementary Figure S3b). Two SNPs, rs1042711 and rs1042714, were additionally selected, since the significant associations of these SNPs with obesity had been previously reported.[7–9,18] These nine SNPs were genotyped for 694 Oceanic subjects (Supplementary Material, Supplementary Table S1). Although a significant deviation from the Hardy-Weinberg equilibrium was observed at rs1042714 in the Kusaghe subjects (Supplementary Material, Supplementary Table S1), their genotype data were included in the following statistical analyses since the Hardy-Weinberg P-value of 0.04 for rs1042714 in Kusaghe was not markedly small.

A multiple logistic regression analysis adjusted for age, sex, and population was performed for each of the nine SNPs (Table 2 and Figure 1b). The results revealed that five SNPs, rs34623097, rs11959427, rs1042711, rs1042714 (Gln27Glu), and rs1042720, were significantly associated with obesity. Among these five SNPs, rs34623097-A was the strongest risk factor for obesity ($P = 5.6 \times 10^{-4}$, odds ratio [OR] = 2.5, 95% confidence interval [CI] = 1.5–4.2). After the significance level α was corrected by the

Table 2. Association of ADRB2 polymorphisms with obesity (BMI > 27 kg/m^2)

SNP	Derived allele	Tonga (Ha'apai)		Tonga (Nuku'alofa)		Munda		Kusaghe		Ravaki		P-value[a]	Odds ratio (95%CI)
		Case	Control	Case	Control	Case	Control	Case	Control	Case	Control		
rs17778257	T	0.25	0.25	0.39	0.41	0.45	0.42	0.48	0.48	0.32	0.34	0.75157	1.05 (0.79–1.38)
rs34623097	A	0.08	0.00	0.04	0.00	0.04	0.04	0.11	0.05	0.21	0.10	0.00056	2.48 (1.48–4.16)
rs2895795	A	0.58	0.71	0.52	0.56	0.45	0.45	0.31	0.31	0.47	0.53	0.19106	0.84 (0.64–1.09)
rs2053044	A	0.14	0.00	0.09	0.03	0.11	0.13	0.20	0.21	0.21	0.13	0.08165	1.41 (0.96–2.07)
rs11959427	T	0.85	1.00	0.93	1.00	0.96	0.96	0.89	0.95	0.79	0.88	0.00178	0.45 (0.27–0.74)
rs1042711	T	0.85	1.00	0.95	1.00	0.95	0.96	0.89	0.95	0.79	0.88	0.00167	0.45 (0.27–0.74)
rs1042713	A	0.23	0.21	0.42	0.41	0.50	0.51	0.57	0.64	0.33	0.36	0.74218	0.96 (0.73–1.25)
rs1042714	C	0.94	1.00	0.97	1.00	0.97	0.97	0.89	0.96	0.82	0.88	0.01293	0.51 (0.30–0.87)
rs1042720	A	0.60	0.79	0.60	0.65	0.51	0.55	0.41	0.48	0.49	0.54	0.03326	0.75 (0.57–0.98)

[a]A logistic regression analysis adjusted for age, sex, and population was performed to assess the association of each SNP with obesity.
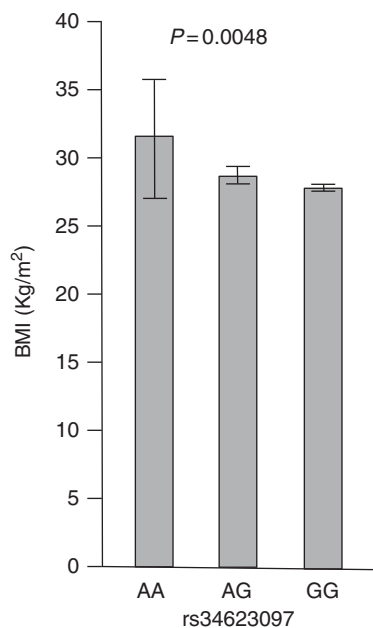

Figure 2. The means and standard errors of BMI, stratified by rs34623097 genotypes. Mean BMI was compared among AA, AG, and GG genotypes at rs34623097 using the analysis of covariance (ANCOVA) by adjusting for age, gender, and population. The significant difference in BMI among three genotypes was detected (P = 0.0048).

rs34623097-A allele-specific binding protein was inhibited by unlabeled oligonucleotide probes (Figure 3b). We further conducted an in-silico search with the TFSEARCH software[21] to examine whether rs34623097 is located in any putative mammalian transcription factor-binding sites. The results indicated no putative transcription factor whose binding affinity is affected by rs34623097.

Implications for the origin of rs34623097-A
The rs34623097-A allele is a derived allele since the homolog sites in other mammalian genomes, including the chimpanzee genome, have G. The rs34623097-A allele is found among Oceanic Islanders, Asians, and the Finnish, but it is not found in Africans (Supplementary Material, Supplementary Figure S6). Of particular interest, the Neandertal genome has A at the corresponding site (Supplementary Material, Supplementary Figure S6).[23] These

observations imply that a G > A mutation at rs34623097 occurred in the Neandertal genome, and then the rs34623097-A allele flowed into the ancestors of present-day humans. To examine this possibility, based on the genotype data retrieved from the 1000 Genomes Project database,[22] we analyzed the LD structure around rs34623097 in Asian and Finnish people (Supplementary Material, Supplementary Figure S7a). Next, we estimated the frequencies of haplotypes bearing SNPs that had a minor allele frequency of more than 0.1 and were in LD (D' > 0.9) with rs34623097 (Supplementary Material, Supplementary Figure S7b). In this analysis, we considered only SNPs whose sequences of the Neandertal genome were available in the UCSC database (http://genome.ucsc.edu/). A comparison of the haplotypes in human populations with the Neandertal genome revealed that the haplotype bearing rs34623097-A in present-day humans had the same allele (ie, sequence) at each SNP as the Neandertal haplotype (Supplementary Material, Supplementary Figure S7b). In other words, Neandertals and present-day humans share rs34623097-A on the same 21-kb genomic segment. Although only genomes from three Neandertals were analyzed, and the read depth obtained by using next generation sequencing was very low,[23] all Neandertal individuals seem to have the same segment or haplotype bearing rs34623097-A, and the population frequency is estimated to have been high in Neandertals. These results suggest that a G > A mutation at rs34623097 occurred in Neandertals and then the rs34623097-A allele flowed into the common ancestors of the Oceanic Islanders, Asians, and Finnish.

DISCUSSION
To our knowledge, this is the first study in which a significant association of rs34623097 with obesity or BMI was detected. Functional analyses indicated that rs34623097-A reduced the transcriptional activity of the luciferase reporter gene compared to rs34623097-G. Our results suggest that the lower expression of ADRB2 caused by rs34623097-A increases the risk for obesity. It has been reported that ADRB2 expression is decreased in omental adipose tissues obtained from obese subjects as compared to those from lean subjects.[25] Taken together, these observations indicate that decreased expression of ADRB2 in adipose tissue is one of the underlying causes of obesity. ADRB2 would be a potential therapeutic target for the development of anti-obesity drugs.

Since the gene flow between Neandertals and present-day humans is considered to have occurred before the divergence of non-African populations,[23] rs34623097-A would have existed for at least 50 000 years in ancestors of Asians, Oceanic Islanders, and the Finnish. This period could be long enough for rs34623097-A to increase in frequency due to positive selection, if it operates.
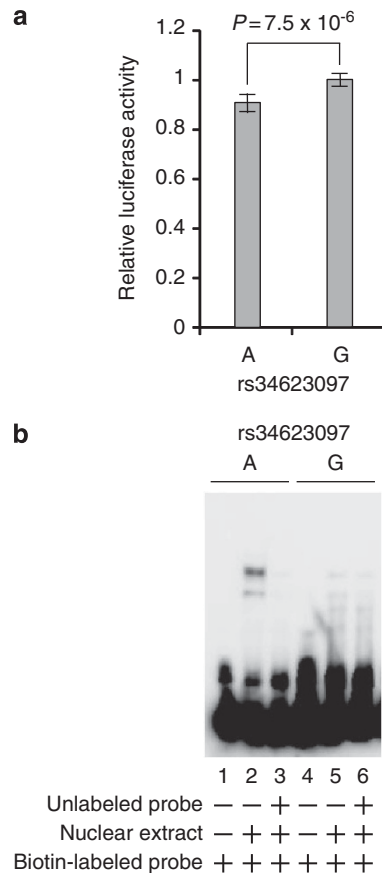
rs34623097-A was not increased in the Polynesians compared to the other Oceanic populations (Supplementary Material, Supplementary Figure S6). The present observation does not seem to support Neel's 'thrifty genotype' model for Oceanic populations. To evaluate 'thrifty genotype' model for the Oceanic populations and obtain conclusive evidence, more polymorphisms associated with obesity are required to be identified.

In this study, we showed that rs34623097-A upstream of *ADRB2* was significantly associated with an increased risk for obesity in the Oceanic populations. The functional analysis revealed that rs34623097-A leads to lower expression of the luciferase reporter gene, suggesting lower expression of *ADRB2*. The lower expression of *ADRB2* may be a risk for obesity in the Oceanic populations. A previous meta-analysis reported that the 27Glu allele significantly increased the risk for obesity in Asians, Pacific Islanders, and Native Americans, but not in Europeans.[12] The 27Glu allele is commonly observed in worldwide populations (Supplementary Material, Supplementary Figure S8), whereas rs34623097-A is observed in Asian and Oceanic populations, but not in Europeans except for the Finnish (Supplementary Material, Supplementary Figure S5). Since rs34623097 is in LD with Gln27Glu (rs1042714) in CHB, CHS, JPT, and FIN populations (Supplementary Material, Supplementary Figure S7) as well as in the Oceanic populations (Supplementary Material, Supplementary Figure S4), the association of 27Glu with obesity that has been found in populations of Asian ancestry[12] may have come from the positive LD between rs34623097-A and 27Glu. Since Europeans do not have rs34623097-A, an apparent association of 27Glue with obesity, as found in Asian populations, may have not been detected in populations of European ancestry in a meta-analysis.[12] This should be investigated in future studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Figure 3. Functional analysis of rs34623097. (**a**) Luciferase reporter assay of *ADRB2* rs34623097 SNP. The reporter gene construct with rs34623097-A or rs34623097-G was transfected in HEK293T cells. Transcriptional activity was measured using Renilla luciferase as a reporter and was normalized using Firefly luciferase activity. The experiment was replicated five times, with quintuplicate assays performed within each replicate. The results of a total of 25 assays of each allele are divided by the mean of rs34623097-G. The values presented are mean ± s.e. after the division (ie, relative values against rs34623097-G). The *P*-value was obtained by ANCOVA adjusted for replication. The significant difference in transcriptional activity between rs34623097-A and rs34623097-G was detected ($P = 7.5 \times 10^{-6}$). (**b**) Electrophoretic mobility shift assay (EMSA) of *ADRB2* rs34623097 SNP. The biotin-labeled probes carrying rs34623097-A and rs34623097-G were incubated with reaction mixtures containing no nuclear extracts (lanes 1 and 4) and with those containing 6 μg of nuclear extracts from HEK293T cells (lanes 2 and 5); lanes 3 and 6: as lanes 2 and 5 plus 200-fold molar excess of unlabeled probes carrying rs34623097-A and rs34623097-G.

However, the frequency of rs34623097-A is still low in present-day human populations, and no extended LD is observed for rs34623097-A (Supplementary Material, Supplementary Figure S7a). Thus, it would be reasonable to postulate that rs34623097-A has not been subjected to strong positive selection in present-day human populations, although we do not exclude the possibility that rs34623097-A was beneficial for Neandertals.

It has been considered that obesity in Oceanic populations may be explained by Neel's 'thrifty genotype' model[26] in which genotypes responsible for improved energy storage conferred a selective advantage during their voyage to Oceania; however, in modern societies with a constant abundance of food, these genotypes increase the risk for obesity. In this model, the frequencies of alleles associated with the risk for obesity are expected to be higher in populations that have experienced long distance voyages, such as the Polynesians. The allele frequency of

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