ONLINE METHODS

Study subjects. Characteristics of each case-control group are shown in **Supplementary Table 1**. All subjects with atopic dermatitis were diagnosed by physicians according to the criteria of Hanifin and Rajka[31]. All individuals were Japanese and gave written informed consent to participate in the study. This research project was approved by the ethical committees at the Institute of Medical Science, the University of Tokyo and the RIKEN Yokohama Institute.

BioBank Japan cases. The BioBank Japan project has been running since 2003, aiming at the collection of basic information for application to personalized medicine[32]. We selected case samples from the subjects who participated in the BioBank Japan, and a total of 1,472 cases for the GWAS and 940 cases for the validation study were recruited from several medical institutes, including the Fukujuji Hospital, Iizuka Hospital, Juntendo University, Hospital Iwate Medical University School of Medicine, National Hospital Organization Osaka National Hospital, Nihon University, Nippon Medical School, Shiga University of Medical Science, Cancer Institute Hospital of the Japanese Foundation for Cancer Research, Tokushukai Hospital and Tokyo Metropolitan Geriatric Hospital in Japan.

RIKEN cases. For the validation study, a total of 916 cases were recruited from the Takao Hospital, Kyushu University Hospital, University of Tokyo Hospital, Keio University Hospital, University of Tsukuba Hospital and several other hospitals.

BioBank controls. We used genome-wide screening data from subjects in the BioBank Japan project for the controls. Individuals with bronchial asthma and atopic dermatitis were excluded from the controls. Controls for the GWAS consisted of 6,042 cases in BioBank Japan with 1 of 5 diseases (cerebral aneurysm, esophageal cancer, endometrial cancer, chronic obstructive pulmonary disease and glaucoma), 1,023 healthy volunteers from members of the Rotary Club of Osaka-Midosuji District 2660 Rotary International in Japan[33] and 906 healthy subjects from the PharmaSNP Consortium.

A total of 5,547 cases registered in BioBank Japan with 1 of 4 diseases (epilepsy, urolithiasis, nephrotic syndrome and Graves' disease) were recruited for the validation study.

RIKEN controls. A total of 1,474 healthy volunteers were recruited from several medical institutes in Japan, including the Japanese Red Cross Wakayama Medical Center, Fukui University and Tsukuba University. Individuals with bronchial asthma and atopic dermatitis were excluded from the control group.

Genotyping and quality control. For the GWAS, we genotyped 1,491 cases and 7,983 controls using the Illumina Human OmniExpress BeadChip. We excluded a total of 19 cases and 12 controls because allele sharing analysis revealed that they were closely related, paired samples. We performed PCA of genotype data from the subjects along with data from European (CEU), African (YRI) and east Asian (Japanese (JPT) and Han Chinese (CHB) individuals obtained from the Phase 2 HapMap database by using smartpca[34]. The PCA plot indicated that cases and controls were genetically matched, with minimal evidence of population stratification (**Supplementary Fig. 1a,b**). We excluded samples with a call rate for autosomal SNPs of <0.98. We also excluded SNPs with minor allele frequencies of less than 0.01 from both cases and controls. SNPs having call rates of ≥99% in both cases and controls were used for the association study. We conducted exact Hardy-Weinberg equilibrium analysis, and SNPs with P values less than the cutoff value for the Hardy-Weinberg equilibrium test ($P < 1 \times 10^{-6}$ in controls) were excluded from the analysis.

In the validation study, we genotyped SNPs using the TaqMan assay (Life Technologies) or the multiplex PCR–based Invader assay (Third Wave Technologies). The genotype concordance rates for the eight SNPs in **Table 1** between samples genotyped using the Illumina Human OmniExpress BeadChip and those same samples genotyped with the TaqMan assay or multiplex PCR–based Invader assay were 1.000 and 1.000, respectively.

Statistical analysis. In the GWAS and validation study, the statistical significance of the association with each SNP was assessed using a 1-degree-of-freedom Cochran-Armitage trend test. We assessed association of SNPs on chromosome X by a meta-analysis with the Mantel-Haenszel method for two 2 × 2 allele frequency tables within male and female subjects. Odds ratios and confidence intervals were calculated from a 2 × 2 allele frequency table. We combined data from the GWAS and validation study by the Mantel-Haenszel method. Heterogeneity across the studies was examined using the Breslow-Day test[35]. Regional association plots were generated using LocusZoom[36].

Imputation. Imputation provides a high-resolution view of an associated region. Genotype imputation within the GWAS was performed using minimac. Association tests were performed with mach2dat using the fractional dosages output[37,38]. We used individuals from the 1000 Genomes Project (phased JPN, CHB and Han Chinese South (CHS) data, June 2011) as reference populations[39]. SNPs with a minor allele frequency of <5% and low quality of imputation ($r^2 < 0.7$) were excluded.

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Meta-analysis identifies multiple loci associated with kidney function–related traits in east Asian populations

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Chronic kidney disease (CKD), impairment of kidney function, is a serious public health problem, and the assessment of genetic factors influencing kidney function has substantial clinical relevance. Here, we report a meta-analysis of genome-wide association studies for kidney function–related traits, including 71,149 east Asian individuals from 18 studies in 11 population-, hospital- or family-based cohorts, conducted as part of the Asian Genetic Epidemiology Network (AGEN). Our meta-analysis identified 17 loci newly associated with kidney function–related traits, including the concentrations of blood urea nitrogen, uric acid and serum creatinine and estimated glomerular filtration rate based on serum creatinine levels (eGFRcrea) ($P < 5.0 \times 10^{-8}$). We further examined these loci with *in silico* replication in individuals of European ancestry from the KidneyGen, CKDGen and GUGC consortia, including a combined total of ~110,347 individuals. We identify pleiotropic associations among these loci with kidney function–related traits and risk of CKD. These findings provide new insights into the genetics of kidney function.

Chronic kidney disease—the impairment of kidney function—constitutes a serious public health burden on society worldwide, with increased risks of mortality and morbidity[1,2]. Biochemical measures of kidney function that are commonly used in clinical practice include the concentrations of blood urea nitrogen, serum creatinine and uric acid and glomerular filtration rate (GFR). Heritability estimates have shown that genetic factors contribute significantly to interindividual variance in kidney function[3], and recent developments in genome-wide association studies (GWAS) have identified a number of genetic loci associated with measurements of kidney function[4–13]. However, most of these studies were conducted in populations of European ancestry[4,6–8,10–13], and the extension of GWAS approaches to non-European populations would provide an opportunity to discover additional loci. We report a large-scale meta-analysis of GWAS and a replication study of kidney function–related traits involving 71,149 east Asian subjects performed by AGEN[9,14–16] in which 11 cohorts participated (BBJ, SP2, SiMES, SINDI, SCES, KARE, HEXA, TWSC, TWT2D, GenSalt and CAGE; Online Methods and **Supplementary Note**).

In this study, we evaluated four kidney function–related traits (**Supplementary Tables 1** and **2**): the concentrations of blood urea nitrogen (n = 57,178), uric acid (n = 33,074) and serum creatinine (n = 61.919) and eGFRcrea (n = 62,087). Blood urea nitrogen concentration reflects the amount of nitrogen in the blood and is related to protein metabolism, including excretion by the kidneys[17]. Uric acid is the end product of purine metabolism, and impaired renal excretion of uric acid leads to hyperuricemia. Epidemiological studies suggest that uric acid is a risk factor for various diseases, including gout and myocardial infarction[13]. Serum creatinine levels and eGFRcrea are the most common kidney function measures used for the definition of CKD[1,2], for which extensive genetic studies in European populations have been conducted[4,6–8].

The GWAS meta-analysis included 51,327 east Asian individuals and evaluated approximately 2.4 million autosomal SNPs with a minor allele frequency (MAF) of ≥0.01. These SNPs were obtained by imputation of genotypes on the basis of HapMap Phase 2 panels (**Supplementary Tables 3** and **4**). The inflation factors of the test statistics were modest (λ_{GC} = 1.060, 1.072, 1.079 and 1.031 for blood urea nitrogen, serum creatinine, eGFRcrea and uric acid, respectively), which suggested that population structures did not have a substantial

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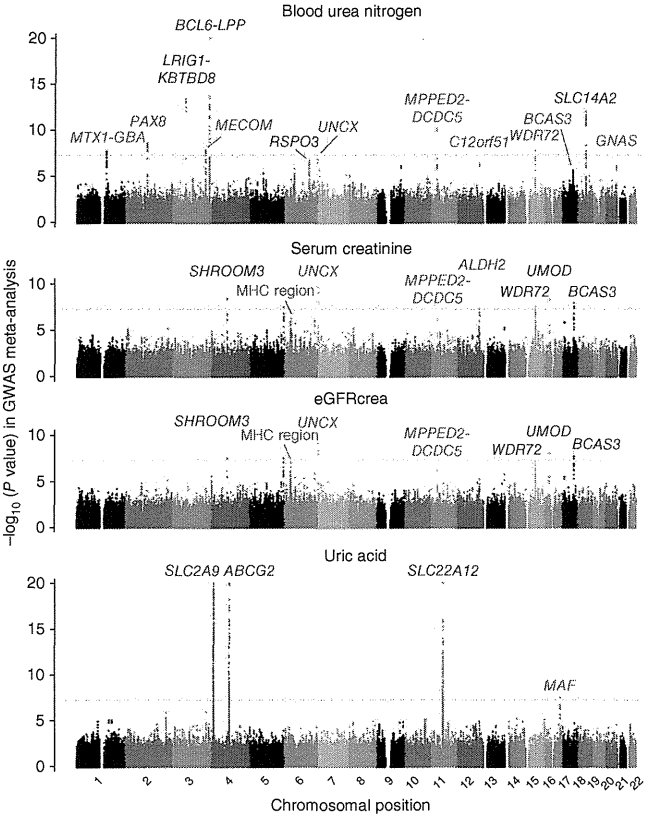


Figure 1 Manhattan plots of the GWAS meta-analysis for kidney function–related traits. Shown are the $-\log_{10}$ (*P* values) of the SNPs for the concentrations of blood urea nitrogen, serum creatinine and uric acid, and for eGFRcrea. The genetic loci that satisfied the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ (gray horizontal dotted line) in the combined study of the GWAS meta-analysis and replication are labeled for each of the traits. The newly identified loci are colored red, and the previously known loci are colored blue. The SNPs for which the *P* value was smaller than 1.0×10^{-20} are indicated at the upper limit of each plot.

impact on the results of the meta-analysis. Quantile-quantile plots of the *P* values indicated notable discrepancies in their tails from those anticipated under the null hypothesis of no association, indicating the presence of significant associations in the meta-analysis (**Supplementary Fig. 1**). We identified 25 associations that satisfied the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$. Of these, eight, seven, six and four genetic loci were found to associated with blood urea nitrogen, serum creatinine, eGFRcrea and uric acid, respectively (**Supplementary Table 5**).

We then performed an *in silico* replication study using data from an additional 19,822 east Asians for the loci that associated at $P < 5.0 \times 10^{-6}$ in the GWAS meta-analysis. Through the combined study of the GWAS meta-analysis and replication, we identified 32 significant associations at $P < 5.0 \times 10^{-8}$ (13, 8, 7 and 4 loci for blood urea nitrogen, serum creatinine, eGFRcrea and uric acid, respectively; **Fig. 1** and **Supplementary Table 5**). We found that seven of these newly associated loci were associated with both serum creatinine concentration and eGFRcrea, with the same landmark SNPs involved at each locus, which reflects the close relationship between these two phenotypes ($R^2 = 0.76$ for common log-transformed values; **Supplementary Table 6**)[1,2]. Among the loci identified in the combined analysis, associations at 15 loci were previously reported[4–13]: *LRIG1-KBTBD8*, *BCL6-LPP*, *RSPO3* and *SLC14A2* for blood urea nitrogen concentration (smallest $P = 8.8 \times 10^{-30}$ at *BCL6-LPP*); *SHROOM3*, *WDR72*, *UMOD* and *BCAS3* for serum creatinine concentration (smallest $P = 1.2 \times 10^{-13}$ at *WDR72*); *SHROOM3*, *WDR72*, *UMOD* and *BCAS3* for eGFRcrea (smallest $P = 6.0 \times 10^{-13}$ at *WDR72*); and *SLC2A9*, *ABCG2* and *SLC22A12* for uric acid concentration (smallest $P = 1.6 \times 10^{-65}$ at *SLC2A9*). At the *UMOD* locus, the rs12917707 variant associated with eGFRcrea in Europeans[4,7] had a low MAF (<0.01) and was not evaluated in our GWAS meta-analysis. However, we identified another variant that showed a significant association with eGFRcrea ($P = 3.6 \times 10^{-10}$ at rs11864909; MAF = 0.19; $r^2 = 0.02$ with rs12917707).

In addition, we identified 17 loci newly associated with kidney function–related traits (**Table 1** and **Supplementary Fig. 2**). Namely, we identified associations at nine loci for blood urea nitrogen concentration (*MTX1-GBA*, *PAX8*, *MECOM*, *UNCX*, *MPPED2-DCDC5*, *C12orf51*, *WDR72*, *BCAS3* and *GNAS* at 1q22, 2q13, 3q26, 7p22, 11p14, 12q24.13, 15q21, 17q23 and 20q13, respectively; smallest $P = 4.5 \times 10^{-16}$ at rs10767873 in *MPPED2-DCDC5*), four loci for serum creatinine concentration (the major histocompatibility (MHC) region, *UNCX*, *MPPED2-DCDC5* and *ALDH2* at 6p21, 7p22, 11p14 and 12q24.2, respectively; smallest $P = 4.6 \times 10^{-11}$ at rs10277115 in *UNCX*), three loci for eGFRcrea (the MHC region, *UNCX* and *MPPED2-DCDC5* at 6p21, 7p22 and 11p14, respectively; smallest $P = 1.0 \times 10^{-10}$ at rs10277115 in *UNCX*) and one locus for uric acid concentration (*MAF* at 16q23, $P = 1.1 \times 10^{-9}$ at rs889472). Combinations of these identified loci explained 1.3%, 0.54%, 0.55% and 2.3% of interindividual variance in blood urea nitrogen, serum creatinine, eGFRcrea and uric acid, respectively.

To determine whether the associations that we observed were relevant to populations of European ancestry, we evaluated the newly associated loci (at $P < 5.0 \times 10^{-8}$ in our combined meta-analysis) in Europeans by using the results of studies by the KidneyGen (n = 23,812 for serum creatinine concentration)[6], CKDGen (n = 67,093 for eGFRcrea)[7] and GUGC (n = 110,347 for uric acid concentration; A. Köttgen *et al.*, personal communication) consortia. Nine of the 15 loci that reached $P < 5.0 \times 10^{-8}$ for serum creatinine, eGFRcrea and uric acid measures in our study also showed significant associations in the European study ($P < 0.05/15 = 0.0033$, Bonferroni correction for the number of loci with available results), including the *MPPED2-DCDC5* locus for eGFRcrea ($P = 5.3 \times 10^{-8}$ at rs963837; **Supplementary Table 5**).

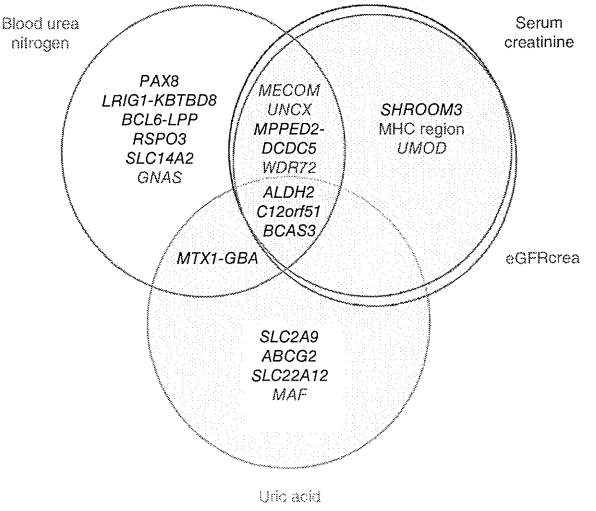
We also evaluated the loci previously reported to be associated with kidney function measures, after excluding the 14 loci that had already been identified in our study (**Supplementary Table 7**)[4–13]. Of the 31 loci evaluated, we replicated associations at 8 loci in our study ($P < 0.05/31 = 0.0016$, Bonferroni correction for the number of loci), including in *CPS1*, *RGS14*, *STC1*, *RNASEH2C-OVOL1* and *SLC6A13* for eGFRcrea and *GCKR*, *LRP2* and *LRRC16A-SLC17A1* for uric acid concentration.

As the evaluated phenotypes reflect both common and unique biological aspects of kidney status, it is of interest to understand whether the loci associated with kidney function traits show pleiotropic patterns of associations[18]. We evaluated the associations of the identified loci within the evaluated kidney function–related traits and risk of stage 3+ CKD (defined as eGFRcrea of <60 ml/min/1.73 m^2; **Fig. 2**, **Table 2** and **Supplementary Table 8**)[1,2]. Of 21 unique loci, 9 yielded significant associations with three or more phenotypes ($P < 0.05/21 = 0.0024$, Bonferroni correction for the number of loci). In particular, the *ALDH2*, *C12orf51* and *BCAS3* loci had significant associations with all of the evaluated kidney function–related traits.

Table 1 Loci newly associated with kidney function–related traits

rsID[a]	Chr.	Position (bp)	Band	Gene	A1/A2[b]	Freq.[c]	GWAS meta-analysis β (SE)[d]	GWAS meta-analysis P	Replication study β (SE)[d]	Replication study P	Combined study β (SE)[d]	Combined study P
Blood urea nitrogen (n = 39,717 for GWAS meta-analysis, n = 17,461 for replication)												
rs2049805	1	153461604	1q22	*MTX1-GBA*	T/C	0.17	0.0072 (0.0013)	1.9×10^{-8}	0.0072 (0.0017)	2.3×10^{-5}	0.0072 (0.0010)	1.8×10^{-12}
rs11123170	2	113695411	2q13	*PAX8*	G/C	0.35	0.0059 (0.0010)	2.4×10^{-9}	0.0035 (0.0014)	0.014	0.0051 (0.0008)	3.3×10^{-10}
rs16853722	3	170633326	3q26	*MECOM*	C/T	0.29	0.0059 (0.0010)	1.3×10^{-8}	0.0072 (0.0014)	2.7×10^{-7}	0.0064 (0.0008)	2.7×10^{-14}
rs10275044	7	1240371	7p22	*UNCX*	T/A	0.34	0.0079 (0.0015)	1.3×10^{-7}	0.0053 (0.0019)	0.0056	0.0069 (0.0012)	4.3×10^{-9}
rs10767873	11	30725254	11p14	*MPPED2-DCDC5*	C/T	0.69	0.0068 (0.0010)	7.0×10^{-11}	0.0068 (0.0014)	1.4×10^{-6}	0.0068 (0.0008)	4.5×10^{-16}
rs2074356	12	111129784	12q24.13	*C12orf51*	A/G	0.23	0.0064 (0.0013)	4.4×10^{-7}	0.0063 (0.0020)	0.0012	0.0064 (0.0011)	1.8×10^{-9}
rs17730281	15	51695240	15q21	*WDR72*	G/A	0.58	0.0054 (0.0010)	1.5×10^{-8}	0.0046 (0.0013)	4.5×10^{-4}	0.0051 (0.0008)	3.0×10^{-11}
rs11868441	17	56594003	17q23	*BCAS3*	G/A	0.75	0.0062 (0.0013)	2.1×10^{-6}	0.0055 (0.0015)	2.2×10^{-4}	0.0059 (0.0010)	2.1×10^{-9}
rs6026584	20	56902468	20q13	*GNAS*	T/C	0.32	0.0055 (0.0011)	7.1×10^{-7}	0.0046 (0.0016)	0.0033	0.0052 (0.0009)	8.8×10^{-9}
Serum creatinine (n = 42,257 for GWAS meta-analysis, n = 19,662 for replication)												
rs3828890	6	31548648	6p21	MHC region	G/C	0.11	0.0074 (0.0015)	5.3×10^{-7}	0.0060 (0.0018)	0.0011	0.0069 (0.0012)	2.6×10^{-9}
rs10277115	7	1251721	7p22	*UNCX*	T/A	0.35	0.0060 (0.0009)	2.2×10^{-10}	0.0034 (0.0015)	0.022	0.0052 (0.0008)	4.6×10^{-11}
rs963837	11	30705666	11p14	*MPPED2-DCDC5*	T/C	0.64	0.0036 (0.0007)	7.5×10^{-7}	0.0040 (0.0012)	0.0013	0.0037 (0.0006)	3.4×10^{-9}
rs671	12	110726149	12q24.2	*ALDH2*	A/G	0.27	0.0047 (0.0009)	5.0×10^{-8}	0.0040 (0.0013)	0.0015	0.0045 (0.0007)	2.8×10^{-10}
eGFRcrea (n = 42,451 for GWAS meta-analysis, n = 19,636 for replication)												
rs3828890	6	31548648	6p21	MHC region	G/C	0.11	−0.0091 (0.0017)	9.8×10^{-8}	−0.0062 (0.0020)	0.0018	−0.0079 (0.0013)	1.2×10^{-9}
rs10277115	7	1251721	7p22	*UNCX*	T/A	0.35	−0.0066 (0.0011)	7.3×10^{-10}	−0.0039 (0.0016)	0.014	−0.0058 (0.0009)	1.0×10^{-10}
rs963837	11	30705666	11p14	*MPPED2-DCDC5*	T/C	0.64	−0.0041 (0.0008)	6.3×10^{-7}	−0.0048 (0.0013)	3.8×10^{-4}	−0.0043 (0.0007)	1.1×10^{-9}
Uric acid (n = 21,417 for GWAS meta-analysis, n = 11,657 for replication)												
rs889472	16	78203490	16q23	*MAF*	C/A	0.57	0.0758 (0.0136)	2.8×10^{-8}	0.0584 (0.0227)	0.010	0.0711 (0.0117)	1.1×10^{-9}

Chr., chromosome; SE, standard error, Freq., frequency.
[a]SNPs in the newly identified loci associated with kidney function–related traits. [b]The allele that increased blood urea nitrogen, serum creatinine or uric acid concentration or that decreased eGFRcrea was defined as allele 1 (A1) and is indicated on the basis of the forward strand of NCBI Build 36. [c]Frequency of allele 1 in the GWAS meta-analysis. [d]Effect size of allele 1 on common log-transformed values of blood urea nitrogen or serum creatinine concentration, eGFRcrea or non-transformed values of uric acid concentration.



We also observed significant risk for CKD at several loci, including in *MECOM*, the MHC region, *UNCX*, *WDR72*, *UMOD*, *MAF* and *GNAS*. Because of the definition of CKD[1,2], previous studies assessed CKD risk primarily at the loci associated with serum creatinine concentration and eGFRcrea[4,6–8]. However, our results suggest that genetic risk for CKD would also be contributed to by other kidney function–related loci, such as *MAF* and *GNAS*. Recent studies suggested the superiority of eGFR based on serum cystatin C concentration (eGFRcys) relative to eGFRcrea, especially for predicting GFR in subjects with normal or mildly reduced GFR, and assessment of the genetic factors underlying eGFRcys in east Asians would thus be warranted.

Figure 2 Venn diagram of pleiotropic associations of the identified loci. Genetic loci identified in the study are classified on the basis of the results of the pleiotropic association study of kidney function–related traits (**Table 2** and **Supplementary Table 8**). Genes that showed significant associations with risk for stage 3+ CKD are colored red.

Table 2 Pleiotropic associations of the identified loci with kidney function–related traits and CKD risk

					Blood urea nitrogen (n = 57,178)	Serum creatinine (n = 61,919)	eGFRcrea (n = 62,087)	Uric acid (n = 33,074)	CKD (8,805 cases and 35,259 controls)
					P^b	P^b	P^b	P^b	P^b
rs2049805	1	153461604	1q22	*MTX1-GBA*	**1.8×10^{-12}**	0.027	0.046	**0.0022**	0.75
rs11123170	2	113695411	2q13	*PAX8*	**3.3×10^{-10}**	0.0059	0.0082	0.079	0.0058
rs13069000	3	66881640	3p14	*LRIG1-KBTBD8*	**1.4×10^{-19}**	0.24	0.42	0.0093	0.94
rs16853722	3	170633326	3q26	*MECOM*	**2.7×10^{-14}**	**9.4×10^{-4}**	**8.3×10^{-4}**	0.76	**9.9×10^{-4}**
rs10937329	3	189196412	3q27	*BCL6-LPP*	**8.8×10^{-30}**	0.90	0.93	0.36	0.40
rs3775948	4	9604280	4p16	*SLC2A9*	0.061	0.40	0.36	**1.6×10^{-65}**	0.13
rs13146355	4	77631164	4q21	*SHROOM3*	0.043	**9.4×10^{-12}**	**6.6×10^{-11}**	0.16	0.090
rs2725220	4	89178946	4q22	*ABCG2*	0.20	0.18	0.23	**4.2×10^{-30}**	0.44
rs3828890	6	31548648	6p21	MHC region	0.14	**2.6×10^{-9}**	**1.2×10^{-9}**	0.051	**0.0016**
rs1936800	6	127477757	6q22	*RSPO3*	**1.2×10^{-11}**	0.64	0.57	0.78	0.72
rs10277115	7	1251721	7p22	*UNCX*	**1.9×10^{-9}**	**4.6×10^{-11}**	**1.0×10^{-10}**	0.014	**1.7×10^{-6}**
rs10767873	11	30725254	11p14	*MPPED2-DCDC5*	**4.5×10^{-16}**	**4.3×10^{-7}**	**1.8×10^{-7}**	0.012	0.0055
rs504915	11	64220661	11q13	*SLC22A12*	0.68	0.32	0.39	**3.3×10^{-63}**	0.74
rs671	12	110726149	12q24.2	*ALDH2*	**1.3×10^{-5}**	**2.8×10^{-10}**	**7.8×10^{-8}**	**1.6×10^{-6}**	0.16
rs2074356	12	111129784	12q24.13	*C12orf51*	**1.8×10^{-9}**	**1.9×10^{-9}**	**6.5×10^{-8}**	**1.6×10^{-5}**	0.14
rs17730281	15	51695240	15q21	*WDR72*	**3.0×10^{-11}**	**3.6×10^{-14}**	**1.3×10^{-13}**	0.29	**1.3×10^{-8}**
rs11864909	16	20308340	16p12	*UMOD*	0.0058	**1.1×10^{-10}**	**3.6×10^{-10}**	0.87	**7.0×10^{-4}**
rs889472	16	78203490	16q23	*MAF*	0.30	0.29	0.30	**1.1×10^{-9}**	**0.0012**
rs11868441	17	56594003	17q23	*BCAS3*	**2.1×10^{-9}**	0.010	0.0098	0.0089	0.062
rs9895661	17	56811371	17q23	*BCAS3*	0.65	**7.4×10^{-11}**	**4.8×10^{-11}**	**9.3×10^{-4}**	0.0060
rs7227483	18	41441128	18q12	*SLC14A2*	**6.7×10^{-18}**	0.32	0.32	0.033	0.11
rs6026584	20	56902468	20q13	*GNAS*	**8.8×10^{-9}**	0.19	0.10	0.0041	**0.0022**

Detailed results of the analysis are provided in **Supplementary Table 8**.
[a]Indicated on the basis of the forward strand of NCBI Build 36. [b]P values that satisfied the Bonferroni correction based on the number of loci ($\alpha = 0.05$, $n = 21$; $P < 0.0024$) are shown in bold.

In this study, we identified new associations at *MTX1-GBA*, *PAX8*, *MECOM*, the MHC region, *UNCX*, *MPPED2-DCDC5*, *ALDH2*, *C12orf51*, *WDR72*, *MAF*, *BCAS3* and *GNAS* with kidney function–related traits. *MTX1* has an essential role in embryonic development, and *GBA* encodes glucocerebrosidase, an enzyme mediating glycolipid metabolism[19]. Both are known as causal genes in Gaucher disease[19], a lysosomal storage disease, although kidney function decline has not been implicated in pathogenesis. *PAX8* is a member of the PAX gene family and is widely expressed in renal tissues[20]. *MECOM* (also known as *EVI1*) encodes a transcriptional regulator involved in hematopoiesis[21]. The MHC region contains a large number of genes related to the immune system, including human leukocyte antigen (HLA) genes. The SNP that was found to be associated with serum creatinine concentration and eGFRcrea (rs3828890) was located in the MHC class I region[22] and was in moderate linkage disequilibrium with the *HLA-DRB1*1302* and *HLA-DQB1*0604* alleles ($D' > 0.65$ and $r^2 > 0.40$ for both alleles)[23]. *UNCX* encodes a paired-type homeobox transcription factor that has essential roles in skeleton formation and kidney development[24]. The function of *MPPED2* is as yet unknown, and *DCDC5* encodes a protein with two doublecortin domains, which serve as protein-interaction platforms[25]. It is noteworthy that the *MTX1-GBA*, *MECOM* and *MPPED2-DCDC5* loci have been reported to influence serum magnesium levels[26], which are maintained by renal regulation of magnesium reabsorption. The loci in *ALDH2*, *WDR72* and *BCAS3* have been reported to be associated with some kidney function measures[5,7], although the biological roles of these genes in renal homeostasis have not been substantially explored. Although the function of the protein encoded by *C12orf51* has not been examined, this locus was reported to be associated with serum lipid and liver enzyme concentrations in east Asians[9]. *MAF* encodes a leucine zipper transcription factor and has been implicated in the pathogenesis of minimal-change nephrotic syndrome (MCNS)[27]. Defects in *MAF* cause juvenile-onset pulverulent cataract as well as congenital cerulean cataract (CCA4)[28]. *GNAS* encodes the heterotrimeric G protein $G_s\alpha$, and the associated locus in this gene is also associated with multiple metabolic traits, including blood pressure, in Europeans[29]. Nevertheless, other genes near each of the loci could also be candidates, and further functional assessment is desirable.

In conclusion, in this large-scale meta-analysis in east Asian populations, we identified multiple loci newly associated with kidney function–related traits and pleiotropic associations. Our study should make an important contribution to the enhanced understanding of the genetic architecture of kidney function.

URLs. International HapMap Project, http://hapmap.ncbi.nlm.nih.gov/; MACH and mach2qtl software, http://www.sph.umich.edu/csg/abecasis/MACH/index.html; IMPUTE and SNPTEST software, http://www.stats.ox.ac.uk/~marchini/software/gwas/gwas.html; BEAGLE software, http://faculty.washington.edu/browning/beagle/beagle.html; PLINK software, http://pngu.mgh.harvard.edu/~purcell/plink/; R statistical software, http://cran.r-project.org/; SNAP software, http://www.broadinstitute.org/mpg/snap/index.php.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

Y.O. and T. Tanaka designed the overall study. Y.O., X.S., M.J.G., C.-H.C., D.G., F.T. and P.C. analyzed GWAS data. Y.O. performed meta-analysis and other statistical analysis. Y.O., A.T., S.M., T. Tsunoda, K.Y., M.K., Y.N., N. Kamatani and T. Tanaka managed GWAS data of BBJ. X.S., P.C., S.-C.L., T.-Y.W., J.L., T.L.Y., T.A., M.S., Y.-Y.T. and E.-S.T. managed the GWAS data from SP2, SiMES, SINDI and SCES. M.J.G., Y.J.K., J.-Y.L., B.-G.H., D.K. and Y.S.C. managed the GWAS data from KARE and HEXA. C.-H.C., F.-J.T., L.-C.C., S.-J.C.F., Y.-T.C. and J.-Y.W. managed the GWAS data from TWSC and TWT2D. D.G., H.M., D.C.R., J.E.H., S.C. and J.H. managed the GWAS data from GenSalt. F.T., T.K., M.I., T.O. and N. Kato managed the GWAS data from CAGE. J.C.C., W.Z. and J.S.K. managed the data from the KidneyGen Consortium. E.A. managed the data from the GUGC consortium. Y.O., T. Tanaka, E.-S.T., Y.S.C., J.-Y.W., J.H. and N. Kato directed the study and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. The 71,149 subjects included in the GWAS meta-analysis for kidney function–related traits (n = 57,178, 61,919, 62,087 and 33,074 for blood urea nitrogen, eGFRcrea and uric acid, respectively) were obtained from 18 studies conducted in the following 11 population-, hospital- or family-based cohorts of east Asian populations through the collaborations of AGEN[9,14–16]: the BioBank Japan Project (BBJ), the Singapore Prospective Study Program (SP2), the Singapore Malay Eye Study (SiMES), the Singapore Indian Study (SINDI), the Singapore Chinese Eye Study (SCES), the Korea Association Resource project (KARE), the Health Examinee shared control study (HEXA), the Taiwan Super Control Study (TWSC), the Taiwan Type 2 Diabetes Consortium (TWT2D), the Genetic Epidemiology Network of Salt Sensitivity (GenSalt) and Cardio-metabolic Genome Epidemiology (CAGE). Of these, 51,327 subjects were enrolled in the GWAS meta-analysis, and 19,822 subjects were enrolled in the *in silico* replication study. Some of the subjects were included in previous studies of east Asian populations[9,14–16]. All participants in each cohort provided written informed consent for participation in the study, as approved by the ethical committees of each of the institutional review boards. Each study established a consensus on subject participation and phenotype definition and analytical protocol for the project. Detailed descriptions of the participating cohorts and the characteristics of the subjects are provided in **Supplementary Tables 1** and **2** and in the **Supplementary Note**. Details of the European studies enrolled by the KidneyGen (n = 23,812 for serum creatinine concentration), CKDGen (n = 67,093 for eGFRcrea) and GUGC (n = 110,347 for uric acid concentration) consortia, including subject details and the study designs, have been described at length elsewhere (refs 6,7 and A. Köttgen *et al.*, personal communication).

Genotyping and quality control. Genotyping platforms and quality control criteria, including exclusion of closely related subjects and outliers in terms of ancestry and cutoff values for sample call rate, SNP call rate, MAF and Hardy-Weinberg equilibrium P value are provided for each study (**Supplementary Table 3** and **Supplementary Note**). Genotype imputation was performed on the basis of the HapMap Phase 2 panels (Japanese in Tokyo, Japan (JPT) and Han Chinese in Beijing, China (CHB) populations, except for SiMES and SINDI, for which JPT, CHB, Yoruba in Ibadan, Nigeria (YRI) and Utah residents of Northern and Western European ancestry (CEU) populations were adopted) by using MACH, IMPUTE or BEAGLE software (see URLs). After imputation, we excluded SNPs with MAF of <0.01 or imputation quality score of R^2 of <0.5 from each study.

Phenotype modeling. Clinical information on the subjects, including age, gender and mean ± s.d. values for the kidney function–related traits, are provided (**Supplementary Table 2**). Collection methods for the clinical information in each of the cohorts are described (**Supplementary Note**). In this study, eGFRcrea was estimated on the basis of serum creatinine levels, using the Japanese coefficient-modified CKD Epidemiology Collaboration (CKD-EPI) equation[2]. We excluded subjects who were <18 or >85 years old, those who had eGFRcrea of <15 ml/min/1.73 m^2 and those who had undergone renal replacement therapy. Subjects with gastrointestinal bleeding, systemic infection or hepatic failure and subjects who had undergone uric acid–lowering therapy (alloprinol, benzbromarone or probenecid) were also excluded from the analyses for blood urea nitrogen and uric acid concentration, respectively.

Genome-wide association study. Associations of SNPs with common log-transformed values of blood urea nitrogen (mg/dl), serum creatinine (mg/dl), eGFRcrea (ml/min/1.73 m^2) or non-transformed values of uric acid concentration (mg/dl) were assessed by linear regression models assuming additive effects of the allele dosages of the SNPs using mach2qtl, SNPTEST, PLINK or R statistical software (see URLs). For the subjects in the family-based cohort, generalized linear mixed models accounting for the family structure were applied. In the regression model, gender, age, drinking status (current drinker or not), smoking status (previous or current smoker or not), body mass index and other cohort-specific variables were incorporated as covariates (**Supplementary Note**).

GWAS meta-analysis. In the GWAS meta-analysis, we included autosomal SNPs that satisfied quality control criteria in three or more GWAS for each of the traits, which yielded between 2.2 and 2.4 million SNPs (**Supplementary Table 4**). Information about the SNPs, including the coded alleles, was oriented to the forward strand of the NCBI Build 36 reference sequence. GWAS meta-analysis was performed using an inverse variance–weighted method, assuming a fixed-effects model for study-specific effect estimates (β) and standard errors (SE) of the coded alleles of the SNPs, using a Java source code implemented by the authors[30,31]. Genomic control corrections were carried out on test statistics from each of the GWAS using study-specific inflation factors (λ_{GC}) and were applied again to the results of the GWAS meta-analysis (**Supplementary Fig. 1**)[32].

***In silico* replication study.** The *in silico* replication study was conducted using additional independent east Asian subjects (**Supplementary Tables 1** and **2**) for the loci that satisfied $P < 5.0 \times 10^{-6}$ in the GWAS meta-analysis for each of the traits (17, 14, 14 and 6 loci for blood urea nitrogen, serum creatinine, eGFRcrea and uric acid, respectively; **Supplementary Table 5**). For each of the loci, the SNP that showed the most significant association was selected. The associations of the SNPs were assessed in the same manner as in the GWAS. The combined study of the GWAS meta-analysis and replication was conducted using an inverse variance method, assuming a fixed-effects model[30,31]. The SNPs that satisfied $P < 5.0 \times 10^{-8}$ in the combined study were considered to be significantly associated with the relevant kidney function–related trait, and the associations of these SNPs were further evaluated using data in European populations from the KidneyGen, CKDGen and GUGC consortia (refs 6,7 and A. Köttgen *et al.*, personal communication).

Estimation of explained variance. The interindividual variance in kidney function–related traits explained by the combination of the identified loci ($P < 5.0 \times 10^{-8}$ for each phenotype) was estimated using a genetic risk score model. We calculated the scores of the subjects enrolled in the *in silico* replication study by the BioBank Japan Project[33] (BBJ_5 and BBJ_6; **Supplementary Table 2**) by summing the dosages of the effect alleles carried by the subjects, which were weighted by the effect sizes of the SNPs obtained from the GWAS meta-analysis. The explained variance was estimated from linear regression models on the covariate-adjusted phenotypes by the scores.

Pleiotropic association analysis for kidney function–related phenotypes. For the genetic loci that showed associations at $P < 5.0 \times 10^{-8}$ in the combined study, pleiotropic associations with the kidney function–related traits and with risk for stage 3+ CKD (defined as eGFRcrea of <60 ml/min/1.73m^2)[1,2] were assessed. Associations with CKD risk were assessed using logistic regression models, incorporating the covariates using the subjects obtained from the BioBank Japan Project[33] (BBJ_1– BBJ_6; **Supplementary Table 2**).

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nature genetics

A genome-wide association study identifies two new susceptibility loci for lung adenocarcinoma in the Japanese population

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Lung adenocarcinoma is the most common histological type of lung cancer, and its incidence is increasing worldwide. To identify genetic factors influencing risk of lung adenocarcinoma, we conducted a genome-wide association study and two validation studies in the Japanese population comprising a total of 6,029 individuals with lung adenocarcinoma (cases) and 13,535 controls. We confirmed two previously reported risk loci, 5p15.33 (rs2853677, $P_{combined} = 2.8 \times 10^{-40}$, odds ratio (OR) = 1.41) and 3q28 (rs10937405, $P_{combined} = 6.9 \times 10^{-17}$, OR = 1.25), and identified two new susceptibility loci, 17q24.3 (rs7216064, $P_{combined} = 7.4 \times 10^{-11}$, OR = 1.20) and 6p21.3 (rs3817963, $P_{combined} = 2.7 \times 10^{-10}$, OR = 1.18). These data provide further evidence supporting a role for genetic susceptibility in the development of lung adenocarcinoma.

Lung cancer is the leading cause of cancer-related death in most countries[1]. Lung cancer consists of three major histological types: adenocarcinoma, squamous-cell carcinoma and small-cell carcinoma[1–3]. Adenocarcinoma is the most common type, comprising ~40% of all cases of lung cancer, and its incidence is increasing in both Asian and Western countries. The development of lung adenocarcinoma is more weakly associated with smoking than are the developments of squamous and small-cell carcinomas, indicating that the mechanisms of carcinogenesis differ among these histological types. A better understanding of the genetic factors underlying the development of lung adenocarcinoma is strongly needed to elucidate the etiology of disease and identify high-risk individuals for targeted screening and/or prevention. In particular, the proportion of females and never smokers among patients with lung adenocarcinoma is considerably higher in Asians than in Europeans[2,3], suggesting that genetic factors contribute differently to disease in the two populations.

Genome-wide association studies (GWAS) of lung cancer with a full range of histological types have been conducted in European populations, and associations at 15q25.1, 5p15.33 and 6p21.33 have been identified[4–8]. Variants at these regions have been defined in European populations by a meta-analysis of GWAS according to histological types, and rs2736100 in *TERT* at 5p15.33 was found to be associated with risk of lung adenocarcinoma[9]. However, no additional loci reached genome-wide significance in the study; therefore, GWAS focusing on lung adenocarcinoma were greatly needed[9]. A recent GWAS on lung adenocarcinoma risk in the Japanese and Korean populations identified a new locus, 3q28 (*TP63*)[10]. Subsequently, a significant but weaker association of 3q28 variations with lung adenocarcinoma risk was validated in Europeans[11]. Notably, the association of this locus with cancer risk was supported by a recent GWAS on lung cancer with a full range of different histological types in the Chinese population[12]. These results indicate that there may be differences in the magnitude of the contribution of these loci to lung cancer susceptibility by ethnicity. Here, to further elucidate the genetic factors contributing to the development of lung adenocarcinoma, we performed a GWAS focusing on lung adenocarcinoma in the Japanese population and expanded the scale of our previous study in terms of both sample size and SNP coverage[10].

Using Illumina Omni1-Quad and OmniExpress chips, we genotyped 1,722 cases and 5,846 controls for 709,857 SNPs (**Supplementary Table 1**). Based on the results of a stringent quality-control analysis, we chose 538,166 autosomal SNPs, 1,695 cases and 5,333 control subjects for our GWAS analyses (Online Methods and

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Table 1 Summary of the GWAS and validation studies and the combined analyses

dbSNP locus	Gene	Allele [risk allele]	Stage	Cases Total	Cases RAF	Controls Total	Controls RAF	P^a	OR (95% CI)	P_{het}
rs2853677	*TERT*	T/C	GWAS	1,695	0.384	5,333	0.308	8.66×10^{-17}	1.41 (1.30–1.53)	
5p15.33	intron 2	[C]	First validation	2,955	0.374	7,036	0.297	8.62×10^{-21}	1.43 (1.32–1.54)	
			Second validation	1,373	0.360	1,132	0.290	5.88×10^{-6}	1.35 (1.19–1.54)	
			Combined validation[b]	4,328	0.370	8,168	0.296	3.90×10^{-25}	1.42 (1.32–1.50)	0.49
			Combined all[b]	6,023	0.374	13,501	0.300	2.80×10^{-40}	1.41 (1.32–1.50)	0.79
rs2736100	*TERT*	T/G	GWAS	1,695	0.458	5,329	0.391	7.31×10^{-12}	1.32 (1.22–1.42)	
5p15.33	intron 2	[G]	First validation	2,954	0.458	7,036	0.385	2.13×10^{-19}	1.39 (1.29–1.49)	
			Second validation	1,343	0.432	1,166	0.368	1.79×10^{-4}	1.27 (1.12–1.44)	
			Combined validation[b]	4,297	0.450	8,202	0.383	3.97×10^{-22}	1.36 (1.28–1.44)	0.22
			Combined all[b]	5,992	0.452	13,531	0.386	2.50×10^{-32}	1.34 (1.28–1.41)	0.39
rs10937405	*TP63*	C/T	GWAS	1,695	0.728	5,333	0.677	1.10×10^{-8}	1.29 (1.18–1.40)	
3q28	intron 1	[C]	First validation	2,953	0.714	7,036	0.663	9.22×10^{-10}	1.27 (1.18–1.37)	
			Second validation	1,375	0.704	1,166	0.682	1.22×10^{-1}	1.11 (0.97–1.26)	
			Combined validation[b]	4,328	0.711	8,202	0.666	8.17×10^{-10}	1.23 (1.15–1.31)	0.076
			Combined all[b]	6,023	0.715	13,535	0.670	6.92×10^{-17}	1.25 (1.19–1.32)	0.15
rs7216064	*BPTF*	A/G	GWAS	1,695	0.747	5,333	0.706	1.07×10^{-5}	1.22 (1.12–1.34)	
17q24.3	intron 9	[A]	First validation	2,955	0.736	7,036	0.708	7.72×10^{-5}	1.17 (1.08–1.27)	
			Second validation	1,376	0.744	1,166	0.708	4.70×10^{-3}	1.21 (1.06–1.39)	
			Combined validation[b]	4,331	0.739	8,202	0.708	1.34×10^{-6}	1.18 (1.10–1.26)	0.65
			Combined all[b]	6,026	0.741	13,535	0.707	7.40×10^{-11}	1.20 (1.13–1.26)	0.76
rs3817963	*BTNL2*	A/G	GWAS	1,695	0.363	5,331	0.327	5.54×10^{-5}	1.18 (1.09–1.28)	
6p21.3	intron 4	[G]	First validation	2,951	0.347	7,028	0.310	1.59×10^{-5}	1.18 (1.09–1.27)	
			Second validation	1,376	0.358	1,166	0.329	2.41×10^{-2}	1.16 (1.02–1.32)	
			Combined validation[b]	4,327	0.350	8,194	0.313	1.14×10^{-6}	1.17 (1.10–1.25)	0.86
			Combined all[b]	6,022	0.354	13,525	0.318	2.69×10^{-10}	1.18 (1.12–1.24)	0.97

RAF, risk allele frequency; P_{het}, P value for heterogeneity.
[a]Adjusted for age and gender. [b]The combined meta-analysis was performed using a fixed effect model.

Supplementary Fig. 1). We generated a quantile-quantile plot using the results of a logistic regression trend test (**Supplementary Fig. 1d**). The genomic inflation factor ($\lambda_{1,000}$)[13] was 1.021, indicating a low possibility of false-positive associations resulting from population stratification or genotype misclassification (**Supplementary Fig. 2**).

In the GWAS, two loci reached genome-wide significance for association ($P < 5 \times 10^{-8}$; **Supplementary Fig. 1e**); these two loci have been reported in previous GWAS (rs2736100 at 5p13.33 and rs10937405 at 3q28)[9,10]. We also identified a significant association for a SNP (rs2853677 at 5p13.33) that was not examined in our previous GWAS (**Table 1**). In addition, we examined associations of other previously reported loci with lung cancer risk (**Supplementary Table 2**). We found one locus (rs2131877 at 3q29)[14] to be associated with lung adenocarcinoma risk, but we could not confirm the associations between lung adenocarcinoma risk and the other loci identified in a recent GWAS of the European and Han Chinese populations[12]. These results are probably the result of the lower statistical power in our GWAS than in the previous GWAS (**Supplementary Table 2**). In addition, most of the earlier GWAS were performed in lung cancer representing a full range of histological types and in subjects of European descent. Therefore, differences in genetic modifiers and/or environmental factors in different histological types and populations might have contributed to the differing results.

To investigate additional susceptibility loci, we conducted a validation study using two independent sample sets consisting of 2,955 cases and 7,036 controls (first validation cohort) and 1,379 cases and 1,166 controls (second validation cohort) (**Supplementary Table 1**). Among 125 SNPs with a logistic regression trend of $P < 1 \times 10^{-4}$ in our GWAS, we selected 78 SNPs, excluding 38 SNPs within the same locus ($r^2 > 0.8$) and nine SNPs located at the previously reported loci, 5p13.33 and 3q28. We successfully genotyped all 78 SNPs in the first validation set using the multiplex PCR-based Invader assay, and 8 SNPs had ORs with a significance of $P < 0.05$ in the same direction of association (**Supplementary Table 3**). We then subjected these eight SNPs to the second validation set analysis. When we combined the results of both validation sets using a fixed effects model, two SNPs, rs7216064 at 17q24.3 and rs3817963 at 6p21.3, showed significant associations after Bonferroni correction ($P < 6.4 \times 10^{-4}$, calculated as 0.05/78) in addition to three SNPs at the two known loci described above (**Table 1**). When we combined the results of the GWAS and the validation study, both of the newly discovered loci reached genome-wide significance (rs7216064, $P = 7.4 \times 10^{-11}$, OR = 1.20; rs3817963, $P = 2.7 \times 10^{-10}$, OR = 1.18) (**Table 1**). The ORs were similar between the GWAS and the validation study, with no heterogeneity (**Table 1**). The strengths of the associations remained similar after adjustment for smoking (**Supplementary Table 4**). In a subgroup analysis (**Supplementary Table 5**), there was no clear association between the two newly discovered loci and gender or smoking behavior, and there was also no such association for the two known loci[10].

We next performed imputation analyses using the Japanese in Tokyo (JPT) and Han Chinese in Beijing (CHB) reference sets from the 1000 Genomes Project database (June 2010 release) (Online Methods), and we examined the associations between 1,665 putative SNPs and lung adenocarcinoma risk. We found a series of signals in high linkage disequilibrium (LD) with a marker SNP at 17q24.3 (rs7216064), and we observed significant associations with lung adenocarcinoma risk for 33 of the imputed SNPs (**Fig. 1a** and **Supplementary Table 6**). However, none of the SNPs in LD at 6p21.3 reached the P value of our marker SNP (**Fig. 1b**).

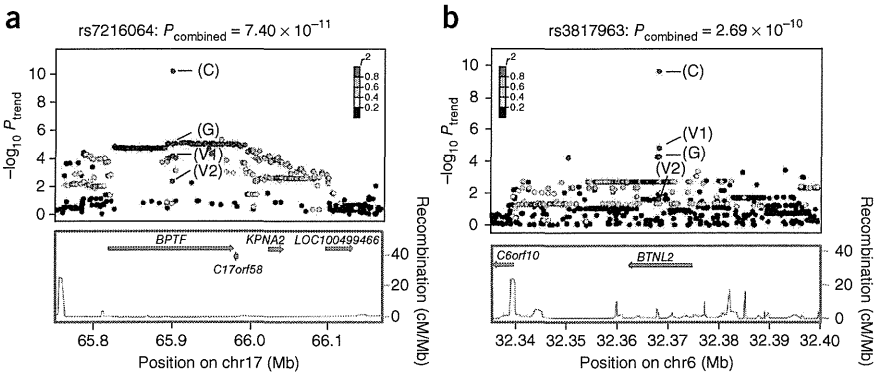

Figure 1 Regional plots of the identified marker SNPs. (**a**) rs7216064 at 17q24.3. (**b**) rs3817963 at 6p21.3. The marker SNP is shown in purple, and the r^2 values for the other SNPs are indicated by different colors. The correlations were estimated using data from the 1000 Genomes Project. The genes within the region of interest are annotated and are indicated by arrows. The blue lines indicate the recombination rates in cM per Mb. The $-\log_{10} P_{trend}$ values of the marker SNPs are shown for the GWAS (G), the first validation study (V1), the second validation study (V2) and the combined study (C).

SNP rs7216064 resides within intron 9 of *BPTF* (encoding bromodomain PHD finger transcription factor) at 17q24.3. Other imputed SNPs in this locus showing similarly significant associations were also synonymous (not resulting in amino acid changes in translated proteins). Based on the regional plot and recombination rates, we found that rs7216064 represented an LD region that includes three genes: *BPTF*, *C17orf58* (encoding a protein without known domains) and *KPNA2* (encoding karyopherin α 2) (**Fig. 1a**). Thus, to address the biological importance of 17q24.3 variants, we examined the mRNA expression levels of these three genes in 314 noncancerous lung tissues by real-time quantitative PCR (**Supplementary Note**). We detected expression of *BPTF*, but not of *C17orf58* or *KPNA2*, in these lung tissues. The expression of *BPTF* was marginally different depending on the genotype of the rs7216064 SNP ($P = 0.02$), implying low expression from the risk (G) allele (**Supplementary Table 7**). *BPTF* encodes a chromatin remodeling factor that regulates transcription through the specific recognition of methylated histone proteins[15]. Recently, chromatin remodeling genes have been implicated as tumor suppressors in lung[16] and other cancers[17]. Therefore, a low level of *BPTF* mRNA being associated with the risk allele might lead to an elevated risk for lung adenocarcinoma through decreased transcriptional regulation. However, further studies are needed to conclude whether *BPTF* is responsible for lung adenocarcinoma susceptibility.

SNP rs3817963 is located in intron 4 of *BTNL2* (encoding butyrophilin-like 2) at 6p21.3 (**Fig. 1b**). Based on the regional plot and recombination rates, rs3817963 represents an LD region that includes only a single gene, *BTNL2*. The top ten SNPs (genotyped or imputed), including rs3817963, were synonymous. The effects of the SNPs on the expression of *BTNL2* could not be assessed because of the low or absent expression of this gene in noncancerous lung tissues. *BTNL2* encodes a T cell co-stimulatory molecule, and associations between *BTNL2* SNPs and risk have been reported in several immune-related diseases, including asthma[18], vitiligo[19] and ulcerative colitis[20,21]. Therefore, *BTNL2* might affect lung adenocarcinoma risk by affecting immune responses against tumor cells. However, 6p21.3 is a part of the extended major histocompatibility complex (MHC) region, whose association with lung cancer risk has previously been reported[5]. The previously identified marker SNPs, rs3117582 and rs3131379, located 700 kb from the *BTNL2* locus, were monomorphic in our study populations. Therefore, it is possible that the association at 6p21.3 identified in the present study is not new, and further studies are warranted.

We here provide further evidence for the existence of genetic susceptibility in the development of lung adenocarcinoma through the identification of two candidate susceptibility loci, 17q24.3 and 6p21.3, at genome-wide significance. rs7216064 at 17q24.3 showed a tendency of association in the same direction as lung cancer risk in Europeans, although this association did not reach statistical significance, whereas rs3135353 at 6p21.3, which is in LD with rs3817963, showed a statistically significant association with lung cancer risk in European and American populations (**Supplementary Table 8**)[7,9]. Therefore, these loci might be involved in lung cancer risk in individuals of European descent. Further studies of these loci in multiple populations, including those with other histological types of lung cancers, will help to elucidate the etiology of lung adenocarcinoma.

URLs. The BioBank Japan project, http://biobankjp.org/; R, http://cran.r-project.org/; PLINK statistical software v1.06, http://pngu.mgh.harvard.edu/~purcell/plink/; Primer3 v0.3.0, http://frodo.wi.mit.edu/primer3/; UCSC Genome Browser, http://genome.ucsc.edu/; LocusZoom, http://csg.sph.umich.edu/locuszoom/; a catalog of genome-wide association studies, http://www.genome.gov/gwastudies/; SNPinfo Web Server, http://manticore.niehs.nih.gov/index.html; Illumina's IconDB resource, http://www.illumina.com/science/icontroldb.ilmn.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

K.S., J.Y., M.K. and T.K. designed the study. A.T., K.A., S.O., N.K. and A.S. analyzed the GWAS and replication study. H.S., Y.S., T.Y. and K.S. performed the genotyping for the GWAS and the replication study. H.K., K.G., S.W. and K.T. recruited

subjects and participated in diagnostic evaluations. K.S. and T.K. wrote the manuscript. M.K., Y.D., T.Y. and Y.N. contributed to the overall GWAS design.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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ONLINE METHODS

Study design and subjects. We performed a three-stage GWAS of lung adenocarcinoma in the Japanese population using independent samples. The characteristics of each case-control group are shown in **Supplementary Table 1**. The discovery GWAS samples consisted of 1,722 cases from the National Cancer Center Hospital (NCCH) and 5,846 controls from the BioBank Japan project[22], Osaka-Midosuji Rotary Club (MRC) and the Pharma SNP consortium (PSC). The BioBank Japan project (see URLs) was begun in 2003 for the collection of genomic DNA, serum and clinical information from 300,000 individuals diagnosed with any of 47 diseases by a collaboration network of 66 hospitals in all areas of Japan[22]. The subjects from MRC were 1,018 healthy volunteers, and the subjects from PSC were 906 Japanese healthy volunteers from whom immortalized B lymphoblast cell lines were established by the PSC. The cell lines were obtained from the Japan Health Sciences Foundation (JHSF)/Health Science Research Resources Bank (HSRRB). Individuals with any cancer were excluded from the control group.

The validation study consisted of two independent cohorts. The first validation cohort included 2,955 cases with lung adenocarcinoma and 7,036 controls. The cases included 1,747 subjects from the BioBank Japan project and 1,208 subjects from NCCH. All control subjects were from the BioBank Japan project. Individuals with any cancer were excluded from the control group. The second validation cohort included 1,379 cases with lung adenocarcinoma from the NCCH and 1,166 controls (cancer-free volunteers) from the NCCH and the Keio University in Tokyo[23]. Individuals with any cancer were excluded from the control group.

All of the cases with lung adenocarcinoma were diagnosed by cytological and/or histological examination according to the World Health Organization classification[24]. The cases from NCCH in the GWAS and first validation sets consisted of cases for which enough DNA for large-scale SNP analyses were available, and the cases in thee second validation set consisted of cases for which less DNA were available, for example, because only small noncancerous tissues were available for DNA extraction. Controls from the BioBank Japan project were genotyped previously. Genome-wide genotyping data obtained before and after 2010 were used as the controls for the GWAS and the first validation study described below, respectively, and thus there was no specified rationale for the selection of control subjects other than time of genotyping. Eight controls and 1,529 cases with lung adenocarcinoma from BioBank Japan and 906 control subjects from the Osaka-MRC were used in the previous GWAS[10]. All the participants provided written informed consent. This project was approved by the ethical committees of each participating institution.

Sample preparation and genotyping. Genomic DNA was extracted from peripheral blood leukocytes or noncancerous lung tissues using standard methods.

In the GWAS, we genotyped 1,722 cases with lung adenocarcinoma from the NCCH using the Illumina HumanOmni1-Quad Chip. For the controls in the GWAS, we used genome-wide data from 5,846 individuals with cerebral aneurysms, chronic obstructive pulmonary disease or glaucoma, which were genotyped using the Illumina Human OmniExpress Genotyping BeadChip.

In the study of the first validation cohort, we genotyped 2,955 cases with lung adenocarcinoma using the multiplex PCR-based Invader assay (Third Wave Technologies), as previously described[10]. The control group consisted of genome-wide data from 7,036 individuals with epilepsy, nephrosis syndrome, atopic dermatitis, urinary tract stone disease or Basedow's (Graves') disease, which were genotyped using the Illumina Human OmniExpress Genotyping BeadChip. The same quality control criteria were applied as in the GWAS (see below) to confirm that no unexpected duplicates or probable relatives were present between the control subjects of the GWAS and those of the first validation study.

In the study of the second validation cohort, we genotyped 1,379 cases with lung adenocarcinoma and 1,166 controls using the TaqMan method, according to the protocol for the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems).

Quality control. Systemic quality control was performed on the raw genotyping data from 709,857 SNPs in DNA samples obtained from 7,568 subjects, consisting of 1,722 cases and 5,846 controls, using PLINK (v 1.06)[25]. Forty-four subjects were excluded because they showed gender discrepancies based on their X chromosome genotypes (7,524 subjects remained). Next, SNPs were excluded according to the following criteria: (i) 19,993 SNPs were not mapped on autosomal chromosomes; (ii) 102,929 SNPs had a minor allele frequency <0.01; and (iii) a total of 48,769 SNPs had a call rate <0.99 and genotype distributions that clearly deviated from those expected by Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-6}$). Together, 538,166 SNPs in autosomal chromosomes passed the quality-control filters and were used for the GWAS.

Next, an additional 75 unexpected duplicates or probable relatives in the GWAS were excluded based on pairwise identity by state according to their PI_HAT values in PLINK (all PI_HAT > 0.25) (7,449 subjects remained). Heterozygosity rates were calculated using PLINK, and more than 6 s.d. from the mean was used as the exclusion criterion. A principal components analysis (PCA) was performed on the genotype data from the samples, along with European (CEU), African (YRI) and east Asian (Japanese (JPT)) and Han Chinese (CHB)) individuals obtained from the phase II HapMap database using smartpca[26]. The PCA revealed no evident population substructure (**Supplementary Fig. 1a**) and identified seven outliers for exclusion (7,442 subjects remained). Most subjects fell into a known main cluster (Hondo) of the Japanese population (**Supplementary Fig. 1b**), and 414 subjects that fell far from the Hondo cluster[27] were excluded. The remaining 7,028 subjects, consisting of 1,695 cases and 5,333 control subjects, were used for the GWAS. The lack of population substructure between the cases and controls was validated by PCA of the subjects in the Hondo cluster (**Supplementary Fig. 1c**).

Statistical analyses. In the GWAS and the validation study, the statistical significance of the association with each SNP was assessed using a logistic regression trend test in the R program. Age and gender were included as covariates. Heterogeneity across the two stages was examined using the Breslow-Day test[28].

Imputation. We performed SNP imputation for each individual in the GWAS using the IMPUTE7 program[29]. The 1000 Genomes Project database (June 2010 release) was used as a reference panel. After excluding imputed SNPs with a low genotype information content (<0.5), posterior probability score (<0.90), call rate (<0.90), minor allele frequency (<0.01) or Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-7}$), imputed SNPs with $r^2 > 0.3$, residing 200–500 kb upstream or downstream of the two newly identified marker SNPs, were subjected to association analyses.

Software. For general statistical analyses, we used the R statistical environment version 2.6.1 or PLINK1.06 (ref. 25). We used LocusZoom to plot regional association plots[30].

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Research article

Different circulating brain-derived neurotrophic factor responses to acute exercise between physically active and sedentary subjects

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Running Head: Circulating BDNF and acute exercise

Abstract

Although circulating brain-derived neurotrophic factor (BDNF) level is affected by both acute and chronic physical activity, the interaction of acute and chronic physical activity was still unclear. In this study, we compared the serum and plasma BDNF responses to maximal and submaximal acute exercises between physically active and sedentary subjects. Eight active and 8 sedentary female subjects participated in the present study. Both groups performed 3 exercise tests with different intensities, i.e. 100% (maximal), 60% (moderate) and 40% (low) of their peak oxygen uptake. In each exercise test, blood samples were taken at the baseline and immediately, 30 and 60 min after the test. The serum BDNF concentration was found to significantly increase immediately after maximal and moderate exercise tests in both groups. In maximal exercise test, the pattern of change in the serum BDNF concentration was different between the groups. While the serum BDNF level for the sedentary group returned to the baseline level during the recovery phase, the BDNF levels for the active group decreased below the baseline level after the maximal exercise test. No group differences were observed in the pattern of plasma BDNF change for all exercise tests. These findings suggest that regular exercise facilitates the utilization of circulating BDNF during and/or after acute exercise with maximal intensity.

Key words: Serum BDNF, plasma BDNF, acute exercise.

Introduction

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors. In addition to its neurotrophic and synaptotrophic actions, such as the promotion of growth and survival of neurons (Aloe and Calza, 2004; Thoenen, 1995) and learning and memory (Ma et al., 1998), BDNF may play important metabotrophic roles such as the regulation of food intake (Xu et al., 2003), glucose and lipid metabolism, and energy homeostasis (Chaldakov, 2011; Nakagawa et al., 2000; Noble et al., 2011; Tsuchida et al., 2002). BDNF is present in the nervous system and peripheral tissues, and is also found in blood (Fujimura et al., 2002; Radka et al., 1996; Rosenfeld et al., 1995). Chronic treatment with subcutaneous BDNF administration significantly decreased food intake and improved the glucose uptake in skeletal muscle (Yamanaka et al., 2007) in diabetic mice, and increased glucose transporter 4 expression in normal mice (Suwa et al., 2010). In humans, the level of circulating BDNF is associated with depression (Duman, 2004), Alzheimer's disease (Tapia-Arancibia et al., 2008), obesity (Suwa et al., 2006), glucose and lipid metabolism (Levinger et al., 2008; Suwa et al., 2006), type 2 diabetes mellitus (Suwa et al., 2006) and metabolic syndrome (Chaldakov et al., 2004). Although it has been generally accepted that the neurotrophins act by paracrine or autocrine mechanisms (Davies, 1996), evidence also indicates that circulating BDNF may exert endocrine action to reveal or execute physiologic functioning.

BDNF is present in human serum and plasma, and is much more concentrated in the serum (Radka et al., 1996). Because more than 90% of blood BDNF is stored in the platelets and is released during the clotting process (Fujimura et al., 2002), serum BDNF seems to reflect both the platelet-stored BDNF and the freely-circulating BDNF in the blood, while plasma BDNF seems to reflect only the freely-circulating *BDNF* (Lommatzsch et al., 2005).

Regular exercise is well known to have many health benefits, including the prevention and improvement of obesity (Wing and Hill, 2001), type 2 diabetes mellitus (Orozco et al., 2008) and Alzheimer's disease (Heyn et al., 2004). Several animal studies have shown that $mRNA^{BDNF}$ and BDNF protein levels increase with acute and chronic voluntary wheel running in the hippocampus (Neeper et al., 1996; Gomez-Pinilla et al., 2011), and improved learning and memory (Vaynman et al., 2004). In addition, the $mRNA^{BDNF}$ and BDNF protein expression levels in skeletal muscle have been shown to be enhanced in response to muscle contraction, which is associated with enhanced lipid oxidation (Matthews et al., 2009). Collectively, these results raise the possibility that BDNF mediates, at least in part, the adaptation to exercise.

There have been several studies examining circulating BDNF responses to acute endurance exercise (Ferris et al., 2007; Gold et al., 2003; Gustafsson et al., 2009; Matthews et al., 2009; Rasmussen et al., 2009; Rojas Vega et al., 2006; Zoladz et al., 2008). In the majority of these studies, serum (Ferris et al., 2007; Gold et al., 2003; Matthews et al., 2009; Rojas Vega et al., 2006) and plasma (Gustafsson et al., 2009; Rasmussen et al., 2009) BDNF levels increased following acute exercise. On the other hand, we (Nofuji et al., 2008) and Chan et al. (2008) showed that regular physical activity affected the resting serum BDNF level. Therefore, it appears that the circulating BDNF level is affected by both acute and chronic

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physical activity. However, the interaction of acute and chronic physical activity was still unclear.

Therefore, the aim of this study was to clarify the effect of chronic physical activity on the circulating BDNF responses to acute exercise. In the present study, we simultaneously measured the serum and plasma BDNF concentrations before and after three exercise tests with different intensities for the physically active and sedentary subjects.

Methods

Subjects

Eight physically active and 8 sedentary female Japanese subjects participated in this study. "Active" was defined as performing regular sports activities more than 3 times per week for more than 3 years. The active group included distance runners (n = 3), basketball players (n = 3), and badminton players (n = 2). The sedentary subjects had not performed any regular exercise for at least 1 year. All participants were non-smokers, free from any diseases, and not taking any medications. This study was conducted in accordance with the Declaration of Helsinki, and approved by the ethics committee of the Institute of Health Science, Kyushu University, Fukuoka, Japan. Written informed consent was obtained from all participants prior to their participation.

Exercise tests

All subjects performed 3 different exercise tests in 3 separate days. At Day1, they performed the graded exercise test (maximal) to determine their volume of peak oxygen uptake (VO_2peak). After 15 min of seated rest, the subjects started pedaling at 0 W (for the sedentary group) or 30 W (for the active group). The workload was increased by 30 W in every 4 min until a 4.0 mmol·L^{-1} of blood lactate level was obtained. After that, the workload was increased by 15 W in every 1 min until exhaustion. "The blood for measuring the lactate concentration was obtained from an ear lobe and blood lactate level was measured using the Lactate Pro instrument (Lactate Pro LT-1710, ARKRAY, Kyoto, Japan) in every 3 min and immediately after exercise test." The heart rate (HR) was monitored using an electrocardiogram telemetry system (DS-3140, Fukuda Denshi, Tokyo, Japan). The VO_2 peak was defined as the highest VO_2 obtained during a maximal exercise test.

Two submaximal exercise tests were conducted at Day2 or 3 in random order. Trials consisted of a 30-min cycle ergometry (Monark 828E) at a constant load of 60% (moderate) or 40% (low) of the subject's VO_2peak, preceded by a 15 min of seated rest. The HR and VO_2 were recorded during each exercise test.

The subjects were instructed to refrain from heavy exercise the day before each exercise test. All exercise tests were conducted at 9:00-10:30 to diminish the effect of circadian changes in circulating BDNF levels (Piccinni et al., 2008).

Physical activity level

The daily physical activity level was evaluated with an accelerometer (Lifecorder, Suzuken Co., Nagoya, Japan). This device comprises an acceleration sensor, an amplifier, a microprocessor and memory, and was employed to ensure different physical activity levels between the two groups. All participants attached the accelerometer for 1 week just before the Day1.

Anthropometric measurements

Anthropometric measurements were conducted at Day1. The percentage of body fat was measured by bioelectrical impedance analysis device (Tanita, Tokyo, Japan).

Blood collection and biochemical analysis

In each exercise test, blood samples were taken from an antecubital vein in a sitting position at the baseline time, and immediately, 30 min and 60 min after the exercise. The blood samples were drawn into additive-free containers (serum) or heparinized containers (plasma). After kept at room temperature for 1 hour, the serum samples were centrifuged at 2000 × g for 10 min at 4°C. Plasma samples were immediately centrifuged. Supernatants were stored at -80°C until the analyses were performed. The serum and plasma BDNF concentrations were measured using an enzyme-linked immunoassay (ELISA) kit (Promega, Madison, WI).

Statistical analysis

The anthropometric measurements and physiological responses to maximal exercise tests between the active and sedentary groups were compared using Student's unpaired t-test. The comparisons of physical responses during moderate and low exercise tests in each group and serum BDNF level at rest between the groups were performed using the paired t-test. The changes in BDNF responses were assessed by two-way (4 time point × 2 groups) repeated measures analysis of variance (ANOVA). If an interaction was significant, one-way ANOVA was performed. A Dunnett's test was employed for all post-hoc tests. The alpha-level was set at 0.05.

Results

Characteristics of the subjects

The subject characteristics are summarized in Table 1. There were no significant differences in any anthropometric variables between the two groups. The daily physical activity level was significantly higher in the active group compared to the sedentary group ($p < 0.05$).

Table 1. Characteristics of the subjects. The data are expressed as the means (± SD).

	Sedentary	Active
Age (years)	22.8 (1.9)	21.6 (3.0)
Height (m)	1.59 (.06)	162.9 (6.8)
Weight (kg)	50.8 (6.7)	54.5 (7.5)
Body mass index (kg·m^{-2})	20.0 (2.0)	20.5 (1.9)
Body fat (%)	23.6 (5.9)	21.8 (2.0)
Total energy expenditure (kJ·day^{-1})	7451 (793)	8749 (842)**
Moving-related energy expenditure (kJ·day^{-1})	1115 (379)	1970 (640)**
Step count (steps·day^{-1})	10890 (2950)	14961 (4188)*

* $p < 0.05$, ** $p < 0.01$

Table 2. Physical parameters at the end of the low and moderate exercise tests. The data are expressed as the means (± SD).

	Low exercise		Moderate exercise	
	Sedentary	Active	Sedentary	Active
VO_2 (ml·kg^{-1}·min^{-1})	10.8 (2.6)*	14.7 (2.4)*	20.0 (4.4)	22.9 (2.7)
%VO_2 (%)	30.8 (5.6)*	35.2 (8.0)*	58.4 (8.9)	54.8 (5.1)
Heart rate (bpm)	99 (12)*	100 (13)*	141 (16)	130 (14)
Workload (W)	39 (11)*	66 (8)*	77 (16)	107 (12)

* Significantly different from the moderate exercise ($p < 0.05$)

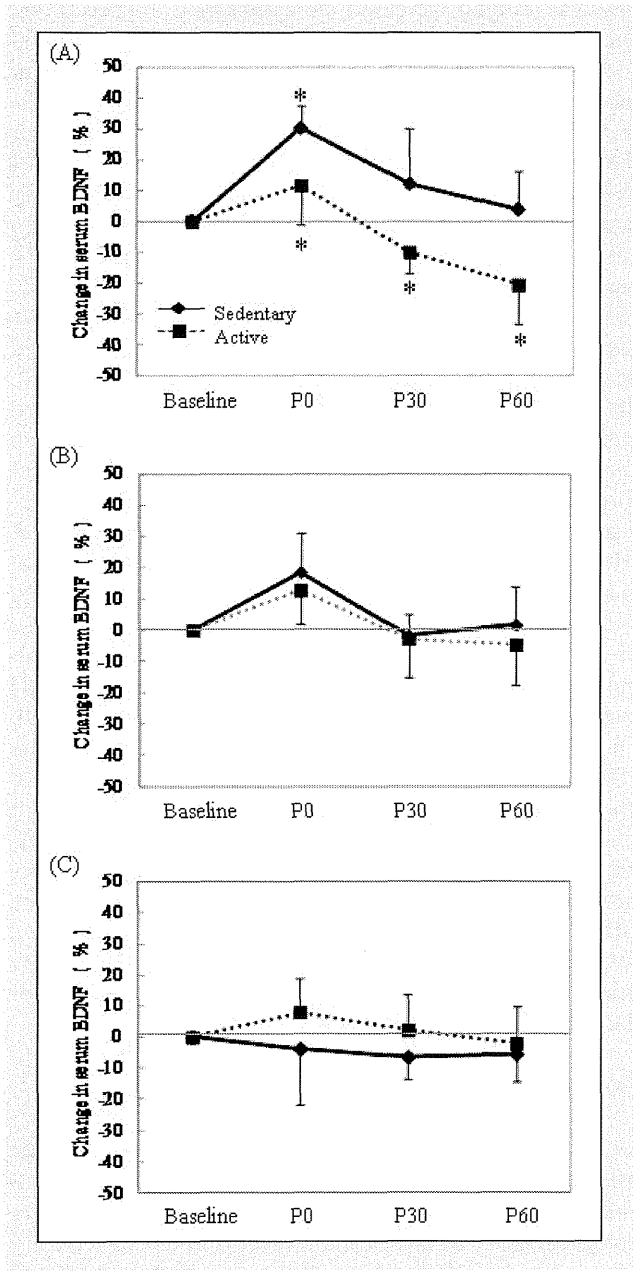

Figure1. Level of serum BDNF concentrations before maximal (A), moderate (B), or low (C) exercise tests (baseline), immediately after (P0), 30 min after (P30), and 60 min after (P60) the exercise session. The data are expressed as the means ± SD. * $p < 0.05$ vs. baseline. The changes in BDNF responses for the groups were assessed by two-way repeated ANOVA. As an interaction and main effect of time were significant, one-way ANOVA followed by a Dunnett's post-hoc test was performed.

Physical parameters in the exercise test

The VO_2peak and workload at the end of maximal exercise in the active group (42.3 ± 4.5 ml·kg^{-1}·min^{-1}, 199 ± 16 W, respectively) was significantly higher than that in the sedentary group (34.7 ± 4.0 ml·kg^{-1}·min^{-1}, 147 ± 16 W, respectively, < 0.01). There were no significant differences in the HR (Sedentary 183 ± 5 bpm, Active 179 ± 12 bpm, $p = 0.45$) and blood lactate level (Sedentary 9.6 ± 0.8 mmol·L^{-1}, Active 8.6 ± 1.4 mmol·L^{-1}, $p = 0.14$) between the groups at the end of the maximal exercise test. Table 2 shows the physical parameters for two submaximal exercise tests. All parameters were significantly higher at the moderate exercise test than at the low exercise test. There was no group difference in the average BDNF level at rest (Sedentary; 11.9 ng·ml^{-1}, Active; 12.5 ng·ml^{-1}, $p = 0.49$).

Change in the serum BDNF concentration

For the maximal exercise test, a two-way ANOVA for repeated measures on serum BDNF levels revealed significant interactions of the factors ($F(3, 42) = 7.01$, $p < 0.01$). A subsequent one-way ANOVA for repeated measures revealed a significant effect of time ($F(3, 45) = 24.8$, $p < 0.01$). The serum BDNF concentrations significantly increased immediately after the maximal exercise test in both groups (Sedentary; +30% $p < 0.01$, Active; +11% $p < 0.01$ vs. baseline, Figure 1A). While BDNF levels in the sedentary group returned to the baseline level during the recovery phase (30 min; +12% $p = 0.06$, 60 min; +4% $p = 0.80$, Figure 1A), the BDNF levels in the active group decreased below the baseline level (30 min; -15% $p < 0.01$, 60 min; -25% $p < 0.01$ vs. baseline, Figure 1A).

For the moderate exercise, neither interactions ($F(3, 42) = 0.68$, $p = 0.57$) nor the effect of groups ($F(1, 14) = 0.86$, $p = 0.37$) on the BDNF response was observed, although the effect of time was significant ($F(3, 42)=18.7$, $p < 0.01$). The serum BDNF concentrations in both groups increased immediately after the exercise tests (+16%, $p < 0.01$ vs. baseline, Figure 1B) and returned to the baseline level during the recovery phase (30 min; -2% $p = 0.84$, 60 min; -2% $p = 0.94$ vs. baseline, Figure 1B).

The low exercise did not affect the BDNF concentration in either group (time × group; $F(3, 42) = 1.19$, $p = 0.33$, time; $F(3, 42) = 1.17$ $p = 0.33$, group; $F(1, 14) = 4.06$ $p = 0.06$, Figure 1C).

Change in the plasma BDNF concentration

For the maximal and moderate exercise test, interactions ($F(3, 42) = 1.85$, $p = 0.15$, $F(3, 42) = 1.19$, $p = 0.33$, respectively) nor the effect of groups ($F(1, 14) = 1.40$, $p = 0.26$, $F(1, 14) = 0.67$, $p = 0.43$, respectively) on the plasma BDNF response were detected. Although the effect of time were significant ($F(3, 42) = 4.24$, $p = 0.01$, $F(3, 42) = 5.40$, $p < 0.01$, respectively), a subsequent Dunnett's post-hoc test showed no significant difference in plasma BDNF between baseline and each time point

(maximal; 0min +33% p = 0.10, 30min +10% p = 0.87, 60min -9% p = 0.89 vs. baseline, Figure 2A, moderate; 0min +11% p = 0.58, 30min -11% p = 0.59, 60min -19% p = 0.17 vs. baseline, Figure 2B).

No interaction (F(3, 42) = 0.65, p = 0.59) or main effects (time; F(3, 42) = 0.77, p = 0.52, group; F(1, 14) = 0.03, p = 0.86, Figure 2C) were found in the low exercise.

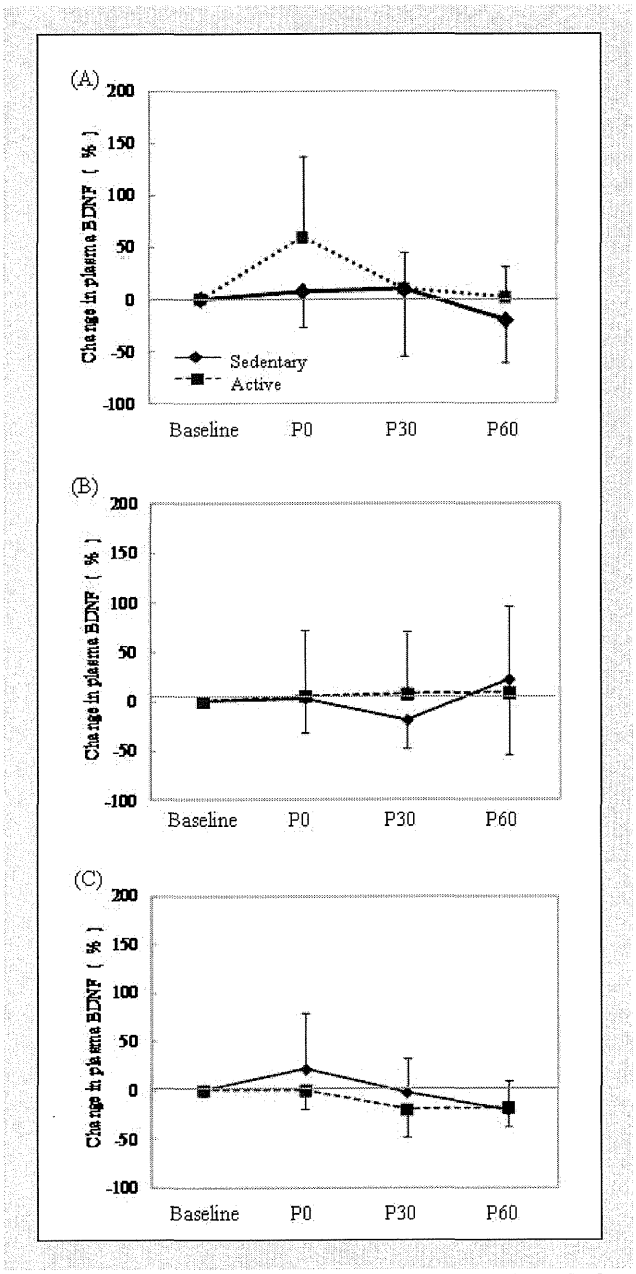

Figure 2. Level of plasma BDNF concentrations before maximal (A), moderate (B), or low (C) exercise tests (baseline), immediately after (P0), 30 min after (P30), and 60 min after (P60) the exercise session. The data are expressed as the means ± SD.

Discussion

We investigated the differences in the serum and plasma BDNF responses to acute maximal and submaximal exercises between the active and sedentary subjects. One of the novel findings of the present study was that the serum BDNF responses to maximal exercise were different between active and sedentary subjects. Especially, serum BDNF levels in the active group decreased below the baseline level during the recovery phase, while it was not the case in the sedentary group. A possible mechanism for this excessive reduction of serum BDNF in the active group is an enhanced utilization mediated by the upregulation of BDNF TrkB (tyrosine protein kinase) receptor in the peripheral tissues. Previous studies demonstrated that physical training increased the expression of TrkB in the spinal cord (Skup et al., 2000), brain (Widenfalk et al., 1999) and soleus muscle (Gómez-Pinilla et al., 2002) in rats. Although the physiological significance of the decreases in BDNF after exercise remains unknown, one of the possible roles of BDNF utilization is the repair of exercise-induced muscle damage. Ninety percent of circulating BDNF is stored in the platelets, where are also epidermal growth factor (EGF) (Oka and Orth, 1983), vascular endothelial growth factor (VEGF) (Tischer et al., 1989), and platelet-derived growth factor (PDGF) (Antoniades et al. 1979), all of which play a role in wound healing. In the current and previous studies (Ferris et al., 2007; Rojas Vega et al., 2006), serum BDNF increased with moderate- to high-intensity exercise, which has been shown to induce muscle damage (Kuipers, 1994). Therefore, it is possible that the increased BDNF during exercise contributes to the repair of skeletal muscle damaged. Although there are no direct reports demonstrating that circulating BDNF acts in the repair of exercise-induced muscle damage, BDNF treatment suppressed the release of creatine kinase and prostaglandin E2, which are common indicators of muscle cell damage in the rat muscle exposed to oxidative stress *in vivo* (Lian et al., 1998). Furthermore, the delayed regeneration of muscle fibers after injury was observed in muscle-specific BDNF knockout mice, suggesting that BDNF plays an important role in the regeneration of muscle fibers (Clow and Jasmin, 2010). Based on the potential wound-healing functions of BDNF, it is proposed that the utilization of serum BDNF during exercise may help muscle regeneration following exercise-induced damage and that the active group may have adapted to utilize circulating BDNF for the promotion of muscle repair.

Conclusion

In conclusion, the circulating BDNF responses to acute maximal exercise were different between active and sedentary groups. While serum BDNF levels in the sedentary group returned to the baseline level during the recovery phase, the BDNF levels in the active group decreased below the baseline level after high-intensity exercise. These results raise the possibility that regular exercise facilitates the utilization of circulating BDNF after acute exercise with maximal intensity. Limitations of this study were the small sample size. Additional studies with large sample size are called for. Likewise, further studies should clarify the mechanisms and physiological significance of the exercise-induced responses to circulating BDNF.

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Key points

- In maximal exercise test, the pattern of change in the serum BDNF concentration was different between the groups.
- While the serum BDNF level for the sedentary group returned to the baseline level during the recovery phase, the BDNF levels for the active group decreased below the baseline level after the maximal exercise test.
- No group differences were observed in the pattern of serum BDNF change for moderate or low exercise tests.
- No group differences were observed in the pattern of plasma BDNF change for all exercise tests.

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The Role of the Supraspinal Center during Soleus Stretching Reflexes with Simultaneous Vibration

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Abstract. [Purpose] We examined whether monoaminergic brain stem centers contribute to reflexive soleus (Sol) activity when vibration is applied to ankle joints on a moving platform. [Methods] Ten male subjects (23–35 years) stood with their eyes closed on a movable platform. Vibrators (92 Hz) were applied to the malleolus and Achilles' tendon. Sol electromyographic (EMG) responses of short- (SLR) and medium-latency reflexes (MLR) during platform movement were collected under the control, Sol vibration (SV), and malleolus vibration (MV) conditions. The SLR, MLR areas and their latencies were measured. [Results] The Sol SLR and MLR onsets were significantly delayed under the SV and MV conditions compared to the control condition. The intercept of the regression line under the MV conditions was significantly greater than under the SV condition. [Conclusion] Delays of SLR and MLR onset under the SV and MV conditions might correspond to the length of time required for temporal summation of α-motoneurons due to inhibition of afferent fibers. A rise in the intercept of the regression line under the MV condition means an increase of MLR area. That is, the monoaminergic brain stem centers compensated for stimulation of the group II interneuron via ankle joint afferents acting against the inhibition of the stimulation of Sol α-motoneurons.
Key words: Ankle joint, Group II fibers, Medium latency reflex

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INTRODUCTION

Signals mediated by Ia and group II afferent fibers from peripheral leg muscles contribute to two component reflexive homonymous muscle responses during static and dynamic standing. It has been shown that a sudden toe-up rotation on a platform being used for standing produces two peak electromyogram (EMG) responses mediated by Ia and group II afferents during stretching of the soleus (Sol) and flexor digitorum brevis (FDB) muscle [1–3]. The first component is a short-latency reflex (SLR) that takes place at about the latency of the monosynaptic reflex arc. It originates in the spindle primaries and is mediated by group Ia large afferent fibers. The second component is a medium-latency reflex (MLR) that is transmitted to the spinal cord from spindle secondary terminations by group II afferent fibers[3]. Several studies have reported abnormal SLR and MLR activities of Sol and FDB in the legs of subjects who have peripheral neuropathy cause standing postural instability [4–6]. These studies concluded that delayed and decreased SLR and MLR activities are the result of dysfunction of Ia and group II afferent fibers in mediating signals from muscle spindles and cause large postural sway. It is known that afferent signals from sole cutaneous receptors, other than Ia and group II afferent signals from muscle spindles in peripheral leg muscles, contribute to peripheral muscle activity in upright stance [7, 8]. According to the major results of recent studies of reflexive Sol and FDB activities during static standing, Sol and FDB activities are affected by not only afferent signals from homonymous muscle spindles, but also by sole cutaneous afferent signals. However, little is known about the influence of afferent signals from ankle joints on reflexive peripheral muscle activity in the upright posture. One study examined whether afferent signals decreased by local anesthesia of the ankle joint affected the stability of upright posture. Errors of passive position sense were significantly increased in comparison to active position sense errors, and the difference in stability of one-leg standing between the pre- and post-anaesthetic block was not significant[9]. Thus, the afferent signals from ankle joint may thus play a dominant role on passive position sense, to which afferent signals from the muscle spindle do not contribute, even though muscle spindle afferents are predominantly involved in the stability of static standing. No study has yet examined the changes in SLR and MLR activities of peripheral muscles in the leg while standing on one-leg with the ankle joint under