

MPT, a DPS-20 digital force gauge (Imada, Northbrook, IL, USA) was used to measure the level of force when the subjects felt pain, with a wooden sphere attached to the tip of the instrument so that the subjects could feel pain on a single point of the finger (Supplementary Fig. 1: available in the online version only). The basal sensitivity to pain was evaluated as detailed in the Supplementary Materials and Methods (Supplementary Table 2). Additionally, the TCI was used to assess the personality profiles of all of the subjects (35). The TCI used in the present study was based on the shortened 125-item questionnaire of the longer 240-item Japanese version of the TCI. The usage of the TCI for the analysis in the present study is detailed in the Supplementary Materials and Methods and a previous report (33).

For the subjects included in the study on the susceptibility to nicotine dependence, the results of the questionnaire, especially the questions related to smoking, were used in the analysis. The questionnaire included the Fagerström Test for Nicotine Dependence (FTND; a test that yields a continuous measure of nicotine dependence) (41) and Tobacco Dependence Screener [TDS; a screening questionnaire for tobacco/nicotine dependence according to the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10), Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (DSM-III-R), and Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV)], which consists of 10 questions (42). The questionnaire also included questions about the number of cigarettes smoked per day (CPD), the participants' age when they began smoking, how many times current-smokers tried to quit smoking [i.e., the number of trials for smoking cessation in current-smokers (NTC)], and how many times ex-smokers tried to quit smoking before succeeding [i.e., the number of trials for smoking cessation in ex-smokers (NTE)]. In the present study, the FTND, TDS, CPD, NTC, and NTE were used as measures of nicotine dependence and severity (Supplementary Table 3).

Genotyping and linkage disequilibrium (LD) analysis

Genomic DNA was extracted from whole-blood samples using a QIAamp DNA BloodMaxi kit (Qiagen, Hamburg, Germany) or a Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions and extracted from oral mucosa samples as described in the Supplementary Materials and Methods and a previous report (43).

To initially analyze SNPs within and around the *KCNJ6* gene region, genotype data for approximately 300,000 SNP markers that resulted from whole-genome

genotyping with the orthognathic surgery samples as described previously and in the Supplementary Materials and Methods (33) were basically used, and the genotype data for all of the SNPs with *KCNJ6* gene annotation were extracted. For additional analyses, a TaqMan allelic discrimination assay was conducted to genotype the candidate Tag SNP, rs2835859, which was selected by LD analysis in the *KCNJ6* gene and flanking region and association analysis with the orthognathic surgery samples for further analyses using the other samples.

Of the 65 SNPs with minor allele frequencies above 0.001 that were located within the exon and intron regions and approximately within the 10 kbp 5'- and 3'-flanking regions of the *KCNJ6* gene, SNPs for the association studies were selected based on recently advanced tagging strategies (44–46). To identify relationships between the SNPs used in the study, an LD analysis was performed for 127 of the 355 samples using Haploview v. 4.1 (47). To estimate the LD strength between the SNPs, the commonly used D' and r^2 values were pairwise calculated using the genotype dataset of each SNP. Linkage disequilibrium blocks were defined among the SNPs with minor allele frequencies above 0.05 that showed "strong LD" based on the default algorithm of Gabriel et al. (48), in which the upper and lower 95% confidence limits on D' for strong LD were set at 0.98 and 0.7, respectively. Tag SNPs in the LD block were consequently determined using the Tagger software package with default settings, which is incorporated in Haploview and has been detailed in a previous report (46).

To perform the TaqMan assay with a LightCycler 480 (Roche Diagnostics, Basel, Switzerland), we used TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA, USA) that contained sequence-specific forward and reverse primers to amplify the polymorphic sequence and two probes labeled with VIC and FAM dye to detect both alleles of the rs2835859 SNP (Assay ID: C_16076710_10), as detailed in the Supplementary Materials and Methods.

Statistical analysis

Among the 355 subjects who underwent painful cosmetic surgery, one subject lacked postoperative clinical data; thus, a total of 354 subjects were used for the initial LD and association analyses (126 and 228 subjects for the exploratory and confirmatory analyses, respectively). As an index of opioid sensitivity, postoperative PCA fentanyl use during the first 24-h postoperative period was used because analgesic requirements likely reflect the efficacy of fentanyl in each individual. Prior to the analyses, the quantitative values of postoperative fentanyl requirements ($\mu\text{g}/\text{kg}$) were natural-log–

transformed for approximation to the normal distribution as described in the Supplementary Materials and Methods. To explore the associations between the SNPs and phenotypes, analysis of variance (ANOVA) was performed for trichotomized comparisons between each genotype of the SNPs in the exploratory stage of the analysis, in which 24-h postoperative fentanyl use ($\mu\text{g}/\text{kg}$, log-transformed) and the genotype data for each SNP were incorporated as dependent and independent variables, respectively, and the SNPs that showed $P < 0.05$ in the analysis were considered nominally significant and selected for further analysis. In the following confirmatory stage of the analysis, dichotomized comparisons were made, in addition to the trichotomized comparisons, in which dominant and recessive genetic models for the minor allele of each SNP were also considered. In this stage, the Q -values of the false discovery rate were calculated to correct for multiple testing, in addition to the P -values based on previous reports (49, 50). The SNPs that showed $Q < 0.05$ in the analysis were considered significant for the entire SNP set in the *KCNJ6* gene region. All of the statistical analyses were performed using gPLINK v. 2.050, PLINK v. 1.07 (51), and Haploview v. 4.1 (47).

To corroborate the possible association between the SNPs and opioid sensitivity observed in the subjects who underwent painful cosmetic surgery, additional analyses were subsequently conducted for those SNPs. The samples included in these analyses were obtained from healthy volunteers with pain sensitivity and personality profile data and patients with smoking behavior data. For all of the genotype data used in these analyses, the distributions were checked using the χ^2 test, and the absence of significant deviation from the theoretical distribution expected from Hardy-Weinberg equilibrium was confirmed. For all of the statistical analyses described below, SPSS 18.0J for Windows (International Business Machines Corporation, Armonk, NY, USA) was used. The criterion for significance was set at $P < 0.05$.

For the analysis of the pain sensitivity data from healthy volunteer subjects, quantitative values of both the average latency (s) to pain perception in the CPT and average weight (kg) when the subjects perceived pain in the MPT were natural-log-transformed for approximation to the normal distribution as described in the Supplementary Materials and Methods. For the analysis of personality profile data from healthy volunteer subjects, raw TCI scores were processed according to a previous report (33). The score on each subscale in each dimension was averaged, in which the total score was divided by the number of items in each subscale. The average score on each subscale was averaged to

calculate the overall score on each dimension, in which the sum of the average score on each subscale was divided by the number of subscales in each dimension, which was used as the endpoint in the association study. Prior to the analysis, quantitative values of the overall score on each dimension (ranging from 0 to 1) were natural-log-transformed for approximation to the normal distribution as described in the Supplementary Materials and Methods. To explore the association between the rs2835859 SNP and phenotypes, Student's t -test or the Welch test and ANOVA were performed for dichotomized and trichotomized comparisons between genotypes, respectively, in which the endpoint values in the pain tests and TCI scores (log-transformed) and genotype data of the SNP were incorporated as dependent and independent variables, respectively. Corrections for multiple testing for the analyses of the two and seven phenotypes for the pain tests and TCI, respectively, were not performed in this additional exploratory study.

For the analysis of smoking behavior data in the patients, quantitative values of the smoking period (years), FTND, TDS, CPD, NTC, and NTE were natural-log-transformed for approximation to the normal distribution as described in the Supplementary Materials and Methods. To explore the association between the rs2835859 SNP and phenotypes, Student's t -test or Welch test and ANOVA were performed for dichotomized and trichotomized comparisons between genotypes, respectively, in which the phenotype values (log-transformed) and genotype data of the SNP were incorporated as dependent and independent variables, respectively. Corrections for multiple testing for the analyses of the six phenotypes were not performed in this additional exploratory study.

Corrections for multiple testing for the many phenotypes examined were not performed in the present study because they may not be necessarily required in exploratory studies, such as the present study, meaning that the associations between the SNPs and phenotypes have not yet been reported. Considering that the likelihood of type II errors is increased by corrections for multiple testing, such as Bonferroni adjustments, and considering that truly important differences may not be deemed significant (52), such adjustments were not done in the present study. Corrections for multiple testing would be too conservative for genetic association studies (53). Indeed, in similar previous studies, such corrections were not performed (54, 55).

Results

Identification of a potent SNP associated with postoperative analgesia

After whole-genome genotyping, an LD analysis was initially conducted using the genotype data from 126 samples in a total of 355 samples from subjects who underwent painful cosmetic surgery (Supplementary Table 1). As a result, a total of 11 LD blocks (LD1 – LD11) were observed within and around the *KCNJ6* gene region, and 27 Tag SNPs were selected in this region (Supplementary Figs. 2 and 3: available in the online version only). Of these Tag SNPs, only one SNP, rs2835859, was found to be nominally significant ($P < 0.05$) in the initial exploratory association analysis between the SNPs and postoperative fentanyl requirements (Supplementary Table 4: available in the online version only). A further analysis of the remaining 228 samples to confirm the association observed in the exploratory association analysis indicated that the rs2835859 SNP was significantly associated with postoperative analgesic use after false discovery rate correction ($Q = 0.0353$, Supplementary Table 4). The carriers of the C allele in this SNP required less analgesics

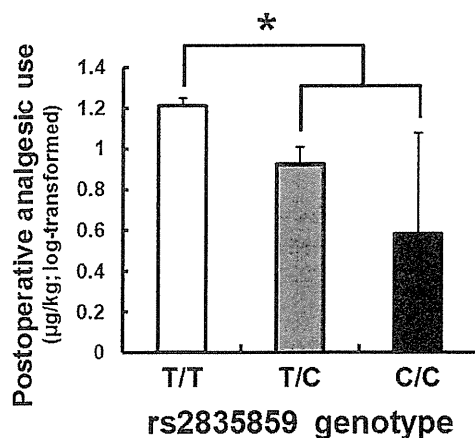


Fig. 1. Association analysis between opioid analgesic requirements during the 24-h postoperative period after cosmetic orthognathic surgery and *KCNJ6* SNPs. The results are shown for the rs2835859 SNP. * $P < 0.05$, significantly lower dose of analgesics administered in the combined T/C and C/C genotype than in the T/T genotype in the confirmatory study. The data are expressed as the mean \pm S.E.M.

compared with non-carriers (Table 1, Fig. 1). Therefore, this SNP was selected for further analysis of associations between SNP genotype and other demographic and clinical characteristics. The number of subjects carrying the T/T, T/C, and C/C genotypes of this SNP was 305, 45, and 4, respectively, and the distribution was not significantly different from the theoretical Hardy-Weinberg equilibrium value in the 355 patient subjects used in the association analyses ($\chi^2 = 2.39$, $P = 0.12$).

Association between rs2835859 SNP and pain sensitivity in healthy subjects

The observed association between the rs2835859 SNP and postoperative analgesia suggested that the subjects with the C allele of the SNP required less analgesics than the subjects without this allele, likely attributable to the increased effectiveness of opioid analgesics in this cohort. These C allele carriers may have presented higher sensitivity than non-carriers to an exogenous opioid, fentanyl. To examine whether the possible difference between genotypes in the sensitivity to exogenous opioids can be extended to the difference in the sensitivity to endogenous opioids, we compared basal pain sensitivity between the genotypes of this SNP in healthy volunteer subjects (Supplementary Table 2). In the association analysis of the CPT data, a significant difference was found between the T/T subgroup and combined T/C and C/C genotype subgroup in the average latency to pain perception, and C allele carriers had a longer latency compared with non-carriers ($t_{495} = -2.762$, $P = 0.006$; Fig. 2A). Interestingly, a similar result was obtained in the association analysis of the MPT data, in which a significant difference was found between the T/T subgroup and combined T/C and C/C genotype subgroup in the average weight when the subjects perceived pain, and C allele carriers had a greater weight at which they perceived pain compared with non-carriers ($t_{495} = -2.107$, $P = 0.036$; Fig. 2B). However, significant associations were not found in the association analyses for any of the seven dimensions of TCI scores [novelty seeking (NS): $t_{495} = 0.067$, $P = 0.947$; harm avoidance (HA): $t_{495} = 1.851$, $P = 0.065$; reward dependence (RD): $t_{495} = -0.812$, $P = 0.417$; persistence (P): $t_{495} = -1.877$, $P = 0.061$; self-directedness (SD): $t_{495} = -1.054$, $P = 0.292$; cooperativeness (C): $t_{495} = -1.039$, $P = 0.299$; self-

Table 1. Results of confirmatory association analysis between the *KCNJ6* rs2835859 SNP and postoperative analgesia

SNP	CHR ^a	Position ^b	Location	Genotypic			Dominant			Recessive		
				BETA	STAT ^c	P	BETA	STAT ^c	P	BETA	STAT ^c	P
rs2835859	21	37940032	intron 3	NA	5.81	0.0548	-0.3145	-2.391	0.0177*	-0.4007	-1.1	0.2725

^aCHR: chromosome number, ^bPosition: chromosomal position (bp), ^cSTAT: *F*-statistic or *t*-statistic, *corrected $P < 0.05$.

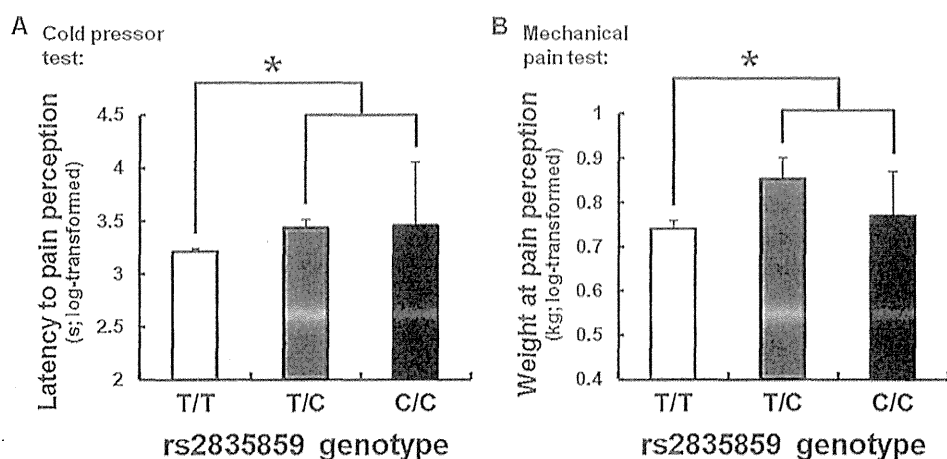


Fig. 2. Association analysis between basal pain sensitivity and the rs2835859 SNP. The results from the cold pressor-induced pain test (CPT) (A) and mechanically induced pain test (MPT) (B) are shown for the *KCNJ6* rs2835859 SNP. In panel A, *: significantly longer latency for the combined T/C and C/C genotype compared with T/T genotype. In panel B, *: significantly greater weight to perceive pain for the combined T/C and C/C genotype compared with the T/T genotype. The data are expressed as the mean \pm S.E.M.

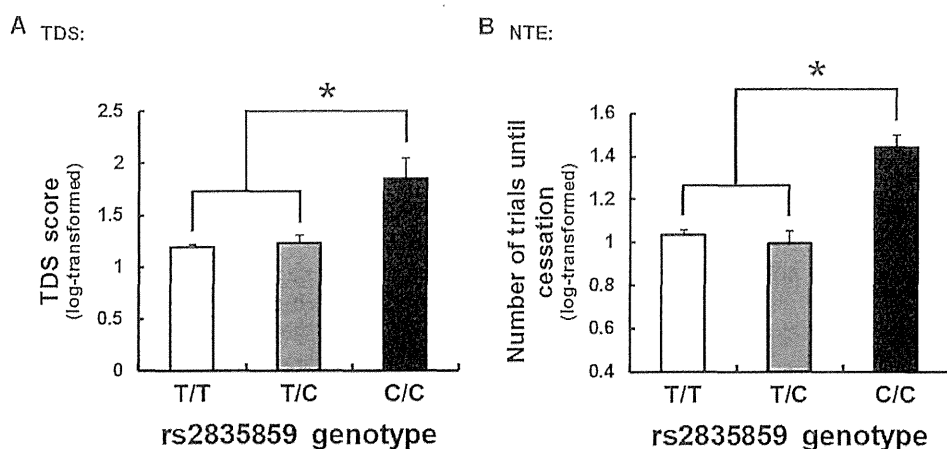


Fig. 3. Association analysis between susceptibility to nicotine dependence and the rs2835859 SNP. Tobacco Dependence Screener (TDS) scores (A) and the number of trials for smoking cessation in ex-smokers (NTE) (B) are shown for the *KCNJ6* rs2835859 SNP. In panel A, *: significantly greater score for the C/C genotype compared with the combined T/T and T/C genotype. In panel B, *: significantly greater number of trials for the C/C genotype compared with the combined T/T and T/C genotype. The data are expressed as the mean \pm S.E.M.

transcendence (ST): $t_{495} = 0.037$, $P = 0.970$]. These results suggest that C allele carriers were less sensitive to both cold pressor-induced and mechanically induced pain. The number of subjects carrying the T/T, T/C, and C/C genotypes of this SNP was 436, 60, and 2, respectively, for the CPT data, and 435, 60, and 2, respectively, for the MPT data; and the distribution was not significantly different from the theoretical Hardy-Weinberg equilibrium values in the entire subjects used in the association analyses ($\chi^2 < 0.01$, $P = 0.97$, and $\chi^2 < 0.01$, $P = 0.96$, respectively).

Association between rs2835859 SNP and susceptibility to nicotine dependence

The results further suggested that the subjects with the C allele in the rs2835859 SNP required less analgesics and were less sensitive to cold and mechanical pain than the subjects without the allele, attributable to the increased effectiveness of not only exogenous but also endogenous opioids in both cohorts. Given the fact that the opioid system is involved in both analgesic and

rewarding effects, one may hypothesize that increased opioid sensitivity reflects the increased rewarding effects of addictive substances or behaviors and greater liability to serious dependence. To test this hypothesis, we investigated the contribution of the rs2835859 SNP to the vulnerability to substance dependence in additional subjects with various smoking habits (Supplementary Table 3). In the association analysis of the TDS data, a significant difference was found between the combined T/T and T/C subgroup and C/C genotype subgroup in TDS scores, and homozygous C allele carriers had a higher TDS than non-carriers ($t_{510} = -2.130$, $P = 0.034$; Fig. 3A). A significant difference was found between the combined T/T and T/C subgroup and C/C genotype subgroup in the NTE, and homozygous C allele carriers had a higher NTE than non-carriers ($t_{382} = -1.948$, $P = 0.002$; Fig. 3B). However, no significant associations were found in the association analyses for smoking period, FTND, CPD, or NTC (Supplementary Table 5: available in the online version only). These results suggest that homozygous C allele carriers had higher

susceptibility to nicotine dependence and required a greater number of trials until they achieved successful smoking cessation. The number of subjects carrying the T/T, T/C, and C/C genotypes of this SNP was 443, 65, and 4, respectively, for the TDS scores, and 331, 49, and 4, respectively, for the NTE scores; and the distribution was not significantly different from the theoretical Hardy-Weinberg equilibrium values in the entire subjects used in the association analyses ($\chi^2 = 0.87$, $P = 0.35$, and $\chi^2 = 1.96$, $P = 0.16$, respectively).

Discussion

To our knowledge, the present study is the first to comprehensively explore SNPs of the *KCNJ6* gene with regard to associations between these SNPs and all of the phenotypes related to opioid actions such as outcomes in clinical pain management, basal pain sensitivity, and smoking behavior, simultaneously in humans. A novel result of the present study was that the rs2835859 was found to be potently associated with opioid analgesic sensitivity, in which carriers of the C allele of this SNP required less analgesics compared with non-carriers (Table 1, Fig. 1). The subsequent association study indicated that this SNP was also associated with sensitivity to two different pain modalities, in which carriers of the C allele of this SNP were less sensitive to both cold and mechanical pain (Fig. 2: A and B). The examination of patient subjects with clinical data related to smoking behavior indicated that homozygous carriers of the C allele of this SNP had higher susceptibility to nicotine dependence and required a greater number of trials to achieve successful smoking cessation (Fig. 3: A and B). This result appears to be consistent with previous studies, which demonstrated that nicotine-induced antinociception and rewarding effects were modulated by opioids (56–58), suggesting the involvement of opioid-cholinergic interactions (58). To our knowledge, the associations found between SNPs of the *KCNJ6* gene and nicotine dependence in the present study are novel findings in human studies that used various indices, including TDS and NTE. Altogether, the present results suggest that the rs2835859 SNP may affect individual differences in exogenous and endogenous opioid sensitivity. Carriers of the C allele, especially homozygous carriers, have higher sensitivity, and non-carriers have the opposite sensitivity, resulting in less postoperative analgesic requirements, less pain sensitivity, and a higher liability to develop nicotine dependence, possibly because of increased analgesic and rewarding effects of opioids. The decreased pain sensitivity observed in carriers of the C allele in the present study is not necessarily caused by the facilitation of endogenous

opioid sensitivity. Such an effect could also be caused by other factors. For example, GIRK2 is activated with other $G_{i/o}$ protein-coupled receptors, including α_2 -adrenergic (11) and 5-HT_{1A} receptors (12), which play pivotal roles in descending pain pathways. Furthermore, the decreased pain sensitivity associated with the rs2835859 SNP after cosmetic orthognathic surgery may reduce the doses of postoperative PCA-fentanyl that are necessary for adequate pain relief.

In the present study, differences existed in the models (i.e., dominant model and recessive model) used to find associations between the rs2835859 SNP and phenotypes (i.e., postoperative opioid use, pain sensitivity, nicotine dependence, and difficulty in quitting smoking). The differences in these models may suggest differences in the involvement of the genes that underlie various phenotypes, which was shown in a previous study that investigated the influence of the 118A > G polymorphism in the *OPRM1* gene, which encodes the human μ -opioid receptor gene (59). Patients homozygous for the variant G allele of this SNP needed more morphine to achieve pain control compared with individuals heterozygous and homozygous for the A allele. However, the patients heterozygous for the 118A > G polymorphism had significantly more pain than patients with other genotypes (59). Similarly, the required doses of postoperative PCA-fentanyl and basal pain sensitivity were reduced even in the T/C heterozygous subgroup, whereas the TDS score and NTE were changed only in the C/C homozygous subgroup and not in the T/C heterozygous subgroup in the present study. These findings suggest that the difference in nicotine dependence may not necessarily be caused by only alterations in the endogenous opioid system. Other reward-related G-protein-coupled receptors (GPCRs), such as dopamine D₂/D₄ receptors (10), may also be involved.

To investigate the susceptibility to nicotine dependence, we adopted several indices because associations between SNPs of the *KCNJ6* gene and nicotine dependence have not been previously reported in studies that used these indices. The lack of consistent associations across these nicotine-dependence phenotypes observed in the present study may be attributable to different characteristics between these indices. In our preliminary study, the intercorrelations between the FTND and TDS, between the FTND and CPD, and between the TDS and NTE were found to be significant (Kasai et al., in preparation). However, we did not find a significant association between the rs2835859 SNP and FTND. Although the most frequently used instrument is the FTND, it may have several limitations. For example, it fails to include important aspects of dependence as defined by the DSM-IV and ICD-10 (60, 61), and several

items of the FTND are difficult to apply in relatively light smokers (60). The TDS is reported to have better screening performance for ICD-10, DSM-III-R, and DSM-IV diagnoses than the Fagerström Tolerance Questionnaire (FTQ) (42), which is the previous version of the FTND. Thus, we consider that investigating the TDS and NTE as well as the other indices and conducting analyses of these various aspects of nicotine dependence are important.

We previously explored *KCNJ6* gene variations in the exon regions, exon-intron boundary regions (approximately 30 bp), and putative promoter regions (approximately 1.8 kbp) and found that the A1032G SNP (rs2070995) and a haplotype that consisted of two alleles of the A1032G and G-1250A SNPs (rs6517442) were significantly associated with postoperative analgesic requirements after major abdominal surgery (31). In the present study that more comprehensively targeted SNPs in and around the gene, the rs2070995 SNP was included in the investigated region but not incorporated into the LD1 – LD11 blocks (Supplementary Fig. 2). The rs6517442 SNP was included in the investigated region and tagged by the rs7275707 SNP, which was in absolute LD with the rs6517442 SNP ($r^2 = 1$; Supplementary Figs. 2 and 3). Although we distinctly examined the association between these SNPs and postoperative analgesic requirements after painful cosmetic surgery in the present study, no significant associations were observed in the dichotomous analysis or trichotomous analysis (rs2070995: $P = 0.662$; rs6517442 or rs7275707: $P = 0.866$). The present results indicate that these SNPs are not useful for predicting opioid analgesic sensitivity in the case of cosmetic orthognathic surgery, although these SNPs may be useful for predicting opioid analgesic sensitivity in the case of major abdominal surgery. Although the causal factors cannot be easily identified, differences in pain, innervated neurons by which pain signals are transmitted, the analgesics mainly used, and the required amount of analgesics may affect the degree of associations found in both studies.

To date, only a few studies have examined the relationship between genetic variations in GIRK channels and phenotypic differences related to opioid actions in humans. Most of these studies analyzed the *KCNJ6* gene (29 – 32). In a study that targeted over 300 candidate genes and analyzed 3713 SNPs in 1,050 cases and 879 controls of European ancestry, the rs6517442 SNP was found to be among the top candidates for an association with nicotine dependence (32). Although this SNP was not investigated for associations with nicotine dependence in the present study, such analyses would be worthwhile in future studies. Lotsch et al. reported a tendency toward less opioid analgesic

effectiveness and addiction in the A/A genotype in the rs2070995 SNP (30), which was consistent with our previous study in terms of analgesia (31). In these studies, the rs2835859 SNP was not investigated. In another recent study, Bruehl et al. (29) revealed that eight *KCNJ6* SNPs were significantly associated with the pain-related phenotype in Caucasian patients who underwent total knee arthroplasty (TKA) with post-surgical oral opioid analgesic medication (29). However, these SNPs and other SNPs reportedly tagged by these SNPs were not included in our candidate SNPs in the exploratory stage of the present study (Supplementary Table 4). The results might suggest that the SNPs that greatly contributed to pain or analgesia may not be consistent for different phenotypic traits or populations. Therefore, evaluating potential SNPs with respect to each phenotype and population is important. Notably, both Bruehl et al. and the present study identified promising SNPs associated with pain-related phenotypes in the *KCNJ6* gene region. These results underscore the significant role played by this gene in the sensitivity to pain, which was previously shown in animal studies (4, 23). Moreover, the present study demonstrated that some *KCNJ6* SNPs also affected individual differences in the sensitivity to analgesia and susceptibility to dependence. In particular, to our knowledge, the present study is the first to find an association between the rs2835859 SNP and nicotine dependence evaluated by the TDS and NTE. Future studies should be performed to more clearly confirm the important contribution of *KCNJ6* SNPs to analgesia sensitivity and dependence susceptibility.

The best candidate SNP identified in the present study, rs2835859, is located in the third intronic region of the *KCNJ6* gene (Supplementary Table 4). This SNP is in absolute LD with the rs2835860 SNP and falls on the LD1 block (Supplementary Fig. 2). However, all of the SNPs in the LD1 block are located in intron 3 and are apparently not in strong LD with other SNPs located in exon or putative regulatory regions (Supplementary Table 4 and Supplementary Fig. 2), which excludes the possibility that phenotypic alterations related to the rs2835859 SNP found in the present study are attributable to alterations in the function or expression of *KCNJ6* caused by other SNPs that are in strong LD with these SNPs in the LD1 block. Considering that intronic SNPs can affect enhancer or some other activities of the gene, future studies are needed to clarify the underlying mechanisms by which the effects of opioids are modulated by this SNP. Such studies are important because they will clarify the pharmacological and neurobiological mechanisms that underlie the associations found in the present study. Our findings

may provide some insights for future investigations.

Although the observed associations in the present study might be restricted to the Japanese population and the underlying mechanism remains to be fully elucidated, the present results indicate that the rs2835859 SNP may serve as a marker that predicts increased opioid sensitivity and open new avenues for the personalized treatment of pain and dependence.

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Conflicts of Interest

KI has received support from Eisai for a project unrelated to this research and speaker's fees from Taisho Pharmaceutical Co., Ltd. and Japan Tobacco, Inc. The authors declare no other conflict of interest.

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Case Report

Extensive goblet cell metaplasia of the peripheral lung may harbor precancerous molecular changes: Comparison of two cases

Shogo Tajima,¹ Nobuya Kurabe,² Koji Okudela,³ Kiyoshige Yajima,⁴ Tsuyoshi Takahashi,⁴ Hiroshi Neyatani,⁴ Haruhiko Sugimura² and Kenji Koda⁵

¹Department of Pathology, Shizuoka Saiseikai General Hospital, ²Department of Tumor Pathology, Hamamatsu University School of Medicine, Departments of ⁴Chest Surgery and ⁵Pathology, Fujieda Municipal General Hospital, Shizuoka and ³Department of Pathology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

We present two cases of extensive goblet cell metaplasia in the peripheral lung. The first case was a 72-year-old male with a nodule (12 × 10 mm) detected by CT. Macroscopically, it contained abundant mucin; histopathologically, goblet cells were predominant, whereas ciliated and basal cells were sparse. A *KRAS* G12V mutation was detected. In the pulmonary background of this case, scattered tiny foci of goblet cell metaplasia were present. The second case was a 71-year-old female with a nodule (7 × 5 mm) detected by CT. It contained abundant mucin, and microscopically, various cell types were intermingled. Although the nodule mainly comprised goblet cells, ciliated and basal cells were also easily identified. No *KRAS* mutation was found in this patient. Dispersed minute foci of goblet cell metaplasia were identified in the pulmonary background. Therefore, we suspect that airway irritants may have contributed to the changes that occurred in the pulmonary background of both cases. We propose that the tendency of goblet cells to overwhelm other cell types in the metaplastic epithelium may be an indicator of precancerous molecular changes.

Key words: goblet cell metaplasia, *KRAS*, lung, precancerous

Small airways consist of membranous and respiratory bronchioles with a terminal bronchiole located between them. Respiratory bronchioles participate in gas exchange through alveoli attached to their walls. The small airways can sustain injuries due to inhaled tobacco smoke, minerals, dust, and intrinsic and extrinsic allergens. In general, there are several

histopathological reactions of small airway injuries that have similar morphological features.¹ Chronic irritation of the small airways is the most common type of injury and is usually accompanied by metaplastic epithelium, such as bronchiolar, goblet cell, and squamous metaplasia. Peribronchiolar alveolar epithelial metaplasia manifests as cuboidal, bronchiolar, goblet cell, and squamous metaplasias.¹ As for goblet cell metaplasia, Clara cells are thought to transdifferentiate into goblet cells,^{2,3} whereas goblet cell proliferation is thought to occur less often.⁴

Goblet cell metaplasia is confined to regions near the small airways and usually does not form macroscopic nodules. Here, we report two cases of mucinous nodules of the peripheral lung. They were predominantly composed of goblet cells as an epithelial component with abundant mucin in the air spaces. We attempted to identify the molecular characteristics of these peculiar lesions.

CLINICAL SUMMARY

Case 1

A 72-year-old male was referred from a nearby hospital for a definite diagnosis of a nodule present in the right S6 of the lung. The nodule had slightly increased in size over the previous months on CT; he had no complaints. Although he stopped smoking when he was 47 years, his Brinkman index was 500. PET-CT was performed to evaluate the nodule in detail. CT images displayed a nodule with an irregular border measuring 12 × 10 mm (Fig. 1a), whereas PET revealed no accumulation. Additional tiny lesions with increased densities were also present in the left and right lower lobes. However, the tumor markers CEA, CA19-9, CYFRA, and ProGRP were not elevated. Although malignancy was not highly suspected,

Correspondence: Shogo Tajima, MD, Department of Pathology, Shizuoka Saiseikai General Hospital, 1-1-1 Oshika, Suruga-ku, Shizuoka 422-8021, Japan. Email: stjima-tky@umin.ac.jp

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