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Screening study for genetic polymorphisms affecting pharmacokinetics of talniflumate

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Talniflumate is a phthalidyl ester of niflumic acid, which has potent analgesic and anti-inflammatory effects and is widely used to treat inflammatory disorders, such as rheumatoid arthritis. To screen the possible genetic factors affecting the pharmacokinetics (PK) of talniflumate, 23 male Korean volunteers were enrolled from two separate bioequivalence studies. All subjects received 740 mg (two tablets) talniflumate in a standard 2x2 cross-over model in a randomized order. For the genetic study, PK parameters of the reference drug were used. We used Illumina Human610Quad v1.0 DNA Analysis BeadChip for whole genome single nucleotide polymorphism (SNP) analysis and whole genome genotyping data were processed by linear regression analysis for PK parameters. Whole genome analysis revealed 1498 significant SNPs ($P < 0.0001$) for Cmax, 65 significant SNPs ($P < 0.0001$) for T_{max}, and 1491 significant SNPs ($P < 0.0001$) for $AUC_{inf.}$ For clinical pharmacological purposes, we selected SNPs from drug metabolizing enzymes and transporters, and analyzed the PK parameters of various genotypes. Two SNPs (rs11165069 from ABCA4 (p=0.00002); rs17847036 from CYP2C9 (p=0.000001)) showed significant associations with talniflumate C_{max}. In the T_{max} group, two SNPs (rs3787555 from CYP24A1 (p=0.00035); rs2275034 from ABCA4 (p=0.000587)) showed significant associations with talniflumate T_{max}. In the AUC_{inf} group, two SNPs (rs11165069 from ABCA4 (p=0.00002); rs12461006 from SLC1A6 (p=0.00008)) exhibited significant associations with talniflumate absorption. These results show that genetic factors could affect the PK parameters, and provide information that may be used in the development of personalized talniflumate therapy.

Introduction

Talniflumate, 3-pyridinecarboxylic acid 2-{[(3-trifluoromethyl) phenyl]amino}-1,3-dihydro-3-oxo-1-soenzofuranyl ester, is a phthalidyl ester of niflumic acid and is currently used to treat osteoarthritis and rheumatoid arthritis.[1,2] Niflumic acid shows rapid absorption followed by extensive metabolism, involving hydroxylation or glucuronidation.[3] However, the occurrence of side effects such as gastrointestinal irritation has been reported both in experimental animals and in clinical use. In order to lower ulcergenic activity, talniflumate was synthesized by esterification of the carboxyl group of niflumic acid.[4] Many genetic and non-genetic factors such as age, sex, life style, organ function, concomitant therapy, and the nature of the disease influence the effects of medications.[5] Following the Human Genome Project, it is understood that genetic factors can influence the response of an individual to a drug.[6] In clinical pharmacogenomics, drug-metabolizing enzymes.[7,8] drug transporters.[9] and other genes have been known to influence the individual differences in drug response.

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation, and are proven to significantly

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affect drug absorption, distribution, biotransformation, and excretion.[10] Our previous studies showed that the pharmacokinetics (PK) of simvastatin and pioglitazone are affected by the genotype of each individual.[11,12]

The purpose of this study was to screen possible candidate genes affecting the PK of talniflumate. A total of 23 male Korean volunteers were recruited from two bioequivalence studies after approval by the Institutional Review Board (IRB) of Kyung Hee University Hospital. In this study, DNA from each subject was analyzed using Illumina Human610Quad v1.0 DNA Analysis BeadChip. Linear regression analysis was performed for significant SNPs affecting PK parameters, such as maximum measured plasma concentration (C_{max}), time of the maximum measured plasma concentration (T_{max}), and area under the plasma concentration-time curve from zero to infinity (AUC_{inf}). For clinical pharmacological purposes, further analysis was performed on significant SNPs encoding phase I and II drug metabolizing enzymes to examine their effect on the PK of talniflumate.

Methods

Subjects

Volunteers were healthy Korean males who participated in two talniflumate bioequivalence tests at Kyung Hee Clinical Medical Research Institute of Kyung Hee University Hospital. The clinical protocol was approved by the IRB of Kyung Hee University Hospital and volunteers were recruited by direct call. Twenty-three out of 60 subjects from these previous tests participated in this pharmacogenetics study after providing written informed consent. The demographic characteristics of the volunteers are summarized in Table 1. Subjects ranged in age from 20 to 35 years (26 ± 3.6 years), in weight from 57.0 to 90.0 kg (68.7 ± 8.3 kg), and in height from 160.0 to 184.0 cm (173.3 ± 4.8 cm) (Table 1).

Previous bioequivalence studies

Previous studies were based on two talniflumate bioequivalence tests. Each bioequivalence study of two 370 mg talniflumate formulations (reference drug, Somalgen tablet 370 mg, Kunhwa Co., Ltd.) was conducted in healthy male Korean volunteers after a single dose administration in a randomized cross-over study with a washout period of at least one week. The subjects were hospitalized (Kyung Hee University Hospital, Seoul, Korea) and fasted overnight (10 h) and for 4 h after each drug administration. The doses were administered at 8.00 a.m. of each dosing day along with 240 ml of tap water. No food was allowed until 4 h after dose administration. Approximately 7 ml of blood for talniflumate and active metabolite assays were drawn into heparinized tubes through indwelling cannula before (0 h) and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 15 h after dosing. Blood samples were centrifuged at 3,000 rpm for 10 min; plasma was separated and kept frozen at -70°C until assayed. Plasma was analyzed for talniflumate concentration using a validated high-performance liquid chromatography method.

Pharmacokinetics analysis

PK parameters (C_{max}, T_{max}, AUC_t and AUC_{inf}) were calculated by noncompartmental models in WinNonlin v5.2 (Table 1).

DNA extraction

DNA extraction was performed as previously described.[11] From May to August 2008, blood samples were obtained from 23 participants. After obtaining informed consent, approximately 10 ml of whole blood was collected from each participant. Blood samples were drawn into sterile tubes containing ethylenediaminetetraacetic acid (EDTA) and stored at -70°C until the isolation of genomic DNA by a standard phenol chloroform extraction method.

Genotyping

SNPs of 23 healthy male volunteers were analyzed by Standard Illumina procedures using Illumina BeadStation 500G (Illumina Human610Quad v1.0 DNA Analysis BeadChip) as previously described.[11,12] Intensity files (*.idat) were processed by BeadStudio GT module 3.3.4 with default analysis settings. Each SNP was analyzed independently to cluster and identify genotypes. Genotype calls were generated by comparing experimental data with those in the supplied cluster file (*.egt). The SNP set was filtered on the basis of genotype call rates (≥ 95%), and minor allele frequency (MAF ≥ 0.05).

Statistical analysis & data analysis

Statistical analysis was performed as previously described.[11,12] Hardy Weinberg equilibrium (HWE) was calculated for individual SNPs using an exact test. All of the SNPs reported in this manuscript have HWE P-values > 0.001. To calculate the degree (Beta), 95% confidence intervals (CI) and P-value while controlling for age, height and weight as covariants in a linear regression analysis with significant SNPs on individual PK parameters of talniflumate, unadjusted genotypic association with additive, dominant and recessive models were tested by calculating the Beta and P-value using PLINK version 1.06 (Shaun Purcell, USA). When the additive model was used, each genotype was independently coded as 0, 1, or 2 and analyzed. In the dominant model, a homozygote major allele and another two genotypes were coded as 0 and 1 and analyzed. In the recessive model, a homozygote minor allele and another two genotypes were coded a 0 and 1 and analyzed. After filtering, SNPs were analyzed on chromosome 1 through chromosome 22 for each group.

Significant SNPs were identified from each PK parameter (C_{max}, T_{max} and AUC_{inf}). These included SNPs of phase I and II drug metabolizing enzymes, which were selected to further analyze their effect on the PK parameters (two SNPs in C_{max}, two SNPs in T_{max} and two SNPs in AUC_{inf}).

Table 1. Demographic characteristics and reference talniflumate pharmacokinetic parameters of volunteers

NO.	Age	Weight /kg	Height /cm	AUC_t	AUC_{inf}	C_{max}	T_{max}
T1	25	66	180	906.13	999.72	159.44	6.0
T2	26	90	184	622.32	622.32	123.95	4.0
T3	26	69	175	779.54	779.54	145.57	4.0
T4	28	75	171	2347.19	2443.90	437.40	3.0
T5	26	64	168	1517.40	1593.58	250.04	2.5
T6	25	61	171	517.70	583.46	113.53	2.0
T7	28	66	160	472.36	472.36	137.56	1.5
T8	20	57	171	1062.67	1143.30	239.89	4.0
T9	24	58	174	1343.76	1397.23	349.84	2.0
T10	24	68	173	1478.44	1589.52	218.99	6.0
T11	22	72	174	1984.33	2102.38	357.11	4.0
T12	30	68	170	1120.51	1201.74	257.67	3.0
T13	24	85	176	1493.98	1571.46	394.48	4.0
T14	22	62	170	836.85	882.83	164.70	3.0
T15	22	71	177	1087.64	1087.64	178.06	3.0
T16	30	66	175	458.10	517.74	87.99	3.0
T17	35	80	176	600.89	152.32	152.32	2.5
T18	27	73	178	628.44	628.44	193.06	1.5
T19	34	70	168	1798.92	1901.22	489.93	1.5
T20	26	68	174	501.71	501.71	145.15	2.5
T21	24	61	172	447.39	545.75	235.83	2.5
T22	25	58	173	596.64	596.64	125.06	2.0
T23	25	73	177	1743.96	2007.03	355.20	2.5
Mean±S.D	26±3.6	68.7±8.3	173.3±4.8	1058.6±557.1	1100.9±621.9	230.9±113.5	3.0±1.2

Abbreviations: C_{max}, maximum measured plasma concentration; T_{max}, time of the maximum measured plasma concentration; AUC_t, area under the plasma concentration-time curve from time zero to time of last measurable concentration; AUC_{inf}, area under the plasma concentration-time curve from zero to infinity; SD, standard deviation.

Table 2. Highly significant SNPs identified in C_{max} group following regression analysis (P<0.0001)

Name	Chr	Gene	Location	g1	n1	m1	s1	g2	n2	m2	s2	P VALUE
rs11165069	1	ABCA4	intron	CC	22	175.40	95.28	TC	1	831.52		0.000001
rs7637682	3	SLC9A10	intron	AA	22	175.40	95.28	AG	1	831.52		0.000001
rs4234409	3	SLC9A10	intron	AA	22	175.40	95.28	AC	1	831.52		0.000001
rs4434123	3	SLC9A10	missense	AA	22	175.40	95.28	AG	1	831.52		0.000001
rs17446282	3	SLC9A10	intron	AA	22	175.40	95.28	AG	1	831.52		0.000001
rs2291550	12	ABCC9	synonymous	CC	22	175.40	95.28	TC	1	831.52		0.000001
rs1051640	17	ABCC3	synonymous	AA	22	175.40	95.28	AG	1	831.52		0.000001
rs757220	19	SLC25A42	intron	CC	22	175.40	95.28	TC	1	831.52		0.000001
rs12150890	19	SLC7A9	synonymous	AA	22	175.40	95.28	AG	1	831.52		0.000001
rs17724104	20	SLC9A8	intron	AA	22	175.40	95.28	AG	1	831.52		0.000001
rs6781844	3	SLC9A10	missense	TG	1	831.52		TT	22	175.40	95.28	0.000001
rs9855755	3	SLC9A10	intron	AG	1	831.52		GG	22	175.40	95.28	0.000001
rs4646196	5	SLC22A4	intron	AG	1	831.52		GG	22	175.40	95.28	0.000001
rs17847036	10	CYP2C9	synonymous	AG	1	831.52		GG	22	175.40	95.28	0.000001
rs7248399	19	SLC25A42	intron	AG	1	831.52		GG	22	175.40	95.28	0.000001
rs10417974	19	SLC25A42	3UTR	TC	1	831.52		TT	22	175.40	95.28	0.000001
rs215096	16	ABCC1	intron	TC	3	527.07	264.40	TT	20	155.45	73.41	0.000015

Abbreviations: Chr, chromosome; N, number; g1, genotype 1; n1, number 1; s1, standard deviation 1; g2, genotype 2; n2, number 2; s2, standard deviation 2. *Table only shows several significant SNPs from a total of 1498 highly significant SNPs.

Results

Pharmacokinetics analysis

The PK parameters of talniflumate are shown in Table 1. C_{max} is 230.9 ± 113.5 μg/L, T_{max} is 3.0 ± 1.2 h, AUC_t is 1058.6 ± 557.1 μg·h/L, and AUC_{inf} is 1100 ± 621.9 μg/L. These data were used for linear regression analysis.

SNP analysis

Linear regression analysis was performed on the PK parameters of talniflumate (C_{max}, T_{max}, and AUC_{inf}). In this analysis, 25,361 significant SNPs in the C_{max} group, 24,067 significant SNPs in the T_{max} group, and 23,604 significant SNPs in the AUC_{inf} group showed significant associations (P<0.05). Among those significant SNPs, 1,498 SNPs from the Cmax group, 65 SNPs from the T_{max} group, and 1,491 SNPs from the AUC_{inf} group showed highly significant associations with each parameter (Table 2, 3 and 4, P<0.0001). All data were adjusted for age, height, and weight. For clinical pharmacological purposes, significant SNPs encoding phase I and II drug metabolizing enzymes were selected for further analysis.

We selected two SNPs for further analysis from the C_{max} group (rs11165069 from ABCA4; rs17847036 from CYP2C9 Table 2), the T_{max} group (rs3787555 from CYP24A1; rs2275034 from ABCA4, Table 3), and the AUC_{inf} group (rs11165069 from ABCA4; rs12461006 from SLC1A6, Table 4).

ABCA4 (rs11165069, P = 0.000001 in C_{max} group; rs2275034, P = 0.000587 in the T_{max} group; rs11165069, P = 0.00002 in AUC_{inf} group) showed statistically significant changes by genotype (Fig. 1, 2, and 3 and Table 5). ABCA4 gene polymorphisms affect talniflumate PK parameters. These data show that genetic polymorphism of ABCA4 has a pro-

Table 3. Highly significant SNPs identified in T_{max} group following regression analysis (P<0.0001)

Name	chr	Gene	Location	g1	n1	m1	s1	g2	n2	m2	s2	g3	n3	m3	s3	P VALUE
rs2072219	7	DNAH11	intron	AC	4	6.00	0.00	CC	19	2.84	0.94					0.00000
rs2084789	16	A2BP1	intron	AA	3	6.00	0.00	AG	2	5.00	1.41	GG	18	2.78	0.93	0.00000
rs8008333	14	RAD51L1	intron	AA	18	2.78	0.93	AG	5	5.60	0.89					0.00001
rs4370152	4	LOC729112	intron	AA	13	2.46	0.85	AG	8	4.25	1.16	GG	2	6.00	0.00	0.00001
rs770297	5	CDH18	3flanking	CC	2	6.00	0.00	TC	3	5.33	1.15	TT	18	2.78	0.93	0.00001
rs10899786	10	RASGEF1A	intron	AA	7	5.00	1.29	AG	10	3.10	0.91	GG	6	2.00	0.45	0.00001
rs2503853	10	RASGEF1A	intron	AA	7	5.00	1.29	AG	11	3.05	0.88	GG	5	1.90	0.42	0.00001
rs3781832	11	SORL1	intron	GG	8	2.13	0.64	TG	10	3.50	1.15	TT	5	5.20	1.10	0.00002
rs2452600	4	PDLIM5	missense	CC	6	2.08	0.38	TC	14	3.39	1.16	TT	3	6.00	0.00	0.00002
rs7794797	7	CACNA2D1	intron	AA	11	4.55	1.21	AG	12	2.33	0.75					0.00003
rs6534295	4	LOC729112	intron	CC	10	2.25	0.79	TC	10	3.95	1.21	TT	3	5.33	1.15	0.00004
rs1158024	4	LOC729112	intron	CC	3	5.33	1.15	TC	10	3.95	1.21	TT	10	2.25	0.79	0.00004
rs12646248	4	LOC729112	intron	AA	10	2.25	0.79	AG	10	3.95	1.21	GG	3	5.33	1.15	0.00004
rs13119523	4	LOC729112	intron	CC	10	2.25	0.79	TC	10	3.95	1.21	TT	3	5.33	1.15	0.00004
rs12403933	1	C1orf125	intron	AA	13	2.54	0.85	AG	8	4.13	1.36	GG	2	6.00	0.00	0.00004
rs3781834	11	SORL1	intron	AA	9	2.17	0.61	AG	11	3.86	1.27	GG	3	5.33	1.15	0.00004
rs17125523	11	SORL1	intron	AA	9	2.17	0.61	AG	11	3.86	1.27	GG	3	5.33	1.15	0.00004
rs12287339	11	SORL1	intron	CC	3	5.33	1.15	TC	11	3.86	1.27	TT	9	2.17	0.61	0.00004
rs722074	16	XYLT1	intron	CC	2	6.00	0.00	TC	10	3.95	1.30	TT	11	2.41	0.80	0.00005
rs6848730	4	MIST	intron	GG	2	6.00	0.00	TG	5	4.50	1.50	TT	16	2.72	0.93	0.00005
rs187985	7	IGF2BP3	intron	CC	1	6.00		TC	6	4.83	1.33	TT	16	2.69	0.93	0.00006
rs7728604	5	SLIT3	intron	CC	12	2.54	0.89	TC	7	3.64	1.31	TT	4	5.50	1.00	0.00007
rs532841	8	DLC1	missense	CC	5	5.20	1.10	TC	12	3.29	1.16	TT	6	2.08	0.74	0.00008
rs2136638	2	CDKL4	intron	CC	14	2.57	0.83	TC	8	4.50	1.41	TT	1	6.00		0.00008
rs2302677	16	RPGRIP1L	missense	CC	18	2.83	0.97	TC	5	5.40	1.34					0.00009
rs9934800	16	RPGRIP1L	intron	TC	5	5.40	1.34	TT	18	2.83	0.97					0.00009

Abbreviations: Chr, chromosome; N, number; g1, genotype 1; n1, number 1; s1, standard deviation 1; g2, genotype 2; n2, number 2; s2, standard deviation 2; g3, genotype 3; n3, number 3; s3; standard deviation. 3 *Table only shows several SNPs from a total of 65 highly significant SNPs.

Table 4. Highly significant SNPs identified in AUC_{inf} group following regression analysis (P<0.0001)

Name	chr	Gene	Location	g1	n1	m1	s1	g2	n2	m2	s2	P VALUE
rs11165069	1	ABCA4	intron	CC	22	908.30	573.63	TC	1	4073.42		0.00002
rs1051640	17	ABCC3	synonymous	AA	22	908.30	573.63	AG	1	4073.42		0.00002
rs2291550	12	ABCC9	synonymous	CC	22	908.30	573.63	TC	1	4073.42		0.00002
rs17847036	10	CYP2C9	synonymous	AG	1	4073.42		GG	22	908.30	573.63	0.00002
rs12461006	19	SLC1A6	intron	AG	2	3049.93	1447.43	GG	21	855.06	529.17	0.00008
rs4646196	5	SLC22A4	intron	AG	1	4073.42		GG	22	908.30	573.63	0.00002
rs757220	19	SLC25A42	intron	CC	22	908.30	573.63	TC	1	4073.42		0.00002
rs7248399	19	SLC25A42	intron	AG	1	4073.42		GG	22	908.30	573.63	0.00002
rs10417974	19	SLC25A42	3UTR	TC	1	4073.42		TT	22	908.30	573.63	0.00002
rs4980343	15	SLC28A1	intron	AG	1	4073.42		GG	22	908.30	573.63	0.00002
rs12150890	19	SLC7A9	synonymous	AA	22	908.30	573.63	AG	1	4073.42		0.00002
rs7637682	3	SLC9A10	intron	AA	22	908.30	573.63	AG	1	4073.42		0.00002
rs4234409	3	SLC9A10	intron	AA	22	908.30	573.63	AC	1	4073.42		0.00002
rs4434123	3	SLC9A10	missense	AA	22	908.30	573.63	AG	1	4073.42		0.00002
rs17446282	3	SLC9A10	intron	AA	22	908.30	573.63	AG	1	4073.42		0.00002
rs6781844	3	SLC9A10	missense	TG	1	4073.42		TT	22	908.30	573.63	0.00002
rs9855755	3	SLC9A10	intron	AG	1	4073.42		GG	22	908.30	573.63	0.00002
rs17724104	20	SLC9A8	intron	AA	22	908.30	573.63	AG	1	4073.42		0.00002

Abbreviations: Chr, chromosome; N, number, g1, genotype 1, n1, number 1; s1, standard deviation 1; g2, genotype 2; n2, number 2; s2, standard deviation 2 *Table only shows several SNPs from a total of 1498 highly significant SNPs.

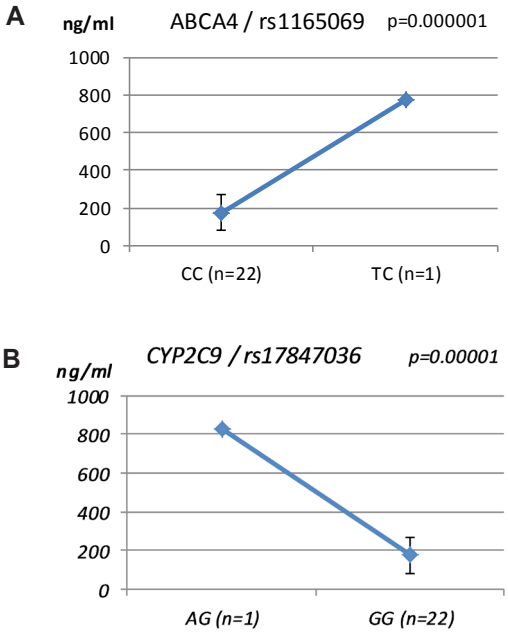

Figure 1. Results of regression analysis of C_{max} group.

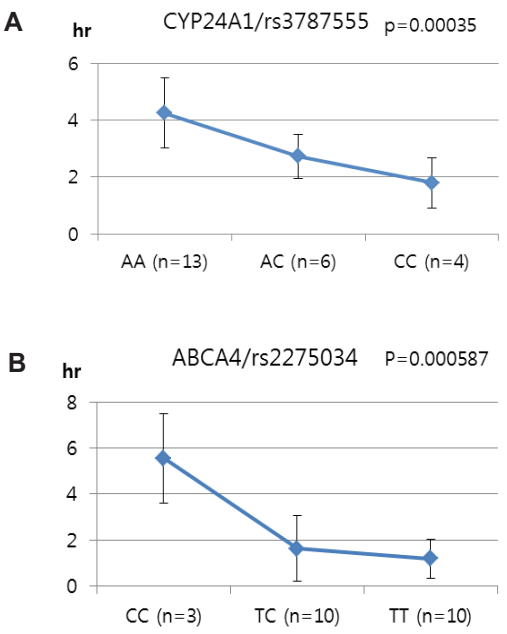

Figure 2. Results of regression analysis of T_{max} group.

Table 5. Summary of regression Analysis

A. Results of C_{max} group (ng/ml)

	ABCA4 / rs1165069	
	CC (n=22)	TC (n=1)
mean	175.4	775.532
stdev	95.28	

	CYP2C9 / rs17847036	
	AG (n=1)	GG (n=22)
mean	831.52	175.4
stdev		95.28

B. Results of T_{max} group (Hhr)

	CYP24A1		
	AA (n=13)	AC (n=6)	CC (n=4)
mean	4.25	2.73	1.8
stdev	1.23	0.78	0.89

	GSTZ1/rs2270422		
	CC (n=3)	TC (n=10)	TT (n=10)
mean	5.56	1.625	1.188
stdev	1.95	1.43	0.85

C. Results of AUC_{inf} group (hr·ng/ml)

	ABCA4 / rs1165069	
	CC (n=22)	TC (n=1)
mean	908.3	4073.42
stdev	573.63	

	SCL1A6 / rs12461006	
	AG (n=2)	GG (n=21)
mean	3049.93	855.06
stdev	1447.43	529.17

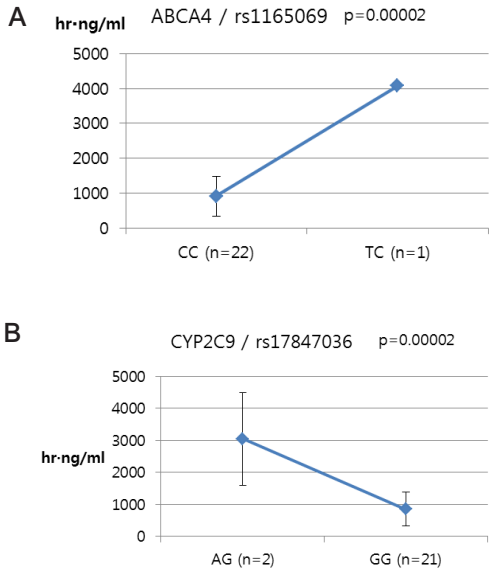

Figure 3. Results of regression analysis of AUC_{inf} group.

found effect on the PK of talniflumate.

In the C_{max} group, CYP2C9 rs17847036 showed statistically significant changes according to genotype; the AG genotype exhibited a greater C_{max} value (Fig. 1 and Table 5).

In the T_{max} group, CYP24A1 rs37875551 with an AA genotype showed more than 2-fold increase in T_{max} than the CC genotype. ABCA4 rs2275034, with a CC genotype showed a 2-fold increase in T_{max} compared to the TT genotype (Fig. 2 and Table 5).

In the AUC_{inf} group, ABCA4 rs11165069 with a TC genotype showed a greater than 4-fold increase in AUC_{inf} than the CC genotype. SLC1A6 rs12461006 with an AG genotype showed a 3-fold increase in AUC_{inf} group compared to the GG genotype (Fig. 3 and Table 5).

Discussion

Many factors affect talniflumate blood concentration, such as diet, age, liver function and disease condition. The genes involved in the process of drug absorption, distribution, metabolism and excretion may affect the PK of talniflumate [10,13,14] but the effect of pharmacogenetics factors has yet to be investigated. In this study, we performed whole genome association studies and regression analysis to investigate the genetic factors influencing the PK of talniflumate.

Regression analysis of significant SNPs and SNPs of candidate genes revealed several SNPs which significantly affect the PK of talniflumate. Interestingly, although SNPs of ABCA4 have low frequency, their effect was significant, resulting in increases in $C_{max,}$ T_{max} and $AUC_{inf.}$ Although these results were only obtained via screening studies, they are the first data concerning the influence of genes, including those responsible for metabolizing enzymes and transporters, on the PK of talniflumate.

We identified the significant association of SNP rs11165069 (P=0.000001) (Fig. 1A) in intron 24 of the transporter gene ABCA4 with the C_{max} and AUC_{inf} of talniflumate. In our study, ABCA4 (rs1165069) with a TC increased the absorption of talniflumate by approximately four times compared to the CC genotype. Therefore, the TC genotype resulted in greater C_{max} (0.000001) and AUC_{inf} (P=0.00002) values. However, because of the low frequency of the TC genotype of ABCA4 rs11165069, further analysis is required. Furthermore, subjects in the T_{max} group with the minor C allele of the intron 37 SNP rs2275034 (P=0.00058) (Fig. 2B) of ABCA4 showed a more than 2-fold delayed T_{max}. From the result, it can be assumed that ABCA4 can affect the absorption of talniflumate and it is possible that the PK parameters can vary according to genotype.

Subjects with the minor A allele of the synonymous exon 2 SNP rs17847036 (P=0.000001) of the phase I enzyme gene CYP2C9 showed an increase in C_{max} (Fig. 1B). It is well understood that CYP2C9 influences the metabolism of drug types [15] and these results indicate that the genotype of CYP2C9 could influence talniflumate plasma concentration.

Subjects with the minor A allele of the synonymous exon 2 SNP rs17847036 (P=0.000001) of the phase I enzyme gene CYP2C9 showed an increase in C_{max} (Fig. 1B). It is well understood that CYP2C9 influences the metabolism of drug types [15] and these results indicate that the genotype of CYP2C9 could influence talniflumate plasma concentration.

Other genes, such as CYP24A1 (rs3787555) and SLC1A6 (rs12461006), showed significant associations with the PK of talniflumate, influencing T_{max} and AUC_{inf}, respectively.

To the best of our knowledge, this is the first whole genome association study regarding the PK of talniflumate. However, the limitations of this study must be acknowledged. As this was a screening study, we performed only univariate analysis between genotypes and PK parameters. Therefore, further confirmative study on the PK of talniflumate with subgroup analysis according to genotypes is required. In conclusion, this study is useful in explaining why the PK of talniflumate may vary depending on the individual. As several additional drugs and enzymes may also influence talniflumate metabolism, we propose that genetic differences are major contributing factors in the PK of this drug.

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Conflicts of interests

-Authors: The authors declare no conflict of interest.
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