Evaluating the association between single nucleotide polymorphisms in the stonin 2 (*STON2*) gene and keratoconus in a Han Chinese population

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Background: A recent genome-wide association study (GWAS) identified a significant association between the single nucleotide polymorphism (SNP) rs2371597 in the stonin 2 gene (*STON2*) and keratoconus (KCTN) susceptibility. The current study further explored the association between *STON2* and KCTN susceptibility in an independent Han Chinese population.

Methods: Three SNPs (rs2371597, rs8004137, and rs8008602) located in the *STON2* gene were examined in 164 Han Chinese patients with KCTN and 239 age- and gender-matched healthy subjects. The TaqMan SNP genotyping assays were performed, and the LDlink, RegulomeDB, and PLINK package were applied for data analyses. The gene expression levels of *STON2* were investigated in various murine organ tissues using quantitative real-time polymerase chain reaction (qRT-PCR).

Results: The SNP rs2371597 was significantly associated with KCTN risk in this Han Chinese population. The frequency of the C allele in KCTN patients was significantly higher than that in healthy subjects [34.8% *vs*. 26.6%; odds ratio (OR) =1.47; 95% confidence interval (CI): 1.08 to 2.02; P=0.01409]. The genotype distribution of the SNP rs2371597 was also significantly different between KCTN patients and controls. The other two genotyped SNPs allele and genotypic frequencies were not remarkably different between the KCTN group and the control group. However, the haplotype CAT formed by the three SNPs was substantially associated with the risk of KCTN (P=0.04101). Also, gene expression pattern analysis showed a relatively higher expression of *STON2* in the cornea in comparison to other tissues.

Conclusions: The current study demonstrated that SNPs in the *STON2* gene were associated with an increased risk of developing KCTN in this Han Chinese population, suggesting that the *STON2* gene may play an important role in the etiology of KCTN.

Keywords: Stonin 2 gene (*STON2*); keratoconus (KCTN); single nucleotide polymorphism (SNP); association study; Han Chinese population

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Introduction

Keratoconus (KCTN) is a major indication for cornea transplantation in developed countries (1). It is characterized by progressive corneal thinning and conical protrusion, leading to severe visual disturbances (2). KCTN has an average prevalence of 1:2,000 globally but varies across different ethnicities. Asians are reported to have a remarkably higher incidence of KCTN than Caucasians, suggesting that ethnic differences might have considerable impacts on this disease (3). The clinical management of KCTN varies depending on the clinical stages. In the early stages, collagen UV cross-linking or contact lenses can stabilize the cornea biomechanics, thereby controlling KCTN progression (4). Therefore, early diagnosis of KCTN based on a clear understanding of its etiology may help guide clinical interventions.

The etiology of KCTN is multifactorial, combining both genetic and non-genetic factors. Environmental factors including UV exposure (5), contact lens wear (6), atopy (7), and constant eye rubbing (8) can all act as triggers of the condition in genetically predisposed individuals. However, more importantly, a strong genetic susceptibility underlying KCTN pathogenesis has been clearly demonstrated in familial aggregation studies (9,10), twins studies (11), linkage analyses, and genome-wide association studies (GWASs) (4,12). So far, over 150 single polynucleotide polymorphisms (SNPs) in more than 60 loci have been identified to be linked with KCTN susceptibility (13-20). Some SNPs were discovered in GWASs examining central corneal thickness (CCT), which is a critical quantitative trait locus (QTL) associated with increased KCTN risk. These identified loci include *MDPZ-NF1B*, *FOXO1*, *FND3B*, *COL4A3*, *COL4A4*, and *COL5A1* (21-23). While many of these reported loci have been detected in several other ethnicities, inconsistent results exist across different cohorts, rendering their contribution to KCTN risk inconclusive (18,24-27).

Recent GWASs identified the stonin 2 gene (*STON2*) as a susceptibility gene for CCT (21,23). *STON2* encodes a membrane protein that is involved in regulating endocytotic complexes. It is one of the clathrin-associated adaptor molecules which ensures that specific proteins are internalized (28). Hosoda *et al.* (23) discovered that the *STON2* allele rs2371597, which was associated with increased CCT, was also associated with an increased risk of KCTN in the Japanese population. However, due to the underlying genetic heterogeneity of KCTN susceptibility, it remains unknown whether this SNP also confers the risk of KCTN susceptibility in other cohorts. It is also possible that other additional SNPs located in the same locus might contribute to KCTN susceptibility. Therefore, an extensive investigation on this locus is warranted. Herein, a replication study on the reported SNP rs2371597, together with two potential regulatory SNPs within the same locus, was carried out to explore the contribution of the *STON2* locus to KCTN susceptibility in an independent Han Chinese population.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/atm-20-6654).

Methods

Subjects

A total of 164 Han Chinese patients with KCTN were randomly selected from the Department of Ophthalmology at the Eye and ENT Hospital of Fudan University between October 2015 and November 2018. All patients were self-reported as Han Chinese ethnicity. Patients were diagnosed with KCTN if they presented with at least one KCTN symptom, such as corneal stromal thinning, Fleischer's ring, Vogt's striae, or Munson's sign, and videokeratography showed refractive errors or signs. Patients with KCTN and syndromic disease (such as Down's syndrome and Leber optic atrophy) were excluded. A total of 239 healthy individuals with no signs of ocular disorder were included as the control group.

The study was conducted following the Declaration of Helsinki (as revised in 2013) and has been approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University (2015044-1). Informed consent was obtained from all participants.

Genomic DNA extraction

Genomic DNA was extracted from the peripheral blood monocytes using the QIAGEN FlexiGene DNA kit (Qiagen, Hamburg, Germany) following the manufacturer's protocol. DNA concentration was measured with a NanoDrop spectrophotometer (NanoDrop Technologies, DE, USA).

Genotyping of SNPs

Three SNPs (rs2371597, rs8004137, and rs8008602) located in the *STON2* gene were selected for investigation, utilizing

Table 1 Basic characteristics of patients with KCTN and healthy controls

Features	KCTN (n=164)	Controls (n=239)
Gender (female/male)	40/124	89/150
Average age (years)*	23.64±6.17	30.02±5.36
Age range (years)	13–48	15–41
Disease onset age (years)*	21.04±4.72	NA
Visual activity*	OS: 0.53±0.35; OD: 0.32±0.27	NA

*, average age and patients' visual activity are presented as mean ± SD. KCTN, keratoconus; OS, oculus sinister; OD, oculus dexter; SD, standard deviation.

the TaqMan SNP Genotyping Assays (C__2791741_10 for rs2371597, C__29283383_10 for rs8004137, and C__29283385_10 for rs8008602). The genotyping assay was performed using real-time polymerase chain reaction (PCR; Applied Biosystems VII, USA) with the standard genotyping protocol. Each reaction contained 2 μL DNA template, 5 μL of MasterMix buffer (2× concentration; ThermoFisher), 2.5 μL double distilled water (ddH_2O), and 0.25 μL probe (40× concentration). Genotypes were automatically determined using the ratio of VIC and FAM fluorescent signals from the TaqMan assay.

Data analysis

Data analysis was conducted with the PLINK package (29). SNP frequency in case and control group was tested for departure from HWE with an exact test. A χ^2-test was used to calculate the difference in SNP allele frequency between cases and controls. The logistic regression model, adjusted by gender and age, was used to calculate the odds ratios (ORs) and the 95% confidence intervals (CIs). P values less than 0.05 were considered statistically significant. The LDlink package was used to calculate the linkage disequilibrium (LD) within SNPs (30). The RegulomeDB (31) tool was applied to annotate SNPs with known or predicted regulatory elements. Meta-analysis was conducted by weighting effect size estimates with the inverse of the standard errors. The SNP association's statistical significance was calculated using the Z-test, and a pooled P value <0.05 was considered significant.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Male Balb/c mice (6–8 weeks old) were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences (CAS). Mice were anesthetized by pentobarbital sodium and perfused by intracardiac injection of phosphate-buffered saline (PBS). Murine organ tissues, including the cornea, retina, heart, liver, lung, kidney, brain, and lymph nodes, were isolated. Total RNA was extracted with RNA Simple Total RNA Kit (TIANGEN, Beijing, China). The first-strand cDNA was reverse transcribed with the FastKing RT Kit (TIANGEN, Beijing, China). Quantitative real-time PCR was performed with a QuantiNova SYBR Green PCR Kit (Qiagen, Hamburg, Germany) according to the manufacturer's protocols. Primer sequences used are listed below.

Mouse glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene:

Forward 5'-CATCACTGCCACCCAGAAGACTG-3',
Reverse 5'-ATGCCAGTGAGCTTCCCGTTCAG-3';
Mouse *STON2* gene:
Forward 5'-GTATGAGCACGCCTTCAACTCC-3',
Reverse 5'- GGCAAATCTGGAAGGCACTTCC-3'.

All reactions were performed in quadruplicate, and the experiments were replicated three times independently. Relative gene expression levels were determined using the delta Ct method. Animal experiments were performed under a project license (2015044-2) granted by the Ethics Committee of the Eye and ENT Hospital of Fudan University, in compliance with the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research.

Results

A total of 164 patients with KCTN and 239 healthy subjects were recruited in this study. The average age of patients with KCTN was 23.64±6.17 years, and 75.6% of this cohort were male (*Table 1*). The healthy controls had an average

Table 2 A summary of the three SNPs in the *STON2* gene

SNP	Coordinate (GRCh38.p12)	Allele	RegulomeDB score	HWE P values	
				Cases	Controls
rs2371597*	Chr14:81407033	C/G	2b (likely to affect binding)	0.8604	0.865
rs8004137	Chr14:81418513	A/G	2b (likely to affect binding)	0.6990	0.7294
rs8008602	Chr14:81423827	T/A	2b (likely to affect binding)	0.5767	0.8673

*, shown as the reported SNP in the Nagahama study (23). SNP, single nucleotide polymorphisms; HWE, Hardy-Weinberg equilibrium.

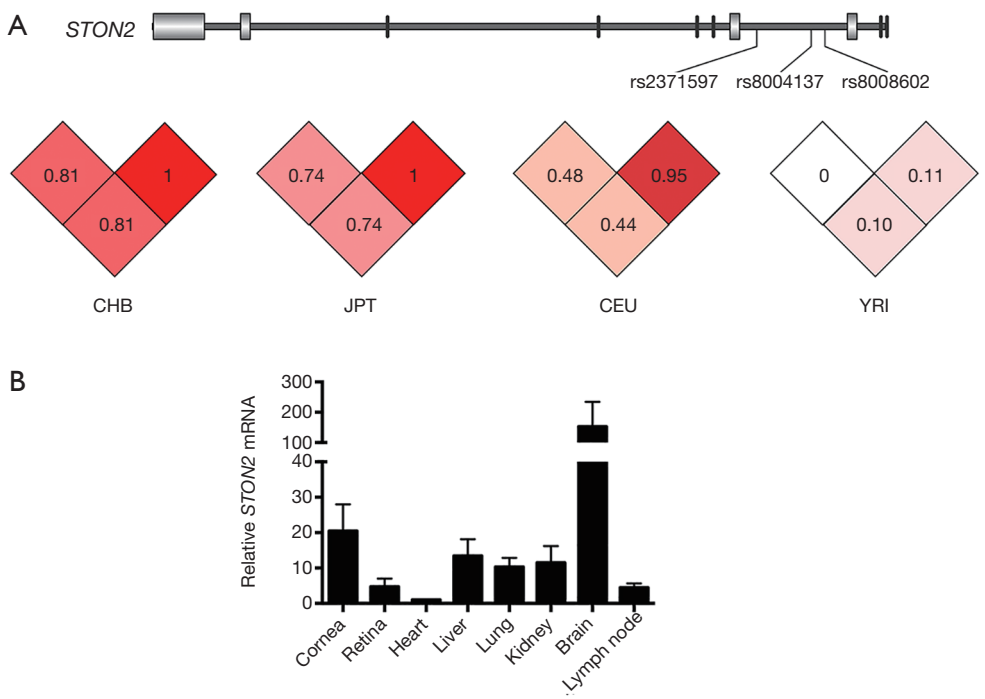

Figure 1 LD of the three SNPs in the *STON2* gene and *STON2* mRNA expression in various organ tissues. (A) LD of the three SNPs in the *STON2* gene, shown as pairwise r^2 values in CHB (Chinese Han in Beijing), JPT (Japanese in Tokyo), CEU (Caucasian), and YRI (Yoruba in Ibadan, Nigeria), from the 1000G Project Phase 3. (B) Relative *STON2* mRNA expression in various adult mouse tissues measured by qRT-PCR. Data is shown as mean ± SD. The experiments were performed three times, and each experiment included five mice. LD, linkage disequilibrium; SNP, single nucleotide polymorphism; qRT-PCR, quantitative real-time polymerase chain reaction; SD, standard deviation.

age of 30.02±5.36 years, and 62.7% were male. These characteristics were comparable to that of the KCTN group.

Three SNPs in the *STON2* gene were genotyped. SNP rs2371597 has been shown in a GWAS to affect KCTN susceptibility in the Japanese population (23). The current study aimed to determine whether it had a similar effect on the Han Chinese population. The other two SNPs were selected based on in-silico functional annotation. Briefly, SNPs showing LD (r^2≥0.8 in East Asian cohort from 1000 Genomes Project Phase 3) with rs2371597 were subjected to RegulomeDB annotation to examine if they overlapped with some known or predicted regulatory elements. Consequently, two other SNPs, namely rs8004137 and rs8008602, were selected for replication, given their predicted roles in affecting transcription factor binding (*Table 2*, *Figure 1A*).

The average genotyping call rate was 95.0% for the three genotyped SNPs, and all satisfied the Hardy-Weinberg equilibrium (HWE) in the controls (*Table 2* and Table S1).

Table 3 Association analyses of the three SNPs in the *STON2* gene

SNP	Allele	MAF (case), %	MAF (control), %	χ^2	P value	OR (95% CI)	Meta P value	Meta OR (95% CI)
rs2371597	C/G	34.8	26.6	6.027	0.01409	1.47(1.08–2.02)	0.005694	1.37 (1.11–1.62)
rs8004137	A/G	30.3	26.6	1.222	0.2690	1.20 (0.88–1.67)	–	–
rs8008602	T/A	31.2	27.6	1.198	0.2737	1.19 (0.87–1.64)	–	–

95% CI: lower/upper bound of 95% CI for OR; meta-analysis was performed on association results from a previous Japanese cohort (23) and this Chinese cohort. SNP, single nucleotide polymorphisms; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

Table 4 Association analyses on SNP genotypes with KCTN susceptibility

SNP/group	Frequency (%)			P	Dominant model		Recessive model	
	Genotype 1	Genotype 2	Genotype 3		OR (95% CI)	P	OR (95% CI)	P
rs2371597	CC	CG	GG	0.05007	CC&CG *vs*. GG		CC *vs*. CG&GG	
Cases	11.6	46.4	41.9		1.59 (1.06–2.41)	0.02601	1.85 (0.90–3.79)	0.08984
Controls	6.6	39.8	53.5					
rs8004137	AA	AG	GG	0.4265	AA&AG *vs*. GG		AA *vs*. AG&GG	
Cases	10.0	40.7	49.3		1.17 (0.77–1.77)	0.4645	1.62 (0.76–3.46)	0.2106
Controls	6.4	40.4	53.2					
rs8008602	TT	TA	AA	0.4241	TT&TA *vs*. AA		TT *vs*. TA&AA	
Cases	10.8	40.7	48.4		1.15 (0.77–1.73)	0.4896	1.59 (0.78–3.24)	0.2033
Controls	7.1	40.9	52.0					

SNP, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

The association result showed that only the C allele of SNP rs2371597 was significantly associated with KCTN susceptibility (OR =1.47, 95% CI: 1.08 to 2.02, P=0.01409, P=0.04227 with Bonferroni correction, *Table 3*). A meta-analysis on the association results of the Japanese cohort (23) and this Chinese cohort further supported the association of rs2371597 with KCTN risk (P_meta =0.005694, OR_meta =1.37, 95% CI: 1.11 to 1.62). Genotype association analysis showed that, within the dominant model, genotype CC and CG of rs2371597 revealed an OR of 1.59 (95% CI: 1.06 to 2.41, P=0.02601, *Table 4*). The association results between the *STON2* gene haplotype and the risk of KCTN are listed in *Table 5*. The haplotype CAT was significantly associated with the risk of KCTN (P=0.04101). The other two SNPs allele and genotypic frequencies did not reveal substantial differences between the cases and the controls.

Furthermore, gene expression pattern analysis among different murine organ tissues showed that *STON2* was widely expressed in all mouse tissues examined and presented a relatively higher expression in the cornea (*Figure 1B*).

Discussion

This study demonstrated the association of the *STON2* locus with KCTN susceptibility in a Han Chinese population. This concurs with a previous study in a Japanese population (23), and meta-analysis data supported this observation (P_meta 0.005694, OR_meta =1.37, 95% CI: 1.11 to 1.62). These results support the notion that *STON2* contributes to KCTN susceptibility among Asians.

Segregation analyses have suggested that KCTN is a complicated trait affected by many factors, including genes, variable penetrance, and environmental triggers (32). Although some efforts have been made (4) to explore the genetic factors contributing to KCTN risk, the understanding of KCTN genetics is still in its infancy. In contrast to other complex diseases that usually recruit over thousands of participants, the sample sizes in GWASs examining KCTN have generally been quite small, thus limiting the statistical power for detecting novel association signals. Alternatively, the involvement of the endophenotype CCT is efficient in identifying additional susceptibility genes for KCTN (21). CCT is a highly heritable

Table 5 Results of the *STON2* haplotype analysis

Haplotype (rs2371597/rs8004137/rs8008602)	MAF (case), %	MAF (control), %	χ^2	P value	OR (95% CI)
C/A/T	27.9	21.8	4.175	0.04101	1.46 (0.89–2.32)
G/A/T	2.9	4.5	1.088	0.2969	0.67 (0.23–1.94)
C/G/A	6.1	4.8	0.7589	0.3837	1.36 (0.57–3.31)
G/G/A	62.2	68.1	2.753	0.09708	0.79 (0.50–1.18)

MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

quantitative trait, with heritability estimates ranging from 0.68 to 0.95 (33,34). Thinner CCT is often observed in common ocular diseases such as KCTN (35). With such an approach, several recent GWASs have successfully identified that some of these CCT loci were also associated with KCTN susceptibility. *STON2* was one such gene, and therefore it was the focus of this current study. The successful validation of the association between *STON2* SNPs and KCTN risk in both the Japanese and the Han Chinese populations supports future investigations into other newly defined CCT loci and their association with KCTN susceptibility (21).

The current study demonstrated the association between *STON2* and KCTN susceptibility among Asians. Interestingly, the effect size of SNP rs2371597 differed between the Chinese and the Japanese cohorts (OR =1.47 in the Chinese cohort, and OR =1.27 in the Japanese cohort), which may be partially due to the different minor allele frequencies between the two cohorts (C =32.5% in the Chinese cohort and C =25.5% in the Japanese cohort). Thus, it would be of interest to further examine the contribution of *STON2* SNP(s) to KCTN susceptibility in other ethnicities, such as the Caucasian population. Furthermore, three SNPs with predicted roles in affecting binding were genotyped. However, two of them did not show any substantial association with KCTN in this cohort, although some trends were detected. Herein, in-depth replication studies with larger sample size are warranted. Lastly, the relationship between genetic variants and cellular abnormalities is still unclear. A previous Japanese study showed strong expression of *STON2* in basal cells compared to superficial cells in the corneal epithelium, with minimal expression in the corneal stroma and endothelium layer. Our findings also highlighted a relatively high expression of *STON2* in the cornea compared to other organ tissues. Deficiencies in *STON2* may increase cell vulnerability to physical damage or immunological changes, thereby affecting KCTN development via interacting with extracellular matrix modeling. However, direct biological evidence will be required to confirm this hypothesis. Future systematically functional investigations should be conducted to elucidate further the mechanisms by which susceptible variants affect *STON2* gene function and finally contribute to KCTN development.

In conclusion, this study explored the association between SNPs in the *STON2* gene and KCTN susceptibility in a Han Chinese population. The results verified that the *STON2* gene contributes to KCTN susceptibility. This study provided novel insights related to the genetic basis underlying KCTN pathogenesis, which will pave the way for future advances in prevention and treatment.

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Footnote

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conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Eye and ENT Hospital of Fudan University (2015044-1) and informed consent was obtained from all participants. Animal experiments were performed under a project license (2015044-2) granted by the Ethics Committee of the Eye and ENT Hospital of Fudan University, in compliance with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

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Supplementary

Table S1 Individual genotype for SNPs rs2371597, rs8004137, rs8008602 of the included 164 cases and 239 controls

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
11F	Control	C	G	A	G	T	A
11M	Control	G	G	G	G	A	A
12F	Control	C	G	A	G	T	A
12M	Control	G	G	G	G	A	A
13M	Control	C	G	A	G	T	A
14F	Control	G	G	A	G	T	A
14M	Control	C	C	A	G	T	A
15F	Control	G	G	0	0	0	0
15M	Control	G	G	G	G	A	A
16F	Control	G	G	G	G	A	A
16M	Control	G	G	G	G	A	A
18F	Control	C	C	A	G	T	A
18M	Control	G	G	G	G	A	A
19F	Control	G	G	G	G	A	A
19M	Control	C	C	A	G	T	A
1F	Control	C	G	0	0	T	A
1M	Control	C	G	G	G	T	A
20F	Control	G	G	G	G	A	A
20M	Control	C	G	G	G	A	A
21F	Control	C	G	A	G	T	A
21M	Control	G	G	0	0	0	0
22M	Control	C	G	G	G	A	A
23F	Control	G	G	G	G	A	A
23M	Control	G	G	G	G	A	A
24F	Control	C	G	A	G	T	A
24M	Control	C	G	G	G	T	A
25F	Control	G	G	G	G	A	A
25M	Control	G	G	G	G	A	A
26F	Control	C	C	A	G	T	A
26M	Control	G	G	G	G	A	A
27F	Control	G	G	0	0	0	0
27M	Control	G	G	G	G	A	A
28F	Control	G	G	G	G	A	A
28M	Control	G	G	G	G	A	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
29F	Control	C	G	A	G	T	A
2F	Control	G	G	G	G	A	A
2M	Control	C	G	A	G	T	A
30F	Control	G	G	0	0	A	A
30M	Control	G	G	G	G	A	A
31F	Control	C	G	A	A	A	A
31M	Control	G	G	A	A	T	T
33F	Control	C	C	A	A	T	T
33M	Control	C	G	A	G	T	A
36F	Control	C	G	A	A	T	T
36M	Control	C	G	A	G	T	A
37F	Control	C	C	G	G	T	T
37M	Control	G	G	G	G	T	T
38F	Control	0	0	0	0	A	A
38M	Control	G	G	G	G	A	A
39F	Control	0	0	0	0	T	A
39M	Control	G	G	G	G	A	A
3F	Control	G	G	G	G	A	A
3M	Control	G	G	G	G	A	A
40F	Control	G	G	A	G	T	T
40M	Control	C	G	A	G	T	A
41F	Control	G	G	G	G	A	A
41M	Control	C	G	A	G	T	A
42F	Control	G	G	G	G	A	A
42M	Control	C	G	A	G	T	A
43F	Control	G	G	G	G	A	A
43M	Control	G	G	G	G	A	A
44F	Control	G	G	G	G	A	A
44M	Control	G	G	G	G	A	A
45F	Control	C	G	A	G	0	0
45M	Control	C	G	A	G	T	A
46F	Control	G	G	G	G	A	A
46M	Control	G	G	G	G	A	A
47F	Control	C	G	A	G	T	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
47M	Control	G	G	G	G	A	A
48F	Control	C	G	A	G	T	A
48M	Control	G	G	G	G	A	A
4F	Control	C	G	A	G	T	A
4M	Control	C	G	A	G	T	A
50F	Control	C	G	A	G	T	A
50M	Control	G	G	G	G	A	A
51F	Control	C	G	G	G	A	A
51M	Control	C	G	G	G	A	A
52F	Control	G	G	G	G	A	A
52M	Control	C	G	A	G	T	A
54F	Control	G	G	0	0	A	A
54M	Control	G	G	G	G	A	A
55F	Control	G	G	G	G	A	A
55M	Control	G	G	G	G	A	A
56M	Control	G	G	G	G	A	A
58F	Control	G	G	G	G	A	A
58M	Control	C	G	A	G	T	A
59F	Control	C	G	A	G	T	A
59M	Control	G	G	G	G	A	A
5F	Control	G	G	G	G	A	A
5M	Control	G	G	G	G	A	A
60M	Control	G	G	0	0	A	A
61F	Control	C	G	G	G	A	A
61M	Control	G	G	G	G	A	A
62F	Control	C	C	A	A	T	T
62M	Control	C	G	A	G	T	A
66F	Control	G	G	G	G	A	A
66M	Control	G	G	A	G	T	A
67F	Control	C	G	G	G	A	A
67M	Control	G	G	G	G	A	A
68F	Control	G	G	G	G	A	A
68M	Control	G	G	G	G	A	A
69F	Control	G	G	G	G	A	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
69M	Control	G	G	G	G	A	A
6F	Control	C	G	A	A	T	T
6M	Control	C	G	A	G	T	A
70F	Control	C	G	A	G	T	A
70M	Control	C	G	A	G	T	A
71F	Control	G	G	G	G	A	A
71M	Control	C	C	A	A	T	T
72F	Control	G	G	G	G	A	A
72M	Control	C	C	A	G	T	A
73F	Control	C	G	A	G	T	A
73M	Control	G	G	G	G	A	A
74F	Control	G	G	G	G	A	A
74M	Control	G	G	A	G	T	A
75F	Control	C	G	A	G	T	A
75M	Control	C	G	G	G	A	A
76F	Control	C	G	A	G	T	A
76M	Control	G	G	G	G	A	A
77F	Control	C	G	A	G	T	A
77M	Control	G	G	G	G	A	A
78F	Control	C	G	G	G	A	A
78M	Control	C	G	A	G	T	A
79F	Control	G	G	G	G	A	A
79M	Control	G	G	G	G	A	A
7F	Control	C	G	A	G	T	A
7M	Control	C	G	A	G	T	A
80F	Control	C	G	A	G	T	A
80M	Control	G	G	G	G	A	A
81F	Control	C	C	A	A	T	T
81M	Control	G	G	A	G	T	A
82F	Control	G	G	A	G	T	A
82M	Control	C	G	A	G	T	A
8F	Control	C	G	A	G	T	A
9F	Control	G	G	0	0	A	A
9M	Control	C	G	A	G	T	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
CD10F	Control	C	G	A	G	T	A
CD10M	Control	G	G	G	G	A	A
CD7F	Control	G	G	0	0	A	A
CD7M	Control	C	G	A	G	T	A
CD81F	Control	G	G	G	G	A	A
CD81M	Control	C	C	A	A	T	T
HC001	Control	0	0	0	0	T	A
HC002	Control	0	0	0	0	0	0
HC003	Control	G	G	A	G	T	A
HC004	Control	G	G	G	G	A	A
HC005	Control	G	G	G	G	A	A
HC006	Control	C	G	A	A	T	T
HC007	Control	C	G	A	G	T	A
HC008	Control	C	G	A	G	T	A
HC009	Control	G	G	A	G	T	A
HC010	Control	C	G	G	G	T	A
HC011	Control	G	G	G	G	A	A
HC012	Control	G	G	G	G	A	A
HC013	Control	G	G	G	G	A	A
HC014	Control	G	G	G	G	A	A
HC015	Control	C	G	A	G	T	A
HC016	Control	G	G	G	G	A	A
HC017	Control	C	G	G	G	T	A
HC018	Control	C	G	G	G	A	A
HC019	Control	G	G	G	G	A	A
HC020	Control	C	G	A	G	T	A
HC021	Control	C	G	A	G	T	A
HC022	Control	G	G	G	G	A	A
HC023	Control	C	G	A	G	T	A
HC024	Control	G	G	0	0	A	A
HC025	Control	C	G	A	A	T	T
HC026	Control	C	G	A	G	T	A
HC027	Control	C	C	A	A	T	T
HC028	Control	G	G	G	G	A	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
HC029	Control	C	G	A	G	T	A
HC030	Control	C	G	A	G	T	A
HC031	Control	C	G	A	G	T	A
HC032	Control	C	G	G	G	A	A
HC033	Control	G	G	G	G	A	A
HC034	Control	G	G	G	G	A	A
HC035	Control	G	G	G	G	A	A
HC036	Control	G	G	G	G	A	A
HC037	Control	G	G	G	G	A	A
HC038	Control	G	G	G	G	A	A
HC039	Control	G	G	G	G	A	A
HC040	Control	G	G	A	G	T	A
HC041	Control	G	G	G	G	A	A
HC042	Control	G	G	G	G	A	A
HC043	Control	0	0	0	0	0	0
HC044	Control	C	C	A	A	T	T
HC045	Control	G	G	G	G	A	A
HC046	Control	0	0	0	0	0	0
HC047	Control	G	G	G	G	A	A
HC048	Control	C	G	A	G	T	A
HC049	Control	0	0	0	0	0	0
HC050	Control	0	0	A	G	0	0
HC051	Control	G	G	A	G	T	A
HC052	Control	C	G	A	G	T	A
HC053	Control	G	G	G	G	A	A
HC054	Control	G	G	G	G	A	A
HC055	Control	C	G	G	G	A	A
HC056	Control	G	G	G	G	A	A
HC057	Control	C	G	A	G	T	A
HC058	Control	G	G	G	G	A	A
HC059	Control	G	G	G	G	A	A
HC060	Control	G	G	G	G	A	A
HC061	Control	G	G	G	G	A	A
HC062	Control	C	G	A	G	T	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
HC063	Control	C	G	A	G	T	A
HC064	Control	C	G	A	G	T	A
HC065	Control	G	G	G	G	A	A
HC066	Control	C	C	A	G	T	A
HC067	Control	G	G	G	G	A	A
HC068	Control	G	G	G	G	A	A
HC069	Control	C	C	A	A	T	T
HC070	Control	G	G	G	G	A	A
HC071	Control	C	G	G	G	A	A
HC072	Control	G	G	A	G	T	A
HC073	Control	G	G	G	G	A	A
HC074	Control	G	G	G	G	A	A
HC075	Control	G	G	G	G	0	0
HC076	Control	C	G	G	G	A	A
HC077	Control	G	G	A	G	T	A
HC078	Control	C	G	A	G	T	A
HC079	Control	C	G	A	G	T	A
HC080	Control	G	G	A	G	T	A
HC081	Control	C	G	A	G	T	A
HC082	Control	C	G	G	G	A	A
HC083	Control	C	G	A	G	T	A
HC084	Control	C	G	A	G	T	A
HC085	Control	C	G	A	G	T	A
HC086	Control	G	G	G	G	A	A
K1	Case	C	G	A	G	T	A
K10	Case	C	G	A	G	T	A
K2	Case	C	G	A	G	T	A
K3	Case	C	G	A	G	T	A
K4	Case	C	G	A	G	T	A
K5	Case	C	G	A	G	T	A
K6	Case	0	0	0	0	0	0
K7	Case	C	G	A	G	T	A
K8	Case	C	G	0	0	0	0
K9	Case	G	G	0	0	0	0

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
KC001	Case	C	G	A	G	T	A
KC002	Case	G	G	G	G	A	A
KC003	Case	C	G	A	G	T	A
KC004	Case	C	G	A	G	T	A
KC005	Case	G	G	G	G	A	A
KC006	Case	G	G	A	G	T	A
KC007	Case	C	G	A	G	T	A
KC008	Case	G	G	G	G	A	A
KC009	Case	C	G	A	G	T	A
KC010	Case	G	G	G	G	A	A
KC011	Case	C	C	A	A	T	T
KC012	Case	C	G	G	G	A	A
KC013	Case	C	G	G	G	A	A
KC014	Case	G	G	G	G	A	A
KC015	Case	G	G	G	G	A	A
KC016	Case	G	G	G	G	A	A
KC017	Case	G	G	G	G	A	A
KC018	Case	C	G	A	G	T	A
KC019	Case	C	C	A	A	T	T
KC020	Case	G	G	G	G	A	A
KC021	Case	G	G	G	G	A	A
KC022	Case	G	G	A	G	T	A
KC023	Case	C	C	A	G	T	A
KC024	Case	C	C	A	A	T	T
KC025	Case	C	G	A	G	T	A
KC026	Case	G	G	G	G	A	A
KC027	Case	C	C	0	0	T	T
KC028	Case	C	G	A	G	T	A
KC029	Case	C	G	A	A	T	T
KC030	Case	G	G	G	G	A	A
KC031	Case	C	G	A	G	T	A
KC032	Case	C	G	A	A	T	T
KC033	Case	G	G	G	G	A	A
KC034	Case	C	G	A	G	T	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
KC035	Case	C	G	A	A	T	T
KC037	Case	C	G	A	G	T	A
KC038	Case	C	C	A	A	T	T
KC039	Case	G	G	G	G	A	A
KC040	Case	C	G	G	G	A	A
KC041	Case	G	G	A	G	T	A
KC042	Case	C	C	A	A	T	T
KC043	Case	G	G	G	G	A	A
KC044	Case	C	C	A	G	T	A
KC045	Case	G	G	G	G	A	A
KC046	Case	G	G	G	G	A	A
KC047	Case	C	G	G	G	A	A
KC048	Case	C	G	A	G	T	A
KC049	Case	C	G	A	G	T	A
KC050	Case	G	G	G	G	A	A
KC051	Case	G	G	G	G	A	A
KC052	Case	C	C	A	G	T	A
KC053	Case	G	G	G	G	A	A
KC054	Case	G	G	G	G	A	A
KC055	Case	G	G	G	G	A	A
KC056	Case	G	G	G	G	A	A
KC058	Case	G	G	G	G	A	A
KC059	Case	C	G	A	G	T	A
KC060	Case	C	G	G	G	A	A
KC061	Case	G	G	G	G	A	A
KC062	Case	C	G	A	G	T	A
KC063	Case	C	G	A	G	T	A
KC064	Case	G	G	G	G	A	A
KC065	Case	G	G	G	G	A	A
KC066	Case	G	G	G	G	A	A
KC067	Case	G	G	G	G	A	A
KC068	Case	C	G	A	G	T	A
KC069	Case	G	G	G	G	A	A
KC070	Case	C	G	G	G	A	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
KC071	Case	G	G	A	G	T	A
KC072	Case	C	G	A	G	T	A
KC073	Case	C	G	A	G	T	A
KC074	Case	C	G	A	A	T	T
KC075	Case	C	G	A	G	T	A
KC076	Case	C	G	A	G	T	A
KC077	Case	G	G	G	G	A	A
KC078	Case	C	C	A	A	T	T
KC079	Case	G	G	G	G	A	A
KC080	Case	G	G	G	G	A	A
KC081	Case	G	G	G	G	A	A
KC082	Case	G	G	G	G	A	A
KC083	Case	C	C	G	G	A	A
KC084	Case	C	G	0	0	T	A
KC085	Case	C	G	A	G	T	A
KC086	Case	G	G	G	G	A	A
KC087	Case	C	C	G	G	T	T
KC088	Case	G	G	G	G	A	A
KC089	Case	C	G	A	G	T	T
KC090	Case	C	G	A	G	T	A
KC091	Case	C	C	A	A	T	T
KC092	Case	C	G	G	G	A	A
KC093	Case	C	C	A	A	T	A
KC094	Case	C	C	0	0	T	A
KC095	Case	0	0	0	0	A	A
KC096	Case	0	0	0	0	A	A
KC097	Case	0	0	0	0	A	A
KC098	Case	0	0	0	0	0	0
KC099	Case	0	0	0	0	T	A
KC100	Case	G	G	G	G	A	A
KC101	Case	0	0	0	0	0	0
KC102	Case	0	0	0	0	0	0
KC103	Case	G	G	G	G	A	A
KC104	Case	0	0	0	0	0	0

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
KC105	Case	C	G	A	G	T	A
KC106	Case	G	G	G	G	A	A
KC107	Case	C	G	A	G	T	A
KC108	Case	C	G	A	G	T	A
KC109	Case	C	G	A	G	T	A
KC110	Case	G	G	G	G	A	A
KC111	Case	G	G	G	G	A	A
KC112	Case	G	G	G	G	A	A
KC115	Case	C	G	A	G	T	A
KC116	Case	C	G	A	G	T	A
KC117	Case	C	G	A	G	T	A
KC118	Case	G	G	G	G	A	A
KC119	Case	C	G	A	G	T	A
KC120	Case	G	G	G	G	A	A
KC121	Case	C	G	A	G	T	A
KC122	Case	G	G	G	G	A	A
KC123	Case	G	G	G	G	A	A
KC124	Case	C	G	G	G	A	A
KC125	Case	G	G	G	G	A	A
KC126	Case	G	G	G	G	A	A
KC127	Case	C	G	A	G	T	A
KC129	Case	C	G	G	G	A	A
KC130	Case	G	G	G	G	A	A
KC131	Case	C	G	A	G	T	A
KC132	Case	C	C	A	A	T	T
KC133	Case	C	C	A	A	T	T
KC134	Case	C	G	A	G	T	A
KC135	Case	C	G	A	G	T	A
KC136	Case	G	G	G	G	A	A
KC137	Case	C	G	A	G	T	A
KC138	Case	C	C	A	A	T	T
KC139	Case	G	G	G	G	A	A
KC140	Case	G	G	G	G	A	A
KC141	Case	G	G	G	G	A	A

Table S1 (*continued*)

http://dx.doi.org/10.21037/atm-20-6654

Table S1 (*continued*)

Sample ID	Case/control	rs2371597		rs8004137		rs8008602	
		Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
KC142	Case	C	G	A	G	T	A
KC143	Case	C	G	G	G	A	A
KC144	Case	C	G	A	G	T	A
KC145	Case	C	G	A	G	T	A
KC146	Case	C	G	A	G	T	A
KC147	Case	C	G	A	G	T	A
KC148	Case	C	G	A	G	T	A
KC149	Case	C	G	A	G	T	A
KC150	Case	G	G	G	G	A	A
KC151	Case	G	G	G	G	A	A
KC152	Case	G	G	G	G	A	A
KC153	Case	G	G	G	G	A	A
KC154	Case	C	G	A	G	T	A
KC155	Case	C	G	G	G	A	A
KC156	Case	G	G	G	G	A	A
KC157	Case	G	G	G	G	A	A
KC158	Case	C	G	G	G	A	A
KC159	Case	C	G	A	G	T	A
N1	Control	C	G	A	G	T	A
N10	Control	G	G	0	0	0	0
N11	Control	0	0	A	G	T	A
N2	Control	0	0	0	0	0	0
N3	Control	C	G	A	G	T	A
N4	Control	0	0	A	G	T	A
N5	Control	0	0	0	0	0	0
N6	Control	C	G	A	G	T	A
N7	Control	C	G	A	G	T	A
N8	Control	C	G	A	G	T	A
N9	Control	0	0	0	0	0	0

http://dx.doi.org/10.21037/atm-20-6654