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Variants at potential loci associated with Sjogren's syndrome in Koreans: A genetic association study

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ABSTRACT

Sjogren's syndrome (SS), a chronic autoimmune disease, typically causes or involves inflammation in the salivary and lacrimal glands. Although recent genetic association studies have contributed to the discovery of SS susceptible genes, few studies have reported on the Korean population. Here, we did a genetic association study of SS in Korean patients using whole-exome sequencing data of 15 patients and 100 healthy controls. In addition to confirming previously described SS susceptibility loci *MSH5* ($p = 1.67 \times 10–5$) and *RELN* ($p = 4.91 \times 10–6$), we also validated *PRAMEF13* ($p = 2.28 \times 10–5$), *TARBP1* ($p = 1.87 \times 10–5$), *UGT2B28* ($p = 1.33 \times 10–5$), *TRBV5-6* ($p = 2.27 \times 10–5$) and *NAPB* ($p = 3.73 \times 10–5$) as novel susceptibility loci for SS. Furthermore, we identified *UGT2B28*, *TARBP1* and *PRAMEF13* as associated with human immune function. These findings may provide useful insight into to the pathways and pathogenesis contributing to SS susceptibility in the Korean population.

1. Introduction

Autoimmune diseases result from an organism's own antibodies working against healthy tissues. Sjogren's syndrome (SS), a common autoimmune systemic disease, causes chronic inflammation in both the salivary and lacrimal glands, leading to dry eyes and mouth. It is expressed nine times more commonly in women than in men, particularly among women between 30 and 50 years old. About 2% of adults suffer from SS worldwide [1], half of them being late diagnosed.

In 1937, Lisch suggested a hereditary link in SS [2] for the first time and Chang-Fu Kuo et al. observed an increasing risk of SS development in first-degree relatives of SS patients, as well as of other autoimmune diseases [3]. By the late 1970's, the relationship between SS pathogenesis and immune response genes was recognized [4–6]. In recent decades, several studies have identified genes associated with SS using a simple candidate gene approach; more recently, genome-wide association studies (GWAS) have enabled much more specific genetic analyses. Larger scale studies have started to reveal race-dependent SS biomarkers, as seen in GWAS studies including over 10,000 subjects from European [7] and Han Chinese populations [8]. As one would expect, some genes which were significant in Europeans were not significant in Chinese, suggesting that the two populations may express different SS risk-associated genes. However, questions remain because although the patients shared similar symptoms such as xerophthalmia and xerostomia, not many of their symptoms had a clear pathogenesis. Next generation sequencing, introduced to address these questions, has yielded a glimpse of the relation between SS symptoms and associated biomarker genes.

Whole-exome sequencing (WES) recognizes the exons or protein-coding regions of genes simultaneously using next-generation sequencing techniques. Rare variations not found in the general population can be detected in patients' DNA sequences by comparing the patients' exomes with a normal reference sequence. One may thus analyze genes to determine whether they are functionally related to a patient's symptom phenotype. WES therefore addresses a shortcoming of previous GWAS studies, insensitivity to rare mutations and structural variability, through its highly effective approach for identifying both homozygous and heterozygous, de novo, germinal, and novel rare

Abbreviations: SS, Sjogren's syndrome; GWAS, genome-wide association studies; WES, Whole-exome sequencing; IRB, Institutional Review Boards; QC, quality control; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; OR, odds ratio; CHROM, chromosome; POS, basepair position; REF, reference allele; ALT, alternative allele; AA, amino acid; MAF, minor allele frequency; CI, confidence interval; P, *p* value of association analyses; IN, Insensitive; NO, Normal

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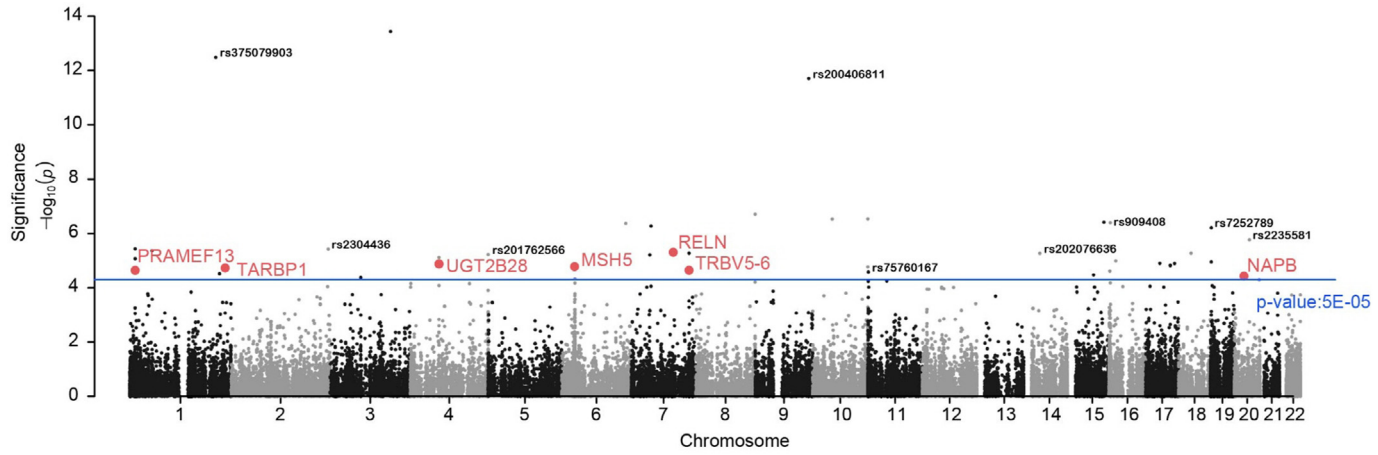

Fig. 1. Summary of genome-wide association results. The –log10P value for each variant is plotted according to chromosome and base-pair position. Seven loci (red points) exceeded the GWS of Pmeta $< 5 \times 10^{-5}$ (blue-dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coding variants [9].

In this first genetic association study of SS in a Korean population based on WES data, we analyzed the genetic variants of both homozygotes and heterozygotes to find variants at potential loci associated with SS as well as to match those variants to each symptom of SS phenotype. We also tried to find the pathogenesis of SS by using bioinformatic tools such as gene set enrichment analysis.

2. Materials and methods

2.1. Subjects

Patients who had been diagnosed with SS in the Rheumatology Clinic of Severance Hospital and presented to the Oral Medicine Clinic in Yonsei Dental Hospital participated in this study. Clinical medical records and signed consent documents were obtained for all patients in this study. We excluded patients who had been diagnosed with a history of other combined autoimmune syndromes.

2.2. Clinical assessment and measurements

2.2.1. Medical examination forms

The following information was collected on the medical examination form: clinical signs, symptoms, complications and results of laboratory examinations. All patients were interviewed to determine whether they were experiencing the symptoms associated with SS, including feeling sand or grit in the eyes, dryness in the eyes, and dryness in mouth and throat. Clinical laboratory data such as Schirmer's I test, Rose Bengal score, unstimulated salivary flow and antibodies to RO (SS-A) or La (SS-B) antigens were provided by the Department of Rheumatology.

2.2.2. Unstimulated salivary flow

The patients were asked to relax several minutes in a chair and to refrain from drinking and eating for at least one hour before the saliva collection. Patients placed their tongue to the upper incisors and spit gently in a beaker. Saliva was collected for 15 min, and then the amount of saliva in the unstimulated condition (sitting and not moving) per minute was measured. Unstimulated flow < 0.1 ml/min was considered hyposalivation [10], > 0.2 ml/min was regarded as normal, and 0.1–0.2 ml/min as low [11].

2.2.3. Schirmer's I test

Without anesthetic, the Schirmer strips were inserted into the lower conjunctival sac at the junction of the lateral and middle thirds, avoiding the cornea. After 5 min the length of wetting strips was recorded in millimeters. The test results were considered hypofunction if the length of wetting obtained was < 5 mm in 5 min and normal when > 5 mm in 5 min.

2.2.4. Gustometry

Patients who had completed medical examination forms underwent gustometry using six different concentrations of chemical liquid and recorded scores for the four basic taste sensations (sweet, bitter, salty and sour) [12]. The chemical liquids used in the test were: sucrose (sweet), sodium chloride (salty), citric acid (sour) and quinine hydrochloride (bitter). The concentration used in sucrose was 0.00316–1.0 g/l; in sodium chloride, 0.00316–1.0 g/l; in citric acid, 0.0316–0.0001 g/l and in quinine hydrochloride in the range of 0.00000316–0.001 g/l. The solutions were applied directly on the tongue by dropper. We considered < 0.05 g/l in sucrose solutions, 0.158 g/l in sodium chloride, 0.00063 g/l in citric acid and 0.00005623 g/l in quinine HCl solutions as normal taste responses. The Institutional Review Boards (IRB) of Yonsei University College of Dentistry approved this study (approval number: 2-2017-0026).

2.3. DNA collecting

2 ml of saliva were collected by the patients using the Oragene DNA Self-Collection Kit (DNA Genotek Inc. Ottawa, Canada) (Fig. 1), which contains a solution that allows saliva samples to remain stable at room temperature. Further analysis besides DNA extraction and DNA analysis was carried out by DNA Link Inc., Seoul, South Korea.

2.4. Control group

We ascertained 100 healthy exome sequencing controls from 3703 Ansan-Ansung cohort subjects using data provided by Korea BioBank, Center for Genome Science, National Institute of Health, Korea Centers for Disease Control and Prevention.

2.5. WES on HISEQ2500 using sureselect all exon kit 50Mb

With an OD260/280 ratio of 1.8–2, DNA should be as intact as possible. We confirmed the quality of DNA by 1% agarose gel electrophoresis and PicoGreen® dsDNA Assay (Invitrogen). A Bravo automated liquid handler was used to prepare, SureSelect sequencing libraries according to the manufacturer's instructions (Agilent SureSelect All Exon 50 MB Kit). 3 μg of genomic DNA in 120 ml EB buffer was fragmented to a median size of 150 bp using the Covaris-S2 instrument

Table 1
SNPs in the exome-wide association analysis.

Gene	SNP	CHROM	POS	REF	ALT	MAF		Discovery		AA change	SIFT Prediction
						case	control	OR (95% CI)	P		
RELN	rs362691	7	103,251,161	G	C	0.3333	0.065	7.192 (2.797–18.5)	4.91E-06	Leu997Val	Tolerated
UGT2B28	rs72552704	4	70,160,342	T	C	0.4333	0.12	5.608 (2.424–12.97)	1.33E-05	Cys469Arg	Tolerated
UGT2B28	r s72552705	4	70,160,343	G	C	0.4333	0.12	5.608 (2.424–12.97)	1.33E-05	Cys469Ser	Damaging
MSH5	rs2075789	6	31,708,328	C	T	0.3929	0.09302	6.309 (2.523–15.77)	1.67E-05	Pro29Ser	Damaging
TARBP1	rs117698521	1	234,614,390	G	C	0.8333	0.35	9.286 (2.942–29.31)	1.87E-05	Arg154Gly	Tolerated
TRBV5–6	No_id	7	142,131,702	A	G	0.2333	0.035	8.391 (2.702–26.06)	2.27E-05	Leu18Ser	
PRAMEF13	rs200801174	1	13,448,547	T	C	0.4444	0.09412	7.7 (2.66–2.29)	2.28E-05	Met310Val	Tolerated
NAPB	rs140977227	20	23,401,975	T	C	0.2692	0.04444	7.921 (2.586–24.27)	3.73E-05	Ls22Arg	Tolerated

SNP, single-nucleotide polymorphism; OR, odds ratio; CHROM, chromosome; POS, basepair position; REF, reference allele ALT, alternative allele; AA, amino acid; MAF, minor allele frequency; CI, confidence interval; P, p value of association analyses.

(Covaris) with the following settings: duty cycle 10%, intensity 5, cycles per burst 200, and mode frequency sweeping for 360 s at 4. The fragmentation efficiency was evaluated by using capillary electrophoresis on DNA 1000 chips (Bioanalyzer, Agilent). Sequencing adapters were ligated on the DNA fragments following the manufacturer's protocol (Agilent). The ligated adapters were then amplified by PCR. The quality of the PCR products was evaluated by capillary electrophoresis (Bioanalyzer, Agilent). SureSelect hyb #1, #2, #3, and #4 reagents (Agilent) were mixed to make the hybridization buffer. The amplified DNA fragments were concentrated to 500 ng in 3.4 ul with SureSelect block #1, #2, and #3 reagents (Agilent) added together. The DNA blocker mix and hybridization buffer were incubated for 5 min at 95° and then for 10 min at 65° in a thermal cycler. RNase block (Agilent) was then added to the SureSelect oligo capture library (Agilent) and incubated for 2 min at 65°. The hybridization buffer with the DNA blocker mix was added to the capture library and the mixture was incubated for 24 h at 65° in a thermal cycler. 50 ul of streptavidin coated with the Dynal MyOne Streptavidin T1 (Invitrogen) were washed by 200 ml SureSelect binding buffer (Agilent) 3 times and resuspended in 200 ml of the binding buffer. The hybridization mixture was added to the bead suspension and incubated for 30 min at RT with mixing. The beads were washed with 500 ml SureSelect wash buffer #1 (Agilent) for 15 min at RT, and three times with 500 ml SureSelect wash buffer #2 (Agilent) for 10 min at 65°. DNA was eluted with 50 ml SureSelect elution buffer (Agilent) for 10 min at RT. 50 ml of SureSelect neutralization buffer (Agilent) was added to the eluted DNA. The reaction product was purified with AMPure XP beads (Beckman). The captured library was amplified to add index tags using Herculase II Fusion DNA Polymerase (Finnzymes). The quality of the amplified libraries was verified by capillary electrophoresis (Bioanalyzer, Agilent). After fulfilling QPCR using SYBR Green PCR Master Mix (Applied Biosystems), we combined 6 libraries, index tagged in equimolar amounts in the pool. Cluster generation occurred in the flow cell on the cBot automated cluster generation system (Illumina). After that, the flow cell was loaded on the HISEQ 2500 sequencing system (Illumina), with sequencing by 2x100bp read length.

2.6. Statistical assessment of exomic variants

For quality control (QC) of variants, SNPs below 1% of minor allele frequency (MAF) were excluded using PLINK v1.90 [13] and 64,293 eligible SNPs were chosen. To assess the statistical association between the variant and SS, chi-square test was carried out to compare cases' allele and genotype frequencies with controls. Consequently, 48 variants with the association $P < 5 \times 10–5$ were considered to be statistically significant. Fisher's exact test was used as appropriate for between-group comparisons. Odds ratio with 95% confidence interval was calculated for hyposalivation and hypofunction and the four insensitive gustometry subgroups separately. The statistical software used was SPSS V.22.0 (SPSS, Chicago, IL, USA), and significance was defined as $p < 0.05$.

2.7. Functional enrichment analysis of exomic variants

DAVID v6.8 [14] was used to perform GO term enrichment for SS-associated genes. A total 38 genes that aggregated from the 48 variants were covered by DAVID and analyzed. The following categories were used in DAVID: for 'pathway': 'KEGG'; for 'Protein_Domains': 'INTERPRO' and 'PFAM'; for 'Gene_Ontology': 'GOTERM_BP_DIRECT', 'GOTERM_MB_DIRECT', and 'GOTERM_CC_DIRECT. Table 3 presents functions significantly enriched in the SS-associated genes with P-value < 0.05.

2.8. Functional enrichment analysis of overlapping gene functions

DAVID v6.8 [14] was used to perform GO term enrichment for SS-associated genes. The following categories were used in DAVID: for 'pathway': 'KEGG'; for 'Protein_Domains': 'INTERPRO' and 'PFAM'; for 'Gene_Ontology': 'GOTERM_BP_DIRECT', 'GOTERM_MB_DIRECT', and 'GOTERM_CC_DIRECT. Table 3 presents functions significantly enriched in the SS-associated genes with Benjamini corrected p-value < 10-3. To analyze the functional terms and pathways associated with the variants, we expanded the list of genes based on functional association using STRING v11.0 [15]. We used multiple proteins query with three search options: "all active interaction sources," "confidence level 700" and "1st shell with no more than 50 interactors". As a result, genes from Korean, Han Chinese and European population studies expanded to 57, 58, and 66 SS associated genes and their neighbor genes (Supplement Table S1).

2.9. Functional impact analysis of exomic variants

To validate the functional impact of variants on protein-coding regions, we filtered out variants with missense mutations using SnpEff [16]. The total eight missense variants are presented in Table 1. SIFT [17] was used to sort out two variants for additional inspection of highly-impactful variants.

3. Results

3.1. Patient characteristics

The characteristics of the 15 SS patients on whom WES analysis was performed are shown in the Table 2. The mean age of our patients, all female, was 61.3. Almost 80.0% (hyposalivation 60.0%, low 20.0%) of these patients showed abnormal unstimulated salivary flow. In the gustometry test, except for citric acid stimuli, > 70% of patients scored in the normal range on the other three stimulus results and no patient showed sensitivity, especially to citric acid. Twelve patients could identify sucrose and three patients were insensitive. Normal sodium

Table 2
Characteristics of the fifteen patients.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Age (years)	62	47	58	70	66	56	56	58	61	76	48	56	76	62	67
Duration of disease (months)	27	8	20	16	17	13	12	8	14	20	15	12	24	20	18
Unstimulated salivary flow (ml/min)	0.05	0.292	0.018	0.198	0.046	0.046	0.029	0.05	0.173	0.02	0.22	0.185	0.237	0.092	0.087
Gustometry (Sucrose) (1–6 grade)	4	3	2	3	4	3	3	2	4	2	3	6	4	4	6
Gustometry (Sodium chloride)(1–6 grade)	X	5	3	3	3	3	3	X	3	2	2	3	4	3	4
Gustometry (Citric acid)(1–6 grade)	3	2	3	3	3	2	3	4	4	1	3	4	4	4	4
Gustometry (Quinine hydrochloride)(1–6 grade)	3	3	3	3	3	3	5	1	5	3	4	4	3	3	4
Schirmer's I test (< 5 mm in 5 min)	–	–	–	–	✓	✓	–	–	–	–	✓	–	✓	✓	✓
Antibodies to Ro (SSA) or La (SSB)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	–	✓	✓	✓	–
Minor salivary gland biopsy focus score (> 1)	–	–	–	–	–	–	–	–	–	–	✓	–	–	–	–
Abnormal salivary scintigraphy	–	✓	–	–	–	✓	✓	✓	✓	–	✓	–	✓	✓	✓

X - no response, ✓-there is a response.

chloride responses were observed in thirteen patients and two showed insensitivity to sodium chloride. Five patients scored in the normal range for citric acid, ten patients being insensitive. Thirteen patients demonstrated a normal bitter response to quinine HCL stimulus whereas two were insensitive. Twelve patients had an unstimulated salivary flow below the cut-off level (0.2 ml/min). Of those, nine showed hyposalivation (< 0.1 ml/min) and three showed a low value (0.1–0.2 ml/min). Only three patients produced > 0.2 ml/min, demonstrating normal unstimulated salivary flow. Six of the 15 patients scored abnormal on the bilateral Schirmer's test, being < 5 mm in 5 min (hypofunction), the other nine patients showing > 5 mm in 5 min.

3.2. Exome-wide association analysis

In order to identify the risk loci influencing SS, we identified 7 genes which include missense variants significantly associated with SS (Table 1). The results of the genetic model analysis are summarized in a Manhattan plot (Fig. 1). Specifically, *PRAMEF13*, *TARBP1*, *UGT2B28*, *MSH5*, *RELN*, *TRBV5-6* and *NAPB* showed P-values < 5 × 10–5 with a high odds ratio (Table 1). Among them, our data showed *TARBP1* SNP rs17698521 expressed the highest odds ratio 9.286 (95% CI 2.942–29.31). SNP rs362691 in *RELN* had the lowest association P-values (4.91E-06), the next most significant variants (rs72552704 and rs72552705) being found in *UGT2B28* (P = 1.33E-05) (Table 1). The minor allele frequencies (MAF) of seven SNPs in our study were comparable to those controls. The MAFs in our research were twice as high as those reported in the controls. The variants in *UGT2B28* (rs72552705) and *MSH5* (rs2075789) were predicted to be damaging (SIFT Prediction) (Table 1). Among these 7 variants, two (*MSH5* and *RELN*) have been previously linked with SS. In a genome-wide association study, Christopher J. Lessard et al. observed a peak association in the *MSH5* gene among SS patients [18]. Moreover, a mutation in the *RELN* gene has been observed in patients with SS [19].

3.3. Function enrichment analysis of effect genes

The analysis results yielded three notable categories: GO, INTERPRO and PFAM. We found 5 GO terms, 2 INTERPRO, and 1 PFAM with a p-value cut-off < 0.05. The details of these GO terms are shown in Table 3. The top overrepresented term was 'intracellular protein transport.' Moreover, 13 genes (28.3%) had annotations of GO terms 'plasma membrane' and 9 genes (19.6%) had annotations of 'membrane'. This test indicated that almost half of the genes are involved in cellular components rather than molecular function or biological process.

3.4. Genes identified in subgroup patients

Because the allelic heterogeneity and homogeneity of these seven variants are significant for SS but do not contribute in all the tests, there may be subgroup-specific associations, as well as frequencies that vary by subgroups. A summary of the genes in the subgroups of unstimulated salivary flow, gustometry and Schirmer's I test are listed in Tables 4, 5 and 6. When combining the hyposalivation group results with those of low and normal groups, we found the three genes *MSH5, PRAMEF13*, and *NAPB* were not identified in the low salivary flow group (Table 4). *TARBP1, MSH5* and *RELN* showed the highest odds ratio in the hyposalivation group of any group. In Schirmer's I Test, *UGT2B28* occupied the largest proportion in both hypofunction and normal groups (Table 5). However, *MSH5* and *PRAMEF13* expressed the highest odds ratio. In gustometry, *NAPB* expressed the highest odds ratio in the sucrose test, at nearly 10.000. On the contrary, *TRBV5-6* was not identified in the sucrose insensitive group nor was *NAPB* in the sodium insensitive group. Moreover, on the sodium test, the gene *RELN* only occurred in the normal group (Table 6).

4. Discussion

In this study, we performed a case-control genetic association analysis by using data from 15 patients who were treated for SS and from 100 healthy individuals as a control. According to our best understanding, this is the first gene approach research using WES in a Korean population with SS. In addition to validating the previously described susceptibility loci *MSH5* and *RELN*, we also identified *PRAMEF13*, *TARBP1, UGT2B28, TRBV5-6,* and *NAPB* as novel susceptibility loci for SS. As these five new genes have not been linked with SS in previous studies, this finding may represent a milestone in the search for the genetic pathogenesis of SS.

Although the pathogenesis of these genes for SS on the molecular level remains unknown, we did find additional testimony to an association between genetic variants and immune functions. Among the genes, *RELN* showed significant P values (4.91E-06), with the highest odds ratio (OR = 7.102) (Table 1). As shown in previous studies, the genes in the focal adhesion pathway play a central role as intermediaries between endothelial cell scaffold and extracellular matrix. In a genetic association database, *RELN*, a known risk gene of autoimmune diseases, occurs in this pathway [20], indicating the focal adhesion pathway may play a part in the pathophysiology of SS. Further research into this pathway may help elucidate its connection with SS and finally lead to a mode of treatment involving its manipulation.

UGT2B28 mainly functions in connecting with liver and mammary glands [21], and has previously been shown to be related to Addison's disease [22], which is also a distructive autoimmune disease. Among our female patients, average age 60.9, almost all have amenorrhea, as noted in previous research, *UGT2B28* plays an important role in regulating steroid hormones [23]. Certain autoimmune disorders besides SS are significantly influenced by sex steroid hormones. *UGT2B28* has the intrinsic capacity to conjugate some bile acids and estrogen [21]. Steroid hormones influence immune cell function and inflammation [24]. Therefore, we can speculate that the association between

Table 3
Function enrichment analysis of genes.

Category	Term	Count	%	Genes	P-value
GOTERM_BP_DIRECT	GO:0006886~intracellular protein transport	5	0.116	TBC1D3C, TBC1D3H, AP3D1, NAPB, RAMP1	1.00×10^{-3}
PFAM	PF00488:MutS domain V	2	0.046	MSH5-SAPCD1, MSH5	9.92×10^{-3}
INTERPRO	IPR007696:DNA mismatch repair protein MutS, core	2	0.046	MSH5-SAPCD1, MSH5	1.06×10^{-2}
INTERPRO	IPR000432:DNA mismatch repair protein MutS, C-terminal	2	0.046	MSH5-SAPCD1, MSH5	1.06×10^{-2}
GOTERM_MF_DIRECT	GO:0030983~mismatched DNA binding	2	0.046	MSH5-SAPCD1, MSH5	1.94×10^{-2}
GOTERM_CC_DIRECT	GO:0016020~membrane	9	0.209	KREME2, HLA-DRB1, SLC39A11, FANCI, PKMYT1, AP3D1, CLIC1, GLE1, MMP25	2.74×10^{-2}
GOTERM_CC_DIRECT	GO:0005886~plasma membrane	13	0.302	TPC1D3C, ADCY1, KREMEN2, HLA-DRB1, SLC39A11, TBC1D3H, TGM2, RELN, GLE1, CLIC1, RAMP1, MARK1, MMP25	3.20×10^{-2}
GOTERM_MF_DIRECT	GO:0005524~ATP binding	7	0.162	ADCY1, MSH5SAPCD1, MSH5, TGM2, PKMYT1, MARK1, ABCA13	4.48×10^{-2}

Table 4
Summary of the genes in unstimulated salivary flow subgroups.

Gene	Hyposalivation (total = 9)	Normal (total = 3)	OR	95% CI	P
TARBP1	10 (83.3%)	1 (33.3%)	10.000	0.584–171.202	0.154
RELN	8 (66.7%)	1 (33.3%)	4.000	0.273–58.562	0.525
UGT2B28	8(66.7%)	2 (66.7%)	1.000	0.068–14.640	1.000
MSH5	3 (25.0%)	3 (100.0%)	4.000	1.501–10.658	0.044
PRAMEF13	3 (25.0%)	2 (66.7%)	0.167	0.011–2.563	0.242
NAPB	3 (25.0%)	1 (33.3%)	0.667	0.043–10.253	1.000
TRBV5-6	4 (33.3%)	2 (66.7%)	0.250	0.017–3.660	0.525

OR, odds ratio; CI, confidence interval; P, p value of association analyses.

Table 5
Summary of the genes in Schirmer's I test subgroups.

Gene	Hypofunction (total = 6)	Normal (total = 9)	OR	95% CI	P
UGT2B28	5 (83.3%)	6 (66.7%)	2.500	0.194–32.194	0.604
MSH5	3 (50.0%)	2 (22.2%)	3.500	0.372–32.971	0.329
TARBP1	4 (66.7%)	6 (66.7%)	1.000	0.112–8.947	1.000
PRAMEF13	3 (50.0%)	2 (22.2%)	3.500	0.372–32.971	0.329
RELN	3 (50.0%)	5 (55.6%)	0.800	0.101–6.347	1.000
TRBV5-6	2 (33.3%)	5 (55.6%)	0.400	0.047–3.424	0.608
NAPB	1 (16.7%)	2 (22.2%)	0.700	0.049–10.014	1.000

OR, odds ratio; CI, confidence interval; P, p value of association analyses.

UGT2B28 and immune function might also be regulatory, possibly via steroid hormones. However, due to the lack of sufficient data, the relationship of *UGT2B28* to autoimmunity is not obvious at this time.

According to a previous study, the *TARBP1* gene expresses an autoimmunity signature which associated with two autoimmune diseases, multiple sclerosis and systemic lupus erythematosus [25]. Additionally, our results showed an association between the unstimulated salivary flow test and *TARBP1* in SS patients. The *TARBP1* gene clearly occurred more often in the hyposalivation salivary flow patients than in the normal salivary flow patients (Table 4), with the highest odds ratio being nearly 10. This suggests that *TARBP1* may be linked with salivary flow. Previous studies have indicated the pathogenesis of SS involves an increase in epithelial cell apoptosis [26,27]. *TARBP1* has been linked with negative regulation of proliferation [25] and uncontrolled proliferation, which may accompany a high level of apoptosis [28] and play a role in producing anti-RO/SSA and anti-La/SSB, followed by progressive plasma and lymphocytic cell infiltration in the salivary glands [26,27]. The salivary gland infiltrates are predominantly comprised of T cells [27,29], in which the transcription of *TARBP1* mainly occurs [30]. Although there is no direct evidence that TARBP1 is involved in dry mouth development, this gene seems to be an interesting candidate for dry mouth.

Our results also suggest genetic heterogeneity between hypofunction and normal groups in Schirmer's I test. *MSH5* and *PRAMEF13* (odds ratio: 3.500) seemed to be specific to those patients in the hypofunction group (Table 5). As a member of a protein family engaged in DNA mismatch repair (Table 3), *MSH5* has been closely associated with systemic lupus erythematosus [31]. *PRAMEF13*, member 13 of the PRAME family, can be recognized by autologous cytolytic T lymphocytes (CTLs) [32–34], which, depending on the disease, can cause cell destruction. Recent evidence suggests that a higher level of CTLs tends to accompany more severe autoimmune diseases [35] involving connective tissues of the mucous membrane, glands, and so forth [36–40]. Hence, the cornea, conjunctiva, and lacrimal gland tissues of the ocular surface also become immune attack sites, leading to dry eye.

To identify those genes specific to SS pathways where most changes occurred, we further applied GO analysis. While we acknowledge the analysis may seem overstretched, we believe it may serve as a basis for classification in several domains of molecular and cellular biology in

Table 6
Summary of the genes in gustometry subgroups.

	Sucrose					Sodium				Citric acid				Quinine hydrochloride				
	IN	NO	OR	95% CI	P	IN	NO	OR	95% CI	P	IN	NO	OR	95% CI	P	IN	NO	OR	95% CI	P
TARBP1	3	8	0.667	0.447–0.995	0.516	1	9	0.444	0.022–9.032	1.000	7	4	0.583	0.044–7.661	1.000	1	10	0.300	0.014–6.382	0.476
RELN	2	6	2.000	0.141–28.416	1.000	0	8	0.385	0.193–0.765	0.200	6	2	2.250	0.251–20.131	0.608	0	8	0.385	0.193–0.765	0.200
PRAMEF13	2	4	4.000	0.273–58.562	0.525	1	3	3.333	0.157–70.906	0.476	3	3	0.286	0.030–2.692	0.329	1	5	1.600	0.081–31.771	1.000
NAPB	2	2	10.000	0.584–171.202	0.154	0	3	0.769	0.571–1.036	1.000	3	1	1.7141	0.131–22.513	1.000	0	4	0.692	0.482–0.995	1.000
UGT2B28	1	8	0.250	0.017–3.660	0.525	1	7	0.857	0.044–16.851	1.000	6	3	1.000	0.112–8.947	1.000	0	9	0.308	0.136–0.695	0.143
MSH5	0	7	0.417	0.213–0.814	0.200	1	3	3.333	0.157–70.906	0.476	5	2	1.500	0.170–13.225	1.000	0	7	0.462	0.257–0.830	0.467
TRBV5-6	0	7	0.417	0.213–0.814	0.200	1	3	3.333	0.157–70.906	0.476	3	4	0.107	0.008–1.407	0.119	0	7	0.462	0.257–0.830	0.467

IN, Insensitive; NO, Normal; OR, odds ratio; CI, confidence interval; P, p value of association analyses.

larger-0scale studies. The GO term which showed the most significant association is “intracellular protein transport,” annotated with the gene *NAPB* in our research (Table 3). The GO terms “protein transport” and “intracellular transport” have been reported to be significantly enriched in salivary glands of SS [41]. Moreover, autoantibodies against DNA repair proteins such as “DNA mismatch repair proteins” which showed significant association in our research have also been found in autoimmune patients [42].

Our GO analysis appears to be in line with these previous findings on SS. Moreover, in order to detect correlations with other lineages mentioned in the previous studies we compared the variants found from three different studies of Sjogren's syndrome in Korean, Han Chinese, and European populations [7,8]. Despite the lack of significant overlap between the different populations due to insufficient number of the genes with associated variants (Korean $N = 7$, Han Chinese N = 7, European $N = 16$) (Supplement Table S1), we found that several functional terms were enriched in the variants of different populations. Our analysis found an overlap of several functions and pathways in the three populations which might be relevant to the disease etiology. Specifically, in the Korean and Han Chinese studies, five functional terms representing Golgi membrane, vesicle transport, and protein binding were found (corrected p-value $<$ 10-3) (Supplement Table S2-1). In addition, the enriched functions in the Korean and European studies overlapped with similar functional terms found in the comparison of Korean and Han Chinese populations (Supplement Table S2-2). Our further analysis focused on overlapping gene functional terms in the populations and identified the Golgi network as a common term in all three (Supplement Table S2). Most autoantibodies directed against autoantigens of nuclear or cytoplasmic components have been well characterized, including anti-Golgi autoantibodies. The association of Golgi autoantigen with SS in previous studies [43–46], consistent with our findings, indicates that the Golgi network may appear in the disease processes at the cellular level. Although we did not investigate the Golgi network in detail, further research into its function may help elucidate the pathogenesis of the disease. We anticipate that genes contributing to the robust enrichment signals among different populations may inform the design of future studies of SS pathogenesis. Furthermore, knowledge of these functions may help clinicians make hypotheses before diagnoses.

We acknowledge potential limitations of the present research. First, the 15 patient cases we collected in this study yielded a relatively limited number of case samples for subgroup analyses, with no sufficient power to ferret out risk genes which are less frequent or are related with weaker genetic effects. Second, as SS occurs more often in women, all the patients in our study were female. It is possible that men would differ from women in terms of final results.

5. Conclusion

This study utilized whole genome sequencing to find two previously proven SS risk genes and five novel variants associated with Sjogren's syndrome in a Korean population. Despite the small number of samples, this study may support future research based on a larger reference population by elucidating more precisely which genes are related to certain subtypes of complications. Our data provides basic information on the pathogenesis of SS, including biomarkers which may serve as targeted genes for clinical precision medicine. We hope our discovery will eventually enable clinicians to treat patients based on their individual genetic characteristics.

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Authors' contributions

The study was conceived and designed by JHL. HJA was responsible for the selection and clinical evaluation of patients. QXS analyzed the

data and wrote the manuscript. KHL, SKH and SUK performed data analysis and reviewed the manuscript. All authors reviewed and approved the manuscript.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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