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A high-throughput sequence analysis of Japanese patients revealed 11 candidate genes associated with type 1 autoimmune pancreatitis susceptibility

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ABSTRACT

The pathogenesis of autoimmune pancreatitis is unknown. In the present study we used high-throughput sequencing with next generation sequencing to identify the candidate genes associated with AIP. A total of 27 type 1 AIP patients and 30 healthy blood donors were recruited, and DNA samples were isolated from their mononuclear cells. A high-throughput sequencer with an original custom panel of 1031 genes was used to detect the genetic variants in each sample. Polymorphisms of CACNA1S (c.4642C > T), rs41554316, rs2231119, rs1042131, rs2838171, P2RX3 (c.195delG), rs75639061, SMAD7 (c.624delC) and TOP1 (c.2007delG), were identified as candidate genetic variants in patients with type 1 AIP. P2RX3 and TOP1 were significantly associated with AIP, even after adjusting bay means of Bonferroni's correction. In addition, we also identified eight candidate genetic variants that were associated with the relapse of type 1 AIP, namely: rs1143146, rs1050716, HLA-C (c.759_763delCCCCCinsTCCCG), rs1050451, rs4154112, rs1049069, CACNA1C (c.5996delC) and CXCR3 (c.630_631delGC). Finally polymorphisms of rs1050716 and rs111493987 were identified as candidate genetic variants associated with extra-pancreatic lesions in patients with type 1 AIP. These candidates might be used as markers of AIP susceptibility and could contribute to the pathogenesis of type 1 AIP.

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1. Introduction

Autoimmune pancreatitis (AIP) is a condition which involves the chronic inflammation of pancreas. It is characterized radiologically by pancreatic enlargement with delayed enhancement and strictures or narrowing of the main pancreatic duct without marked upstream dilation; serologically by elevation of serum immunoglobulin G fraction 4 (IgG4); histologically by lymphoplasmacytic infiltration and fibrosis; and therapeutically by a dramatic response to steroids [1]. The International Consensus Criteria for AIP [1] proposed two subtypes of the disease. The majority of Japanese patients with AIP are classified as type 1; type 2 AIP is more common in Western countries. Type 1 AIP is also recognized as a pancreatic manifestation of IgG4-related disease [2]. Conversely, type 2 AIP is frequently complicated by inflammatory bowel diseases [1]. The pathogenesis of type 1 AIP is unknown but it is considered to be a multifactorial disease which is associated with genetic and environmental factors. Haruta et al. reported that a mouse model of AIP was established by persistent exposure to heat-killed *Escherichia coli*, suggesting that the chronic activation of the innate immune system, triggered by intestinal flora, might cause AIP [3]. In addition, gastric infection by *Helicobacter pylori* was suspected to contribute to the pathogenesis of AIP, because it shows significant homology with human carbonic anhydrase 2 and the alpha-carbonic anhydrase of *Helicobacter pylori* [4].

On the other hand, the genetic factors associated with AIP have also been reported. Kawa et al. reported that the human leukocyte

Abbreviations: AIP, autoimmune pancreatitis; IgG4, immunoglobulin G fraction 4; HLA, human leukocyte antigen; HV, healthy volunteer; DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism

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antigen (HLA) DRB1*04:05-DQB1*0401 haplotype was associated with AIP [5]. Subsequently, some of the genes that are associated with AIP susceptibility were identified by direct sequencing or a Taqman assay, these include: ATP-binding cassette sub-family F1 [6], Fc receptor-like 3 [7], cytotoxic T lymphocyte antigen 4 [8], KCNA3 [9] and PPRS1 [10].

Because multiple genes are usually associated with susceptibility to a multifactorial disease (including unknown genes), a high-throughput sequence analysis is necessary for the investigation of genes that confer AIP susceptibility. Oguchi et al. performed a genome-wide association study to investigate genes that conferred susceptibility to the triggering of dacryoadenitis or sialadenitis in Japanese AIP patients; however, they did not show the genes that were associated with AIP susceptibility [11]. In the present study, we performed high-throughput sequencing with next generation sequencing, which targeted more than 1000 genes.

2. Material and methods

2.1. Patients and clinical diagnosis

A total of 27 patients with type 1 AIP and 30 healthy blood donors were consecutively diagnosed and recruited from January 2013 to September 2014 at Asahikawa Medical University or its affiliated institutions. All of the AIP patients and healthy blood donors were Japanese and provided written informed consent for inclusion in the high-throughput sequencing analysis. The patients with a definite or probable diagnosis of AIP based on the Clinical Diagnostic Criteria for Autoimmune Pancreatitis 2011 [12] were enrolled in this study.

2.2. Primer design for custom amplicon sequencing

Because the present study aimed to identify new genetic variants that reflect AIP susceptibility, the genes associated with inflammatory and autoimmune diseases, hematological and metabolic disorders, and oxidative stress in the gastrointestinal tract, liver, pancreas and biliary tract were selected as candidates. Consequently, 883 genes that are associated with inflammatory and autoimmune diseases and 209 genes that are associated with metabolic disorders and oxidative stress were identified. After excluding the overlapping genes, 1031 genes were investigated in the present study. We designed multiple primer sets which targeted 1031 genes (total of 12,609 amplicons) using the Ion AmpliSeqTM Designer software program (https://www.ampliseq.com/browse.action) (Life Technologies, Carlsbad, CA, USA). These primer sets were provided as five primer pools. The 12,609 amplicons and the targeted lesions are described in Supplemental Table 1.

2.3. Sample preparation for amplicon sequencing

Peripheral blood samples from each of the patients or healthy volunteers (HVs) were processed for mononuclear cell isolation by Ficoll gradient centrifugation. The genomic deoxyribonucleic acid (DNA) was then extracted and purified using DNeasy Blood & Tissue Kits (Qiagen, Venlo, Netherlands). The DNA concentrations were determined by a QubitTM Fluorometer (Life Technologies, Carlsbad, CA, USA). The quality of the genomic DNA was assessed by agarose gel electrophoresis.

2.4. High-throughput sequencing

Using 50 ng of each DNA sample, an ultra-high multiplex polymerase chain reaction (PCR) was performed and a DNA fragments library (5 primer pools per sample) was generated, using an Ion AmpliSeqTM Library Kit 2.0 (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions in order to perform custom amplicon sequencing. The concentration and quality of the DNA fragments library was evaluated with an Agilent 2200 Tape station (Agilent Technologies, Santa Clara, CA, USA). The DNA fragment libraries were then processed for an emulsion PCR using an Ion OneTouchTM System and an Ion OneTouch 200 Template Kit v3 (Life Technologies, Carlsbad, CA, USA). Template-positive Ion SphereTM Particles from the sequencing reaction were enriched and purified with an Ion OneTouchTM ES system (Life Technologies, Carlsbad, CA, USA). The template-positive Ion SphereTM Particles were then applied on Ion PI™ Chips (Life Technologies, Carlsbad, CA, USA), and the high throughput sequencing reaction was carried out using an Ion Proton™ Semiconductor sequencer (Life Technologies, Carlsbad, CA, USA).

2.5. The data analysis to detect genetic variants

All of the sequencing data were mapped on a human reference genome sequence (GRCh37/hg19) using the Torrent Suite Software program (Life technologies, Carlsbad, CA, USA). The genetic variants were then detected by a Torrent Variant Caller plug-in for the software program (Life technologies, Carlsbad, CA, USA). In this program, alleles with frequencies of (the percentage of reads which possessed a variant) $>10\%$, with a coverage (the number of reads which had a variant) of >5 and a quality score of >15 were regarded as significant variants. The variant information for each sample was imported into the CLC Genomics Workbench software system (CLC bio, Aarhus, Denmark), and Fisher's exact test was performed to determine the significance of the differences among the samples. Strand bias was defined according to the following numerical formula: strand bias$=$max (VpCm, VmCp)/VpCm$+$VmCp (Cp, the number of reads from the plus direction in the known sequence; Cm, the number of reads from the minus direction in the known sequence; Vp, the number of reads from the plus direction in the variant sequence; Vm, the number of reads from the minus direction in the variant sequence).

2.6. Statistical analysis

In the amplicon sequencing analysis, the candidate genetic variants were filtered using the *P*-values determined by Fisher's exact test. The age between AIP patients and HVs was compared using the Mann-Whitney *U* test. *P* values of <0.05 were considered to indicate statistical significance.

2.7. Ethics statement

The present study was approved by the institutional review board of Asahikawa Medical University. Written informed consent was obtained from all of the subjects after a full explanation of the study.

3. Results

3.1. The demographics of the AIP patients and HVs

A total of 57 participants, including 27 patients with type 1 AIP (male, n$=$22; female, n$=$5) and 30 HVs (male, n$=$17; female, n$=$13) were enrolled in this study. The median age of the AIP patients at the time of blood collection was 73 years (range: 55–87). The median age of the HVs at the time of blood collection was 29.5 years (range: 22–49). The HVs were significantly younger

Table 1
Status information of the patients with AIP and HVs.

		AIP	HVs	*P* value
Gender;	Male	22 (81.5%)	17 (56.7%)	0.0522
	Female	5 (18.5%)	13 (43.3%)	
Median age (range)		73 (55–87)	29.5 (20–49)	< 0.01
Comorbidity	Diabetes mellitus	13 (48.1%)	0 (0%)	< 0.01
	Hypertension	9 (33.3%)	3 (10%)	0.0489
	Hyperlipidemia	9 (33.3%)	1 (3.3%)	< 0.01
	Asthma bronchiale	4 (14.8%)	1 (3.3%)	0.1791
	Cataract	4 (14.8%)	0 (0%)	0.0444
	Hyperuricemia	2 (7.4%)	0 (0%)	0.2199
	Heart failure	2 (7.4%)	0 (0%)	0.2199
	Aortic aneurysm	2 (7.4%)	0 (0%)	0.2199
	Allergic rhinitis	1 (3.7)	3 (10%)	0.6135
Past history	Appendectomy	7 (25.9%)	1 (3.3%)	0.021
	Maligancy	3 (11.1%)	0 (0%)	0.10
	Biliary stone	3 (11.1%)	0 (0%)	0.10
	Urolithiasis	0 (0%)	2 (6.7%)	0.4925
	Pancretic disease	0 (0%)	0 (0%)	1.0

Table 2
Clinical findings of the patients with AIP.

The median age at the time of onset (range)		64 years old (50–82)	
Obstructive Jaundice		10/27 (37%)	
Enlargement of the pancreas [Diffuse: Focal]		25/27 (92.6%) [15/25(60%): 10/25(40%)]	
Serum IgG4	> 135 mg/dl	25/26 (96.2%)	
	Median(range)	462 (57–3245)	
Serum IgG	> 1800	15/27 (55.6%)	
	Median(range)	1857 (1327–6348)	
Hypergammaglobulinemia		17/26 (65.4%)	
Anti-nuclear antibody		11/25 (44%)	
Rheumatoid factor		1/19 (5.3%)	
Pathological diagnosis		4/26 (15.4%)	
Extra-pancreatic lesions	Sclerosing cholangititis	18/27 (66.7%)	17/18 (94.4%)
	Sialadenitis		2/18 (11.1%)
	Dacryoadenitis		3/18 (16.7%)
	Interstitial nephritis		2/18 (11.1%)
	Swelling of lymph node		8/18 (44.4%)
Treatment of steroids		24/27 (88.9%)	
Relapse		7/27 (25.9%)	

than the AIP patients at the time of blood collection ($p < 0.01$), while the difference in the gender ratio of the two groups was not statistically significant (Table 1). The comorbidities of the patients in both of the groups are shown in Table 1; the frequency of diabetes mellitus (AIP, n=13 [48.1%]; HVs, n=0 [0%], p < 0.01), hypertension (AIP, n=9 [33.3%]; HVs, n=3 [10%], p=0.0498), hyperlipidemia (AIP, n=9 [33.3%]; HVs, n=1 [3.3%], p < 0.01) and cataracts (AIP, n=4 [14.8%]; HVs, n=0 [0%], p=0.0444) was significantly higher in the AIP patients than in the HVs. Furthermore, a significantly higher percentage of the AIP patients had a past history of appendectomy for appendicitis than the HVs (AIP, n=7 [25.9%]; HVs, n=1 [3.3%], p=0.021).

The median age at the time of AIP onset was 64 years (range: 50–82 years), which was in line with previous reports [13]. Obstructive jaundice, enlargement of the pancreas and irregular narrowing of the main pancreatic duct was observed in 10 (37%), 25 (93%; focal, n=10; diffuse, n=15) and 27 (100%) of the AIP patients, respectively. The elevation of serum IgG4 (more than 135 mg/dl) and serum IgG (more than 1800 mg/dl) was observed in 25 (96%) and 15 patients (56%), respectively. The median serum IgG4 and IgG values were 462 mg/dl (range: 57–3245 mg/dl) and 1857 mg/dl (range: 1327–6348 mg/dl), respectively. Hypergammaglobulinemia, anti-nuclear antibody and rheumatoid factor were detected in 17 (65%), 11 (44%) and one (5%) patient, respectively.

Although pathological examinations were performed in 26 patients, only four patients were pathologically diagnosed with AIP (15.4%). Extra-pancreatic lesions (n=18, 67%), sclerosing cholangititis (n=17, 94.4%), sialadenitis (n=2, 11.1%), dacryoadenitis (n=3, 16.7%), interstitial nephritis (n=2, 11.1%) and lymph node swelling (n=8, 44.4%) were observed. Prednisolone was administered to 24 patients (89%), all of whom showed radiological improvement. Seven patients (29%) relapsed during dose tapering or after the termination of steroid therapy (Table 2).

3.2. *The candidate genetic variants identified in patients with type 1 AIP*

DNA was isolated from mononuclear cells in the blood samples from the 27 AIP patients and the 30 HVs. The quality check showed that all of the samples were of sufficient quality to allow the genetic variants to be analyzed. These DNA samples were amplified using 5 primer pools and were subsequently sequenced using a high-throughput sequencer.

The mean depth of coverage, number of variants and mapped reads and ratio of output reads/mapped reads (on target) are shown in Supplemental Table 2. The mean depth of coverage was 241.70 (SD: 206.53) and 225.28 (SD: 142.18) in the HVs and the AIP patients, respectively. The mean on-target value was 96.02% (SD:

Table 3
Identified candidates of genetic variants associated with type 1 AIP.

Chr	Position type (SNP rs)	A	Frequency		Candidate Gene	Gene description	*P* value (Pc value)
			AIP(%) N=27	HVs(%) N=30			
1	201,016,269 SNP (new)	G > A	7 (25.9)	0(0)	CACNA1S	Calcium channel in skeleton muscle	0.0034 (0.85)
6	29,912,315 SNP (rs41554316)	A > C	11 (40.7)	4(13.3)	HLA-A	The class 1 major histocompatibility complex	0.020 (1)
6	29,912,856 SNP (rs2231119)	A > T	23 (85.2)	18(60)	HLA-A	The class 1 major histocompatibility complex	0.033 (1)
6	33,048,602 SNP (rs1042131)	C > A	24 (88.9)	18(60)	HLA-DPB1	The class 2 major histocompatibility complex	0.014 (1)
7	151,945,051 SNP (rs2838171)	A > G	12 (44.4)	2(6.7)	MLL3	Methylation of histon, Altering antibody effector function	0.0011 (0.20)
11	57,114,093 Deletion	G > -	11 (40.7)	0(0)	P2RX3	Purinergic receptor of pain perception	0.000071 (0.015)
15	78,789,672 SNP (rs75639061)	A > G	9 (33.3)	3(10)	IREB2	Regulation of iron homeostasis	0.033 (1)
18	46,474,797 Deletion	G > -	5 (18.5)	0(0)	SMAD7	Inhibitory protein of TGFB1 signaling	0.019 (0.83)
20	39,750,392 Deletion	G > -	12 (44.4)	0(0)	TOP1	DNA topoisomerases 1 catalyze the breaking DNA	0.000025 (0.0010)

Chr: Chromosome, SNP: single nucleotide polymorphism, A: Allele, AIP: autoimmune pancreatitis, HV: Healthy volunteer, TGFB1: Transforming growth factor beta-1, Pc value: *P* value after adjustment by Bonferroni's correction.

Table 4
Identified candidates of genetic variants associated with relapse of type 1 AIP.

Chr	Position type (SNP rs)	Allele	Frequency		Candidate gene	Gene description	*P* value
			Relapse (%) N=27	Non relapse (%) N=30			
6	29,910,358 SNP (rs1143146)	C > G	7(100)	11(55)	HLA-A	The class 1 major histocompatibility complex	0.036
6	31,238,230 SNP (rs1050716)	G > C	7(100)	10(50)	HLA-C	The class 1 major histocompatibility complex	0.022
6	31,238,230–31,238,234 MNP	GGGGG > CGGGA	6(85.7)	8(40)	HLA-C	The class 1 major histocompatibility complex	0.048
6	31,239,802 SNP (rs1050451)	C > G	6(85.7)	8(40)	HLA-C	The class 1 major histocompatibility complex	0.048
6	32,629,920 SNP (rs41544112)	C > T	3(42.9)	1(5)	HLA-DQB1	The class 2 major histocompatibility complex	0.042
6	32,632,770 SNP (rs1049069)	A > G	5(71.4)	5(25)	HLA-DQB1	The class 2 major histocompatibility complex	0.043
12	2,797,824 Deletion	C > -	3(42.9)	1(5)	CACNA1C	A voltage dependent calcium channel associated with Long QT syndrome	0.042
X	70,836,832–70,836,833 Deletion	GG > -	4(57.1)	3(15)	CXCR3	The chemokine receptor expressed by helper T cell	0.050

Chr: Chromosome, SNP: single nucleotide polymorphism, MNP: multiple nucleotide polymorphism.

0.4764) and 95.30% (SD: 0.8082) in the HVs and AIP patients, respectively. Therefore, the coverage in this study was thought to be sufficient [14].

We first profiled the differences in the frequencies of genetic variants in the AIP patients and the HVs. Among a total of 4206 variants, 16 variants were extracted by Fishers' exact test ($P < 0.05$) as significant candidates, when we focused on the variants with amino-acid modification. In this study, we set the cut-off value for the strand bias at < 0.60, and 11 of variants remained as candidates. Among these 11 candidates, there were seven known single nucleotide polymorphisms (SNPs, rs4973986, rs41554316, rs2231119, rs1042131, rs2838171, rs75639061, rs17576) and four new variants (CACNA1S (c.4642C > T), P2RX3 (c.195delG), SMAD7 (c.624delC), TOP1 (c.2007delG)). Rs4973986 and rs17576 were excluded because almost all Japanese individuals are known to have these variants [15]. Finally, we identified nine candidate variants associated with type 1 AIP (Table 3).

3.3. The candidate genetic variants identified as being associated with the relapse of type 1 AIP

AIP relapse occurred in seven patients. Next, we profiled the difference in the frequencies of genetic variants in the AIP patients with/without relapse. Among a total 2631 variants, 13 variants were extracted as significant candidates by Fishers' exact test ($P < 0.05$) after excluding the variants without amino-acid modifications in which the strand bias was > 0.60. Among these 13 candidates, there were ten known single nucleotide polymorphisms (rs1143146, rs1050716, rs1050451, rs41544112, rs1049069, rs328, rs238238, rs16027, rs2108622, rs2071747) and three new variants (HLA-C (c.759_763delCCCCCinsTCCCG), CACNA1C (c.5996delC), CXCR3 (c.630_631delGC)). Rs328, rs238238, rs16027, rs2108622 and rs2071747 were excluded because almost all Japanese individuals are known to have these variants [15]. Finally we identified eight candidate variants associated with the relapse of AIP (Table 4).

3.4. The identified candidate genetic variants associated with extra-pancreatic lesions of type 1 AIP

Among the 24 AIP patients, 18 had extra-pancreatic lesions. Finally, we investigated the difference in the frequencies of genetic variants in the AIP patients with/without extra-pancreatic lesions. Among a total 3319 variants, five known SNPs were extracted as significant candidates by Fishers' exact test ($p < 0.05$) after excluding the variants without amino-acid modification or in which the strand bias was > 0.60, we identified rs2228001, rs78422529/rs116229259/rs17413706/rs1050716, rs111493987, rs3736032 and rs1800470. Rs2228001, rs3736032 and rs1800470 because they are known to exist in almost all Japanese individuals [15]. Finally, we identified two known SNPs as candidate gene variants associated with extra-pancreatic lesions (Table 5).

4. Discussion

The high-throughput sequencing analysis using next generation sequencing identified nine, eight and two candidate variants associated with the development of type 1 AIP, AIP relapse and extra-pancreatic lesions in type 1 AIP patients, respectively. Most of the previous studies to investigate the genes associated with susceptibility to AIP [6–10] did not involve comprehensive analyses, rather, they focused on target lesions that were reported to be associated with autoimmune disease or which were extracted by a microsatellite genotyping analysis. This might have been caused by the classical sequencing procedures. In contrast, our

Table 5
Identified candidates of genetic variants associated with extra-pancreatic lesions of type 1 AIP.

Chr	Position type (SNP rs)	A	Frequency		Candidate gene	Gene description	*P* value (Pc value)
			with EPL(%) N=18	w/o EPL(%) N=9			
6	31,238,230 SNP (rs1050716)	G > C	14 (77.8)	3 (33.3)	HLA-C	The class 1 major histocompatibility complex	0.034
7	151,935,871 SNP (rs111493987)	C > A	14 (77.8)	4 (13.3)	MLL3	Methylation of histon, Altering antibody effector function	0.034

Chr: Chromosome, SNP: single nucleotide polymorphism, A: Allele, EPL: extra-pancreatic lesions, w/o: without.

study used high-throughput sequencing. A recent genome-wide association study did not reveal the genes that are associated with AIP susceptibility [11]. The present study is therefore the first comprehensive analysis of the genes related to AIP susceptibility.

AIP is considered to be an autoimmune disorder. Indeed, carbonic anhydrase, lactoferrin, pancreatic secretory trypsin inhibitor, amylase-alpha, ubiquitin-protein ligase E3, SPINK and TRY1 have been identified as the autoimmune antigens associated with AIP [16,17]. We therefore hypothesized that there might be commonality in the genetic background of AIP patients with regard to the genes associated with the immune system.

Among the nine candidate genetic variants that were found to be associated with type 1 AIP, P2RX3 (c.195delG) and TOP1 (c.2007delG) showed a highly significant association with AIP, even after adjustment by Bonferroni's correction. P2RX3, which constitutes a positive autocrine signal for insulin release in the pancreatic beta cells [18,19] and contributes to the pancreatic pain caused by chronic pancreatitis [20], is associated with the immune system. A recent study reported that an enlarged spleen, which corresponds to an increase in the numbers of lymphocytes and macrophages, and hypocellularity of the thymus and bone marrow were observed in P2RX2 and P2RX3 knockout mice, suggesting that they resulted from compensatory changes in a compromised immune system [21]. TOP1, which encodes DNA topoisomerase 1 that catalyzes the breaking and rejoining of single-strand DNA and which functions normally during transcription [22], is also associated with various autoimmune diseases, including scleroderma, systemic lupus erythematosus and rheumatoid arthritis [23,24]. Besides these two candidates, CACNA1 S and MLL3 also play a role in the immune system. CACNA1S, which encodes the L-type calcium channel alpha 1 subunit, is expressed on T-lymphocytes and regulates the calcium current, which can induce the activation of T cell antigen receptors [25]. MLL3 forms a complex with MLL4 and subsequently initiates the transcription of downstream switch regions at the immunoglobulin heavy-chain locus in B-lymphocytes, leading to defective immunoglobulin class switching [26]. Although, these candidates might contribute to the pathogenesis of AIP, the influence of these candidate genetic variants on the immune system remains unknown. Further studies to investigate the relationship between these candidate genetic variants and the autoantibodies associated with AIP are needed.

Transforming growth factor beta (TGF-β) is known to be an important regulating factor in the maintenance of immune homeostasis. A previous study suggested that a loss of TGF-β signaling contributed to AIP [27]. In the present study, we identified a deletion in SMAD7 as a candidate genetic factor associated with the pathogenesis of AIP. Thus SMAD7 could be suitable marker of AIP; however, it is necessary to further evaluate TGF-β signaling under the genetic condition of SMAD7 deletion (c.624delC).

We also identified eight candidate genetic variants that are associated with the relapse of type 1 AIP. Six candidates were HLAs, which are closely associated with some autoimmune diseases, including AIP [5]. In particular, HLA-DQB1 was reported to be associated with the relapse of AIP in a Korean study [28], which suggests that HLA-DQB1 could be a strong candidate gene associated with susceptibility to AIP relapse.

In this study, we also explored the genes that were associated with susceptibility to extra-pancreatic lesions, including sclerosing cholangitis, sialadenitis, dacryoadenitis, interstitial nephritis and lymph node swelling, and identified two candidates. Oguchi et al. performed a genome-wide association study and extracted ten candidates associated with susceptibility to dacryoadenitis or sialadenitis in the Japanese patients with AIP, while no genes associated with all extra-pancreatic lesions was identified [11]. In this study, because sialadenitis and dacryoadenitis were only observed in two and three patients, respectively, we could not identified the genes that were specifically associated with dacryoadenitis or sialadenitis susceptibility in AIP patients. Indeed, AIP is a rare disease with a prevalence rate of 4.6 per 100,000 population and an annual incidence rate of 1.4.per 100,000 population [13]. A nationwide or worldwide study will therefore be needed to validate the candidates associated with susceptibility to AIP.

5. Conclusion

In the present study, we performed a high-throughput sequencing analysis using next generation sequencing to comprehensively investigate 1031 genes. Our analysis identified nine, eight and two candidate variants associated with type 1 AIP, the relapse of AIP and extra-pancreatic lesions, respectively. These candidates might be used as markers for the diagnosis of AIP and extra-pancreatic lesions as well as for predicting the relapse of AIP.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.03.005.

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