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Association Study of *MAFA* and *MAFB* Genes Related to Organ-Specific Autoimmunity, with Susceptibility to Type-1 Diabetes in Japanese and Caucasian Populations

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Abstract

The transcriptional factor MAFA is specifically expressed in beta cells of pancreatic islets, and activates tissue-specific transcription of insulin. We previously reported that MAFA is also expressed in the thymus and regulates intra-thymic expression of insulin in the mouse. In humans, we identified a functional polymorphism of *MAFA*, Gly346Cys, which was suggested to be associated with type 1 diabetes. This study aimed to validate the association of *MAFA* with type 1 diabetes in a larger number of subjects. In addition, molecular scanning of *MAFB* another member of the large MAFA transcription family, and an association study with type 1 diabetes were also performed. A total of 1733 subjects, including newly recruited Japanese (346 controls and 532 cases) and Caucasians (223 controls and 228 cases), were studied. In newly recruited Japanese subjects, the minor allele frequency of *MAFA*Gly346Cys was lower in cases than in controls (2.9 vs. 5.1%, odds ratio [95%CI]: 0.56 [0.34-0.91], p=0.02). Meta-analysis with our previous data showed a significant association of *MAFA* with type 1 diabetes (summary odds ratio [95%CI]: 0.49 [0.32-0.76], p=0.0013). When cases were limited to subjects with a risk genotype of *INS*, the association was further strengthened (odds ratio [95%CI]: 0.47 [0.30-0.74], p=0.00097). In the Caucasian population, the difference in minor allele frequency of *MAFA* between cases and controls was not significant (6.4% vs. 5.4%, odds ratio [95%CI]: 1.14 [0.66-1.99], NS). When data from Japanese and Caucasians were combined, summary odds ratio was 0.68 [95%CI: 0.48-0.95] (p<0.03). P value for heterogeneity, however, reached statistical significance (p<0.05), suggesting genetic heterogeneity between the two populations. For *MAFB* two novel variants (-632C>G and 618C>A) were identified, but neither was significantly associated with type 1 diabetes. In conclusion, *MAFA* Gly346Cys is associated with type 1 diabetes, especially in the Japanese population, which possesses the high-risk *INS* genotype.

Keywords: Type 1 Diabetes; Organ-Specific Autoimmunity; *MAFA*; *MAFB*; Insulin; Association study; Meta-analysis

Introduction

Type 1 diabetes is an organ-specific autoimmune disease against pancreatic islets, characterized by targeted destruction of insulin-producing beta cells by infiltrated lymphocytes in genetically susceptible individuals [1]. A meta-analysis of genome-wide association studies revealed that over 40 chromosomal loci are associated with type 1 diabetes risk in Caucasian populations [2]. However, only few genes are functionally identified as responsible genes for these susceptibility loci. The strongest susceptibility genes are located within the HLA region of the major histocompatibility complex (MHC) on chromosome 6p21, termed *IDDM1*, accounting for approximately 45% of the familial clustering of type 1 diabetes [3]. Non-MHC genes, such as insulin (*INS*) lymphoid tyrosine phosphatase (*PTPN22*) and cytotoxic T lymphocyte antigen 4 (*CTLA4*) were initially identified by candidate gene approach [4-6]. The functions of LYP (Lymphoid-specific tyrosine phosphatase: a protein of *PTPN22*) and CTLA4 are related to regulation of immune response in general, but not to beta-cell-specific autoimmune response as evidenced by association with multiple autoimmune diseases including Graves' disease and Rheumatoid Arthritis (RA) [7,8]. In contrast to these immune-regulating genes, the insulin gene (*INS*), located at the *IDDM2* locus on chromosome 11p15, is a unique susceptibility gene showing association with type 1 diabetes only, suggesting a function related to beta-cell-specific autoimmunity. The insulin gene is known to play an important role in beta-cell-specific autoimmunity through the maturation of T cells in the thymus. Various self-antigens are ectopically expressed in the thymus for the induction of central tolerance to self-antigens [9]. Lack of intra-thymic expression of a certain self-antigen therefore causes breakdown of central tolerance and survival of autoreactive T cells, leading to an autoimmune response against the tissue expressing the self-antigen. Insulin, as a primary autoantigen of type 1 diabetes is also expressed in both pancreatic beta cells and the thymus in a normal condition [10]. Pugliese et al. reported that a Variable Number of Tandem Repeats (VNTR) in the promoter region of *INS* is associated

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with the expression level of inulin in the thymus and susceptibility to type 1-diabetes [4]. Reduced expression of insulin is observed in the thymus of individuals with the risk genotype of *INS* (VNTR class I/I) in comparison with those without.

The transcriptional factor MafA, a member of the large MafA transcription family, is specifically expressed in beta cells of pancreatic islets, and activates beta-cell-specific transcription of insulin [11]. We previously reported that MafA, but not Pdx1 or NeuroD, is expressed in the thymus and regulates intra-thymic expression of insulin in the mouse [12]. Molecular scanning of the mouse Mafa gene (*Mafa*) revealed that the entire nucleotide sequence of mouse *MafA* was identical among control strains (BALB/c, C3H, and CTS mice) and a mouse model of type 2 diabetes (Nagoya-Shibata-Yasuda, NSY mice), but identified unique variants in the promoter region of *Mafa* in a mouse model of type 1 diabetes (Non-Obese Diabetic, NOD mice) [13]. The promoter activity of *Mafa* was significantly lower in the NOD mouse than in wild type, so that the expression of MafA and insulin were reduced in the thymus of the NOD mouse. Systemic disruption of the MafA gene resulted in reduced expression of insulin in the thymus, indicating that MafA is a key regulator of insulin expression in the thymus. In humans, we identified a novel functional polymorphism of the MafA gene (*MAFA* Gly346Cys) that affects transcriptional activity of MafA itself. An association study with a relatively small number of subjects suggested that the polymorphism was associated with type 1 diabetes, but not with autoimmune thyroid disease (Graves' disease and Hashimoto's thyroiditis) suggesting that MafA is involved in beta-cell-specific autoimmunity in humans as well as in mice [12].

MafBv-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian), is another member of the large MafA transcription family, and is selectively expressed in alpha cells of mouse pancreatic islets [14]. Recent studies in humans, however revealed that MafB is expressed in both alpha and beta cells of human islets suggesting the possibility that not only *MAFA*, but also the MafB gene (*MAFB*), contributes to type 1 diabetes susceptibility [15,16].

The present study was performed to clarify the contribution of the *MAFA* and *MAFB* to susceptibility to type 1- diabetes. To this end, we studied the association of *MAFA* Gly346Cys with susceptibility to type 1-diabetes in a larger number of subjects, using the 2nd panel of Japanese population and Caucasian population by meta-analysis. In addition, we re-sequenced human *MAFB* and newly identified variants were studied for the association with type 1 diabetes.

Materials and Methods

Subjects

A total of 1733 subjects (832 control subjects and 901 patients with type 1-diabetes) were studied for meta-analysis in the Japanese and Caucasian populations. The data of the 1st panel were derived from our previous report (263 control subjects and 139 patients with type 1 diabetes from western Japan) [12]. The 2nd panel (346 control subjects and 534 patients with type 1 diabetes, clinical characteristics were shown in supplementary subjects) was newly recruited from three geographic areas in Japan (two from eastern Japan and one from western Japan) (table 1). The 3rd panel (223 control subjects and 228 patients with type 1 diabetes), as the Caucasian population, was provided by Professor George S. Eisenbarth, the Barbara Davis Center for Childhood Diabetes, Colorado, USA. A total of 126 subjects with alopecia areata were recruited in the Japanese population (110 subjects were provided by Associate Professor Naoki Oiso, Department of Dermatology, Kinki University School of Medicine, Osaka, Japan and 16 subjects by Professor Tamio Suzuki, Department of Dermatology, Yamagata University School of Medicine, Yamagata, Japan). This study was approved by the appropriate ethics committees, and informed consent was obtained from all participants.

Genotyping and direct sequencing: Restriction fragment length polymorphism analysis using ApaLI (New England Biolab) was performed to genotype the Gly346Cys polymorphism (rs62521874) of *MAFA*, and a part of subjects was confirmed using the Taqman system (Applied Biosystems). The *INS* variable number of tandem repeat (VNTR) class I/class III status was estimated by genotyping the -23 HphI(New England Biolab) single nucleotide polymorphism as previously described [17].

The entire sequence of the human MAFB gene (4511 base pairs) was amplified by Polymerase Chain Reaction (PCR) using 10 pairs of primer sets (Supplementary Methods) (table 2). Direct sequencing was performed using an ABI PRISM Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). Restriction fragment length polymorphism analysis using RsaI and TseI (New England Biolab) was performed for genotyping the variants of *MAFB*(-632C>G and 618C>A), respectively.

Statistical analysis: Allele frequency was estimated by direct counting. The significance of differences in the distribution of alleles was determined by Fisher's direct probability test. Observed and

	Control	Type 1 diabetes
Sex (Female/Male)	365/244	223/311
Age (year)	46.3 ± 9.9	
Range of age (year)	22-78	
Age at onset (year)		8.1 ± 17.5
Range of age at onset (year)		1-75

Mean ± SD

Table 1: Supplementary subjects: Clinical characteristics of patients with type 1 diabetes and control subjects.

#	Name of oligo	5'-3'
1	hMAFB-1F	GCCCTCCCCAACATACAAAT
2	hMAFB-1R	TTTATCGCCTCCTGATGTCAC
3	hMAFB-2F	GAACAAAGGGCCCAGGAG
4	hMAFB-2R	CGTTGCTCGCTCTCTAGCTC
5	hMAFB-3F	CATCAGGAGGCGATAAAAGG
6	hMAFB-3R	TTCACGTCGAACTTGAGCAG
7	hMAFB-4F	TGGCCATGGAGTATGTCAAC
8	hMAFB-4R	GTGGCTAGTGGGCAGCTGTT
9	hMAFB-5F	CATCACCATCATCACCAAGC
10	hMAFB-5R	GGGGTAGTCTGGGACTAGGG
11	hMAFB-6F	CCTCCTCTCCCGAGTTCTTT
12	hMAFB-6R	AGGTCCGTCTGTCCTTCCTT
13	hMAFB-7F	GGAAAGGAGAGGGACTCTGG
14	hMAFB-7R	GTGTGCCCCAAGACAAAGTT
15	hMAFB-8F	AAGAAAAAGTTCCGGGCATT
16	hMAFB-8R	CGCACTTGAAAGTTGCAAAA
17	hMAFB-9F	CACTGCACTGAACCAACTGC
18	hMAFB-9R	GCAGGGTTTGAAATTGATCC
19	hMAFB-10F	TGTCCTGCATCAGAAACGAG
20	hMAFB-10R	ATCCAGTGCTGGGTTGTACC

Table 2: Supplementary methods. Primer sets for PCR amplification of human MafB gene.

expected genotype frequencies were compared by Hardy-Weinberg equilibrium using chi-squared analysis. No significant deviation from equilibrium was observed in this study. Meta-analysis was performed with our previous data [12]. For calculation of the summary odds ratio according to the genotype groups from case-control studies, we adopted a fixed model using the Mantel-Haenszel method [18]. The p value for heterogeneity in meta-analysis was calculated by Breslow-Day test [19]. Statistical significance was defined as $p<0.05$.

Results

Association of *MAFA* Gly346Cys with type 1 diabetes in Japanese population

In newly recruited Japanese subjects (2nd panel), the minor allele (Cys allele) frequency of *MAFA* Gly346Cys in type 1 diabetic patients was significantly lower than that in control subjects (2.9% vs. 5.1%, odds ratio [95%CI]: 0.56 [0.34-0.91], p=0.02, Fisher's direct probability test (Table 3). Meta-analysis of the 2nd panel with our previous data (1st panel, Supplementary Table 1) showed that the *MAFA*Gly346Cys polymorphism was significantly associated with susceptibility to type 1 diabetes in the Japanese population (summary odds ratio [95%CI]: 0.49 [0.32-0.76], p=0.0013, p value for heterogeneity: p=0.84) (Table 4). When the subjects were limited to type 1 diabetic patients with the risk genotype of *INS* (VNTR class I/I) as "high-risk type 1 diabetes", the association was concentrated in the Japanese population with the high risk *INS* genotype (odds ratio [95%CI]: 0.47 [0.30-0.74], p=0.00097, p value for heterogeneity: p=0.15 (Table 5).

Association of *MAFA* Gly346Cys with type 1 diabetes in Caucasian population

In the Caucasian population (3rd panel), the difference in minor allele frequency between control subjects and patients with type 1 diabetes was not statistically significant (5.6% vs. 6.4%, odds ratio [95%CI]: 1.14 [0.66-1.99], NS (Table 6). Minor allele frequency of control subjects was comparable with that in high-risk type 1 diabetes patients who possess the high-risk genotype of *INS*(5.6% vs. 6.6%, odds ratio [95%CI]: 1.18 [0.67-2.08], NS).

Meta-analysis of association studies of *MAFA* with type 1 diabetes in Japanese and Caucasian populations

When data of the Japanese (1st and 2nd panels) and Caucasian populations (3rd panel) were combined, the difference in minor allele frequencies between control subjects and patients with type 1 diabetes was still significant (summary odds ratio: 0.68 [95%CI: 0.48-0.95], p=0.02, Table 2), but the p value for heterogeneity also reached significance (p<0.05, Breslow-Day test) (Table 7). When cases were limited to patients with the high-risk *INS* genotype, a similar tendency was observed in comparison with control subjects (summary odds ratio: 0.67 [95%CI: 0.47-0.94], p=0.022, p value for heterogeneity: p<0.03 (Table 8).

	Control		Type 1 diabetes				
	n = 346	(%)	n = 534	(%)	OR	95%CI	P value
Genotype							
Gly / Gly	312	(90.2)	503	(94.2)			
Gly / Cys	33	(9.5)	31	(5.8)			
Cys / Cys	1	(0.3)	0	(0.0)			0.05
Allele							
Gly	657	(94.9)	1037	(97.1)			
Cys	35	(5.1)	31	(2.9)	0.56	0.34-0.91	0.02

OR: odds ratio, CI: confidence interval
Fisher's exact probability test

Table 3: Association of *MAFA* Gly346Cys with susceptibility to type 1 diabetes in Japanese population (2nd panel).

	Control		Type 1 diabetes				
	n = 263	(%)	n = 139	(%)	OR	95%CI	P value
Genotype							
Gly / Gly	236	(89.7)	134	(96.4)			
Gly / Cys	27	(10.3)	5	(3.6)			
Cys / Cys	0	(0.0)	0	(0.0)	0.33	0.13-0.83	<0.02
Allele							
Gly	499	(94.9)	273	(98.2)			
Cys	27	(5.1)	5	(1.8)	0.34	0.13-0.85	0.02

OR: odds ratio, CI: confidence interval
Fisher's exact probability test

Table 4: Association of *MAFA* Gly346Cys with susceptibility to type 1 diabetes in Japanese population (1st panel).

	Control		High-risk T1DM*				
	n	MAF (%)	n	MAF (%)	OR	95%CI	P value
1st panel	263	(5.1)	124	(1.2)	0.23	0.08-0.71	0.001
2nd panel	346	(5.1)	507	(3.0)	0.57	0.35-0.94	0.026
Summary	609	(5.1)	631	(2.6)	0.47	0.30-0.74	0.00097

P value for heterogeneity: p= 0.15
*High-risk type 1 diabetes: *INS-VNTR* classes I / I at *IDDM2* locus, MAF: minor allele frequency, OR: Odds ratio

Table 5: Meta-analysis of association studies of *MAFA* Gly346Cys with susceptibility to high-risk type 1 diabetes in Japanese population.

	Control		Type 1 diabetes				
	n = 223	(%)	n = 228	(%)	OR	95%CI	P value
Genotype							
Gly / Gly	198	(88.8)	200	(87.7)			
Gly / Cys	25	(11.2)	27	(11.8)			
Cys / Cys	0	(0.0)	1	(0.4)			0.60
Allele							
Gly	421	(94.4)	427	(93.6)			
Cys	25	(5.6)	29	(6.4)	1.14	0.66-1.99	0.68

OR: odds ratio, CI: confidence interval
Fisher's exact probability test

Table 6: Association of *MAFA* Gly346Cys with susceptibility to type 1 diabetes in Caucasian population (3rd panel).

		Control		Type 1 diabetes				
	Ethnic group	n	MAF (%)	n	MAF (%)	OR	95%CI	P value
1st panel	Japanese	263	(5.1)	139	(1.8)	0.34	0.13-0.83	0.022
2nd panel	Japanese	346	(5.1)	534	(2.9)	0.56	0.34-0.91	0.028
3rd panel	Caucasian	223	(5.6)	228	(6.4)	1.14	0.66-1.99	0.68
Summary		832	(5.2)	901	(3.6)	0.68	0.48-0.95	0.023

P value for heterogeneity: p< 0.05
MAF: minor allele frequency, OR: Odds ratio

Table 7: Meta-analysis of association studies of *MAFA* Gly346Cys with susceptibility to type 1-diabetes in Japanese and Caucasian populations.

Difference in association of *MAFA* with three subtypes of type 1 diabetes in Japanese population

The data from the 2nd panel in the Japanese population were stratified into three subtypes of type 1 diabetes: Fulminant (F), Slowly Progressive (SP) and Acute onset (A) type 1-diabetes. The association of *MAFA* Gly346Cys was most apparent in acute-onset type 1 diabetes

		Control		High-risk T1DM*				
	Ethnic group	n	MAF (%)	n	MAF (%)	OR	95%CI	P value
1st panel	Japanese	263	(5.1)	124	(1.2)	0.23	0.08-0.71	0.001
2nd panel	Japanese	346	(5.1)	507	(3.0)	0.57	0.35-0.94	0.026
3rd panel	Caucasian	223	(5.6)	159	(6.6)	1.18	0.67-2.08	0.57
Summary		832	(5.2)	829	(3.6)	0.67	0.47-0.94	0.022

P value for heterogeneity: p< 0.03
*High-risk type 1 diabetes: *INS-VNTR*class I / I at *IDDM2* locus, MAF: minor allele frequency, OR: Odds ratio

Table 8: Meta-analysis of association studies of *MAFA* Gly346Cys with susceptibility to high-risk type 1 diabetes in Japanese and Caucasian populations.

(minor allele frequency: 2.7%, odds ratio [95%CI]: 0.53 [0.30-0.92], p<0.03 but was not significant in slowly progressive type 1 diabetes (minor allele frequency: 3.1%, odds ratio [95%CI]: 0.61 [0.29-1.28], NS) or fulminant type 1 diabetes (minor allele frequency: 4.0%, odds ratio [95%CI]: 0.78 [0.18-3.34], NS) (Table 9).

Association study of *MAFA* with susceptibility to alopecia areata

A total of 126 patients with alopecia areata, an organ-specific autoimmune disease against hair follicles, were newly recruited for further investigation of the association with other organ-specific autoimmune disease. *MAFA* Gly346Cys was not significantly associated with susceptibility to alopecia areata (odds ratio [95%CI]: 0.69 [0.34-1.40], NS (Table 10).

Identification of rare variants of *MAFB* and association study with type 1 diabetes

By direct sequencing of *MAFB* in 16 control subjects and 16 patients with type 1 diabetes, two novel rare variants: a SNP located in the 5' upstream region (-632C>G, minor allele frequency in control subjects: 1.3%) and a non-synonymous SNP at exon 1 (618C>A, Ser206Arg, minor allele frequency in control subjects: 0.2%) were identified. Case-control studies of these variants did not show a significant association of these variants with susceptibility to type 1 diabetes (Tables 11 and 12).

Discussion

The present data reproduced the significant association of a functional polymorphism *MAFA* Gly346Cys with susceptibility to type 1-diabetes in an independent 2nd panel of the Japanese population. Meta-analysis of the 1st and 2nd panels further validated the significant association of *MAFA* with type 1 diabetes in the Japanese population. As we previously reported, the association was concentrated in type 1 diabetes with the risk genotype of *INS* (*IDDM2*) (summary odds ratio: 0.47, 95%CI: 0.30-0.74, p=0.00097, Table 1), suggesting a gene-gene interaction between the transcriptional factor *MAFA* and its target gene, insulin (*INS*) (Table 5). The Cys allele (minor allele) of *MAFA* Gly346Cys increased the transcriptional activity of MAFA response element (MARE) and showed strong linkage disequilibrium with one repeat (1R) of VNTR in promoter region of *MAFA*, which showed higher promoter activity of *MAFA* itself [12]. These observations suggest that the Cys allele of *MAFA* Gly346Cys causes higher insulin expression in the thymus, leading to the induction of central tolerance to insulin and a protective effect against the development of type 1 diabetes. In clear contrast to the strong association with type 1 diabetes in the Japanese population, we did not observe a significant association of *MAFA* with type 1 diabetes in the Caucasian population (odds ratio: 1.14, 95%CI: 0.66-1.99, NS). Even if the patients were limited to those possessing the high-risk genotype of *INS*, there was no difference in minor allele frequency between all patients and high-risk patients with type 1 diabetes (6.4% vs. 6.6%). The association of *INS* with type 1 diabetes was initially reported in the Caucasian population and reproduced in the Japanese population [17]. The incidence of type 1

Control		Type 1 diabetes													
		Fulminant		Slowly Progressive		Acute-onset		C vs. F		C vs. SP		C vs. A			
n=346	(%)	n=25	(%)	n=143	(%)	n=366	(%)	P value	OR	P value	OR	P value	OR		
Genotype															
Gly / Gly		312	(90.2)		23	(92.0)		134	(93.7)			346	(94.5)		
Gly / Cys		33	(9.5)		2	(8.0)		9	(6.3)			20	(5.5)		
Cys / Cys		1	(0.3)		0	(0.0)		0	(0.0)			0	(0.0)		
Allele															
Gly		657	(94.9)	48	(96.0)	277	(96.9)	712	(97.3)						
Cys		35	(5.1)	2	(4.0)	9	(3.1)	20	(2.7)	NS	0.78	NS	0.61	<0.03	0.53

OR: Odds ratio

Table 9: Association of *MAFA* Gly346Cys with susceptibility to three subtypes of type 1 diabetes: Fulminant (F), Slowly Progressive (SP) and Acute-onset type 1 diabetes (A).

	Control		Alopecia areata				
	n=263	(%)	n=126	(%)	OR	95%CI	P value
Genotype							
Gly / Gly	236	(89.7)	117	(92.9)			
Gly / Cys	27	(10.3)	9	(7.1)			
Cys / Cys	0	(0.0)	0	(0.0)	0.67	0.31-1.47	NS
Allele							
Gly	499	(94.9)	243	(96.4)			
Cys	27	(5.1)	9	(3.6)	0.68	0.32-1.47	NS

OR: odds ratio, CI: confidence interval
Fisher's exact probability test

Table 10: Association study of *MAFA* Gly346Cys with susceptibility to alopecia areata in Japanese population.

	Control		Type 1 diabetes				
	n=194	%	n=134	%	OR	95% CI	P value
Genotype							
CC	189	97.4	129	96.3			
CG	5	2.6	5	3.7			
GG	0	0.0	0	0.0	1.47	0.42-5.14	NS
Allele							
C	383	98.7	263	98.1			
G	5	1.3	5	1.9	1.46	0.42-5.05	NS

OR: Odds ratio, CI: confidence interval
Fisher's exact probability test

Table 11: Association study of *MAFB* -632C>G with susceptibility to type 1 diabetes.

	Control		Type 1 diabetes				
	n=206	%	n=137	%	OR	95% CI	P value
Genotype							
CC	205	99.5	137	100.0			
AC	1	0.5	0	0.0			
AA	0	0.0	0	0.0	1.34	0.04-39.85	NS
Allele							
C	411	99.8	274	100.0			
G	1	0.2	0	0.0	1.33	0.04-39.52	NS

OR: Odds ratio, CI: confidence interval
Fisher's exact probability test

Table 12: Association study of *MAFB* 618C>A with susceptibility to type 1 diabetes.

diabetes in the Japanese population (1.4-2.2 per 100,000 per year) is relatively lower than that in the European population (5.0-36.8 per 100,000 per year) in 0-14 years of age [20]. Despite the low incidence of type 1 diabetes in the Japanese population, more than 90% of the Japanese general population possesses the high-risk genotype of *INS*, suggesting a reduction of intra-thymic expression of insulin and breakdown of central tolerance to insulin in most of the Japanese population [21]. This might be a reason why the Cys allele of *MAFA* Gly346Cys contributes to protection against the development of type 1 diabetes, especially in subjects with the high-risk genotype of *INS* in the Japanese population, in that the protective allele of *MAFA*, which increases insulin expression in the thymus, plays a more important role in subjects with the high risk genotype of *INS*, which decreases insulin expression in the thymus. Meta-analysis of Japanese (1st and 2nd panels) and Caucasian (3rd panel) populations revealed heterogeneity among the three panels (p value for heterogeneity: $p<0.05$, Breslow-Day test), although no heterogeneity was observed between the two panels in Japanese populations (1st and 2nd panel, $p=0.15$), suggesting genetic heterogeneity in the association of the *MAFA* polymorphism with type 1 diabetes between the Japanese and Caucasian populations.

Type 1 diabetes is a clinically and etiologically heterogeneous disorder, and is classified into three subtypes: typical acute-onset, slowly progressive and fulminant type 1 diabetes. Fulminant type 1 diabetes is characterized by an extremely acute onset of diabetes and absence of islet-related autoantibodies accounting for up to 20% of type 1-diabetes in Japan [22,23]. Slowly progressive type 1 diabetes is characterized by positivity for islet-related autoantibodies, but a long insulin-independent stage with gradual loss of beta cells, leading ultimately to an insulin-dependent stage [24]. Association studies of HLA genes with these three subtypes of type 1 diabetes revealed that associations of HLA genes with fulminant type 1 diabetes are qualitatively different from those with other subtypes of type 1 diabetes, whereas those of slowly progressive type 1 diabetes are qualitatively similar to, but quantitatively different from, those of acute-onset type 1 diabetes [25]. The present study showed that the association with *MAFA* is most apparent in acute-onset type 1 diabetes, suggesting that *MAFA* is involved in the pathogenesis of typical beta-cell-specific autoimmunity.

We previously reported that *MAFA* is significantly associated with susceptibility to type 1 diabetes (1st panel), but no evidence of an association with autoimmune thyroid disease (Graves' disease and Hashimoto's thyroiditis) was observed [12]. A similar result to that in autoimmune thyroid disease was observed in alopecia areata, an organ-specific autoimmune disease against hair follicles suggesting the contribution of *MAFA* to the pathogenesis of beta-cell-specific autoimmunity, but not other organ-specific autoimmune diseases against thyroid tissue and hair follicles [26] (Table 9).

Recent studies showed a difference in expression profiles of transcriptional factors in pancreatic islets between rodents and humans [15]. For example, v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (avian) (MafB), another member of the large Maf transcription family, is detected at equal levels in human pancreatic alpha and beta cells, whereas MafB expression is absent in adult mouse beta cells [16]. In order to clarify the contribution of the MafB gene (*MAFB*) to the development of type 1 diabetes, we performed re-sequencing of human *MAFB* in 32 Japanese subjects, and identified two novel rare variants (-632C>G and 618C>A). These variants were not significantly associated with susceptibility to type 1 diabetes suggesting that *MAFB* does not play a major role in susceptibility to type 1 diabetes, although further analysis is needed to validate the association (Tables 10 and 11).

In conclusion, our data demonstrated that a functional polymorphism of *MAFA* is associated with type 1 diabetes especially in subjects who possess the risk genotype of *INS* in the Japanese population. The association of *MAFA* with type 1 diabetes, but not with other autoimmune diseases, suggests that the function of MafA is related to organ-specific autoimmunity, as is reported for the insulin gene. *MAFB* in contrast was not associated with type 1 diabetes, although further studies are needed to clarify the contribution of MafB to beta-cell autoimmunity. It is important to study susceptibility genes related to beta-cell-specific autoimmunity to further recognize the mechanisms of selective destruction of beta cells by insulitis, in order to establish safe and effective treatment of type 1 diabetes with minimal adverse effects by beta-cell-specific immunotherapy.

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