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

Shinsuke Noso1, Yumiko Kawabata1, Naru Babaya1, Yoshihisa Hiromine1, Eiji Kawasaki2, Takuya Awata3, Taro Maruyama4, Sunanda Babu5, Naoki Oiso6, Akira Kawada6, Tamio Suzuki7, George S Eisenbarth5 and Hiroshi Ikegami6*

1Department of Endocrinology, Metabolism and Diabetes, Kinki University Faculty of Medicine, Osaka, Japan
2Department of Metabolism / Diabetes and Clinical Nutrition, Nagasaki University Hospital, Nagasaki, Japan
3Department of Endocrinology and Diabetes, Faculty of Medicine, Saitama Medical University, Saitama, Japan
4Department of Internal Medicine, Saitama Social Insurance Hospital, Saitama, Japan
5Barbara Davis Center for Childhood Diabetes, University of Colorado, Denver, CO, USA
6Department of Dermatology, Kinki University Faculty of Medicine, Osaka, Japan
7Department of Dermatology, Faculty of Medicine, Yamagata University, Yamagata, Japan

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

*Corresponding author: Hiroshi Ikegami, Department of Endocrinology, Metabolism and Diabetes, Kinki University Faculty of Medicine, 377-2 Ohno-higashi, Osaka-sayama, Osaka 589-8511, Japan, Tel: +81-72-366-0246 (ext. 3125). Fax: +81-72-366-2095; E-mail: ikegami@med.kindai.ac.jp

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with the expression level of insulin 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 Maf 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 Maf 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 (MAPA 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 MabB is expressed in both alpha and beta cells of human islets suggesting the possibility that not only MAFA, but also the MabB gene (MAFB), contributes to type 1 diabetes susceptibility [15,16].

The present study was performed to clarify the contribution of the MAFB and MAF to susceptibility to type 1 diabetes. To this end, we studied the association of MAFB 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 a 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 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 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 MAFB, and a part of subjects was confirmed using the Taqman system (Applied Biosystems). The INS variable number of tandem repeat (VNTR) class I/class II 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 Rsal and Tsel (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
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).
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1. **Table 8:** Meta-analysis of association studies of MAFA Gly346Cys with susceptibility to high-risk type 1 diabetes in Japanese and Caucasian populations.

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Control High-risk T1DM*</th>
<th>n</th>
<th>MAF (%) n</th>
<th>MAF (%) OR 95%CI P value for heterogeneity p&lt; 0.03</th>
<th>*High-risk type 1 diabetes: INS VNTR class 1/1 at IDDM2 locus, MAF: minor allele frequency, OR: Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st panel Japanese</td>
<td>263 (5.1) 124 (1.2)</td>
<td>0.23</td>
<td>0.08-0.71</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>2nd panel Japanese</td>
<td>346 (5.1) 507 (3.0)</td>
<td>0.57</td>
<td>0.35-0.94</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>3rd panel Caucasian</td>
<td>223 (5.6) 159 (6.6)</td>
<td>1.18</td>
<td>0.67-2.08</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>Summary</td>
<td>832 (5.2) 829 (3.6)</td>
<td>0.67</td>
<td>0.47-0.94</td>
<td>0.022</td>
<td></td>
</tr>
</tbody>
</table>

P value for heterogeneity: p< 0.03

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

2. **Table 9:** Association study of MAFA Gly346Cys with susceptibility to alopecia areata.

| Allele | Fulminant Slowly Progressive Acute-onset C vs. F C vs. SP C vs. A |
|--------|--------------------------------------------------|----------------|----------------|----------------|----------------|
| Gly / Gly | 312 (90.2) 23 (92.0) 134 (93.7) | 346 (94.5) | 0.78 NS 0.61 <0.03 0.53 |
| Gly / Cys | 33 (9.5) 2 (8.0) 9 (6.3) | 20 (5.5) | 0 (0.0) |
| Cys / Cys | 1 (0.3) 0 (0.0) 0 (0.0) | 0 (0.0) |

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).

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

<table>
<thead>
<tr>
<th>Allele</th>
<th>Control</th>
<th>Alopecia areata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly / Gly</td>
<td>236 (89.7) 117 (92.9)</td>
<td>0.67 0.31-1.47 NS</td>
</tr>
<tr>
<td>Gly / Cys</td>
<td>27 (10.3) 9 (7.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Cys / Cys</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

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.
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 MAF is most apparent in acute-onset type 1 diabetes, suggesting that MAF is involved in the pathogenesis of typical beta-cell-specific autoimmunity.

We previously reported that MAF 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 MAF 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 transcriptional 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 MAF is associated with type 1 diabetes especially in subjects who possess the risk genotype of INS in the Japanese population. The association of MAF 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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