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Functional polymorphism in Z-DNA–forming motif of promoter of *SLC11A1* gene and type 1 diabetes in Japanese subjects: Association study and meta-analysis

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Abstract

The association of the polymorphism of the Z-DNA–forming repeats in the promoter region of *SLC11A1* (solute carrier family 11 member 1), formerly designated *NRAMP1* (natural resistance associated macrophage protein 1), with type 1 diabetes was studied in a total of 244 Japanese subjects. Three alleles were detected in Japanese subjects. In diabetic patients, allele 2 was less frequent and allele 3 was more frequent, albeit not significantly, than in control subjects. Allele 2 was significantly ($P < .024$) less frequent whereas allele 3 was more, albeit not significantly, frequent in the younger onset group than in the control subjects. In patients with a susceptible HLA allele, *DRB1*0405* or *DRB1*0901*, the frequency of allele 2 was significantly ($P < .013$) lower and that of allele 3 tended to be higher than that in patients without either *DRB1*0405* or *DRB1*0901*. The protective effect of allele 2 against type 1 diabetes and other autoimmune diseases was confirmed by meta-analysis (summary odds ratio, 0.71, 95% confidence interval, 0.53-0.96). Because allele 2 was shown to be associated with low expression of *SLC11A1* and protection against another autoimmune disease, rheumatoid arthritis, the negative association of allele 2 with autoimmune type 1 diabetes in the present study suggests that a less active immune system in subjects with allele 2 may protect individuals from autoimmune diseases.

1. Introduction

Type 1 diabetes is a multifactorial autoimmune disease, the susceptibility to which is determined by environmental and genetic factors [1-3]. In addition to the strongest component (*IDDM1*) located in the HLA region on chromosome 6 [4,5], it is known that non-HLA genes contribute to the disease susceptibility [6].

Among them, distal chromosome 2q has been demonstrated to be an important region for disease susceptibility, where several susceptibility genes, *IDDM7*, *IDDM12*, and *IDDM13*, have been mapped by linkage studies [7-10]. In our previous study [11], we investigated the association between type 1 diabetes and the gene for IA-2 on 2q35-q36.1, which has been proposed to be an autoantigen in type 1 diabetes [12]. The results demonstrated that the *IA-2* gene is not involved in susceptibility to, or heterogeneity of, type 1 diabetes in Japanese subjects [11]. Therefore, which gene in this region is responsible for disease susceptibility remains to be elucidated.

Recently, a gene encoding solute carrier family 11 member 1 (*SLC11A1*), which was formerly designated natural resistance associated macrophage protein 1 (NRAMP1), was assigned to chromosome region 2q35. *SLC11A1* regulates priming and activation of macrophages [13]. Because macrophages are known to play a role in the pathogenesis of autoimmune diseases [14], including type 1 diabetes, *SLC11A1* is a functional as well as a positional candidate gene for type 1 diabetes.

The promoter region of the *SLC11A1* gene possesses a Z-DNA–forming repeat, whereas Z-DNA is associated with actively transcribing genes [15]. In previous studies, the Z-DNA–forming repeat of the *SLC11A1* gene was shown to be polymorphic [16] and to directly affect its promoter activity [17]. One allele, designated “allele 3,” of the

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Z-DNA–forming repeat polymorphism was shown in vitro to be associated with increased expression of SLC11A1 protein in macrophages [17]. Several studies have demonstrated an association of allele 3 with protection against chronic infectious diseases and with risk for susceptibility to rheumatoid arthritis [14]. In contrast, another allele, "allele 2," which was shown to be associated with lower promoter activity [17], was reported to be associated with susceptibility to infectious diseases and with protection against rheumatoid arthritis. Thus, the Z-DNA–forming polymorphism may be involved in the genetic predisposition to infectious diseases and autoimmune diseases through its effect on altered expression of *SCL11A1*.

In this study, as a positional and functional candidate, we examined the association of a polymorphism of the Z-DNA–forming repeats in the promoter region of the *SLC11A1* gene with type 1 diabetes in Japanese subjects. To further confirm the contribution of the *SLC11A1* locus to genetic susceptibility to autoimmune diseases, meta-analysis of available data was also performed.

2. Materials and methods

One hundred fourteen unrelated Japanese patients (47 males, 67 females) with type 1 diabetes and 130 unrelated healthy control subjects (73 males, 57 females) were studied. Median age at onset of the disease was 12.0 years (interquartile range, 12.5 years) and its range was 2 to 59 years. The diagnosis of type 1 diabetes was defined by the following criteria: ketosis-prone and lacking endogenous insulin secretion as judged by urinary C-peptide level of less than 3.3 nmol/d, and needing more than 4 insulin injections per day. Control subjects consisted of medical staff in our department who had normal glucose tolerance and no family history of type 1 diabetes or other autoimmune diseases. Mean (±SD) age of the control subjects was 42.0 (±10.9) years. All patients and control subjects were of Japanese origin and resided in the Osaka area (western Japan). Informed consent was obtained from all subjects.

The polymorphic Z-DNA–forming repeats of the *SLC11A1* gene were investigated using a fluorescence-based method, as reported previously [18,19]. Briefly, forward polymerase chain reaction (PCR) primers were labeled with 6-FAM and the PCR products were electrophoresed in 4% denaturing polyacrylamide gel, using an ABI Prism Genetic Analyzer (Applied Biosystems, Foster City, Calif) with Genescan 350 ROX (Applied Biosystems) as an internal lane size standard. Polymerase chain reaction fragments were sized with GeneScan Analysis software (Applied Biosystems), genotyped with Genotyper software (Applied Biosystems), and alleles were identified using a histogram as reported previously [20].

Allele 1, corresponding to a PCR product size of 116 base pairs (bp) in Caucasian, is apparently the same size, but has a different sequence from an allele 7 reported in a Japanese population [17,21]. Because alleles 1 and 7 were indistinguishable in the present study analysis, these two were combined and dealt with as allele 1 [7].

Given that younger age at onset of type 1 diabetes is associated with predisposition to the disease, as suggested in family [22,23] and case-control [24] studies, the diabetic patients were divided into 2 subgroups according to age at onset at puberty: (age at onset <11 years, n = 42; ≥11 years, n = 64).

To investigate the possible interaction between *SLC11A1* and *IDDM1*, the strongest component of susceptibility, the *HLA-DRB1* allele was determined by PCR-RFLP method as reported previously [25]. Statistical analysis was performed by χ^2 test or Fisher exact probability test.

To perform a meta-analysis, the published literature was searched using PubMed with the following key words: SLC11A1 or NRAMP1, and autoimmunity, followed by a complimentary search in the reference list of the selected articles. Articles in which genotype and/or allele data were described in unrelated individuals according to autoimmune disease status were analyzed, as reported previously [26]. A random effect model was adopted to assess the summary odds ratio and its 95% confidence interval (CI).

Table 1
Allele frequencies of *SLC11A1* gene polymorphism in Japanese subjects

Present study			Allele 1 [7]	Allele 2	Allele 3	*P*
Type 1 diabetes	n = 228	n	20	21	187	
		%	8.8	9.2	82	
	Age at onset of type 1 diabetes					
	<11 (n = 84)	%	9.5	4.8*	85.7	*<.024 vs control
	≥11 (n = 128)	%	7.8	11.7	80.5	
	Susceptible HLA *DRB1*0405* or *DRB1*0901*					
	(+)	n (%)	17 (8.8)	3 (7.2*)	19 (84.0)	*<.013 vs (−)
	(−)	n (%)	3 (8.8)	7 (20.6)	24 (70.6)	
Control	n = 260	n	19	36	205	
		%	7.3	13.8	78.9	

Table 2
Meta-analysis of *SLC11A1* gene polymorphism in patients with autoimmune disease

Disease	Population	Phenotype	n	Allele				n	Genotype					
				Allele 2 (+) (%)	Allele 2 (−) (%)	Allele 3 (+) (%)	Allele 3 (−) (%)		Allele 2 (+/+) (%)	Allele 2 (+/−) (%)	Allele 2 (−/−) (%)	Allele 3 (+/+) (%)	Allele 3 (+/−) (%)	Allele 3 (−/−) (%)
Type 1 diabetes (present study)	Japanese	Disease(+)	228	21 (9.2)	207 (90.8)	187 (82.0)	41 (18.0)	114	0 (0)	21 (18.4)	93 (81.6)	76 (66.7)	35 (30.7)	3 (2.6)
		Disease(−)	260	36 (13.8)	224 (86.2)	205 (78.8)	55 (21.2)	130	2 (1.5)	32 (24.6)	96 (73.8)	83 (63.9)	39 (30.0)	8 (6.2)
Type 1 diabetes (Bassuny et al [27])	Japanese	Disease(+)	412	49 (11.9)	363 (88.1)									
		Disease(−)	400	65 (16.3)	335 (83.8)									
Type 1 diabetes (Takahashi et al [28])	Japanese	Disease(+)	190	22 (11.6)	168 (88.4)	150 (78.9)	40 (21.0)							
		Disease(−)	448	69 (15.4)	379 (84.6)	359 (80.1)	89 (19.9)							
Inflammatory bowel disease (Kojima et al [21])	Japanese	Disease(+)	430	65 (15.1)	365 (84.9)	317 (73.7)	113 (26.3)	215	4 (1.9)	57 (26.5)	154 (71.6)	117 (54.4)	83 (38.6)	15 (7.0)
		Disease(−)	648	96 (14.8)	552 (85.2)	520 (80.2)	128 (19.8)	324	6 (1.9)	84 (25.9)	234 (72.2)	206 (63.6)	108 (33.3)	10 (3.1)
Juvenile rheumatoid arthritis (Sanjeevi et al [30])	Latvian	Disease(+)	238	38 (16.0)	200 (84.0)	200 (84.0)	38 (16.0)	119	6 (5.0)	25 (21.0)	88 (73.9)	88 (73.9)	25 (21.0)	6 (5.0)
		Disease(−)	222	64 (28.8)	158 (71.2)	156 (70.3)	66 (29.7)	111	15 (13.5)	35 (31.5)	61 (54.9)	60 (54.1)	35 (31.5)	16 (14.4)
Rheumatoid arthritis (Rodoriguez et al [31])	Spanish	Disease(+)	282	91 (32.2)	191 (67.7)	189 (67.0)	93 (33.0)	141	18 (12.8)	55 (39.0)	68 (48.2)	66 (46.8)	57 (40.4)	18 (12.8)
		Disease(−)	388	107 (27.6)	281 (72.4)	278 (71.6)	110 (28.4)	194	15 (7.7)	77 (39.7)	102 (52.6)	100 (51.5)	78 (40.2)	16 (8.2)
Multiple sclerosis (Kotze et al [32])	African Americans	Disease(+)	208	41 (19.7)	167 (80.3)	160 (76.9)	48 (23.1)							
		Disease(−)	658	223 (33.9)	435 (66.1)	434 (66.0)	224 (34.0)							

3. Results

In total, 3 PCR products (116, 118, and 120 bp) were detected in the present analysis, which were assigned to alleles 1 [7], 2 and 3, respectively. The allele distribution of our controls was not significantly different from that of each reported in the Japanese population ($P = .36$ [21], $P = .61$ [27] and $P = .26$ [28], respectively, χ^2 test with 2 degrees of freedom).

To investigate the association of the polymorphism with type 1 diabetes, the allele distribution of *SLC11A1* was compared between diabetic patients and control subjects. In the diabetic patients, allele 2 was less frequent and allele 3 was more frequent than in the control subjects, but the differences were not statistically significant (Table 1).

To study the association of the polymorphism with type 1 diabetes in patients with stronger predisposition to the disease, the diabetic patients were divided into 2 subgroups according to onset at puberty (11 years old), which has been suggested to be biologically related to a high incidence of diabetes [29]. The frequency of allele 2 was significantly lower in the younger onset group than in the control subjects ($P < .024$), whereas that of allele 3 was higher, albeit not significantly, in diabetic individuals than in control subjects (Table 1).

Because of the well-known contribution of *IDDM1* in the HLA region to genetic susceptibility to type 1 diabetes, it is important to consider the possible genetic interaction of the polymorphism with *IDDM1*. Hence, the patients were stratified according to the presence or absence of disease-associated HLA alleles in Japanese subjects. HLA class II distribution per se was not significantly different between younger and older onset groups (data not shown). In patients with susceptible HLA alleles, *DRB1*0405* or *DRB1*0901*, the frequency of allele 2 was significantly lower than that in patients without either *DRB1*0405* or *DRB1*0901* ($P < .013$), whereas the frequency of allele 3 was higher, albeit not significantly, in patients with the susceptible HLA (Table 1).

Because the association of the *SLC11A1* polymorphism with type 1 diabetes in the present study was marginal, a meta-analysis was performed. From 49 articles detected by PubMed search, 7 sets of data, including the present result, in total [21,27,28,30-32] were available (Table 2). In a random effect model, allele 2 was negatively associated with autoimmune diseases, with a summary odds ratio of 0.71 (95% CI, 0.53-0.96). When analysis was restricted to type 1 diabetes ([27,28] including the present results), allele 2 was negatively associated with type 1 diabetes mellitus, giving a summary odds ratio of 0.68 (95% CI, 0.52-0.90).

4. Discussion

In the present study in Japanese patients with type 1 diabetes, allele 2 of the functional polymorphism of *SLC11A1*, which was shown to be associated with decreased expression of *SLC11A1*, was significantly less frequent in patients with younger onset of the disease and in patients with a susceptible HLA than in controls. Because patients with younger onset and with a susceptible HLA are expected to have a predisposition to type 1 diabetes, these data suggest that polymorphism in the Z-DNA–forming motif of the *SLC11A1* gene may be involved in the genetic preposition to type 1 diabetes as a modifier of other susceptibility genes, such as *IDDM1* in HLA. Another possibility is that there is more genetic heterogeneity in type 1 diabetes in the Japanese than has been appreciated previously, and that other, non-HLA genes are accounting for a larger proportion of genetic susceptibility in the older onset subjects.

SLC11A1 is found in macrophages and plays roles in the killing of bacteria within lysozomes [33]. The promoter region of human *SLC11A1* possesses a dinucleotide repeat polymorphism within a possible enhancer element of a Z-DNA–forming motif. A previous in vitro study [17] demonstrated that the polymorphism is functional, associated with altered gene expression. Because macrophages were shown to play an important role in the development of type 1 diabetes in the NOD mouse, chronic hyperactivation of macrophages associated with higher expression of *SLC11A1*, possibly promoted by allele 3, may well promote the autoimmune disease process leading to β-cell destruction, whereas lower expression of *SLC11A1*, associated with allele 2, may be protective against the development of autoimmune diabetes [17].

Accumulating lines of evidence have pointed to a triggering role of some viral infections in the development of type 1 diabetes [34]. As *SLC11A1* is expressed in macrophages and is involved in killing bacteria, it may have little effect on defense against viral infections. Therefore, the functional alteration of *SLC11A1* related to allele 2, if any, may have less effect on triggering type 1 diabetes.

The present results suggested that the Z-DNA–forming DNA repeat may be responsible for *IDDM13*. The *IDDM13* region on human chromosome 2q is syntenic to mouse chromosome 1, where a susceptibility locus for type 1 diabetes, *Idd5*, was mapped. A recent congenic mapping in the NOD mouse demonstrated that a previously identified susceptibility gene, *Idd5*, is composed of multiple components, *Idd5.1* and *Idd5.2* [35,36]. The *Idd5.2* congenic interval (9.4 cM) contains *Slc11a1*, the mouse ortholog of human *SLC11A1*. Thus, the gene encoding SLC11A1 is a functional and positional candidate for *IDDM13* in human beings and for *Idd5.2* in mice. A recent study by Ueda et al [37] demonstrated that the gene encoding CTLA4 is responsible for *IDDM12*, a susceptibility gene for type 1 diabetes on chromosome 2q. Furthermore, extensive studies on the NOD mouse demonstrated that *Ctla4*, the mouse ortholog of human *CTLA4*, is responsible for type 1 diabetes susceptibility, *Idd5.1* [35-37]. These data indicate the importance of studies on orthologs in mice and human beings in determining susceptibility genes for type 1

diabetes. The localization of the gene for SLC11A1 in the *IDDM13* region in human beings and the *Idd5.2* region in mice therefore suggests this gene as a strong candidate for type 1 diabetes susceptibility.

Although the present study suggested the association of the *SLC11A1* polymorphism with type 1 diabetes, the association was marginal and in fact no longer significant when corrected for multiple comparisons, probably because of relatively small sample size. We therefore performed a meta-analysis to confirm the association suggested in the present study. Meta-analyses have been shown to be powerful for robust estimation of genetic effects by increasing the effective sample size [38]. From the present meta-analysis, allele 2 was shown to be negatively associated with autoimmune diseases. As the summary odds ratio of the allele 2 for type 1 diabetes (0.68) was comparable to that for autoimmune diseases (0.71), the protective effect of the allele 2 against an autoimmune disease was consistent throughout a wide spectrum of autoimmune diseases. These analyses suggest that the protective effect of the allele 2 against autoimmune disease is not organ-specific, but may be through immune regulation common to several autoimmune diseases.

In our present case-control analysis, a statistically significant difference was not detected in allele frequencies per se, which could be because the sample size was not large enough. However, the present observation revealed quite similar associations of allele 2 with type 1 diabetes among studies and helped increase the power of the present meta-analysis, resulting in robust estimation of the genetic effect.

In the last century, marked improvement in hygiene and great advances in the treatment of classic infectious diseases, such as malaria and tuberculosis, have led to a large reduction in mortality due to infectious diseases. Under the present hygiene system, an overactive immune system may lead to excessive immune reaction, which may predispose individuals to autoimmune diseases. Because allele 2 of *SLC11A1* was shown to be associated with low expression of *SCL11A1* and protection against another autoimmune disease, rheumatoid arthritis, the negative association of allele 2 with autoimmune type 1 diabetes in the present study suggests that a less active immune system in subjects with allele 2 may be protective against autoimmune disease and lack of allele 2 may predispose individuals to autoimmune disease under the present hygiene system.

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References

[1] Castano L, Eisenbarth GS. Type-I diabetes: a chronic autoimmune disease of human, mouse, rat. Annu Rev Immunol 1990;8:647-79.
[2] Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 1994;331:1428-36.
[3] Tisch R, McDevitt HO. Insulin-dependent diabetes mellitus. Cell 1996;85:291-7.
[4] Todd JA, Bell JI, McDevitt HO. *HLA-DQβ* gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 1987;329:599-604.
[5] She J-X. Susceptibility to type 1 diabetes, HLA-DQ and DR revisited. Immunol Today 1996;17:323-9.
[6] Todd JA, Farrall M. Panning for gold: genome-wide scanning for linkage in type 1 diabetes. Hum Mol Genet 1996;5:1443-8.
[7] Copeman JB, Cucca F, Hearne CM, et al. Linkage disequilibrium mapping of a type 1 diabetes susceptibility gene (*IDDM7*) to chromosome 2q31-q33. Nat Genet 1995;9:80-5.
[8] Morahan G, Huang D, Tait BD, et al. Markers on distal chromosome 2q linked to insulin-dependent diabetes mellitus. Science 1996;272:1811-3.
[9] Nistico L, Buzzetti R, Pritchard LE, et al. The *CTLA4* gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Hum Mol Genet 1996;5:1075-80.
[10] Marron MP, Raffel LJ, Garchon HJ, et al. Insulin-dependent diabetes mellitus (IDDM) is associated with *CTLA4* polymorphism in multiple ethic groups. Hum Mol Genet 1997;6:1275-82.
[11] Nishino M, Ikegami H, Kawaguchi Y, et al. Polymorphism in gene for islet autoantigen, IA-2, and type 1 diabetes in Japanese subjects. Hum Immunol 2001;62:518-22.
[12] Leslie RDG, Atkinson MA, Notkins AL. Autoantigens IA-2 and GAD in type 1 (insulin-dependent) diabetes. Diabetologia 1999;42:3-14.
[13] Blackwell JM. Structure and function of the natural-resistance-associated macrophage protein 1 (NRAMP1), a candidate protein for infectious and autoimmune disease susceptibility. Mol Med Today 1996;2:205-11.
[14] Shaw MA, Clayton D, Atkinson SE, et al. Linkage of rheumatoid arthritis to the candidate gene *NRAMP1* on 2q35. J Med Genet 1996;33:672-7.
[15] Rich A, Zhang S. Z-DNA: The long road to biological function. Nat Rev Genet 2003;4:566-72.
[16] Blackwell JM, Barton CH, White JK, et al. Genomic organization and sequence of the human *NRAMP* gene: identification and mapping of promoter region polymorphism. Mol Med 1995;1:194-205.
[17] Searle S, Blackwell JM. Evidence for a functional repeat polymorphism in the promoter of the human NRAMP1 gene that correlates with autoimmune versus infectious disease susceptibility. J Med Genet 1999;36:295-9.
[18] Reed PW, Davis JL, Copeman JB, et al. Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 1994;7:390-5.
[19] Fu J, Ikegami H, Yamato E, et al. Age- and HLA-dependent heterogeneity in the association of a marker on chromosome 2q with insulin-dependent diabetes mellitus. Diabetes Res 1996;31:215-22.
[20] Prichard LE, Kawaguchi Y, Reed PW, et al. Analysis of the CD3 region and type 1 diabetes: application of fluorescence-based technology to linkage disequilibrium mapping. Hum Mol Genet 1995;4:197-202.
[21] Kojima Y, Kinouchi Y, Takahashi S, et al. Inflammatory bowel disease is associated with a novel promoter polymorphism of natural resistance-associated macrophage protein 1 (NRAMP1) gene. Tissue Antigens 2001;58:379-84.
[22] Hyttinen V, Kaprio J, Kinnunen L, et al. Genetic liability of type 1 diabetes and the onset age among 22,650 young Finnish twin pairs. Diabetes 2003;52:1052-5.

[23] Redondo MJ, Yu L, Hawa M, et al. Heterogeneity of type 1 diabetes: analysis of monozygotic twins in Great Britain and the United States. Diabetologia 2001;44:354-62.
[24] Caillat-Zucman S, Garchon HJ, Timsit J, et al. Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. J Clin Invest 1992;90:2242-50.
[25] Kawabata Y, Ikegami H, Kawaguci Y, et al. Asian-specific HLA haplotypes reveal heterogeneity of the contribution of HLA-DR and-DQ haplotypes to susceptibility to type 1 diabetes. Diabetes 2002;51:545-51.
[26] Fujisawa T, Ikegami H, Kawaguchi Y, et al. Meta-analysis of association of insertion/deletion polymorphism of angiotesin I-converting enzyme gene with diabetic nephropathy and retinopathy. Diabetologia 1998;41:47-53.
[27] Bassuny WM, Ihara K, Matsuura N, et al. Association study of the *NRAMP1* gene promoter polymorphism and early-onset type 1 diabetes. Immunogenetics 2002;54:282-5.
[28] Takahashi K, Satoh J, Kojima Y, et al. Promoter polymorphism of *SLC11A1* (formerly *NRAMP1*) confers susceptibility to autoimmune type 1 diabetes mellitus in Japanese. Tissue Antigens 2004;63:231-6.
[29] Pozzilli P, Visalli N, Buzzetti R, et al. Metabolic and immune parameters at clinical onset of insulin-dependent diabetes: a population based study. Metabolism 1998;47:1205-10.
[30] Sanjeevi CB, Miller EN, Dabadghao P, et al. Polymorphism at *NRAMP1* and *D2S1471* loci associated with juvenile rheumatoid arthritis. Arthritis Rheum 2000;43:1397-404.
[31] Rodríguez MR, González-Escribano MF, Aguilar F, et al. Association of *NRAMP1* promoter gene polymorphism with the susceptibility and radiological severity of rheumatoid arthritis. Tissue Antigens 2002;59:311-5.
[32] Kotze MJ, Villiers JNP, Rooney RN, et al. Analysis of *NRAMP1* gene implicated in iron transport: association with multiple sclerosis and age effects. Blood Cells Mol Dis 2001;27:44-53.
[33] Blackwell JM, Searle S, Mohamed H, et al. Divalent cation transport and susceptibility to infectious and autoimmune disease: continuation of Ity/Lsh/Bcg/Nramp1/Slc11a1 gene story. Immunol Lett 2003; 85:197-203.
[34] Hyöty H, Taylor KW. The role of viruses in human diabetes. Diabetologia 2002;45:1353-61.
[35] Hill NJ, Lyons PA, Armitage N, et al. NOD *Idd5* locus controls insulitis and diabetes and overlaps the orthologous CTLA4/IDDM12 and NRAMP1 loci in humans. Diabetes 2000;49:1744-7.
[36] Wicker LS, Chamberlain G, Hunter K, et al. Fine mapping, gene content, comparative sequencing, and expression analyses support *Ctla4* and *Nramp1* as candidates for *Idd5.1* and *Idd5.2* in the nonobese diabetic mouse. J Immunol 2004;173:164-73.
[37] Ueda H, Howson JMM, Esposito L, et al. Association of T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 2003;423:506-11.
[38] Lohmueller KE, Pearce CL, Pike M, et al. Meta-analysis of genetic association studies supports a contribution of common variants susceptibility to common disease. Nat Genet 2003;33:177-82.