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A low-frequency *GLIS3* variant associated with resistance to Japanese type 1 diabetes

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ABSTRACT

The role of low-frequency variants in type 1 diabetes (T1D) susceptibility still remains to be clarified. In the present study, we analyzed low-frequency variants of the T1D candidate genes in Japanese. We first screened for protein-changing variants of 24 T1D candidate genes in 96 T1D patients and 96 control subjects, and then the association with T1D was tested in 706 T1D patients and 863 control subjects recruited from the collaborating institutions in Japan. In total, 56 protein-changing variants were discovered; among them, 34 were low-frequency variants (allele frequency < 5%). The association analysis of the low-frequency variants revealed that only the A908V variant of *GLIS3* was strongly associated with resistance to T1D (Haldane's odds ratio = 0.046, $p = 8.21 \times 10^{-4}$, and $p_c = 2.22 \times 10^{-2}$). GLIS3 is a zinc finger transcription factor that is highly expressed in pancreatic beta cells, and regulates beta cell development and insulin gene expression. GLIS3 mRNA is also moderately expressed in the human thymus. The precise mechanism responsible for the association is unclear at present, but the A908V variant may affect autoimmunity to the GLIS3 protein itself; the 908V containing epitope may induce central or peripheral tolerance more efficiently than that of 908A.

1. Introduction

A number of type 1 diabetes (T1D) susceptibility loci have been revealed in Caucasians. Among them, HLA is the strongest, and contributes about one-half of the genetic component of T1D. Previous candidate gene studies have identified several non-HLA loci, which include the gene encoding insulin (*INS*), *CTLA4*, *PTPN22*, and *IL2RA* (or *CD25*) [1,2]. Furthermore, recent genome-wide association studies (GWAS) identified many weaker susceptibility loci. However, proportion of heritability explained for T1D by the confirmed T1D loci is still between two-thirds and three-quarters [2].

In Japanese subjects, it was revealed that HLA class II is also a very strong susceptibility factor with the mostly specific susceptibility haplotypes to Asian populations [3,4]. *INS* is another confirmed T1D susceptible loci in Japanese, although the susceptible class 1 allele is predominant over the protective class 3 allele; the frequency of the class 3 is less than 5% both in the T1D patients and control subjects [5]. We have also obtained evidence for an association with the *CTLA4* [6], *IL2RA* [7], *ERBB3* [8], *CLEC16A* [8] and *IL7R* [9] loci, however, it was revealed that the cumulative effect of these non-HLA susceptibility loci was much weaker than that of the HLA class II loci [9]. Thus, taking into account the much higher recurrence risk in siblings of Japanese T1D patients compared to Caucasian patients [10], non-HLA susceptibility T1D loci largely remain to be elucidated in Japanese.

It is reasonable to postulate that low-frequency variants (allele frequency < 5%) may contribute to the high risk in siblings of patients in Japanese, since they are abundant and more likely to be functional than common SNPs [11–13]. In the present study,

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we analyzed low-frequency variants of the T1D candidate genes and their potential association with T1D in Japanese.

2. Materials and methods

2.1. Subjects

A total of 1569 Japanese subjects, consisting of 706 patients with T1D and 863 control subjects recruited from the collaborative institutions were studied. The patients consisted of 418 females and 288 males with a mean (±SD) age-at-onset of 31.5 (±17.7) years. The control subjects consisted of 332 females and 531 males with a mean (±SD) age of 31.5 (±12.8) years. An ethics committee from each institute approved the study, and informed consent was obtained from all subjects.

2.2. Screening for variants by next-generation sequencing

We resequenced 24 candidate genes by a next-generation sequencer in DNA of 96 T1D patients and 96 control subjects; these subjects were among those recruited from the hospital or school of Saitama Medical University. The selected candidate genes were shown in Table 1. We selected 16 genes from the confirmed type 1 diabetes loci, four from genes responsible for monogenic diabetes, three from autoantigens, and one from genes responsible for the BB rat. Briefly, four DNA pools of 48 patients or 48 controls were prepared, and all exons and splice sites of the candidate genes were PCR-amplified, then the DNA samples were mixed and sequenced with an Illumina Genome Analyzer IIx. The obtained sequence reads (approximately 2.5 billion reads for each DNA pool) were mapped to the genes to identify variants using the software MAQ (http://maq.sourceforge.net/). Theoretically, resequencing 96 subjects provides 98% and 85% probability of detecting variants at 2% and 1% frequency, respectively.

2.3. Estimated frequencies of the variant and wild-type alleles based on sequence reads from DNA pools

In the present study, we focused on the variants that change coding proteins. For the identified variants, we first estimated the allele frequencies by the sequence reads (variant and wild-type alleles) generated from the DNA pools containing 96 chromosomes of T1D patients or control subjects each. Before the large-scale genotyping, we validated the accuracy of the frequency estimates in the DNA pools by next-generation sequencing in 14 variants by comparing allele frequencies in individually genotyped DNA samples.

2.4. Association analysis of low-frequency variants

Next, we conducted association analysis of low-frequency variants in 706 T1D patients and 863 control subjects enrolled from collaborating institutions. Individual genotypes of variants were determined by DigiTag2 assay [14] and/or TaqMan SNP Genotyping Assay (Applied Biosystems; Foster City, CA). Theoretically, association test based on 706 cases (1412 alleles) and 863 controls (1726 alleles) has 86% power to detect association at false-positive rate $\alpha = 0.05$ for allele frequency 2% and OR = 2 or 61% power for allele frequency 1% and OR = 2 (GraphPad StatMate 2.00, GraphPad Software, Inc., USA).

2.5. Statistical analysis

The correlation of the frequency estimates in the DNA pools and allele frequencies in individually genotyped DNA samples were assessed by Pearson's correlation coefficient (r). The differences in the allele frequencies determined by individually genotyping were assessed by two-sided Fisher's exact test, and each p-value was corrected by multiplying the number of analyzed variants (p_c-value; Bonferroni correction). The odds ratios (OR) for the variant allele were calculated using Haldane's method. StatsDirect Ver. 2.6.5 (StatsDirect, Cheshire, UK) were used for these tests. Statistical significance was defined as $p_c < 0.05$.

3. Results

3.1. Screening for variants by next-generation sequencing

As shown in Table 2, 56 variants that change coding proteins (55 non-synonymous and one splice site) were discovered in 17 among the 24 genes studied by next-generation sequencing: the average coverage of depth for the positions of these variants was 10536x. Among them, 34 (61%) were low-frequency variants both in T1D patients and control subjects.

3.2. Validation for the frequency estimates in the sequenced DNA pools

We analyzed the correlation of the frequency estimates in the DNA pools and allele frequencies in individually genotyped DNA samples in 14 variants. A good correlation was found between these, in the total variants (Fig. 1A) and in the low-frequency variants only (Fig. 1B) ($r = 0.98$ in both).

3.3. Association analysis low-frequency variants

Thirty-four low-frequency variants were genotyped by the DigiTag2 assay and/or the TaqMan SNP assay; the DigiTag2 assay was reported to have high accuracy and reproducibility [14]. Actually, we genotyped five variants of this study by both DigiTag2 assay and TaqMan SNP assay, and found that 99.9% of the typing results were identical. Seven variants (*CCR5* M49V, *CLEC16A* T773P, *CLEC16A* D1053A, *ERBB3* Y603S, *SLC30A8* S230AR, *WFS1* I421M and *WFS1* H763P) were excluded from association analysis, since these variants were not detected by the DigiTag2 assay (possibly due to typing failure), assuming that the variant allele frequencies were at

Table 1
Studied genes.

Category	Gene
Type 1 diabetes loci	*CCR5, CD226, CD28, CLEC16A, CTLA4, ERBB3, GLIS3, IFIH1, IL2, IL21, IL2RA, IL7R, INS, PTPN2, PTPN22, SH2B3*
Autoimmune polyglandular syndrome (APS) type 1	*AIRE*
Wolfram syndrome	*WFS1*
Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome	*FOXP3*
Neonatal diabetes	*KCNJ11*
Type 1 diabetes autoantigens	*GAD2, PTPRN, SLC30A8*
BB rat	*IAN4L1*

Table 2
Non-synonymous or splice site variants identified by next-generation sequencing.

Category	Gene	Variant
Type 1 diabetes loci	*CCR5*	K26R[b], M49V[b], R223Q
	CD226	T257A[b], R279K, G307S
	CLEC16A	T773P[b], T846P[b], D1053A[b]
	CTLA4	T17A
	ERBB3	N126D[b], R453H[b], Y603S[b], G908R[b], R967K[b], R1127H[b]
	GLIS3[a]	H824R[b], S893F[b], A908V[b], R918H[b]
	IFIH1	R705S[b], H843R, N930S[b], IVS14 + 1[b], A946T
	IL2RA	M113V[b]
	IL7R	V138I, T244I, I356V, T414M
	INS	A23T
	SH2B3	A536T[b]
Autoimmune polyglandular syndrome (APS) type 1	*AIRE*	S278R
Wolfram syndrome	*WFS1*	V412L[b], V412A[b], I421M[b], C426S[b], R456H, G576S, R611H, A616C[b], R708C[b], I720V, E737K, H763P[b], D866N[b]
Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome	*FOXP3*	G52V
Neonatal diabetes	*KCNJ11*	E23K, I337V
Type 1 diabetes autoantigens	*SLC30A8*	S230R[b], R325W, R325Q[b], I349F[b]
BB rat	*IAN4L1*	H219Y[b], V253G, W267G[b]

[a] Long isoform.
[b] Low-frequency variant (allele frequency < 0.05 in both type 1 diabetes patients and control subjects).

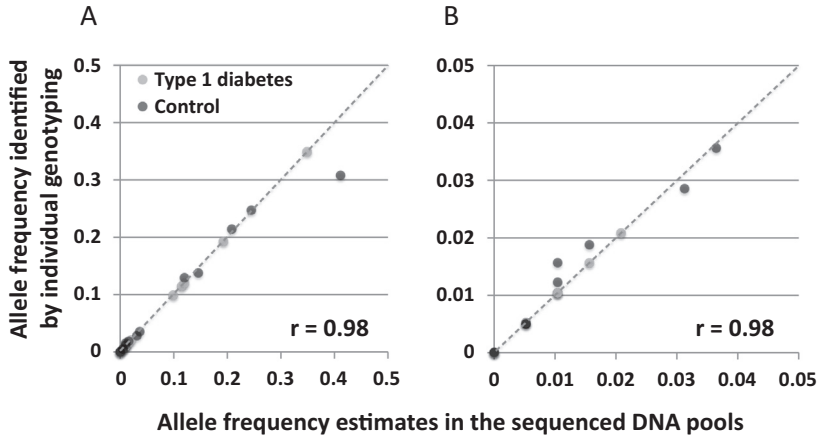

Fig. 1. The correlation between the frequency estimates in the sequenced DNA pools and the allele frequencies in individually genotyped DNA samples. A good correlation was found between these frequencies, in both the total variants (A) and in the low-frequency variants (B) ($r = 0.98$ in both).

least very low in both T1D patients and control subjects. As shown in Table 3, the association analysis in the subjects recruited from the collaborating institutions revealed that the significant association was only observed with the *GLIS3* A908V variant. *GLIS3* A908V was significantly associated with resistance to T1D with a substantial significance (Haldane's odds ratio = 0.046, $p = 8.21 \times 10^{-4}$, and $p_c = 2.22 \times 10^{-2}$): the 908V allele was absent in 706 patients, while it was present in 12 control subjects heterozygously, and the frequency was 0.7% in the controls. The G-to-T substitution (codon change: GCT-to-GTT) of the *GLIS3* A908V variant were confirmed by direct sequencing in the heterozygote subjects.

4. Discussion

In the present Japanese collaboration study, we performed a resequencing study of candidate genes for T1D. We focused on protein-changing and low-frequency variants which are likely to be functional. A significant association of such variants was only observed in the gene encoding GLIS3. GLIS3 is a highly expressed zinc finger transcription factor in pancreatic beta cells, which regulates beta cell development and insulin gene expression [15–17]. Mutations in human *GLIS3* were found to cause sporadic neonatal diabetes [18], and GLIS3-deficiency in mice leads to congenital diabetes and neonatal lethality [15]. In addition, *GLIS3*has been identified as a susceptibility locus for the risk of both T1D [19] and T2D [20]. It has been reported that *GLIS3* variants are associated with fasting glucose level [20] and glucose-stimulated beta cell function [21] in non-diabetic adults. Furthermore, Yang recently reported that normal *GLIS3* expression is required for beta cell function and mass maintenance during adulthood [22]. Therefore, the central role of GLIS3 in beta cell function could explain the link between *GLIS3* and T2D susceptibility.

However, the susceptibility of *GLIS3* to T1D is likely to have a different explanation, because arecent large-scale meta-analysis has revealed that the T1D-risk allele of a SNP at the *GLIS3* locus was protective against T2D [23] i.e. the opposite direction of association was observed between T1D and T2D. We favor the hypothesis that GLIS3 may be a T1D autoantigen [24]. Since *GLIS3* mRNA is moderately expressed in the human thymus [25], the GWAS-identified T1D risk allele may decrease *GLIS3* expression in the thymus leading to T1D autoimmunity by GLIS3-reactive T cells that escaped negative selection. Alternatively, diabetogenic T cell response after physiological or pathological beta cell apoptosis may be enhanced by the *GLIS3* risk allele. In the present study, the current low-frequency A908V variant conferred strong

Table 3
Association analysis of the twenty-seven non-synonymous or splice site low-frequency variants identified by the resequencing analysis.

				Variant Allele Frequency				
Gene	Variant	Variant allele	SIFT[a]/PolyPhen-2[b]	Type 1 diabetes (n = 1412)	Control (n = 1726)	Haldane's OR	p-value[c]	p_c-value[d]
CCR5	K26R	R	−/−	0.0022	0.0025	0.93	1	1
CD226	T257A	A	−/−	0.0007	0.0000	3.60	0.455	1
CLEC16A	T846P	P	−/−	0.0007	0.0000	3.60	0.455	1
ERBB3	N126D	D	−/−	0.0007	0.0000	3.59	0.455	1
	R453H	H	−/++	0.0022	0.0038	0.63	0.520	1
	G908R	R	+/++	0.0007	0.0000	3.60	0.455	1
	R967K	K	−/++	0.0036	0.0030	1.20	0.763	1
	R1127H	H	+/++	0.0189	0.0194	0.97	1	1
GLIS3	H824R	R	−/−	0.0205	0.0238	0.87	0.627	1
	S893F	F	+/++	0.0028	0.0041	0.73	0.764	1
	A908V	V	−/−	0.0000	0.0070	0.046	8.21×10^{-4}	2.22×10^{-2}
	R918H	H	−/+	0.0064	0.0081	0.80	0.676	1
IFIH1	R705S	S	+/++	0.0036	0.0052	0.72	0.597	1
	N930S	S	−/+	0.0007	0.0000	3.76	0.444	1
	IVS14 + 1	Frame-shift		0.0345	0.0351	0.98	1	1
IL2RA	M113V	V	−/−	0.0007	0.0000	3.53	0.460	1
SH2B3	A536T	T	−/+	0.0090	0.0161	0.57	0.099	1
WFS1	V412L	L	−/−	0.0147	0.0087	1.67	0.166	1
	V412A	A	+/++	0.0103	0.0068	1.50	0.320	1
	C426S	S	+/−	0.0007	0.0000	3.54	0.459	1
	A616C	C	−/++	0.0014	0.0030	0.55	0.467	1
	R708C	C	+/++	0.0014	0.0000	6.07	0.204	1
	D866N	N	−/+	0.0121	0.0195	0.63	0.161	1
SLC30A8	R325Q	Q	−/−	0.0452	0.0474	0.95	0.793	1
	I349F	F	+/++	0.0015	0.0050	0.35	0.122	1
IAN4L1	H219Y	Y	−/−	0.0015	0.0000	5.70	0.219	1
	W267G	G	−/+	0.0000	0.0006	0.40	1	1

[a] SIFT: $^{-}$ tolerated, $^{+}$ not tolerated.
[b] PolyPhen-2 (HumDiv): $^{-}$ benign, $^{+}$ possibly damaging, $^{++}$ probably damaging.
[c] Fisher's exact test (two-sided).
[d] Bonferroni correction.

protectionagainst T1D despite the fact that it is unlikely to be functional according to the SIFT (http://sift.jcvi.org/www/SIFT_BLink_submit.html) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml) as shown in Table 3. It is possible that the 908V containing epitope may induce central or peripheral tolerance more efficiently than 908A, thereby reducing autoimmune reactions to beta cells. However, further immunological and/or functional studies are required to confirm whether this is the case and to elucidate the precise mechanisms of the link between *GLIS3* and T1D.

By resequencing and follow-up genotyping a large number ofpatients and controls in Caucasians, it was discovered that low-frequency loss-of-function *IFIH1* variants confer strong protection [26], subsequent to the report that a common *IFIH1* SNP (T946A) was reported to be associated with resistance to T1D [27,28]. It was assumed that normal function of the IFIH1 protein is associated with T1D and variants reducing IFIH1 function would decrease the T1D risk. In contrast, in Japanese subjects, we previously reported that common T946A was not significantly associated with T1D [9], and in the present study, the low-frequency variants were also not significantly associated; it is worth noting that among the present low-frequency *IFIH1*variants, IVS14 + 1 (rs3573203) was also described in the above resequencing studywith the T allele being protective against T1D (allele frequency = 0.0351 in control subjects and odds ratio = 0.74) [26], albeit R705S and N930S have not yet been reported in Caucasians. The reason for the small contribution of the *IFIH1* locus to Japanese T1D susceptibility is unclear but may be related to some distinction in he etiology of T1D between Japanese and Caucasians including preceding viral infections, since *IFIH1* encodes an intracellular helicase, MDA5, that recognizes RNA of picornaviruses (including enteroviruses, such as coxsackie visuses) and mediates immune activation [29]. However, further large studies are required.

In conclusion, in the present study, a low-frequency variant in *GLIS3* wasfound to be protective against Japanese T1D. The precise mechanism responsible for the association is unclear, but the variant may affect autoimmunity to the GLIS3 protein itself. Although the power of the present study is limited, the contribution of low-frequency variants in the known candidate genes for Japanese T1D susceptibility appears to be small. Thus, we are currently conducting a whole exome sequencing analysis to identify the genes with a substantial effect.

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References

[1] F. Pociot, B. Akolkar, P. Concannon, H.A. Erlich, C. Julier, G. Morahan, C.R. Nierras, J.A. Todd, S.S. Rich, J. Nerup, Genetics of type 1 diabetes: what's next?, Diabetes 59 (2010) 1561–1571
[2] C. Polychronakos, Q. Li, Understanding type 1 diabetes through genetics: advances and prospects, Nat. Rev. Genet. 12 (2011) 781–792.
[3] T. Awata, T. Kuzuya, A. Matsuda, Y. Iwamoto, Y. Kanazawa, Genetic analysis of HLA class II alleles and susceptibility to type 1 (insulin-dependent) diabetes mellitus in Japanese subjects, Diabetologia 35 (1992) 419–424.
[4] Y. Kawabata, H. Ikegami, T. Awata, A. Imagawa, T. Maruyama, E. Kawasaki, S. Tanaka, A. Shimada, H. Osawa, T. Kobayashi, T. Hanafusa, K. Tokunaga, H. Makino, Differential association of HLA with three subtypes of type 1 diabetes: fulminant, slowly progressive and acute-onset, Diabetologia 52 (2009) 2513–2521.
[5] T. Awata, E. Kawasaki, H. Ikegami, T. Kobayashi, T. Maruyama, K. Nakanishi, A. Shimada, H. Iizuka, S. Kurihara, M. Osaki, M. Uga, Y. Kawabata, S. Tanaka, Y. Kanazawa, S. Katayama, Insulin gene/IDDM2 locus in Japanese type 1 diabetes: contribution of class I alleles and influence of class I subdivision in

susceptibility to type 1 diabetes, J. Clin. Endocrinol. Metab. 92 (2007) 1791–1795.
[6] H. Ikegami, T. Awata, E. Kawasaki, T. Kobayashi, T. Maruyama, K. Nakanishi, A. Shimada, S. Amemiya, Y. Kawabata, S. Kurihara, S. Tanaka, Y. Kanazawa, M. Mochizuki, T. Ogihara, The association of CTLA4 polymorphism with type 1 diabetes is concentrated in patients complicated with autoimmune thyroid disease: a multicenter collaborative study in Japan, J. Clin. Endocrinol. Metab. 91 (2006) 1087–1092.
[7] E. Kawasaki, T. Awata, H. Ikegami, T. Kobayashi, T. Maruyama, K. Nakanishi, A. Shimada, M. Uga, S. Kurihara, Y. Kawabata, S. Tanaka, Y. Kanazawa, K. Eguchi, Genetic association between the interleukin-2 receptor-alpha gene and mode of onset of type 1 diabetes in the Japanese population, J. Clin. Endocrinol. Metab. 94 (2009) 947–952.
[8] T. Awata, E. Kawasaki, S. Tanaka, H. Ikegami, T. Maruyama, A. Shimada, K. Nakanishi, T. Kobayashi, H. Iizuka, M. Uga, Y. Kawabata, Y. Kanazawa, S. Kurihara, M. Osaki, S. Katayama, Association of type 1 diabetes with two Loci on 12q13 and 16p13 and the influence coexisting thyroid autoimmunity in Japanese, J. Clin. Endocrinol. Metab. 94 (2009) 231–235.
[9] H. Yamashita, T. Awata, E. Kawasaki, H. Ikegami, S. Tanaka, T. Maruyama, A. Shimada, K. Nakanishi, K. Takahashi, T. Kobayashi, Y. Kawabata, Y. Miyashita, S. Kurihara, T. Morita-Ohkubo, S. Katayama, Analysis of the HLA and non-HLA susceptibility loci in Japanese type 1 diabetes, Diabetes Metab Res Rev 27 (2011) 844–848.
[10] H. Ikegami, T. Ogihara, Genetics of insulin-dependent diabetes mellitus, Endocr. J. 43 (1996) 605–613.
[11] W. Bodmer, C. Bonilla, Common and rare variants in multifactorial susceptibility to common diseases, Nat. Genet. 40 (2008) 695–701.
[12] I.P. Gorlov, O.Y. Gorlova, M.L. Frazier, M.R. Spitz, C.I. Amos, Evolutionary evidence of the effect of rare variants on disease etiology, Clin. Genet. 79 (2011) 199–206.
[13] J.A. Tennessen, A.W. Bigham, T.D. O'Connor, W. Fu, E.E. Kenny, S. Gravel, S. McGee, R. Do, X. Liu, G. Jun, H.M. Kang, D. Jordan, S.M. Leal, S. Gabriel, M.J. Rieder, G. Abecasis, D. Altshuler, D.A. Nickerson, E. Boerwinkle, S. Sunyaev, C.D. Bustamante, M.J. Bamshad, J.M. Akey, G.O. Broad, G.O. Seattle, N.E.S. Project, Evolution and functional impact of rare coding variation from deep sequencing of human exomes, Science 337 (2012) 64–69.
[14] N. Nishida, T. Tanabe, M. Takasu, A. Suyama, K. Tokunaga, Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay, Mol. Cell. Biol. 29 (2009) 6366–6379.
[15] H.S. Kang, Y.S. Kim, G. ZeRuth, J.Y. Beak, K. Gerrish, G. Kilic, B. Sosa-Pineda, J. Jensen, C.E. Pierreux, F.P. Lemaigre, J. Foley, A.M. Jetten, Transcription factor Glis3, a novel critical player in the regulation of pancreatic beta-cell development and insulin gene expression, Mol. Cell. Biol. 29 (2009) 6366–6379.
[16] Y. Yang, B.H. Chang, V. Yechoor, W. Chen, L. Li, M.J. Tsai, L. Chan, The Kruppel-like zinc finger protein GLIS3 transactivates neurogenin 3 for proper fetal pancreatic islet differentiation in mice, Diabetologia 54 (2011) 2595–2605.
[17] H.S. Kang, G. ZeRuth, K. Lichti-Kaiser, S. Vasanth, Z. Yin, Y.S. Kim, A.M. Jetten, Gli-similar (Glis) Kruppel-like zinc finger proteins: insights into their physiological functions and critical roles in neonatal diabetes and cystic renal disease, Histol. Histopathol. 38 (2006) 682–687.
[18] V. Senee, C. Chelala, S. Duchatelet, D. Feng, H. Blanc, J.C. Cossec, C. Charon, M. Nicolino, P. Boileau, D.R. Cavener, P. Bougneres, D. Taha, C. Julier, Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism, Nat. Genet. 38 (2006) 682–687.
[19] J.C. Barrett, D.G. Clayton, P. Concannon, B. Akolkar, J.D. Cooper, H.A. Erlich, C. Julier, G. Morahan, J. Nerup, C. Nierras, V. Plagnol, F. Pociot, H. Schuilenburg, D.J. Smyth, H. Stevens, J.A. Todd, N.M. Walker, S.S. Rich, C. Type 1 Diabetes Genetics, Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes, Nat. Genet. 41 (2009) 703–707.
[20] J. Dupuis, C. Langenberg, I. Prokopenko, R. Saxena, N. Soranzo, A.U. Jackson, E. Wheeler, N.L. Glazer, N. Bouatia-Naji, A.L. Gloyn, C.M. Lindgren, R. Magi, A.P. Morris, J. Randall, T. Johnson, P. Elliott, D. Rybin, G. Thorleifsson, V. Steinthorsdottir, P. Henneman, H. Grallert, A. Dehghan, J.J. Hottenga, C.S. Franklin, P. Navarro, K. Song, A. Goel, J.R. Perry, J.M. Egan, T. Lajunen, N. Grarup, T. Sparso, A. Doney, B.F. Voight, H.M. Stringham, M. Li, S. Kanoni, P. Shrader, C. Cavalcanti-Proenca, M. Kumari, L. Qi, N.J. Timpson, C. Gieger, C. Zabena, G. Rocheleau, E. Ingelsson, P. An, J. O'Connell, J. Luan, A. Elliott, S.A. McCarroll, F. Payne, R.M. Roccasecca, F. Pattou, P. Sethupathy, K. Ardlie, Y. Ariyurek, B. Balkau, P. Barter, J.P. Beilby, Y. Ben-Shlomo, R. Benediktsson, A.J. Bennett, S. Bergmann, M. Bochud, E. Boerwinkle, A. Bonnefond, L.L. Bonnycastle, K. Borch-Johnsen, Y. Bottcher, E. Brunner, S.J. Bumpstead, G. Charpentier, Y.D. Chen, P. Chines, R. Clarke, L.J. Coin, M.N. Cooper, M. Cornelis, G. Crawford, L. Crisponi, I.N. Day, E.J. de Geus, J. Delplanque, C. Dina, M.R. Erdos, A.C. Fedson, A. Fischer-Rosinsky, N.G. Forouhi, C.S. Fox, R. Frants, M.G. Franzosi, P. Galan, M.O. Goodarzi, J. Graessler, C.J. Groves, S. Grundy, R. Gwilliam, U. Gyllensten, S. Hadjadj, et al., New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk, Nat. Genet. 42 (2010) 105–116.
[21] T.W. Boesgaard, N. Grarup, T. Jorgensen, K. Borch-Johnsen, T. Hansen, O. Pedersen, G.Meta-Analysis of, C. Insulin-Related Trait, Variants at DGKB/TMEM195, ADRA2A, GLIS3 and C2CD4B loci are associated with reduced glucose-stimulated beta cell function in middle-aged Danish people, Diabetologia 53 (2010) 1647–1655.
[22] Y. Yang, B.H. Chang, L. Chan, Sustained expression of the transcription factor GLIS3 is required for normal beta cell function in adults, EMBO Mol Med 5 (2013) 92–104.
[23] A.P. Morris, B.F. Voight, T.M. Teslovich, T. Ferreira, A.V. Segre, V. Steinthorsdottir, R.J. Strawbridge, H. Khan, H. Grallert, A. Mahajan, I. Prokopenko, H.M. Kang, C. Dina, T. Esko, R.M. Fraser, S. Kanoni, A. Kumar, V. Lagou, C. Langenberg, J. Luan, C.M. Lindgren, M. Muller-Nurasyid, S. Pechlivanis, N.W. Rayner, L.J. Scott, S. Wiltshire, L. Yengo, L. Kinnunen, E.J. Rossin, S. Raychaudhuri, A.D. Johnson, A.S. Dimas, R.J. Loos, S. Vedantam, H. Chen, J.C. Florez, C. Fox, C.T. Liu, D. Rybin, D.J. Couper, W.H. Kao, M. Li, M.C. Cornelis, P. Kraft, Q. Sun, R.M. van Dam, H.M. Stringham, P.S. Chines, K. Fischer, P. Fontanillas, O.L. Holmen, S.E. Hunt, A.U. Jackson, A. Kong, R. Lawrence, J. Meyer, J.R. Perry, C.G. Platou, S. Potter, E. Rehnberg, N. Robertson, S. Sivapalaratnam, A. Stancakova, K. Stirrups, G. Thorleifsson, E. Tikkanen, A.R. Wood, P. Almgren, M. Atalay, R. Benediktsson, L.L. Bonnycastle, N. Burtt, J. Carey, G. Charpentier, A.T. Crenshaw, A.S. Doney, M. Dorkhan, S. Edkins, V. Emilsson, E. Eury, T. Forsen, K. Gertow, B. Gigante, G.B. Grant, C.J. Groves, C. Guiducci, C. Herder, A.B. Hreidarsson, J. Hui, A. James, A. Jonsson, W. Rathmann, N. Klopp, J. Kravic, K. Krjutskov, C. Langford, K. Leander, E. Lindholm, S. Lobbens, S. Mannisto, et al., Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes, Nat. Genet. 44 (2012) 981–990.
[24] J.A. Todd, Etiology of type 1 diabetes, Immunity 32 (2010) 457–467.
[25] Y.S. Kim, G. Nakanishi, M. Lewandoski, A.M. Jetten, GLIS3, a novel member of the GLIS subfamily of kruppel-like zinc finger proteins with repressor and activation functions, Nucleic Acids Res. 31 (2003) 5513–5525.
[26] S. Nejentsev, N. Walker, D. Riches, M. Egholm, J.A. Todd, Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes, Science 324 (2009) 387–389.
[27] D.J. Smyth, J.D. Cooper, R. Bailey, S. Field, O. Burren, L.J. Smink, C. Guja, C. Ionescu-Tirgoviste, B. Widmer, D.B. Dunger, D.A. Savage, N.M. Walker, D.G. Clayton, J.A. Todd, A genome-wide association study of nonsynonymous SNPs identifies a type 1 diabetes locus in the interferon-induced helicase (IFIH1) region, Nat. Genet. 38 (2006) 617–619.
[28] S. Liu, H. Wang, Y. Jin, R. Podolsky, M.V. Reddy, J. Pedersen, B. Bode, J. Reed, D. Steed, S. Anderson, P. Yang, A. Muir, L. Steed, D. Hopkins, Y. Huang, S. Purohit, C.Y. Wang, A.K. Steck, A. Montemari, G. Eisenbarth, M. Rewers, J.X. She, IFIH1 polymorphisms are significantly associated with type 1 diabetes and IFIH1 gene expression in peripheral blood mononuclear cells, Hum. Mol. Genet. 18 (2009) 358–365.
[29] H. Kato, O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, S. Uematsu, A. Jung, T. Kawai, K.J. Ishii, O. Yamaguchi, K. Otsu, T. Tsujimura, C.S. Koh, C. Reis e Sousa, Y. Matsuura, T. Fujita, S. Akira, Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses, Nature 441 (2006) 101–105.