

<https://helda.helsinki.fi>

Genetic Discrimination Between LADA and Childhood-Onset Type 1 Diabetes Within the MHC

Mishra, Rajashree

2020-02

Mishra , R , Akerlund , M , Cousminer , D L , Ahlqvist , E , Bradfield , J P , Chesi , A , Hodge , K M , Guy , V C , Brillon , D J , Pratley , R E , Rickels , M R , Vella , A , Ovalle , F , Harris , R I , Melander , O , Varvel , S , Hakonarson , H , Froguel , P , Lonsdale , J T , Mauricio , D , Schloot , N C , Khunti , K , Greenbaum , C J , Yderstraede , K B , Tuomi , T , Voight , B F , Schwartz , S , Boehm , B O , Groop , L , Leslie , R D & Grant , S F A 2020 , ' Genetic Discrimination Between LADA and Childhood-Onset Type 1 Diabetes Within the MHC ' , Diabetes Care , vol. 43 , no. 2 , pp. 418-425 . <https://doi.org/10.2337/dc19-0986>

<http://hdl.handle.net/10138/327246>

<https://doi.org/10.2337/dc19-0986>

publishedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.



Genetic Discrimination Between LADA and Childhood-Onset Type 1 Diabetes Within the MHC

Diabetes Care 2020;43:418–425 | <https://doi.org/10.2337/dc19-0986>

Rajashree Mishra,^{1,2} Mikael Åkerlund,³ Diana L. Cousminer,^{1,4} Emma Ahlqvist,³ Jonathan P. Bradfield,⁵ Alessandra Chesi,¹ Kenyatta M. Hodge,¹ Vanessa C. Guy,¹ David J. Brillon,⁶ Richard E. Pratley,⁷ Michael R. Rickels,⁸ Adrian Vella,⁹ Fernando Ovalle,¹⁰ Ronald I. Harris,¹¹ Olle Melander,³ Stephen Varvel,¹² Hakon Hakonarson,^{5,13} Phillippe Froguel,^{14,15} John T. Lonsdale,¹⁶ Didac Mauricio,¹⁷ Nanette C. Schloot,¹⁸ Kamlesh Khunti,¹⁹ Carla J. Greenbaum,²⁰ Knud B. Yderstræde,²¹ Tiinamajja Tuomi,^{22,23,24} Benjamin F. Voight,^{4,8,25,26} Stanley Schwartz,²⁷ Bernhard O. Boehm,^{28,29} Leif Groop,^{3,24} Richard David Leslie,³⁰ and Struan F.A. Grant^{1,4,5,8,13}

OBJECTIVE

The MHC region harbors the strongest loci for latent autoimmune diabetes in adults (LADA); however, the strength of association is likely attenuated compared with that for childhood-onset type 1 diabetes. In this study, we recapitulate independent effects in the MHC class I region in a population with type 1 diabetes and then determine whether such conditioning in LADA yields potential genetic discriminators between the two subtypes within this region.

RESEARCH DESIGN AND METHODS

Chromosome 6 was imputed using SNP2HLA, with conditional analysis performed in type 1 diabetes case subjects ($n = 1,985$) and control subjects ($n = 2,219$). The same approach was applied to a LADA cohort ($n = 1,428$) using population-based control subjects ($n = 2,850$) and in a separate replication cohort (656 type 1 diabetes case, 823 LADA case, and 3,218 control subjects).

RESULTS

The strongest associations in the MHC class II region (rs3957146, β [SE] = 1.44 [0.05]), as well as the independent effect of MHC class I genes, on type 1 diabetes risk, particularly *HLA-B*39* (β [SE] = 1.36 [0.17]), were confirmed. The conditional analysis in LADA versus control subjects showed significant association in the MHC class II region (rs3957146, β [SE] = 1.14 [0.06]); however, we did not observe significant independent effects of MHC class I alleles in LADA.

CONCLUSIONS

In LADA, the independent effects of MHC class I observed in type 1 diabetes were not observed after conditioning on the leading MHC class II associations, suggesting that the MHC class I association may be a genetic discriminator between LADA and childhood-onset type 1 diabetes.

Latent autoimmune diabetes in adults (LADA) is typically defined as initial insulin independency for at least 6 months after diagnosis and the presence of diabetes-associated autoantibodies (1). Despite such features, autoantibody screening is not carried out in routine clinical practice, resulting in frequent misdiagnosis. For instance, in a cohort of apparent type 2 diabetes cases, as many as 8–10% can actually represent misdiagnosed autoimmune diabetes cases (2,3). Hence, there is a need to identify biomarkers to aid in accurately diagnosing LADA as well as other diabetes subtypes (4).

A comprehensive analysis of the genetic etiology of LADA has, until recently, not been performed (5). Previous genetic studies have suggested that the condition

¹Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA

²Graduate Group in Genomics and Computational Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

³Lund University Diabetes Centre, Department of Clinical Sciences Malmö, Lund University, Skåne University Hospital, Malmö, Sweden

⁴Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

⁵Center for Applied Genomics, Children's Hospital of Philadelphia, Philadelphia, PA

⁶Weill Cornell Medical College, New York City, NY

⁷Translational Research Institute for Metabolism and Diabetes, Florida Hospital, FL

⁸Institute for Diabetes, Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

⁹Mayo Clinic, Rochester, MN

¹⁰University of Alabama at Birmingham, Birmingham, AL

¹¹Geisinger Health System, Danville, PA

¹²Health Diagnostic Laboratory, Inc., Richmond, VA

¹³Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

¹⁴CNRS 8199, Université Lille Nord de France, Pasteur Institute, Lille, France

¹⁵Department of Genomics of Common Disease, Imperial College London, London, U.K.

¹⁶National Disease Research Interchange, Philadelphia, PA

¹⁷Hospital de la Santa Creu i Sant Pau, CIBERDEM, Barcelona, Spain

¹⁸German Diabetes Center, Düsseldorf, German

¹⁹Diabetes Research Centre, University of Leicester, Leicester, U.K.

comprised both type 1 diabetes and type 2 diabetes components either because it is an intermediate form of diabetes or because it is a mixture of type 2 diabetes in a cohort of predominantly type 1 diabetes owing to a high false positive detection rate with use of autoantibodies when screening. There is some debate as to whether LADA is in fact a distinct clinical entity or simply a category imposed on continuous features such as age of onset and time to insulin. However, since LADA is currently defined as a slowly progressive form of type 1 diabetes (6), it is crucial to define genetic differences between childhood-onset type 1 diabetes and LADA if we are to clarify the clinical utility of identifying adult-onset autoimmune diabetes.

Previous genetic studies in LADA have shown a strong association signal in the MHC, although with diminished effect sizes compared with observations in childhood-onset type 1 diabetes (5,7). The MHC region is located on chromosome 6 and harbors >400 genes, with two main classes, MHC class I and MHC class II, which together harbor classic HLA genes (*HLA-A*, *HLA-B*, *HLA-C*, and *HLA-DRB*, *HLA-DQA*, *HLA-DQB*, *HLA-DPA*, and *HLA-DPB*, respectively). The HLA encodes cell surface proteins for antigen presentation and accounts for ~50% of the genetic heritability of type 1 diabetes, with susceptibility principally harbored within the MHC class II genes *HLA-DQB1* and *HLA-DRB1*. However, in addition to class II genes, MHC Class I genes in susceptibility to type 1 diabetes have also been suggested in previous studies (8–10); in particular, variation within the MHC class I genes *HLA-A* and *HLA-B* has been shown through conditional analysis to further increase type 1 diabetes risk (11). MHC class I markers have also been shown to be associated with younger age

at diagnosis in type 1 diabetes, and given the adult-onset phenotype of LADA, we hypothesized that this genetic variation will be less enriched in LADA.

First, we aimed to recapitulate the independent effects of MHC class I variants using the SNP2HLA imputation tool followed by stepwise forward logistic regression in the same type 1 diabetes cohort that participated in a previous study (11). In addition, we set out to identify distinguishing features within the MHC between childhood-onset type 1 diabetes and adult-onset LADA by performing the same conditional analysis followed by a replication attempt in a second case/control set. Finally, we compared β regression coefficients for each disease to determine whether effect sizes differ among LADA case and control subjects versus type 1 diabetes case and control subjects.

RESEARCH DESIGN AND METHODS

Study Populations

LADA Case Subjects

A total of 1,492 LADA cases were derived from multiple cohorts across the U.K., Germany, and the U.S. Details on the participants can be found in Supplementary Table 1. All participants were diagnosed with LADA if they fulfilled the following criteria: age at diagnosis 30–70 years, testing positive for at least one diabetes-associated autoantibody (most case subjects were positive for GAD autoantibodies), and not on insulin treatment for at least 6 months after diagnosis.

Control Subjects

The LADA population-based control subjects comprised of two cohorts ($n = 2,979$). The first cohort consisted of 1,296 children and adolescents of European ancestry who did not have diabetes, aged 5–20 years, and who were enrolled in the Bone

Mineral Density in Childhood Study (BMDCS) (12). The second control cohort consisted of 1,683 adults of European ancestry from a non-Hodgkin lymphoma genome-wide association study (GWAS), available in dbGaP (www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000818.v2.p1) (13). Details on the control cohorts can be found in Supplementary Table 1.

Recapitulating a Previous Study

We also leveraged 3,000 healthy adult British Birth Cohort control subjects, 2,000 individuals with childhood-onset type 1 diabetes, and 1,999 individuals with type 2 diabetes from the Wellcome Trust Case Control Consortium (WTCCC) (14) to recapitulate observations in a previous study (11). Individual data from the WTCCC are available through the consortium's Data Access Committee (<http://www.wtccc.org.uk>). More details on cohort information can be found in Supplementary Table 1.

Replication

A cohort of individuals from the All New Diabetics In Scania (ANDIS) and Scania Diabetes Registry (SDR) studies was used for further recapitulation and replication, including type 1 diabetes case subjects ($N = 656$), LADA case subjects ($n = 823$), and population-based control subjects ($N = 3,218$). Details on the participants can be found in Supplementary Table 1. See flowchart for overview of data sets and workflow (Supplementary Fig. 1).

Genotyping

All samples, except the WTCCC data, were genotyped using the Illumina OmniExpress genotyping chip. WTCCC type 1 diabetes and type 2 diabetes case subjects were genotyped using Affymetrix 500K, and WTCCC control subjects were genotyped

²⁰Benaroya Research Institute, Seattle, WA

²¹Odense University Hospital, Odense, Denmark

²²Department of Endocrinology, Helsinki University Hospital, Helsinki, Finland

²³Folkhälsan Research Centre, Helsinki, Finland, and Research Programs Unit, Clinical and Molecular Metabolism, University of Helsinki, Helsinki, Finland

²⁴Institute for Molecular Medicine Finland, Helsinki, Finland

²⁵Department of Systems, Pharmacology and Translational Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

²⁶Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

²⁷Main Line Health System, Wynnewood, PA

²⁸Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, and Imperial College London, London, U.K.

²⁹Department of Internal Medicine I, Ulm University Medical Centre, Ulm, Germany

³⁰Department of Immunobiology, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, U.K.

Corresponding author: Struan F.A. Grant, grants@email.chop.edu

Received 16 May 2019 and accepted 16 November 2019

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc19-0986/-/DC1>.

R.M., M.Å., and D.L.C. made equal contributions.

R.M. is currently affiliated with GlaxoSmithKline, Collegeville, PA. N.C.S. is currently affiliated with Lilly Germany, Bad Homburg vor der Höhe, Germany. V.C.G. is currently affiliated with Science 37, Los Angeles, CA.

© 2019 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at <http://www.diabetesjournals.org/content/license>.

on the Illumina 1.2M BeadChip. Quality control was performed using PLINK (15). Individuals with ambiguous sex, genotype missingness >5%, genome-wide heterozygosity (3 SDs from the mean), duplicates, and related individuals were excluded (see Supplementary Table 1 for details). Principal component analysis was performed using PLINK, and outliers were removed to exclude individuals with non-European ancestry. Single nucleotide polymorphisms (SNPs) with missing rate <5%, minor allele frequency (MAF) <1%, and Hardy-Weinberg equilibrium exact test P value $<1 \times 10^{-5}$ were removed before HLA imputation.

HLA Imputation

Starting from the genotyped SNPs, we imputed chromosome 6 using the HLA imputation software SNP2HLA along with the Type 1 Diabetes Genetics Consortium (T1DGC) reference panel (16). A marker window size of 1,000 base pairs and a posterior probability (gprob) threshold of 0.5 were used. The HLA alleles of LADA case subjects ($n = 1,428$) and WTCCC type 1 diabetes case subjects ($n = 1,985$) were imputed to both two-digit resolution and four-digit resolution for increased coverage and resolution of HLA alleles. In total, there were 5,698 SNPs, 424 HLA alleles, and 1,276 HLA amino acids. In this study, we focused on a subset of SNPs and HLA alleles that had an MAF >1% in all three control cohorts (159 HLA alleles and 5,506 SNPs remained).

Power Calculations

Power calculations were performed using the Genetic Association Study (GAS) Power Calculator (<http://csg.sph.umich.edu/abecasis/cats/>). Assumptions included a multiplicative model, a disease incidence of 0.0036, 1,428 case and 2,979 control subjects, and a significance level of 8.83×10^{-6} , based on a Bonferroni correction for the 5,665 variants tested (Supplementary Table 2).

Recapitulation of a Previously Published Conditional Analysis for Type 1 Diabetes

Logistic regression using SNPTTEST (17) was used to test all HLA alleles and SNPs with MAF >1% in all three control cohorts. Sex and the 12 broad geographical regions, provided by the WTCCC, were included as covariates in the analysis. The analyses were performed in the WTCCC

type 1 diabetes versus control data sets using forward stepwise conditional logistic regression until there were no significant signals remaining after correction for multiple testing.

Conditional Analysis in Subjects With LADA Versus Population-Based Control Subjects

Conditional logistic regression was performed using SNPTTEST in the LADA versus population-based control subjects, including sex and the first four principal components as covariates.

Replication

To further validate MHC class I independent effects in type 1 diabetes and lack of MHC class I independent effects in LADA, we implemented approximate conditional and joint (COJO) analysis in GCTA (18) on summary statistics from the Swedish replication cohort. Association analysis was performed using SNPTTEST, and sex and the first four principal components were used as covariates. There were 656 case subjects with type 1 diabetes vs. 3,218 population-based control subjects and 823 case subjects with LADA vs. 3,211 population-based control subjects.

Sensitivity Analysis

We performed sensitivity analysis to determine whether the lack of independent type 1 diabetes-associated signals in MHC class I genes in LADA case subjects could be due to a lack of power. We randomly sampled 1,428 type 1 diabetes case subjects and 714 type 1 diabetes cases subjects (subsets equating to the same size as the LADA cohort and half the size of the LADA cohort, respectively) and 2,219 control subjects to determine whether the type 1 diabetes-associated signals could be still be detected. Stepwise conditional logistic regression using SNPTTEST was performed as described above. To test the hypothesis that LADA is a mixture of type 1 diabetes and type 2 diabetes cases, we performed a conditional analysis in 714 randomly sampled type 1 diabetes cases and 714 randomly sampled type 2 diabetes cases (total $n = 1,428$ cases) and 2,219 WTCCC control subjects.

Further Validating Independent Signals

PLINK was used to calculate pairwise linkage disequilibrium (LD) between

variants to further validate that the associated variants were truly independent of each other. For confirmation of the independent association of *HLA-B*39*, the specific *HLA-B*39* subtype *HLA-B*3906* was tested in the WTCCC type 1 diabetes case subject ($n = 1,985$) versus control subject ($n = 2,219$) data set with *DQB1*0402* and *DQB1*0501* as covariates.

Ethics Approval

This study was approved by local institutional ethics review boards.

RESULTS

Confirming Independent Effects of MHC Class I Signals in WTCCC Subjects With Type 1 Diabetes Versus Control Subjects

Before conditioning, we observed rs3957146 as the strongest association signal in analysis of subjects with type 1 diabetes versus WTCCC control subjects ($P = 8.94 \times 10^{-165}$) (Fig. 1A). rs3957146 is in strong LD with a classical HLA subtype allele, *HLA-DQB1*0302* ($r^2 = 0.99$). After conditioning on the top signal, rs3957146, and subsequent independent MHC class II signals (*HLA-DQB1*0201* and rs9268633), we observed the reported independent significant association of MHC class I variants rs1610649 (*HLA-G*, $P = 6.89 \times 10^{-23}$) and *HLA-B*39* ($P = 6.89 \times 10^{-23}$) (Fig. 1B, Table 1, Supplementary Table 3, and Supplementary Fig. 2). Conditioning on these variants in addition to the MHC class II variants also demonstrated significant association with the *HLA-A* locus (rs9259852, $P = 2.04 \times 10^{-8}$).

Conditional Analysis in Subjects With LADA Versus Population-Based Control Subjects

We then went on to perform stepwise conditional analysis in 1,428 LADA case and 2,979 control subjects. Similar to observations in the type 1 diabetes versus WTCCC control data set, before conditioning on any variants, the strongest association signal in LADA case subjects versus population-based control subjects was also rs3957146 ($P = 1.80 \times 10^{-68}$) (Fig. 2A). Although we had 98% power to detect *HLA-B*39* with an allele frequency of 2% and an odds ratio of 2.5 (Supplementary Table 2), when conditioning on the most highly significant MHC class II alleles (rs3957146, *HLA-DRB1*03*, rs9269081, *DRB1*0404*, and

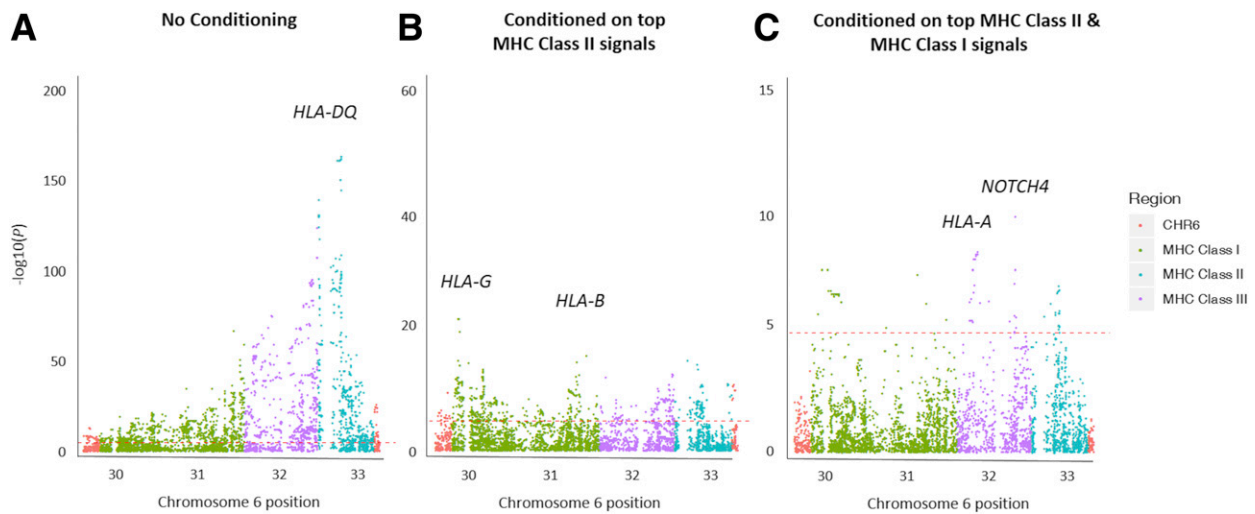


Figure 1—Conditional analysis in 1,985 type 1 diabetes case and 2,219 WTCCC control subjects. **A:** Logistic regression analysis without conditioning on MHC class II alleles. **B:** Logistic regression analysis conditioning on MHC class II alleles. **C:** Logistic regression analysis conditioning on MHC class II and MHC class I signals.

*DQB1*0602*), there were no remaining independent signals in the MHC class I region reaching significance after correction for multiple comparisons ($P < 8.83 \times 10^{-6}$) (Fig. 2B). Furthermore, we also noted independent effects in the MHC class III region ($rs2143462, P = 8.24 \times 10^{-8}$) and the MHC class II region (*HLA-DPA1*02, P = 1.62 \times 10^{-6}, and *HLA-DPB1* variant $rs3130192, P = 5.32 \times 10^{-6}$), which*

are known to be associated with type 1 diabetes (19). Here, *HLA-DPB1* variant is in strong LD with $rs2301225 (r^2 = 0.85)$ and is independently associated with type 1 diabetes. MHC class I variants were not observed to be independently associated with LADA after correction for multiple comparisons (Table 1, Supplementary Fig. 2, and Supplementary Table 4).

Sensitivity Analysis in Reduced Sample of Subjects With Type 1 Diabetes Versus Control Subjects

To ensure that the lack of significant associations with MHC class I genes in the LADA cohort was not explained by reduced power, we conducted a sensitivity analysis by systematically decreasing the sample size of the type 1 diabetes versus WTCCC control cohort to match the size

Table 1—Comparison of β -coefficients between conditional analyses in type 1 diabetes and LADA cohorts

SNP/HLA allele	Locus	Position	Alleles (risk/other)	WTCCC type 1 diabetes case vs. WTCCC control subjects				LADA case vs. control subjects				P
				RAF, case subjects	RAF, control subjects	β	SE	RAF, case subjects	RAF, control subjects	β	SE	
$rs3957146$	<i>HLA-DQA2</i>	32789508	T/C	0.385	0.113	1.44	0.05	0.251	0.101	1.14	0.06	1.22×10^{-4}
$DQB1*0201$	<i>HLA-DQB1</i>	32739039	P/A	0.338	0.140	1.56	0.06	0.209	0.119	1.05	0.07	3.17×10^{-8}
$rs9268633$	<i>HLA-DRA</i>	32514451	G/A	0.983	0.803	1.46	0.10	0.919	0.812	0.83	0.06	6.58×10^{-8}
$rs1610649$	<i>HLA-G</i>	29876896	G/A	0.616	0.582	0.61	0.06	0.592	0.586	0.16	0.05	8.33×10^{-9}
B*39	<i>HLA-B</i>	31431272	P/A	0.043	0.016	1.36	0.17	0.023	0.019	0.58	0.19	2.22×10^{-3}
$DRB1*0404$	<i>HLA-DRB1</i>	32660042	P/A	0.082	0.048	1.04	0.13	0.037	0.035	1.01	0.14	0.88
$rs17427599$	<i>HLA-DQB1</i>	32775342	T/C	0.849	0.755	0.59	0.09	0.818	0.775	0.31	0.07	1.41×10^{-2}
$rs2301225$	<i>HLA-DPA1</i>	33143838	T/C	0.941	0.891	0.72	0.11	0.924	0.886	0.42	0.08	2.74×10^{-2}
$rs397081$	<i>NOTCH4</i>	32300595	T/C	0.095	0.045	0.79	0.12	0.075	0.054	0.64	0.10	0.34
$rs9262545$	<i>MUC22</i>	31101041	A/G	0.913	0.881	0.67	0.11	0.862	0.858	0.09	0.07	8.65×10^{-6}
$rs9262547$	<i>MUC22</i>	31101206	T/A	0.135	0.119	1.59	0.19	0.137	0.142	-0.09	0.07	1.07×10^{-16}
$rs9259852$	<i>HLA-A</i>	30004400	C/T	0.977	0.959	1.00	0.18	0.968	0.963	0.19	0.13	2.64×10^{-4}
$rs9269081$	<i>HLA-DRA</i>	32549078	A/C	0.890	0.735	0.57	0.11	0.821	0.685	0.71	0.05	0.25
$rs1978029$	<i>HLA-DQB2</i>	32839688	C/T	0.651	0.537	0.36	0.08	0.565	0.475	0.39	0.05	0.75

β -Coefficients and SEs are calculated for the risk allele frequency (RAF) from stepwise regression conditional on all SNP/HLA alleles (first column) in 1,985 type 1 diabetes case vs. 2,219 control subjects. β -Coefficients and SEs for LADA case vs. control subjects correspond with maximum effect size in conditional analysis. P values are derived from two-sample Z test to formally test whether β -coefficients are significantly different. Forest plot is shown in Supplementary Fig. 2. Three independent signals appeared in both type 1 diabetes and LADA conditional analyses ($rs3957146, HLA-DRB1*0404,$ and $rs9269081$); however, of these three signals, only $rs3957146$ had a significant difference in effect size between type 1 diabetes and LADA (interaction P value = 4.15×10^{-10}).

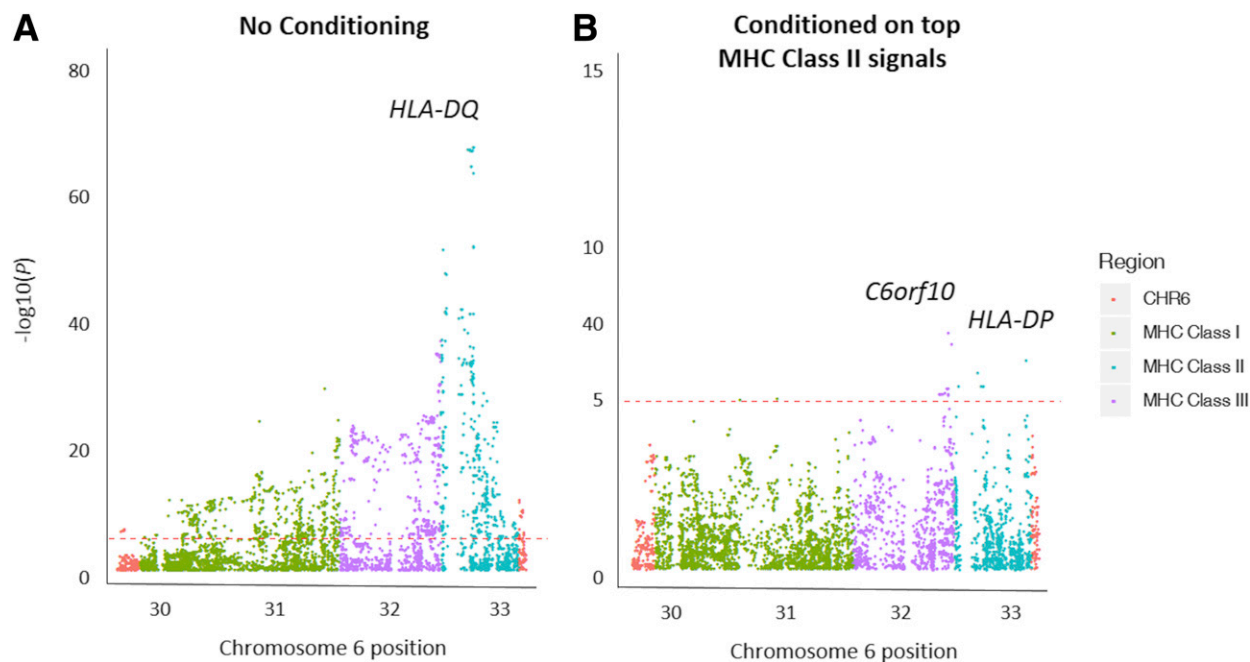


Figure 2—Conditional analysis in 1,428 LADA case and 2,979 WTCCC control subjects. A: Logistic regression analysis without conditioning on MHC class II alleles. B: Logistic regression analysis conditioning on MHC class II alleles.

of the discovery LADA cohort ($n = 1,428$ type 1 diabetes case and 2,219 control subjects) and performing conditional analysis. Independent significant association signals at *HLA-G* ($P = 1.37 \times 10^{-17}$), *HLA-B* ($P = 5.58 \times 10^{-14}$), and *MUC22* (rs9262545, $P = 3.36 \times 10^{-9}$; rs9262547, $P = 9.69 \times 10^{-14}$) were still observed in this reduced type 1 diabetes sample size (Supplementary Table 5), although these signals were missing in the comparatively sized LADA versus control subjects data set. Similarly, independent significant association signals at *HLA-B* ($P = 1.26 \times 10^{-10}$), *HLA-G* ($P = 0.002$), and *MUC22* (rs9262545, $P = 1.57 \times 10^{-5}$) remained after further reduction of the type 1 diabetes cohort size to equate with half the LADA cohort size (Supplementary Table 6).

Sensitivity Analysis in a Randomly Mixed Cohort of Type 1 Diabetes and Type 2 Diabetes Case Subjects Versus Control Subjects

Another explanation for the lack of independent, significant associations across MHC class I genes in LADA could be that the LADA cohort simply represents an ~50/50 mixture of misdiagnosed type 1 diabetes and type 2 diabetes cases. Therefore, we randomly sampled 714 type 1 diabetes case subjects, 714

type 2 diabetes case subjects, and 2,219 control subjects, creating a “mixture” cohort. We performed the same conditional analysis described above and observed that the *HLA-B*, *HLA-G*, and two *MUC22* signals in the MHC class I regions remained independently significant in this mixed cohort, driven by the type 1 diabetes case subset (Supplementary Table 7).

Replication

We leveraged summary statistics data from Swedish cohorts to attempt replication of our findings. In type 1 diabetes case versus control subjects, the strongest association was rs9275206 ($P = 6.35 \times 10^{-89}$), which is in strong LD with *HLA-DQB1*0302* ($r^2 = 0.99$). After conditioning on rs9275206 and subsequent top signals (Supplementary Table 8), we again observed significant association signals at the *HLA-G* ($P = 1.74 \times 10^{-10}$) and *HLA-B* (1.10×10^{-9}) loci. However, when conditional analysis was performed in LADA case versus control subjects, there were no such signals across MHC class I genes and very sparse signals in the MHC class II region (Supplementary Table 9). Furthermore, we observed a significant association signal at the *NOTCH4* (rs397081, $P = 1.11 \times 10^{-10}$) locus, the *MUC22* locus (rs9262545, $P = 7.83 \times 10^{-11}$, and

rs9262547, $P = 7.17 \times 10^{-17}$), and the *HLA-A* locus (rs9259852, $P = 5.84 \times 10^{-14}$) (Fig. 1C). Notably, rs9259852 is in strong LD with the classic HLA subtype allele, HLA-A*32 ($r^2 = 0.96$).

Further Validating HLA*B*39

It has been shown that the *HLA-B*3906* allele is associated with a high risk of diabetes only for specific HLA-DR/DQ haplotypes, *DRB1*0801-DQB1*0402* and *DRB1*0101-DQB1*0501* (20). With specific conditioning on these HLA-DR/DQ haplotypes in the WTCCC type 1 diabetes case and control cohort, the independent significant association of the more specific *HLA-B*3906* subtype still remained (odds ratio 4.57 [95% CI 3.08–6.80]; $P = 5.84 \times 10^{-14}$).

CONCLUSIONS

The main objective of this study was to perform conditional analysis of the HLA region in LADA, which has been underexplored to date in this disease context. The few genetic studies in LADA (5,21,22) only focused on the HLA class II *DRB1* and *DQB1* haplotypes. Such studies, in populations of both European and Chinese ancestry, show that type 1 diabetes risk haplotypes are less frequent in LADA compared with childhood-onset type 1 diabetes case subjects, whereas type 1

diabetes protective haplotypes are more frequent in LADA, suggesting that LADA is a genetically attenuated form of type 1 diabetes. By extending the analysis of HLA in LADA beyond the MHC class II region, we were able to observe further genetic differences between LADA and childhood-onset type 1 diabetes.

Although previous studies have reported MHC class I independent effects in type 1 diabetes, those studies directly used HLA-typed case and control subjects. Given the cost and challenges of direct HLA typing, we utilized the imputation tool SNP2HLA on genotyping data. SNP2HLA has been commonly used in the field to assess the genetics of autoimmune diseases (16,23–25). Furthermore, given that this approach differs from that of Nejentsev et al. (11), it was crucial to first ensure that we could recapitulate the previously reported type 1 diabetes observations in the same cohort. First, we leveraged the WTCCC type 1 diabetes case and control data set, as a positive control, with previous studies identifying MHC class I independent type 1 diabetes associations in the MHC class I region (8,10,11,26). Since these studies were reported, imputation tools have allowed the analysis of the HLA region more cheaply and, in general, more practically. Before investigating MHC class II independent LADA associations in the MHC class I region, given the difference in our analytical approach, we recapitulated the observations in previous studies (10,11) by leveraging the same WTCCC type 1 diabetes and control data sets. We confirmed that MHC class I variants are significantly associated with type 1 diabetes, independent of the MHC class II region using this imputation-based approach followed by stepwise conditional logistic regression. The conditional analysis was repeated in the LADA cohort, which consisted of case and population-based control subjects. Crucially, there were no significant independent effects in the MHC class I region remaining after correction for multiple comparisons; furthermore, this observation was replicated in a separate Swedish cohort of type 1 diabetes case, LADA case, and population-based control subjects.

The MHC class I variant *HLA-B*39* is an established locus associated with type 1 diabetes risk (10,11,27). More specifically, studies suggested a strong association with type 1 diabetes for the

subtype *HLA-B*3906*, which is now used in type 1 diabetes genetic risk scores to predict type 1 diabetes diagnosis (23). It has also been shown that the *B*3906* allele significantly enhances the risk of type 1 diabetes when present on specific *HLA-DR/DQ* haplotypes (e.g., *DRB1 0801-DQB1 0402* and *DRB1 0101-DQB1 0501*). The frequency of *HLA-B*3906* is different among different populations and here did not survive our filter of having an $MAF > 1\%$ in the replication control cohort of Swedes. Thus, it was excluded in the analyses across the three cohorts. However, we confirmed that the *HLA-B*3906* allele remained significantly associated with type 1 diabetes after conditioning on the presence of the *DRB1 0801-DQB1 0402* and *DRB1 0101-DQB1 0501* haplotypes. Additionally, *HLA-B*3906* is associated with younger age-at-diagnosis in type 1 diabetes (9,11). A recent study using a NOD mouse model showed that *HLA-B*3906* mediates the development of $CD8^+$ T cells required for type 1 diabetes onset; moreover, in the context of reduced immunological tolerance to insulin, *HLA-B*3906*-transgenic NOD mice develop type 1 diabetes at an accelerated rate (28). The lack of an independent *HLA-B*39* association observed in the adult-onset phenotype of LADA further confirms the link between *HLA-B*39* with autoimmune progression with earlier onset of clinical disease.

HLA-B associations have been confirmed in a previous study (26), as well as associations around *HLA-G*, which is expressed in human pancreas (29) and may play a role in autoimmune progression (30). However, the MHC class I variant rs1619379, located in *HLA-G* and ~100 kb telomeric of *HLA-A*, may be less informative compared with *HLA-A* variants in predicting type 1 diabetes risk (10). This particular MHC class I variant was independently significant in the down-sampled type 1 diabetes cohort, but is in strong LD with *HLA-G* variants, rs1610649 and rs2735028, which were significantly associated in the full type 1 diabetes set, the mixture cohort consisting of type 1 diabetes and type 2 diabetes case subjects, and the type 1 diabetes Swedish replication cohort. Additionally, the MHC class I variants located in the *MUC22* locus have not been replicated in separate cohorts and likely form haplotypes with HLA class I alleles.

One limitation of this study was that we only tested variants with an $MAF > 1\%$ in all three control cohorts, which resulted in filtering out many informative alleles such as *HLA-B*3906*. By filtering to include only common alleles, we limited potential discrepancies between populations and were able to replicate our observations across cohorts with different frequencies of known risk variants. Furthermore, our study is limited in power to assess the underlying continuous traits of age at onset, time to insulin, and autoantibody titer; future well-designed, large studies are needed to enable those analyses. With larger and more complete data in individuals, we can then formally test the many competing hypotheses regarding the state of LADA in the field. The hypothesis that LADA exists as a disease different from type 1 diabetes with both overlapping genes and distinct genes is unlikely, as we did not clearly observe distinct susceptible loci that were unique to LADA in this study or our previous GWAS (5). Future studies leveraging case subjects diagnosed with type 1 diabetes and LADA across the age of onset range will be crucial to test the remaining hypotheses, which are: 1) LADA is type 1 diabetes with misdiagnosed type 2 case subjects who are false positive for autoantibodies, 2) LADA cases are essentially type 1 diabetes at later onset with lower rates of progression, 3) LADA is a form of diabetes where case subjects have both type 1 and type 2 risk alleles present at the individual level. This first hypothesis (LADA is type 1 diabetes with misdiagnosed type 2 case subjects who are false positive for autoantibodies) motivated the sensitivity analysis, in which we randomly sampled case subjects from the WTCCC type 1 diabetes and type 2 diabetes cohort to create a random LADA cohort under the assumption that the LADA group would be a “mixture” of actual type 1 diabetes and type 2 diabetes case subjects. In this analysis, we still observed the same independent effects of MHC class I variants, showing that the type 1 diabetes signature remained in the “mixture” cohort despite not being observed in LADA. However, we recognize that type 1 diabetes case subjects were sampled from the cohort of childhood-onset type 1 diabetes and that properly testing this hypothesis would require that LADA cases be ascertained from another cohort but also further stratified by autoantibody titer.

Although the underlying populations from which the type 1 diabetes and LADA sets were derived are the same, we addressed whether the LD structure in the HLA region could be different between the two sample sets, which in turn could have resulted in inaccurate imputation. However, when we calculated LD for the MHC region in these data sets, we found it to be highly correlated (Pearson correlation coefficient = 0.97). Future studies are needed to address this question in-depth as well as validate these findings in a cohort directly typed for MHC class II and MHC class I HLA alleles. Additionally, for further delineation of this putative distinguishing genetic feature between LADA and childhood-onset type 1 diabetes, it will be crucial to investigate how the HLA profile compares across the diabetes age continuum stratified for different autoantibody positivity status. A previous study observed different independent effects of MHC class I variants to GAD autoantibodies and insulinoma-associated antigen-2 autoantibodies (31). Additionally, studies have shown that children with type 1 diabetes who are positive for a single autoantibody are more like to show type 2 diabetes features (32,33), for instance, a significant association with type 2 diabetes GWAS-implicated variants. Overall, our results point to key differences in the genetic signature in the MHC region, especially class I markers, between LADA and childhood-onset type 1 diabetes. This study highlights the clinical utility of genetic screening in adult-onset diabetes that may be autoimmune in origin. The potential of defining these subjects who are at risk for rapid loss of insulin secretion using genetic characteristics could enable targeted immune-based, disease-modifying therapy.

Acknowledgments. The authors thank all the patients and control subjects for their participation. The authors thank JDRF, German Research Council (DFG), German Diabetes Foundation, EUPF5 (Action LADA), ANDIS, SDR, and Malmö Diet and Cancer Study–Cardiovascular Cohort (MDC-CC) for providing samples for this research. The authors also thank the BMDCS, a multicenter, longitudinal study of bone accrual in healthy children (authors listed in Supplementary Data). This study makes use of data generated by the WTCCC; a full list of the investigators who contributed to the generation of the data is available from www.wtccc.org.uk, and funding for the project was provided by the Wellcome Trust under

award 076113. The authors acknowledge Stephen S. Rich (University of Virginia), Marcella Devoto (University of Pennsylvania/Children's Hospital of Philadelphia), and Andrew Wells (University of Pennsylvania/Children's Hospital of Philadelphia) for fruitful discussions.

Funding. D.L.C. is supported by the National Institutes of Health (1K99HD099330-1). D.M. is supported by CIBERDEM, Instituto de Salud Carlos III (Spain). B.O.B. is supported by the German Research Council (SFB 518, A1), Lee Kong Chian School of Medicine's Ong Tiong Tat Chair of Diabetes Research, the state of Baden-Württemberg, and a Ministry of Education, Singapore, startup grant. S.F.A.G. is supported by the National Institutes of Health (R01-DK-085212) and the Children's Hospital of Philadelphia Daniel B. Burke Endowed Chair for Diabetes Research.

Duality of Interest. R.M. is currently affiliated with GlaxoSmithKline. V.C.G. is currently affiliated with Science 37. N.C.S. is currently affiliated with Lilly Germany. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. R.M., M.Å., D.L.C., E.A., J.P.B., R.D.L., and S.F.A.G. contributed to study concept and design. R.M., M.Å., D.L.C., E.A., A.C., N.C.S., B.F.V., B.O.B., R.D.L., and S.F.A.G. contributed to drafting and critical revision of the manuscript. R.M., M.Å., D.L.C., R.D.L., and S.F.A.G. contributed to analysis and interpretation of data. M.Å., K.M.H., V.C.G., D.J.B., R.E.P., M.R.R., A.V., F.O., R.I.H., S.V., H.I.H., P.F., J.T.L., D.M., N.C.S., K.K., C.J.G., K.B.Y., T.T., B.O.B., L.G., R.D.L., and S.F.A.G. were responsible for resources. S.S., B.O.B., R.D.L., and S.F.A.G. obtained funding. All authors contributed to the final version of the manuscript. R.M. and S.F.A.G. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this study were presented at the 54th Annual Meeting of the European Association for the Study of Diabetes, Berlin, Germany, 1–5 October 2018.

References

- Tuomi T, Carlsson A, Li H, et al. Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. *Diabetes* 1999;48:150–157
- Hawa MI, Kolb H, Schloot N, et al.; Action LADA Consortium. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. *Diabetes Care* 2013;36:908–913
- Mishra R, Hodge KM, Cousminer DL, Leslie RD, Grant SFA. A global perspective of latent autoimmune diabetes in adults. *Trends Endocrinol Metab* 2018;29:638–650
- Ahlqvist E, Storm P, Karajamaki A, et al. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. *Lancet Diabetes Endocrinol* 2018;6:361–369
- Cousminer DL, Ahlqvist E, Mishra R, et al.; Bone Mineral Density in Childhood Study. First genome-wide association study of latent autoimmune diabetes in adults reveals novel insights linking immune and metabolic diabetes. *Diabetes Care* 2018;41:2396–2403

6. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539–553

7. Mishra R, Chesni A, Cousminer DL, et al.; Bone Mineral Density in Childhood Study. Relative contribution of type 1 and type 2 diabetes loci to the genetic etiology of adult-onset, non-insulin-requiring autoimmune diabetes. *BMC Med* 2017;15:88

8. Noble JA, Valdes AM, Bugawan TL, Apple RJ, Thomson G, Erlich HA. The HLA class I A locus affects susceptibility to type 1 diabetes. *Hum Immunol* 2002;63:657–664

9. Valdes AM, Erlich HA, Noble JA. Human leukocyte antigen class I B and C loci contribute to type 1 Diabetes (T1D) susceptibility and age at T1D onset. *Hum Immunol* 2005;66:301–313

10. Howson JMM, Walker NM, Clayton D, Todd JA; Type 1 Diabetes Genetics Consortium. Confirmation of HLA class II independent type 1 diabetes associations in the major histocompatibility complex including HLA-B and HLA-A. *Diabetes Obes Metab* 2009;11(Suppl. 1):31–45

11. Nejentsev S, Howson JM, Walker NM, et al.; Wellcome Trust Case Control Consortium. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature* 2007;450:887–892

12. Kalkwarf HJ, Zemel BS, Gilsanz V, et al. The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. *J Clin Endocrinol Metab* 2007;92:2087–2099

13. Berndt SI, Skibola CF, Joseph V, et al. Genome-wide association study identifies multiple risk loci for chronic lymphocytic leukemia. *Nat Genet* 2013;45:868–876

14. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–678

15. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575

16. Jia X, Han B, Onengut-Gumuscu S, et al. Imputing amino acid polymorphisms in human leukocyte antigens. *PLoS One* 2013;8:e64683

17. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nat Genet* 2007;39:906–913

18. Yang J, Ferreira T, Morris AP, et al.; Genetic Investigation of Anthropometric Traits (GIANT) Consortium; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet* 2012;44:369–375, S1–S3

19. Varney MD, Valdes AM, Carlson JA, et al.; Type 1 Diabetes Genetics Consortium. HLA DP1, DPB1 alleles and haplotypes contribute to the risk associated with type 1 diabetes: analysis of the type 1 diabetes genetics consortium families. *Diabetes* 2010;59:2055–2062

20. Baschal EE, Baker PR, Eyring KR, Siebert JC, Jasinski JM, Eisenbarth GS. The HLA-B 3906 allele imparts a high risk of diabetes only on specific

- HLA-DR/DQ haplotypes. *Diabetologia* 2011;54:1702–1709
21. Luo S, Lin J, Xie Z, et al. HLA genetic discrepancy between latent autoimmune diabetes in adults and type 1 diabetes: LADA China Study No. 6. *J Clin Endocrinol Metab* 2016;101:1693–1700
22. Desai M, Zeggini E, Horton VA, et al. An association analysis of the HLA gene region in latent autoimmune diabetes in adults. *Diabetologia* 2007;50:68–73
23. Sharp SA, Rich SS, Wood AR, et al. Development and standardization of an improved type 1 diabetes genetic risk score for use in newborn screening and incident diagnosis. *Diabetes Care* 2019;42:200–207
24. Karnes JH, Shaffer CM, Bastarache L, et al. Comparison of HLA allelic imputation programs. *PLoS One* 2017;12:e0172444
25. Hu X, Deutsch AJ, Lenz TL, et al. Additive and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. *Nat Genet* 2015;47:898–905
26. Eike MC, Becker T, Humphreys K, Olsson M, Lie BA. Conditional analyses on the T1DGC MHC dataset: novel associations with type 1 diabetes around HLA-G and confirmation of HLA-B. *Genes Immun* 2009;10:56–67
27. Noble JA, Valdes AM, Varney MD, et al.; Type 1 Diabetes Genetics Consortium. HLA class I and genetic susceptibility to type 1 diabetes: results from the Type 1 Diabetes Genetics Consortium. *Diabetes* 2010;59:2972–2979
28. Schloss J, Ali R, Racine JJ, Chapman HD, Serreze DV, DiLorenzo TP. HLA-B*39:06 efficiently mediates type 1 diabetes in a mouse model incorporating reduced thymic insulin expression. *J Immunol* 2018; 200:3353–3363
29. Cirulli V, Zalatan J, McMaster M, et al. The class I HLA repertoire of pancreatic islets comprises the nonclassical class Ib antigen HLA-G. *Diabetes* 2006;55:1214–1222
30. Shiroishi M, Tsumoto K, Amano K, et al. Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLA-G. *Proc Natl Acad Sci U S A* 2003;100: 8856–8861
31. Howson JMM, Stevens H, Smyth DJ, et al. Evidence that HLA class I and II associations with type 1 diabetes, autoantibodies to GAD and autoantibodies to IA-2, are distinct. *Diabetes* 2011;60:2635–2644
32. Redondo MJ, Muniz J, Rodriguez LM, et al. Association of TCF7L2 variation with single islet autoantibody expression in children with type 1 diabetes. *BMJ Open Diabetes Res Care* 2014;2: e000008
33. Redondo MJ, Geyer S, Steck AK, et al.; Type 1 Diabetes TrialNet Study Group. TCF7L2 genetic variants contribute to phenotypic heterogeneity of type 1 diabetes. *Diabetes Care* 2018;41:311–317