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ARTICLE

Type 2 diabetes susceptibility gene variants predispose to adult-onset autoimmune diabetes

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Abstract
Aims/hypothesis Latent autoimmune diabetes in adults (LADA) is phenotypically a hybrid of type 1 and type 2 diabetes. Genetically LADA is poorly characterised but does share genetic predisposition with type 1 diabetes. We aimed to improve the genetic characterisation of LADA and hypothesised that type 2 diabetes-associated gene variants also predispose to LADA, and that the associations would be strongest in LADA patients with low levels of GAD autoantibodies (GADA).
Methods We assessed 41 type 2 diabetes-associated gene variants in Finnish (phase I) and Swedish (phase II) patients with LADA (n=911) or type 1 diabetes (n=406), all diagnosed after the age of 35 years, as well as in non-diabetic control individuals 40 years or older (n=4,002).
Results Variants in the *ZMIZ1* (rs12571751, $p=4.1\times10^{-5}$) and *TCF7L2* (rs7903146, $p=5.8\times10^{-4}$) loci were strongly associated with LADA. Variants in the *KCNQ1* (rs2237895, p=0.0012), *HHEX* (rs1111875, p=0.0024 in Finns) and *MTNR1B* (rs10830963, p=0.0039) loci showed the strongest association in patients with low GADA, supporting the hypothesis that the disease in these patients is more like type 2 diabetes. In contrast, variants in the *KLHDC5* (rs10842994, $p=9.5\times10^{-4}$ in Finns), *TP53INP1* (rs896854, p=0.005), *CDKAL1* (rs7756992, $p=7.0\times10^{-4}$; rs7754840, $p=8.8\times10^{-4}$) and *PROX1* (rs340874, p=0.003) loci showed the strongest

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association in patients with high GADA. For type 1 diabetes, a strong association was seen for *MTNR1B* (rs10830963, $p=3.2\times10^{-6}$) and *HNF1A* (rs2650000, $p=0.0012$).
Conclusions/interpretation LADA and adult-onset type 1 diabetes share genetic risk variants with type 2 diabetes, supporting the idea of a hybrid form of diabetes and distinguishing them from patients with classical young-onset type 1 diabetes.

Keywords Adult-onset type 1 diabetes · GADA · GAD autoantibodies · Genetics · HNF1A · LADA · Latent autoimmune diabetes in adults · MTNR1B · TCF7L2 · ZMIZ1

Abbreviations

ANDIS	All New Diabetics in Scania
DIREVA	Diabetes Registry in Vasa
GADA	GAD autoantibodies
LADA	Latent autoimmune diabetes in adults
MDC	Malmö Diet and Cancer Study
SNP	Single-nucleotide polymorphism

Introduction

Diabetes is a complex disease with two main subtypes: type 1 diabetes, characterised by autoimmune destruction of the insulin-producing beta cells, and type 2 diabetes, characterised by a combination of insulin resistance and reduced insulin secretion. The subdivision into type 1 and type 2 diabetes is especially intricate in adults, in whom the disease is more like a spectrum of phenotypes. This spectrum also includes the subtype termed latent autoimmune diabetes in adults (LADA).

LADA accounts for about 10% of European patients initially diagnosed with type 2 diabetes in whom type 1 diabetes-associated autoantibodies are present [1–3]. The most prevalent autoantibodies among these adults are GAD autoantibodies (GADA), present in 90%. Other autoantibodies, such as those to protein tyrosine phosphatase IA-2 or zinc transporter 8 are reported in only 1–2% of GADA-negative patients [3, 4]. The level of autoantibodies, particularly GADA, has a clear influence on the phenotype of LADA. A high GADA level is associated with a phenotype more like type 1 diabetes, including reduced beta cell function, increased need for insulin treatment, lower BMI and lower prevalence of dyslipidaemia (reviewed by [5, 6]).

The genetic component of diabetes has been intensively studied in patients with type 2 diabetes and in patients with type 1 diabetes diagnosed in childhood, which has resulted in identification of several susceptibility gene variants [7]. Genetic studies in LADA comprising >100 patients are rare but indicate that LADA shares a genetic predisposition with childhood-onset type 1 diabetes. Association with *HLA-DQB1* and *PTPN22* risk alleles has consistently been shown, while association with *INS* is disputed. Furthermore, the frequency of these type 1 diabetes risk alleles has consistently been shown to correlate with GADA level among LADA patients (reviewed by [6]). For type 1 diabetes the genetic predisposition seems to be similar in children and adults diagnosed predominantly at >15 years of age; only for *HLA-DQB1* and *IL2RA* have effects related to age at onset been reported [8–10].

Whether type 2 diabetes-associated variants predispose to adult-onset type 1 diabetes or LADA is largely unknown. To date, consistent association has only been reported for *TCF7L2* and LADA [11, 12]. We hypothesised that LADA, and possibly adult-onset type 1 diabetes, is a genetic hybrid of type 1 and type 2 diabetes with increased frequency of type 2 diabetes risk genotypes. Further, we expected the type 2 diabetes-associated gene variants to show strongest association in LADA patients with low GADA levels, whose disease is phenotypically more like type 2 diabetes. In this study, we assessed the association of type 2 diabetes susceptibility gene variants in LADA and adult-onset type 1 diabetes diagnosed at age >35 years, to explore the degree of genetic overlap in adult-onset forms of diabetes.

Methods

Study participants

We applied a two-stage study design with Finnish (phase I) and Swedish (phase II) data sets. The Finnish data set comprised individuals from the Diabetes Registry in Vasa (DIREVA), the Botnia Study [13, 14] and the FinnDiane Study [15]. The Swedish data set comprised individuals from the Scania Diabetes Registry [16], the diabetes registry (DiabNorth) in the population-based Västerbotten Study [17] and the Malmö Diet and Cancer Study (MDC) [18] (Fig. 1). The studies were approved by the local ethics committees, and informed consent was obtained from all study participants. The 25 patients included in both the Botnia Study and DIREVA were analysed as part of the Botnia Study.

We included patients with LADA or type 1 diabetes diagnosed at >35 years of age and non-diabetic control individuals. The control individuals were aged ≥40 years at the time of investigation (Finnish/Swedish, mean±SD, 56.6±10.3/56.8±6.2 years), had a BMI of 26.5±4.0/25.4±3.7 kg/m^2 and included 46.1/48.8% men.

Diagnosis of LADA was based on presence of GADA and sufficient beta cell function at the time of diagnosis, indicated by no insulin treatment and/or C-peptide level >0.2 nmol/l. The majority of the LADA patients had initially been diagnosed with type 2 diabetes. Diagnosis of type 1 diabetes was based on initial diagnosis of type 1 diabetes made by the

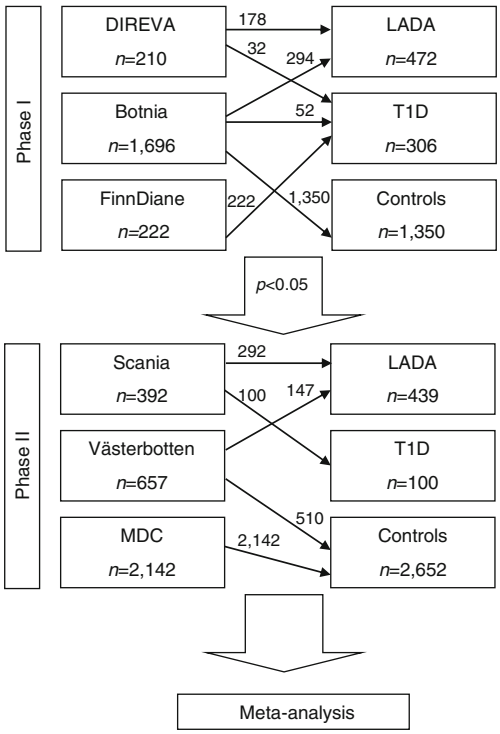

Fig. 1 The two-staged study design, with indication of the contribution of patients and control individuals from each of the cohorts. T1D, adult-onset type 1 diabetes

treating physician, a fasting serum C-peptide concentration <0.2 nmol/l at the time of investigation and initiation of permanent insulin treatment within 6 months from diagnosis.

Measurements and assays

C-peptide concentrations were determined by a radioimmunoassay (Human C-peptide RIA; Linco, St Charles, MO, USA; or Peninsula Laboratories, Belmont, CA, USA), or with Cobas e411 (Roche Diagnostics, Mannheim, Germany). GADA were measured with an ELISA assay (RSR, Cardiff, UK) in the DIREVA patients (positive cut-off: 10 IU/ml) and with radiobinding assays using ^{35}S-labelled protein [2] in the rest of the patients (positive cut-off: 5 RU or 32 IU/ml). The radiobinding assay showed 62–88% sensitivity and 91–99% specificity in workshops (Combinatorial Autoantibody or Diabetes/Islet Autoantibody Standardization Programs) from 1998 to 2013, and the ELISA assay showed 72% sensitivity and 99% specificity in the 2013 Islet Autoantibody Standardization Program workshop.

To assess the relationship between gene variants and GADA level, the LADA patients were stratified into $LADA_{high}$ and $LADA_{low}$ according to the median GADA level among Finnish LADA patients. The same cut-off values (Botnia, 20.4 RU or 73.0 IU/ml; DIREVA, 59.0 IU/ml) were applied to the Swedish patients (65.9% were $LADA_{high}$) as they were typed using the same methods.

Genotyping

All cases (n=1,317) and a subset of the controls (n=987) were genotyped with a single-nucleotide polymorphism (SNP) panel, custom-designed for the All New Diabetics in Scania (ANDIS) study in Sweden (http://snd.gu.se/en/catalogue/study/EXT0057; accessed 7 April 2014), including SNPs reported to be associated with type 2 diabetes or related phenotypes. Genotyping was performed by matrix-assisted laser desorption–ionisation time-of-flight mass spectrometry on a MassARRAY platform (Sequenom, San Diego, CA, USA). We excluded SNPs with a minor allele frequency (MAF) <5%, with genotyping success rate <90% or disagreeing with Hardy–Weinberg equilibrium (p<0.00029, corresponding to 0.05/No. of SNPs). Individuals with missing genotypes for >5% of the SNPs were also excluded. Altogether, 143 SNPs (genotyping success rate >99%), 1,272 cases and 905 control individuals passed the quality control.

The rest of the controls (Botnia, n=873; MDC, n=2,142) had been genotyped with the Metabochip [19], where genotypes were called with GenomeStudio (Illumina, San Diego, CA, USA). SNPs included both on the Metabochip and in the ANDIS panel numbered 105; of these SNPs, 46 were in or near loci previously associated with type 2 diabetes in Europeans [20] and 40 passed the quality control (electronic supplementary material [ESM] Table 1). In addition, we included a SNP in *GLIS3*, as this is one of the few loci that have shown an association with both type 1 and type 2 diabetes (type 2 diabetes association only in Asians). Hence, 41 SNPs were analysed.

Statistical analysis

Statistical Package for Social Science software (version 19.0; SPSS, Chicago, IL, USA) was applied to compare clinical characteristics between patient groups by the non-parametric Mann–Whitney U test or the χ^2 test. Genetic association analyses were performed with PLINK v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/ [21]). Differences in allele frequencies between cases and controls were assessed with logistical regression adjusted for the effect of age, sex and BMI. ORs are presented for the reported type 2 diabetes-associated alleles. For the association tests in the Finnish phase I data set a p value of <0.0012 was considered statistically significant, corresponding to a Bonferroni correction for the number of SNPs tested (n=41). RevMan v5.2.6 (http://tech.cochrane.org/revman/download) was applied to conduct fixed-effect meta-analyses estimating the combined effect sizes for the Finnish and Swedish data sets. Cochran's Q test was used to assess the heterogeneity between data sets in the meta-analyses.

The possible effect of *HLA-DQB1* risk genotypes (*0302 or *0302/02) on association results was tested in part of the

Finnish data set with available *HLA-DQB1* genotype data (LADA, n=264; type 1 diabetes, n=245; control individuals, n=503). *HLA-DQB1* genotype was included as covariate in logistical regression analyses; the main results were similar with this adjustment (data not shown).

Results

Clinical characteristics

Compared with the Swedish LADA patients, the Finnish LADA patients were older at the time of investigation and had longer disease duration as well as lower levels of fasting serum C-peptide and fasting plasma glucose (Table 1). Moreover, the Finnish patients included a smaller proportion of patients with high GADA levels. The two groups of LADA patients were similar with respect to age at diagnosis, glycaemic control and BMI. Among patients with type 1 diabetes, the Finnish patients were younger at diagnosis, had longer disease duration and higher BMI than the Swedish patients (Table 1). C-peptide, lipid levels and age at investigation were similar for Swedish and Finnish patients with type 1 diabetes.

Phase I: Finnish patients

LADA Nominal association was observed for 11 variants (Table 2 and ESM Table 1). Applying the conservative Bonferroni-corrected cut-off, association remained for two loci, namely *ZMIZ1*, which is a novel finding in LADA (rs12571751, OR [95% CI]: 1.35 [1.15, 1.60], $p=3.2\times10^{-4}$), and the previously reported *TCF7L2* (rs7903146, 1.44 [1.18, 1.77], $p=3.8\times10^{-4}$).

When the LADA patients were stratified according to GADA level, nominal association was seen for ten variants (Fig. 2) with either $LADA_{low}$ (GADA level below the median) or $LADA_{high}$ (GADA above the median). The strongest evidence for association in the $LADA_{low}$ patients was observed for *HHEX* (rs1111875: 1.43 [1.13, 1.80], p=0.0024) and *CDKN2A/B* (rs10811661: 1.65 [1.16, 2.35], p=0.0057). For *KLHDC5* (rs10842994: 1.65 [1.23, 2.22], $p=9.5\times10^{-4}$), *TP53INP1* (rs896854: 1.36 [1.12, 1.66], p=0.0023), *CDKAL1* (rs7756992: 1.39 [1.13, 1.72], p=0.0020; rs7754840: 1.32 [1.08, 1.62], p=0.0075) and *ANKRD55* (rs459193: 1.28 [1.02, 1.61], p=0.030) association was restricted to $LADA_{high}$ patients. Although statistically significant association for *MTNR1B* was limited to $LADA_{high}$ patients (rs10830963: 1.27 [1.03, 1.56], p=0.028), the risk-allele frequency was similar in $LADA_{high}$ and $LADA_{low}$ patients. The *ZMIZ1* ($LADA_{low}$, 1.47 [1.17, 1.85], p=0.0011; $LADA_{high}$, 1.30 [1.06, 1.59], p=0.011) and *TCF7L2* ($LADA_{low}$ 1.46 [1.11, 1.93], p=0.0073; $LADA_{high}$, 1.42 [1.11, 1.82], p=0.0052) associations were independent of GADA level (Fig. 2 and ESM Table 1).

Adult-onset type 1 diabetes Nominal association was observed for nine variants (Table 3). Applying the Bonferroni-corrected cut-off, association remained for three loci:

Table 1 Clinical characteristics

Characteristic	LADA			Type 1 diabetes		
	Finnish	Swedish	p value	Finnish	Swedish	p value
Men, n (%)	472 (48.1)	439 (57.8)	0.005	306 (49.7)	100 (45.0)	
$LADA_{high}$, %	244 (51.7)	335 (65.9)	<0.0001	NA	NA	
Age at diagnosis, years	55.9 (11.6)	56.8 (10.9)		42.5 (7.2)	48.4 (9.9)	<0.0001
Age at investigation, years	63.5 (11.2)	59.4 (11.3)	<0.0001	54.3 (9.4)	55.6 (11.4)	
Duration, years	7.8 (7.6)	3.7 (5.6)	<0.0001	11.9 (8.9)	7.2 (5.9)	<0.0001
fP-glucose, mmol/l	9.2 (3.8)	10.3 (4.1)	<0.0001	NA	12.8 (4.8)	
HbA_{1c}, %	7.3 (1.6)	7.6 (2.4)		8.4 (1.4)	8.5 (2.5)	
HbA_{1c}, mmol/mol	56.7 (17.6)	59.6 (26.7)		68.6 (15.0)	69.9 (27.2)	
BMI, kg/m^2	28.5 (5.2)	28.1 (5.3)		25.3 (3.7)	23.3 (3.6)	<0.0001
fS C-peptide, nmol/l	0.63 (0.56)	0.84 (0.60)	<0.0001	0.03 (0.05)	0.04 (0.07)	
Total cholesterol, mmol/l	5.24 (1.13)	5.55 (1.17)	<0.0001	5.05 (0.93)	5.20 (1.02)	
Triacylglycerol, mmol/l	1.61 (1.13)	1.79 (1.44)	0.032	1.13 (0.68)	1.10 (0.48)	
HDL-cholesterol, mmol/l	1.34 (0.38)	1.22 (0.38)	<0.0001	1.49 (0.47)	1.49 (0.40)	

Data are number (%) or mean (SD)

Only significant p values are shown

fP, fasting plasma; fS, fasting serum; $LADA_{high}$, LADA patients with GADA above the median, defined based on the patients from the Botnia Study; NA, not applicable

Table 2 Association results: LADA

SNP – Candidate gene	RAF: controls/cases	OR (95% CI)	*p* value
rs12571751 – *ZMIZ1*	A: 0.49/0.56	1.35 (1.15, 1.60)	3.2×10^{-4}[a]
rs7903146 – *TCF7L2*	T: 0.17/0.22	1.44 (1.18, 1.77)	3.8×10^{-4}[a]
rs10842994 – *KLHDC5*	C: 0.81/0.85	1.44 (1.15, 1.80)	0.0016
rs896854 – *TP53INP1*	T: 0.46/0.52	1.28 (1.09, 1.50)	0.0026
rs108116661 – *CDKN2A/B*	T: 0.82/0.87	1.42 (1.11, 1.80)	0.0046
rs10830963 – *MTNR1B*	G: 0.29/0.34	1.27 (1.07, 1.51)	0.0055
rs1111875 – *HHEX*	C: 0.52/0.56	1.23 (1.05, 1.46)	0.013
rs2237895 – *KCNQ1*	C: 0.48/0.52	1.20 (1.02, 1.41)	0.024
rs7578597 – *THADA*	T: 0.94/0.96	1.49 (1.03, 2.16)	0.034
rs7756992 – *CDKAL1*	G: 0.27/0.31	1.20 (1.01, 1.43)	0.044
rs7754840 – *CDKAL1*	C: 0.30/0.34	1.19 (1.01, 1.40)	0.047

Loci obtaining a p value <0.05 in the Finnish data set, with logistical regression adjusted for age, sex and BMI

[a] Significant below the Bonferroni cut-off ($p<0.0012$)

RAF, risk allele (reported for type 2 diabetes) frequency

MTNR1B (rs10830963: 1.59 [1.32, 1.93], $p=1.7\times10^{-6}$), *PROX1* (rs340874; 0.68 [0.56, 0.82], $p=6.1\times10^{-5}$) and *HNF1A* (rs2650000; 1.43 [1.19, 1.72], $p=1.7\times10^{-4}$).

Phase II: Swedish patients and meta-analyses

SNPs obtaining a p value <0.05 (adjusted for age, sex and BMI) in phase I were tested in the Swedish phase II data set.

LADA The *ZMIZ1* association was replicated in the Swedish LADA patients (1.27 [1.08, 1.50], $p=0.0049$), but driven by $LADA_{high}$ (1.40 [1.15, 1.70], $p=7.2\times10^{-4}$; $LADA_{low}$: 1.02 [0.76, 1.37], p=NS). The *TCF7L2* risk allele was significantly increased in the Swedish $LADA_{low}$ patients (1.55 [1.13, 2.13], $p=0.0066$), but failed to reach statistical significance in the whole group. None of the other associations observed in Finnish LADA patients were replicated in the Swedish patients (ESM Table 1).

Combining the Finnish and the Swedish data sets in a meta-analysis, both *ZMIZ1* ($p=4.1\times10^{-5}$) and *TCF7L2* ($p=5.8\times10^{-4}$) remained significant below the Bonferroni-corrected threshold in the whole LADA group, without heterogeneity between the data sets (Fig. 3). Variants in *KCNQ1* (rs2237895, 1.17 [1.06, 1.31]), *CDKAL1* (rs7756992, 1.19 [1.06, 1.34]; rs7754840, 1.19 [1.06, 1.32]), *KLHDC5* (rs10842994, 1.23 [1.07, 1.42]) and *CILP2* (rs10401969, 1.27 [1.07, 1.50]) obtained p values <0.005 and heterogeneity was only observed with respect to *CILP2* ($p=0.02$).

In meta-analyses where LADA patients were stratified based on GADA level, association with $LADA_{low}$ was accentuated for *TCF7L2* ($p=2.4\times10^{-4}$) and *KCNQ1* ($p=0.0012$) (Fig. 3) and suggestive association was observed for *MTNR1B* (1.27 [1.08, 1.50], $p=0.0039$). With respect to $LADA_{high}$, *ZMIZ1* ($p=1.1\times10^{-4}$) and *CDKAL1* (rs7756992, $p=7.0\times10^{-4}$; rs7754840, $p=8.8\times10^{-4}$) showed significant association below the Bonferroni-corrected threshold (Fig. 3) and suggestive association was seen for *PROX1* (rs340874, 1.22 [1.07, 1.39], $p=0.003$) and *TP53INP1* (rs896854, 1.21 [1.06, 1.37], $p=0.005$). Only the *TP53INP1* SNP showed signs of heterogeneity between the data sets ($p=0.09$).

Adult-onset type 1 diabetes None of the loci associated with adult-onset type 1 diabetes in the Finnish patients were replicated in the Swedish data set (ESM Table 1) but in the meta-analysis *MTNR1B* ($p=3.2\times10^{-6}$) and *HNF1A* ($p=0.0012$) remained significant, although with heterogeneity between data sets (Fig. 4). Suggestive association was observed for *CDKAL1* (rs7756992, 1.27 [1.08, 1.50], $p=0.0040$) and *CENDT2* (rs1552224, 0.77 [0.65, 0.92], $p=0.0042$).

Discussion

To improve the genetic characterisation of LADA and to address the question of genetic overlap in the adult-onset forms of diabetes, we studied type 2 diabetes-associated gene variants in patients with LADA or adult-onset type 1 diabetes diagnosed at >35 years of age. We show that both subgroups of adult-onset autoimmune diabetes share genetic risk variants with type 2 diabetes. The reported associations show effect sizes and direction of effect consistent with those reported for type 2 diabetes, which indicate that the associations are true. With respect to LADA, the level of GADA had a distinct effect on the genetic associations. Previously, we have assessed type 1 diabetes-associated gene variants in the LADA patients included in the current study and reported association with *HLA-DQB1* and *PTPN22*, although with lower risk-allele frequencies in the LADA patients than in patients with young and adult-onset type 1 diabetes [9, 11]. LADA, thus, seems to be a genetic hybrid of type 1 and type 2 diabetes distinct from classical young-onset type 1 diabetes.

A great strength of our study is the inclusion of a unique collection of patients with adult-onset type 1 diabetes

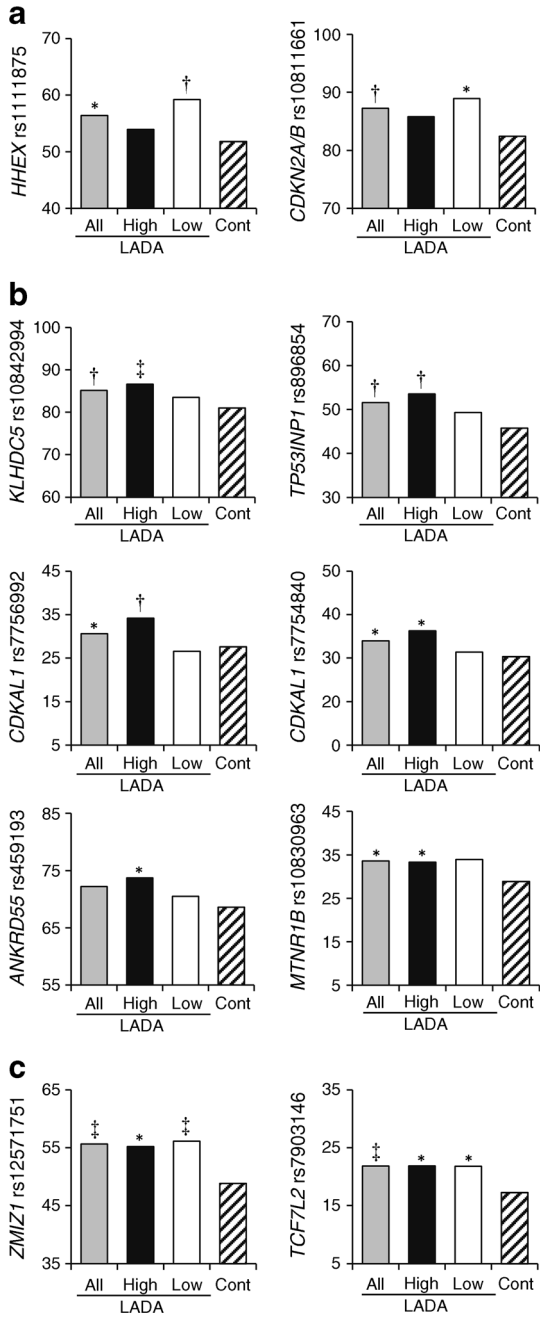

Fig. 2 Risk-allele frequencies (%) for variants that show association with $LADA_{low}$ (**a**), $LADA_{high}$ (**b**) or both (**c**). *$p<0.05$, †$p<0.005$ and ‡$p<0.0012$, risk-allele frequency for patients with LADA (grey bars), $LADA_{low}$ (white bars) or $LADA_{high}$ (black bars) vs control individuals (hatched bars)

diagnosed after the age of 35 years, and the largest published collection of genetically characterised LADA patients, numbering over 900. Further, we analysed LADA and adult-onset type 1 diabetes as separate phenotypes since type 1 diabetes risk variants show different frequencies in the two diabetes subtypes. Even though this stratification reduces the sample size in the groups analysed, the more precise phenotyping may pinpoint associations that would be missed in the combined group of unspecified adult-onset autoimmune diabetes.

The sample size is a limitation of our study, especially when considering the reported effect sizes of variants associated with type 2 diabetes ranging from OR of 1.05 to 1.38 [20]. Thus, we can detect the variants showing strongest association with LADA or adult-onset type 1 diabetes but lack statistical power to exclude association with other variants. This problem was accentuated in the analyses of GADA-level subgroups. Another limitation is the fact that our study is based on current knowledge of the genetic predisposition to type 2 diabetes. For the great majority of associated loci the causal variant is still unknown and therefore most of the examined variants represent statistical association and not necessarily biological association. The lack of knowledge about the causal variant within each locus is reflected in the difficulties of pinpointing the mechanistic links between genetic variants and disease.

Variants in *TCF7L2* and *ZMIZ1* showed strong evidence of association with LADA. In Europeans, the intronic *TCF7L2* rs7903146 variant is the strongest identified genetic risk factor for type 2 diabetes [22]. Association has also been shown between *TCF7L2* and LADA [12] in work encompassing a subset of the patients included in the current study [11]. *TCF7L2* encodes a transcription factor, which is involved in maintaining the secretory function of beta cells and has been suggested to be a key determinant of beta cell mass (reviewed in [23]). The rs7903146 variant is associated with impaired insulin secretion, although the exact mechanistic link is unclear [24]. The variant most likely imposes a non-autoimmune reduction in insulin secretion, which in LADA patients exacerbates the presumably already reduced beta cell function caused by autoimmune destruction.

In agreement with the Italian Non-Insulin Requiring Autoimmune Diabetes (NIRAD) study [25], the meta-analysis showed stronger *TCF7L2* association in patients with low GADA level. This GADA-level effect was, however, driven by the Swedish data set, while the association in the Finnish data set was independent of GADA level. This discrepancy may reflect differences in the differentiation between LADA and type 1 diabetes in Finnish and Swedish patients. The frequency of the *TCF7L2* risk T allele among the adult-onset type 1 diabetes patients and control individuals was similar, in line with previous reports in a mixture of patients with adult-onset autoimmune diabetes [8] and in childhood-onset type 1 diabetes [26]. Thus, *TCF7L2* represents a distinguishing factor between LADA and type 1 diabetes, irrespective of age at diagnosis, and the T allele seems to protect against type 1 diabetes possibly by sufficient impairment of insulin synthesis, resulting in reduced antigenic burden suggested to be operative in type 1 diabetes.

Importantly, the association of *ZMIZ1* rs12571751 with LADA is novel. An association was seen both in Finnish

Table 3 Association results: adult-onset type 1 diabetes

SNP – Candidate gene	RAF: controls/cases	OR (95% CI)	*p* value
rs10830963 – *MTNR1B*	G: 0.29/0.39	1.59 (1.32, 1.93)	1.7×10^{-6}[a]
rs340874 – *PROX1*	C: 0.49/0.40	0.68 (0.56, 0.82)	6.1×10^{-5}[a]
rs2650000 – *HNF1A*	A: 0.35/0.43	1.43 (1.19, 1.72)	1.7×10^{-4}[a]
rs7754840 – *CDKAL1*	C: 0.30/0.36	1.26 (1.05, 1.52)	0.015
rs7756992 – *CDKAL1*	G: 0.27/0.33	1.26 (1.04, 1.53)	0.019
rs10811661 – *CDKN2A/B*	T: 0.82/0.86	1.38 (1.05, 1.80)	0.020
rs864745 – *JAZF1*	T: 0.48/0.42	0.81 (0.67, 0.97)	0.025
rs10401969 – *CILP2*	C: 0.08/0.05	0.64 (0.43, 0.96)	0.030
rs7957197 – *HNF1A*	T: 0.74/0.79	1.27 (1.02, 1.57)	0.035

Loci obtaining a p value <0.05 in the Finnish data set, with logistic regression adjusted for age, sex and BMI

[a] Significant below the Bonferroni cut-off ($p<0.0012$)

RAF, risk allele (reported for type 2 diabetes) frequency

and Swedish LADA patients, but in the Swedish patients it was strongest in those with a high GADA level. Other intronic *ZMIZ1* variants have been associated with a variety of autoimmune diseases, including childhood-onset type 1 diabetes [27] and coeliac disease [28]. Even though we observed no association between the variant and adult-onset type 1 diabetes, the *ZMIZ1* loci seems to play a key role in the development of autoimmune diseases.

ZMIZ1 is an interesting candidate gene for LADA as the encoded protein is a transcriptional co-activator interacting with Smad3 and p53 [29, 30]. Both these transcription factors are involved in pathways possibly linked to diabetes susceptibility. Smad3 modulates the TGF-β signalling pathway, which is linked to immune tolerance, inhibition of autoimmune diabetes and induction of anti-inflammatory responses [31, 32]. On the other hand, p53 is linked to apoptosis, which may be a determining factor for beta cell mass but also in the autoimmune process where apoptosis may induce the formation of autoantibodies by increasing the exposure to autoantigens [33]. The p53 link is also interesting in light of the suggestive association between *TP53INP1* and $LADA_{high}$, as *TP53INP1* is a direct target gene for p53 and has been

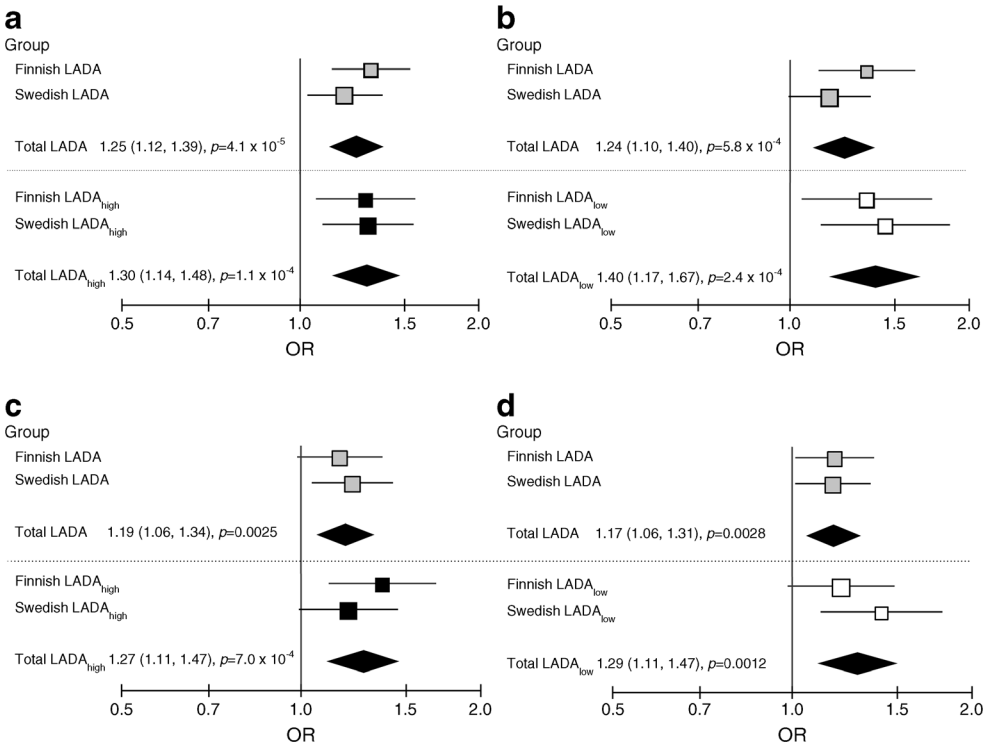

Fig. 3 Forest plots for loci obtaining $p\leq0.0012$ in the meta-analyses of LADA, $LADA_{high}$ or $LADA_{low}$. The OR (95% CI) is indicated for the Finnish and Swedish data sets (squares), as well as for the meta-analysis (diamonds). Associations are shown for: (**a**) *ZMIZ1* rs12571751 with LADA and $LADA_{high}$ with no heterogeneity between data sets ($p=0.350$ and $p=0.946$, respectively); (**b**) *TCF7L2* rs7903146 with LADA and $LADA_{low}$ with no heterogeneity between data sets ($p=0.251$ and $p=0.693$, respectively); (**c**) *CDKAL1* rs7756992 with LADA and $LADA_{high}$ with no heterogeneity between data sets ($p=0.677$ and $p=0.364$, respectively); (**d**) *KCNQ1* rs2237895 with LADA and $LADA_{low}$ with no heterogeneity between data sets ($p=0.950$ and $p=0.328$, respectively)

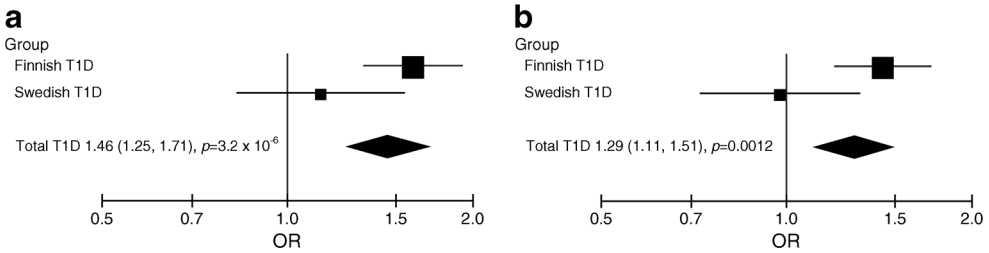

Fig. 4 Forest plots for the variants obtaining $p \leq 0.0012$ in the meta-analyses of adult-onset type 1 diabetes. The OR (95% CI) is indicated for the Finnish and Swedish data sets (squares), as well as for the meta-analysis (diamonds). (**a**) *MTNR1B* rs10830963; (**b**) *HNF1A* rs2650000. Both loci showed heterogeneity between data sets ($p=0.064$ and $p=0.032$, respectively). T1D, adult-onset type 1 diabetes

suggested to regulate p53-dependent apoptosis [34]. Moreover, reduced activity of transcription factor 7-like 2 has been linked to increased expression of *TP53INP1* and beta cell apoptosis [23]. Thus, p53 and apoptosis could provide a molecular connection between three LADA-associated genes.

We hypothesised that among LADA patients, those with low GADA levels would have a genotype more similar to type 2 diabetes (i.e. they show higher frequencies of the type 2 diabetes risk variants than those with high GADA levels). However, only a few type 2 diabetes variants supported this hypothesis and the results were inconsistent in the Finnish and Swedish data sets. This heterogeneity in GADA-level association may, of course, be explained by the small sample size. An alternative explanation is that the associations, which seem to be GADA-level dependent, are mediated by factors that correlate with the GADA level, such as BMI, and that the type 2 diabetes variants are not directly linked to the autoimmune process in LADA. Considering LADA, it is, however, very interesting that some of the type 2 diabetes variants are associated only with $LADA_{high}$, as this supports LADA being a true hybrid of type 1 diabetes and type 2 diabetes and not just a mixed group of misclassified patients with type 1 diabetes or type 2 diabetes.

With respect to type 1 diabetes, we hypothesised that non-autoimmune mechanisms may contribute to disease development, especially in adult-onset type 1 diabetes where the autoimmune disease process seems less pronounced. A genetic overlap between type 1 and type 2 diabetes is supported by the reported associations for variants in *ZMIZ1* and *GLIS3* with both subtypes of diabetes [20, 27, 35] and possibly also by associations for variants in *HHEX* and *PPARG*, for which inconsistent results have been published [36, 37]. Our data showed no support for these previous findings, as none of the four loci were associated with adult-onset type 1 diabetes and only *ZMIZ1* was associated with LADA. Of note, with the current sample size we were unable to rule out association for these loci. However, in support of the hypothesis of a genetic overlap between type 1 and type 2 diabetes, we identified novel associations between adult-onset type 1 diabetes and variants in two type 2 diabetes-associated loci, *MTNR1B* and *HNF1A*. Both associations were driven by the Finnish patients, but remained significant in the meta-analysis. The discrepancy between the data sets may be due to the small sample size or the significant difference between Finnish and Swedish patients with respect to age at diagnosis.

The intronic *MTNR1B* rs10830963 variant has been associated with type 2 diabetes, elevated fasting glucose level and impaired insulin response to oral and intravenous glucose. *MTNR1B* encodes the melatonin receptor 1B (MT2), which is expressed in human pancreatic islets and beta cells and plays a key role in the regulation of circadian rhythms. Disruption of the circadian rhythms has been linked to metabolic disorders such as type 2 diabetes (reviewed by [38]). Furthermore, melatonin has been shown to be an immune stimulator (reviewed by [39]) and seems to be directly involved in the modulation of insulin secretion. However the effect of melatonin on insulin secretion is debatable, as melatonin has been shown to inhibit insulin secretion in vivo and in vitro in rodent models, whereas in one study it enhanced insulin secretion in human islets [40, 41].

The functional link between *MTNR1B* and type 2 diabetes is unclear. The mechanism has been suggested to be gain-of-function, as the rs10830963 risk allele is associated with increased mRNA expression of the MT2 receptor in islets from non-diabetic individuals [40]. In contrast, studies of rare coding *MTNR1B* variants point to loss of receptor function as the causal mechanism linked to type 2 diabetes [42].

A role for *MTNR1B* in the pathogenesis of autoimmune diabetes is further supported by a possible association between the *MTNR1B* variant and LADA, especially in the Finnish patients, in whom the risk-allele frequency was similar in $LADA_{high}$ and $LADA_{low}$ patients. However, as no association has been observed between the variant and childhood-onset type 1 diabetes [43], these data may indicate that *MTNR1B* is a candidate gene only for the adult-onset forms of diabetes. This hypothesis is supported by the observed age-related reduction in endogenous melatonin levels. Reduced melatonin levels have been linked to immunosenescence, a process that is thought to contribute to autoimmune diseases in elderly people (reviewed by [39]).

Rare mutations in *HNF1A* cause MODY [44], and the common variants have been linked to LDL-cholesterol (rs2650000) and type 2 diabetes (rs7957197) [45, 46]. *HNF1A* encodes a transcription factor essential for pancreatic beta cell

development and function [47] and is thus a good candidate gene for diabetes in general. This *HNF1A*–type 1 diabetes association, together with the majority of the LADA associations, point to compromised beta cell mass as a key mechanism contributing to insulin deficiency also in adult-onset autoimmune diabetes.

Our study adds to the genetic knowledge about the much-debated diabetes subtype LADA. However, so far all genetic studies of LADA have been candidate-gene based and have focused on type 1 or type 2 diabetes-associated variants, thus it is still unknown whether LADA harbours its own unique risk variants. A genome-wide association study in a larger sample of LADA patients will be needed to answer this question. A larger sample size would also permit extended stratification of the LADA patients according to HLA genotype, which could reveal interesting genetic interactions, and according to the presence of additional autoantibodies, which could reveal genetic differences as carriers of multiple autoantibodies show a more rapid disease progression.

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Contribution statement The study was planned by MKA, OR, P-HG, LG and TT. MS, TF, AK, CF, KL and PMN contributed to phenotype and genotype data acquisition. MKA and TT analysed and interpreted the data and drafted the initial version of the manuscript. All co-authors reviewed the manuscript and approved the submitted version. MKA is the guarantor of this work.

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