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SHORT COMMUNICATION

Attenuated humoral responses in *HLA-A*24*-positive individuals at risk of type 1 diabetes

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Abstract
Aims/hypothesis The rate of progression from islet autoimmunity to clinical type 1 diabetes depends on the rate of beta cell destruction. The *HLA-A*24* gene is associated with early diabetes onset, but previous studies have shown attenuated humoral responses to islet antigens in individuals with both recent and long-standing type 1 diabetes carrying *HLA-A*24*. We aimed to establish whether *HLA-A*24* is also associated with attenuated humoral responses in individuals at high risk of type 1 diabetes.
Methods We established *HLA-A*24, DQ* and rs9258750 (an *HLA-A*24* tagged single-nucleotide polymorphism) genotype, as well as GAD, zinc transporter 8 (ZnT8), insulin, islet antigen-2 (IA-2), and IA-2β autoantibody status in 373 islet cell antibody-positive first-degree relatives participating in the European Nicotinamide Diabetes Intervention Trial.
Results Univariate regression analyses showed that humoral responses to GAD, ZnT8 and insulin were less common in relatives carrying *HLA-A*24*. The prevalence of GAD and ZnT8 autoantibodies remained negatively associated with *HLA-A*24* and rs9258750 after adjusting for age, sex, proband relationship and HLA class II genotype.
*Conclusions/interpretation HLA-A*24* is associated with attenuated humoral responses in individuals at high risk of type 1 diabetes, and this may reflect a distinct phenotype of rapid beta cell loss.

Keywords Autoantibodies · *HLA-A*24* · Type 1 diabetes

Abbreviations

ENDIT	European Nicotinamide Diabetes Intervention Trial
GADA	GAD autoantibodies
IA-2A	Islet antigen-2 autoantibodies
IA-2βA	Islet antigen-2β autoantibodies
IAA	Insulin autoantibodies
ICA	Islet cell autoantibodies
IQR	Interquartile range
PCR-SSP	PCR sequence-specific primers
SNP	Single-nucleotide polymorphism
ZnT8A	Zinc transporter 8 autoantibodies
ZnT8RA	Zinc transporter 8 autoantibodies, arginine residue variant
ZnT8WA	Zinc transporter 8 autoantibodies, tryptophan residue variant

Introduction

The strongest genetic determinants of type 1 diabetes are in the HLA region. The onset of islet autoimmunity is characterised by the appearance of autoantibodies to insulin (IAA), GAD (GADA), zinc transporter 8 (ZnT8A) and islet antigen-2 (IA-2A). Development of multiple islet autoantibodies usually precedes clinical onset of type 1 diabetes, with IA-2A and ZnT8A commonly appearing later in the prodrome than IAA and GADA [1]. The rate of beta cell destruction is heterogeneous among multiple islet autoantibody-positive

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individuals, but the reasons for this are poorly defined. The main type 1 diabetes susceptibility alleles are in the HLA class II region, but the class I allele *HLA-A*24* has been linked with accelerated diabetes progression, acute diabetes onset and early and complete beta cell destruction [2].

Islet autoantibody characteristics in *HLA-A*24* individuals with diabetes have proved counterintuitive; one might expect an overwhelming islet autoantibody response to be associated with rapid progression, but the opposite appears true. In patients with newly diagnosed diabetes, autoantibodies to ZnT8, IA-2 and IA-2β, which are usually associated with disease progression, are less common in *HLA-A*24* carriers [3]; negative associations between *HLA-A*24* and IA-2A have also been observed in patients with long-standing diabetes [4]. In addition, the minor G allele of an *HLA-A*24*-tagged single-nucleotide polymorphism (SNP), rs9258750, was found to be negatively associated with ZnT8A prevalence [5].

Collectively these data suggest a distinct humoral response in *HLA-A*24*-positive patients. We hypothesised that islet autoantibody responses would also be attenuated in 'at-risk' individuals carrying *HLA-A*24*. The aim of this study therefore was to investigate the association between *HLA-A*24*, rs9258750 and islet autoantibody responses in a well-characterised 'at-risk' population.

Methods

Study population The European Nicotinamide Diabetes Intervention Trial (ENDIT) has been described previously [6]. Briefly, 552 first-degree relatives who were positive for islet cell autoantibodies (ICA) of ≥20 Juvenile Diabetes Foundation (JDF) units, with an ICA result of ≥5 JDF units in a second sample and a non-diabetic OGTT at baseline were randomised to treatment with nicotinamide or placebo for 5 years or until diagnosis of diabetes. DNA samples were available for 373 individuals (68%) (median age 15.61 years; interquartile range [IQR] 10.6–33.0).

HLA and autoantibody status, including IAA, GADA, ZnT8A (to both arginine [R] and tryptophan [W] isoforms), IA-2A and IA-2βA were analysed using PCR sequence-specific primers (PCR-SSP) and radioimmunoassay, respectively [3, 7]. HLA class II haplotypes were defined as *HLA-DQA1*0501-DQB1*0201 (DQ2)*, *HLA-DQA1*0301-DQB1*0302 (DQ8)*, *HLA-DQA1*01-DQB1*0602 (DQ6)*, or none of the above (X).

***HLA-A*24* and rs9258750 typing** Participants were initially screened for *HLA-A*24* using PCR-SSP, as published previously [7]. HLA-A genotype was determined as *HLA-A*24/HLA-A*24* or *HLA-A*24/HLA-A*Y* (where *Y* is any other HLA-A allele). *HLA-A*24* four digit typing was not performed since the majority (98%) of people of European descent carry *HLA-A*2402* [8]. rs9258750 genotype was determined using a Taqman genotyping assay on the StepOne plus system (Life Technologies, Paisley, UK).

Statistical analyses Univariate analyses were used to compare autoantibody prevalence by *HLA-A*24* genotype. HLA class II genetic risk was ranked as high risk (*DQ2/DQ8*), intermediate risk (*DQ8/DQ8, DQ8/X, DQ2/X* and *DQ2/DQ2*), low risk (*X/X*), or protective (at least one *DQ6* haplotype), where *X* refers to any other haplotype. Logistic regression models were fitted to determine the prevalence of islet autoimmune responses and *HLA-A*24* /rs9258750 genotype association after adjusting for age, sex, relationship to proband (sibling, parent or child) and HLA class II genetic risk. Kruskal–Wallis testing was used to compare the levels of islet autoantibodies in *HLA-A*24*-positive and *HLA-A*24*-negative individuals. Statistical analyses were performed using SPSS Statistics, version 21 (IBM, Chicago, IL, USA). A p value of ≤0.05 was considered statistically significant.

Results

The distribution of islet autoantibodies, *HLA-A*24,* HLA class II genetic risk and sex in 373 ICA-positive first-degree relatives is shown in Table 1. Of 373 relatives, 60 (16.1%) were positive for at least one *HLA-A*24* allele. There were no significant differences in age, sex or frequencies of islet autoantibodies between the population studied and the original 552 participants in ENDIT (data not shown).

As illustrated in Fig. 1, univariate analysis showed that GADA prevalence was lower in *HLA-A*24*-positive than in *HLA-A*24*-negative individuals (40.0% vs 59.4%, respectively, p=0.005). A similar reduction was observed in *HLA-A*24* carriers for ZnT8A (25.0% vs 44.1%, respectively, p=0.006) and IAA (28.3% vs 43.5%, respectively, p=0.029). *HLA-A*24*-positive and *HLA-A*24*-negative individuals were not significantly different in terms of the prevalence of IA-2A (31.7% vs 40.0%, respectively, p=0.228) and IA-2βA (15.0% vs 25.0%, respectively, p=0.096). Similar patterns were observed for rs9258750 in that carriers of the minor G allele (vs those homozygous for the major A allele) had a lower prevalence of GADA (47.1% vs 60.7%, p=0.013), ZnT8A (29.8% vs 46.4%, p=0.002), and IA-2βA (15.7% vs 27.0%, p=0.016). No differences were found in IAA (36.4% vs 43.3%, p=0.205) or IA-2A (32.2% vs 41.7%, p=0.080) prevalence according to rs9258750 genotype.

After adjusting for age, sex, relationship to the proband and HLA class II genetic risk, *HLA-A*24* remained negatively associated with the prevalence of GADA (OR 0.49, 95% CI: 0.27, 0.90, p=0.022) and ZnT8A (OR 0.45, 95% CI: 0.23, 0.89, p=0.021). Prevalence of IAA (OR 0.51, 95% CI: 0.25, 1.01, p=0.055), IA-2A (OR 0.82, 95% CI: 0.43, 1.55,

Table 1 The distribution of sex, islet autoantibodies, *HLA-A*24* genotype, rs9258750 and HLA class II risk in 373 ICA-positive ENDIT participants analysed in this study

Variable	Number (%)
Sex	
Male	192 (51.5)
Female	181 (48.5)
Autoantibody	
IAA	153 (41.0)
GADA	210 (56.3)
IA-2A	144 (38.6)
IA-2βA	87 (23.3)
ZnT8A	153 (41.0)
ZnT8WA	120 (32.2)
ZnT8RA	133 (35.7)
ICA+ at least one autoantibody	238 (63.8)
*HLA-A*24*	
*A*24/A*24*	9 (2.4)
*A*24/Y*	51 (13.7)
*A*Y/A*Y*	313 (83.9)
rs9258750	
AA	252 (67.6)
AG	108 (29.0)
GG	13 (3.4)
HLA class II genotype	
High risk *(DQ2/DQ8)*	68 (18.2)
Moderate risk *(DQ2/DQ2, DQ8/DQ8, , DQ2/X, DQ8/X)*	207 (55.4)
Low risk *(X/X)*	64 (17.2)
Protective *(DQ2/DQ6, DQ6/DQ6, DQ6/DQ8, DQ6/X)*	34 (9.1)

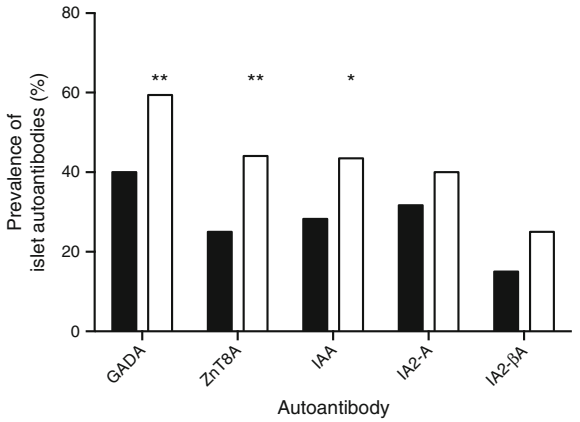

Fig. 1 The prevalence of islet autoantibodies in at-risk individuals with or without *HLA-A*24*. Black bars represent *HLA-A*24* carriers, white bars represent non-*HLA-A*24* carriers. GADA, ZnT8A and IAA were less common in *HLA-A*24* carriers *$p \leq 0.05$, **$p \leq 0.01$ vs *HLA-A*24* non-carriers

$p=0.54$) and IA-2βA (OR 0.58, 95% CI: 0.25, 1.33, $p=0.20$) did not vary between the *HLA-A*24*-positive and *HLA-A*24*-negative groups. The minor G allele of rs9258750 was associated with a lower prevalence of GADA (OR 0.60, 95% CI: 0.38, 0.97, $p=0.036$), ZnT8A (OR 0.50, 95% CI: 0.30, 0.84, $p=0.008$) and IA-2βA (OR 0.52, 95% CI: 0.28, 0.97, $p=0.041$), but not IAA (OR 0.78, 95% CI: 0.47, 1.32, $p=0.361$) or IA-2A (OR 0.73, 95% CI: 0.45, 1.21, $p=0.226$).

*HLA-A*24* carriers also had lower autoantibody levels than non-carriers in response to GAD (median [IQR]: 0.73, [0.36–18.85] vs 9.10 [0.47–66.15] DK units/ml, respectively, $p<0.001$), ZnT8R (1.02 [0.76–1.54] vs 1.39 [0.92–26.09] DK units/ml, respectively, $p=0.008$) and ZnT8W (1.11 [0.82–1.46] vs 1.26 [0.88–12.20] DK units/ml, respectively, $p=0.042$). However, IAA (0.00 [0.00–0.54] vs 0.00 [0.00–1.15], respectively, $p=0.055$), IA-2A (0.54 [0.37–3.61] vs 0.60, [0.39–72.02] DK units/ml, respectively, $p=0.138$) and IA-2βA (2.00 [0.00–87.00] vs 15 [0.00–123.50] DK units/ml, respectively, $p=0.296$) levels did not vary between the two groups.

Consistent with *HLA-A*24*, levels of GADA, ZnT8RA and ZnT8WA were significantly lower in participants carrying the G allele of rs9258750 compared with individuals homozygous for the A allele but IAA, IA-2A and IA-2βA levels were not different by rs9258750 genotype (data not shown).

GADA levels remained lower in *HLA-A*24* carriers even after GADA-negative relatives were excluded (median [IQR]: 26.24 [6.58–63.86] vs 60.91 [25.46–74.25] DK units/ml, $p=0.007$), although this effect was not observed by rs9258750 genotype (data not shown).

Discussion

Humoral responses to GAD and ZnT8 were less frequent in *HLA-A*24*-positive first-degree relatives at risk of type 1 diabetes, even after adjustment for age, sex, relationship to proband and HLA class II genetic risk. In addition, IA-2βA were less frequent in relatives carrying the minor G allele of the *HLA-A*24*-tagged SNP rs9258750.

The strength of this study is the well-characterised high-risk population analysed. All participants in the ENDIT trial were selected for ICA positivity and thus the immunogenetic interactions observed reflect ongoing islet autoimmunity. A comprehensive autoantibody profile including ICA, IAA, GADA, ZnT8A, IA-2A and IA-2βA status was established in participants.

In contrast to previous studies of patients with newly diagnosed and long-standing type 1 patients [3, 4], we did not observe negative associations between *HLA-A*24* and IA-2A/IA2-βA in at-risk relatives. This could be because ICA screening preferentially selects individuals positive for IA-2A or IA-2βA. Furthermore, as GADA also

contributes to ICA staining, we may have underestimated the effect of *HLA-A*24* on lowering autoantibody prevalence in individuals at risk of type 1 diabetes. The low frequency of IA-2βA-positive participants may have meant that the study was underpowered to detect an effect of *HLA-A*24* on IA-2βA prevalence [9]. Similarly, as many ENDIT participants were adults, only a minority had IAA and this may have limited our ability to identify subtle effects of *HLA-A*24* on IAA prevalence and levels [10]. In contrast with other studies of at-risk relatives [2], we did not observe accelerated progression in *HLA-A*24*-positive ENDIT participants. The individuals at highest risk, including *HLA-A*24* carriers, may have developed diabetes between ICA screening and study entry. *HLA-A*24* was previously found to be less frequent in ICA-positive patients [11], which may have reduced our ability to detect effects on progression.

The negative association of GADA prevalence and levels with *HLA-A*24* was not observed at diagnosis or in long-standing cases. This indicates that the effect of *HLA-A*24* in attenuating humoral autoimmune responses to islet antigens during the type 1 diabetes prodrome may be more widespread than originally thought. Longitudinal sampling, not available in the ENDIT cohort, will be required to address the natural history of attenuated islet autoantibody responses in *HLA-A*24*-positive individuals.

Taken together, this study and others [3, 4] demonstrate a negative association between *HLA-A*24* and islet autoantibodies throughout the natural history of type 1 diabetes. This could suggest a distinct subtype of pathogenesis in *HLA-A*24* individuals.

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Contribution statement JY, AJKW and KMG initiated and designed the study, reviewed the data and drafted the manuscript. AEL, JAP, and PJB contributed to the acquisition, analysis and interpretation of data and to writing the manuscript. HT contributed to the analysis and interpretation of the data and to writing the manuscript. All authors approved the final version for publication. KMG is responsible for the integrity of the work as a whole.

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