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Research: Metabolism

Glutamic acid decarboxylase and islet antigen 2 antibody profiles in people with adult-onset diabetes mellitus: a comparison between mixed ethnic populations in Singapore and Germany

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Abstract

Aim To gain insight into the presence of islet cell autoimmunity in an ethnic Asian compared with a white European population.

Methods For this cross-sectional study we recruited people with adult-onset diabetes (age of diagnosis 20–60 years), at tertiary referral centres in Germany (n=1020) and Singapore (n=1088). Glutamic acid decarboxylase and islet antigen 2 antibodies were measured according to Islet Autoantibody Standardization Program protocols.

Results The prevalence of glutamic acid decarboxylase antibody positivity was 13.9% (95% CI 12.1–16.0; P<0.001) in the white European cohort compared with 6.8% (95% CI 5.5–8.4; P<0.001) in the Asian cohort. Glutamic acid decarboxylase antibody positivity was 11.4% (95% CI 7.7–16.6) in Indian, 6.0% (95% CI 3.6–9.9) in Malay and 5.8% (95% CI 4.3–7.7; P<0.001) in Chinese participants. In the white European participants, the prevalence of islet antigen 2 antibody positivity was 7.8% (95% CI 6.4–9.4) compared with 14.8% (95% CI 12.8–17.0; P<0.001) in the Asian cohort as a whole, and among the three ethnicities in the Asian cohort it was 12.4% (95% CI 8.6–17.7) in Indian, 16.8% (95% CI 12.6–22.2) in Malay and 15.7% (95% CI 13.2–18.6) in Chinese participants. Double antibody positivity was seen in 5.7% (95% CI 4.5–7.1) of white European participants compared with 1.6% (95% CI 1.0–2.5; P<0.01) of Asian participants. In the white European cohort, those who were glutamic acid decarboxylase autoantibody-positive had a lower BMI than those who were autoantibody-negative, but this trend was absent in the Asian cohort.

Conclusions A marked prevalence of islet cell autoimmunity was observed in people with adult-onset diabetes. While glutamic acid decarboxylase antibodies were more frequent in the European cohort, islet antigen 2 antibody positivity was highest in the three ethnic groups in Singapore, suggesting ethnic-specific differences in antibody profiles.

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Introduction

Diabetes mellitus is prevalent worldwide and has been on the rise in Singapore, with the Asian population becoming both more affluent and obese over the last 25 years [1]. A report by the International Diabetes Federation, released in 2015, revealed that Singapore has the second-highest proportion of people with diabetes among developed nations [1]; however, the degree of disease heterogeneity in Asian people with adult-onset diabetes is not clear, particularly with regard to the presence of islet-cell autoimmunity [1,2]. Data on the prevalence of adult-onset autoimmune diabetes gathered from population-based studies in Asia are sparse [3]. Singapore, with its mixed ethnic population, provides a unique environment to look for the existence of islet cell antibodies in three different ethnic groups; namely, Chinese, Malay and Indian

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What's new?

- Data on the prevalence of adult-onset autoimmune diabetes gathered from population-based studies in Asia are sparse. We assessed the presence of islet cell antibodies [glutamic acid decarboxylase (GAD) and islet antigen 2 (IA2) antibodies] in three ethnic Asian groups from Singapore as compared with a white European population with diabetes.
- In the Asian cohort, IA2 antibodies were most prevalent, across the three ethnicities, whereas GAD antibodies were more frequent in the white European cohort. In the white European cohort, those who were GAD autoantibody-positive had a lower BMI than those who were GAD autoantibody-negative, but this trend was absent in the Asian cohort.

people. To enable a trans-ethnic comparison, we also recruited from another affluent environment: a cohort of white Europeans from the southern part of Germany, which we used, in view of the existing literature, as a reference cohort [4]. We determined antibodies against the two major islet cell autoantigens, glutamate decarboxylase (GAD) and islet antigen 2 (IA2), which are considered to be key humoral markers of islet cell autoimmunity.

Research Design and Methods

Participants

The study enrolled participants with adult-onset diabetes attending tertiary referral centres in Germany and Singapore, with an age of diabetes diagnosis ranging from 20 to 60 years. There was no significant difference between the cohorts in the mean age of disease onset or proportion of men: German cohort (n = 1020) 42.0 ± 1.2 years, 623 men (61.07%), and Asian cohort (n =1088) 43.8 ± 0.4 years, 701 men (64.4%).

Inclusion criteria were diabetes diagnosis according to American Diabetes Association criteria [5], with no insulin treatment. Exclusion criteria included prior insulin therapy, ketosis at diagnosis, pregnancy and presence of any other severe disease. The study was approved by the ethics review committee/institutional review board and written informed consent was obtained from all participants before screening and inclusion in the study, in both Singapore and Germany. Blood samples were taken by venipuncture up to 2 years after the diagnosis of diabetes was made and stored at –80°C until required for assay.

Immunoprecipitation of *in vitro* translated proteins

The GAD and IA2 antibodies were analysed at Lee Kong Chian School of Medicine [Islet Autoantibody Standardization Program (IASP) 2015 Laboratory ID 1501] by radioligand assay according to the IASP [6–8]. In brief, GAD and the intracellular domain of IA2, IA2ic [9], was transcribed into RNA and translated into protein *in vitro* using the TNT T7 Quick Coupled System (Promega, Madison, WI, USA), according to the manufacturer's instructions, in the presence of [^{35}S]-methionine (Perkin Elmer, Waltham, MA, USA). The protein-bound radiolabel was separated from the unincorporated label through a NAP5 Sephadex G25 DNA Grade column (GE Healthcare, Little Chalfont, UK). A concentration of 20 000/min [^{35}S]-GAD antibodies in 25 µl assay buffer (TBS/0.05% Tween) was added to 2 µl serum in 96-deep-well microtitre plates and incubated for 16 h on ice. The immune complexes were further incubated with a suspension of 50 µl of 50% Protein A Sepharose (SciMed, Singapore) in a 96-deep-well plate for 1 h at 4°C with shaking. They were then washed extensively with assay buffer and transferred into a 96-well scintillation plate, containing 200 µl scintillation fluid (Perkin Elmer), and counts/min were measured in a scintillation counter for radioactivity (TopCount NXT Microplate Scintillation and Luminescence Counter; Perkin Elmer). All samples were measured in duplicate, and 20% of samples were randomly selected and repeatedly tested to confirm their antibody status. GAD and IA2 antibody positivity was determined as the 99.5th percentile of 1192 healthy white European control participants (age range 18–70 years; mean age 39.7 years) and 145 healthy ethnic Asians control participants (age range 20–69 years; mean age 49.1 years). Sera obtained from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) were used as positive controls, as described by The Environmental Determinants of Diabetes in the Young (TEDDY) Study Group [10,11]. Sera with ≥21 arbitrary DK units/ml for GAD antibodies and ≥5 arbitrary DK units/ml for IA2 antibodies were considered to be antibody-positive.

The sensitivity and specificity of GAD antibodies were 76.0% and 87.8%, and the sensitivity and specificity of IA2 antibody assays were 76.0% and 94.4%, respectively, as evaluated in the 4th Diabetes IASP 2015 (laboratory ID 1501).

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (version 6). Data were expressed as frequencies (%) ± 95% CI, to one decimal point. Student's t-tests were used to compare the means between two (ethnic or age-adjusted) groups as appropriate. Gender groups were analysed using chi-squared tests. Two-way ANOVA and Tukey tests were used to determine the differences between the white European and ethnic Asian cohorts. A linear regression model was used to test associations between antibody-positive frequency and age of onset.

Results

Positivity for GAD antibodies was detected in 13.9% (95% CI 12.1–16.0) of white European participants as compared

Table 1 Islet cell antibody prevalence and mean BMI in the three main ethnic Asians ethnic groups (Chinese, Malay, Indian) compared with white European participants

	Mean (± sd) BMI, kg/m^2	GAD antibodies, % (95% CI)	Mean (± SD) BMI, kg/m^2 GAD antibody-positive	IA2 antibody, % (95% CI)	Mean (± SD) BMI, kg/m^2 IA2 antibody-positive	GAD and/or IA2ic antibodies, % (95% CI)	Mean (± SD) BMI, kg/m^2 GAD and/or IA2 antibody-positive	GAD and IA2antibodies, % (95% CI)	Mean (± SD) BMI, kg/m^2 GAD and IA2 antibody-positive
White European cohort (*n*=1020)	**25.2 (± 0.5)**	**13.9 (12.1–16.0)**	**22.9 (± 0.5)**††	**7.8 (6.4–9.4)**	**24.2 (± 0.4)****	**14.3 (12.4–16.4)**	**24.2 (± 0.5)****	**5.7 (4.5–7.1)**	**24.0 (± 0.3)****
Male (*n*=623)	25.3 (± 0.6)	15.7 (13.2–18.5)	23.5 (± 0.5)	8.5 (6.6–10.8)	25.2 (± 0.3)	16.1 (13.6–19.0)	25.3 (± 0.6)	5.8 (4.3–7.8)	25.0 (± 0.3)
Female (*n*=397)	25.0 (± 0.5)	11.3 (8.8–14.5)	21.7 (± 0.7)	6.7 (4.8–9.3)	22.5 (± 0.4)	11.5 (9.0–14.7]	24.9 (± 0.5)	5.4 (3.7–7.9)	22.7 (± 0.4)
Ethnic Asian cohort (*n*=1088)	**27.7 (± 0.6)**	**6.8 (5.5–8.4)**†	**28.0 (± 0.5)**	**14.8 (12.8 - 17.0)**†	**26.1 (± 0.6)**	**17.4 (15.2 - 19.6)**†	**26.1 (± 1.2)**	**1.6 (1.0 - 2.5)***	**26.1 (± 0.6)**
Men (*n*=701)	27.4 (± 0.5)	7.2 (5.5–9.3)	27.6 (± 0.5)	12.7 (10.4–15.4)	25.7 (± 0.5)	19.5 (16.8–22.6)	27.6 (± 0.5)	1.9 (1.2–3.2)	25.7 (± 0.5)
Women (*n*=387)	28.2 (± 0.7)	6.1 (4.2–8.8)	28.7 (± 0.3)	18.6 (15.0–22.8)	26.4 (± 0.7)	22.0 (18.1–26.4)	28.6 (± 0.7)	1.4 (0.7–3.1)	26.4 (± 0.7)
Chinese (*n*=655)	**27.0 (± 0.5)**	**5.8 (4.3–7.7)**	**27.2 (± 0.5)**	**15.7 (13.2–18.6)**	**25.9 (± 0.5)**	**19.4 (16.6–22.4)**	**27.0 (± 0.5)**	**2.1 (1.3–3.6)**	**25.9 (± 0.5)**
Men (*n*=455)	27.0 (± 0.5)	5.9 (4.1–8.4)	27.3 (± 0.4)	14.9 (12.0–18.4)	25.7 (± 0.5)	18.5 (15.3–22.2)	27.0 (± 0.5)	2.3 (1.3–4.1)	25.7 (± 0.5)
Women (*n*=200)	27.0 (± 0.5)	5.5 (3.2–9.2)	27.1 (± 0.6)	17.3 (13.0–22.6)	26.3 (± 0.5)	21.1 (16.4–26.7)	27.0 (± 0.5)	1.7 (0.7 to 4.3)	26.3 (± 0.5)
Malay (*n*=232)	**29.9 (± 0.5)**	**6.0 (3.6–9.9)**	**29.7 (± 0.5)**	**16.8 (12.6–22.2)**¶	**29.3 (± 0.5)**	**21.1 (16.4–26.8)§**	**29.8 (± 0.5)**	**1.3 (0.4–3.7)**§	**29.3 (± 0.5)**
Men (*n*=125)	29.5 (± 0.6)	7.2 (3.8–13.1)	29.0 (± 0.5)	16.0 (10.6–23.4)	27.7 (± 0.5)	21.6 (15.3–29.6)	29.6 (± 0.6)	1.6 (0.4 - 5.7)	27.7 (± 0.5)
Women (*n*=107)	30.4 (± 0.6)	4.7 (2.0–10.5)	30.6 (± 0.5)	17.8 (11.7–26.1)	30.9 (± 0.4)	20.6 (14.0–29.2)	30.3 (± 0.6)	1.9 (0.5–6.6)	30.9 (± 0.4)
Indian (*n*=201)	**27.8 (± 0.5)**	**11.4 (7.7–16.6)**¶	**28.0 (± 0.4)**	**12.4 (8.6–17.7)**¶	**28.7 (± 0.5)**	**23.4 (18.1–29.7)**§	**28.2 (± 0.5)**	**0.5 (0.1–2.8)**§	**28.7 (± 0.5)**
Men (*n*=121)	27.1 (± 0.4)	12.4 (7.7–19.5)	27.3 (± 0.5)	9.1 (5.2 to 15.6)	26.6 (± 0.6)	20.7 (14.4–28.7)	27.7 (± 0.4)	0.8 (0.2–4.5)	26.6 (± 0.6)
Women (*n*=80)	28.9 (± 0.7)	10.0 (5.2–18.5)	29.2 (± 0.5)	17.5 (10.7–27.3)	30.0 (± 0.7)	27.5 (18.9–38.1)	28.9 (± 0.7)	0	0

GAD, glutamic acid decarboxylase; IA2, islet antigen 2.
Ratios for GAD antibodies and IA2 antibody positivity were 13.9: 5.7: 6: 11.4 and 7.8: 15.7: 16.8: 12.4 for white European: Chinese: Malay: Indian participants, respectively.*$P < 0.01$, †$P < 0.001$ compared with white European cohort. Among ethnic Asians ethnic groups, §$P < 0.01$, ¶$P < 0.001$ in comparison with ethnic Chinese. Among white Europeans, **$P < 0.01$, ††$P < 0.001$ compared with white Europeans mean BMI. All P values obtained using the t-test.

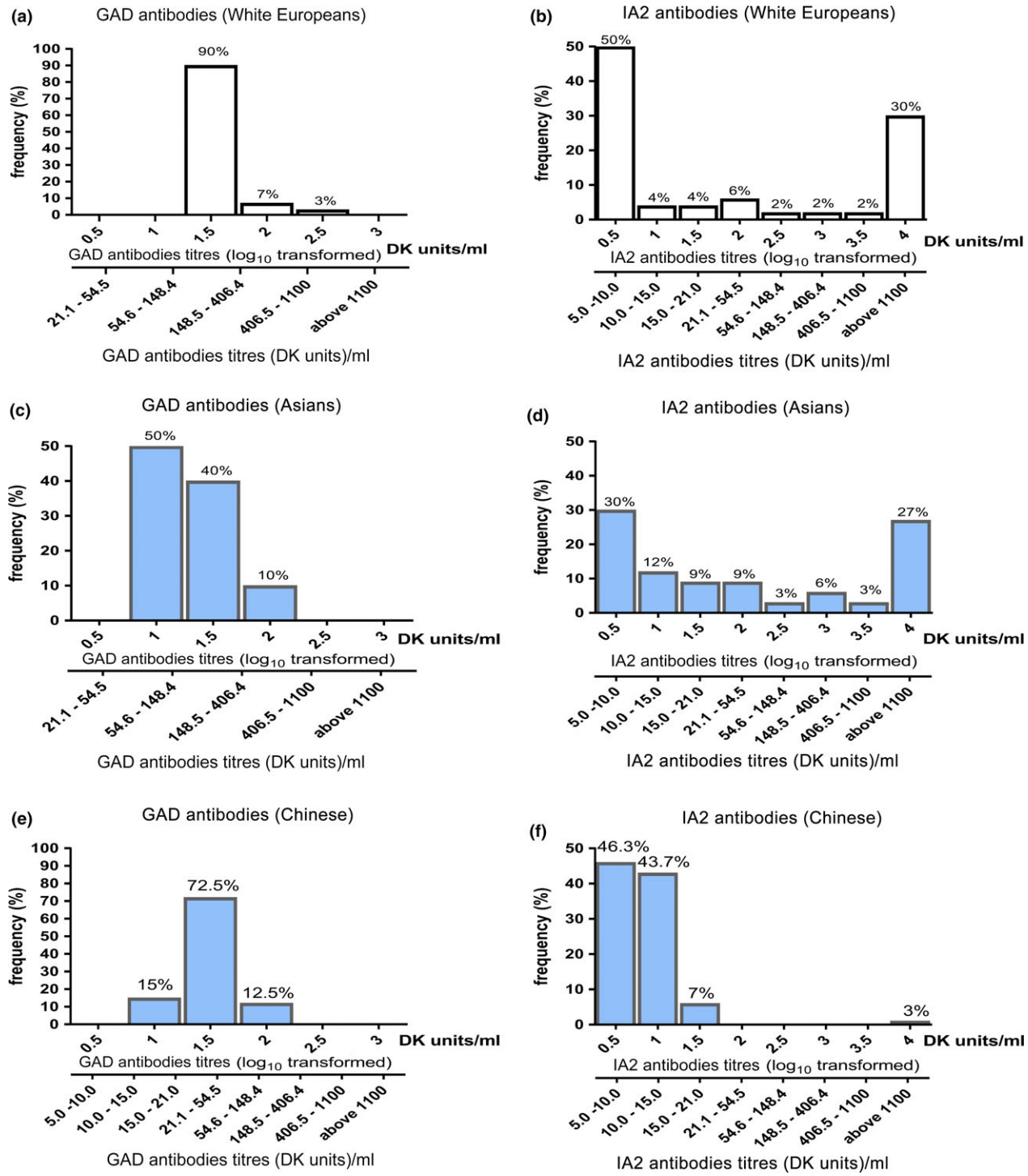

FIGURE 1 Islet cell antibody prevalence in Asian and white European participants with diabetes. (a) Glutamic acid decarboxylase (GAD) antibodies and (b) islet antigen 2 (IA2) antibody distribution in white European participants. (c) GAD antibodies and (d) IA2 antibody distribution in ethnic Asian participants. Ethnic-specific GAD antibodies positivity for Chinese (e), Malay (g) and Indian (i) participants; IA2 antibody distribution for Chinese (f), Malay (h) and Indian (j) participants. *$P < 0.01$, **$P < 0.001$ compared with white European cohort using the *t*-test. DK units, arbitrary National Institute of Diabetes and Digestive and Kidney Diseases units.

with 6.8% (95% CI 5.5–8.4; $P<0.001$) of ethnic Asian participants. Among the three ethnic groups from Singapore, GAD antibody positivity was significantly higher in Indian participants [11.4% (95% CI 7.7–16.6)] as compared with both Chinese (5.8%; 95% CI 4.3–7.7; $P<0.001$) and Malay participants (6.0%; 95% CI 3.6–9.9; $P<0.001$; Table 1).

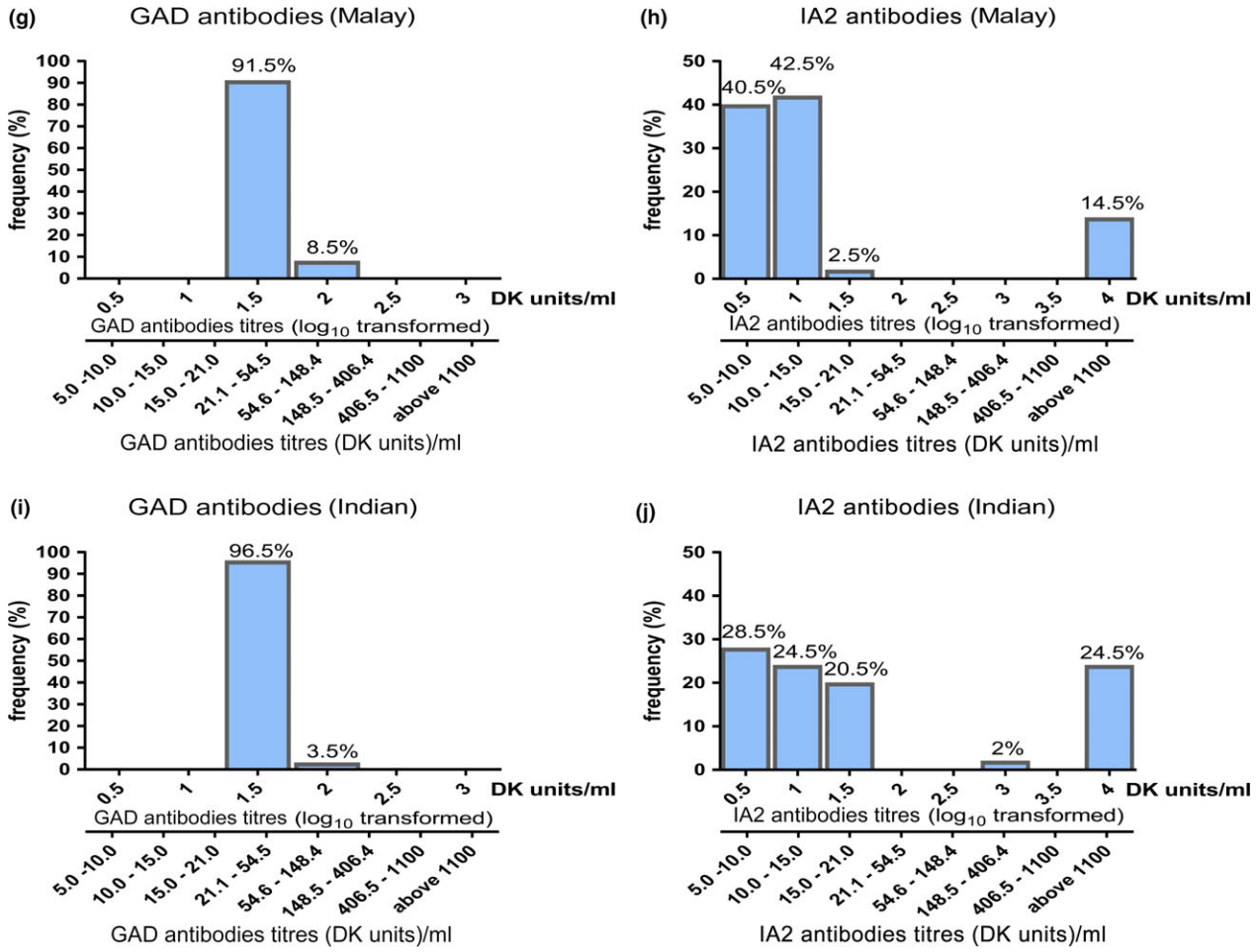

FIGURE 1 Continued

Positivity for IA-2 antibodies was detected in 7.8% (95% CI 6.4–9.4) of white European participants as compared with 14.8% (95% CI 12.8 -17.0; P<0.001) of ethnic Asian participants. Amongst the ethnic groups from Singapore, 15.7% (95% CI 13.2–18.6) of Chinese, 16.8% (95% CI 12.6–22.2; non-signficant) of Malay and 12.4% (95% CI 8.6–17.7; non-significant) of Indian participants had similar rates of IA-2 antibody positivity.

The presence of GAD or IA2 autoantibodies was common both in the white European as well as the ethnic Asians cohort: 14.3% (95% CI 12.4–16.4) vs 18.7% (95% CI 16.5 - 21.0; Table 1). Double antibody positivity was significantly higher in white European participants (5.7%; 95% CI 4.5–7.1) as compared with Asian participants (1.6%; 95% CI 1.0–2.5; P<0.001). Amongst the three ethnic groups from Singapore, 2.1% (95% CI 1.3–3.6) of Chinese, 1.3% (95% CI 0.4–3.7) of Malay and 0.5% (95% CI 0.1–2.8) of Indian participants were double antibody-positive (Table 1).

White European participants who were GAD antibody- and/or IA2 antibody-positive had a lower mean BMI compared with antibody-negative participants (24.2 ± 0.5 kg/m^2 vs 27.2 ± 0.6 kg/m^2; P=0.01); however, the mean BMI of antibody-positive participants in the Singapore cohort was not different from the antibody-negative participants (26.1 ± 1.2 kg/m^2 vs 27.8 ± 1.1 kg/m^2; P=0.35). A significant difference was also found in the mean BMI of GAD antibody-positive vs IA2 antibody-positive participants (22.9 kg/m^2 vs 24.2 kg/m^2) in the white European cohort (P<0.05). Again, no such difference was observed in the Asian cohort (Figures S1–S3).

Antibody titres were also found to be different among the four ethnic groups. The majority of GAD antibody-positive white Europeans had a GAD antibody titre range of 54.6–148.4 arbitrary NIDDK units/ml [12], whereas 3% had a GAD antibody titre of >406.4 arbitrary NIDDK units/mL (Fig. 1a). GAD antibody-positive Asian participants had GAD antibody titres of 21.1–54.6 arbitrary NIDDK units/ml (50% of the cohort), 54.6–148.4 arbitrary NIDDK units/mL (40% of the cohort) and >148.4 arbitrary NIDDK units/mL (10% of the cohort; Fig. 1c). The three Asian ethnic groups had similar GAD antibodies titre (Fig. 1e, g and i) and IA2antibody titre distributions (Fig. 1f, h and j).

There was a different pattern of age of disease onset in relation to antibody positivity and antibody levels in white European compared with Asian participants

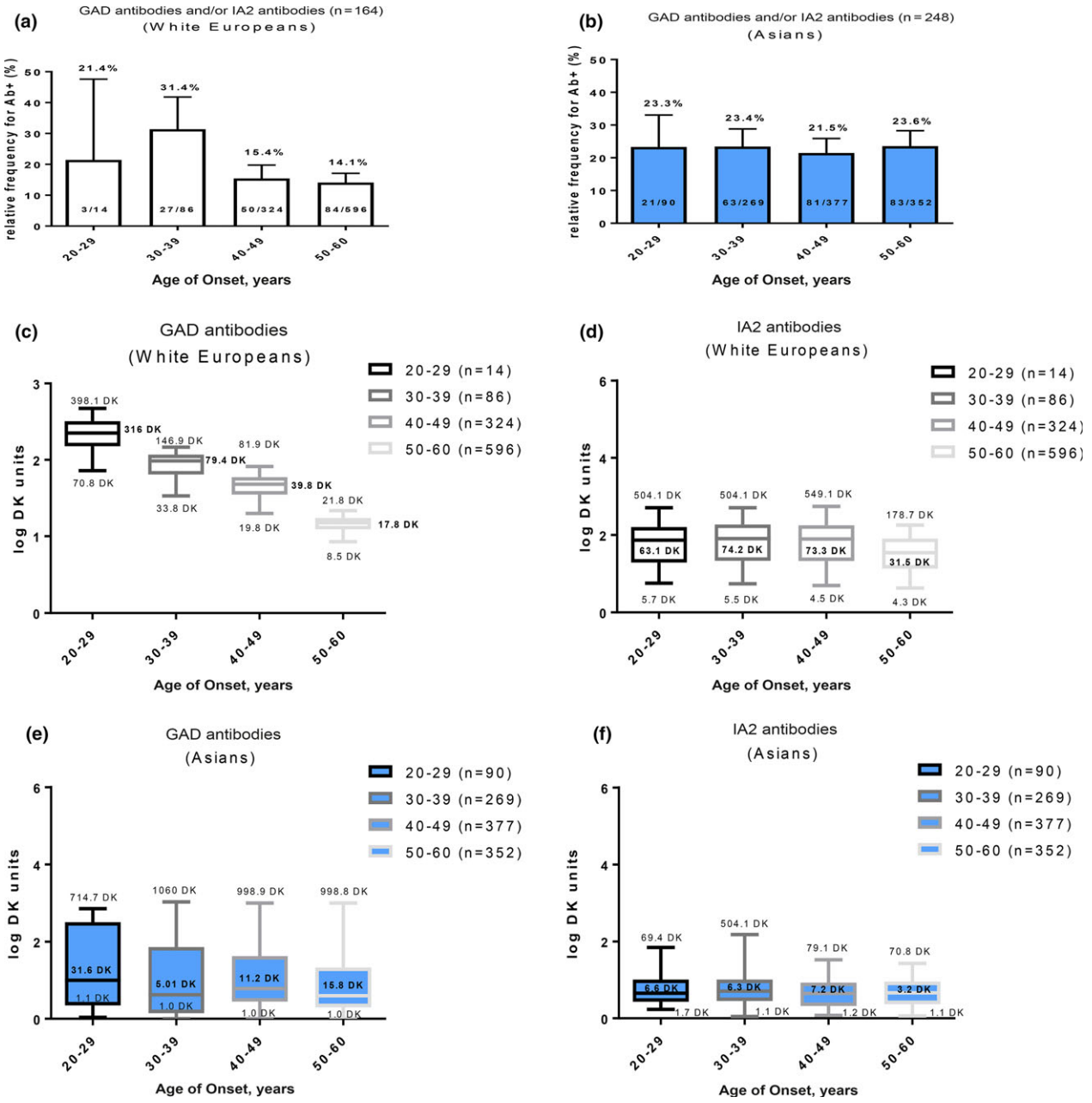

FIGURE 2 Islet cell antibody prevalence of glutamic acid decarboxylase (GAD) and/or islet antigen 2 (IA2) antibodies in relation to age of disease onset. (a) White European (linear regression slope= -0.38±0.03) and (b) ethnic Asian (linear regression slope= -0.012±0.05) participants with increasing age of onset. Distribution box plots showing antibody titre distribution in participants with increasing age of onset: (c) GAD antibodies in white European participants (P<0.001) and (d) IA2 antibodies in white European participants (P=0.096; non-significant) in comparison with (e) GAD antibodies in the Singapore cohort (P=0.534; non-significant) and (f) IA2 antibodies in the Singapore cohort (P= 0.399; non-significant). Total population in each age group=100%. P values compared with cohort in 20–29-year age group using one-way ANOVA. DK units, arbitrary National Institute of Diabetes and Digestive and Kidney Diseases units.

(Fig. 2). In white European participants, antibody prevalence and antibody titre for both GAD and IA-2 antibodies was lower after the interval of 30–39 years of disease onset. (Fig. 2a, c and d). Among the Asian participants, such an age-dependency in prevalence and antibody titre was not observed (Fig. 2b, e and f). In addition, the distribution of antibody titres and antibody positivity among the three ethnic groups was similar (Supporting Information).

Discussion

This is the first study to investigate GAD and IA2 antibody positivity and antibody levels in people with non-insulin-

dependent adult-onset diabetes from four different ethnic groups. The predominant islet cell antigen in Asian participants with adult-onset diabetes was IA2, while it was GAD in the white European participants. The prevalence of GAD antibody positivity within the four ethnic groups (Table 1) was consistent with the GAD antibody positivity rate of 7% reported in the UK Prospective Diabetes Study [14,15] and also with the results from the China LADA Consortium of 5.9% [16].

Age of onset dependency for islet cell antibody positivity had been reported previously, as a characteristic of adult-onset autoimmune diabetes [16,17], in various European [13,15–19] and US cohorts [20]. This association with age of onset was not seen in Asian participants with diabetes.

The presence of single islet cell antibody positivity was a unique feature for the Asian cohorts [21], whereas double antibody positivity was largely confined to white Europeans. White European participants were leaner and had a lower BMI with increasing GAD titre [15], while this trend was not seen in Asian participants [21,22]. Notably, the mean BMI of GAD antibody-positive participants was lower than that of IA2 antibody-positive participants within the white European cohort. Again, there was a lack of this association within the various Asian cohorts (Figure S3).

The present study has some limitations. The cohorts analysed were recruited at tertiary referral centres, and therefore more severe clinical cases of the disease spectrum were probably captured, which may have had an impact on the likelihood of autoantibody positivity [13]. In addition, participants were not characterized according to their β-cell (C-peptide) reserve, unlike in other studies [23], or followed for their long-term treatment methods [13,15].

Strengths of the present study include the application of Diabetes IASP methods [6–8], and the comparison of patient cohorts from similar socio-economic environments, as determined by income per capita.

To learn more about the clinical impact of islet cell autoimmunity in different ethnic groups, follow-up studies involving larger cohorts are needed. These studies should take into consideration, in particular, ethnic-specific differences in antibody patterns and clinical outcomes among people with islet cell autoimmunity in comparison to participants without islet cell antibodies [14,16,17,24–26].

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Competing interests

None declared.

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Physicians involved in the Adult-Onset Autoimmune Diabetes Mellitus Singapore Consortium (ADAMS) (see Appendix for the full list).

Figures 2a and b and Figs S1a and b were presented as a poster for the SingHealth Duke-NUS Scientific Congress 2016.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Autoantibody (GAD, IA2) titres in (A, B) white European (non-significant), (C,D) Chinese (P<0.001), (E, F) Malay (P<0.001) and (G,H) Indian (P <0.001) participants with increasing age of onset. P values compared with white European cohort using the t-test.
Figure S2. Autoantibody (GAD, IA2) titres in (A, B) German (non-significant), (C, D) Chinese (P <0.001), (E, F) Malay (P <0.001) and (G, H) Indian (P <0.001) participants with increasing BMI. P values compared with white European cohort using the t-test.
Figure S3. BMI values of islet cell antibody-negative and islet cell antibody-positive subgroups. (A) Mean BMI for white European islet cell antibody-negative participants was 27.2 (95% CI 25.2–29.2). BMI of GAD antibody-positive Europeans was 22.9 (95% CI 19.3–27.3; P<0.001), for IA2 antibody-positive participants 24.2 (95% CI 20.7–28.4; P<0.001) and double antibody-positive participants 24.0 (95% CI 19.9–28.1; P<0.001). (B) Mean BMI for Asians without islet cell antibodies was 27.8 (95% CI 27.4–28.1). BMI of Asian GAD antibody-positive participants was 28.0 (95% CI 27.0–28.9; P=non-significant), for IA2 antibody-positive participants 27.2 (95% CI 26.4–28.0; P=non-significant), and double antibody-positive participants 26.1 (95% CI 24.7–28.6; P<0.01). Mean BMI for ethnic Chinese GAD antibody positives was 27.2 (95% CI 25.6–28.9; P=non-significant), IA2 antibody-positive participants 26.3 (95% CI 25.3–27.2; P<0.01). Mean BMI for ethnic Malay GAD antibody-positive participants was 29.8 (95% C.I. 27.4–30.9; P<0.001) and 29.4 (95% CI 27.5–30.5; P<0.001) for IA2 antibody-positive participants. Mean BMI for ethnic Indian GAD antibody-positive participants was 28.0 (95% CI 25.5–30.1; P=non-significant), IA2 antibody-positive participants had a BMI of 28.7 kg/m^2 (95% CI 26.4–30.9; P=non-significant). P values compared with antibody-negative participants within each cohort using the t-test.

Appendix

List of physicians involved in the Adult-Onset Autoimmune Diabetes Mellitus Singapore Consortium (ADAMS).

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