Characteristic	ADDRESS-2	StartRight
n	1,644	449
Female (%)	704 (43)	221 (49)
Non-european descent (%)	108 (6.6)	22 (5)
Age of Diagnosis (years)	21 (13, 31)	34 (26, 46)
Duration of Diabetes at Antibody testing (weeks)	11 (6, 18)	15 (6, 32)
Hospital Admission (%)	1,251 (76)	267 (60)
DKA (%)	687 (43)	156 (35)
Polyuria (%)	1,562 (96)	411 (92)
Weight Loss (%)	1,393 (87)	386 (86)
HbA1c (mmol/mol)	80 (59, 107)	64 (50, 87)
BMI (kg/m^2)	23.4 (21.1, 26.2)	24.5 (21.8, 27.2)
C-Peptide (picomol/L)	-	464 (278, 684)
Parent with Diabetes (%)	260 (16)	88 (20)
Other autoimmune condition (%)	125 (8)	68 (15)
T1D-GRS	0.274 (0.255, 0.292)	-
***HLA-DR3-DQ2* (%)**	861 (52)	-
***HLA-DR4-DQ8* (%)**	880 (54)	-
Number of positive autoantibodies		
One (%)	475 (29)	160 (36)
Two (%)	565 (34)	126 (28)
Three (%)	604 (37)	163 (36)
GADA positive (%)	1,364 (83)	404 (90)
IA-2A positive (%)	1,099 (67)	237 (53)
ZnT8A positive (%)	954 (58)	260 (58)

ESM Table 1: Clinical characteristics for the study cohorts. '-' indicates unavailable data for this cohort. Values expressed as median (interquartile range) unless stated.

Method	Antibody	%Sensitivity	%Specificity	IASP Workshop
RBA	GADA	74	96.7	2015
RBA	IA-2A	72	100	2015
RBA	ZnT8RA	60	100	2015
RBA	ZnT8WA	46	100	2015
RSR ELISA	GADA	74.0	98.9	2020
RSR ELISA	IA-2A	72.0	98.9	2020
RSR ELISA	ZnT8A	74.0	98.9	2020

ESM Table 2: IASP Workshop performance for each islet autoantibody assay. The radiobinding assays were all conducted centrally in Bristol (U.K.) by the Diabetes and Metabolism Group. The RSR ELISA assays were all conducted centrally by the Academic Department of Clinical Biochemistry (Royal Devon and Exeter NHS Trust) in Exeter (U.K.). RBA: Radiobinding immunoassay; ELISA: Enzyme-linked immunosorbent assay.

Characteristic	High Level GADA	Low Level GADA	High vs Low Level P value	GADA Negative‡	Low vs GADA Negative P value
n (% of GADA positives)	604 (44)	760 (56)		268 (26)	
GADA level (DK U/ml)	723 (594, 877)	149 (76, 274)		7 (0, 18)	
Female (%)	317 (52)	282 (37)	$1x10^{-8}$*	97 (36)	0.79
Non-european descent (%)	45 (7)	51 (7)	0.6	0 (0)	$1x10^{-5}$*
Age of Diagnosis (years)	27 (17, 38)	19 (13, 29)	$9x10^{-17}$*	14 (10, 21)	$2x10^{-12}$*
Duration of Diabetes (weeks)	10 (6, 17)	11 (6, 18)	0.29	14 (8, 21)	$2x10^{-4}$*
Hospital Admission (%)	434 (72)	577 (76)	0.1	231 (87)	$3x10^{-4}$*
DKA (%)	250 (42)	323 (43)	0.74	110 (41)	0.58
Polyuria (%)	580 (97)	712 (95)	0.21	258 (98)	0.09
Weight Loss (%)	522 (88)	641 (86)	0.23	220 (85)	0.7
HbA1c (mmol/mol)	87 (64, 110)	81 (58, 107)	0.01	72 (54, 104)	0.08
BMI, kg/m2	23.7 (21.4, 26.7)	23.2 (20.9, 26.2)	0.04	23.0 (21.0, 25.1)	0.25
Parent with Diabetes (%)	121 (20)	104 (14)	0.002*	35 (13)	0.83
Other autoimmune condition (%)	77 (13)	42 (6)	$3x10^{-6}$*	6 (2)	0.03
T1D-GRS	0.273 (0.256, 0.292)	0.275 (0.255, 0.292)	0.48	0.275 (0.258, 0.294)	0.57
HLA-DR3-DQ2 (%)	353 (58)	387 (51)	0.006	113 (42)	0.01
HLA-DR4-DQ8 (%)	291 (48)	401 (53)	0.09	184 (69)	$6x10^{-6}$*
Number of positive autoantibodies			0.45		$6x10^{-42}$*
One (%)	165 (27)	195 (26)		107 (40)	
Two (%)	183 (30)	217 (29)		161 (60)	
Three (%)	256 (42)	348 (46)		0 (0)	
IA-2A (%)	372 (62)	484 (64)	0.43	235 (88)	$2x10^{-13}$*
IA-2A level (DK U/ml)#	254 (91, 340)	255 (124, 338)	0.66	246 (95, 316)	0.1
High level IA-2A (%)	265 (44)	362 (48)	0.17	168 (63)	$2x10^{-5}$*
ZnT8A (%)	323 (53)	429 (56)	0.27	194 (72)	$4x10^{-6}$*
ZnT8A level (AU/ml) #	43 (13, 83)	36 (12, 76)	0.13	24 (8, 75)	0.08
High level ZnT8A (%)	180 (30)	215 (28)	0.54	78 (29)	0.8

ESM Table 3: Low GADA level comparison to those negative for GADA but positive for IA-2A and/or ZnT8A. Bimodal GADA level distribution was divided into two groups using the nadir between the two modes at 450 DK U/ml (High vs Low level GADA). Values expressed as median (interquartile range) unless stated. Autoantibody levels were assessed in those people who were positive for that antibody. ‡Other islet autoantibody positive. *indicates a p value lower than threshold the p value for multiple comparisons (0.05/22 =0.0023)

Characteristic	High Level IA-2A	Low Level IA-2A	High vs Low Level P value	IA-2A Negative‡	Low vs IA-2A Negative P value
n (%)	803 (73)	296 (27)		502 (63)	
IA-2A level (DK U/ml)	295 (237, 359)	24 (8, 65)		0 (0, 0)	
Female (%)	355 (44)	117 (40)	0.16	210 (42)	0.52
Non-european descent (%)	48 (6)	17 (6)	0.88	0 (0)	$6x10^{-8}$*
Age of Diagnosis (years)	17 (12, 25)	23 (13, 34)	$3x10^{-7}$*	28 (18, 38)	$6x10^{-6}$*
Duration of Diabetes (weeks)	12 (7, 18)	11 (6, 18)	0.2	10 (6, 18)	0.77
Hospital Admission (%)	650 (81)	217 (74)	0.01	346 (69)	0.19
DKA (%)	350 (44)	131 (45)	0.88	190 (39)	0.11
Polyuria (%)	767 (97)	279 (96)	0.21	476 (95)	0.92
Weight Loss (%)	679 (87)	244 (86)	0.2	431 (88)	0.11
HbA1c (mmol/mol)	76 (56, 101)	83 (60, 114)	0.01	87 (63, 111)	0.46
BMI	23.2 (21.2, 25.9)	23.7 (21.5, 26.6)	0.07	23.4 (20.9, 26.2)	0.13
Parent with Diabetes (%)	110 (14)	47 (16)	0.37	93 (19)	0.33
Other autoimmune condition (%)	45 (6)	24 (8)	0.13	51 (10)	0.33
T1D-GRS	0.275 (0.257, 0.293)	0.275 (0.254, 0.294)	0.73	0.273 (0.256, 0.292)	0.8
HLA-DR3-DQ2 (%)	354 (44)	165 (56)	$6x10^{-4}$*	316 (63)	0.05
HLA-DR4-DQ8 (%)	533 (66)	149 (50)	$1x10^{-6}$*	187 (37)	$3x10^{-4}$*
Number of positive autoantibodies			$9x10^{-9}$*		$7x10^{-78}$*
One (%)	40 (5)	38 (13)		360 (72)	
Two (%)	283 (35)	134 (45)		142 (28)	
Three (%)	480 (60)	124 (42)		0 (0)	
GADA (%)	627 (78)	229 (77)	0.8	469 (93)	$4x10^{-11}$*
GADA level (DK U/ml)	323 (117, 698)	374 (149, 671)	0.19	382 (136, 679)	0.74
High level GADA (%)	265 (33)	107 (36)	0.33	212 (42)	0.09
ZnT8A (%)	616 (77)	153 (52)	$1x10^{-15}$*	175 (35)	$3x10^{-6}$*
ZnT8A level (AU/ml)	44 (16, 84)	26 (7, 54)	$3x10^{-6}$*	18 (6, 65)	0.65
High level ZnT8A (%)	348 (43)	60 (20)	$2x10^{-12}$*	65 (13)	0.01

ESM Table 4: Low IA-2A level comparison to those negative for IA-2A but positive for GADA and/or ZnT8A. Bimodal IA-2A level distribution was divided into low and high level groups using the nadir between the two modes at 125 DK U/ml. Values expressed as median (interquartile range) unless stated. Autoantibody levels were assessed in those people who were positive for that antibody. ‡Other islet autoantibody positive. *indicates a p value lower than threshold the p value for multiple comparisons (0.05/22 =0.0023)

Characteristic	High Level ZnT8A	Low Level ZnT8A	High vs Low Level P value	ZnT8A Negative‡	Low vs ZnT8A Negative P value
n (%)	477 (50)	477 (50)		630 (57)	
ZnT8A level (AU/ml)	78 (54, 110)	11 (5, 21)		0.23 (0.16, 0.43)	
Female (%)	207 (43)	196 (41)	0.47	270 (43)	0.56
Non-european descent (%)	27 (6)	21 (4)	0.37	0 (0)	$1x10^{-7}$*
Age of Diagnosis (years)	17 (12, 26)	19 (12, 30)	0.06	25 (15, 37)	$6x10^{-10}$*
Duration of Diabetes (weeks)	11 (6, 18)	11 (6, 19)	0.74	11 (6, 18)	0.25
Hospital Admission (%)	372 (78)	385 (81)	0.3	439 (70)	2x10-5
DKA (%)	196 (42)	218 (46)	0.16	242 (39)	0.02
Polyuria (%)	462 (98)	457 (97)	0.29	587 (94)	0.04
Weight Loss (%)	400 (85)	410 (87)	0.38	530 (86)	0.6
HbA1c (mmol/mol)	78 (59, 101)	79 (58, 112)	0.35	85 (61, 109)	0.4
BMI	23.4 (21.3, 26.5)	23.3 (20.9, 25.5)	0.11	23.5 (21.3, 26.4)	0.06
Parent with Diabetes (%)	61 (13)	69 (15)	0.49	116 (19)	0.08
Other autoimmune condition (%)	37 (8)	32 (7)	0.54	50 (8)	0.45
T1D-GRS	0.276 (0.257, 0.293)	0.273 (0.255, 0.292)	0.54	0.274 (0.256, 0.293)	0.98
HLA-DR3-DQ2 (%)	256 (54)	233 (49)	0.14	335 (53)	0.15
HLA-DR4-DQ8 (%)	264 (55)	283 (59)	0.21	314 (50)	0.002
Number of positive autoantibodies			$5x10^{-6}$*		$2x10^{-129}$*
One (%)	12 (3)	25 (5)		401 (64)	
Two (%)	127 (27)	186 (39)		229 (36)	
Three (%)	338 (71)	266 (56)		0 (0)	
GADA (%)	395 (83)	357 (75)	0.003	556 (88)	$6x10^{-9}$*
GADA level (DK U/ml)	371 (146, 728)	298 (111, 675)	0.03	378 (141, 666)	0.06
High level GADA (%)	180 (38)	143 (30)	0.01	255 (40)	$3x10^{-4}$*
IA-2A (%)	408 (86)	361 (76)	$1x10^{-4}$*	303 (48)	$2x10^{-20}$*
IA-2A level (DK U/ml)	290 (215, 359)	245 (117, 326)	$1x10^{-5}$*	177 (23, 301)	$1x10^{-6}$*
High level IA-2A (%)	348 (73)	268 (56)	$6x10^{-8}$*	169 (27)	$4x10^{-23}$*

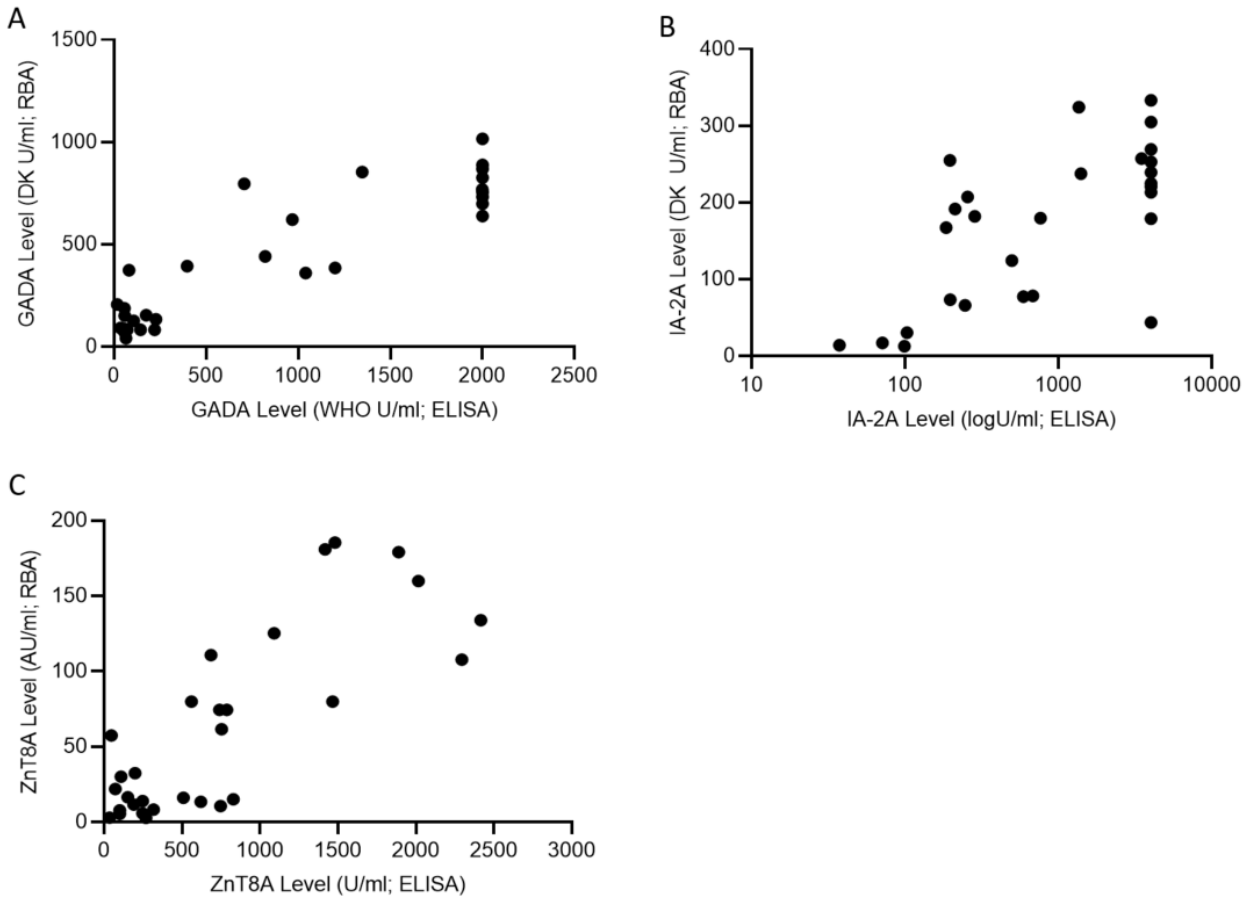
ESM Table 5: Low ZnT8A level comparison to those negative for ZnT8A but positive for GADA and/or IA-2A. ZnT8A level distribution was divided into two groups using the median of the distribution at 35.6 AU/ml. Values expressed as median (interquartile range) unless stated. Autoantibody levels were assessed in those people who were positive for that antibody. ‡Other islet autoantibody positive. *indicates a p value lower than threshold the p value for multiple comparisons (0.05/22 =0.0023)

Characteristic	Low Level GADA	High Level GADA	P value
n (%)	207 (51)	197 (49)	
GADA level (U/ml)	94 (46, 309)	2001 (1830, 2001)	
Female (%)	88 (43)	118 (60)	$5x10^{-4}$*
Non-european descent (%)	10 (5)	11 (6)	0.73
Age of Diagnosis (years)	30 (24, 38)	40 (31, 53)	$6x10^{-13}$*
Duration of Diabetes (weeks)	15 (6, 32)	15 (7, 29)	0.85
Hospital Admission (%)	129 (63)	113 (57)	0.28
DKA (%)	70 (34)	71 (36)	0.67
Polyuria (%)	192 (93)	178 (90)	0.39
Weight Loss (%)	169 (82)	178 (90)	0.01
HbA1c (mmol/mol)	65 (51, 87)	64 (50, 89)	0.94
BMI (kg/m2)	24.2 (21.4, 27.0)	24.7 (21.9, 27.5)	0.25
C-Peptide (picomol/L)	480 (273, 684)	457 (275, 683)	0.94
Parent with Diabetes (%)	38 (18)	42 (21)	0.46
Other autoimmune condition (%)	23 (11)	42 (21)	0.005
Number of positive autoantibodies			0.95
One (%)	65 (31)	61 (31)	
Two (%)	60 (29)	55 (28)	
Three (%)	82 (40)	81 (41)	
IA-2A (%)	106 (51)	99 (50)	0.85
IA-2A level (U/ml)	360 (86, 1950)	210 (40, 1575)	0.11
High level IA-2A (%)	22 (21)	17 (17)	0.51
ZnT8A (%)	118 (57)	118 (60)	0.56
ZnT8A level (AU/ml)	233 (93, 542)	279 (97, 592)	0.62
High level ZnT8A (%)	57 (48)	62 (53)	0.52

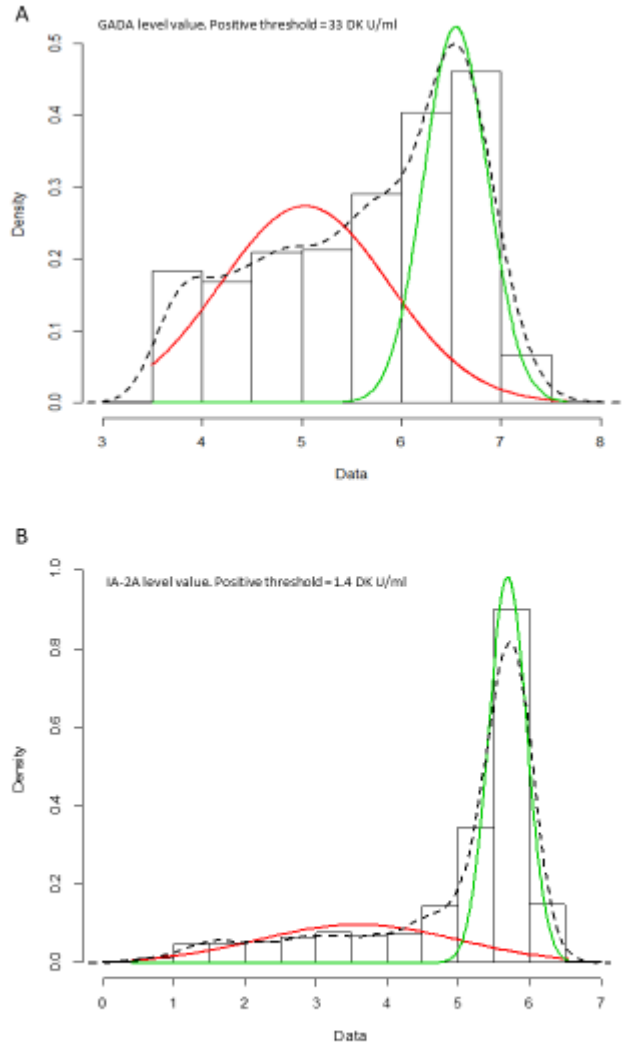
ESM Table 6: Comparison of clinical characteristics between high and low level GADA groups for positive GADA T1D cases in StartRight study. Bimodal GADA level distribution was divided into two groups using the nadir between the two modes at 937 WHO U/ml. Values expressed as median (interquartile range) unless stated. Autoantibody levels were assessed in those people who were positive for that antibody. * indicates a p value lower than threshold the p value for multiple comparisons (0.05/20 =0.0025)

Characteristic	Low Level IA-2A	High Level IA-2A	P value
n (%)	192 (81)	45 (19)	
IA-2A level (U/ml)	141 (38, 553)	4001 (3999, 4001)	
Female (%)	87 (45)	25 (56)	0.22
Non-european descent (%)	7 (4)	3 (7)	0.36
Age of Diagnosis (years)	33 (25, 46)	28 (22, 48)	0.27
Duration of Diabetes (weeks)	15 (7, 35)	11 (7, 17)	0.04
Hospital Admission (%)	119 (62)	30 (67)	0.59
DKA (%)	72 (38)	16 (36)	0.79
Polyuria (%)	176 (92)	43 (96)	0.38
Weight Loss (%)	166 (86)	33 (74)	0.03
HbA1c (mmol/mol)	63 (49, 86)	66 (57, 91)	0.36
BMI (kg/m2)	24.6 (22.3, 27.0)	25.3 (21.2, 29.2)	0.55
C-Peptide (picomol/L)	482 (296, 637)	443 (291, 756)	0.75
Parent with Diabetes (%)	30 (16)	8 (18)	0.72
Other autoimmune condition (%)	31 (16)	6 (13)	0.64
Number of positive autoantibodies			0.91
One (%)	17 (9)	4 (9)	
Two (%)	44 (23)	9 (20)	
Three (%)	131 (68)	32 (71)	
GADA (%)	166 (86)	39 (87)	0.97
GADA level (U/ml)	910 (91, 2001)	439 (76, 2001)	0.97
High level GADA (%)	82 (49)	17 (44)	0.51
ZnT8A (%)	140 (73)	34 (76)	0.72
ZnT8A level (AU/ml)	279 (114, 606)	533 (335, 1088)	0.002
High level ZnT8A (%)	74 (53)	27 (79)	0.005

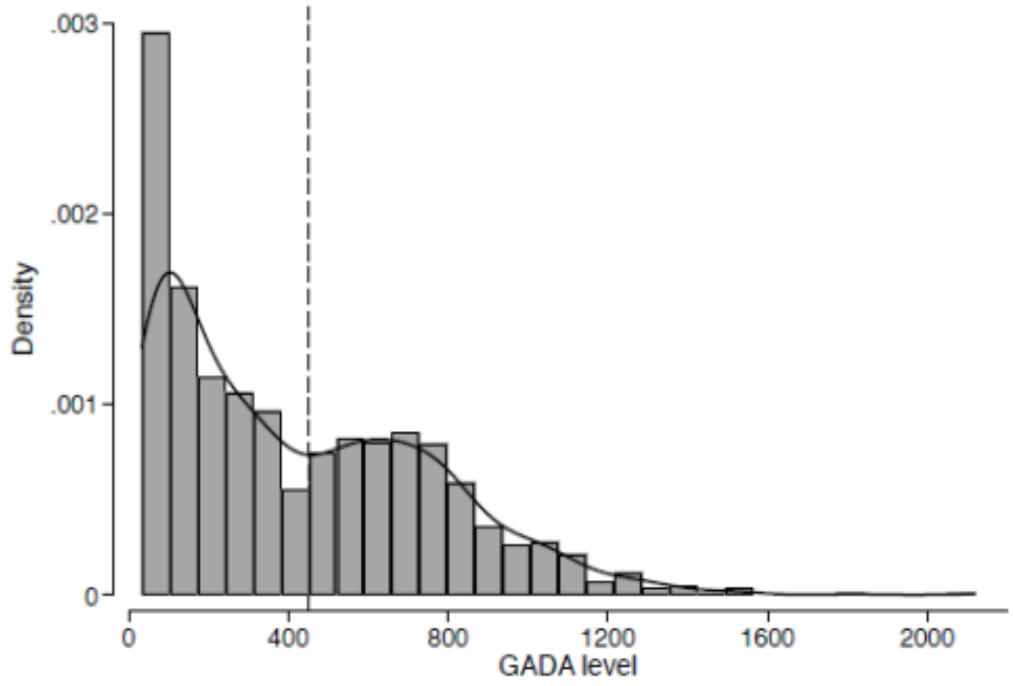
ESM Table 7: Comparison of clinical characteristics between high and low level IA-2A groups for positive for IA-2A type 1 diabetes cases in StartRight study. Bimodal IA-2A level distribution was divided into low and high level groups using the nadir between the modes at 2756 WHO U/ml. Autoantibody levels were assessed in those people who were positive for that antibody. Values expressed as median (interquartile range) unless stated. *Indicates a p value lower than threshold the p value for multiple comparisons (0.05/20=0.0025)



ESM Figure 1: Scatter plots of islet autoantibody levels measured by radiobinding assay and ELISA assay for each autoantibody on the same samples from the IASP 2018 Workshop. The data is presented for the samples which were positive on the both assays**.** All three autoantibodies show high level of correlation between radiobinding assay and ELISA assays with Pearson's correlation coefficient of 0.91 (n=32, 95%CI 0.82-0.96) for GADA (A), 0.81(n=30, 95% CI 0.63-0.91) for ZnT8A (C) and slightly lower 0.56 (n=28 95%CI 0.24-0.77) for IA-2A (B).

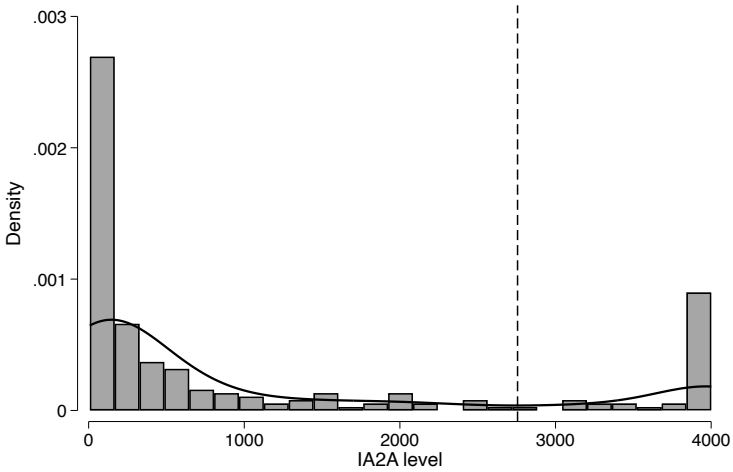


ESM Figure 2: Log autoantibody level density plots showing the two best fitting normal densities for GADA and IA-2A level distributions. A) Log GADA level density plot. Histogram and black dashed line show observed density. Red and green lines show the two best fitting normal densities which are used in the calculation of the likelihood ratio test. Likelihood ratio test: normal distribution versus two component mixture distribution: Log(GADA) ~ $N(\theta, \sigma^2)$ vs. Log(GADA) ~ $w_1N(\theta_1, \sigma_1^2)+w_2N(\theta_2, \sigma_2^2)$. LRT = 352 on 3 degrees of freedom, $p<5x10^{-76}$. B) Log IA-2A level density plot. Histogram and black dashed line show observed density. Red and green lines show the two best fitting normal densities which are used in the calculation of the likelihood ratio test**.** Likelihood ratio test: univariate normal distribution versus two component mixture distribution: Log(IA-2A) ~ $N(\theta, \sigma^2)$ vs. Log(IA-2A) ~ $w_1N(\theta_1, \sigma^2)+w_2N(\theta_2, \sigma^2)$. LRT = 1219 on 3 degrees of freedom, p-value $<5x10^{-264}$.



ESM Figure 3: Histogram with kernel density curve showing the distribution of glutamate decarboxylase autoantibody levels in patients with type 1 diabetes at diagnosis and no autoimmune thyroid disease. Histogram of GADA level at diagnosis measured using radiobinding assay for type 1 diabetes cases who were positive for GADA. GADA level exhibits a bimodal distribution when those with autoimmune thyroid disease was removed. The nadir value of 450 DK U/ml between the two modes is highlighted with the black dashed line.

A


.003
.002
.001
0
Density
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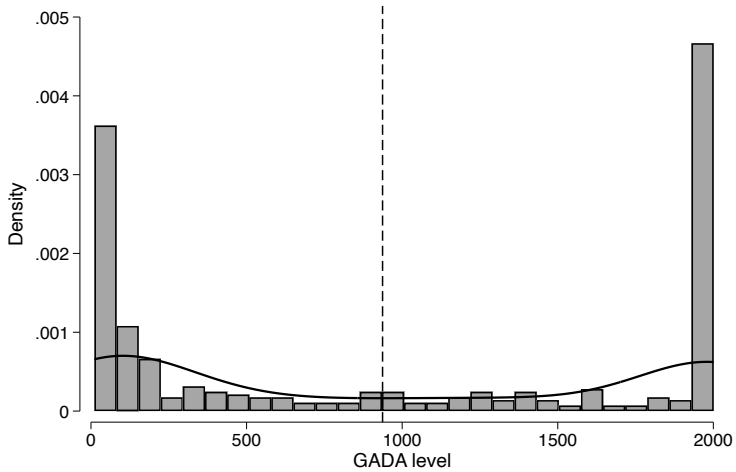
1000
2000
3000
4000
IA2A level

B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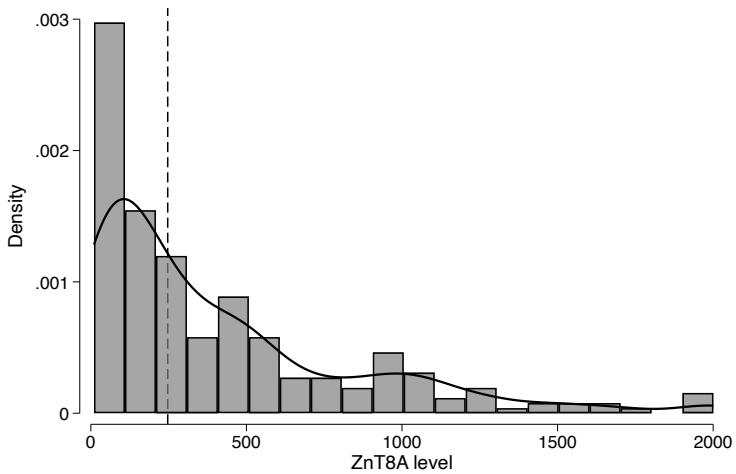
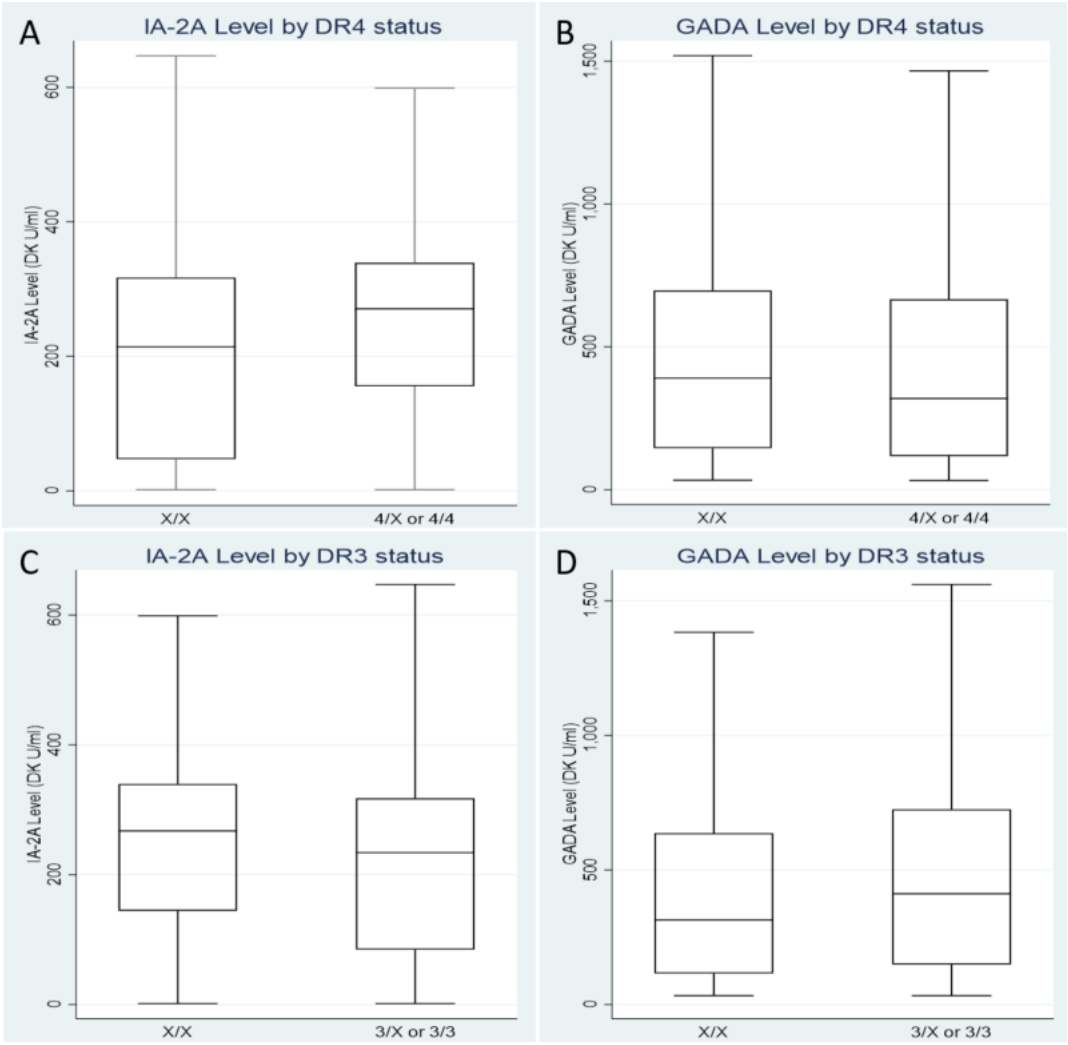
.004
.003
.002
.001
0
Density
0
500
1000
1500
2000
GADA level

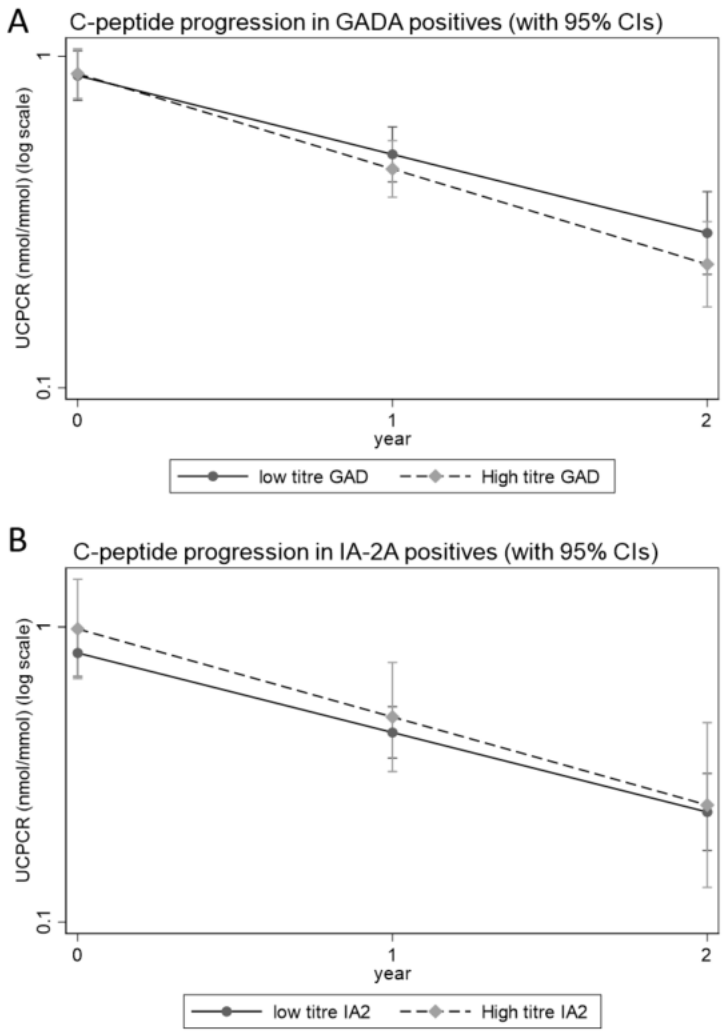
C


.003
.002
.001
0
Density
0
500
1000
1500
2000
ZnT8A level

ESM Figure 4: Histograms with kernel density curves showing the distribution of islet autoantibody levels at diagnosis measured using ELISA assay in patients with type 1 diabetes in the replication cohort (StartRight cohort). A) Histogram of GADA level for type 1 diabetes cases who were positive for GADA (n=404) at diagnosis. GADA levels exhibit a bimodal distribution. The ELISA assay was calibrated to maximum value of 2000 WHO U/ml. The nadir value of 937 WHO U/ml between the two modes is highlighted with black dashed line and used to defined high level group (≥937 WHO U/ml) and low level group (<937 WHO U/ml). B) Histogram of IA-2A level for type 1 diabetes cases who were positive for IA-2A (n=237) at diagnosis. IA-2A levels exhibit a bimodal distribution. The ELISA assay was calibrated to maximum value of 4000 WHO U/ml. The nadir value of 2756 WHO U/ml between the two modes is highlighted with black dashed line and used to defined high level group (≥2756 WHO U/ml) and low level group (<2756 WHO U/ml). C) Histogram of ZnT8A level for type 1 diabetes cases positive for ZnT8A (n=260) at diagnosis show a right skewed distribution. The ELISA assay was calibrated to maximum value of 2000 WHO U/ml. Median value of the distribution (247 AU/ml) is highlighted with black dashed lines.



ESM Figure 5: Box plots of GADA and IA-2A levels by HLA-DR4-DQ8 and HLA-DR3-DQ2. A-B) Higher IA-2A levels (371 DK U/ml [155, 339]) and lower GADA levels (319 DK U/ml [118, 667]) were observed in those with HLA-DR4-DQ8 compared to those without HLA-DR4-DQ8 (IA-2A: 214 DK U/ml [47, 317], $p=2x10^{-7}$ and GADA: 391 [146, 697], p=0.011). **C-D)** Those with HLA-DR3-DQ2 had higher GADA levels (412 DK U/ml [149, 725] vs. 314 DK U/ml [116, 63], p=0.0002) and lower IA-2A levels (234 DK U/ml [85, 318] vs. 267 DK U/ml [144, 340], p=0.0006) compared to those without HLA-DR3-DQ2.



ESM Figure 6: Urinary C-Peptide /Creatinine Ratio (UCPCR) decline in the first 2 years following diagnosis of type 1 diabetes in StartRight Study. [A] Log-linear c-peptide decline in those with high and low GADA levels. [B] Log-linear c-peptide decline in those with high and low IA-2A levels. High level GADA/IA-2A: dotted lines. Low level GADA/IA-2A: solid line.