SLC30A8 polymorphism and BMI complement *HLA-A*24* as risk factors for poor graft function in islet allograft recipients

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Abstract

Aims/hypothesis *HLA-A*24* carriership hampers achievement of insulin independence in islet allograft recipients. However, less than half of those who fail to achieve insulin independence carry the allele. We investigated whether genetic polymorphism at the recipients' zinc transporter 8-encoding *SLC30A8* gene (rs13266634) could complement their *HLA-A*24* status in predicting functional graft outcome.

Methods We retrospectively analysed data of a hospital-based patient cohort followed for 18 months post transplantation. Forty C-peptide-negative type 1 diabetic individuals who received >2 million beta cells (>4000 islet equivalents) per kg body weight in one or two intraportal implantations under similar immunosuppression were genotyped for *SLC30A8*. Outcome measurements included achievement and maintenance of graft function. Metabolic benefit was defined as <25% CV of fasting glycaemia in the presence of >331 pmol/l C-peptide, in addition to achievement of insulin independence and maintenance of C-peptide positivity.

Results In multivariate analysis, *HLA-A*24* positivity, presence of *SLC30A8* CT or TT genotypes and BMI more than or equal to the group median (23.9 kg/m²) were independently associated with failure to achieve insulin independence ($p = 0.015$ – 0.046). The risk increased with the number of factors present ($p < 0.001$). High BMI interacted with *SLC30A8* T allele carriership to independently predict difficulty in achieving graft function with metabolic benefit ($p = 0.015$). Maintenance of C-peptide positivity was mainly associated with older age at the time of implantation. Only *HLA-A*24* carriership independently predicted failure to maintain acceptable graft function once achieved ($p = 0.012$).

Conclusions/interpretation *HLA-A*24*, the *SLC30A8* T allele and high BMI are associated with poor graft outcome and should be considered in the interpretation of future transplantation trials.

Trial registration [ClinicalTrials.gov](https://clinicaltrials.gov) NCT00798785 and NCT00623610

Keywords Body mass index · Genetics of type 1 diabetes · HLA class I · Human islet transplantation · Zinc transporter 8

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Research in context

What is already known about this subject?

- We previously reported that carrying *HLA-A*24* hampers achievement of insulin independence in islet allograft recipients; however, less than half of those who failed to achieve insulin independence carry the allele
- Both BMI and genetic polymorphism (rs13266634) at the zinc transporter 8-encoding *SLC30A8* gene impact diabetes risk and insulin secretion, action and/or clearance

What is the key question?

- Do the rs13266634 polymorphism and recipients' BMI at baseline impact graft function, and could they complement *HLA-A*24* for prediction of functional outcome?

What are the new findings?

- *HLA-A*24* positivity, presence of *SLC30A8* CT or TT genotypes, and BMI greater than or equal to the group median (23.9 kg/m²) were independently associated with failure to achieve insulin independence, and risk increased with the number of factors present
- High BMI interacted with *SLC30A8* T allele carriership to independently predict difficulty in achieving graft function with a metabolic benefit
- Only *HLA-A*24* carriership independently predicted failure to maintain this graft function once achieved

How might this impact on clinical practice in the foreseeable future?

- These results may have practical implications for the selection, preparation and treatment of participants in future transplantation trials, the design of study protocols, and the interpretation of their results

Abbreviations

BW	Body weight
CVfg	CV of fasting glycaemia
IEQ	Islet equivalents
ZnT8	Zinc transporter 8

Introduction

In our clinical transplant protocol with cultured islet cell grafts (>2 million beta cells per kg body weight [BW] or >4000 islet equivalents [IEQ] per kg BW), *HLA-A*24*⁺ recipients less frequently achieved insulin independence within 6 months post implantation compared with *HLA-A*24*[−] individuals [1]. However, *HLA-A*24* was only present in less than half of the recipients who failed to achieve insulin independence. We therefore searched for additional baseline characteristics of recipients that could complement their *HLA-A*24* status in predicting graft function.

Since zinc transporter 8 (ZnT8) is a major antigenic target for autoreactive antibodies and CD8⁺ T lymphocytes in type 1 diabetes [2–4], we examined a correlation of graft outcome with genetic polymorphism at the *SLC30A8* gene, which encodes for this transmembrane protein that facilitates Zn²⁺

efflux from cells and sequestration into intracellular compartments [5, 6]. This protein is abundantly expressed in secretory granules of pancreatic beta cells [2] but is also present in pancreatic alpha cells and adipose tissue [7, 8]. A non-synonymous SNP in *SLC30A8* (rs13266634) encodes for either Arg (C allele) or Trp (T allele) at aa₃₂₅ in the intracellular carboxy-terminal domain of ZnT8. This polymorphic region appears highly immunogenic, as the nature of aa₃₂₅ determines the specificity of the autoimmune response in type 1 diabetes [2, 9]. We therefore hypothesised that ZnT8 polymorphism might influence functional graft outcome by modulating auto- and/or alloimmune responses. In addition, rs13266634 alleles and various *SLC30A8* mutations have been associated with differences in ZnT8 activity, insulin secretion, insulin sensitivity and clearance, and risk of type 2 diabetes and post-transplantation diabetes mellitus in renal allograft recipients [10–14]. Moreover, interactions between environmental factors and *SLC30A8* polymorphisms appear to modulate the risk of obesity in humans and in animal models [15, 16].

In the context of our previous short communication [1], we observed that in *HLA-A*24*[−] allograft recipients both failure to achieve insulin independence and development of an autoantibody surge post transplantation were associated with a higher BMI ($p = 0.005$ and $p = 0.019$, respectively) (unpublished observations from our previous analysis in reference

[1]). Excess BMI has also been proposed as a risk factor for type 1 diabetes development in autoantibody-positive relatives, and as a driver of beta cell loss in individuals with recent-onset type 1 diabetes [17, 18].

Given the importance of ZnT8 as a diabetes autoantigen and the reported interactions of its variants with components of the metabolic syndrome [13–16], we investigated whether, in recipients of an islet allograft, rs13266634 polymorphism and BMI at baseline impacted graft function and could complement *HLA-A*24* for prediction of functional outcome. To test this hypothesis, we determined rs13266634 polymorphism in recipients and donors included in a previous publication [1]. We therefore retrospectively analysed the impact of the two candidate genetic risk factors and BMI on graft function during an 18 month observation period post transplantation. In addition to achievement of insulin independence and maintenance of C-peptide positivity used in our previous report [1], we included achievement and maintenance of acceptable graft function (defined as basal C-peptide >331 pmol/l) associated with the metabolic benefit of a low variability in fasting glucose (defined as <25% CV of fasting glycaemia [CVfg]) as outcome criteria [19].

Methods

Participants Forty C-peptide-negative type 1 diabetic individuals (median age 45 years) who were beginning to have chronic complications and/or hypoglycaemic unawareness, with no pre-existing HLA class I or II antibodies [20, 21], received 2.3–9.0 million beta cells (~4600–18,000 IEQ) per kg BW in one ($n = 10$) or two ($n = 30$) intraportal implantations, with ≤ 6 months between the first and second grafts (ClinicalTrials.gov NCT00798785 and NCT00623610), under anti-thymocyte globulin–mycophenolate mofetil–tacrolimus immunosuppression [19, 22]. Data and DNA samples collected during the above clinical transplantation trials were made available by the principal investigator (BK) for additional analysis in our previous report [1] (database and analysis of HLA class I alleles including *HLA-A*24*) and in the present study (database and analysis of *SLC30A8* gene polymorphism). Compared with our previous study [1], one participant was excluded because immunosuppression had to be stopped due to the development of a melanoma 12 months post implantation. The study was approved by the Institutional Review Board of the University Hospital of the Vrije Universiteit Brussel; written informed consent was obtained from all participants [19, 22].

Assays and outcome measures Plasma C-peptide and proinsulin (to correct for proinsulin interference in the C-peptide assay) were measured by time-resolved fluorescence immunoassay on AutoDELFIA (PerkinElmer, Turku, Finland) [23,

24]. HbA_{1c} levels were determined by HPLC (Tosoh Bioscience, Tessenderlo, Belgium), and lymphocyte subclasses by an Epics XL flow cytometer (Beckman Coulter, Miami, FL, USA). C-peptide negativity [1] and acceptable graft function with a metabolic benefit [19] were defined as before. The time of achieving insulin independence was defined as the beginning of a period of at least 14 days without insulin injections. Autoantibodies against GAD, insulinoma-associated antigen-2 and ZnT8 were determined by liquid-phase radiobinding assays [25] at various time points within 6 months after the first islet implantation [1]. Significant autoantibody surges were defined as previously described [1, 26]. *HLA-DQ2/DQ8* and *HLA-A*24* were determined by allele-specific oligotyping [27], and ZnT8 genotypes by allelic discrimination (7900HT Fast Real-Time PCR System, TaqMan SNP Genotyping Assays, catalogue no. 4351379, and TaqMan Genotyping Master Mix, catalogue no. 4371355; Applied Biosystems, Foster City, CA, USA) [28]. The hyperglycaemic clamp procedure was performed as before [22]. C-peptide measurements at 120, 135 and 150 min of hyperglycaemia (10 mmol/l) were used to calculate C-peptide AUC [1]. Not all participants underwent the hyperglycaemic clamp test at 6, 12 and/or 18 months after the first implantation, because the procedure was implemented in the protocol when the follow-up of certain participants had already begun or was completed.

Statistical analysis The data were analysed with the Mann–Whitney U test, χ^2 test or Fisher’s exact test. Kaplan–Meier analysis with the logrank test was used to assess achievement of insulin independence, achievement and maintenance of acceptable graft function and maintenance of C-peptide positivity. The tested factors were compared with previously reported determinants for their capacity to act as independent predictors of insulin independence, achievement and maintenance of acceptable graft function and maintenance of C-peptide positivity, by means of time-dependent Cox regression analysis performed by a forward stepwise method. Only variables with p values <0.1 in the univariate analysis were entered into multivariate models with respect to the maximum number of variables allowed according to the Vittinghoff criterion [29]. All tests were performed two-sided using IBM SPSS for Windows, version 24.0 (IBM, Armonk, NY, USA). A p value <0.05 was considered significant.

Results

Occurrence of investigated risk factors among recipients Subgroups were defined on the basis of the occurrence of three selected risk factors: *HLA-A*24* status, *SLC30A8* polymorphism and BMI. Twelve recipients carried *HLA-A*24*. The CC, CT and TT *SLC30A8* genotypes at rs13266634 were

present in 19, 17 and four participants, respectively. Since carriers of the CT and TT genotypes did not differ in frequency of developing insulin independence (electronic supplementary material [ESM] Fig. 1), we used a dominant model with respect to the T allele (CC vs CT+TT). To investigate the impact of BMI at baseline, the study population was stratified according to the median (P50; 23.9 kg/m²) of the whole group (BMI <P50 vs BMI ≥P50).

Association of selected risk factors with probability of achieving graft function At post-transplantation month 2, HbA_{1c} levels were <47 mmol/mol (6.5%) in all recipients. Eighteen achieved insulin independence which lasted for a median period (range) of 16.5 (3.5–44.2) months. Within 12 months after the first implantation, 34 participants achieved CVfg <25% in the presence of random C-peptide >331 pmol/l, and in 18 participants this was sustained for ≥18 months after achieving the functional endpoint. Low HbA_{1c} levels (<47 mmol/mol [6.5%]) persisted less long, as all but four individuals presented with HbA_{1c} values ≥47 mmol/mol (6.5%) 18 months after the first implantation (not shown). *HLA-A*24*[−] individuals (*n* = 28) displayed a higher rate of achieving insulin independence compared with *HLA-A*24*⁺ individuals (*n* = 12; *p* = 0.036) (Fig. 1a), but metabolic outcome in terms of reduction in CVfg (to <25%) and of random C-peptide >331 pmol/l was similar for both subgroups (Fig. 1b). Progression rate to insulin independence was also higher in carriers of the *SLC30A8* CC genotype (*n* = 19) than

in CT- or TT-positive recipients (*n* = 21) (*p* = 0.022) (Fig. 1c), while again no significant difference was observed in the rate of achieving the two additional endpoints (Fig. 1d). The higher rate of reaching insulin independence in the presence of CC than in the presence of CT or TT could not be explained by differences in the degree of mismatching with the donors' *SLC30A8* genotype; indeed, the frequency of mismatches was higher for CC-positive recipients (0–83%) than for CT-positive recipients (0%), but lower than for TT carriers (67–100%). However, CT and TT carriers had similar functional outcomes, which were worse for both groups than for CC carriers (ESM Fig. 1 and ESM Table 1). The rate of reaching insulin independence was higher in participants with BMI <P50 (*n* = 20) than in those with BMI ≥P50 (*n* = 20); this was also the case for reaching the additional functional endpoints (*p* = 0.044 and *p* = 0.016, respectively) (Fig. 1e, f).

Association of selected risk factors with probability of maintaining graft function *HLA-A*24*[−] recipients tended to maintain C-peptide positivity longer during follow-up (*p* = 0.05) (Fig. 2a). Among recipients who achieved graft function with a metabolic benefit (CVfg <25% and random C-peptide >331 pmol/l), those who lacked *HLA-A*24* tended to maintain this good functional outcome longer compared with carriers of this allele (*p* = 0.007) (Fig. 2b). Maintenance of C-peptide positivity or graft function was not affected by *SLC30A8* genotype or BMI (Fig. 2c–f). Carriers of *HLA-A*24* needed more insulin (*p* = 0.035–0.003 depending on the time point)

Fig. 1 Development of insulin independence (a,c,e) and achievement of CVfg <25% and C-peptide >331 pmol/l (b,d,f) in islet allograft recipients. (a, b) *HLA-A*24*[−] (solid line) vs *HLA-A*24*⁺ participants (dashed line) (number of events and total number of participants per group: a, 16/28 vs 2/12; b, 24/28 vs 10/12) (c, d) *SLC30A8* CC (solid line) vs CT and TT (dashed line) (number of events and total number of participants per group: c, 12/19 vs 6/21; d, 18/19 vs 16/21). (e, f) BMI <P50 (solid line) vs BMI ≥P50 (dashed line) (number of events and total number of

participants per group: e, 12/20 vs 6/20; f, 19/20 vs 15/20). None of the 18 participants who became insulin-independent returned to C-peptide negativity within 18 months after the first transplantation. CVfg remained <25% in 15 of those 18 participants during 18 months of follow-up (not shown). Compared with our previous study [1], one participant was excluded because immunosuppression had to be stopped owing to the development of a melanoma 12 months post implantation. **p* < 0.05

Fig. 2 C-peptide-positive survival in all allograft recipients (**a,c,e**) and maintenance of CVfg <25% and C-peptide >331 pmol/l in recipients who achieved acceptable graft function (**b,d,f**). (**a, b**) *HLA-A*24*⁺ (solid line) vs *HLA-A*24*[−] participants (dashed line) (number of events and total number of participants per group: **a**, 24/28 vs 7/12; **b**, 16/24 vs 2/10). (**c, d**) *SLC30A8* CC (solid line) vs CT + TT (dashed line) (number of events and total number of participants per group: **c**, 16/19 vs 15/21; **d**, 10/18 vs 8/16). (**e, f**) BMI <P50 (solid line) vs BMI ≥P50 (dashed line)

(number of events and total number of participants per group: **e**, 16/20 vs 15/20; **f**, 12/19 vs 6/15). None of the 18 participants who became insulin-independent, returned to C-peptide negativity within 18 months after the first transplantation. CVfg remained <25% in 15 of those 18 participants during 18 months after reaching a clinically satisfactory graft function (not shown). Compared with our previous study [1], one participant was excluded because immunosuppression had to be stopped owing to the development of a melanoma 12 months post implantation. ** $p < 0.01$

(Table 1), and presented higher HbA_{1c} values and glycaemic variability compared with *HLA-A*24*[−] recipients during follow-up (Table 1). Also, their hyperglycaemic clamp-derived C-peptide AUC tended to decline more rapidly during follow-up (Table 1). None of these metabolic variables were significantly affected by the recipients' *SLC30A8* genotype or BMI (data not shown).

Complementary contribution of selected risk factors to failure of islet cell implants In the joint absence of the three risk factors (*HLA-A*24*, *SLC30A8* T allele and BMI ≥P50), six out of seven recipients achieved insulin independence; all seven recipients achieved the set criteria for metabolic graft function and six out of seven maintained it (Table 2). Interestingly, none exhibited a rise in autoantibodies following implantation, whereas such a rise occurred in ten out of 33 recipients when one of these risk factors was present (Table 2).

Interference with favourable outcome by one of the three examined risk factors was higher when at least one additional factor was present (Table 2). The complementarity of the selected risk factors in hampering the achievement or maintenance of the selected outcome measures was also evident when Kaplan–Meier analysis was performed according to the number of factors present (ESM Fig. 2 and ESM Fig. 3, respectively). In particular, we noticed that high BMI and presence of the *SLC30A8* T allele interacted to increase the risk of failure to achieve insulin independence ($p = 0.005$) and

satisfactory graft function ($p = 0.022$) in Kaplan–Meier analysis (ESM Fig. 4). Post-transplantation autoantibody surges tended to occur predominantly in recipients presenting with two or more risk factors (7/19 vs 3/21 in all other recipients; $p = 0.100$) (not shown).

Assessment of predictive power of tested risk factors vs that of previously reported determinants

All graft and recipient characteristics listed in ESM Tables 2–5 were first tested in univariate Cox regression analysis (data not shown). Only the variables that were significant in univariate analysis and a selection of previously reported potentially confounding risk factors at baseline are shown in Tables 3 and 4. BMI ≥P50, *HLA-A*24* positivity and presence of *SLC30A8* CT or TT genotypes were all independently associated with failure to achieve insulin independence (Table 3). In multivariate analysis, the risk increased with the number of risk factors (HR 0.31 [95% CI 0.16, 0.74] for zero to three risk factors; $p < 0.001$) (Table 3). Higher BMI was the only independent predictor of difficulty in achieving acceptable graft function (HR 0.46 [95% CI 0.23, 0.93]; $p = 0.029$) (Table 3); in multivariate analysis it was outperformed by the joint presence of higher BMI and *SLC30A8* T allele (HR 0.32 [95% CI 0.13, 0.80]; $p = 0.015$) (not shown). In the univariate analysis, maintenance of C-peptide positivity was mainly associated with older age at the time of implantation, in line with the 9th Annual Collaborative Islet Transplant Registry report [30];

Table 1 Insulin dose, HbA_{1c}, variability of fasting glycaemia, and C-peptide release during hyperglycaemic clamp tests at 6, 12 and 18 months after the first islet transplantation according to the presence or absence of *HLA-A*24*

Characteristic	<i>HLA-A*24</i> [−] (<i>n</i> = 24)	<i>HLA-A*24</i> ⁺ (<i>n</i> = 10)	<i>p</i> value
Insulin dose, U kg ^{−1} day ^{−1} ; P50 (IQR)			
6 months	0.02 (0.00–0.20)	0.20 (0.12–0.27)	0.035
12 months	0.09 (0.00–0.22)	0.35 (0.24–0.48)	0.003
18 months	0.14 (0.07–0.29)	0.40 (0.28–0.46)	0.035
HbA _{1c} , mmol/mol; P50 (IQR)			
6 months (<i>n</i> = 1) ^a	46 (43–50)	56 (46–65)	0.054
12 months (<i>n</i> = 1) ^a	44 (40–47)	47 (37–58)	NS
18 months (<i>n</i> = 1) ^a	49 (43–54)	59 (52–73)	0.010
HbA _{1c} , %; P50 (IQR)			
6 months (<i>n</i> = 1) ^a	6.4 (6.1–6.8)	7.3 (6.4–8.1)	0.054
12 months (<i>n</i> = 1) ^a	6.2 (5.9–6.5)	6.5 (5.6–7.5)	NS
18 months (<i>n</i> = 1) ^a	6.7 (6.1–7.1)	7.6 (7.0–8.9)	0.010
CVfg, %; P50 (IQR)			
6 months (<i>n</i> = 0) ^a	11 (8–21)	15 (10–20)	NS
12 months (<i>n</i> = 0) ^a	14 (9–24)	30 (17–42)	0.007
18 months (<i>n</i> = 2) ^a	18 (13–23)	27 (17–35)	0.075
AUC C-peptide ^b , pmol l ^{−1} min ^{−1} ; P50 (IQR)			
6 months (<i>n</i> = 14) ^a	818 (655–917)	494 (322–701)	0.013
12 months (<i>n</i> = 14) ^a	721 (511–874)	341 (60–530)	0.032
18 months (<i>n</i> = 11) ^a	466 (387–602)	190 (51–615)	0.089

Data are expressed as median (interquartile range [IQR])

Data from participants with acceptable graft function (CVfg <25% and random C-peptide >331 pmol/l)

^a Number of participants with missing data for each variable of interest (one participant had unreliable HbA_{1c} values due to a genetic condition. The two participants with no per cent CVfg data at 18 months after the first implantation lost acceptable graft function before 12 months). Not all participants underwent the hyperglycaemic clamp test at 6, 12 and/or 18 months after the first implantation, because the procedure was implemented in the protocol during or after follow-up

^b AUC for the second phase (120–150 min) of the hyperglycaemic clamp test

multivariate analysis was not performed for this endpoint due to the low number of events (*n* = 9) (not shown). *HLA-A*24* carriership was the only independent predictor of failure to maintain satisfactory graft function (HR 0.28 [95% CI 0.10, 0.75]; *p* = 0.012) (Table 4). Graft and recipient characteristics listed in Tables 3 and 4 did not differ according to the presence or absence of *HLA-A*24* (ESM Table 2), *SLC30A8* T allele (ESM Table 3), BMI ≥P50 (ESM Table 4) or at least one risk factor (ESM Table 5).

Discussion

We previously reported that the presence of *HLA-A*24* was associated with a higher risk of failure to achieve insulin independence after implantation of a beta cell graft. In the present study, we identified two additional risk factors, i.e. presence of the T allele at rs13266634 of the *SLC30A8* gene and high BMI. The three baseline characteristics carry an independent risk which is increased with the number of factors present. All

Table 2 Frequency of achieving or maintaining clinical endpoints, and lack of developing autoantibody surges within 6 months after the first islet implantation, according to the number of risk factors present

Presence of studied risk factors	None	≥1	≥2	<i>p</i> value ^a	<i>p</i> value ^b
Achieving insulin independence	6/7	12/33	2/19	0.017	<0.001
Achieving acceptable graft function	7/7	27/33	14/19	0.221	0.131
Maintaining C-peptide positivity	7/7	24/33	13/19	0.117	0.090
Maintaining acceptable graft function	6/7	12/27	4/14	0.051	0.013
Absence of autoantibody rise	7/7	23/33	12/19	0.093	0.060

Risk factors are *HLA-A*24*⁺, *SLC30A8* T⁺ and/or BMI ≥P50

^a 0 factors vs ≥1 factor

^b 0 factors vs ≥2 factors

Table 3 Cox regression analysis of patient and graft characteristics associated with achieving insulin independence or acceptable graft function within 18 months after the first implantation in 40 recipients of an islet allograft

Independent variable	Achievement of insulin independence ^a			Achievement of acceptable graft function ^{b,c}	
	Univariate <i>p</i> value	Multivariate <i>p</i> value (HR; 95% CI)	Multivariate <i>p</i> value (HR; 95% CI)	Univariate <i>p</i> value	Multivariate <i>p</i> value (HR; 95% CI)
Age at first implantation, years	0.376	NM	NM	0.418	NM
BW, kg	0.753	NM	NM	0.505	NM
T lymphocyte count, CD3 ⁺ cells/mm ³	0.956	NM	NM	0.974	NM
B lymphocyte count, CD19 ⁺ cells/mm ³	0.656	NM	NM	0.892	NM
No. of beta cells, T × 1, ×10 ⁶ /kg	0.894	NM	NM	0.254	NM
No. of beta cells, total, ×10 ⁶ /kg	0.342	NM	NM	0.184	NM
Insulin dose, U ⁻¹ kg ⁻¹ day ⁻¹	0.892	NM	NM	0.589	NM
HbA _{1c} , mmol/mol (<i>n</i> = 1) ^d	0.095	NM	0.524 ^d	0.588	NM
BMI ≥P50 (0, 1) ^e	0.045	0.015 (0.29; 0.10, 0.78)	NM	0.029	0.029 (0.46; 0.23, 0.93)
<i>HLA-A*24</i> ⁺ (0, 1) ^f	0.022	0.032 (0.19; 0.04, 0.87)	NM	0.852	NM
<i>SLC30A8</i> T ⁺ (0, 1) ^g	0.030	0.046 (0.36; 0.13, 0.98)	NM	0.226	NM
<i>HLA-A*24</i> ⁺ / <i>SLC30A8</i> T ⁺ /high BMI (no. of risk factors) ^h	<0.001	NM	<0.001 (0.31; 0.16, 0.74)	0.057	0.464

^a 18 events^b Defined as CVfg <25% and random C-peptide >331 pmol/l^c 34 events^d No. of participants with missing data for each variable of interest; no participants had to be excluded from being entered in the different multivariate analyses because of missing values, except for one participant with missing HbA_{1c} in the second model for insulin independence (this participant had unreliable HbA_{1c} values due to a genetic condition)^e BMI <P50 (0) vs BMI ≥P50 (1)^f *HLA-A*24*⁻ (0) vs *HLA-A*24*⁺ (1)^g *SLC30A8* T allele⁻ (0) vs *SLC30A8* T allele⁺ (1)^h Participants with 0, 1, 2 or 3 risk factors among *HLA-A*24*⁺, *SLC30A8* T⁺ and BMI ≥P50

NM, not selected for entry in the multivariate model

participants lacking the three complementary risk factors achieved and maintained graft function with a metabolic benefit and none experienced a significant post-implantation surge in autoantibody levels, which is considered an indicator of poor functional outcome [1, 31, 32].

A higher BMI—mostly in combination with positivity for the rs13266634 T allele—conferred a lower probability of achieving acceptable graft function (defined as <25% CVfg and >331 pmol/l C-peptide) within the first months post transplantation. By contrast, failure to maintain this level of function was only significantly associated with *HLA-A*24* positivity of the recipient, in parallel with a more rapid decline of hyperglycaemic clamp-derived C-peptide release and metabolic control in this group.

The use of glycaemic variability—a surrogate marker for risk of severe hypoglycaemia and a close and inverse correlate of restored functional beta cell mass [19]—as a functional outcome measure is a strength of the study, as is the homogeneity of the patient cohort. All initially C-peptide-negative participants received a sufficient amount of beta cells to generate plasma C-peptide levels at post-transplantation month 2 under

similar immunosuppression [1, 21]. The relatively small cohort size constitutes a weakness of our study which may have caused some variables to remain non-significant in Cox regression analysis (Tables 3 and 4). Our observations could also have benefited from a larger number of participants in the subgroups, in particular carriers of the *SLC30A8* TT genotype. Our retrospective analysis did not allow us to evaluate insulin sensitivity by euglycaemic–hyperinsulinaemic clamp, nor by surrogate markers such as waist circumference.

Our observations that *HLA-A*24* carriership, presence of the *SLC30A8* T allele and high BMI of the recipients impact transplant outcome, alone or in combination, do not deny the importance of other well-established graft and recipient characteristics, related, but not limited, to islet isolation procedure, islet cell quality and numbers, pre-existing auto- and alloimmunity, and instant blood-mediated inflammatory reaction post transplantation [22, 31, 33–39]. However, we have several reasons to believe that our findings are largely independent of these factors. First, when stratified according to the presence or absence of (a combination of) the three investigated risk factors, participants did not differ according to a large

Table 4 Cox regression analysis of patient and graft characteristics associated with maintenance of acceptable graft function (CVfg <25% and random C-peptide >331 pmol/l) within 18 months after achieving satisfactory graft function in the 34 participants who reached this target after implantation

Independent variable	Maintenance of acceptable graft function ^a	
	Univariate <i>p</i> value	Multivariate <i>p</i> value (HR; 95% CI)
Age at first implantation, years	0.521	NM
BW, kg	0.055	0.065
T lymphocyte count, CD3 ⁺ cells/mm ³	0.494	NM
B lymphocyte count, CD19 ⁺ cells/mm ³	0.718	NM
No. of beta cells, T × 1, ×10 ⁶ /kg	0.836	NM
No. of beta cells, total, ×10 ⁶ /kg	0.843	NM
Insulin dose, U kg ⁻¹ day ⁻¹	0.615	NM
HbA _{1c} , mmol/mol (<i>n</i> = 1) ^b	0.255	NM
BMI ≥P50 (0, 1) ^c	0.225	NM
<i>HLA-A*24</i> ⁺ (0, 1) ^d	0.012	0.012 (0.28; 0.10, 0.75)
<i>SLC30A8</i> T ⁺ (0, 1) ^e	0.989	NM

^a 16 events of loss of acceptable graft function

^b The number of participants with missing data for each variable of interest

^c BMI <P50 (0) vs BMI ≥P50 (1)

^d *HLA-A*24*⁻ (0) vs *HLA-A*24*⁺ (1)

^e *SLC30A8* T⁻ (0) vs *SLC30A8* T⁺ (1)

NM, Not selected for entry in the multivariate model

number of graft and recipient characteristics (ESM Tables 2–5). Second, none of the participants had pre-existing HLA class I and II antibodies, and their transplant outcome was already previously shown to be unaffected by pre-existing islet autoantibody status [1, 31, 36, 37]. Third, all participants received at least 2 million beta cells (4000 IEQ) per kg BW, an amount previously shown to consistently induce graft function with a metabolic benefit [22]. Finally, excessive beta cell loss in the first hour post implantation could not have biased our results, because only two participants showed a disproportionate rise in plasma GAD65 1 h after implantation of their first islet cell graft [38].

Our results confirm and extend our previous observations on the accelerated loss of implanted beta cells in *HLA-A*24*⁺ recipients, regardless of the number of mismatches for this allele with donor HLA [1], and are consistent with reports of an association of the allele with a more rapid progression to clinical onset in autoantibody-positive individuals at risk of type 1 diabetes, and accelerated decline of residual beta cell mass in those with recent-onset type 1 diabetes [27, 40]. Currently, transplantation centres do not preselect recipients on the basis of HLA composition, which limits the value of this observation. Nevertheless, our results re-emphasise that, in order to compare the efficacy of islet allotransplantation protocols or the prognostic value of novel biomarkers, results should be adjusted for the recipients' *HLA-A*24* status. They also raise the question as to whether the immunosuppressive regimen should be adapted for recipients carrying the allele. Although the exact mechanism by which *HLA-A*24* affects

transplant outcome remains unknown, one might envisage testing a therapy that better targets autoimmune aggression than anti-thymocyte globulin, for example a T cell-directed immunodepletion strategy using an anti-CD3 monoclonal antibody (e.g. oteelixizumab) with or without an anti-B cell therapy (e.g. rituximab) [39, 41].

To the best of our knowledge, our finding that the presence of the *SLC30A8* T allele combines with BMI ≥P50 (in our cohort, 23.9 kg/m²) to increase insulin needs in allograft recipients and to hamper the achievement of acceptable graft function is novel. The effect of the recipient's T allele could not be explained by the degree of mismatching with the donor's *SLC30A8* alleles. Indeed, while CT carriers had a lower frequency of mismatches and TT carriers had a higher frequency of mismatches compared with CC-positive recipients, both CT- and TT-positive individuals had overall a worse functional outcome compared with CC-positive participants. Hence, our findings provide no support for the possibility that ZnT8 polymorphism might have impacted allo- or autoimmune responses, as might have been hypothesised from the immunogenicity of the ZnT8 epitope co-determined by rs13266634 [9]. Rather, they may suggest a role for the recipients' ZnT8 activity in modulating insulin sensitivity, and imply that cell types other than beta cells are likely to be involved in the process. Expression of ZnT8 has indeed been reported in pancreatic alpha cells and in adipose tissue [7, 8], and altered levels of transporter activity have been linked with functional or metabolic changes in animal models [7, 16]. It is, however, unknown through which mechanisms altered non-beta cell

ZnT8 activity may impact insulin sensitivity and interact with the effect of raised BMI in humans.

Our observation of a worse functional outcome in allograft recipients carrying the *SLC30A8* T allele is consistent with reports of a less preserved beta cell function, higher insulin needs and lower insulin sensitivity in children and adolescents with type 1 diabetes carrying the TT genotype or the T allele, compared with individuals with the CC genotype [42, 43]. Our results are in apparent contradiction to the reported association of the C allele with increased risk of type 2 diabetes and post-transplantation diabetes in kidney allograft recipients [11, 12]. Based on the available knowledge, it is difficult to find a unifying explanation for the seemingly conflicting available data on the relationship between *SLC30A8* variants, ZnT8 activity and risk of diabetes (progression). Indeed, while the type 2 diabetes risk allele (C) was associated with lowered ZnT8 activity and beta cell function [10, 12, 44], rare loss-of-function mutations in *SLC30A8* were found to confer protection against type 2 diabetes [13]. It has been suggested that the risk of diabetes varies according to the balance between pancreatic hormone secretion and clearance, as influenced by the complex interplay between changes in ZnT8 activity and circulating Zn^{2+} concentrations [10, 14].

Although a BMI >28 or $>26 \text{ kg/m}^2$ (depending on the studies) [33–35] is being used as an exclusion criterion in islet allotransplantation, overweight (BMI $>25 \text{ kg/m}^2$) at baseline failed to predict graft function when considered in isolation, but did so when combined with a family history of type 2 diabetes [45]. Our study shows that even a BMI in the upper-normal range might reduce the probability of achieving insulin independence and an acceptable graft function. As BMI is a continuous variable, further studies on larger cohorts should determine from which threshold onwards the risk starts to increase. In our cohort, the effect of BMI became apparent from 23 kg/m^2 onwards (not shown). Consistent with those of Leitão et al [45], our results in addition indicate that the effect of BMI is amplified by the presence of other risk factors. In particular, failure to achieve insulin independence and acceptable graft function with a metabolic benefit was most manifest in the combined presence of high BMI and the *SLC30A8* T allele (ESM Fig. 4). When participants were stratified according to the presence or absence of high BMI and T allele we observed no difference in baseline insulin needs. Therefore, the latter could not explain the interaction between both risk factors. This is not to deny that the effect of high BMI (greater than or equal to the median of the whole group) on transplant outcome tended to be more pronounced in those with higher insulin needs at baseline (greater than or equal to the median of the whole group) (not shown), in line with previous observations [35, 46]. Our results suggest that candidate recipients of an islet allograft who have a BMI in the upper-normal range or who are slightly overweight should be asked to lose weight before being put on a waiting list.

In conclusion, we identified three recipient characteristics—*HLA-A*24*, the *SLC30A8* T allele and high BMI—that act as independent risk factors for failing to achieve insulin independence following islet allotransplantation and contribute in various ways to hampering the achievement or maintenance of a graft function with metabolic benefit. These results should be confirmed in independent cohorts, as they may have practical implications for the selection, preparation and treatment of participants in future transplantation trials, the design of study protocols and the interpretation of their results.

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Data availability The datasets generated and/or analysed during the current study are available from the principal investigator of the transplantation trial (BK) upon reasonable request.

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Contribution statement EMB, SD, BK and FKG designed the study and acquired, researched and interpreted the data. EMB and SD performed the statistical analyses. DL, PG, RH, UVdV, BJvDA, ZL, BOR and DGP contributed to the data acquisition, analysis and interpretation. EMB and FKG drafted the manuscript and SD, DL, PG, RH, UVdV, BJvDA, ZL, BOR, DGP and BK revised it critically. All authors approved the final version of the manuscript. FKG and BK 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.

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