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Polygenic Risk Scores for Islet Autoantibody Positive Early-Stage Type 1 Diabetes Pre-Screening

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ABSTRACT

Aims: To develop a sex-aligned polygenic risk score (PRS) for early-stage type 1 diabetes (T1D) in children without a first-degree family history of T1D.

Materials and Methods: Global Single Nucleotide Polymorphism (SNP) Array was performed in 16 335 individuals without a first-degree family history of T1D, including 13 956 newborns from the background population of Bavaria, Germany, 240 children with early-stage and 1094 with islet autoantibody-positive Stage 3 T1D from Bavaria as cases and a validation cohort of 695 patients with clinical T1D. Previously developed PRS were assessed and sex-aligned PRS for boys and for girls were developed from 150 SNPs defining 14 HLA DR-DQ haplotypes and 143 T1D genetic susceptibility regions.

Results: PRS were similar between children with early-stage T1D and stage 3 T1D for the GPPAD GRS (median 12.8 vs. 12.8, $p = 0.81$) and the GRS2 (median 13.8 vs. 13.9, $p = 0.1$). At thresholds corresponding to the 99.2 centile of the background population, the estimated risk for early-stage T1D was higher in boys than girls using both the GPPAD GRS (10.7% in boys vs. 6.8% in girls) and GRS2 (16.4% vs. 8.6%). Novel full (150 SNP) and short (38 SNP) sex-aligned early-stage T1D PRS discriminated cases from controls with around 90% area under the receiver operator characteristic curve in boys and girls. Estimated risks for boys and girls at the 99.2 centile of the background population using the sex-aligned PRS converged, but differences were not eliminated (12.6% for boys, 9.3% for girls).

Conclusions: PRS in children with early-stage and Stage 3 T1D are highly comparable, but suggest a sex bias in early-stage Type 1 diabetes risk in children with high PRS. A sex-aligned PRS was predicted to reduce, but not eliminate, sex-related differences in predicted risk. The PRS was developed primarily in a paediatric population of predominantly European ancestry and requires independent external validation, including in other ancestry groups.

1 | Introduction

Type 1 diabetes (T1D) polygenic risk scores (PRS) are used to enrol infants into primary prevention trials [1] and are

proposed as a pre-screening step for identifying early-stage T1D in the general population [2]. Currently used PRS are the GPPAD GRS [1] and the GRS2 [3]. The GPPAD GRS was developed primarily for identifying children with very high risk

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for islet autoimmunity among those with high-risk HLA genotypes and has been used to screen newborns for primary prevention trials [4]. The GRS2 was developed from large-scale clinical (Stage 3) T1D case-control studies and incorporates more sophisticated discrimination of HLA associated risk than the GPPAD GRS. While these are shown to be capable of identifying individuals with elevated risk for early-stage type 1 diabetes, it remains unknown whether the PRS in individuals with early-stage T1D is the same as in individuals who have Stage 3 T1D or whether the PRS needs to be modified for early-stage T1D screening. Furthermore, current PRS do not consider age or sex differences in the disease.

Sex influences the risk of autoimmune diabetes in some animal models [5]. In humans, a slightly increased incidence of T1D is observed in boys as compared to girls in populations of European descent [6]. Although no sex differences are reported for genes contributing to T1D susceptibility, the risk for developing islet autoimmunity in children selected by HLA genetic susceptibility is increased in boys as compared to girls at a young age [7]. In contrast, the rate of progression to Stage 3 T1D in children with islet autoimmunity is faster in girls [8]. Sex, therefore, may modulate the processes leading to T1D.

Here, we compared PRS in children with early-stage and Stage 3 T1D in Bavaria, Germany. We also addressed sex-associated differences in the genetic susceptibility for early-stage T1D and developed sex-aligned PRS for pre-screening children without a first-degree family history of T1D.

2 | Materials and Methods

2.1 | Participants

2.1.1 | Background Population

Newborns from Bavaria, Germany who were genetically screened in the Frederik study in 2018 and 2019 and for whom consent for extended genetic typing was provided were used as the background population [1, 9]. Since the overall objective was to develop a PRS for children without a first-degree family history of T1D, all newborns for whom a first-degree family history of T1D was recorded ($n=364$) were excluded, leaving 13956 newborns with genetic data for analysis (7083 boys, 6873 girls). Newborns from 2018 ($n=8581$) were allocated to the test group and newborns from 2019 ($n=5375$) to the validation group (Table 1). There was no overlap between the test and validation groups. Additionally, 350 newborns with an HLA DR3/DR4-DQ8 or a DR4-DQ8/DR4-DQ8 genotype and no first-degree family history of T1D screened outside the 2018–2019 period were included for the development of a PRS in HLA-selected individuals.

2.1.2 | Early-Stage T1D Population

Children without a first-degree family history from the FrIda study (Bavaria, Germany) who were multiple islet autoantibody-positive and who provided consent and a sample for genetic testing and where genetic typing passed quality assessment ($n=240$;

134 boys, 106 girls; Table 1) were included [10]. The median age at diagnosis of early-stage T1D was 4.3 years (IQR, 3.2–5.4).

2.1.3 | Stage 3 T1D Population

Children and adolescents with Stage 3 T1D in the Bavarian Dimelli study who were aged <18 years at the onset of disease (median, 10.4 years; IQR, 7.0, 13.2), had at least one islet autoantibody, did not have a first-degree family history of T1D and where genetic typing passed quality assessment were included ($n=1094$; 632 boys, 462 girls; Table 1) [11]. As a validation group, the T1D proband parents of children enrolled into the BABYDIAB study were included ($n=695$, 231 males, 464 females) [12]. The median age at diagnosis was 18.4 years (IQR 13.5–23.7). There was no overlap between the test and validation groups.

2.1.4 | POInT Study

The Primary Oral Insulin Trial (POInT) trial enrolled infants with >10% estimated risk for developing early-stage T1D into a primary prevention trial [9]. The trial included 495 children (255 boys, 240 girls) without a first-degree family history of T1D who had HLA DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 and a PRS >14.4 as previously described [1].

2.2 | Genotyping

The Illumina ImmunoChip Array or the Illumina Global Screening Array was used to genotype DNA samples. Additionally, all the infants screened in the background cohorts were genotyped for 51 single nucleotide polymorphisms (SNPs) in a central genotyping lab (LGC group, Teddington, Middlesex, UK [<https://www.lgcgroup.com>]) as previously described [1]. For Illumina array typing, SNPs and samples with a <90% SNP call rate, samples with high heterozygosity ($\pm 3SD$), samples with a sex mismatch between reported and imputed sex and highly related individuals (PI HAT >0.9) were removed. The remaining samples were imputed using the Haplotype Reference Consortium on the Sanger imputation server [13]. The reference panel version was HRC r1.1 2016 (GRCh37/hg19) and phasing was done with Eagle v2.4.1.

Differences between the genotyping arrays were evaluated by assessing the concordance of genotype calls generated by the central genotyping laboratory (LGC Group, Teddington, Middlesex, UK) in a subset of samples that had been genotyped on both platforms. The concordance rate between the central laboratory and the Illumina ImmunoChip Array was 99.46%, whereas the concordance rate between the central laboratory and the Illumina Global Screening Array was 99.83%. Post-imputation checks were performed using ic v1.10.0 (<http://www.well.ox.ac.uk/~wrayner/tools/Post-Imputation.html>). Variants with MAF <0.01, Hardy Weinberg p value (pHWE) <10^{−4} and INFO score <30% were removed.

Seven SNPs for HLA DR-DQ risk genotypes and 171 additional candidate SNPs were selected from previous reports of associations with T1D (Table S1) [3, 4, 14, 15]. Twelve of these SNPs

TABLE 1 | Demographics of Control and Case cohorts used in the study.

Characteristic	Background test N= 8581	Background validation N= 5375	Early-stage T1D N= 240	Stage 3 T1D N= 1094	Stage 3 T1D validation N= 695	Background risk HLA N= 350
Sex						
Female; n (%)	4226 (49%)	2647 (49%)	106 (44%)	462 (42%)	464 (67%)	152 (43%)
Male; n (%)	4355 (51%)	2728 (51%)	134 (56%)	632 (58%)	231 (33%)	198 (57%)
Age ^a ; years (IQR)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	4.3 (3.2, 5.4)	10.4 (7.0, 13.2)	18.4 (13.5, 23.7)	0.0 (0.0, 0.0)
Inclusion in analyses:						
Assessing PRS sex bias	Yes	Yes	Yes	Yes		
Developing PRS	Training	Validation	Training	Training	Validation	
GPPAD-adapted sex-aligned PRS	Yes	Yes	Yes	Yes		Yes

^aAge is given for the sample collection in the Background cohorts and at diagnosis in the early-stage and Stage 2 type 1 diabetes cohorts.

were missing in the imputed data, 4 had a minor allele frequency (MAF) <0.01, and 12 were highly correlated ($R^2 > 0.9$) with other SNPs in the selected list, leaving a total of 150 SNPs in the analysis.

2.3 | Polygenic Risk Scores

The GPPAD GRS and the GRS2 were calculated as previously defined [1, 3]. For the development of sex-aligned PRS, seven SNPs were used to categorize HLA risk genotypes as *DR4-DQ8/DR4-DQ8*, *DR3/DR4-DQ8*, *DR4-DQ7/DR4-DQ7*, *DR3/DR4-DQ7*, *DR4-DQ7/DR4-DQ8*, *DR4-DQ8/DRx P*, *DR4-DQ8/x NP*, *DR4-DQ7/DRx P*, *DR4-DQ7/DRx NP*, *DR3/3*, *DR3/DRx P*, *DR3/DRx NP*, *DRx/DRx P* and *DRx/DRx NP*, where x represents the non-DR3 alleles and non-DR4-DQ8 haplotypes, P and NP represent the presence or absence of protective alleles *DRB1*1501*, *DQB1*0503* or *DRB1*1303*. The remaining 143 SNPs were encoded as 0, 1 or 2 depending on the number of copies of the susceptibility allele present. Full polygenic risk scores (full-PRS) were built using the HLA genotypes and the 143 SNPs independently for males and females. PRS using reduced numbers of SNPs were developed after selection using a penalized logistic regression and a rjMCMC.

2.4 | Statistical Analyses

ROC curves were calculated using the R package pROC version 1.18.5 [16]. All other analyses were performed in R version 4.3.1 [17]. The risk score weights were calculated using a multivariable logistic regression with the 14 HLA genotypes derived from 7 SNPs and the additional 143 SNPs as independent variables. A reduced SNP PRS was developed with selection of the most important variables by a penalized logistic regression (PLR) using the R package Tidymodels version 1.2.0 [18] and a reversible-jump Markov chain Monte Carlo (rjMCMC) as described in Winkler et al. [19] using the R package NIMBLE version 1.2.0 [20]. The 25 highest-ranking variables in importance, obtained using the R package VIP version 0.4.1 [21], in any of the four models (PLR males, PLR females, rjMCMC males, rjMCMC females) were included in the

reduced SNP PRS. For all PRS, the weights assigned to each of the SNPs and variables in the model were evaluated using 5000 bootstrap samples, stratified by outcome group, and summarized using bootstrap medians and percentile-based 95% confidence intervals. Model calibration was assessed using the calibration slope and calibration-in-the-large, while overall predictive accuracy was evaluated using the Brier score. The incremental predictive performance of the new PRS compared with the GRS2 was assessed using the net reclassification improvement (NRI) and integrated discrimination improvement (IDI), calculated with the package PredictABEL version 1.2–4 [22]. Thresholds in the background population for modelling risk were selected as 99.2 centile since this represented the background threshold derived from GPPAD eligibility criteria for the POINt trial [9], and at the 90th centile as an example for pre-selection in general population islet auto-antibody screening. Risk estimates were made using a 0.5% risk for early-stage T1D in the population [23] and calculated as Risk estimate (%) = frequency of cases above the threshold/frequency of controls above the threshold * 0.5. The positive predictive value (PPV) was also calculated using a 0.5% risk for early-stage T1D and calculated as:

$$\begin{aligned} \text{PPV} = & 100 * \text{frequency of cases above the threshold} * 0.5 \\ & / (\text{frequency of cases above the threshold} * 0.5) \\ & + (\text{frequency of controls above the threshold} * 99.5). \end{aligned}$$

3 | Results

3.1 | Genetic Similarity Between Early-Stage and Stage 3 Type 1 Diabetes

To determine whether PRS derived from Stage 3 T1D case-cohort studies are also applicable to early-stage T1D, we compared the GPPAD PRS and the GRS2 between the early-stage T1D ($n=240$) and Stage 3 T1D ($n=1094$) cohorts (Figure 1). The median GPPAD GRS was 12.8 (IQR, 11.7–13.9) in children with early-stage T1D and 12.8 (IQR, 11.8–13.9) in children with Stage 3 T1D ($p=0.8$). Similarly, the median GRS2 was 13.8 (IQR, 12.5–14.9) in children with early-stage T1D and 13.9 (IQR,

12.8–15.2) in children with Stage 3 T1D ($p=0.1$). The AUCs of the ROC curve for Stage 3 vs. early-stage T1D were 50.5% for the GPPAD GRS (95% CI, 46.3%–54.6%) and 53.4% for GRS2 (95% CI, 49.3%–57.5%). Therefore, children identified as early-stage T1D from the general population and without a first-degree family history of T1D were genetically similar to children with Stage 3 T1D.

3.2 | Predicted Risks for T1D Are Higher in Boys

Having established genetic similarity between children with early-stage and Stage 3 T1D, we reasoned that the majority of the children with early-stage T1D would likely develop Stage 3 type 1 diabetes before adulthood and that the early-stage and autoantibody positive people with Stage 3 T1D would form a relatively homogeneous group. We, therefore, proceeded in pooling these as our test case cohort to assess estimated risks in boys versus girls using the GPPAD GRS and the GRS2 (Figure 2a–c). Within the combined background test and validation dataset, eligibility criteria for GPPAD trials defined by a threshold of 14.4 and the presence of either an HLA DR3/DR4-DQ8 or DR4-DQ8/DR4-DQ8 genotype identified 111 (0.8%) of 13956 infants without a first-degree family history as eligible, including 46 (0.6%) of 7083 boys and 65 (0.9%) of 6873 girls ($p=0.1$). The number of cases (early-stage and Stage 3 T1D) with these genetic criteria was 179 (13.4%) of 1334 and included 106 (13.8%) of 766 boys and 73 (12.9%) of 568 girls, yielding a predicted risk of 10.7% for boys and 6.8% for girls.

At the 99.2 centile, the GRS2 threshold was 15.5. A total of 42 (0.6%) of 7083 boys and 69 (1.0%) of 6873 girls from the background population had a GRS2 above this threshold ($p=0.0084$). In comparison, 247 (18.5%) cases had a GRS2 above 15.5, including 19.5% of boys and 17.3% of girls, resulting in predicted risks of 16.4% in boys and 8.6% in girls. Differences between boys and girls remained at lower centiles of the background population (Figure 2c). Since the GRS2 has been proposed for selection of

individuals for islet autoantibody screening, we also examined performance in boys and girls at 90th centile. This threshold (GRS2, 13.4), identified 671 (9.5%) of 7083 boys and 724 (10.5%) of 6873 girls from the background population ($p=0.039$) and 841 (63.0%) cases, including 63.6% of boys and 62.3% of girls, resulting in predicted risks of 3.4% in boys and 3.0% in girls. Furthermore, the estimated PPV of the GRS2 based on a 0.5% population prevalence of early-stage T1D was consistently higher for boys than girls across the whole range of thresholds (Figure 2d) and sensitivities (Figure 2e).

The data suggest that the risk for early-stage T1D is higher in boys than girls after selection for high risk by either the GPPAD GRS or GRS2. The GRS2 has both an HLA class II component and a component from the remainder of the genome. Examining these components separately suggested that the major difference between boys and girls at thresholds corresponding to the 99.2 centile of the background population comes from the HLA Class II gene component (Table S2).

3.3 | Sex-Aligned Polygenic Risk Scores

Since predicted risks at the highest centile of the population differed between boys and girls, we developed sex-aligned PRS using logistic regression on the test case–control groups. A full sex-aligned PRS was developed for 14 HLA genotypes derived from 7 SNPs and a further 143 SNPs (Table S3). It was noted that weights for some of the HLA genotypes and SNPs within the HA region differed substantially between boys and girls. However, no major differences between boys and girls were observed in the MAF for cases or controls (Table S4), suggesting that the differences may be due to mathematic modelling of small numbers in some categories. In addition to the full model scores, a sex-aligned PRS with fewer SNPs (short-PRS) was developed based on variable importance selection using a penalized logistic regression (Figure S1 A,B) and a rjMCMC, which yielded four models (Figure S1 C,D). Variables that fell within

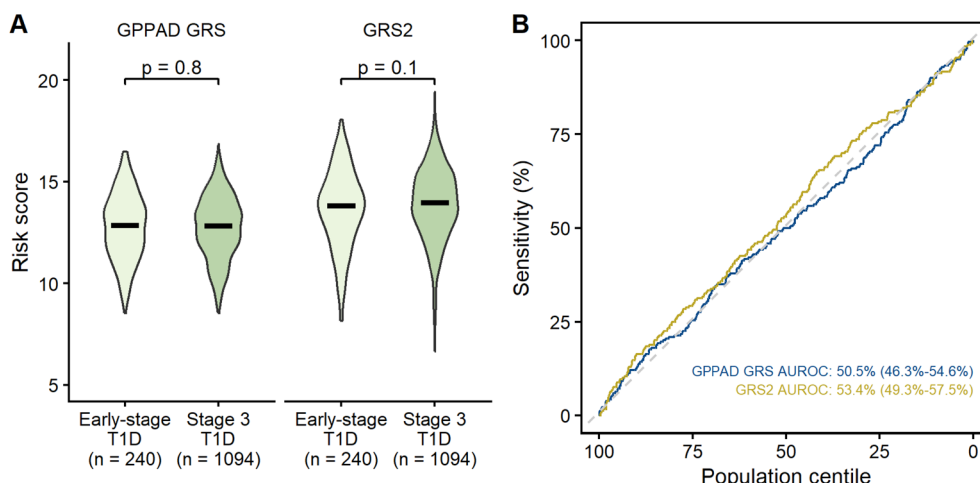


FIGURE 1 | Polygenic risk scores in children with early-stage T1D from the Fr1da study and children with Stage 3 T1D from the Dimelli study. (A) Violin plots of the GPPAD GRS (left) and the GRS2 (right) in the early-stage T1D and Stage 3 T1D cohorts. The median of each curve is indicated. p values compare the distribution in early-stage T1D to Stage 3 T1D. (B) Receiver operator characteristic curves (ROC) for the GPPAD GRS (green) and the GRS2 (brown) where cases are the patients with Stage 3 T1D and controls are the patients with early-stage T1D. The area under the curve (AUROC) and the 95% confidence interval of the AUROC for each curve are indicated. T1D: type 1 diabetes.

the 25 highest-ranking variables in importance in any of these models were kept to build the short-PRS. In total, 45 variables, including 14 HLA DR-DQ genotypes derived from 7 HLA SNPs and 31 additional SNPs for a total of 38 SNPs, were included in the short-PRS (Table S5).

For both males and females, the respective full model and short model scores could discriminate cases from controls in each of the cohorts included in the study (Figure 3 A–B). The full model AUROC was 92.4% (95% CI, 91.5%–93.4%) for boys in the test set and was 89.9% (95% CI, 87.9%–91.9%) in the validation set. For girls, the AUROC was 91.9% (95% CI, 90.8%–92.9%) in the test set and 88.1% (95% CI, 86.4%–89.8%) in the validation set (Figure 3C,D). The AUROC of the short-sex-aligned PRS was 90.8% (95% CI, 89.8%–91.8%) for boys in the test set and was 89.2% (95% CI, 87.2%–91.2%) in the validation set. For girls, the AUROC was 89.3% (95% CI, 88.0%–90.6%) in the test set and 88.6% (95% CI, 87.1%–90.2%) in the validation set (Figure 3C,D). Calibration slopes for the full and short sex-aligned scores in the validation set were all between 0.77 and 0.94 and Brier scores ranged from 0.084 to 0.088 (Table S6). These AUROCs are improved over those obtained for the previous GPPAD GRS and similar to the GRS2 in the same datasets (Table S7).

3.4 | Polygenic Risk Score Performance for General Population Selection of At-Risk Children

We examined how the sex-aligned PRS performed when selecting infants for early intervention trials or for islet autoantibody follow-up using thresholds set to the 99.2 and the 90th centiles, respectively. Using the full-PRS, the frequency of the cases identified at these was 21.9% (RR 27.7) at the 99.2 centile and 77.0% (RR 7.7) at the 90th centile for boys and was 18.0% (RR 22.5) and 73.4% (RR 7.3) for girls (Table S8). With a disease prevalence of 0.5% in the screened population, the estimated risks at the 99.2 and 90th centiles were 13.8% and 3.9% for boys and 11.3% and 3.7% for girls. The performance of the short-sex-aligned PRS was only slightly inferior to the full-sex-aligned PRS with estimated risks at the 99.2 and 90th centiles of 12.3% and 3.6% for boys and 9.2% and 3.3% for girls (Table S8).

3.5 | Adapting the Short-Sex-Aligned PRS to Screening in GPPAD

The GPPAD PRS was based on prior selection of children with high-risk HLA genotypes to obtain estimated risks of 10% in those recruited to trials [9, 24]. The sex-aligned short PRS had similar performances between boys and girls in different HLA risk groups (Figure S2). We, therefore, adapted the short-sex-aligned PRS to the current GPPAD GRS by exchanging some of the SNPs and also further evaluating the score in the background and case children who had HLA DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8 and the infrequent high risk DR4-DQ8/DR4-DQ2 genotypes (Table S9; Figure S3). These genotypes were present in 305 (2.7%) of 11 273 children from the combined test and validation background population and in 422 (31.6%) of

the 1334 case children. The estimated disease risk in children with these genotypes was 5.8%.

To increase the confidence of estimates, an additional set of 350 control children with the high-risk HLA genotypes was included and the sex-aligned GPPAD PRS was applied to a total of 612 control children and the 422 children with early-stage or Stage 3 T1D with DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8 and DR4-DQ8/DR4-DQ2 HLA genotypes. The AUROC was 74.6% (95% CI, 70.6%–78.6%) in boys and 69.4% (95% CI, 64.4%–74.3%) in girls. The thresholds ≥ 18.9 (boys) and ≥ 15.4 (girls) identified 33.8% (boys) and 32.4% (girls) of the children with these genotypes in the background population and 68.5% (boys) and 58.0% (girls) of the cases with these genotypes. This corresponded to background population frequencies of 0.9% (boys) and 0.9% (girls) and case frequencies of 22.2% (boys) and 17.8% (girls). Based on a 0.5% risk for early-stage T1D, the empirically calculated risks are 12.4% for boys and 9.6% for girls.

Finally, we examined the children from the POInT trial to determine whether the new sex-aligned GPPAD GRS would identify the children who developed the primary outcome in the trial (multiple islet autoantibodies or diabetes). Of the 346 without a first-degree family history of T1D who had valid extended genetic data, 29 developed the primary outcome. Of these, 17 (85.0%) of 20 boys had a sex-aligned GPPAD GRS ≥ 18.9 and 8 (88.9%) of 9 girls had a sex-aligned GPPAD GRS ≥ 15.4 . Among those who did not develop the outcome, 98 (68.1%) of boys and 123 (71.1%) of girls were identified by the respective scores.

4 | Discussion

The identification of individuals at risk for early-stage T1D is relevant for primary prevention and for islet autoantibody screening. We show that the distribution of genetic risk scores in children with early-stage T1D is similar to that in patients with Stage 3 T1D. We further show that estimated risks differ between boys and girls in a manner consistent with previous reports of elevated frequency of early-stage T1D in boys as compared to girls in HLA selected infants [7]. Since this affects both risk and eligibility for trials, we developed separate sex-aligned PRS for boys and girls. The predicted performance of these PRS was higher than or similar to previously developed PRS. Their use is expected to reduce, but not eliminate, the bias in risk between boys and girls in the selection of infants from the general population for primary prevention trials.

An uncertainty surrounding screening for early-stage T1D in the general population has been whether the positive predictive value will be similar to that in cohorts with an a priori risk such as first-degree relatives of patients. We observed that children from the general population with early-stage T1D and islet autoantibody positive Stage 3 T1D had very similar distributions in their genetic risk score using two PRS, indicating that early-stage T1D defined by two or more islet autoantibodies in the absence of a prior genetic selection identifies children with a T1D genetic profile. It is, therefore, expected that a diagnosis of early-stage T1D is likely to be effective also when applied to the

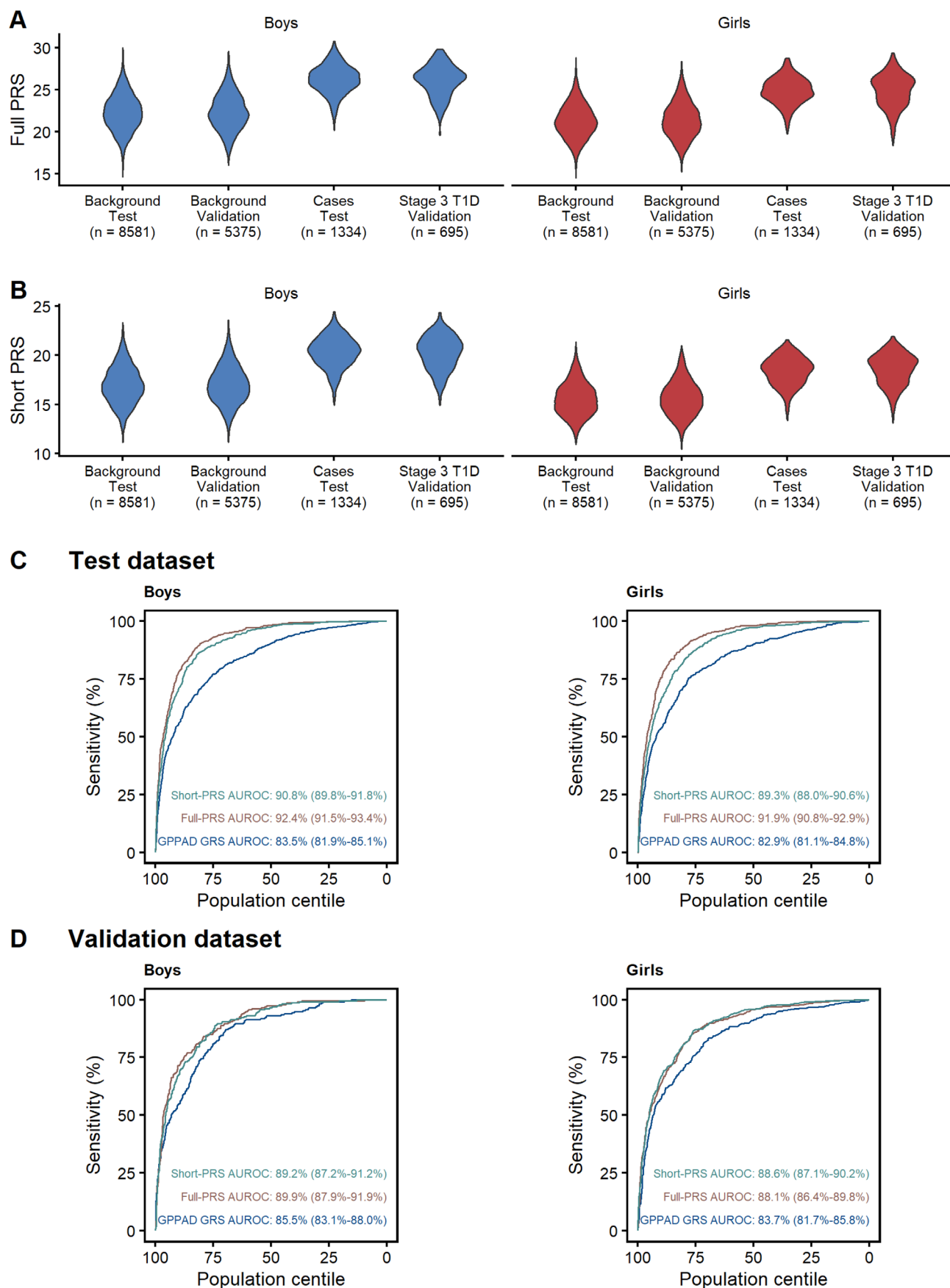


FIGURE 3 | Legend on next page.

FIGURE 3 | Performance of the sex-aligned polygenic risk score (PRS). Violin plots of the distribution of the new full PRS (A) and short PRS (B) in the background and case cohorts separated into boys (blue) and girls (red). Receiver operator characteristic (ROC) curves for the full PRS (brown), short PRS (gold) and previous GPPAD GRS (blue) in boys (left panels) and girls (right panels) for the test cohorts (C) and the validation cohorts (D). The area under the curve (AUROC) and the 95% confidence interval of the AUROC for each curve are indicated.

general population. This is consistent with our observation that progression rates to Stage 3 T1D in children with early-stage T1D are similar in those with and with a first-degree family history of T1D [23].

Sex differences in autoimmunity are frequent and usually with female bias [25]. T1D is one of few autoimmune diseases where there is a slight male preponderance [26]. This is limited to disease development in childhood and adolescence and no genes have been identified that could explain the differences. We were intrigued by the finding from the TEDDY study that showed a difference between boys and girls in the incidence of islet autoantibody development during the first years of life [7]. TEDDY included infants with an elevated risk for developing T1D based on HLA genotypes. Our findings support and partially explain this early sex bias. The differences in risk appeared to be mainly due to a lower frequency of girls with the highest GRS and in particular within the HLA class II risk component of the PRS.

In an attempt to reduce the sex-associated discrepancy, we developed a sex-aligned PRS. The approach taken had some unique features. The training case set included both children with early-stage and autoantibody-positive Stage 3 T1D collected since 2006 and was restricted to cases that did not have a prior first-degree family history of T1D. This should better represent the general population cases that are identified via autoantibody screening than cases previously used for PRS development. A literature search was used to extend the SNPs available for the sex-aligned PRS. The performance of the PRS that included typing at 150 SNPs proved superior to previously developed scores in test and validation cohorts for both boys and girls. Furthermore, a score derived from 38 SNPs was superior to the previous GPPAD GRS and performed similarly to GRS2, which requires 67 SNPs. It was also assessed to have less discrepancy between risks in boys and girls when using high background population centiles as selection criteria than the GRS2 when applied to a German population. Nevertheless, the sex-aligned scores have been developed in an ethnically and regionally restricted population and there will also be bias due to overfitting. Furthermore, the validation case cohort was a historical and older set of patients with T1D, which is a limitation of the study. Therefore, the performance of the PRS is likely to be biased in favour of the sex-aligned PRS as compared to previous PRS, including the GRS2, which has been applied and validated in multiple settings. Therefore, it is important to externally validate the performance of the sex-aligned PRS in other populations, including populations of different ancestries.

One purpose for developing the sex-aligned PRS was to use it for recruitment into GPPAD trials where eligibility for infants without a first-degree family history of T1D has required the presence of certain HLA genotypes. We, therefore, replaced some of the SNPs in the short sex-aligned PRS with those successfully used in the European GPPAD newborn GRS for convenience and reevaluated its performance in children with the highest risk

HLA DR4-DQ8 containing genotypes. Again, predicted risks in boys and girls converged as compared to those predicted for the GPPAD GRS and GRS2. While the sex-aligned full, short and GPPAD PRS were predicted to reduce sex-associated risk differences, they did not eliminate the differences, which remained substantial, suggesting that sex-associated factors modify risk in boys and/or girls with elevated genetic risk. Speculatively, these factors may include early hormonal related differences, which could affect gene function via epigenetic variation similar to what has been suggested for sex differences associated with development and cancer [27] and for protection associated with maternal T1D [28].

The study has several limitations. It is performed in Bavaria, which has a population that is largely of European descent. Therefore, the PRS developed here are unlikely to be as effective when applied to non-European populations. The PRS were developed based on non-adult cases of early-stage and autoantibody positive Stage 3 T1D and may not be appropriate for selection of adults for islet autoantibody screening. Furthermore, the number of cases limited further analyses that could improve the models such as stratification by age [29] and the analysis of interactions between SNPs [30]. The validation cohort of cases was an historical set of patients that included adults. Therefore, further validation in a paediatric population is required. The purpose of our study was to develop sex-aligned PRS to predict early-stage T1D and we cannot assess if they will be able to further improve stratification of progression rates in individuals who are positive for islet autoantibodies as shown for GRS2 [29]. Alternatives to using sex-aligned scores is to weight the GRS by a sex factor using methods such as a sex covariate or recalibration. We did not pursue these approaches since they would likely result in biased selection in favour of boys for trials, which we wanted to avoid. However, since differences in risks were not limited by the sex-aligned scores, our approach may lead to selection of girls with lower overall risk as compared to boys.

In conclusion, our finding that children with early-stage and Stage 3 T1D have very similar T1D PRS distributions suggests that general population screening for islet autoantibodies is likely to identify true early-stage disease with high probability of developing Stage 3 (clinical T1D). This finding also supports the use of PRS for preselection for screening or rescreening. The availability of sex-aligned PRS that reduce sex bias in the risk for early-stage T1D in childhood may further improve selection for primary prevention trials. However, important differences in risk were not eliminated and further evaluation of the sex-aligned scores, in particular in populations other than those of European descent, is required.

Author Contributions

E.B. was the principal investigator and designed the study. J.Z.-G. and E.B. performed the statistical analysis. J.Z.-G. and E.B. drafted the

manuscript. All authors acquired data, contributed to the interpretation of data, and critically reviewed the manuscript. E.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conflicts of Interest

A.-G.Z. served as a member of advisory boards for Sanofi-Aventis, and Novo Nordisk, and received support to give lectures sponsored by Sanofi-Aventis. E.B. received support for travel and accommodation to attend international conferences and to give lectures sponsored by Sanofi-Aventis. No other potential conflicts of interest relevant to this article were reported.

Data Availability Statement

The de-identified individual participant data that underlie the results (text, tables, figures and [Supplementary Appendix](#)) reported in this article can be shared between 9 and 36 months after publication of the article. Requests will be honoured from researchers who provide a methodologically sound proposal and who complete a Data Use Agreement with Helmholtz Munich. Requests should be directed by email to the corresponding author.

Peer Review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.71417>.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** dom71417-sup-0001-Supinfo01.docx. **Table S1:** SNPs included in the study. **Table S2:** Frequencies boys and girls identified by the 99.2 centile of the HLA and non-HLA components of the GRS2 in the background population. **Table S3:** Coefficient weights for each of the HLA genotypes and risk SNP alleles in the Full-PRS performed separately in males and females. **Table S4:** Minor allele

frequencies in test set controls and cases by sex. **Table S5:** Coefficient weights for each of the HLA genotypes and SNP alleles in the Short-PRS performed separately in males and females. **Table S6:** Model calibration and predictive accuracy metrics. **Table S7:** Receiver Operator Curve area under the curve (AUROC) for polygenic risk scores (PRS) and net reclassification improvement (NRI) and integrated discrimination improvement (IDI) using the GRS2 as reference. **Table S8:** Sensitivity and relative risk in boys and girls for polygenic risk scores. **Table S9:** Coefficient weights for each of the HLA genotypes and risk SNP alleles in the sex-aligned GPPAD PRS performed separately in males and females. **Figure S1:** Variable importance for boys and girls. Importance was assessed using a penalized logistic regression (PLR; A & B) and a reversible-jump Markov Chain Monte Carlo (rjMCMC; C & D). The top 25 important features are shown for each of the methods and for boys and girls. **Figure S2:** ROC curves of the sex-aligned short PRS by HLA groups. The ROC curves were performed on the validation cohort separating controls and cases into 4 HLA groups. The HLA groups were defined as follows: high-risk HLA, comprising the DR3/DR4-DQ8, DR4-DQ8/DR4-DQ8 and DR4-DQ2/DR4-DQ8 genotypes (A); other DR4, comprising all genotypes containing a DR4 haplotype, excluding those assigned to the high-risk HLA group (B); DR3 only, comprising genotypes containing a DR3 haplotype that were not included in the high-risk HLA group (C) and non-DR3/DR4, comprising the DRx/x NP and DRx/x P genotypes (D). **Figure S3:** Distribution of the sex-aligned GPPAD PRS in early-stage T1D test and validation cohorts. The sex-aligned GPPAD PRS was calculated in 11 273 children from the background population, 240 with early-stage T1D in the case test set and in an additional validation set of 241 children/adolescents with early-stage T1D from the Frida study (median age, 4.09 years [IQR, 2.90–6.45]; 115 girls). No significant differences were found between the test and validation early-stage cases.