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ORIGINAL ARTICLE

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Clinical features, biochemistry, and *HLA-DRB1* status in youth-onset type 1 diabetes in Mali

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Abstract

Objective: Limited information is available regarding youth-onset diabetes in Mali. We investigated demographic, clinical, biochemical, and genetic features in new diabetes cases in children and adolescents.

Research Design and Methods: The study was conducted at Hôpital du Mali in Bamako. A total of 132 recently-diagnosed cases <21 years were enrolled. Demographic characteristics, clinical information, biochemical parameters (blood glucose, HbA1c, C-peptide, glutamic acid decarboxylase-65 (GAD-65) and islet antigen-2 (IA2) autoantibodies) were assessed. DNA was genotyped for *HLA-DRB1* using high-resolution genotyping technology.

Results: A total of 130 cases were clinically diagnosed as type 1 diabetes (T1D), one with type 2 diabetes (T2D), and one with secondary diabetes. A total of 66 (50.8%) T1D cases were males and 64 (49.2%) females, with a mean age at diagnosis of 13.8 ± 4.4 years (range 0.8–20.7 years) peak onset of 15 years. 58 (44.6%) presented in diabetic ketoacidosis; with 28 (21.5%) IA2 positive, 76 (58.5%) GAD-65 positive, and 15 (11.5%) positive for both autoantibodies. HLA was also genotyped in 195 controls without diabetes. *HLA-DRB1* genotyping of controls and 98 T1D cases revealed that DRB1*03:01, DRB1*04:05, and DRB1*09:01 alleles were predisposing for T1D (odds ratios [ORs]: 2.82, 14.76, and 3.48, *p*-values: 9.68E-5, 2.26E-10, and 8.36E-4, respectively), while *DRB1*15:03* was protective (OR = 0.27; *p*-value = 1.73E-3). No significant differences were observed between T1D cases with and without GAD-65 and IA2 autoantibodies. Interestingly, mean C-peptide was 3.6 ± 2.7 ng/ml (1.2 ± 0.9 nmol/L) in T1D cases at diagnosis.

Conclusions: C-peptide values were higher than expected in those diagnosed as T1D and autoantibody rates lower than in European populations. It is quite possible that some cases have an atypical form of T1D, ketosis-prone T2D, or youth-onset T2D. This study will help guide assessment and individual management of Malian diabetes cases, potentially enabling healthier outcomes.

Stéphane Besançon and Denira Govender contributed equally to this study and recognition as co-first author.

KEYWORDS
autoantibody, childhood diabetes, C-peptide, HLA, Mali

1 | INTRODUCTION

Diabetes in children and adolescents has multiple aetiologies with incidence of both type 1 (T1D) and type 2 diabetes (T2D) varying widely according to geography.[1–3] Considering this variation, it is important to understand the pathophysiology in each part of the world. From this, clinical management and training of healthcare professionals can be tailored to the local situation, and health system resources allocated appropriately.

Our group has recently published information on the rapidly increasing incidence and prevalence of T1D in young people in Mali, a low-income country in west Africa.[4] However, the phenotypic and genotypic features of T1D in Mali remain unclear, and there is evidence from other studies in Africa that patterns can be different from this disease in European-origin populations.[5–8] These include observations in Ethiopia and other countries that found T1D has a later age of onset[5,8] and in Cameroon, autoantibody positivity was not clinically related to disease phenotype,[6] and ketosis-prone T2D also occurred.[7] We therefore conducted a study of 132 new or recent-onset cases of diabetes diagnosed in children and adolescents <21 years of age in Mali, with particular emphasis on investigating demographic, clinical, biochemical features, and *HLA-DRB1* alleles.

2 | METHODS

2.1 | Study site

The study was conducted at the Endocrinology and Diabetes Clinic at the Hôpital du Mali, Yirimadio in Bamako, Mali. The study cases received care through the Malian national health system and the non-government organization Santé Diabète,[9] together with support from the Life for a Child Program.[10] This study was approved by relevant Ethics Committees in Mali and Australia, and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained for all cases prior to enrolment in the study. *HLA-DRB1* genotyping was performed at Children's Hospital Oakland Research Institute (CHORI) with IRB approval.

2.2 | Study cases

A total of 132 cases <21 years of age at diagnosis were enrolled. A total of 82 cases (62.1%) were diagnosed in a consecutive series from February 2014 to October 2015, with 50 (37.9%) cases diagnosed before this period. A total of 17 (12.8%) were assessed in the first week after diagnosis, 10 (7.6%) 1 week to 1 month, 51 (38.6%) 1–6 months and the remaining 54 (40.9%) from 6 months to 2.5 years after. Samples from 100 of the diabetes cases, as well as samples from a set of 200 control cases without diabetes, were *HLA-DRB1* genotyped. Selection criteria for controls were both parents born in Mali, diabetes-negative, and unrelated to any of the cases with diabetes.[11]

2.3 | Demographic data

Date of birth, sex, city, and province of residence at diagnosis, date of diagnosis, as well as distance and travel time to the center were recorded for patients.

2.4 | Clinical parameters

Diabetes was diagnosed according to standard World Health Organization (WHO) criteria.[12] Determination of the type of diabetes was made by the local investigators according to available clinical features and history. The presence of polyuria, polydipsia, weight loss, malnutrition, and diabetic ketoacidosis (DKA) at the time of diagnosis were recorded. DKA was defined as the presence of clinical features (tachypnea, dehydration, confusion, or coma) in combination with marked ketonuria and elevated blood glucose levels.

The following information pertaining to diabetes care was also recorded: date of insulin commencement, number of insulin injections per day, type of insulin used, insulin storage method at homes, use of oral hypoglycemic agents, and other medications or treatments. History of other medical conditions, and family history was also recorded. Body weight and height were measured by electronic scales and stadiometer, respectively, with cases wearing light-weight clothing and without shoes. Body mass index (BMI) was then calculated, with BMI *SD* scores calculated using the WHO standards for those <5 years of age,[13] and for 5–19 years.[14] For those 19–21 years of age, BMI *SD* was calculated using an age of 19.0 years.

2.5 | Sample collection for genotyping

Approximately 200 μl of peripheral blood from the cases with diabetes was preserved by mixing with DNAgard® blood (BioMatrica, San Diego, CA), then dried for storage and shipment to the HLA typing facility. For control subjects, approximately 1 ml of saliva was mixed with 0.5 ml DNAgard® saliva stabilizing reagent (BioMatrica, San Diego, CA) for storage and shipment, again, to the HLA typing facility.

2.6 | Biochemical parameters and serology

Blood glucose was measured with a Automate Maglumi machine (Snibe, Shenzhen, China). HbA1c was measured using a Clover

machine (Infopia, Anyang, Republic of Korea). Fasting C-peptide and autoantibodies against glutamic acid decarboxylase 65 (GAD-65) and islet antigen 2 (IA2) were measured by ELISA in Bamako, as previously described.[11]

2.7 | *HLA-DRB1* genotyping

Samples for 100 diabetes cases and 200 controls were genotyped for *HLA-DRB1* exon 2 using previously-described, next-generation-sequencing-based methods.[15] One diabetes case was removed from the data due to diagnosis of T2D. Genotypes were successfully obtained from 98 T1D cases and 195 controls.

2.8 | Statistics

Demographic and biochemical data were analyzed by Microsoft Excel (Redmond). Mean differences between variables and correlation were calculated by independent *t*-test and Pearson correlation, respectively. DKA rates across two enrolment periods were compared using chi-square test. Statistical analysis was conducted with IBM SPSS Statistics for Windows, Version 27.0. IBM Corporation, Armonk. Tests of association of *DRB1* alleles between T1D and control subjects, along with tests of Hardy–Weinberg equilibrium for *DRB1* genotypes in T1D and control subjects, were conducted using BIGDAWG[12] and as previously described.[15] BIGDAWG was also used to perform overall (kx2) tests of association with T1D subjects positive for autoantibodies to either GAD-65 or IA2.

3 | RESULTS

3.1 | Diagnosis

Of the 132 patient cases, 130 (98.5%) were clinically diagnosed with T1D. One case was diagnosed with T2D, with one having been described as secondary diabetes due to treatment for leukemia. Data for these two patients were excluded from the genetic analyses.

3.2 | Demographic data

Of the 130 cases with T1D, 66 (50.8%) were males and 64 (49.2%) females, reflective of near gender equality. All were Malian Africans. There was no discernible seasonal pattern in disease incidence (data not shown). The mean ± *SD* age of diagnosis of T1D was 13.8 ± 4.4 years (range 0.8–20.7 years). The median age at diagnosis was 14.6 years and the peak age of onset was 15 years (Figure 1). Five cases (3.8%) were diagnosed at 0–4 years (youngest case diagnosed at 10 months), 19 (14.6%) from 5–9 years, 48 (36.9%) from 10 to 14 years, 52 (40.0%) from 15 to 19 years and 6 (4.6%) 20 to 21 years. The singular case with T2D was a female with a BMI of 26.8 diagnosed at 12.6 years of age. The case with secondary diabetes was a male diagnosed at 16.6 years of age. Of all 132 cases,

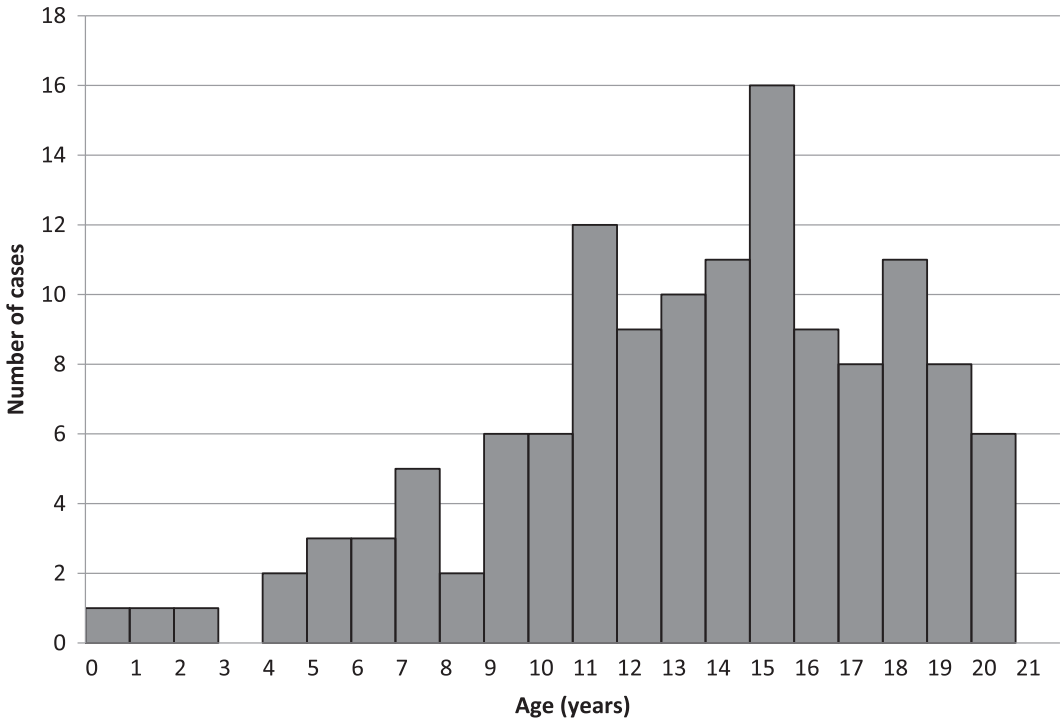

FIGURE 1 The age of onset of type 1 diabetes in young people <21 years of age in Mali.

42 (31.8%) traveled <10 km to access care, 61 (46.2%) 10–50 km, 9 (6.8%) 50–200 km and 20 (15.2%) >200 km.

3.3 | Clinical parameters

The main symptoms preceding diagnosis of T1D were polyuria ($n = 129$, 99.2%), polydipsia ($n = 127$, 97.7%) and weight loss ($n = 128$, 98.5%). A total of 58 (44.6%) cases presented in DKA. The prevalence of DKA at onset in those diagnosed before the consecutive enrolment period was 57.1% compared to 37.0% during the consecutive enrolment ($p = 0.04$).

For the T1D cases, the mean ± *SD* BMI at diagnosis was 18.0 ± 4.4 (range 10.4–30.4). The mean ± *SD* BMI standard deviation score (SDS) was −1.0 ± 1.8 (range −6.4 to 2.7).

Nine T1D cases had a BMI SDS of 2.0–2.7. Of these, five cases were females, one presented in DKA, and one was positive for IA2 autoantibody. Further, five of these nine (55.6%) had a C-peptide of ≥3.1 ng/ml (≥1.03 nmol/L). Five T1D cases had a BMI SDS of <−5.0. Four were males, three presented in DKA, and four had at least one positive T1D associated autoantibody. All five had a C-peptide of ≥3.1 ng/ml (≥1.03 nmol/L).

For the T1D cases, the mean ± *SD* blood glucose at diagnosis was 19.8 ± 6.7 mmol/L (range 7.6–34.0 mmol/L). The mean ± *SD* HbA1c was 10.3 ± 2.3% (89.5 ± 25.1 mmol/mol), with a range 7.0–14.9% (range 53.0–139.3 mmol/mol). Seven T1D cases (5.4%) had other medical conditions: two with asthma, one with growth failure of unknown cause, one with hyperthyroidism, two with an intellectual disability, and one with epilepsy. A total of 21 of the 130 cases (16.2%) diagnosed with T1D had a first degree relative with T1D: five with a brother, four with a sister (including a pair of female twins), one with both a brother and a sister, seven with a father, and four with a mother. All T1D cases were treated with insulin. A total of 122 (93.8%) commenced insulin on the same day as diagnosis, 4 (3.1%) within 1 week, 1 (0.8%) within 1 month and 3 (2.3%) within 3 months. A total of 58 (44.6%) were able to store insulin in a refrigerator at home, with the remaining 72 (55.4%) using traditional cooling methods such as a clay pot and wet sand.[16]

The case with T2D was a female diagnosed at 12.6 years with a BMI *SD* of 2.18 and a height *SD* of 1.35, no DKA at onset, both autoantibodies negative, and C-Peptide 3.9 ng/mL (1.3 nmol/L). She was initially commenced on diet treatment only. The male on treatment for acute lymphoblastic leukemia (ALL) developed diabetes at 16.6 years. Their C-peptide level was 1.66 ng/ml (0.55 nmol/L), IA2 autoantibody was mildly elevated at 38.3 IU/ml and GAD-65 autoantibody was negative.

3.4 | C-peptide

For 80.8% of T1D cases, C-peptide was measured within 1 week of diagnosis (maximum was 15 days after diagnosis). The mean ± *SD* C-peptide for T1D cases was 3.6 ± 2.7 ng/ml (1.2 ± 0.9 nmol/L). A total of 16 (12.3%) had a C-peptide value of <0.8 ng/ml (<0.26 nmol/L), 44 (33.8%) between 0.8 and <3.1 ng/ml (0.26–1.03 nmol/L), and 70 (53.8%) >3.1 ng/ml (>1.03 nmol/L). Table 1 shows relationships between C-peptide and DKA and autoantibody status.

3.5 | Autoantibody results

For T1D cases, 28 (21.5%) were IA2 autoantibody positive, 76 (58.5%) were GAD-65 autoantibody positive, with 15 (11.5%) positive for both (see Table 1 for further relationships). Among seropositive T1D cases the mean ± *SD* titer was 122.7 ± 260.2 IU/ml for GAD-65 autoantibodies and 33.0 ± 44.8 IU/ml for IA2 autoantibodies. Table 1 shows autoantibody relationships with DKA and C-peptide status.

There was no significant difference in C-peptide values between those T1D cases without autoantibodies and with one or two autoantibodies ($p = 0.85$). The mean ± *SD* BMI in those without autoantibodies was 19.7 ± 5.2 and in those with one or two autoantibodies 18.0 ± 4.4 ($p = 0.06$). BMI and C-peptide values were not correlated in T1D cases ($p = 0.51$).

3.6 | HLA genotype and allele analyses

HLA-DRB1 genotypes were successfully generated for 98 cases and 195 control subjects (Table 2). No overall deviations from expected Hardy–Weinberg Equilibrium proportions were detected in T1D patients or controls (*p*-values = 0.0824 and 0.1866, respectively). However, the DRB1*04:05 + DRB1*13:02 genotype was significantly over-represented in T1D cases (6 observed; 2.4 expected; *p*-value = 0.0185), while it was observed once in controls (1 observed; 0.3 expected; *p*-value = 0.1945), and the DRB1*07:01 + DRB1*15:03 genotype was significantly over-represented in controls (10 observed; 3.99 expected; *p*-value = 0.0017), while it was not observed in T1D cases (0 observed; 0.5 expected; *p*-value = 0.671). A list of the 136 unique genotypes found in the data set is shown in Table S1. Association analysis for the DRB1 alleles in the 98 disease cases and 195 controls is shown in.

Predisposing associations were observed for the DRB1*03:01 (odds ratio (OR) = 2.82, *p*-value (*p*) = 9.68E-05), *04:05 (OR = 14.76; $p = 2.26E-10$) and *09:01 (OR = 3.48; $p = 8.06E-4$) alleles, and protective associations were observed for the DRB1*15:03 allele (OR = 0.27; $p = 1.73E-3$), and the Binned category (OR = 0.2; $p = 1.80E-2$). In the latter case, we note that only two alleles were included in the Binned category for T1D cases, versus 19 alleles in T1D controls. As described in the Supplementary material and presented in Table S3, we applied a resampling approach to determine that the protective association with the Binned category is attributable to the ~2:1 size ratio of T1D controls to T1D cases; the Binned category is not actually protective for T1D.

TABLE 1 The relationships of diabetic ketoacidosis, C-peptide, and autoantibodies for 130 T1D cases.

	C-peptide status			Autoantibody status				
	C-peptide <0.8 ng/ml (<0.26 nmol/L) ($n = 16$)	C-peptide 0.8–3.1 ng/ml (0.26–1.03 nmol/L)[a] ($n = 44$)	C-peptide >3.1 ng/ml (>1.03 nmol/L) ($n = 70$)	GAD-65 ≥ 30 ($n = 76$)	IA2 ≥ 30 ($n = 28$)	Both autoantibodies ($n = 15$)	Either/both autoantibodies ($n = 89$)	Neither autoantibodies ($n = 41$)
Diabetic Ketoacidosis ($n = 58$)	7 (12.1%)	19 (32.8%)	32 (55.2%)	43 (74.1%)	11 (19.0%)	8 (13.8%)	46 (79.3%)	12 (20.7%)
C-peptide <0.8 ng/ml (<0.26 nmol/L) ($n = 16$)				13 (81.3%)	1 (6.3%)	1 (6.3%)	13 (81.3%)	3 (18.8%)
C-peptide 0.8–3.1 ng/ml (0.26–1.03 nmol/L)[a] ($n = 44$)				20 (45.5%)	11 (25.0%)	4 (9.1%)	27 (61.4%)	17 (38.6%)
C-peptide >3.1 ng/ml (>1.03 nmol/L) ($n = 70$)				43 (61.4%)	16 (22.9%)	10 (14.3%)	49 (70.0%)	21 (30.0%)

[a]Normal range.

On sub-analysis, no significant differences were found in HLA genotype between T1D cases with no autoantibodies compared to those with one or two autoantibodies ($p = 0.94$).

4 | DISCUSSION

This study investigated the clinical, biochemical, and genetic characteristics of new onset diabetes in children and adolescents in Mali. Its importance relates to a recent study that documented a rapidly increasing incidence and observed prevalence of T1D in young people in Mali.[4] However, the diagnosis of T1D in Mali is almost invariably purely clinical, as measurement of autoantibodies and C-peptide are not routinely available. Data from Cameroon, Ethiopia, and other African countries indicates that patterns of age-of-onset, autoantibodies, C-peptide and HLA can be different to those seen in T1D in European-origin countries, and that some cases do not readily fit the classic definitions of either T1D or T2D.[5–7,17]

The peak age of onset of T1D at 15 years, is older than that seen in European populations,[18] but consistent with other studies in sub-Saharan African populations.[5,17,19] Aside from the possibility of differing aetiologies (see below), it is also possible that the epidemiological shift that has led to an earlier age of onset in developed countries has not yet occurred in Mali. In addition, misdiagnosis may also be contributing: it is thought that many cases in Mali[4] and other countries in sub-Saharan Africa,[17,20,21] die misdiagnosed with malaria, typhoid, pneumonia, gastroenteritis, or another condition and such deaths could be more common in younger children where infectious disease is common and the classic symptoms and signs may be less obvious. The 44.6% rate of DKA at onset is relatively high by international standards,[22] but consistent with other studies in Africa.[17] It is interesting to note that the prevalence of DKA was lower in the consecutive series of patients than in those enrolled at various periods after diagnosis, which suggests diagnosis is being made earlier, consistent with our observation of rapidly increasing diagnosed incidence during this period.[4]

The GAD-65 positivity of 58.5% and particularly the IA2 autoantibody positivity of 21.5% are low compared to European-origin populations,[23] although autoantibody-negative T1D cases are not infrequent in European populations.[23–25] Asanghanwa et al.[6] reported that Cameroonian autoantibody positive cases were older, had higher BMIs, lower autoantibody titers, and were less likely to have multiple autoantibodies than Belgian cases. The most atypical data, compared to European populations, is the number of cases clinically diagnosed with T1D who did not have a low serum C-peptide. External quality assurance of the C-peptide assay was not possible, and it is likely that a number of the samples were taken in the "honeymoon" period where there is a temporary recovery in insulin secretion. Although the C-peptide measurements were fasting, the study did not collect paired glucose measurements. The results are similar to our group's findings in the non-European populations of Pakistan[11] and Bangladesh,[26] whereas the Turkic population in Azerbaijan showed a European pattern.[27] GAD-65 autoantibody was positive in 55.3% of the cases with a C-peptide in the normal range or higher (>0.8 ng/ml [0.26 nmol/L]).

TABLE 2 Association of DRB1 alleles with T1D in Malian cases and controls.

Allele	Case (*N*)	Case (*f*)	Control (*N*)	Control (*f*)	OR	95% CI	*p*-value	Significance
DRB1*01:02	3	0.01531	8	0.02051	0.74	0.13–3.14	6.61E-01	NS
DRB1*03:01	**34**	**0.17347**	**27**	**0.06923**	**2.82**	**1.59–5.03**	**9.68E-05**	*
DRB1*03:02	6	0.03061	26	0.06667	0.44	0.15–1.12	6.99E-02	NS
DRB1*04:01	5	0.02551	3	0.00769	3.38	0.65–21.92	7.95E-02	NS
DRB1*04:05	**26**	**0.13265**	**4**	**0.01026**	**14.76**	**4.99–58.83**	**2.26E-10**	*
DRB1*07:01	16	0.08163	38	0.09744	0.82	0.42–1.56	5.33E-01	NS
DRB1*08:04	14	0.07143	31	0.07949	0.89	0.43–1.78	7.30E-01	NS
DRB1*08:06	4	0.02041	15	0.03846	0.52	0.12–1.67	2.44E-01	NS
DRB1*09:01	**18**	**0.09184**	**11**	**0.02821**	**3.48**	**1.52–8.33**	**8.06E-04**	*
DRB1*10:01	4	0.02041	9	0.02308	0.88	0.2–3.21	8.36E-01	NS
DRB1*11:01	8	0.04082	30	0.07692	0.51	0.2–1.17	9.40E-02	NS
DRB1*11:02	9	0.04592	30	0.07692	0.58	0.24–1.28	1.55E-01	NS
DRB1*13:01	6	0.03061	14	0.0359	0.85	0.26–2.4	7.40E-01	NS
DRB1*13:02	18	0.09184	34	0.08718	1.06	0.55–1.99	8.52E-01	NS
DRB1*13:03	8	0.04082	29	0.07436	0.53	0.21–1.22	1.15E-01	NS
DRB1*13:04	7	0.03571	16	0.04103	0.87	0.3–2.27	7.55E-01	NS
DRB1*14:01	2	0.0102	5	0.01282	0.79	0.07–4.9	7.83E-01	NS
*DRB1*15:03*	*6*	*0.03061*	*41*	*0.10513*	*0.27*	*0.09–0.65*	*1.73E-03*	*
Binned	*2*	*0.0102*	*19*	*0.04871*	*0.2*	*0.02–0.85*	*1.80E-02*	*
Total	196		390					

Note: The *p*-value for the k × 2 chi-squared test of overall locus-level association is 5.8703e-12. Predisposing alleles are shown in boldface. Protective alleles are shown in italics. Significance is evaluated at the 0.05 level. *N*: allele count; *f*: allele frequency: OR: odds ratio; CI: confidence interval; Binned: alleles with expected counts less than 5 in cases or controls that represent less than 20% of contingency table cells (33) were combined into a common category for analysis (5); Total: the total number of alleles is identified in T1D cases and controls.

Insulin resistant cases (using an algorithm based on waist circumference, HbA1c and serum triglyceride) formed 26.4% of the autoantibody-positive cases in the SEARCH study.[24] The 25th–75th percentile C-peptide in the insulin resistant group was 0.3–1.4 ng/ml (0.1–0.5 nmol/L). In another study in the United States, Redondo et al.[25] reported that 38.6% of autoantibody-positive patients were beta-cell positive, with this group having a mean ± *SD* C-peptide of 1.0 ± 0.7 ng/ml (0.3 ± 0.2 nmol/L). These T1D sub-groups would cover some of the cases in this study.

Only one case in this series was clinically diagnosed with type 2 diabetes. It is likely that some of the remaining cases would have T2D if further investigated. Nine cases had a BMI *SD* at onset of ≥2.0. Only one of these presented in DKA and had one autoantibody, and five had elevated serum C-peptide levels. T2D is less common in Mali than many other countries with a prevalence in the 20–79 year-old population of 1.8%[28]. Ketosis-prone T2D is well-described in Africans[7] and African-Americans,[29] and, in the current study, autoantibodies were absent in 20.7% of those cases presenting in DKA.

The incompletely-understood entity of malnutrition-related diabetes has been reported from Ethiopia and other countries,[5] and it is a possible explanation for some of the five cases in this study with very low BMIs. However four of these five had at least one autoantibody, and three presented in DKA – findings not typical of malnutrition-related diabetes.[5]

HLA associations observed in this study are consistent with studies of other populations. The DRB1*04:05 allele has a high OR, whether at high or low frequency, in many populations.[30,31] Risk from DRB1*03:01 varies in magnitude among populations but is nearly always significantly predisposing for T1D.[11,15,26,27] DRB1*15:03 is highly protective for T1D, similar to other DRB1*15 alleles in other populations.[31]

These data show the DRB1*04:05 + DRB1*13:02 genotype to be frequent in cases, consistent with the very high risk demonstrated for this genotype (OR > 34) in African Americans.[31]

The study has various limitations. Due to limited funds and logistic challenges with transfer of samples, autoantibody analysis was limited to two autoantibodies (GAD-65 and IA2), a single HLA gene (*HLA-DRB1*), and a one-time measurement of C-peptide at or soon after diagnosis, with no external quality control. Analysis of other autoantibodies (particularly Zinc transporter-8 antigen [ZnT8A])[3,18,32] may reveal other autoantibody-positive cases, although ZnT8 positivity was low in the Cameroonian study.[6] Furthermore, analysis of other genetic loci would be expected to reveal further genetic risk information. Future plans include full-gene HLA genotyping for all classical HLA loci and perhaps additional disease influencing loci located throughout the genome.

In conclusion, young people diagnosed with T1D in Mali generally present in DKA and have GAD-65 autoantibodies, and yet often do

not have low levels of C-peptide, raising the possibility that atypical T1D and also T2D forms are more common than suspected. This distinction between T1D, atypical T1D and T2D is not only of epidemiological interest, but is critical in individual management. Depending on the pathophysiology, oral agents such as metformin, gliclazide, and also some of the newer agents, may be indicated as a substitution for, or addition to, insulin therapy. Longitudinal studies of Sub-Saharan cohorts are indicated to further understand this heterogeneity, and inform therapeutic guidelines in less-resourced situations. Finally, education efforts about recognition of T1D symptoms, especially in younger children, need to continue in Mali and also be conducted in similar less-resourced countries.

AUTHOR CONTRIBUTIONS

Stéphane Besançon, Assa Traore Sidibé, and Amagara Togo implemented the study in Mali and helped with writing the manuscript. Faizy Kakkat did the preliminary data analysis. Denira Govender did the final data analysis and wrote the initial draft of the manuscript. Mark A. Atkinson and Clive Henry Wasserfall advised on the study protocol, implementation, and analysis and contributed to the manuscript. Steven John Mack performed statistical analysis of HLA data and co-wrote the manuscript, Julie Ann Lane and Gregory G. N. Martin performed HLA genotyping. Graham David Ogle designed and coordinated the study and co-wrote the manuscript. Julie Ann Lane designed and coordinated the genetic portion of the study, including sample collection, *DRB1* genotyping, and HLA analysis, and co-wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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