American Journal of Gastroenterology

EARLY ANTIBODY DYNAMICS IN A PROSPECTIVE COHORT OF CHILDREN AT-RISK FOR CELIAC DISEASE

--Manuscript Draft--

Manuscript Number:	AJG-22-2305R2
Full Title:	EARLY ANTIBODY DYNAMICS IN A PROSPECTIVE COHORT OF CHILDREN AT-RISK FOR CELIAC DISEASE
Article Type:	Brief Communication
Section/Category:	Pediatrics
Abstract:	Objectives: To identify possible serum biomarkers predicting celiac disease (CD) onset in children at risk. Study design: A subgroup from an ongoing, international prospective study of children at risk of CD were classified according to an early trajectory of a deamidated gliadin peptide (DGPs) IgG and clinical outcomes (CD, potential CD and CD autoimmunity). Results: Thirty-eight of 325 children developed anti-tissue transglutaminase IgA antibody (anti-tTG IgA) seroconversion. Twenty-eight out of 38 children (73.6%) showed an increase in aDGPs IgG before their first anti-tTG IgA seroconversion. Conclusions: aDGPs IgG can represent an early pre-clinical biomarker predicting CD onset in children at risk.
Response to Reviewers:	Editor/Editorial Board Comments: The editorial board had invited/expected a resubmission as a Brief Report as outlined in the prior decision letter. The current manuscript does not adhere to that formatting. In addition to addressing the minor reviewer comment below, please also re-submit the content as a Brief Report. As requested by the Editorial Board, we changed the structure of our manuscript to make it a Brief Report. Reviewer #1: Thank you for incorporating the recommended feedback. The HLA classification on p 4 is still confusing to me and I think may be more clear if you include HLA-DQ2.5 and DQ2.2 in the description. One category is currently DQ 2 heterozygous and then isolated DQB*02 as a separate low risk category. Switching between the B chain and DQ description does not make it clear where DQ 2.2/X would fall in this categorization. Thank you for this point. For sake of brevity, we are not mentioning HLA classification at all in the newer version of the paper as editors asked us to pass from original paper to brief report (2,310 words 993 words). However, we modified the table not arising further confusion between the B chain and DQ description. Reviewer #2: No additional comments. Thank you Manuscript Formatting Guide (full submission guidelines are posted online via the "Instructions for Authors" link): Prior to resubmitting your manuscript, we ask that you review the items on the following list carefully. 1. Please be sure that you have included a complete title page. Requirements are listed in the submission guidelines on the Instructions for Authors page online. This information should appear on the title page ONLY and nowhere else in the manuscript. Done

	2. Review the main text of the manuscript thoroughly to confirm that all figures and tables (both primary and supplemental) are cited. If you have removed any figures or tables from the submission, confirm that their citations have been deleted. Done 3. Tables should appear after the main text and before the references in an editable format. Tables must be text-based and cannot contain any graphics or colored text. Review all tables included with the submission to confirm that any abbreviations used are expanded in a footnote for that table.
Order of Authors:	Francesco Valitutti, MD
	Maureen M Leonard, MD
	Victoria Kenyon, MS
	Monica Montuori, MD
	Pasqua Piemontese, MD
	Ruggiero Francavilla, MD
	Basilio Malamisura, MD
	Lorenzo Norsa, MD
	Angela Calvi, MD
	Maria Elena Lionetti, MD
	Mariella Baldassarre, MD
	Chiara Maria Trovato, MD
	Michela Perrone, MD
	Tiziana Passaro, MD
	Naire Sansotta, MD
	Marco Crocco, MD
	Annalisa Morelli, MD
	Lidia Celeste Raguseo, MD
	Federica Malerba, MD
	Luca Elli, MD
	Fernanda Cristofori, MD
	Carlo Catassi, MD
	Alessio Fasano, MD
	CDGEMM Team, MS
Manuscript Classifications:	Celiac Disease; Pediatrics

EARLY ANTIBODY DYNAMICS IN A PROSPECTIVE COHORT OF CHILDREN AT-RISK FOR CELIAC DISEASE

Francesco Valitutti (MD) [1,2], Maureen M Leonard (MD) [3,4], Victoria Kenyon (MS) [3,4], Monica Montuori(MD)[5], Pasqua Piemontese (MD)[6], Ruggiero Francavilla (MD)[7], Basilio Malamisura (MD)[8], Lorenzo Norsa (MD)[9], Angela Calvi (MD)[10], Maria Elena Lionetti (MD)[11], Mariella Baldassarre (MD)[12], Chiara Maria Trovato (MD)[13], Michela Perrone (MD)[6], Tiziana Passaro (MD)[8], Naire Sansotta (MD)[9], Marco Crocco (MD)[10], Annalisa Morelli (MD) [14], Lidia Celeste Raguseo (MD)[7], Federica Malerba (MD)[10], Luca Elli (MD)[15], Fernanda Cristofori (MD)[7], Carlo Catassi (MD)[11], Alessio Fasano (MD) [2,3,4], CD-GEMM Team.*

1)Pediatric Unit, "San Giovanni di Dio e Ruggi d'Aragona" University Hospital, Salerno, Italy; 2) European Biomedical Research Institute of Salerno, Salerno, Italy; 3) Division of Pediatric Gastroenterology and Nutrition, MassGeneral Hospital for Children, Harvard Medical School, Boston, USA; 4) Celiac Research Program, Harvard Medical School, Boston, USA; 5) Pediatric Gastroenterology Unit, Policlinico Umberto I, Sapienza University of Rome, Italy; 6) NICU, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy; 7) Pediatric Unit " Bruno Trambusti", Osp Pediatrico Giovanni XXIII, University of Bari, Italy; 8) Celiac Disease Referral Center, "San Giovanni di Dio e Ruggi d'Aragona" University Hospital, Pole of Cava de' Tirreni, Salerno, Italy; 9) Pediatric Hepatology Gastroenterology and Transplant Unit, Ospedale Papa Giovanni XXIII Bergamo, Italy; 10) Pediatrics, IRCCS Ospedale Giannina Gaslini, Genova, Italy; 11) Pediatrics, Univ. Politec. delle Marche, Ancona, Italy; 12) NICU, University of Bari, Italy; 13) Celiac Disease Referral Center, Bambino Gesù Hospital, Rome, Italy; 14) Pediatric training program, University of Salerno School of Medicine, Salerno; 15) Celiac Disease Referral Center, Ospedale Maggiore Policlinico, Milan, Italy

CD-GEMM team*: Maria Luisa Forchielli (MD), Adelaide Serretiello (MS), Corrado Vecchi (MS), Gemma Castillejo de Villsante (MD), Giorgia Venutolo (MS).

Funding: This manuscript was partially funded by the NIH, National Institute of Diabetes and Digestive and Kidney Diseases Grants DK104344 (to A.F.) and DK109620 (to M.M.L.).
Conflict of interest: all authors declare no conflicts of interests relevant for this paper.
Key words: celiac disease**;** potential celiac disease; anti-transglutaminase antibodies; anti-deamidated gliadin peptides antibodies; infant.
Short title: Early antibody dynamics in pediatric celiac disease
Corresponding author: Prof. Alessio Fasano
Pediatric Gastroenterology and Nutrition
Mucosal Immunology and Biology Research Center
Center for Celiac Research and Treatment
Massachusetts General Hospital
Professor of Pediatrics, Harvard Medical School
afasano@mgh.harvard.edu

ABSTRACT

Objectives: To identify possible serum biomarkers predicting celiac disease (CD) onset in children at risk.

Study design: A subgroup from an ongoing, international prospective study of children at risk of CD were classified according to an early trajectory of a deamidated gliadin peptide (DGPs) IgG and clinical outcomes (CD, potential CD and CD autoimmunity).

Results: Thirty-eight of 325 children developed anti-tissue transglutaminase IgA antibody (anti-tTG IgA) seroconversion. Twenty-eight out of 38 children (73.6%) showed an increase in aDGPs IgG before their first anti-tTG IgA seroconversion.

Conclusions: aDGPs IgG can represent an early pre-clinical biomarker predicting CD onset in children at risk.

INTRODUCTION

Total IgA and anti-tissue transglutaminase IgA (anti-tTG IgA) are widely recommended as initial screening tests for celiac disease (CD) [1,2]. However, the use of anti-deamidated gliadin peptide IgG (aDGPs IgG) for CD screening has been debated . Some studies suggest that aDGPs IgG are better for screening children under age 2 [3,4], while other studies have found no added benefit [5,6].

Through a supplementary study within the Celiac Disease Genomic Environmental Microbiome and Metabolomics (CD-GEMM) study, we sought to assess the early antibody dynamics of aDGPs IgG in the 18 months to 3 years preceding CD onset.

METHODS

CD-GEMM is an international, multicenter prospective study following children from birth at-risk for CD [7]. Study protocol includes DGPs IgG and anti-tTG IgA (Inova Diagnostics Inc., San Diego, CA) conducted every 6 months from age 6-36 months and then annually. With a positive anti-tTG IgA, anti-endomysial antibodies IgA (EMA) are analyzed on the same serum sample using the NOVA Lite Monkey Esophagus IFA Kit (Inova Diagnostics Inc., San Diego, CA). The study protocol (Clinical Trial Identifier: NCT02061306) was approved by local IRBs. CD was diagnosed if the subject tested positive (>10x cut-off) for anti-tTG IgA and positive for EMA on two separate blood samples, according to no-biopsy criteria from ESPGHAN 2020 guidelines or, for anti-tTG IgA values below 10X the cut-off, an upper intestinal endoscopy with duodenal biopsy was performed according to ESPGHAN/NASPGHAN guidelines [1,2]. Potential CD was diagnosed when biopsy confirmation was required, and histological features were not consistent with CD. CD autoimmunity (CDA) was identified if subjects had an elevated anti-tTG IgA on two consecutive time points but had not yet undergone endoscopic evaluation. CD children, potential CD children and CDA children represented a broader group called “CD spectrum.”

For this study, we included children with at least three blood draws over consecutive timepoints. The primary outcome was to assess the predictive capability of the early appearance if aDGPs IgG and CD/CD spectrum onsets.

Analyses were conducted using MedCalc (version 20.0, MedCalc Software, Ostend, Belgium), $p < 0.05$ was considered statistically significant. Receiver-operating characteristics (ROC) curve analysis was performed to determine the best aDGPs IgG cut-off. Logistic regression analysis assessed the association of an early rise in aDGPs IgG with various environmental and demographic factors Covariables were selected for inclusion in the multivariable logistic regression analysis if considered clinically relevant and if they yielded a p-value <0.25 on bivariable analysis.

RESULTS

Three-hundred twenty-five (171 females; mean age 39.5 months; age range 18-60 months) of 552 recruited children fulfilled the inclusion criteria. Of those, 275 were HLA-DQ2+ or HLA-DQ8+ and 38 had anti-tTG IgA seroconversion and were classified as CD spectrum, with 21 CD cases subsequently confirmed. Diagnostic outcomes of this subcohort are summarized in **Figure 1**. Of the 38 children, 28 (73.6%) had a rise in aDGPs IgG at least 6-12 months before their anti-tTG IgA seroconversion, with median age at IgG aDGPs rise of 18 months (age range: 12-48 months).

Rise of aDGPs IgG held a relative risk (RR) of 5.03 (95%CI 3.59 to 7.05; $p< 0.0001$) for CD spectrum after 6 or 12 months. The median rise of these early antibodies in CD spectrum children was 16.9 U (range 10.3-89.9 U). The Area Under the ROC Curve (AUC) for "predictive" aDGPs IgG among the CD spectrum group was 0.86 (95% CI 0.81 to 0.89), and the best cut-off value for this purpose was > 10.0 U (sensitivity 73.7%; specificity 85.4%) as shown in **Figure 2.**

The AUC decreased to 0.82 (95% CI 0.77 to 0.86) in children diagnosed with CD and the best cut-off of aDGPs IgG value was > 10.3 U (sensitivity 71.4%; specificity 82.2%) as also shown in **Figure**

2. The median rise of these antibodies in children with CD prior to diagnosis was 17.2 U (range 10.4-89.9 U). The AUC for our 12 children with CDA overlaps with previous analyses and is shown as a **Supplementary Figure.** Logistic regression analysis did not identify any association between a rise in aDGPs IgG and factors considered in the analysis.

DISCUSSION

Herein we showed that, in children at-risk for CD tested prospectively, a rise in aDGPs IgG predicts a subsequent CD spectrum 6-12 months before disease onset in more than two thirds of the cases. The best cut-off for this predictive feature of aDGPs IgG is >10 UI/ml, which holds a sensitivity of 73.7% and a specificity of 85.4% for the CD spectrum. Thus, aDGPs IgG could represent an earlier signature of break of gluten tolerance in at-risk children and could therefore offer some utility in defining the timeframe for serological re-screening in this setting, such as 6 months after the elevation of aDGPs IgG.

Our findings concur with the known initial phases of CD pathogenesis for which deamidated gliadin is strictly linked [8]. Accordingly, aDGPs have often been considered as earlier markers within the proper clinical frame [3,4] and they have even been proven, albeit seldom, as the isolated serological signature of CD [9].

Our study is in line with previous prospective cohorts of children at-risk for CD [10,11] showing different clinical outcomes over time. Within our cohort, the rise of aDGPs IgG has displayed a similar pattern both in CD and in the larger CD spectrum cohort, with AUCs almost overlapping. Given our data, we speculate that aDGPs IgG could demonstrate a tentative "crawling" during the initial phases of the "CD march," which indeed requires careful monitoring, since early CD could rapidly evolve toward a more severe phenotype [12].

In conclusion, despite earlier literature pointing against aDGPs IgG usefulness, these antibodies may represent an early biomarker for children at risk of developing CD. To our

knowledge, no other marker is available for this purpose that could reduce diagnostic delays and possible clinical complications of pediatric CD. With the confirmation of aDGPS IgG as a single biomarker for CD in early childhood, manipulating the microbiome could be employed as a preventive treatment in the pre-clinical stage of the disease.

List of abbreviations: celiac disease (CD); anti-deamidated gliadin peptide IgG antibodies (aDGPs IgG); IgA anti-transglutaminase antibodies (anti-tTG IgA); CD autoimmunity (CDA); anti-endomysial antibodies (EMA); North American Society for Pediatric Gastroenterology and Nutrition (NASPGHAN); European Society for Pediatric Gastroenterology and Nutrition (ESPGHAN); CD-GEMM study (Celiac Disease Genomic Environmental Microbiome and Metabolomic study); Receiver-operating characteristics (ROC); Area Under the ROC Curve (AUC).

Table: Main epidemiological features of study subcohort

Gender (F/M)	171 / 154
Mean age (age range)	39.5 months (18-60 months)
Country	
US children (%)	170 (52.3%)
Italian Children (%)	155 (47.7%)
Family member with CD	
Father (%)	42 (12.9%)
Mother (%)	183 (56.3%)
Sibling (%)	67 (20.6%)
Two family members affected (%)	33 (10.2%)
HLA	
DQ2 Homozygous	42 (12.9%)
DQ2 Heterozygous	180 (55.4%)
DQ2 / DQ8	27 (8.3%)
DQ8	26 (8%)
Negative for DQ2/DQ8	50 (15.4%)

CD: Celiac Disease; **HLA:** Human Leukocyte Antigens

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó I, et al. European society paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2020. Journal of Pediatric Gastroenterology and Nutrition 2020;70:141-56.
2. Hill ID, Fasano A, Guandalini S, et al. NASPGHAN Clinical Report on the Diagnosis and Treatment of Gluten-related Disorders. J Pediatr Gastroenterol Nutr. 2016;63:156-65.
3. Liu E, Li M, Emery L, et al. Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. J Pediatr Gastroenterol Nutr 2007;45:293-300.
4. Barbato M, Maiella G, Di Camillo C, et al. The anti-deamidated gliadin peptide antibodies unmask celiac disease in small children with chronic diarrhoea. Dig Liver Dis 2011;43:465-9.
5. Gould MJ, Brill H, Marcon MA, et al. In Screening for Celiac Disease, Deamidated Gliadin Rarely Predicts Disease When Tissue Transglutaminase Is Normal. J Pediatr Gastroenterol Nutr 2019;68:20-5.
6. Khan MR, Silvester JA, Sparks B, et al. The Utility of IgA-Based Serologic Markers in Diagnosing Celiac Disease in Children 24 Months of Age or Younger. J Pediatr 2020;224:158-61.
7. Leonard MM, Valitutti F, Karathia H, et al. Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study. Proc Natl Acad Sci U S A 2021;118.
8. van de Wal Y, Kooy Y, van Veelen P, et al. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 1998;161:1585-8.
9. Catassi GN, Pulvirenti A, Monachesi C, et al. Diagnostic Accuracy of IgA Anti-Transglutaminase and IgG Anti-Deamidated Gliadin for Diagnosis of Celiac Disease in Children under Two Years of Age: A Systematic Review and Meta-Analysis. Nutrients 2021;14.
10. Segerstad EMHA, Liu X, Uusitalo U, et al. Sources of dietary gluten in the first two years of life and associations with celiac disease autoimmunity and celiac disease in Swedish genetically predisposed children: TEDDY study. Am J Clin Nutr 2022. Epub 20220408.
11. Auricchio R, Mandile R, Del Vecchio MR, et al. Progression of Celiac Disease in Children With Antibodies Against Tissue Transglutaminase and Normal Duodenal Architecture. Gastroenterology. 2019; 157:413-20.
12. Catassi GN, Vallorani M, Cerioni F, et al. A negative fallout of COVID-19 lockdown in Italy: Life-threatening delay in the diagnosis of celiac disease. Dig Liver Dis 2020;52:1092-3.

FIGURE LEGENDS

Figure 1: Study subgroups as per diagnostic outcomes. Eligible children from CDGEMM cohort who had at least three blood draws assessing both IgG aDGPs and anti-tTG IgA over consecutive timepoints. * CDA for research protocol are those who did not fulfill the ESPGHAN non-biopsy criteria and are awaiting biopsy confirmation or whose parents denied the recommended endoscopy needed to complete CD diagnosis.

Figure 2: Area Under the ROC Curve (AUC) for "predictive" aDGPs IgG among CD spectrum children and among CD children.

EARLY ANTIBODY DYNAMICS IN A PROSPECTIVE COHORT OF CHILDREN AT-RISK FOR CELIAC DISEASE

Francesco Valitutti (MD) [1,2], Maureen M Leonard (MD) [3,4], Victoria Kenyon (MS) [3,4], Monica Montuori(MD)[5], Pasqua Piemontese (MD)[6], Ruggiero Francavilla (MD)[7], Basilio Malamisura (MD)[8], Lorenzo Norsa (MD)[9], Angela Calvi (MD)[10], Maria Elena Lionetti (MD)[11], Mariella Baldassarre (MD)[12], Chiara Maria Trovato (MD)[13], Michela Perrone (MD)[6], Tiziana Passaro (MD)[8], Naire Sansotta (MD)[9], Marco Crocco (MD)[10], Annalisa Morelli (MD) [14], Lidia Celeste Raguseo (MD)[7], Federica Malerba (MD)[10], Luca Elli (MD)[15], Fernanda Cristofori (MD)[7], Carlo Catassi (MD)[11], Alessio Fasano (MD) [2,3,4], CD-GEMM Team.*

1)Pediatric Unit, "San Giovanni di Dio e Ruggi d'Aragona" University Hospital, Salerno, Italy; 2) European Biomedical Research Institute of Salerno, Salerno, Italy; 3) Division of Pediatric Gastroenterology and Nutrition, MassGeneral Hospital for Children, Harvard Medical School, Boston, USA; 4) Celiac Research Program, Harvard Medical School, Boston, USA; 5) Pediatric Gastroenterology Unit, Policlinico Umberto I, Sapienza University of Rome, Italy; 6) NICU, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy; 7) Pediatric Unit " Bruno Trambusti", Osp Pediatrico Giovanni XXIII, University of Bari, Italy; 8) Celiac Disease Referral Center, "San Giovanni di Dio e Ruggi d'Aragona" University Hospital, Pole of Cava de' Tirreni, Salerno, Italy; 9) Pediatric Hepatology Gastroenterology and Transplant Unit, Ospedale Papa Giovanni XXIII Bergamo, Italy; 10) Pediatrics, IRCCS Ospedale Giannina Gaslini, Genova, Italy; 11) Pediatrics, Univ. Politec. delle Marche, Ancona, Italy; 12) NICU, University of Bari, Italy; 13) Celiac Disease Referral Center, Bambino Gesù Hospital, Rome, Italy; 14) Pediatric training program, University of Salerno School of Medicine, Salerno; 15) Celiac Disease Referral Center, Ospedale Maggiore Policlinico, Milan, Italy

CD-GEMM team*: Maria Luisa Forchielli (MD), Adelaide Serretiello (MS), Corrado Vecchi (MS), Gemma Castillejo de Villsante (MD), Giorgia Venutolo (MS).

Funding: This manuscript was partially funded by the NIH, National Institute of Diabetes and Digestive and Kidney Diseases Grants DK104344 (to A.F.) and DK109620 (to M.M.L.).
Conflict of interest: all authors declare no conflicts of interests relevant for this paper.
Key words: celiac disease**;** potential celiac disease; anti-transglutaminase antibodies; anti-deamidated gliadin peptides antibodies; infant.
Short title: Early antibody dynamics in pediatric celiac disease
Corresponding author: Prof. Alessio Fasano
Pediatric Gastroenterology and Nutrition
Mucosal Immunology and Biology Research Center
Center for Celiac Research and Treatment
Massachusetts General Hospital
Professor of Pediatrics, Harvard Medical School
afasano@mgh.harvard.edu

ABSTRACT

Objectives: To identify possible serum biomarkers predicting celiac disease (CD) onset in children at risk.

Study design: A subgroup from an ongoing, international prospective study of children at risk of CD were classified according to an early trajectory of a deamidated gliadin peptide (DGPs) IgG and clinical outcomes (CD, potential CD and CD autoimmunity).

Results: Thirty-eight of 325 children developed anti-tissue transglutaminase IgA antibody (anti-tTG IgA) seroconversion. Twenty-eight out of 38 children (73.6%) showed an increase in aDGPs IgG before their first anti-tTG IgA seroconversion.

Conclusions: aDGPs IgG can represent an early pre-clinical biomarker predicting CD onset in children at risk

INTRODUCTION

Total IgA and anti-tissue transglutaminase IgA (anti-tTG IgA) are widely recommended as initial screening tests for celiac disease (CD) [1,2]. However, the use of anti-deamidated gliadin peptide IgG (aDGPs IgG) for CD screening has been debated . Some studies suggest that aDGPs IgG are better for screening children under age 2 [3,4], while other studies have found no added benefit [5,6].

Through a supplementary study within the Celiac Disease Genomic Environmental Microbiome and Metabolomics (CD-GEMM) study, we sought to assess the early antibody dynamics of aDGPs IgG in the 18 months to 3 years preceding CD onset.

METHODS

CD-GEMM is an international, multicenter prospective study following children from birth at-risk for CD [7]. Study protocol includes DGPs IgG and anti-tTG IgA (Inova Diagnostics Inc., San Diego, CA) conducted every 6 months from age 6-36 months and then annually. With a positive anti-tTG IgA, anti-endomysial antibodies IgA (EMA) are analyzed on the same serum sample using the NOVA Lite Monkey Esophagus IFA Kit (Inova Diagnostics Inc., San Diego, CA). The study protocol (Clinical Trial Identifier: NCT02061306) was approved by local IRBs. CD was diagnosed if the subject tested positive (>10x cut-off) for anti-tTG IgA and positive for EMA on two separate blood samples, according to no-biopsy criteria from ESPGHAN 2020 guidelines or, for anti-tTG IgA values below 10X the cut-off, an upper intestinal endoscopy with duodenal biopsy was performed according to ESPGHAN/NASPGHAN guidelines [1,2]. Potential CD was diagnosed when biopsy confirmation was required, and histological features were not consistent with CD. CD autoimmunity (CDA) was identified if subjects had an elevated anti-tTG IgA on two consecutive time points but had not yet undergone endoscopic evaluation. CD children, potential CD children and CDA children represented a broader group called “CD spectrum.”

For this study, we included children with at least three blood draws over consecutive timepoints. The primary outcome was to assess the predictive capability of the early appearance if aDGPs IgG and CD/CD spectrum onsets.

Analyses were conducted using MedCalc (version 20.0, MedCalc Software, Ostend, Belgium), $p < 0.05$ was considered statistically significant. Receiver-operating characteristics (ROC) curve analysis was performed to determine the best aDGPs IgG cut-off. Logistic regression analysis assessed the association of an early rise in aDGPs IgG with various environmental and demographic factors Covariables were selected for inclusion in the multivariable logistic regression analysis if considered clinically relevant and if they yielded a p-value <0.25 on bivariable analysis.

RESULTS

Three-hundred twenty-five (171 females; mean age 39.5 months; age range 18-60 months) of 552 recruited children fulfilled the inclusion criteria. Of those, 275 were HLA-DQ2+ or HLA-DQ8+ and 38 had anti-tTG IgA seroconversion and were classified as CD spectrum, with 21 CD cases subsequently confirmed. Diagnostic outcomes of this subcohort are summarized in **Figure 1**. Of the 38 children, 28 (73.6%) had a rise in aDGPs IgG at least 6-12 months before their anti-tTG IgA seroconversion, with median age at IgG aDGPs rise of 18 months (age range: 12-48 months).

Rise of aDGPs IgG held a relative risk (RR) of 5.03 (95%CI 3.59 to 7.05; $p< 0.0001$) for CD spectrum after 6 or 12 months. The median rise of these early antibodies in CD spectrum children was 16.9 U (range 10.3-89.9 U). The Area Under the ROC Curve (AUC) for "predictive" aDGPs IgG among the CD spectrum group was 0.86 (95% CI 0.81 to 0.89), and the best cut-off value for this purpose was > 10.0 U (sensitivity 73.7%; specificity 85.4%) as shown in **Figure 2.**

The AUC decreased to 0.82 (95% CI 0.77 to 0.86) in children diagnosed with CD and the best cut-off of aDGPs IgG value was > 10.3 U (sensitivity 71.4%; specificity 82.2%) as also shown in **Figure**

2. The median rise of these antibodies in children with CD prior to diagnosis was 17.2 U (range 10.4-89.9 U). The AUC for our 12 children with CDA overlaps with previous analyses and is shown as a **Supplementary Figure.** Logistic regression analysis did not identify any association between a rise in aDGPs IgG and factors considered in the analysis.

DISCUSSION

Herein we showed that, in children at-risk for CD tested prospectively, a rise in aDGPs IgG predicts a subsequent CD spectrum 6-12 months before disease onset in more than two thirds of the cases. The best cut-off for this predictive feature of aDGPs IgG is >10 UI/ml, which holds a sensitivity of 73.7% and a specificity of 85.4% for the CD spectrum. Thus, aDGPs IgG could represent an earlier signature of break of gluten tolerance in at-risk children and could therefore offer some utility in defining the timeframe for serological re-screening in this setting, such as 6 months after the elevation of aDGPs IgG.

Our findings concur with the known initial phases of CD pathogenesis for which deamidated gliadin is strictly linked [8]. Accordingly, aDGPs have often been considered as earlier markers within the proper clinical frame [3,4] and they have even been proven, albeit seldom, as the isolated serological signature of CD [9].

Our study is in line with previous prospective cohorts of children at-risk for CD [10,11] showing different clinical outcomes over time. Within our cohort, the rise of aDGPs IgG has displayed a similar pattern both in CD and in the larger CD spectrum cohort, with AUCs almost overlapping. Given our data, we speculate that aDGPs IgG could demonstrate a tentative "crawling" during the initial phases of the "CD march," which indeed requires careful monitoring, since early CD could rapidly evolve toward a more severe phenotype [12].

In conclusion, despite earlier literature pointing against aDGPs IgG usefulness, these antibodies may represent an early biomarker for children at risk of developing CD. To our

knowledge, no other marker is available for this purpose that could reduce diagnostic delays and possible clinical complications of pediatric CD. With the confirmation of aDGPS IgG as a single biomarker for CD in early childhood, manipulating the microbiome could be employed as a preventive treatment in the pre-clinical stage of the disease.

List of abbreviations: celiac disease (CD); anti-deamidated gliadin peptide IgG antibodies (aDGPs IgG); IgA anti-transglutaminase antibodies (anti-tTG IgA); CD autoimmunity (CDA); anti-endomysial antibodies (EMA); North American Society for Pediatric Gastroenterology and Nutrition (NASPGHAN); European Society for Pediatric Gastroenterology and Nutrition (ESPGHAN); CD-GEMM study (Celiac Disease Genomic Environmental Microbiome and Metabolomic study); Receiver-operating characteristics (ROC); Area Under the ROC Curve (AUC).

Table: Main epidemiological features of study subcohort

Gender (F/M)	171 / 154
Mean age (age range)	39.5 months (18-60 months)
Country	
US children (%)	170 (52.3%)
Italian Children (%)	155 (47.7%)
Family member with CD	
Father (%)	42 (12.9%)
Mother (%)	183 (56.3%)
Sibling (%)	67 (20.6%)
Two family members affected (%)	33 (10.2%)
HLA	
DQ2 Homozygous	42 (12.9%)
DQ2 Heterozygous	180 (55.4%)
DQ2 / DQ8	27 (8.3%)
DQ8	26 (8%)
Negative for DQ2/DQ8	50 (15.4%)

CD: Celiac Disease; **HLA:** Human Leukocyte Antigens

REFERENCES

1. Husby S, Koletzko S, Korponay-Szabó I, et al. European society paediatric gastroenterology, hepatology and nutrition guidelines for diagnosing coeliac disease 2020. Journal of Pediatric Gastroenterology and Nutrition 2020;70:141-56.
2. Hill ID, Fasano A, Guandalini S, et al. NASPGHAN Clinical Report on the Diagnosis and Treatment of Gluten-related Disorders. J Pediatr Gastroenterol Nutr. 2016;63:156-65.
3. Liu E, Li M, Emery L, et al. Natural history of antibodies to deamidated gliadin peptides and transglutaminase in early childhood celiac disease. J Pediatr Gastroenterol Nutr 2007;45:293-300.
4. Barbato M, Maiella G, Di Camillo C, et al. The anti-deamidated gliadin peptide antibodies unmask celiac disease in small children with chronic diarrhoea. Dig Liver Dis 2011;43:465-9.
5. Gould MJ, Brill H, Marcon MA, et al. In Screening for Celiac Disease, Deamidated Gliadin Rarely Predicts Disease When Tissue Transglutaminase Is Normal. J Pediatr Gastroenterol Nutr 2019;68:20-5.
6. Khan MR, Silvester JA, Sparks B, et al. The Utility of IgA-Based Serologic Markers in Diagnosing Celiac Disease in Children 24 Months of Age or Younger. J Pediatr 2020;224:158-61.
7. Leonard MM, Valitutti F, Karathia H, et al. Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study. Proc Natl Acad Sci U S A 2021;118.
8. van de Wal Y, Kooy Y, van Veelen P, et al. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 1998;161:1585-8.
9. Catassi GN, Pulvirenti A, Monachesi C, et al. Diagnostic Accuracy of IgA Anti-Transglutaminase and IgG Anti-Deamidated Gliadin for Diagnosis of Celiac Disease in Children under Two Years of Age: A Systematic Review and Meta-Analysis. Nutrients 2021;14.
10. Segerstad EMHA, Liu X, Uusitalo U, et al. Sources of dietary gluten in the first two years of life and associations with celiac disease autoimmunity and celiac disease in Swedish genetically predisposed children: TEDDY study. Am J Clin Nutr 2022. Epub 20220408.
11. Auricchio R, Mandile R, Del Vecchio MR, et al. Progression of Celiac Disease in Children With Antibodies Against Tissue Transglutaminase and Normal Duodenal Architecture. Gastroenterology. 2019; 157:413-20.
12. Catassi GN, Vallorani M, Cerioni F, et al. A negative fallout of COVID-19 lockdown in Italy: Life-threatening delay in the diagnosis of celiac disease. Dig Liver Dis 2020;52:1092-3.

FIGURE LEGENDS

Figure 1: Study subgroups as per diagnostic outcomes. Eligible children from CDGEMM cohort who had at least three blood draws assessing both IgG aDGPs and anti-tTG IgA over consecutive timepoints. * CDA for research protocol are those who did not fulfill the ESPGHAN non-biopsy criteria and are awaiting biopsy confirmation or whose parents denied the recommended endoscopy needed to complete CD diagnosis.

Figure 2: Area Under the ROC Curve (AUC) for "predictive" aDGPs IgG among CD spectrum children and among CD children.

Figure 1

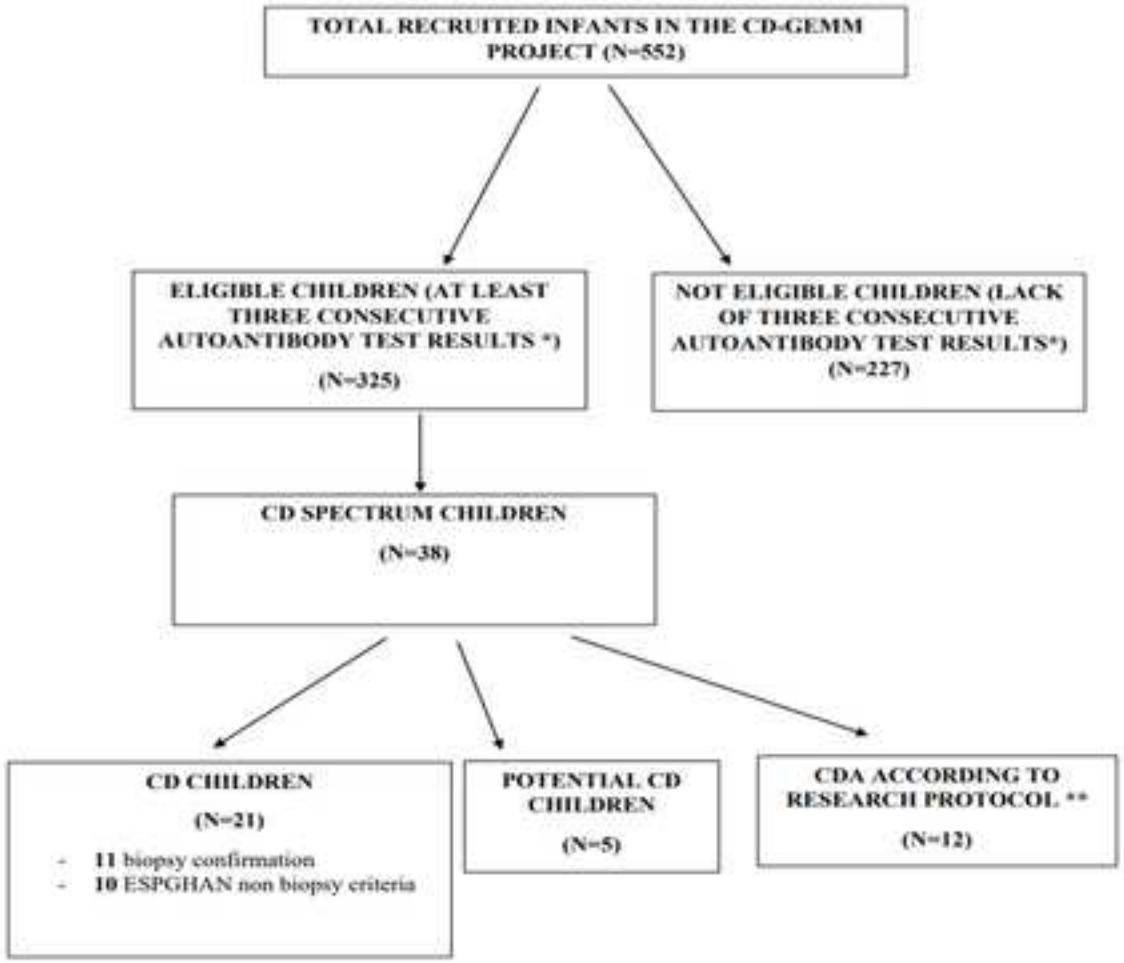
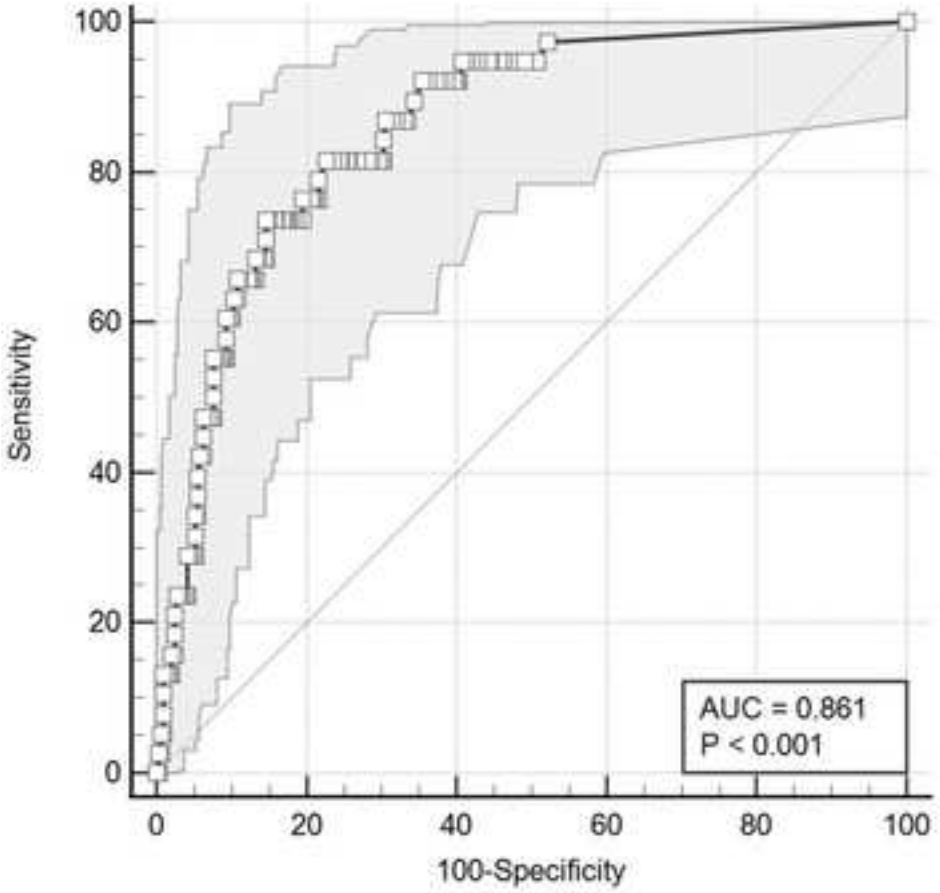
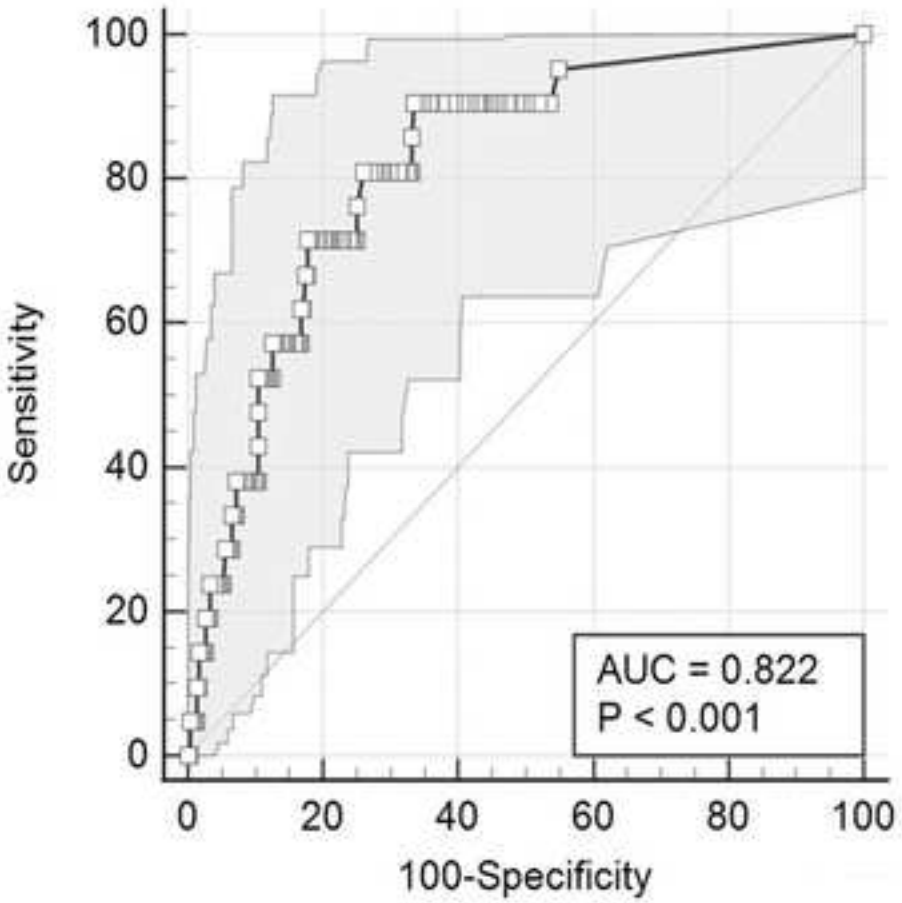


Figure 2



Area Under the ROC Curve (AUC) for "predictive" aDGPs IgG among CD spectrum children



Area Under the ROC Curve (AUC) for "predictive" aDGPs IgG among CD children

Table

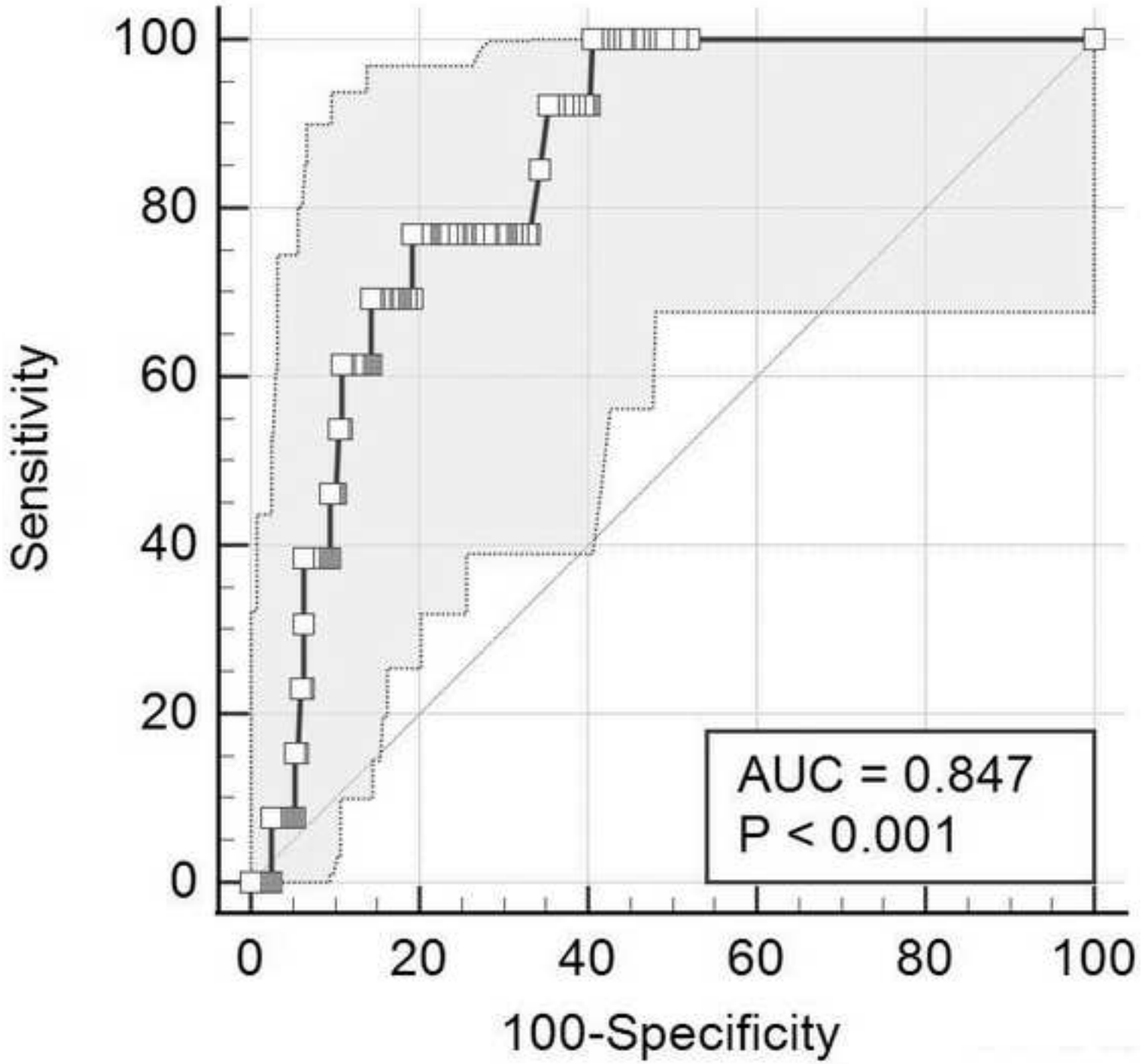
Table: Main epidemiological features of study subcohort

Gender (F/M)	171 / 154
Mean age (age range)	39.5 months (18-60 months)
Country	
US children (%)	170 (52.3%)
Italian Children (%)	155 (47.7%)
Family member with CD	
Father (%)	42 (12.9%)
Mother (%)	183 (56.3%)
Sibling (%)	67 (20.6%)
Two family members affected (%)	33 (10.2%)
HLA	
DQ2 (Homozygous)	42 (12.9%)
DQ2 (Heterozygous)	180 (55.4%)
DQ2 / DQ8	27 (8.3%)
DQ8	26 (8%)
Negative for DQ2/DQ8	50 (15.4%)

CD: Celiac Disease; **HLA:** Human Leukocyte Antigens;

Supplementary Figure

Click here to access/download;Supplementary File;Suppl_Figure.jpg



Editor/Editorial Board Comments:

The editorial board had invited/expected a resubmission as a Brief Report as outlined in the prior decision letter. The current manuscript does not adhere to that formatting. In addition to addressing the minor reviewer comment below, please also re-submit the content as a Brief Report.

As requested by the Editorial Board, we changed the structure of our manuscript to make it a Brief Report.

Reviewer #1: Thank you for incorporating the recommended feedback. The HLA classification on p 4 is still confusing to me and I think may be more clear if you include HLA-DQ2.5 and DQ2.2 in the description. One category is currently DQ 2 heterozygous and then isolated DQB*02 as a separate low risk category. Switching between the B chain and DQ description does not make it clear where DQ 2.2/X would fall in this categorization.

Thank you for this point. For sake of brevity, we are not mentioning HLA classification at all in the newer version of the paper as editors asked us to pass from original paper to brief report (2,310 words → 993 words). However, we modified the table not arising further confusion between the B chain and DQ description.

Reviewer #2: No additional comments.

Thank you

Manuscript Formatting Guide (full submission guidelines are posted online via the "Instructions for Authors" link):

Prior to resubmitting your manuscript, we ask that you review the items on the following list carefully.

1. Please be sure that you have included a complete title page. Requirements are listed in the submission guidelines on the Instructions for Authors page online. This information should appear on the title page ONLY and nowhere else in the manuscript.

Done

2. Review the main text of the manuscript thoroughly to confirm that all figures and tables (both primary and supplemental) are cited. If you have removed any figures or tables from the submission, confirm that their citations have been deleted.

Done

3. Tables should appear after the main text and before the references in an editable format. Tables must be text-based and cannot contain any graphics or colored text. Review all tables included with the submission to confirm that any abbreviations used are expanded in a footnote for that table.

Done

4. Figures must be uploaded separately from the main manuscript text and should be submitted in one of the following formats: TIF, JPG, EPS, or PPT. Include the figure legends in a list in the main manuscript document. Legends should not appear in the figure files. Note that any photomicrographs must have the magnification measurement included in the figure legend.

Done

5. Submit files associated only with the most recent revision. The initial submission manuscript or versions of past revisions and their associated files are not necessary to include with your revision.

Done

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	8
Study size	10	Explain how the study size was arrived at	4,6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding (*b*) Describe any methods used to examine subgroups and interactions (*c*) Explain how missing data were addressed (*d*) If applicable, explain how loss to follow-up was addressed (*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram	6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) Summarise follow-up time (eg, average and total amount)	1,6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6,7

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6,7
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6,7
Discussion			
Key results	18	Summarise key results with reference to study objectives	7,9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8,9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8,9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8,9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1