Genotyping of coeliac-specific human leucocyte antigen in children with type 1 diabetes: does this screening method make sense?

Elisabeth Binder,[1] Martina Loinger,[1] Annelies Mühlbacher,[2] Michael Edlinger,[3] Elisabeth Steichen,[1] Dagmar Meraner,[1] Lorin Loacker,[4] Guenter Weigel,[4] Thomas Müller,[1] Elke Fröhlich-Reiterer,[5] Sabine E Hofer[1]

[1]Department of Paediatrics, Medical University Innsbruck, Innsbruck, Austria
[2]Center of Blood Transfusion and Immunology, Innsbruck, Austria
[3]Department of Medical Statistics, Informatics, and Health Economics, Medical University Innsbruck, Innsbruck, Austria
[4]Institute of Medical and Chemical Laboratory Diagnostics, Medical University Innsbruck, Innsbruck, Austria
[5]Department of Paediatrics, Medical University Graz, Innsbruck, Austria

Correspondence to
Dr Elisabeth Binder, Department of Pediatrics, Medical University of Innsbruck, Anichstrasse 35, Innsbruck 6020, Austria; Elisabeth.Binder@i-med.ac.at

Received 12 July 2016
Accepted 30 November 2016
Published Online First 30 December 2016

ABSTRACT

Objectives Due to a high linkage disequilibrium of diabetes and coeliac-specific human leucocyte antigen (HLA) genotypes, the prevalence of coeliac disease (CD) in children and adolescents with diabetes mellitus type 1 (T1D) is much higher than in the general population. Recently, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) revised new screening guidelines in which genotyping for coeliac-specific HLA alleles is recommended for high-risk patients as patients with T1D. The aim of our study was to investigate the frequency and distribution of coeliac-specific HLA genotypes in paediatric patients with T1D.

Study design HLA genotyping was performed on paediatric patients with T1D, recruited at the Medical University Hospital of Innsbruck and Graz. The test was done by PCR. Statistical analysis was performed with IBM-SPSS V.20.

Results In 121 paediatric patients with T1D (52% male), mean age 13.3 (SD 3.9) years, mean age at diabetes diagnosis 7.4 (SD 3.8) and mean diabetes duration of 5.9 (SD 3.3) years, HLA genotyping was conducted. Ninety-two per cent showed positive HLA DQ2 and/or HLA DQ8 genotypes. Thirty-four per cent carried HLA DQ2, 33% were HLA DQ2+DQ8 positive and 25% of the patients showed positive results for HLA DQ8 alone. Only 8% had no coeliac-specific HLA markers. Four (3%) patients were diagnosed with CD.

Conclusions The majority of paediatric patients with T1D has positive coeliac-specific HLA genotypes DQ2 and/or DQ8. Therefore, genotyping for coeliac-specific HLA alleles as a first-line test in patients with T1D as recommended in the ESPGHAN guidelines does not seem reasonable. Screening for coeliac-specific antibodies needs to be performed on a regular basis for patients with T1D.

What is already known?

- Genotyping for coeliac-specific human leucocyte antigen alleles in patients at risk is recommended by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition to identify asymptomatic patients with type 1 diabetes mellitus (T1D).
- Coeliac disease and diabetes are recognised as associated diseases.
- Patients with T1D are classified as high-risk population for the development of coeliac disease.

What this study adds?

- Frequency and distribution of coeliac-specific human leucocyte antigen (HLA) genotypes in paediatric patients with TID in an Austrian cohort of two centres.
- The majority of children with diabetes in the Austrian population are screened positive for coeliac-specific HLA genotypes.
- HLA genotyping in patients with TID is not useful as a screening method for the development of coeliac disease.

Linked

► http://dx.doi.org/10.1136/archdischild-2016-311544
► http://dx.doi.org/10.1136/archdischild-2017-312714

CrossMark

To cite: Binder E, Loinger M, Mühlbacher A, *et al*. *Arch Dis Child* 2017;**102**:603–606.

INTRODUCTION

Coeliac disease (CD) is an immune-mediated systemic disorder elicited by the protein gluten in genetically susceptible patients[1] and is known to be linked to the human leucocyte antigen (HLA) class II.[2 3] Both type 1 diabetes mellitus (T1D) and CD have overlapping genetic risk factors.[4] The main genetic factors HLA DQ2 and/or HLA DQ8 are well known and account for approximately 40% of the disease heritability,[5 6] whereas non-HLA genetic contribution to CD is weak.[7] Due to a high linkage of coeliac and diabetes-specific HLA genotypes,[8] one has to expect that the genetic susceptibility of CD is much higher in T1D population than in healthy individuals.[2] The HLA-related genes such as HLA DQ2 and DQ8 associated with T1D that reside in HLA class II DR and DQ loci are shared by other autoimmune diseases such as CD.[5]

Although HLA DQ2 and/or HLA DQ8 molecules are expressed in about 30%–40% of the Caucasian population, only about 1% is affected by the gluten-sensitive enteropathy which implicates that the presence of these haplotypes does not necessarily cause the disease.[9] Approximately 90% of patients with T1D and CD present with HLA DQ2 heterodimers,[10] called DQ2.5, encoded by DQA1*05 and DQB1*02.[4]

The CD prevalence in the general population of all ages is 0.3%–1.0%[11] whereas in patients with T1D it ranges from 1.6% to 16.4% in children[4 12 13]

and 6.0% in adults[4] and varies between populations from 6.2% (Sweden),[14] 8.4% (Australia)[15] up to 10.0% reported in ISPAD guidelines[16] and 12.0% reported for Europe by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) group.[17] Being aware of the association between CD in patients with T1D and the fact that the classical intestinal symptoms may be missing in this risk group, the recommendation of paediatric endocrinologists involves active case identification by antibody screening tests performed annually using anti-tissue transglutaminase (TG) two in the first instance followed by anti-endomysial antibody (EMA)[18] as they help to confirm diagnosis of CD due to >99% specificity.[19] The guidelines of the ESPGHAN concerning the methods in screening for CD have recently been changed.[1] HLA genotyping in paediatric patients with T1D is recommended as these patients are characterised as a risk group. The advantage of genotyping as a first-line screening method is that only genetically susceptible patients need further serological testing.[18] Negativity for all antibodies on an unrestricted diet and absence of both HLA DQ2 and DQ8 strongly contradict the diagnosis of CD.

Screening guidelines for diabetes-associated diseases like CD state that due to high linkage disequilibrium only a few patients will benefit from the HLA screening.[16] Therefore, the aim of this study was to investigate the HLA genotypes of all patients with T1D to verify if this screening method as a first-line test could be of any benefit for patients with TID.

METHODS

We report on HLA DQ2 and DQ8 genotyping in a genetically heterogeneous population of paediatric patients with T1D. Patient recruitment took place at the diabetes outpatient services of the Department of Paediatrics, Medical University Hospital Innsbruck and Graz in Austria from August 2014 until March 2015. Patients with T1D were routinely genotyped for HLA DQ2 and DQ8. The study group consists of 121 patients (<22 years of age). Patients with other types of diabetes were excluded. Informed consent for genetic testing was obtained by either patients older than 14 years or their parents (<14 years). As genotyping for HLA risk alleles is a recommended diagnostic procedure and is classified as a routine procedure in screening for CD, there was no explicit approval by the local ethics committee necessary for this retrospective survey. The authors were directly involved in the diagnosis and/or treatment of the included patients and collected and accessed participant information.

As the genetic background might play a role in the frequency of CD, we have considered the immigrant status in our analysis. Immigrant background was defined as both parents born outside of Austria/Germany.

All patients underwent tissue-transglutaminase IgA antibody (tTGA) screening carried out on an annual or biannual basis. Levels of tTGA >10 kU/L were considered as increased. In case of deficiency of IgA, IgG antibodies for tTG were tested. In addition, EMAs were determined in patients with elevated tTGA.

Blood samples were collected by peripheral venepuncture.

Anti-tTG IgA/IgG antibody serum concentrations were measured using an ELISA ('Anti-Tissue-Transglutaminase IgA/IgG' ELISA, Orgentec Diagnostika GmbH, Mainz, Germany) on a BEP 2000 immunoanalyser (Siemens Diagnostics). The lower limit of detection is 1 kU/L. Intra-assay and interassay coefficients of variation were determined to be 3.0%–4.3% and 4.7%–8.1%, respectively.

Anti-EMAs serum levels were determined by the 'Anti-DGP IgA/IgA' ELISA (Orgentec Diagnostika GmbH) on a BEP2000 immunoanalyser (Siemens Diagnostics). The lower limit of detection is 1 kU/L. Intra-assay and interassay coefficients of variation are 4.0%–6.0% and 1.7%–8.7%, respectively. No specific interferences have been reported.

Genomic DNA was extracted from peripheral blood, quantified and used for HLA DQ2/DQ8 typing by multiplex PCR ('Coeliac Strip' PCR assay, Operon, Zaragoza/Spain). The main class I and II HLA haplotypes associated with CD (DQA1*05,02,03 DQB1*02,0301,0302 and DRB1*03,11,12,07,04) are genotyped by using sequence-specific biotin modified primers and subsequent hybridisation of amplified DNA fragments on sequence-specific oligonucleotide probe bound to a nylon membrane. After addition of a streptavidin-peroxidase conjugate, detection of hybridised products is done by conversion of a peroxidase substrate. The pattern of bands corresponds to a specific coeliac HLA haplotype.

A β-globin gene fragment was used as an internal PCR control amplicon. No exceptional specific interferences or hook effect has been reported.

To extract demographic data such as date of birth and sex as well as age at diabetes onset and diabetes duration, the clinical documentation data programme KIS was used. Anthropometric data such as height and weight were not considered.

Statistical analysis was done with SPSS V20.0. Fisher's exact test was used to test for independence of nominally scaled variables and the Kruskal-Wallis test to calculate for differences between phenotypes of patients regarding the age of manifestation of T1D. Two-side p values lower than 0.05 were considered as statistically significant.

RESULTS

One hundred and twenty-one patients with T1D were included in the study: 63 (52%) of them were male and 58 (48%) female. The age of patients ranged from 5 to 22 years with a mean age of 13 years (SD 3.9). The age at disease onset ranged from 1 to 16 years (mean 7, SD 3.8) and the mean diabetes duration in the patient cohort was 6 years (SD 3.3).

HLA genotyping

In 111 patients (92%), positive coeliac-specific HLA DQ genotypes were found. The HLA DQ distribution is shown in figure 1.

Forty-one patients (34%) carried HLA DQ2, 40 (33%) HLA DQ2+DQ8 and 30 (25%) HLA DQ8. Ten patients (8%) had no coeliac-specific HLA markers.

Twenty-three (19%) of the participant patients were classified with immigrant background. They did not reveal any statistically significant differences in the HLA distribution compared with Austrian patients.

Antibody screening

In 17% (20 patients) of the coeliac-specific HLA-positive individuals tTGA could be detected, but one patient was tested positive for tTGA although HLA DQ2 and DQ8 were negative. This patient revealed very low levels of tTGA (0.5 kU/L above the upper limit) and turned negative in follow-up analysis, whereas all patients who were diagnosed with CD had tTGA levels >200 kU/L.

Coeliac disease

In three patients (2%) who had positive screening for tTGA and for coeliac-specific HLA genotypes, endoscopy and diagnostic biopsy were performed. All of them were classified as CD. One patient was diagnosed with likely CD—because of positive

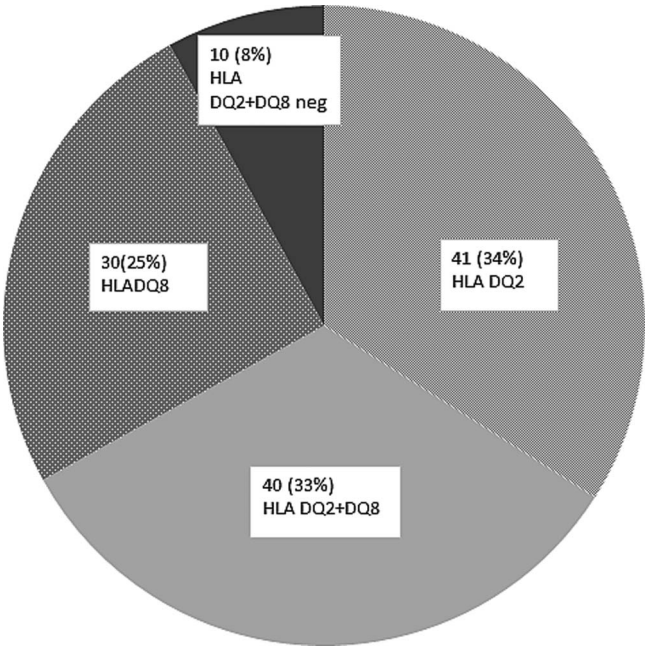

Figure 1 Distribution of HLA DQ2 und DQ8 phenotypes in 121 patients with type 1 diabetes mellitus. HLA, human leucocyte antigen.

tTGA, EMAs and coeliac-specific HLA genotypes but has not undergone biopsy. All four patients labelled as CD were female and were diagnosed with T1D before the age of 3 years. The remaining patients with coeliac-specific HLA genotypes and detected tTGA have not yet undergone biopsy.

DISCUSSION

This study was performed to investigate the presence of HLA genotypes characteristic for CD in patients with T1D in two Austrian paediatric diabetes centres. Until now, in the paediatric department of the Medical University Innsbruck and Graz annual/biannual screening for tTGA is done according to ISPAD guidelines[16] to identify patients who have to undergo further diagnosis for CD. The current ESPGHAN guidelines[1] recommend HLA genotyping as a first-line test in asymptomatic patients that belong to a high-risk group, as patients with T1D do. They emphasise the cost-effectiveness of first-line HLA genotyping because CD can be excluded in 99% of HLA-negative individuals and no further test would be required.[1] Assuming that CD is related to HLA DQ2 and/or DQ8 genotypes, annual laboratory tests of tTGA could be limited to genetically susceptible patients and would not be required in patients without HLA DQ2 or DQ8 genotypes.

We performed HLA DQ2 and DQ8 genotyping in 121 patients with T1D. Ninety-two per cent carried either HLA DQ2 or DQ8. This result might be explained by the high-linkage disequilibrium between diabetes-specific and coeliac-specific HLA genotypes[2 7] and is concordant with published data.[10 19]

In 10 out of 121 patients (8%), neither HLA DQ2 nor HLA DQ8 could be detected. This result leads to the question of whether there are any advantages of HLA genotyping in patients with T1D. Less than 10% of our patients would benefit from these new guidelines, whereas the majority is still in need of further antibody screening tests. Assuming the high occurrence of coeliac-specific HLA genotypes, no saving of expenses could therefore be expected.

Although cost analysis may differ between countries and healthcare systems, we have calculated costs in our setting. Our average costs for HLA genotyping are 60€ versus 10€ for tTG screening. Given these average costs for HLA genotyping and tTG annual screening and the fact that only 8% of our patients with negative HLA genotyping would escape lifelong tTG screening, economical aspects would also not be in favour for routine HLA genotyping. Adding the costs arising from genetic counselling—required by law in Austria—by a medical geneticist for approximately half an hour or more would even stronger argue against HLA genotyping being cost-effective.

Furthermore, calculations performed by a Dutch and a Scottish research group showed that HLA genotyping is rather expensive compared with tTGA testing.[20 21] Additionally, in smaller hospitals the availability of HLA genotyping is questionable and sending blood samples to a specialised laboratory would be elaborate and cost-intensive. Moreover, it is a genetic analysis that needs patients' and parents' consent.

The knowledge about the additional risk of other autoimmune diseases in patients with T1D such as CD is well understood. One has to consider that the detection of certain HLA genotypes, and therefore the predisposition for a greater risk to develop CD could be an additional concern for patients who suffer from T1D. This could lead to uncertainty and anxiety with potential additional psychological burden for the vast majority (92%) of patients while opportunity of providing reassurance to only a few (8%).

The importance of screening for further diabetes-associated autoimmune diseases is broadly acknowledged. It is known that untreated CD in patients with T1D goes along with unstable levels of blood glucose and a higher risk of hypoglycaemia and could lead to an increased risk of developing retinopathy.[22 23] In case of CD diagnosis, a lifelong gluten-free diet is essential to prevent further illness and to counteract the consequences of intestinal malabsorption such as iron deficiency, anaemia, growth retardation and osteoporosis.[24] Comparable to the Dutch investigation by Elias *et al*,[20] our study shows that HLA genotyping in patients with T1D is not sufficiently identifying patients at high risk of CD. The majority still has to undergo regular antibody screening (tTGA/EMA) to detect those with CD. In conclusion, cost benefit, beneficial information and patient release cannot be expected with HLA genotyping in patients with diabetes.

Our study was performed in two University Hospitals that treat children with T1D—one located in western and one located in southeastern Austria. Nineteen per cent of our patients are immigrants—the vast majority from Turkey or southeastern European countries. No significant differences in the HLA distribution between immigrants and the Austrian patients could be detected. Following a Dutch[20] and Scottish[21] investigation with similar sample size, our observation also shows that coeliac-specific HLA genotypes are very common in patients with T1D. The resulting data prompt reconsideration of the ESPGHAN screening guidelines for patients with T1D. HLA genotyping does not seem to be appropriate for this particular population. Testing for tTGA seems to be a more practical screening method.

Contributors We confirm that all authors have participated in the concept and design, analysis and interpretation of the data and have approved this submitted version of the manuscript and take full responsibility for the manuscript. EB, SEH, EF-R and ML collected and researched the data, wrote and edited the manuscript, AM, ES, LL, GW, DM and TM contributed to data interpretation and edited the manuscript, ME performed statistical analyses.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Husby S, Koletzko S, Korponay-Szabó IR, *et al*. European society for pediatric gastoenterology, hepatology, and nutrition. Guidelines for the diagnosis of celiac disease. *J Pediatr Gastroenterol Nutr* 2012;54:136–60.
2 Mäki M, Mustalahti K, Kokkonen J, *et al*. Prevalence of celiac disease among children in Finland. *N Engl J Med* 2003;348:2517–24.
3 Liu E, Lee HS, Aronsson C, *et al*. Risk of pediatric celiac disease according to HLA haplotype and country. *N Engl J Med* 2014;371:42–9.
4 Gutierrez-Achury J, Romanos J, Bekker SF, *et al*. Contrasting the genetic background of type 1 diabetes and celiac disease autoimmunity. *Diabetes Care* 2015;38:37–44.
5 Cohn A, Sofia AM, Kupfer SS. Type 1 diabetes and celiac disease: clinical overlap and new insights into disease pathogenesis. *Curr Diab Rep* 2014;14:517.
6 Megiorni F, Pizzuti A. HLA-DQA1 and HLA-DQB1 in celiac disease predisposition: practical implications of the HLA molecular typing. *J Biomed Sci* 2012;19:88.
7 Tack GJ, Verbeek WH, Schreurs MW, *et al*. The spectrum of celiac disease: epidemiology, clinical aspects and treatment. *Nat Rev Gastroenterol Hepatol* 2010;7:204–13.
8 Ide A, Eisenbarth GS. Genetic susceptibility in type 1 diabetes and its associated autoimmune disorders. *Rev Endocr Metab Disord* 2003;4:243-53.
9 Abadie V, Sollid LM, Barreiro LB, *et al*. Integration of genetic and immunological insights into a model of celiac disease pathogenesis. *Annu Rev Immunol* 2011;29:493–525.
10 Gillespie KM. Type 1 diabetes: pathogenesis and prevention. *CMAJ* 2006;175:165–70.
11 Bai JC, Fried M, Corazza GR, *et al*. World Gastroenterology Organisation global guidelines on celiac disease. *J Clin Gastroenterol* 2013;47:121–6.
12 Pham-Short A, Donaghue KC, Ambler G, *et al*. Screening for celiac disease in type 1 diabetes: a systematic review. *Pediatrics* 2015;136:e170–6.
13 Holmes GK. Coeliac disease and Type 1 Diabetes mellitus—the case for screening. *Diabet Med* 2001;18:169–77.
14 Elfström P, Sundström J, Ludvigsson JF. Systematic review with meta-analysis: association between coeliac disease and type 1 diabetes. *Aliment Pharmacol Ther* 2014;40:1123–32.
15 Doolan A, Donaghue K, Fairchild J, *et al*. Use of HLA typing in diagnosing celiac disease in patients with type 1 diabetes. *Diabetes Care* 2005;28:806–9.
16 Kordonouri O, Klingensmith G, Knip M, *et al*. ISPAD Clinical Practice Consensus Guidelines 2014. Other complications and diabetes-associated conditions in children and adolescents. *Pediatr Diabetes* 2014;15(Suppl 20):270-8.
17 Gujral N, Freeman HJ, Thomson ABR. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol* 2012;18:6036-59.
18 Atherton R, Ross A, Jessop F, *et al*. Coeliac Disease in Children with Type 1 Diabetes—are current guidelines proving difficult to implement in practice? *J Pediatr Gastroenterol Nutr* 2014;59:600–3.
19 Kagnoff MF. Celiac disease pathogenesis of a model immunogenetic disease. *J Clin Invest* 2007;117:41–9.
20 Elias J, Hoorweg-Nijman JJG, Balemans WA. Clinical relevance and cost effectiveness of HLA genotyping in children with Type 1 diabetes mellitus in screening for celiac disease in the Netherlands. *Diabet Med* 2015;32:834-8.
21 Mitchell RT, Sun A, Mayo A, *et al*. Coeliac screening in a Scottish cohort of children with type 1 diabetes mellitus: is DQ typing the way forward? *Arch Dis Child* 2016;101:230-3.
22 Mohn A, Cerruto M, Iafusco D, *et al*. Celiac disease in children and adolescents with type I diabetes: importance of hypoglycemia. *J Pediatr Gastroenterol Nutr* 2001;32:37–40.
23 Fröhlich-Reiterer EE, Kaspers S, Hofer S, *et al*. Anthropometry, metabolic control, follow-up in children and adolescents with type 1 diabetes mellitus and biopsy-proven celiac disease. *J Pediatr* 2011;158:589–93.e2.
24 Mahmud FH, De Melo EN, Noordin K, *et al*. The Celiac Disease and Diabetes-Dietary Intervention and Evaluation Trial (CD_DIET) protocol: a randomised controlled study to evaluate treatment of asymptomatic coeliac disease in type 1 diabetes. *BMJ Open* 2015;5:e008097.

Genotyping of coeliac-specific human leucocyte antigen in children with type 1 diabetes: does this screening method make sense?

Elisabeth Binder, Martina Loinger, Annelies Mühlbacher, Michael Edlinger, Elisabeth Steichen, Dagmar Meraner, Lorin Loacker, Guenter Weigel, Thomas Müller, Elke Fröhlich-Reiterer and Sabine E Hofer

Arch Dis Child 2017 102: 603-606 originally published online December 30, 2016
doi: 10.1136/archdischild-2016-311610

Notes