Screening for celiac disease in Down’s syndrome patients revealed cases of subtotal villous atrophy without typical for celiac disease HLA-DQ and tissue transglutaminase antibodies

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Abstract

AIM: To investigate the prevalence of celiac disease (CD) as well as CD marker antibodies and susceptibility HLA-DQ haplotypes in 134 karyotyped Down’s syndrome (DS) patients.

METHODS: Immunoglobulin A (IgA) and G (IgG) type anti-gliadin antibodies (AGA), IgA type anti-tissue transglutaminase (tTG) antibodies (anti-tTG) with antigen of guinea pig and human source were determined by enzyme-linked immunosorbent assay and endomysium antibodies (EMA) by indirect immunofluorescence test. HLA-DQA1*0501/DQB1*0201 (DQ2) was revealed by polymerase chain reaction. Celiac disease was diagnosed by revised ESPGHAN criteria.

RESULTS: 41% of DS patients had AGA, 6.0% IgA anti-tTG with guinea pig antigen, and 3.0% IgA EMA (all positive for anti-tTG with human tTG). Subtotal villous atrophy was found in 5 out of 9 DS patients who had agreed to small bowel biopsy. One of them had DQA1*0501/DQB1*0201 and anti-tTG and EMA i.e. typical for CD markers (this case also fulfilled the ESPGHAN diagnostic criteria), but other four lacked these markers. Three non-biopsied DS patients had also most probably CD because DQA1*0501/DQB1*0201 and IgA anti-tTG (EMA) were detected. Thus, the prevalence of CD among our DS patients population is 3.0 % (95 % of confidence interval [CI]: 0.1-5.9 %).

CONCLUSION: We confirm the increased frequency of CD among DS patients. In addition, we have revealed a subgroup of patients with subtotal villous atrophy but without characteristic for CD immunological and genetic markers. Whether these cases represent CD (with atypical immunopathogenesis) or some other immune enteropathy, requires further investigations.
loki have been revealed in chromosome 21\textsuperscript{[15, 16]}. The reason for the association of CD and DS, as well as variability of CD frequency in different populations of DS patients, is unknown. It seems that at least one cannot ascribe it to the increased number of polymorphic susceptibility genes on chromosome 21\textsuperscript{[17]} and chromosome 21 located autoimmune regulator (\textit{AIRE}) gene\textsuperscript{[18]}.

Typically, CD is characterized by chronic diarrhoea, weight loss, and failure to thrive. However, in most cases, the symptoms might be mild and non-specific or even absent, which makes it difficult to diagnose. Early diagnosis is needed because the long-term persistence of untreated CD leads to the development of various complications, including malignancy\textsuperscript{[19]}. The gold standard for the diagnosis of CD is small bowel biopsy. According to the revised criteria of the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the diagnosis of CD is based on the results of histological investigations of small bowel mucosa and confirmed by the demonstration of gluten dependence on clinical symptoms\textsuperscript{[20, 21]}. However, in some cases where the small bowel biopsy procedure is not applicable or the investigation results are unequivocal, CD might be exceptionally diagnosed by specific clinical, serological, or HLA data\textsuperscript{[22]}.

Patients

One hundred and thirty-four patients (73 males) with a mean age 11 years (ranging from six months to 45 years) with DS were enrolled in the study. The DS diagnosis was confirmed by chromosome analysis. Regular trisomy was found in 124 patients, translocation in 7 patients (four with 46,XX,der(14;21)(q10;q10),+21 karyotype, one with 46,XY,der(14;21)(q10;q10),+21, and two with 46,XX,der(21;21)(q10;q10),+21), and mosaicism in three cases. One child had translocation between 13;14 chromosomes (46,XY,der(13;14)(q10;q10),+21) with regular trisomy (Table 1). None of the patients had previously been diagnosed with CD and all patients had been on a gluten-containing diet for at least two months. All the studied persons were Caucasians living in Estonia, a country of 45 227 square kilometers and 1.4 million inhabitants. Patients were seen at the Children’s Clinic of the Tartu University Clinics. After written informed consent from the patient and his/her parents or guardian, three blood samples were taken – one for antibody analyses, the second for DNA isolation for immunogenetic analysis, and the third for chromosome analysis.

**Antibody analysis**

In-house enzyme-linked immunosorbent assay (ELISA) was used to detect IgA and IgG AGA using 96-well microtitre plates (Biohit OY, Finland) as described elsewhere\textsuperscript{[23]}. The results were reported in arbitrary units (AU) as a percentage of the optical density of a highly positive serum sample. Values of AU over 59 were considered as a sign for AGA presence. Antiendomysium antibodies of IgA-type were determined by the indirect immunofluorescence test on unfixed frozen sections of the human (blood group 0) umbilical cord using sera from patients and IGA EMA positive and negative controls diluted at 1:10. The serum of a patient was considered positive for IGA EMA if a typical staining pattern was observed around smooth muscle cells of the blood vessels\textsuperscript{[24]}. Smooth muscle antibodies of IgA type were detected using the standard indirect immunofluorescence test with unfixed frozen sections of rat liver, kidney, and mouse stomach in the patients sera by the same procedure as described above. The intracellular staining of smooth muscle cells was designated as IgA SMA\textsuperscript{[25]}. Immunoglobulin A anti-tTG were determined by two assays. The in-house ELISA test\textsuperscript{[26]} was used to determine IgA against the guinea pig tTG (IgA anti-gptTG). The results were reported in arbitrary units (AU) as a percentage of the optical density of a positive serum sample. Test results over 25 AU were considered positive. In order to determine IgA against human tTG (IgA anti-hrTG), the Celkey tTG ELISA kit (Pharmacia and Upjohn Diagnostics, Freiburg, Germany) with human recombinant tTG was used according to the manufacturer’s instructions. Values of IgA anti-hrTG higher than 8 U/ml were considered positive. Antibodies were determined under the external quality control of UK NEQAS (Sheffield, UK).

In the disease control group of consecutive untreated CD patients 100.0% had IgA and/or IgG AGA, 89.0% IgA EMA and IgA anti-tTG (data not shown).

**Materials and methods**

**Patients**

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**Immunogenetic studies**

The HLA-DQA1*0501 and DQB1*0201 alleles, encoding for DQ2 molecule, were determined by PCR-based methodology with allele-specific primers as published elsewhere\textsuperscript{[27]}. Chromosomal analysis

Chromosome preparations were made from peripheral blood lymphocyte cultures. The cytogenetic analysis was performed using GTG banding technique\textsuperscript{[28]}.

**Diagnosis of celiac disease**

Communicating with DS patients and invasive diagnostic
procedures like endoscopy and intestinal biopsy involves considerable difficulty. Therefore, we did not invite all DS patients with whatever CD marker antibodies for small intestinal biopsy procedure to confirm or deny CD, but only those who had most probably CD, that is, all AGA-positive patients with complaints compatible with CD, all patients with IgA EMA and/or anti-tTG, and all seronegative infants with typical CD symptoms (failure to thrive, chronic diarrhea). Biopsy specimens were taken from the proximal part of the mucosa of the small intestine (at the level of ligamentum Treiz) under fluoroscopic control using the Watson capsule. The diagnosis of CD was established on the basis of revised ESPGHAN criteria.[19]

**Ethics**

The study was approved by the Ethics Committee for Medical Investigations at the University of Tartu.

**Statistical analysis**

The SAS/STAT (version 6, 1990, SAS Institute Inc., Cary, NC, USA) statistical package was used for calculations. A P-value of less than 0.05 was taken to be significant.

**RESULTS**

Fifty-five (41.0 %) out of 134 DS patients had a positive test for IgG or/and IgA AGA test (Table 1). Eight (6.0 %) out of 134 DS patients had a positive IgA anti-gptTG test. When the positive sera were retested for IgA anti-hrTG, only four that had also IgA EMA, remained positives. No additional anti-hrTG positive cases were revealed among 2 DS patients with borderline anti-gptTG values (18-25 AU) and 24 randomly selected anti-gptTG negative DS patients (including 16 with the positive IgA AGA test).

Altogether, 11 DS patients with antibodies and gastrointestinal symptoms compatible with CD were invited to small bowel biopsy. In addition, there was a 11-months-old infant with typical CD symptoms (failure to thrive, chronic diarrhea) but without IgA AGA and IgA anti-hrTG (EMA). In all patients cow milk protein allergy, as another possible reason for intestinal villous atrophy, was excluded. Among 9 patients who agreed to the procedure, subtotal villous atrophy (SVA) was revealed in 5 and normal small bowel mucosa in 4 (Table 2). Only one out of these 5 patients had characteristic for CD IgA anti-tTG/EMA (none of them had had total serum IgA below the normal value as evaluated by nephelometry - data not shown) and HLA-DQA1*0501/DQB1*0201, although a clear clinical effect from gluten-free diet-disappearance of chronic diarrhea, abdominal distension and discomfort, and/or failure to thrive with the disappearance of IgA AGA positivity was revealed in four. However, we revealed IgA SMA in 3 out of 5 DS cases with SVA but only in 8 out of 119 DS cases without SVA (P<0.001; Chi-square with Yates correction).

In all 4 IgA anti-hrTG positive cases HLA-DQA1*0501/DQB1*0201 haplotype was revealed. This group of DS patients includes aforementioned patient with biopsy verified CD and 3 patients who had not agreed with intestinal biopsy procedure (two had IgA SMA). All patients had positive clinical effect from gluten free diet and therefore CD was confirmed in all four patients. Thus, we had revealed the CD prevalence at least 3.0 % (95 % CI: 0.1-5.9) among our DS patients.

No significant differences were found in the karyotype characteristics between DS patients with and without antibodies or CD (Table 1).

**DISCUSSION**

Celiac disease deserves special attention as most common gastrointestinal autoimmune associate of DS.[2, 3]. However, the mechanisms underlying the development of CD in DS have remained unknown. We have revealed SVA compatible for CD and/or CD by characteristic IgA anti-tTG and HLA-DQ2 data as well as clinical effect of gluten-free diet in 4 (3.0 %) of studied 134 (95 % confidence interval ([CI]: 0.1-5.9 %) DS patients. This finding 3 or more times exceeds the prevalence of CD in general population.[3]

Similar CD frequencies in DS patients have been revealed in countries of different regions of the world.[4-12, 22]. The only exceptionally high prevalence of CD in DS (16.9 %) was revealed in Sweden by Jansson and Johansson.[5]. However, the selection of DS patients and screening methods, as well as frequency of CD cases in local background population could significantly affect the results. Aforementioned Swedish authors have screened DS patients by AGA test and diagnosed CD in 9 of 19 biopsied patients. In line of their results we have also detected a set of EMA-negative but IgA AGA positive DS patients with SVA (Table 2).

In the present study we used in parallel all the commonly available serological CD screening assays-AGA, EMA and anti-tTG tests – and confirmed three opinions presented in the literature. First, we found a high prevalence (41 %) of IgA and/or IgG AGA among DS patients.[4, 11]. Second, the IgA anti-tTG reactivity is best detected by human tTG.[2, 30, 31]. Third, the IgA EMA and anti-hrTG highly
correlates with the presence of DQ2 haplotype\(^{3, 14}\). However, as a new observation, we revealed a portion of DS patients with SVA but without characteristic for CD serological and genetical markers. One might ask whether these patients have cow milk protein allergy or B-cell immunodeficiency representing other well-known associates of SVA. This was not a case as revealed by the additional clinical inves-tigations in these patients. However, these patients may have severe imbalances in immune regulation leading to the development of this type of enteropathy. As a support to this view we have detected a substantial decr-ease of peripheral blood regulatory T cells (including CD4\(^+\) CD25\(^{hi}\) cells) in one of these SVA patients compared to age-sex mached controls (data not shown). Regulatory T cells play a key role in the maintenance of self-tolerance, thus preventing autoimmune dis-ease, as well as inhibiting harmful inflammatory diseases\(^{32}\).

Noteworthy, in three of five DS patients with SVA SMA were revealed. Smooth-muscle antibodies group may include different antibodies types, antibodies to actin, tubulin, desmin and others\(^{33}\), among which antibodies against actin and desmin have been found in untreated CD patients\(^{34, 35}\). Also a number of autoimmune enteropathy cases have been described to be associated with SMA (reviewed by Russo and Alvarez\(^{36}\)). Whether our patients with SVA but without typical immunologic and genetic characteristics of CD represent an entity of autoimmune enteropathy of DS or just a group of atypical CD cases, needs further investigations. The latter possibility could be easily drawn from the recent studies\(^{5-8}\) where immunologically and immunogenetically atypical CD cases were discovered among DS patients.

What is the actual cause of the rised prevalence of CD in DS patients? According to special analysis there is a number of immune response influencing genes in chromosome 21\(^{36}\). Thus, the abnormal function of these genes (whatever the mechanism) as the cause of general immune dysfunction, including impaired local immunity and high susceptibility to infections, might contribute to the impairment of the integrity of the small bowel and lead to food antigen leakage through the intestinal mucosa. However, some genes responsible for gut mucosa integrity could be involved as well. As indirect evidence for this suggestion, we have revealed AGA in as many as 41% of DS patients. This supports the earlier studies about the high frequency of AGA\(^{37-39}\) and other food antibodies in DS\(^{37}\).

To conclude, the results of our study confirm the earlier reports about an increased prevalence of CD in DS. However, according to our results there are also some DS patients with SVA not fulfilling the typical immunological and genetical criteria for CD. Whether these patients with SVA represent just a subgroup of CD (as judged by the clinical effect of gluten-free diet) but with a deviation in immunopathogenesis, or other types of immune enteropathies (as judged by immunological data), needed to be answered in future studies.

**ACKNOWLEDGEMENTS**

The authors thank Drs Reet Rein and Piret Laidre for their generous help in obtaining patient samples and Ms Kadri Eomäe, Ms Maire Mandel, Ms Jane Urb, Mrs Anu Kaldmaa and Mr Tarmo Peda for technical assistance. Mr. Enn Veldi is greatly appreciated for his help in manuscript preparation. The study was

**REFERENCES**

1. Levy J. The gastrointestinal tract in Down syndrome. *Prog Clin Biol Res* 1991; 373: 245-256
2. Roizen NJ, Patterson D. Down’s syndrome. *Lancet* 2003; **361**: 1281-1289
3. Green PH, Jabri B. Coeliac disease. *Lancet* 2003; **362**: 383-391
4. Storm W. Prevalence and diagnostic significance of gliadin antibodies in children with Down syndrome. *Eur J Pediatr* 1990; **149**: 833-834
5. Jansson U, Johansson C. Down syndrome and celiac disease. *J Pediatr Gastroenterol Nutr* 1995; **21**: 443-445
6. Gale L, Wimalaratna H, Brotodiharjo A, Duggan JM. Down’s
syndrome is strongly associated with celiac disease. Gut 1997; 40: 492-496

7 Zachor DA, Mroczek-Musulman E, Brown P. Prevalence of celiac disease in Down syndrome in the United States. J Pediatr Gastroenterol Nutr 2000; 31: 275-279

8 Book L, Hart A, Black J, Feolo M, Zone JJ, Neuhansen SL. Prevalence and clinical characteristics of celiac disease in Down syndrome in a US study. Am J Med Genet 2001; 98: 70-74

9 Bonamico M, Mariani P, Danesi HM, Crisogionni M, Failla P, Gemme G, Quartinio AR, Giannotti A, Castro M, Balli F, Lecora M, Andria G, Guariso G, Gabrielli O, Catassi C, Lazzari R, Balocco NA, De Virgiliis S, Culasso F, Romano C. Prevalence and clinical picture of celiac disease in Italian down syndrome patients: a multicenter study. J Pediatr Gastroenterol Nutr 2001; 33: 139-143

10 Rumbo M, Chirdo FG, Ben R, Saldungaray I, Villalobos R. Evaluation of coeliac disease serological markers in Down syndrome patients. Dig Liver Dis 2002; 34: 116-121

11 Agardh D, Nilsson A, Carlsson A, Kockum I, Lernmark A, Ivarsson SA. Tissue transglutaminase autoantibodies and human leucocyte antigen in Down’s syndrome patients with coeliac disease. Acta Paediatr 2002; 91: 34-38

12 Hansson T, Dahlbom I, Rogberg S, Nyberg BI, Dahlstro J, Annere G, Klareskog L, Dannaeus A. Antitissue transglutaminase and antithyroid autoantibodies in children with Down syndrome and celiac disease. J Pediatr Gastroenterol Nutr 2005; 40: 170-174; discussion 125-7

13 Solid LM, Thorsby E. HLA susceptibility genes in celiac disease: genetic mapping and role in pathogenesis. Gastroenterology 1993; 105: 910-922

14 Kagnoff MF. Celiac disease pathogenesis: the plot thickens. Gastroenterology 2002; 123: 939-943

15 Liu J, Joo SH, Holopainen P, Terwilliger J, Tong X, Grunn A, Tien A, Hart A, Black J, Feolo M, Zone JJ, Neuhausen SL. Prevalence and clinical characteristics of celiac disease in Down syndrome in the United States. J Pediatr Gastroenterol Nutr 2000; 31: 275-279

16 Babron MC, Nilsson S, Adamovic S, Naluai AT, Wahlstrom J, Ascher H, Ciclitira PJ, Solid LM, Partanen J, Greco L, Clerget-Darpoux F. Meta and pooled analysis of European coeliac disease data. Eur J Hum Genet 2003; 11: 828-834

17 Morris MA, Yiannakou JY, King AL, Brett PM, Biagi F, Vaughan R, Curtis D, Ciclitira PJ. Coeliac disease and Down syndrome: associations not due to genetic linkage on chromosome 21. Scand J Gastroenterol 2000; 35: 177-180

18 Shield JP, Wadsworth EJ, Hassold TJ, Judis LA, Jacobs PA. Is disomic homozygosity at the APECED locus the cause of increased autoimmunity in Down's syndrome? Arch Dis Child 1999; 81: 147-150

19 Kurlemann G, Palm DG. Growth failure secondary to moyamoya syndrome. Arch Dis Child 1990; 65: 1012

20 Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity (‘celiac sprue’). Gastroenterology 1992; 102: 330-354

21 Kaikkonen K, Partanen J, Maki M, Collin P. HLA-DQ typing in the diagnosis of celiac disease. Am J Gastroenterol 2002; 97: 695-699

22 Bonamico M. Which is the best screening test for celiac disease in Down syndrome children? J Pediatr Gastroenterol Nutr 2005; 40: 125-127

23 Bugn-Wolf A, Gaze H, Hadziselimovic F, Huber H, Lentze MJ, Nussle D, Reymond-Berthet C. Antigliadin and antientomysium antibody determination for celiac disease. Arch Dis Child 1991; 66: 941-947

24 Uibo O, Metskula K, Sukk T, Rago T, Uibo R. Results of coeliac disease screening in Estonia in 1990-1994. Acta Paediatr 1996; Suppl 412: 39-41

25 Clemente MG, Musu MP, Troncone R, Volta U, Congia M, Ciacci C, Neri E, Not T, Maggiore G, Strisciuglio P, Corazza GR, Gasbarrini G, Cicotto L, Sole G, Fasano A, De Virgiliis S. Enterocyte actin autoantibody detection: a new diagnostic tool in celiac disease diagnosis: results of a multicenter study. Am J Gastroenterol 2004; 99: 1551-1556

26 Teesalu K, Uibo O, Kalkkinnen N, Jannney P, Uibo R. Increased levels of IgA antibodies against desmin in children with coeliac disease. Int Arch Allergy Immunol 2001; 126: 157-166

27 Ladinsner B, Rossipal E, Pittschuler K. Endomysium antibodies in celiac disease: an improved method. Gut 1994; 35: 776-778

28 Sacchetti L, Sarrantonio C, Pastore L, Carlino V, Calcagno G, Ferrajolo A, Salvatore F. Rapid identification of HLA DQA1*0501, DQB1*0201 and DRB1*04 alleles in celiac disease by a PCR-based methodology. Clin Chem 1999; 45: 2204-2206

29 Mitelman F(ed.). ISCN1995: An international system for human cytogenetic nomenclature. Basel: S. Karger;

30 Wong RC, Wilson RJ, Steele RH, Radford-Smith G, Adelstein S. A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits. J Clin Pathol 2002; 55: 488-494

31 Fabiani E, Peruzzi E, Mandolesi A, Garbuglia G, Fanciulli G, D’Appello AR, Gasparin M, Bravi E, Bearzi I, Galeazzi R, Catassi C. Anti-human versus anti-guinea pig tissue transglutaminase antibodies as the first-level serological screening test for coeliac disease in the general population. Dig Liver Dis 2004; 36: 671-676

32 Allez M, Mayer L. Regulatory T cells: peace keepers in the gut. Inflamm Bowel Dis 2004; 10: 666-667

33 Toh BH. Smooth muscle autoantibodies and autoantigens. Clin Exp Immunol 1979; 38: 621-628

34 Russo P, Alvarez F. Autoimmune enteropathy: A review. Clin Appl Immunol Rev 2002; 2: 203-216

35 Zubillaga P, Vidalles MC, Zubillaga I, Ormaechea V, Garcia-Urka N, Vitoria JC. HLA-DQA1 and HLA-DQB1 genetic markers and clinical presentation in celiac disease. J Pediatr Gastroenterol Nutr 2002; 34: 548-554

36 Gilton Y, Dahmane N, Baik S, Ruiz I Altaba A, Neidhardt L, Scholze M, Herrmann BG, Kahlem P, Benkahla A, Schirrner S, Yildirimman R, Herwig R, Lehrhach H, Yaso M-L. A gene expression map of human chromosome 21 orthologues in the mouse. Nature 2002; 420: 586-590

37 Kanavin O, Scott H, Fausa O, Ek J, Gaarder PI, Brandtzæg P. Immunological studies of patients with Down’s syndrome. Measurements of autoantibodies and serum autoantibodies to dietary antigens in relation to zinc levels. Acta Med Scand 1988; 224: 473-477

S-Editor Guo SY L-Editor Zhang JZ E-Editor Wu M