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RAPID COMMUNICATION

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 immunofluoresence 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.

Key words: Down's syndrome; Subtotal villous atrophy; Celiac disease; Screening; Autoantibodies; Anti-gliadin antibodies; HLA

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INTRODUCTION

Patients with Down's syndrome (DS), trisomy 21, have a variety of gastrointestinal disorders[1] and immunological disturbances that are related to the gastrointestinal tract[2]. However, the mechanisms underlying the complex phenotype of these associations have remained largely unknown.

Celiac disease (CD), characterized by villous atrophy of the small intestine induced by wheat, rye, and barley in the food [3], is the most common immune disease in patients with DS being detected in 1.6% to 16.9% of cases[4 - 12]. In general, susceptibility to CD is associated with the major histocompatibility complex (MHC) genes from extended HLA haplotypes DR3-DQ2 (*DRB1*03, DQA1*0501, DQB1*0201*) or DR5/DR7-DQ2 *(DRB1*11/DRB1*07 or DRB1*12/DRB1*07, DQA1*0501, DQB1*0201*) and about 95% of CD patients have these haplotypes[13, 14]. However, as many as 25-30 % of the general Caucasian population carry DQ2 molecules, showing that other non-HLA genes are also involved[14]. Systematic genome screenings in CD and affected siblings have revealed several other loci possibly involved in CD susceptibility. However, no CD associated

loci have been revealed in chromosome 21[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[17] and chromosome 21 located autoimmune regulator (*AIRE*) gene[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[3]. 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[19, 20]. 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[21]. Patients with DS may be very difficult continent for biopsy due to their mental development retardation, particularly if the the peroral biopsy capsule is used[22].

During the last decades many efforts have been made to find serological markers for CD. Since the 1970s, antigliadin antibodies (AGA) of IgG and IgA types have been used for CD screening, but these antibodies tended to be present also in a number of patients without CD and even in healthy persons[23, 24]. On the other hand, endomysium antibodies (EMA) or antibodies to the EMA's specific target, tissue transglutaminase (tTG), are highly specific for CD[3, 22]. Also, other autoantibodies, including IgA-type anti-smooth muscle (SMA), antiactin and antidesmin antibodies, are frequently detected in patients with CD but revealed in other disease groups as well[25, 26].

The present study aimed to investigate the prevalence of CD, CD marker antibodies and HLA-DQ in DS patients and to compare the results with kariotype and clinical data in these patients.

MATERIALS AND METHODS

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 [24]. 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[27]. 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[26]. Immunoglobulin A anti-tTG were determined by two assays. The in-house ELISA test[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-htTG), the Celikey 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-htTG 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-hutTG (data not shown).

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[28].

Chromosomal analysis

Chromosome preparations were made from peripheral blood lymphocyte cultures. The cytogenetic analysis was performed using GTG banding technique[29].

Diagnosis of celiac disease

Communicating with DS patients and invasive diagnostic

Table 1 Comparative frequency of different karyotypes among 134 Down's syndrome patients and the incidence of EMA, anti-tTG, AGA, and celiac disease in these patients

Karyotype groups	No. of patients	No. with positive AGA	No. with positive anti-tTG (guinea pig antigen)	No. with positive anti-tTG (human antigen) and EMA	No. with celiac disease
Regular trisomy	124	54	8	4	4
Translocation	7	1	0	0	0
Mosaicism	3	0	0	0	0
Total	134	55 (41.0 %)	8 (6.0 %)	4 (3.0 %)	4 (3.0 %)

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 diarrhoea). 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-htTG, only four that had also IgA EMA, remained positives. No additional anti-htTG 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-htTG (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 characterisic 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-htTG 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 characeristic 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[12, 30, 31]. Third, the IgA EMA and anti-htTG highly

Table 2 Profile of 12 Down's syndrome patients (all with regular trisomy) invited for small bowel biopsy

Patient No.	Age (yr) sex	Symptoms	Small bowel biopsy	IgA AGA	IgG AGA	IgA SMA	IgA EMA	IgA anti-gptTG	IgA anti-htTG	HLA-DQB1 *0201	HLA-DQA1 *0501
6	0.9 F	+ #	SVA	-	-	+	-	-	-	-	+
10	13 M	+ ##	SVA	+	+	-	-	-	-	-	-
18	10 M	-	Norm.	+	+	-	-	+	-	-	-
59	1 M	+ ##	SVA	+	+	+	-	-	-	-	-
78	5 M	+ ###	SVA	-	-	-	+	+	+	+	+
91	40 F	+ ####	n. d.	+	+	-	+	+	+	+	+
92	17 M	+ ####	n. d.	+	+	+	+	+	+	+	+
96	18 F	+ ##	SVA	+	+	+	-	-	-	-	-
97	8 M	-	Norm.	-	+	-	-	-	-	n. d.	n. d.
98	7 F	-	Norm.	-	+	-	-	-	-	n. d.	n. d.
105	9 M	+ ####	n. d.	+	+	+	+	+	+	+	+
130	3 F	-	Norm.	-	+	-	-	-	-	n. d.	n. d.

Numbers in bold denote positive values in antibody tests; SVA – subtotal villous atrophy; Norm. – normal mucosa; n. d. – studies not done (biopsy procedure was denied). # - symptoms did not disappear during the four-year gluten-free diet – CD was not diagnosed. ## - symptoms disappeared after introduction of gluten-free diet – CD was not diagnosed due to lack of anti-htTG and HLA-DQ2. ### - symptoms disappeared after introduction of gluten-free diet – CD was diagnosed by morphology data (by ESPGHAN criteria). #### - symptoms disappeared after introduction of gluten-free diet – CD was confirmed without morphology data.

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 enterpathy. As a support to this view we have detected a substantial decr-ease of peripheral blood regulatory T cells (including $CD4^{+}CD25^{high}$ 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 disease, as well as inhibiting harmful inflammatory diseases[32].

Noteworthy, in three of five DS patients with SVA IgA 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[25, 26]. Also a number of autoimmune enteropathy cases have been described to be associated with SMA (reviewed by Russo and Alvarez[34]). 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 invstigations. The latter possibility could be easily drawn from the recent studies[11, 12, 35] 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[5-8] 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 an 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.

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