ERRATUM

Serological screening for coeliac disease in adults with Turner's syndrome: prevalence and clinical significance of endomysium antibody positivity

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The authors and the journal apologize for errors in the above paper, which appeared in **160**(4) 675–679, in which CD was incorrectly expanded to cluster of differentiation throughout the paper. The corrected article is published below.

Abstract

Objective: To investigate the prevalence of coeliac disease (CD) in an adult population with Turner's syndrome (TS).

Design: A clinic population with TS was screened using a serological test for CD.

Methods: Two hundred and fifty six patients with TS were included in the study. Five patients had existing diagnoses of CD. The remaining 251 asymptomatic patients were screened using an IgA endomysium antibody (EMA) test. Positive cases were offered endoscopy with duodenal biopsy. HLA typing was undertaken in existing cases and new EMA-positive cases.

Results: Of the 251 patients screened, eight were found to be EMA positive (3.2%). Seven patients proceeded to duodenal biopsy on which all were confirmed histologically to have CD (2.8%). The prevalence of subclinical CD in the population can therefore be estimated between 2.8 and 3.2%. The total population prevalence of CD, including the previously diagnosed cases, is estimated between 4.7 and 5.1%. Ten patients with histologically confirmed CD underwent HLA typing of which eight were HLA-DQ2 positive, one was HLA-DQ8 positive and one was negative to both HLA-DQ2 and HLA-DQ8.

Conclusions: This study demonstrates an increased prevalence of CD in an adult population with TS over the general population. This is consistent with previous data published in paediatric populations.

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Introduction

The hallmark of Turner's syndrome (TS) is an absent or structurally abnormal X- chromosome in a phenotypic female. It has long been recognized that there is an increased incidence of autoimmune disorders in TS (1), with hypothyroidism being the most common. Many studies have reported an increased prevalence of coeliac disease (CD) in TS (2–9), with prevalences reported of 0–8.1%, compared with 0.5–1.0% in the general population, based on serological screening (10).

Coeliac screening studies in TS have thus far been performed in paediatric populations, and we note that the risk of developing autoimmune thyroid disease increases with age (11). We have therefore undertaken serological screening for CD in an adult population with TS to determine the degree of age progression of this condition.

Recent advances in understanding the pathogenesis of CD have pointed to the modification of wheat gluten proteins by tissue transglutaminase (12), with a key role of HLA-DQ2 in presenting toxic wheat proteins to

T cells. Tissue transglutaminase is the target of the antiendomysial autoantibody response in CD. Antibodies to endomysium (IgA endomysium antibody, EMA) can be measured in serum via indirect immunofluorescence to provide a serological test for CD which has been shown to be highly sensitive and specific, with values for both parameters exceeding 95% in most studies (13). An alternative approach using an enzyme linked immunoabsorbent assay measuring IgA tissue transglutaminase antibody (tTGA) levels has comparable sensitivity and specificity, however, incomplete concordance between the two tests has led some authors to suggest combination screening (14). By contrast, the previously widely used antigliadin tests have been shown to have relatively poor sensitivity and specificity.

While serological screening remains a powerful tool in the diagnosis of CD, it has been demonstrated that EMA sensitivity is reduced in cases of partial rather than total villous atrophy (15), and the gold standard for the diagnosis of CD remains the finding of villous atrophy on duodenal biopsy.

Subjects and methods

Two hundred and fifty six consecutive women with karyotypically proven TS attending the Adult Turner clinic at University College Hospital, London were screened as a routine part of their health surveillance programme. Ethical committee approval was given for clinical notes review.

All subjects were screened using a serological test for EMA positivity. Indirect immunofluorescence analysis was performed using commercially available fixed sections of monkey oesophagus (Biodiagnostics Ltd, Worcestershire, UK) as antigen substrate. Patient serum was diluted 1:10 with PBS. Patient endomysium specific IgA was detected with a FITC-labelled sheep anti-human IgA conjugate (Dako Ltd, Ely, UK F0204). All positive patients were offered a duodenal biopsy. These were undertaken either in our centre or at the patient's local centre, in accordance with routine practice. We accepted routine pathology reports as evidence of CD as we were unable to obtain original slides from all centres to enable a uniform reporting system.

HLA typing was offered to all patients with positive EMA serology or a previous diagnosis of CD. DNA was extracted from EDTA blood using a Qiagen MagAttract DNA Cell Mini Kit by a M48 BioRobot and analysed using the Protrans Domino System HLA CD Association kit.

Results

The median age of the 256 patients with TS was 29 (16–61) years. Five patients had an established diagnosis of CD made prior to the transition to adult care, following a variety of clinical presentations. In order to clarify the effect of ascertainment bias affecting the screening statistics, we have presented prevalence data for CD with and without these cases included. The characteristics of these five cases are presented with those of the newly identified EMA-positive cases (Table 1). The 251 remaining patients with TS were screened for EMA positivity.

EMA were positive in 8/251 (3.2%) patients, none of whom reported symptoms suggestive of CD. Duodenal biopsy was declined by one patient; the remaining seven proceeded to biopsy that confirmed the diagnosis of CD histologically, with partial or total villous atrophy in all cases (7/251 or 2.8%). If the five previously diagnosed cases of CD were included then the total population prevalence of CD would be 12/256 (4.7%).

HLA typing was performed in 10 women with histologically confirmed CD (six newly diagnosed and four previously diagnosed); eight were HLA-DQ2 positive, one was HLA-DQ8 positive and one was negative for both HLA-DQ2 and HLA-DQ8.

In order to place our findings in a wider perspective, we combined our data with that from all published

studies found in a search of Medline (Table 2). The prevalence of EMA positivity detected by EMA/tTGA screening was 27/638 (4.2%). Of the 21 EMA-positive cases who proceeded to biopsy, 18 (85.7%) had histological confirmation of CD, with five studies showing 100% concordance between EMA positivity and biopsy-proven CD.

Discussion

This study confirms the finding of an increased prevalence of CD in TS and, interestingly, the prevalence in an adult population is similar to that reported in paediatric populations (Table 2) (2–9). Combining all studies, the prevalence of screening-detected EMA positivity in TS is 4.2%, which represents a 4 to 8 fold increased risk over the general population. The fact that there appears to be little age-related progression in the prevalence of CD is in contrast to the natural history of hypothyroidism in adults with TS (11) and raises the possibility that a single screening exercise will ascertain most cases. It might be prudent to undertake this during paediatric care, and certainly before treatment with growth hormone is considered, as untreated CD could impact on growth velocity. Reviewing the data in the TS population, serological screening appears to be an effective method of identifying subclinical CD. While HLA screening is a possible alternative, in our population, one HLA-DQ2/8 negative case was identified which raises the possibility of false negatives in the TS population.

It is interesting to speculate on the mechanism for this increased prevalence of CD and other autoimmune diseases in TS. CD is a strongly heritable condition, with a 75% concordance in monozygotic twins compared with 11% in dizygotic twins (16). More than 90% of patients diagnosed with CD have the HLA-DQ2 haplotype, with virtually all the rest having HLA-DQ8 (17). This compares with a European population prevalence of these HLA subtypes of between 20 and 30% (18). There appears to be no increased representation of these genotypes in TS; in the one TS population that has been studied to date, 14/46 (29.1%) were found to be HLA-DQ2 positive, comparable with the general population (6). From these observations, we conclude that a 'second hit' of autoimmune activation of this HLA risk group must be behind the excess of risk of CD in TS. Of note, there was no significant relationship between hypothyroidism (6/13 women with CD) and CD in this audit.

All eight previously reported cases of TS and CD that have been subjected to HLA typing have been found to carry HLA-DQ2 (2, 5, 6). Interestingly, one subject (1/8 or 12.5%) in our study population with positive EMA serology and histologically confirmed CD was HLA-DQ2 and DQ8 negative. This is rare in the general population with CD and is the first case to be reported in the

Table 1 Histological, serological, and genetic characteristics of patients with Turner syndrome with new and existing diagnoses of coeliac disease.

Case no.	Age at diagnosis of TS (years)	Karyotype	Coeliac disease status	Age at diagnosis of CD (years)	Biopsy results (Villous atrophy)	EMA	HLA DQ2	HLA DQ8	Genotype
1	1	45,X/46,X+mar.ish der(X) (DXZ1+)	Previous diagnosis	4	Partial	a	+	—	DRB1*0301
									DRB1*07
									DQA1*0501
									DQA1*0201
									DQB1*0201
									DQB1*0202
2	11	45,X monosomy X	Previous diagnosis	30	Partial	a	+	—	DRB1*0301
									DQA1*0501
									DQB1*0201
3	11	45,X/46,X psu dic(X)(p22.3)	Previous diagnosis	11	*	a	*	*	*
4	20	45,X monosomy X	Previous diagnosis	39	Total	a	+	—	DRB1*0301
									DRB1*07
									DQA1*0501
									DQA1*0201
									DQB1*0201
									DQB1*0202
5	11	45,X/46,X,i(Xq) isochromosome X	Previous diagnosis	20	*	a	—	+	DRB1*04
									DQA1*0301
									DQB1*0302
6	0 (birth)	45,X monosomy X	New diagnosis	31	Partial	+	+	—	DRB1*0301
									DQA1*0501
									DQB1*0201
7	0 (birth)	45,X monosomy X	New diagnosis	29	Partial	+	+	—	DRB1*0301
									DQA1*0501
									DQB1*0201
8	4	45,X monosomy X	New diagnosis	47	Partial	+	+	—	DRB1*0301
									DQA1*0501
									DQB1*0201
9	3	45,X/46,X,i(X) (q10) isochromosome X	New diagnosis	41	Total	+	—	—	DRB1*07
									DQA1*0201
									DQB1*0202
									HLA DQ2/DQ8 haplotypes not detected
10	4	45,X/46,X,idic(X) (p11.1) isochromosome X	New diagnosis	31	Partial	+	+	—	DRB1*0301
									DQA1*0501
									DQB1*0201
11	0 (birth)	45,X monosomy X	New diagnosis	38	Partial	+	+	—	DQA1*0501
									DQB1*0201
									DRB1*0301
									DQA1*0501
									DQB1*0201
12	6	45,X/46,XX	New diagnosis	20	Partial	+	*	*	*
13	7	45,X monosomy X	Refused biopsy	*	*	+	*	*	*

EMA, IgA endomysium antibody; HLA, human leukocyte antigen. *Data not available, a: EMA not performed – gluten free diet established.

Table 2 Summary of serological screening studies for coeliac disease in Turner syndrome. Data from these studies has been modified to only include cases identified by the IgA endomysium antibody (EMA) or tissue transglutaminase antibody (tTGA) screening. Studies using alternative screening methods were not included.

Author, year, study population	Age: median (range) years	No. of patients screened	No. EMA or tTGA positive (%)	No. of positive cases biopsied	No. biopsy positive cases (% of those biopsied)	Prevalence of biopsy-confirmed coeliac disease detected by EMA IgA or tTGA IgA screening	Total population prevalence of coeliac disease, including existing diagnoses
Bonamico (1998) Italy	13.8 (2–27)	35	2 (5.7%)	1	1 (100%)	1/35 (2.9%)	3/37 (8.1%)
Ivarsson (1999) Sweden	* (3–16)	87	4 (4.6%)	3	3 (100%)	3/87 (3.4%)	4/87 (4.6%)
Gillett (1999) Canada	12.4 (5.3–17.6)	18	3 (16.6%)	1	1 (100%)	1/18 (5.6%)	1/18 (5.6%)
Schweizer (2000)	*	100	2 (2.0%)	1	1 (100%)	1/100 (1%)	3/121 (2.5%)
The Netherlands							
Rujner (2001) Poland	* (2–21.7)	46	0 (0.0%)	0	0	0/46 (0%)	2/48 (4.2%)
Doganc (2001) Turkey	* (5–20)	38	1 (2.6%)	1	0 (0%)	0/38 (0%)	0/38 (0%)
Sagodi (2006) Hungary	*	63	7 (11.1%)	7	5 (71.4%)	5/63 (7.9%)	5/63 (7.9%)
Frost (2009) UK	29 (16–61)	251	8 (3.2%)	7	7 (100%)	7/251 (2.8%)	12/256 (4.7%)
Combined data	* (2–61)	638	27 (4.2%)	21	18 (85.7%)	18/638 (2.8%)	30/668 (4.5%)

*Data not available.

Turner's population. In a large European study of 1008 patients with CD, only 61 (6.1%) HLA-DQ2/DQ8 negative cases were described (17). Of these 61 cases, 57 encoded half of the HLA DQ2 heterodimer. It has been suggested that the remaining four cases could carry rare mutations in other HLA-DQ alleles, enabling them to present gliadin to T cells (18). Although only one case of HLA-DQ2/DQ8 negative CD was identified here, it is nevertheless tempting to speculate that this case adds further weight to the notion of an exaggerated 'second hit' in the development of CD in TS. TS therefore provides an important model for the study of the origin of autoimmune disorders, with X-chromosome genes and oestrogen deficiency as possible mechanisms.

When considering screening for subclinical CD in TS, it is important to assess what the clinical significance of the diagnosis may be in this population. Some studies suggest that people with undetected CD have a tendency towards low bone density (19, 20) and mild nutritional deficits (21). Even in our large study, our population with TS and CD was too small to provide sufficient power to address this issue with statistical significance.

There is, however, one scenario when it would be particularly prudent to undertake CD screening and that is in the pre-pregnancy work-up of women with TS seeking ovum donation. Undiagnosed CD at the time of delivery may be associated with pre-term birth, intrauterine growth retardation and low or very low birth weight (22), all of which are already prevalent in TS pregnancies (23). This is in contrast to maternal CD diagnosed before birth which is not associated with adverse foetal outcomes.

In conclusion, our findings confirm an increased prevalence of CD in TS, estimated in our population as lying between 4.7% (biopsy confirmed) and 5.1% (EMA positive). When all studies are combined, a total population prevalence of CD in TS is estimated at 4.5%. The potential advantages to the detection of clinically silent CD in this population, such as beneficial effects on bone mineral density, nutritional status, and pregnancy outcomes remain to be clarified.

Declaration of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

ARF, data collection, manuscript writing; MMB, coordination of HLA typing; GSC, concept, revision of manuscript.

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References

- 1 Fialkow PJ. Autoimmunity and chromosomal aberrations. *American Journal of Human Genetics* 1966 **18** 93–108.
- 2 Bonamico M, Bottaro G, Pasquino AM, Caruso-Nicoletti M, Mariani P, Gemme G, Paradiso E, Ragusa MC & Spina M. Celiac disease and Turner syndrome. *Journal of Pediatric Gastroenterology and Nutrition* 1998 **26** 496–499.
- 3 Ivarsson S-A, Carlsson A, Bredberg A, Alm J, Aronsson S, Gustafsson J, Hagenas L, Hager A, Kristrom B, Marcus C, Moell C, Nilsson KO, Tuvemo T, Westphal O, Albertsson-Wikland K & Aman J. Prevalence of coeliac disease in Turner syndrome. *Acta Paediatrica* 1999 **88** 933–936.
- 4 Gillett PM, Gillett HR, Israel DM, Stewart L, Metzger DL, Chanoine J-P & Freeman HJ. Increased prevalence of celiac disease in girls with Turner syndrome. *Journal of Pediatric Gastroenterology and Nutrition* 1999 **29** 497.
- 5 Schweizer JJ, Mearin ML, Brand R & Wit J-M. Increased frequency of celiac disease in Turner syndrome in the Netherlands. *Journal of Pediatric Gastroenterology and Nutrition* 2000 **31** S144–S145.
- 6 Rujner J, Wisniewski A, Grogorek H, Wozniowicz B, Mlynarski W & Witas HW. Coeliac disease and HLA-DQ2 (DQA1*0501 and DQB1*0201) in patients with Turner syndrome. *Journal of Pediatric Gastroenterology and Nutrition* 2001 **32** 114–115.
- 7 Doganc T, Caliskan L & Vidinlisan S. Celiac disease and HLA-DQ2 association in patients with Turner syndrome. *Journal of Pediatric Gastroenterology and Nutrition* 2001 **33** 352.
- 8 Bonamico M, Pasquino AM, Mariani P, Danesi HM, Cutlasso F, Mazzanti L, Petri A & Bona G. Prevalence and clinical picture of celiac disease in Turner syndrome. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 5495–5498.
- 9 Sagodi L, Solyam E, Tamasi K & Minik K. Prevalence of coeliac disease in Turner syndrome. *Orvosi Hetilap* 2006 **147** 1185–1188.
- 10 Van Heel DA & West J. Recent advances in coeliac disease. *Gut* 2006 **55** 1037–1046.
- 11 Elsheikh M, Wass JAH & Conway GS. Autoimmune thyroid syndrome in women with Turner's syndrome – the association with karyotype. *Clinical Endocrinology* 2001 **55** 223–226.
- 12 Molberg O, Mcadam SM, Korner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P, Lundin KE, Sjoström H & Sollid LM. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nature Medicine* 1998 **4** 713–717.
- 13 Hill ID. What are the sensitivity and specificity of serologic tests for celiac disease? Do sensitivity and specificity vary in different populations? *Gastroenterology* 2005 **128** S25–S32.
- 14 Dickey W, McMillan SA & Hughes DF. Sensitivity of serum tissue transglutaminase antibodies for endomysial antibody positive and negative coeliac disease. *Scandinavian Journal of Gastroenterology* 2001 **36** 511–514.
- 15 Rostami K, Kerchhaert J, Tiemessen R, von Blomberg BM, Meijer JW & Mulder CJ. Sensitivity of antiendomysium and antigliadin antibodies in untreated celiac disease: disappointing in clinical practice. *American Journal of Gastroenterology* 1999 **94** 888–894.
- 16 Greco L, Romino R, Coto I, Di Cosmo N, Percopo S, Maglio M, Paparo F, Gasperi V, Limongelli MG, Cotichini R, D'Agate C, Tinto N, Sacchetti L, Tosi R & Stazi MA. The first large population based twin study of coeliac disease. *Gut* 2002 **50** 624–628.
- 17 Karel K, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, Ciclitera PJ, Sollid LM & Partanen J. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Human Immunology* 2003 **64** 469–477.
- 18 Kipatany A, Toth L, Tumpek J, Csipo I, Sipos E, Woolley N, Partanen J, Szegedi G, Olah E, Sipka S & Korponay-Szab IR. Diagnostic significance of HLA-DQ typing in patients with previous coeliac disease diagnosis based on histology alone. *Alimentary Pharmacology & Therapeutics* 2006 **24** 1395–1402.
- 19 Corazza GR, Di Sario A, Cecchetti L, Jorizzo RA, Di Stefano M, Minguzzi L, Brusco G, Bernardi M & Gasbarrini G. Influence of pattern of clinical presentation and of gluten-free diet on bone mass and metabolism in adult coeliac disease. *Bone* 1996 **18** 525–530.
- 20 Mustalahti K, Collin P, Sievanen H, Salmi J & Maki M. Osteopenia in patients with clinically silent coeliac disease warrants screening. *Lancet* 1999 **354** 744–745.
- 21 Haapalahti M, Kulmala P, Karttunen TJ, Paaanen L, Laurila K, Maki M, Mykkanen H & Kokkonen J. Nutritional status in adolescents and young adults with screen-detected celiac disease. *Journal of Pediatric Gastroenterology and Nutrition* 2005 **40** 566–570.
- 22 Ludvigsson JF, Montgomery SM & Ekblom A. Celiac disease and risk of adverse fetal outcome: a population-based cohort study. *Gastroenterology* 2005 **129** 454–463.
- 23 Abir R, Fisch B, Nahum R, Orvieto R, Nitke S & Ben Rafael Z. Turner's syndrome and fertility: current status and possible putative prospects. *Human Reproduction Update* 2001 **7** 603–610.

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