Normal small bowel biopsy followed by coeliac disease

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Abstract

We report four patients (two children, one adolescent, and one adult) having normal small bowel mucosa shown on a biopsy specimen taken before the initial diagnosis of coeliac disease was made. The first biopsy was undertaken in two cases because of suspected malabsorption, in the third because of suspected dermatitis herpetiformis, and in the fourth as part of a coeliac disease family study. After a further 2·6 to 9 years on a diet containing gluten, small bowel villous atrophy with crypt hyperplasia compatible with coeliac disease was found on a second biopsy specimen. The HLA type of the patients was that typical for coeliac disease; all were DR3 positive. Within the families three other patients with coeliac disease have been diagnosed, two earlier and one at the time the first biopsy was undertaken. Four other HLA-DR3 positive haploidentical first degree relatives were found and had biopsies. All four had normal small bowel villous architecture, one had an increased intraepithelial cell count, and another was positive for reticulin and endomysium antibodies. Coeliac disease may exist latent in patients having normal mucosa when eating a normal diet containing gluten.

It is generally accepted that a normal small bowel biopsy specimen, taken for the first time when the patient is eating a normal diet containing gluten, rules out the diagnosis of coeliac disease once and for all. At present we demand an abnormal small intestinal mucosa (subtotal villous atrophy or flat mucosa) as being essential for a diagnosis of coeliac disease. There are several observations, however, for example, late relapsers on gluten challenge[1] (I Polanco, J Larrauri. Abstract presented at the International Coeliac symposium, St Bartholomew's Hospital, London, 4–6 September, 1988) and late appearing concordancy in monozygotic twins,[2 3] indicating that coeliac disease may exist latent in patients having normal mucosa when eating a normal diet. There are few such patients described in the literature.[4–6] We now report four patients in whom the first biopsy specimen ruled out coeliac disease but who were later found to have the disease. The patients were HLA typed and family members were also studied in greater detail.

Methods

Information on symptoms and signs compatible with coeliac disease was obtained by parental and patient recall and also by reviewing the hospital medical records.

Routine small bowel biopsy was performed with a paediatric or adult Watson capsule and the specimens were studied under light microscopy.

Serum IgA and IgG class gliadin[7] and reticulin[8] as well as IgA class endomysium[9] antibodies were studied as earlier described.

Serological HLA typing of the patients and also of the family members for A, B, C, and DR locus antigens was performed[10] and DR typing confirmed by restriction fragment length polymorphism from DNA samples using Taq I restriction enzyme and DR β probe from the X International Histocompatibility Workshop.[11]

Results

CASE REPORTS

Case 1

This girl was referred to the hospital at 0·9 years of age on account of poor weight gain. She had

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Table 1 Case 1: clinical data and results of serum antibody studies and small bowel biopsy

	Referral	*Follow up*					
Age (years)	0·9	1·2	2·2	3·5†	3·8	4·5	5·4
Gluten in diet	Yes	Yes	Yes	Yes	No	No	No
Abdominal symptoms	No	No	No	No	No	No	No
Height (SD)	−0·5	−0·5	−0·5	−0·5	−0·5	−0·5	−0·5
Weight (SD)	−1·0	−1·0	−0·5	−0·5	−0·5	−0·5	−0·5
Gliadin antibodies*							
IgA	0·12	0·05	<0·05	0·78	0·09	<0·05	<0·05
IgG	9·80	3·32	1·97	37·00	5·30	0·28	0·35
Reticulin antibodies*							
IgA	Negative	Negative	Negative	1:500	Negative	Negative	Negative
IgG	Negative	Negative	Negative	Negative	Negative	Negative	NT
Endomysium antibodies*							
IgA	Negative	NT	NT	1:500	NT	NT	Negative
Biopsy	Normal			Subtotal villous atrophy			

*Reference values: gliadin antibodies (ELISA units/ml), IgA <0·2, IgG <5·0; reticulin antibodies, negative (serum titre <1:10); and endomysium antibodies, negative (serum titre <1:5).
NT=not tested.
†Gluten free diet started after this follow up.

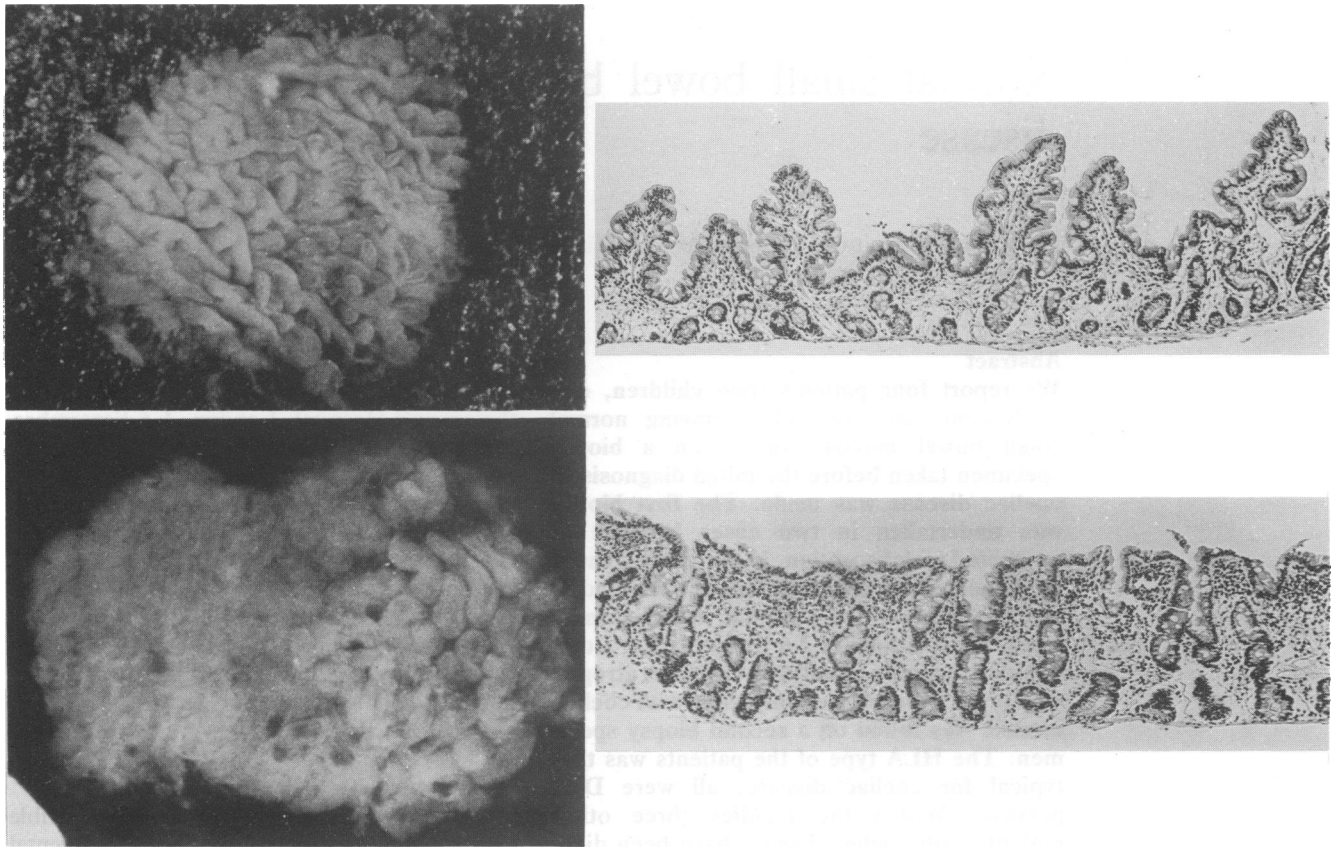

Figure 1 Dissection and findings on light microscopy of small intestinal mucosa of case 1 at the age of 0·9 years (upper figures) and at the age of 3·5 years (lower figures). The patient had been on a normal diet containing gluten from the age of 5 months.

been breast fed for four months, and gluten had been introduced into her diet at 5 months of age. There was no family history of coeliac disease. She had had no abdominal symptoms and the stools were normal. Small bowel biopsy was performed because of slightly positive IgG class gliadin antibodies and poor weight gain (table 1). IgA class reticulin and endomysium antibody tests were at that time negative. In the upper part of fig 1 the dissection and light microscopic findings of the mucosa are shown. The biopsy specimen was interpreted as normal, excluding coeliac disease. On re-evaluation of the specimen the intraepithelial lymphocyte count was found to be 34/100 epithelial cells. She was followed up because of positive gliadin antibody titre. She gained weight on a diet containing gluten and the gliadin antibody titre decreased to negative values. The follow up was continued because of lack of growth and at the visit at 3·5 years of age both IgA and IgG class gliadin antibodies were positive. Also IgA class reticulin and endomysium antibody titres were high. The parents had noticed no change in her well being. A second biopsy specimen showed subtotal villous atrophy (fig 1, lower figures) with some patchiness seen in the dissection microscope. A gluten free diet resulted in the disappearance of gliadin, reticulin, and endomysium antibodies.

Case 2

Small bowel biopsy was performed on this boy at 2·3 years of age because of intermittent loose stools and because his brother had coeliac disease. His weight and height curves were normal. He was on a normal diet containing gluten. The biopsy specimen showed tall villous architecture and coeliac disease was excluded (fig 2). The intraepithelial lymphocyte count was 31/100 epithelial cells. IgA and IgG class reticulin antibodies were not detected. A coeliac disease family study showed at the age of 6·8 years a low IgA class reticulin antibody titre (1:10). Small bowel biopsy was not performed in view of the initial normal biopsy. On follow up at 7·6 years of age the IgA class reticulin antibody titre was 1:1000. He had experienced no abdominal symptoms. The second biopsy specimen showed subtotal villous atrophy (fig 2, lower figure). In table 2 serum gliadin and endomysium antibody results are also shown. After starting a gluten free diet the gliadin antibodies became normal (0·2 years) but IgA class reticulin and endomysium antibodies were positive, although lower.

Case 3

At 11·4 years of age this boy was suspected of having dermatitis herpetiformis because of his skin lesions. He was on a normal diet containing gluten as the other family members. He had no abdominal symptoms and his growth chart showed no deviations. At the same time his younger sister was studied because of short stature at 9 years of age and coeliac disease was diagnosed. Both skin and small bowel biopsies were performed. Reticulin antibodies were negative (table 3). Skin biopsy ruled out dermatitis herpetiformis and small bowel biopsy

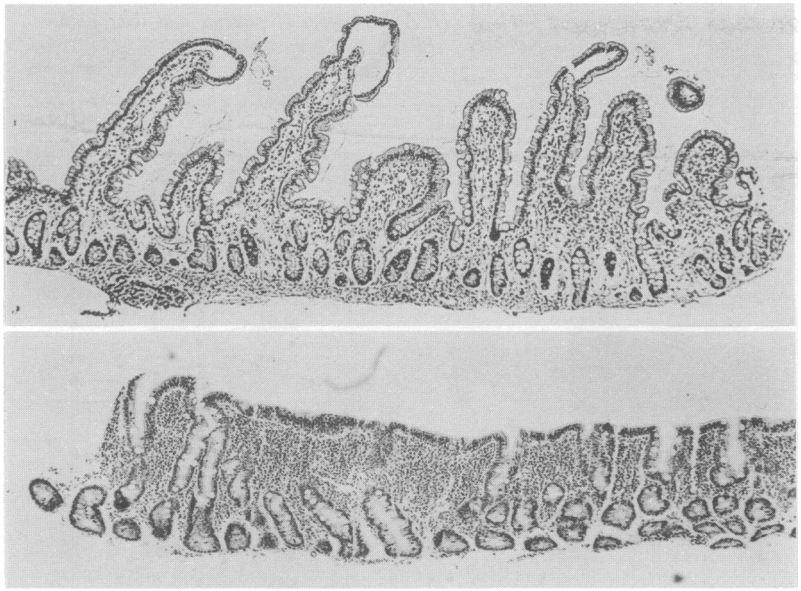

Figure 2 Small bowel mucosal architecture of case 2 at the age of 2·3 years (upper figure) and 7·6 years (lower figure). He had been all the time on a normal diet containing gluten.

was thought to exclude coeliac disease irrespective of technical difficulties (fig 3). The intraepithelial lymphocyte count was at 21/100 epithelial cells. He passed puberty normally on a diet containing gluten. He was enrolled in a coeliac disease family study at 17·5 years of age and a second small bowel biopsy specimen showed subtotal villous atrophy (fig 3, lower figure). IgG class gliadin and IgA class both reticulin and endomysium antibodies were detected. Six months later on a gluten free diet reticulin and endomysium antibodies disappeared, and a control biopsy specimen showed considerable improvement of the villous architecture.

Table 2 Case 2: clinical data and results of serum antibody studies and small bowel biopsy

	Referral	*Follow up*		
Age (years)	2·3	6·8	7·6†	7·8
Gluten in diet	Yes	Yes	Yes	No
Abdominal symptoms	Loose stools	None	None	None
Growth	Normal	Normal	Normal	Normal
Gliadin antibodies*				
IgA	NT	0·07	1·35	0·10
IgG	NT	10·10	21·00	1·45
Reticulin antibodies*				
IgA	Negative	1:10	1:1000	1:100
IgG	Negative	Negative	Negative	Negative
Endomysium antibodies*				
IgA	NT	1:10	1:1000	1:100
Biopsy	Normal		Subtotal villous atrophy	

*Reference values: gliadin antibodies (ELISA units/ml), IgA <0·2, IgG <5·0; reticulin antibodies, negative (serum titre <1:10); and endomysium antibodies, negative (serum titre <1:5).
NT=not tested.
†Gluten free diet started after this follow up.

Table 3 Case 3: clinical data and results of serum antibody studies and small bowel biopsy

	Referral	*Follow up*	
Age (years)	11·4	17·5†	18·0
Gluten in diet	Yes	Yes	No
Growth	Normal	Normal	Normal
Abdominal symptoms	None	None	None
Skin biopsy (suspected dermatitis herpetiformis)	Normal	Normal	NT
Gliadin antibodies*			
IgA	NT	0·15	0·16
IgG	NT	19·10	12·00
Reticulin antibodies*			
IgA	Negative	1:100	Negative
IgG	Negative	Negative	Negative
Endomysium antibodies			
IgA	NT	1:200	Negative
Biopsy	Normal	Subtotal villous atrophy	Partial villous atrophy

*Reference values: gliadin antibodies (ELISA units/ml), IgA <0·2, IgG <5·0; reticulin antibodies, negative (serum titre <1:10); and endomysium antibodies, negative (serum titre <1:5).
NT=not tested.
†Gluten free diet started after this follow up.

Case 4

At 32 years of age the patient was examined because of diarrhoea and suspected ulcerative colitis. She was on a normal diet containing gluten. She had a child with coeliac disease and was enrolled in a coeliac disease family study and found not to have coeliac disease because a small bowel biopsy specimen showed completely normal mucosa (fig 4). Nine years later another family study was performed that included small bowel biopsy. She did not mention that a biopsy had already been taken. She had no abdominal symptoms. Immunological tests were compatible with coeliac disease (table 4) and histologically her small bowel mucosa showed clear deterioration with partial villous atrophy with heavy plasma cell infiltration and a raised intraepithelial lymphocyte count at 41/100 epithelial cells (fig 4, lower figure). During dietary treatment serum antibodies became normal and a control biopsy specimen six months later showed considerable improvement with almost normal mucosa.

HLA RESULTS AND FAMILY MEMBER STUDIES

The results on HLA haplotypes of the patients and their family members are given in table 5. Each of the patients was positive for HLA-DR3. Two of the patients had HLA identical siblings suffering from coeliac disease and one patient had a HLA haploidentical son with the disease. Four other family members had the same HLA-DR3 positive haplotype as the patient. These four family members were on normal diets containing gluten but were also encouraged to eat normal food with at least 10 g of gluten per day for a further three months, after which they were studied for serum gliadin, reticulin, and endomysium antibodies and biopsied.

The mother of case 1 was negative in all antibody tests and her small intestinal mucosa was normal (villous height 521 μm, crypt depth 119 μm, intraepithelial lymphocyte count 16/100 epithelial cells). The father of case 2 was homozygous for DR3 and negative for gliadin antibodies but positive for IgA class both reticulin and endomysium antibodies (low titres 1:10). His mucosal architecture was normal (villous height 417 μm, crypt depth 112 μm, intraepithelial lymphocyte count 18/100 epithelial cells). The father of case 3 was negative for the antibody tests and normal on biopsy (villous height 506 μm, crypt depth 104 μm, slightly raised intraepithelial count 29/100 epithelial cells). The DR3 positive haploidentical son of case 4 had normal antibody titres and normal small bowel mucosal architecture (villous height 461 μm, crypt depth 126 μm, intraepithelial lymphocyte count 17/100 epithelial cells).

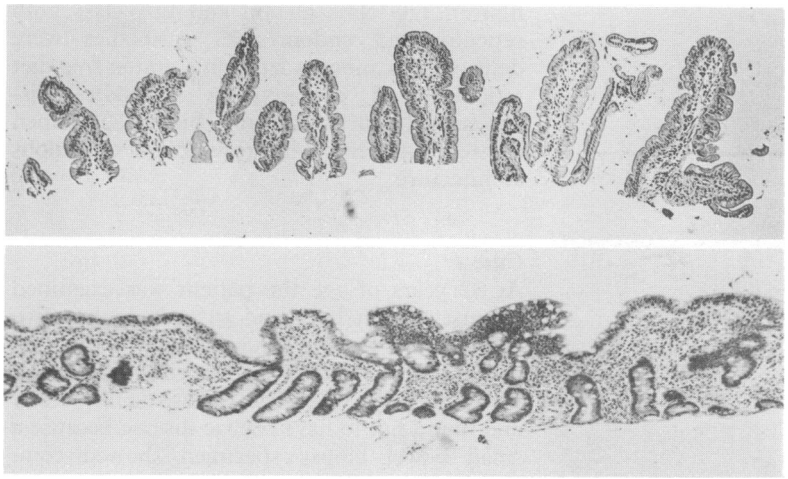

Figure 3 Small bowel mucosal architecture of case 3 at the age of 11·4 years (upper figure) and showing tall villi irrespective of technical difficulties and at 17·5 years (lower figure). with subtotal villous atrophy and crypt hyperplasia.

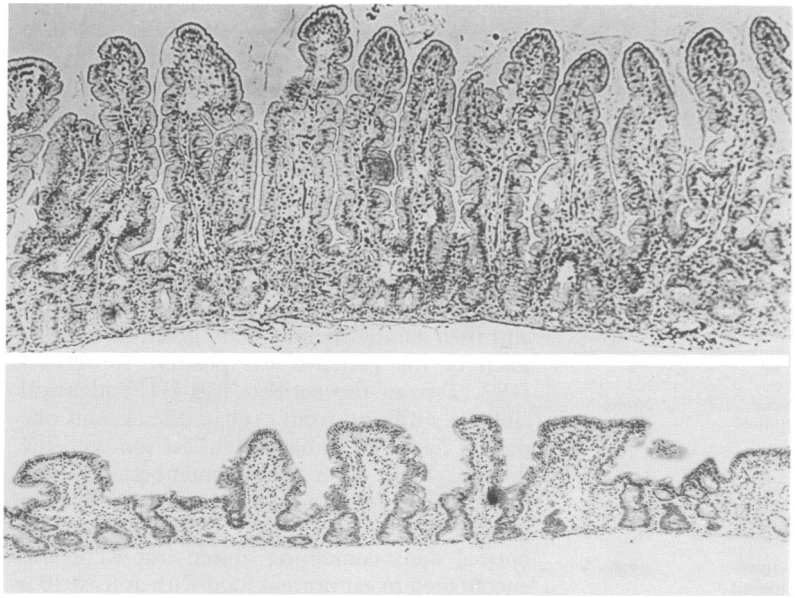

Figure 4 Normal small bowel biopsy appearance of case 4 at the age of 32 years (upper figure). She had been on normal diet since early childhood. A second biopsy specimen at the age of 41 years showed clear deterioration with partial villous atrophy (lower figure).

Table 4 Case 4: clinical data and results of serum antibody studies and small bowel biopsy

	Referral	*Follow up*	
Age (years)	32·0	41·3†	41·8
Gluten in diet	Yes	Yes	No
Abdominal symptoms	Diarrhoea	None	Better
Gliadin antibodies*			
IgA	NT	0·08	<0·05
IgG	NT	62·40	4·10
Reticulin antibodies*			
IgA	NT	1:200	Negative
IgG	NT	Negative	NT
Endomysium antibodies			
IgA	NT	1:100	Negative
Biopsy	Normal	Partial villous atrophy	Normal

*Reference values: gliadin antibodies (ELISA units/ml), IgA <0·2, IgG <5·0; reticulin antibodies, negative (serum titre <1:10); and endomysium antibodies, negative (serum titre <1:5).
NT=not tested.
†Gluten free diet started after this follow up.

Table 5 HLA haplotypes of patients and their family members

Case No	*HLA*				*Family member*	*HLA*			
	A	*B*	*C*	*DR*		*A*	*B*	*C*	*DR*
1	1	8	7	3	Father	2	27	2	4
	2	27	2	4		2	22	1	4
					Mother	2	15	4	8
						1	8	7	3
					Brother	2	22	1	4
						2	15	4	8
2	2	27	2	3	Father	2	27	2	3
	3	7	—	6		3	15	3	3
					Mother	3	7	—	6
						9	27	—	4
					Brother*	2	27	2	3
						3	7	—	6
3	2	8	7	3	Father	2	8	7	3
	11	35	4	6		9	40	3	7
					Mother	11	35	4	6
						2	7	7	2
					Sister	9	40	3	7
						11	35	4	6
					Sister*	2	8	7	3
						11	35	4	6
4	2	5	7	7/9	Son*	3	7	7	2
	1	8	7	3		1	8	7	3
					Son	3	7	—	6
						1	8	7	3
					Son	3	7	7	2
						2	5	2	7/9

*Coeliac disease.

Discussion

Our four cases are identical to those previously described in the literature.[2–6] They were all first diagnosed to be free of coeliac disease and years later found to have the disease. A closer look at the initial biopsy specimens showed minor pathology in an increased count of intraepithelial lymphocyte in some cases, but still with tall villous architecture. Hence they cannot be diagnosed as having coeliac disease and treated as such. Low gluten intake may have influenced the villous architecture in two of the patients as there was one earlier diagnosed case of coeliac disease on a gluten free diet at the time of the first biopsy in the family. In fact, it has been shown that a low gluten diet has no significant effect on gross small bowel mucosal architecture in coeliac disease.[12] Another possible explanation is mucosal patchiness; flat mucosa might have been found even close to the biopsy site. We do not think this is a relevant explanation, however, because the highly sensitive and specific antibody tests used by us[9] were negative at the time of the first biopsy and later positive when mucosal atrophy was manifest.

Late developing mucosal atrophy in coeliac disease (latent coeliac disease) may be much more common than previously assumed. Recent reports indicate that coeliac disease in children is disappearing.[13 14] Our results show that coeliac disease has not disappeared; the clinical picture has changed to a milder form, resulting in an upward shift of age at diagnosis.[15] We have shown by following up birth cohorts to the age of 16 years that coeliac disease exists and appears late in the cohorts.[16] In fact, over 60% of diagnoses here are today made at school age and during adolescence. Also in adults coeliac disease often presents itself with only mild or no abdominal symptoms,[17 18] and a greater awareness of the disease has led to an increasing

number of cases diagnosed annually.[19] It is hardly possible that these patients have been asymptomatic with mucosal atrophy for decades; rather one would think that the disease with mucosal atrophy has developed late. In such cases an earlier mucosal biopsy specimen would have shown normal villous architecture even if these coeliac disease patients had eaten normal amounts of gluten since early childhood.

Each of our patients had the genetic marker, HLA-DR3, which is strongly associated with coeliac disease worldwide, and also among the Finns,[20 21] and we think these patients are genetically typical coeliac disease patients. Two of the patients had HLA identical siblings suffering from the disease and one patient had a son with coeliac disease. The four first degree relatives with the same genetic marker but with normal villous architecture will be further followed up. We believe that among such cases there are those, now called possible latent cases, who will in future develop coeliac disease. One could hypothesise that for example the father of case 2 (table 5) will later develop the disease as he already has low amounts of both reticulin and endomysium antibodies detectable in his serum. On the other hand it is known that many siblings with identical HLA-DR phenotypes to their brothers or sisters with coeliac disease are healthy. It has also been shown that adding extra amounts of gluten to the diet does not produce small intestinal alterations in these healthy siblings.[22]

Taken together it can be hypothesised that factors other than genetic susceptibility and gluten ingestion are at least sometimes needed to induce mucosal damage in coeliac disease. Adenovirus, as suggested by Kagnoff *et al*,[23] could be a trigger needed in latent cases to start the immunological process in the small bowel mucosa leading to crypt hyperplastic villous atrophy typical for coeliac disease. This could also offer an explanation for the phenomenon of coeliac disease relapse over such a large range of time in patients on a normal diet containing gluten.[1 24 25]

A most important future goal is to identify latent coeliac disease and to confirm our results, that is to say, to show the development of mucosal atrophy at older ages. Early treatment of coeliac disease would thus be possible. This is important in many respects, but especially true in the light of the possible development of malignancies in patients with untreated coeliac disease.[26] One line in identifying latent coeliac disease is to measure specific autoantibodies such as IgA class reticulin and endomysium antibodies.[9] In our family study 9% of the first degree relatives of coeliac patients were found to have these antibodies together with villous atrophy.[27] Another 3% were also positive for the antibodies but had at the same time normal mucosa, and these should be followed up. Combining the antibody tests with the use of genetic markers such as DQ typing with oligonucleotides, could pick out the risk groups to be followed. Another approach would be to identify specific markers in 'normal' small intestinal mucosa. It remains to be seen whether cells expressing, for example, gamma-delta receptors are specific for coeliac disease.[28]

On the basis of the present study and the cases found in the literature it now seems that latent coeliac disease does exist. This finding will change our conceptions of this condition. Also the understanding of the heredity of coeliac disease must be re-evaluated.

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