

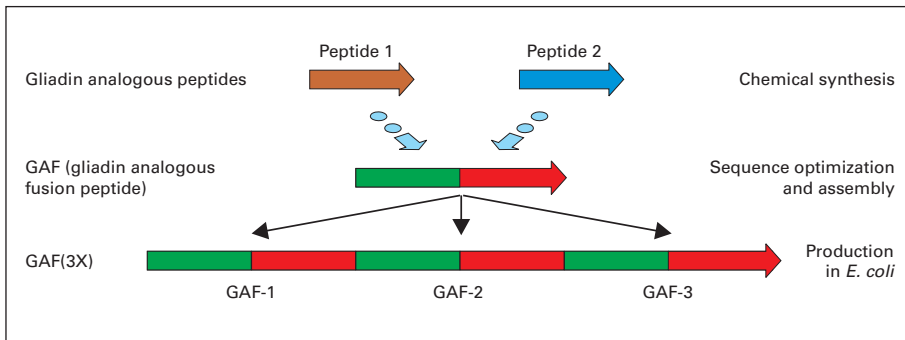
Antibodies against deamidated gliadin peptides as highly valid biomarkers of childhood celiac disease

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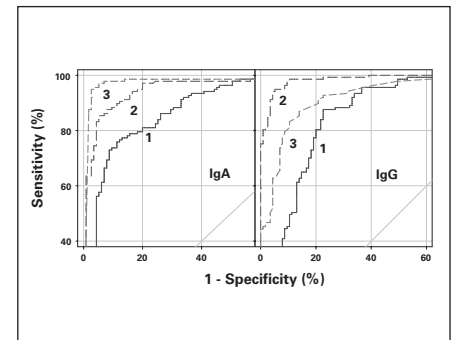
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Schematic illustration of GAF(3X)



ROC curves of six different antibody tests
1 = anti-Gli, 2 = anti-GAF(3X), 3 = anti-tTG

Introduction

Celiac disease (CD) is characterized by IgA and IgG antibodies against native gliadin (anti-Gli) and against tissue transglutaminase (anti-tTG). Generally, anti-Gli have lower diagnostic sensitivity and specificity than anti-tTG. Recently, specific antibodies against synthetic peptides derived from partially deamidated gamma-gliadin were described (Schwertz et al. 2004). Here we describe a simple and highly efficient ELISA for the measurement of antibodies against gliadin analogous fusion peptides (anti-GAF(3X)), which reveals a very high accuracy for CD diagnosis.

Methods

The synthetic DNA coding for GAF(3X), which includes 3 repetitive modified copies of the formerly described glutamine-glutamate substituted peptides PLQPEQFPF and PEQLPQFEE (Schwertz et al. 2004), was cloned into a prokaryotic expression vector and expressed in *E. coli*. Microplates were coated with the GAF(3X) preparation. Bound antibodies were detected with POD-labeled anti-human IgA and IgG. For reference, IgA

and IgG anti-Gli and anti-tTG were measured by ELISA (EUROIMMUN). Sera of 137 biopsy-proven CD patients (56 males, 81 females, mean age 8.0 years) on a normal gluten containing diet (Marsh lesions II or III) and of 115 biopsied disease controls (60 males, 55 females, mean age 9.0 years) were analyzed. The diagnostic validity of the tests was assessed by receiver-operating characteristic (ROC) analysis, and the area under ROC curve (AUC) was calculated. Diagnostic accuracy was defined as the sum of true-positives and true-negatives divided by the total number of individuals (CD patients + controls) tested (in %).

Results

AUC was significantly larger ($p < 0.0001$) for anti-GAF(3X) than for anti-Gli: IgG anti-GAF(3X) 99% versus IgG anti-Gli 86%, and for IgA anti-GAF(3X) 95% versus IgA anti-Gli 89%. There was no significant difference ($p < 0.05$) in AUC between anti-GAF(3X) and IgA anti-tTG (98%). Diagnostic accuracy of IgG Anti-GAF(3X) was 95% and of IgA anti-tTG 96%. There was one IgA deficient CD patient who was negative for IgA anti-tTG but positive for IgG anti-GAF(3X).

Anti-GAF(3X)		Anti-tTG		Number of CD patients (total: 137)
IgA	IgG	IgA	IgG	
+	+	+	+	117
-	+	+	+	10
-	-	+	+	3
-	+	-	-	2
-	-	-	-	5

Anti-GAF(3X)		Anti-tTG		Number of controls (total: 115)
IgA	IgG	IgA	IgG	
+	+	+	+	2
-	+	+	+	1
+	+	-	-	2
+	-	-	-	4
-	-	-	-	106

Discussion

The newly developed Anti-GAF(3X) ELISA applying selectively deamidated gliadin peptides represent a highly valuable tool in the diagnosis of childhood CD. The test overcomes the disadvantage of unspecificity, which is known to be inherent in antibodies against native unmodified gliadin. The diagnostic accuracy of the Anti-GAF(3X) ELISA for IgG antibodies is higher than for the corresponding IgA antibodies and comparable with that of the ELISA for IgA anti-tTG. The use of the new Anti-GAF(3X) ELISA for population screening, early diagnosis, monitoring of diet compliance, and detection of IgA deficient CD patients has to be further investigated.

Literature: Schwertz et al. Serological assay based on gliadin-related nonapeptides as a highly sensitive and specific diagnostic aid in celiac disease. Clin Chem 2004; 50: 2370-2375.

	Anti-Gli		Anti-GAF (3X)		Anti-tTG	
	IgA	IgG	IgA	IgG	IgA	IgG
AUC (%)	88.9	85.9	95.4	98.7	97.6	92.8
Sensitivity (%) at 95% specificity	56.2	X	83.2	94.2	95.6	62.8
Based on cut off calculated for maximum sum of sensitivity plus specificity						
Sensitivity	75.9	87.6	85.4	94.2	94.9	86.9
Specificity	88.7	77.4	94.8	95.7	97.4	86.1
Number of false-negatives	33	17	20	8	7	18
Number of false-positives	13	26	8	5	3	16
Diagnostic accuracy (%)	81.8	82.9	88.9	94.8	96.0	86.5

X: Calculation not possible because IgG anti-Gli only achieve a maximum diagnostic specificity of 94.8%