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Haptoglobin Polymorphism: A Novel Genetic Risk Factor for Celiac Disease Development and Its Clinical Manifestations

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BACKGROUND: Haptoglobin (Hp) α-chain alleles 1 and 2 account for 3 phenotypes that may influence the course of inflammatory diseases via biologically important differences in their antioxidant, scavenging, and immunomodulatory properties. Hp1-1 genotype results in the production of small dimeric, Hp2-1 linear, and Hp2-2 cyclic polymeric haptoglobin molecules. We investigated the haptoglobin polymorphism in patients with celiac disease and its possible association to the presenting symptoms.

METHODS: We studied 712 unrelated, biopsy-proven Hungarian celiac patients (357 children, 355 adults; severe malabsorption 32.9%, minor gastrointestinal symptoms 22.8%, iron deficiency anemia 9.4%, dermatitis herpetiformis 15.6%, silent disease 7.2%, other 12.1%) and 384 healthy subjects. We determined haptoglobin phenotypes by gel electrophoresis and assigned corresponding genotypes.

RESULTS: Hp2-1 was associated with a significant risk for celiac disease ($P = 0.0006$, odds ratio [OR] 1.54, 95% CI 1.20–1.98; prevalence 56.9% in patients vs 46.1% in controls). It was also overrepresented among patients with mild symptoms (69.2%) or silent disease (72.5%). Hp2-2 was less frequent in patients than in controls ($P = 0.0023$), but patients having this phenotype were at an increased risk for severe malabsorption (OR 2.21, 95% CI 1.60–3.07) and accounted for 45.3% of all malabsorption cases. Celiac and dermatitis herpetiformis patients showed similar haptoglobin phenotype distributions.

CONCLUSIONS: The haptoglobin polymorphism is associated with susceptibility to celiac disease and its clinical presentations. The predominant genotype in the celiac population was Hp2-1, but Hp2-2 predisposed to a more severe clinical course. The phenotype-dependent effect of haptoglobin may result from the molecule's structural and functional properties.

Celiac disease is a genetically determined T lymphocyte–mediated chronic inflammatory disorder with an autoimmune component induced by the ingestion of wheat gliadin or related rye and barley proteins. Most affected subjects experience remission after complete removal of gluten from their diet *(1)*. The presence of human leukocyte antigen (HLA)[13]-DQ2 or -DQ8 alleles appears to be necessary but not sufficient for development of disease, and the disease occurs more frequently in females *(2)*. Non-HLA genes are estimated to contribute at least 50% of disease susceptibility, but the putative risks conferred by other proposed candidate genes are substantially lower and controversial *(1, 3)*. Clinical presentation of the disease is highly variable, and little is known about what factors determine its symptoms. Recent epidemiologic studies re-

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[13] Nonstandard abbreviations: HLA, human leukocyte antigen; Hp, haptoglobin; TG, transglutaminase; OR, odds ratio; IFN, interferon; IL, interleukin.

vealed that celiac disease affects approximately 1% of the European and North American populations *(4)* but is underdiagnosed. Increased awareness and the widespread use of serology tests, however, will lead to the recognition of more patients with mild symptoms, and thus to a striking decrease in the duration of symptoms and the prevalence of patients with classic malabsorption *(2)*.

Haptoglobin (Hp) is a potent antioxidant and a positive acute-phase reaction protein whose main function is to scavenge free hemoglobin that is toxic to cells. Hp also exerts direct angiogenic, antiinflammatory, and immunomodulatory properties in extravascular tissues and body fluids. It is able to migrate through vessel walls and is expressed in different tissues in response to various stimuli *(5)*. Furthermore, Hp can also be released from neutrophil granulocytes *(6)* at sites of injury or inflammation and dampens tissue damage locally *(7)*. Hp receptors include CD163 expressed on the monocyte-macrophage system *(8)* and CD11b (CR3) found on granulocytes, natural killer cells, and small subpopulations of lymphocytes. Hp has also been shown to bind to the majority of $CD4^+$ and $CD8^+$ T lymphocytes *(9)*, directly inhibiting their proliferation and modifying the T helper (Th) 1/Th2 balance *(10)*.

The Hp polymorphism is related to 2 codominant allele variants (Hp1 and Hp2) on chromosome 16q22, encoding the Hp α-chain, and resulting in 3 major Hp phenotypes (Hp1-1, Hp2-1, and Hp2-2). The *Hp* gene locus consists of 5 exons encoding the 1 allele or 7 exons encoding the 2 allele. The 2 allele appears to have been generated from the 1 allele by an intragenic duplication of exons 3 and 4, presumably via nonhomologous DNA cross-over. Critical disulfide linkages necessary for covalent cross-linking of Hp monomers to form polymers are found in the regions coded by exons 3 and 4. The Hp1 monomer is monovalent and thus creates dimers. Having a duplicate of exons 3 and 4, Hp2 monomer is bivalent and can associate with 2 different Hp monomers. Thus homozygous Hp1-1 individuals express Hp1-1 at the protein level, which is a single $\alpha 1\beta$ homodimer with a molecular weight of 86 kDa (Fig. 1). Homozygous Hp2-2 individuals express the Hp2-2 phenotype, which consists of cyclic Hp polymers containing 3 or more $\alpha 2\beta$ subunits (170–900 kDa). The haptoglobin molecules synthesized in Hp2-1 heterozygous people are assembled into linear homodimers and multimers from various numbers of $\alpha 2\beta$ subunits flanked by 1 $\alpha 1\beta$ subunit at each terminus (86–300 kDa) *(11)*. Thus the Hp2-1 phenotype is distinctly different from those produced by the homozygotes for the Hp2 or Hp1 gene (Fig. 1). The three Hp protein phenotypes are easily distinguished by gel electrophoresis of hemoglobin-enriched serum samples, and Hp genotypes assigned by this method have shown complete correspondence with those determined by PCR from genomic DNA *(12)*.

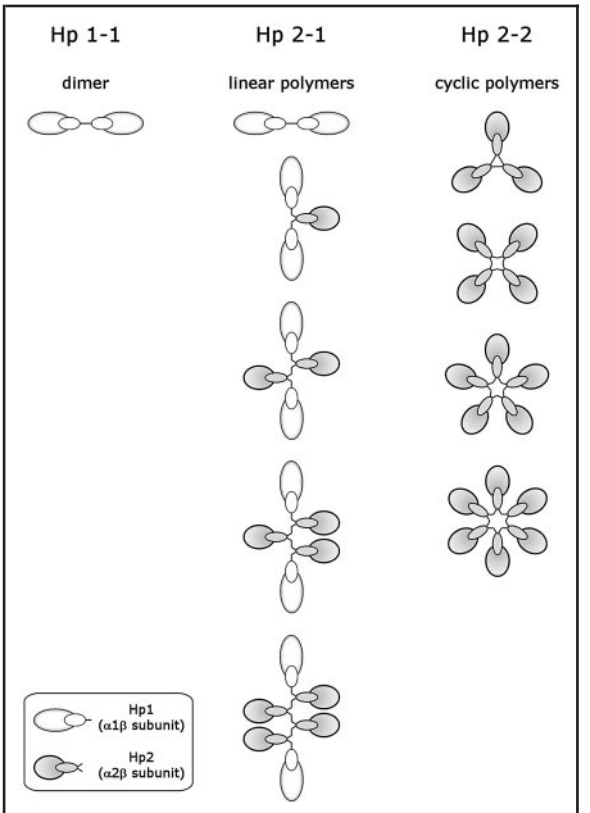

Fig. 1. Structural differences and subunit arrangement of Hp phenotypes.

Critical disulfide linkages necessary for the covalent cross-linking of Hp monomers ($\alpha\beta$ subunit) to form polymers are found on the α-chain of the molecule. The Hp1 monomer ($\alpha 1\beta$) is monovalent and thus can only associate with 1 other Hp monomer, whereas the Hp2 monomer ($\alpha 2\beta$) is bivalent and associates with 2 different Hp monomers. Consequently, the 3 Hp genotypes express entirely different molecular structures at the protein level. The Hp1-1 molecule is a small homodimer composed of 2 $\alpha 1\beta$ subunits (86 kDa). Hp2-2 consists of cyclic polymers carrying different amount of $\alpha 2\beta$ subunits (170–900 kDa). Finally, the Hp2-1 molecule forms a linear polymer series comprising various amounts of $\alpha 2\beta$ subunits flanked by 2 $\alpha 1\beta$ subunits (86–300 kDa) at each end. Although theoretically possible, $\alpha 2\beta$ subunits usually do not associate with each other into cyclic polymers in Hp2-1 individuals owing to the overexpression and excess of $\alpha 1\beta$ subunits.

Phenotype-dependent functional differences exist in the antioxidant, scavenging, and immunoregulatory properties of Hp, and the genetic polymorphism of Hp

has been shown to influence the course of a number of inflammatory and autoimmune diseases *(9, 13)*. Although a cascade of inflammatory events occurs in celiac disease, little is known about what role the Hp polymorphism may play in the pathogenesis or clinical course of celiac disease. A short report in the early 1970s suggested that a higher prevalence of the Hp1-1 phenotype in celiac patients was associated with dermatitis herpetiformis *(14)*.

The aim of this study was to investigate the distribution of Hp polymorphisms in a large cohort of Hungarian celiac disease patients with a broad spectrum of disease and to evaluate possible associations with clinical presentation.

Materials and Methods

PATIENTS

We studied 712 consecutive, unrelated Hungarian patients with biopsy-proven celiac disease (357 children and 355 adults, current median age 17 years [range 2–85], median age at diagnosis 7 years [range 1–85], male/female ratio 233/479). The control group consisted of 384 healthy, ethnically similar and geographically matched individuals (median age 38 years [range 17–69], male/female ratio 192/192) who had normal findings after a thorough medical examination that included blood pressure measurements and laboratory tests for celiac disease (antibodies to transglutaminase [TG]), blood count, serum iron, blood chemistry with liver and kidney function tests, C-reactive protein, and thyroid function. The controls were enrolled in the same clinical centers as the patients and represented both the western and eastern parts of the country (Budapest, n = 110; West Hungary; n = 90, East Hungary; n = 184).

The diagnosis of celiac disease was based on small-bowel biopsy showing severe villous atrophy with crypt hyperplasia (Marsh type III lesions) *(15)* and increased serum levels of antibodies to TG and/or endomysium (EMA) *(16)*. The EMA and anti-TG tests were used actively in the participating centers to recognize celiac disease among patients with suspicious symptoms. IgA and IgG class EMA were investigated on human umbilical cord substrate using the indirect immunofluorescent method as described *(17)*. Anti-TG antibodies were measured by ELISA using human recombinant antigen expressed in *E. coli (18, 19)*. HLA-DQ alleles were determined in 228 celiac patients from whole blood samples by PCR with sequence-specific primers *(20)*. In the absence of initial serology results, we enrolled only patients who had a diagnosis confirmed by traditional gluten challenge *(21)* and the presence of HLA-DQ2 or -DQ8 haplotypes.

Dermatitis herpetiformis was diagnosed by direct immunofluorescence studies of skin biopsy samples showing granular IgA deposition in the dermal papillae *(22)*.

We obtained detailed clinical data from clinicians using a structured questionnaire. Collected data included sex, age, and information related to the clinical disease presentation in the following categories: (*a*) severe generalized malabsorption (presence of at least 4 of the following 5 symptoms: diarrhea, abdominal distension, failure to thrive or weight loss, anemia, hypoproteinemia) or retarded growth; (*b*) nonspecific gastrointestinal symptoms that did not compromise the general condition and somatic development (diarrhea, constipation, bloating, recurrent abdominal pain or vomiting, esophageal reflux); (*c*) iron deficiency anemia without major gastrointestinal complaints; (*d*) dermatitis herpetiformis; (*e*) silent disease (population screening); (*f*) others (autoimmune diseases, reduced bone mineral density, liver disease, brain disease). Patients were assigned to these major presentation categories in a prospective manner, based on clinical findings, routine laboratory results, and growth chart data at diagnosis.

Informed consent was obtained from all patients and controls. The study protocol was approved by the Regional and Institutional Committee of Science and Research Ethics of the University of Debrecen, Medical and Health Science Center (DEOEC RKEB/IKEB: 2601–2007).

HAPTOGLOBIN PHENOTYPE/GENOTYPE ANALYSIS

We determined Hp phenotypes in a blinded fashion from serum samples collected in 2006–2007. SDS-PAGE was run on 5%–10% gradient gel followed by immunoblotting using Millipore polyvinyl-difluoride Immobilon-P transfer membrane (Millipore), polyclonal rabbit antihuman haptoglobin, and goat anti-rabbit horseradish peroxidase antibodies (Dako) in 1:1000 and 1:2000 dilution, respectively, as described *(23)*. Genotypes were reconstructed by comparison to Hp1-1 and Hp2-2 manufacturer standards (Sigma-Aldrich) run in each assay. To evaluate the consistency of the typing method and that of the expression of Hp polymers, we also assayed duplicate or triplicate serum samples taken from the same patients (n = 27) in different time points with a median time interval of 12.8 years (range 0.2–14.8 years). All these samples yielded identical results and indicated the same genotype in all cases.

STATISTICAL ANALYSIS

Variables were tested for normality with the Shapiro Wilk *W* test. We used Student *t* test with separate variance estimates, χ^2 test and Fisher exact test to evaluate

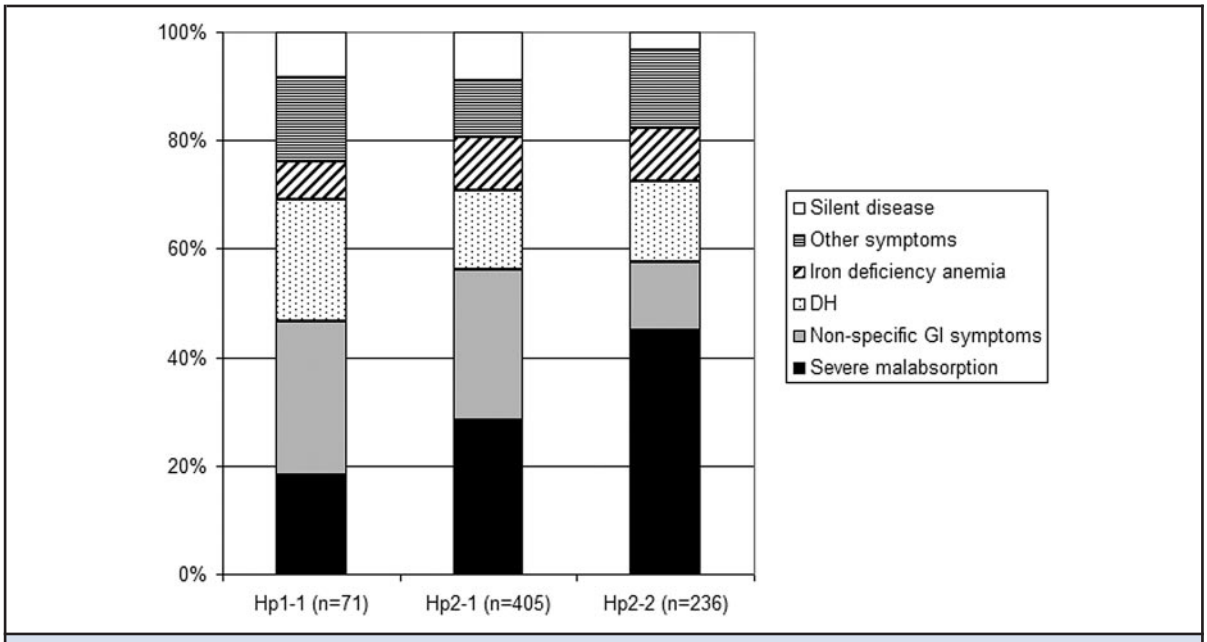

Fig. 2. Clinical presentation of celiac disease in different Hp phenotypes.
DH, dermatitis herpetiformis; GI, gastrointestinal.

differences between celiac patients and controls. Within subgroups of celiac patients, we used χ^2 test and Fisher exact test as appropriate. We used logistic regression to compare genetic and clinical data and expressed results as odds ratios (ORs) with 95% CI. A P value of <0.05 was considered significant. The statistical analysis SPSS13.0 (SPSS Inc.) was performed by P.L.L. with the help of a statistician (Peter Vargha).

Results

We assessed the Hp phenotypes and genotypes in a large cohort of Hungarian celiac patients with diverse clinical manifestations. Of the 712 patients, 234 (32.9%) presented with severe malabsorption or growth failure, 162 (22.8%) with nonspecific or minor gastrointestinal symptoms, 67 (9.4%) with iron deficiency anemia, 111 (15.6%) with dermatitis herpetiformis, and 87 (12.1%) with other symptoms. Fifty-one (7.2%) patients were recognized through population screening.

The frequency of Hp2-1 was significantly higher and that of Hp2-2 was significantly lower in patients with celiac disease compared with the control population and with all groups of historical controls from different regions of Hungary. In addition, Hp1 allele was associated with significantly increased risk for celiac disease compared with 2 of the 4 control groups (Table 1). The Hp phenotype distribution of the healthy control group was similar to that found in previous surveys in the normal Hungarian population, and Hp1 and Hp2 alleles were in Hardy-Weinberg equilibrium and did not differ with age or sex. Of the celiac patients, 211 (30%) were from Budapest, 193 (27%) from West Hungary, and 308 (43%) from East Hungary, but these groups did not show significant differences.

All celiac patients with available HLA results carried at least 1 copy of DQ2 or DQ8, but the prevalence of the high-risk DQ genotypes (DQB1*0201/*0201 [DR3;DQ2/DR3;DQ2] or DQB1*0201/*0202 [DR3;DQ2/DR7;DQ2]) was similar in the 3 Hp groups (Hp1-1 40.0%, Hp2-1 40.7%, Hp2-2 38.8%). This finding shows that HLA-related susceptibility markers were not overrepresented in the Hp2-1 celiac group. HLA-DQ results were not available for the control group.

Clinical presentations of celiac disease were distributed differently in the three Hp genotype groups (Fig. 2, Table 2). Patients with Hp2-2 presented more frequently clinically with an increased risk for severe malabsorption or failure to thrive (OR 2.21, 95% CI 1.60–3.07), and accordingly, less often with only nonspecific or minor gastrointestinal symptoms (OR 0.38, 95% CI 0.25–0.58). Patients with this genotype were less often clinically silent (OR 0.35 95% CI 0.16–0.76) than patients in other Hp groups (Table 3). Of all celiac patients carrying Hp2-2, 44.9% were diagnosed be-

Table 1. Hp phenotypes and Hp allele frequencies in patients with celiac disease and healthy controls.

	Celiac disease n = 712	Comparison with healthy controls, this study n = 384			Comparison with the normal Hungarian population in previous surveys *(24)*								
					Budapest n = 22 842			West Hungary n = 857			East Hungary n = 1740		
	Frequency, n (%)	Frequency, n (%)	OR (95% CI)	*P*	Frequency, n (%)	OR (95% CI)	*P*	Frequency, n (%)	OR (95% CI)	*P*	Frequency, n (%)	OR (95% CI)	*P*
Hp1-1	71 (10.0)	44 (11.5)	0.86 (0.57–1.30)	0.44	2925 (12.8)	0.75 (0.59–0.97)	0.03	118 (13.8)	0.70 (0.50–0.95)	0.02	188 (10.8)	0.91 (0.69–1.22)	0.54
Hp2-1	405 (56.9)	177 (46.1)	**1.54** (1.20–1.98)	**0.0006**	10 349 (45.3)	**1.59** (1.37–1.85)	**<0.0001**	388 (45.3)	**1.60** (1.31–1.95)	**<0.0001**	789 (45.3)	**1.59** (1.33–1.89)	**<0.0001**
Hp2-2	236 (33.1)	163 (42.4)	**0.67** (0.52–0.87)	**0.0023**	9568 (41.9)	**0.69** (0.59–0.82)	**<0.0001**	351 (40.9)	**0.72** (0.58–0.88)	**0.002**	763 (43.9)	**0.64** (0.53–0.76)	**<0.0001**
Hp1 allele	0.3841	0.3451	1.18 (0.98–1.42)	0.3265	0.3533	1.18 (1.05–1.31)	**0.004**	0.3641	1.19 (0.98–1.30)	0.10	0.3347	1.28 (1.13–1.46)	**<0.0001**

Values showing significant differences are shown in bold.

cause of malabsorption or failure to thrive, which was significantly higher than in celiac patients overall (32.9%, $P = 0.0008$) or with other Hp genotypes (26.9%, $P < 0.0001$) (Fig. 2). Likewise, 45.3% of all celiac cases that presented with severe malabsorption or failure to thrive carried Hp2-2 (Table 2), despite the underrepresentation of Hp2-2 among celiac patients compared with the general population (Table 1).

In patients who presented with nonspecific or minor gastrointestinal symptoms and were discovered through screening, there was a higher prevalence of Hp2-1 (69.1% and 72.5%) compared with the entire celiac group, whereas Hp2-2 occurred less frequently (Table 2).

Dermatitis herpetiformis tended to be more frequent in the Hp1-1 group (22.5%) compared with other Hp groups (14.8%; $P = 0.118$) or among celiac patients overall (15.6%; $P = 0.131$), but this difference was not statistically significant. Only 14.4% of all dermatitis herpetiformis cases carried Hp1-1, whereas the majority of this group also had Hp2-1 (Table 2).

The rate of iron deficiency anemia and other clinical symptoms, for disease presentation, were similar among the three Hp phenotypes, and their distribution was not found to be different from that of the entire celiac population. However, the limited number of subjects in some patient groups may have limited the statistical power of our studies to detect such differences.

No correlation was noted between Hp phenotypes and age at diagnosis. Male/female ratios were found to be similar in all Hp groups (Table 2).

Discussion

To our knowledge, this is the first report on the role of Hp genetic polymorphism in relation to disease susceptibility and clinical presentation in a large cohort of celiac patients. We applied gel electrophoresis to identify Hp phenotypes, but it is widely accepted that Hp phenotypes accurately indicate underlying Hp genotypes *(12)*. In our study, we found the frequency of the Hp2-1 phenotype/genotype to be significantly higher in the Hungarian celiac patients than in Hungarian control subjects. Except for one clinical study dealing with ovarian cancer patients *(25)*, no report exists on any disease association with the Hp2-1 phenotype. Disease associations, in contrast, have been seen with Hp1-1 (bronchial asthma, atopic dermatitis, or chronic hepatitis C infection) and Hp2-2 (vascular complications in diabetic patients, essential hypertension, or advanced and therapy-resistant tuberculosis). The few reports on other autoimmune disorders are based on small patient samples and are often contradictory *(26, 27)*.

Table 2. Distribution of Hp phenotypes among celiac patients with different clinical manifestations.

	Hp1-1	Hp2-1	Hp2-2	Total
Celiac disease (total)	71 (10.0)	405 (56.9)	236 (33.1)	712 (100.0)
Male/female	22/49	126/279	85/151	233/479
Mean age at diagnosis, years (SD)	10.79 (10.04)	8.24 (7.86)	8.61 (8.98)	8.61 (8.52)
Clinical presentation				
Severe malabsorption/failure to thrive	13 (5.6)[a]	115 (49.1)[a]	106 (45.3)[a]	234 (100)
Nonspecific gastrointestinal symptoms	20 (12.4)[b]	112 (69.1)[b]	30 (18.5)[b]	162 (100)
Iron deficiency anemia	5 (7.5)	39 (58.2)	23 (34.3)	67 (100)
Dermatitis herpetiformis	16 (14.4)	60 (54.1)	35 (31.5)	111 (100)
Other	11 (12.6)	42 (48.3)	34 (39.1)	87 (100)
Silent disease (population screening)	6 (11.8)[c]	37 (72.5)[c]	8 (15.7)[c]	51 (100)

Data are n (%) unless noted otherwise. [a] $P < 0.0001$, [b] $P < 0.0001$, [c] $P = 0.005$ by χ^2 or Fisher exact test comparing phenotype distribution frequencies between total celiac disease group and each clinical presentation group.

Recent data suggesting that Hp plays a modulating role on the Th1/Th2 balance may be of interest to help in understanding celiac disease. The activation of lamina propria $CD4^+$ T cells by gliadin peptides in the context of HLA-DQ2 or -DQ8 molecule is one of the key events in the pathogenesis of celiac disease, with interferon-γ (IFN-γ) as the dominant cytokine (Th1 skewed response) *(28)*. Hp promotes a predominately Th1 cellular response in vitro via an inhibitory effect on Th2 cytokine release, a finding that has been further corroborated in vivo, using $Hp^{-/-}$ mice *(10)*. These experiments were carried out with Hp mix purified from individuals with Hp1-1 and Hp2-1 phenotypes, but who lacked the Hp2-2 molecule. The Hp1 alleles were also more frequent in a large cohort of Hungarian patients with Crohn's disease; this latter group also showed a higher prevalence of the Hp2-1 phenotype in the subgroup with inflammatory behavior than the subgroup that developed strictures *(23)*. Interestingly, disease-predisposing allele variations in the myosin IXB gene initially identified in celiac patients were similarly found in Crohn's disease *(29)*, presumably owing to their proinflammatory properties. Hp1-1 alone, in contrast, may instead predispose for Th2-associated diseases, and in vitro, significantly induces greater amounts of antiinflammatory interleukin (IL)-6 and IL-10 than Hp2-2 *(30)*.

The association of celiac disease with heterozygotes having both Hp1 and Hp2 alleles is rather unusual, and further explanation is needed for the predisposition of the heterozygous state for disease. It should be noted that the linear haptoglobin polymers produced in Hp2-1 individuals differ considerably from both Hp2-2 and Hp1-1 in their molecular structure, and may also be functionally different. Both in vitro and in vivo data support the distinct antioxidant, scav-

Table 3. Risk for the development of different clinical presentations among celiac patients with different Hp genotypes.

	Hp1-1 vs non–Hp1-1			Hp2-1 vs non–Hp2-1			Hp2-2 vs non–Hp2-2		
Clinical presentation	OR	95% CI	*P*	OR	95% CI	*P*	OR	95% CI	*P*
Severe malabsorption/failure to thrive	0.43	0.23–0.79	0.005	0.63	0.46–0.86	0.0035	2.21	1.60–3.07	<0.0001
Nonspecific or minor gastrointestinal symptoms	1.38	0.80–2.39	NS	1.97	1.35–2.65	0.0004	0.38	0.25–0.58	<0.0001
Iron deficiency anemia	0.71	0.27–1.82	NS	1.06	0.64–1.77	NS	1.06	0.62–1.8	NS
Dermatitis herpetiformis	1.67	0.92–3.04	NS	0.873	0.58–1.31	NS	0.92	0.59–1.42	NS
Other	1.36	0.69–2.71	NS	0.67	0.43–1.06	NS	1.34	0.85–2.13	NS
Silent disease (population screening)	1.22	0.50–2.98	NS	2.104	1.12–3.97	0.0192	0.35	0.16–0.76	0.005

ORs were calculated from 4-fold contingency tables and give the odds for patients in each Hp group to present with the given symptom in comparison with the patients belonging to the other 2 Hp phenotypes. NS, not significant.

enging, and immunomodulatory properties of different Hp phenotypes. Unfortunately, most of the functional studies compared Hp1-1 with Hp2-2, and only a few studies were applied to purified Hp2-1 *(10)*. Thus, little is known about real behavior of Hp2-1. Hp2-1 manifests certain features characteristic of both Hp1-1 and Hp2-2. Like Hp2-2, Hp1-1 consists of a series of polymers with high molecular weights, but it also contains an $\alpha 1\beta$ subunit at each end. These structural features may display a behavior similar to either Hp1-1 or Hp2-2 in certain conditions, whereas opposite behaviors may be evident in other conditions. New functional studies are clearly needed to further clarify the role of Hp2-1 in celiac disease and in the inflammatory process in general.

The clinical manifestations of celiac disease are highly variable, and the disease may present either in childhood or during adult life and may involve multiple organ systems. For certain times in the natural course of celiac disease, the disease may not be associated with obvious clinical signs and symptoms *(4)* but it may later progress to overt disease. Furthermore, the disease manifestations may also change over time, making the task of evaluating the factors that contribute to clinical presentation even more difficult and challenging. For these reasons, we have used only few, obviously different and prospectively assigned subgroups in our study. We evaluated a sufficiently large patient cohort in which not only malabsorption patients, but also cases detected on the basis of screening tests and other mild forms, were sufficiently represented owing to the active diagnostic approach in the clinical centers involved. In this celiac cohort, the frequency of the Hp2-2 phenotype was significantly lower than in the general population ($P = 0.0023$), but patients with this phenotype showed an increased risk for generalized malabsorption or failure to thrive. These severe disease manifestations were once the "classic" features of the disease, but currently they only represent the tip of the celiac iceberg *(31)*. At the same time, Hp2-2 was not associated with the diagnosis at an earlier age, but this could also be explained by the high number of young children who have been diagnosed with celiac disease in recent years while the disease is still relatively mild.

Hp2-2 was rare among celiac patients with nonspecific and mild symptoms or silent disease; rather, Hp2-1 (69.1%–72.5%) in the group with mild manifestations was more prevalent than in the entire celiac patient group. Given that silent cases are estimated to be up to 7 times more common than symptomatic cases *(31)*, this finding further supports the primary association of celiac disease with Hp2-1. However, these results need to be reevaluated in other populations from geographically diverse regions.

The disease-modifying effect of Hp2-2 might be related to its lower efficacy both in preventing oxidative tissue damage *(32)* and to downregulation of the inflammatory processes in general. This is also evidenced clinically in the significant association of Hp2-2 with vascular complications in diabetic subjects *(33)*. Hp2-2–hemoglobin complexes are eliminated less efficiently and more slowly via the CD163 receptor than those complexes that contain Hp1-1. Additionally, Hp2-2 was independently associated with a significant decrease in the expression of CD163 *(34)*. The CD163 receptor constitutes the only route for the clearance of Hp–hemoglobin complexes in extravascular sites. Thus subjects with Hp2-2 might experience higher levels of reactive oxygen species generated in tissues.

Recent data suggest that the CD163 receptor of macrophages not only functions as a scavenger receptor for the Hp–hemoglobin complex but also has an immunomodulatory action with pivotal anti-inflammatory properties leading to the synthesis of IL-10, heme oxygenase, and bilirubin *(35)*. It has been also demonstrated that, upon binding of Hp2-2–hemoglobin complexes to CD163, significantly lower amounts of the antiinflammatory IL-10 are released than with Hp1-1 *(36)*. IL-10 has an immunoregulatory effect on gliadin-dependent T-cell activation, interferes with antigen presentation, and induces hyporesponsive gliadin-specific T cells *(37)*. Hp2-2 is also associated with stronger immune reactivity, weaker inhibitory effect on prostaglandin synthesis *(38, 39)*, and higher B cell and $CD4^+$ T lymphocyte counts in the peripheral blood *(40)*.

Our patient material included a very large cohort of patients with dermatitis herpetiformis (15.6% of all cases). However, we could not confirm the observation of Marks et al. from 1971 (14) on the higher incidence of Hp1-1 phenotype in a small cohort of dermatitis herpetiformis compared with celiac disease patients. These authors did not publish details on the exact number of the subjects or on the frequency of Hp phenotypes. We found a slightly higher percentage of Hp1-1 among our 111 dermatitis herpetiformis cases, but this result was not statistically significant and only 14% of these patients carried Hp1-1, a frequency similar to the general population.

In conclusion, the Hp2-1 phenotype was significantly more frequent in Hungarian patients with celiac disease compared with the general population. When Hp2-2 was present, it predisposed to a more severe clinical course. These findings suggest a role for Hp polymorphism or related polymorphisms in disease susceptibility and modification in celiac disease. Our data also call for additional basic research on Hp in innate and adaptive immunity.

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