

Research article

Chronic Atrophic Gastritis: Analysis and Genetic Basis in a Large Family

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ABSTRACT

Chronic atrophic gastritis (CAG) is a known histopathologic entity caused by autoantibodies against intrinsic factor and gastric parietal cells. Genetic factors are less known and are more difficult to quantify.

This work analyzes a large family affected by CAG with a first objective of establishing a relationship with previous pathologies, laboratory abnormalities and HLA. The second objective was to fit a genetic basis that allows us extend the study by establishing the influence of genetics on the etiology of CAG.

The mother and 11 siblings of a family with a high incidence of CAG were studied. Six of them were diagnosed by endoscopy and biopsy. Also in this group we observed elevated Gastrin levels and low Pepsinogen I levels. We found elevated Gastrin levels in three of the asymptomatic siblings. Of special relevance to our target was the relationship with HLA-DQ6.2 that could be a contributing factor to the disease.

Keywords: Chronic atrophic gastritis; Anti-intrinsic factor antibodies; HLA; Gastrin; Pepsinogen I

Introduction

Under the concept of chronic atrophic gastritis (CAG) it is included any type of inflammation of the gastric mucosa where a cellular inflammatory infiltrate composed mostly of lymphocytes and plasma cells with very few neutrophils are identified. There are various classification systems based on the histological features and the degree of gastric atrophy, and also based on the predominant location, being type A primarily located in the gastric body whose etiology would be autoimmune; and type B, preferably located at the antrum that would have more relationship with *Helicobacter pylori*.

Type A gastritis is the less common form and is associated with pernicious anemia and the presence of circulating antibodies against parietal cells and intrinsic factor, hence the name of autoimmune gastritis. Such antibodies are found in over 90% of patients with pernicious anemia and up to 50% of patients with type A gastritis.[1]

Autoantibodies anti-parietal cells and atrophic gastritis are identified in 20% of individuals over 60 years in relatives of patients with pernicious anemia. Thyroid disease is common in these patients, so that half of them have antibodies against thyroid antigens and about 30% of patients with thyroid disease have antibodies anti-parietal cells. Also there has been a significant incidence of specific haplotypes of familial histocompatibility as the HLA B8 and DR3.[2,3]

Parietal cells injury has two consequences: Firstly aclorhidria with secondary hypergastrinemia which can cause hyperplasia of enterochromaffin cells, and the development of gastric carcinoid tumors. Secondly, a lack of intrinsic factor with deficiency of vitamin B12 and its consequences: pernicious anemia, neurodegenerative problems, cardiovascular disease and gastrointestinal problems

Material and Methods

We conducted a cross-sectional study of a mother and 11 siblings (1 sibling do not participate and the father had died). After given informed consent an epidemiological survey was used on the base of their personal history, with special emphasis on problems related to anemia, gastrointestinal problems and diseases with autoimmune characteristics. Data on their previous and current treatments were also collected.

For the entire family group under study a sample of whole blood was drawn to determine complete blood count and the following biochemical and autoimmune parameters:

- Total protein and lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) by spectrophotometry.
- Proteinogram by capillary electrophoresis and densitometry
- Homocysteine by chemiluminescence.
- Gastrin, reference values (13-115 pg/mL) and Pepsinogen I, reference values (≥ 25 ng/mL) by inmunoluminiscence.
- Immunoglobulins levels (isotypes IgG, IgA and IgM), and prealbumin by nephelometry.
- C-reactive protein by immunoturbidimetry.
- TSH, ferritin, B12 and folate by chemiluminescence immunoassay of paramagnetic particles.
- Antimicrosomal antibodies (positive>4.1) and anti-

intrinsic factor (positive if >25 U, weak positive between 20-25 U, negative if <20 U) by ELISA Anti-parietal cell antibodies were examined by indirect immunofluorescence on triple rodent tissue (positive if titles ≥ 1/40).

- Complete typing of HLA-DQA1 and DQB1 alleles by polymerase multiplex chain reaction (PCR) and SSO (sequence specific oligonucleotide) with reverse hybridization membrane.
- In the mother and two selected siblings, a low sensitivity full typing of HLA class I and II (A, B, C and DRB1) was performed that allowed set the extended haplotypes of maternal and paternal origin.

Statistical Analysis

Inter-groups differences between biological and clinical parameters (presence of gastritis and presence of a second haplotype of paternal origin DQ6.2) were analyzed by nonparametric tests (Kruskal Wallis). Values are expressed as median and range. Nonparametric bivariate correlations (Spearman) were also performed among the various variables in patients with gastritis. The variables with numerical values were expressed as median and range in brackets.

Results

In the present study we were able to have almost all member of a generation within an extended family, offering to participate to clarify the possible genetic basis of CAG.

At the time of sample collection, the participants had ages between 43 and 63 years, and the mother had 85 years old. The participants were 8 men and 3 women. One descendent was not involved in the study for residing in a far location. Among the personal antecedents we found a discreet hypercholesterolemia in a patient without criteria of heterozygous familial hypercholesterolemia, being the other antecedents of isolated character and without a genetic etiological component.

The diagnosis of macrocytic anemia due to atrophic gastritis was considered established based on the analytical data and the results of gastroscopy and biopsy, so that 6 siblings had the process (Table 1). Three women had the disease and only 3 out of 8 men were affected by atrophic gastritis. As relevant data we note that the time for diagnosis was relatively recent, less than 3 years of evolution in all cases. The age at diagnosis was higher in women than in men (59 [6] years vs. 47 [8] years; p=0.05).

A general analysis was performed of blood count, routine biochemistry, determinations of vitamin B12 and folic acid, total protein and protein electrophoresis, thyroid study (TSH and antimicrosomal antibodies), immunoglobulins and specific determinations of Gastrin, Pepsinogen I, anti-parietal cells and anti-intrinsic factor antibodies.

We found no remarkable alteration in blood count in none of studied cases, or even in changes of the mean corpuscular volume. Iron metabolism was also normal in all patients as well as levels of vitamin B12 and folic acid, although there are statistically significant differences in the ferritin values of patients with and without gastritis (24.3 [35.2] mg/dL vs. 84.9 [65.5] mg/dL; p=0.032). We detect only slight increases in cholesterol levels that were already known in the individual survey.

Immunoglobulin levels showed no significant alterations, although we observed IgM values in the lower limits of normal virtually in all studied subjects and even discreetly below the reference level in the mother and 4 cases. Also discreetly high values of IgA were observed in 3 of the 11 siblings studied. Prealbumin and homocysteine levels, and proteinogram did not show significant alterations in any case, although we did positively observe lower prealbumin values in the group with gastritis (26.75 [4.9] mg/dL vs. 32.65 [14.5] mg/dL; p=0.033) (Table 2).

Especially important was the determination of Gastrin and Pepsinogen I (Table 3), distinguishing three different groups based on levels: first, those who had very high levels of Gastrin and a strong inhibition of Pepsinogen I (cases 2, 3, 4, 6, 9 and 12, in addition to the mother); a second group with more moderate Gastrin values without inhibition of Pepsinogen I (cases 1, 5 and 8); and a third group with normal levels of Gastrin and

Table 1: Summary of the patients participating in the study.

	Sex	Age	CGA confirmed	Endoscopy	Biopsy	H. pylori	Treatment B12	Age of diagnosis
1	M	63	NO	NO				
2	F	62	**YES**	YES	CGA	NO	YES	61
3	F	60	**YES**	YES	CGA	YES	YES	59
4	F	58	**YES**	YES	CGA	NO	YES	55
5	M	56	NO	NO				
6	M	54	**YES**	YES	CGA	NO	YES	51
7	M	53	NO	NO				
8	M	51	NO	NO				
9	M	49	**YES**	YES	CGA	NO	YES	47
10	M	47	NO	NO				
11				No data				
12	M	43	**YES**	YES	CGA	NO		43
Mother	F	85	NO					

CGA: Chronic Atrophic Gastritis

Table 2: Immune and proteinogram study.

	IgG (600-1700)	IgA (60-300)	IgM (60-350)	Pre-albumin (20-40)	Homocysteine (0-20)	Total protein (6-8.5)	Albumin (4-4.8)	Alfa1G (0.2-0.35	Alfa2G (0.51-0.85)	BetaG (0.6-0.94)	GammaG (0.8-1.35)
1	974	177	105	28.40	12.10	7.30	4.60	0.33	0.70	0.76	0.96
2	1190	**354**	86	25.40	11.80	7.00	4.10	0.25	0.69	0.90	1.09
3	653	172	**57**	25.30	9.20	6.60	4.10	0.31	0.70	0.81	0.63
4	919	269	**50**	25.50	11.20	6.80	4.30	0.27	0.63	0.81	0.86
5	880	289	72	29.90	12.30	6.60	4.10	0.26	0.62	0.76	0.82
6	1080	**320**	96	28.00	14.10	6.80	4.30	0.26	0.51	0.77	1.05
7	1050	243	70	42.90	10.20	7.00	4.40	0.22	0.61	0.74	0.98
8	1070	246	68	35.00	8.40	6.70	4.20	0.27	0.58	0.68	0.92
9	1140	265	65	30.20	13.30	7.20	4.50	0.23	0.58	0.80	1.06
10	931	**367**	**53**	30.30	12.90	6.80	4.40	0.25	0.48	0.79	0.92
11						No data					
12	795	322	**45**	28.50	11.60	6.40	4.10	0.26	0.57	0.70	0.79
Mother	974	306	47	24.20	14.40	6.80	4.00	0.34	0.83	0.74	0.90

Table 3: Hormonal study.

	Antimicrosomal antibodies (Positive>4.1)	Anti-parietal cells antibodies	Anti-intrinsic factor antibodies	TSH (0.4-5.9 mUI/L)	Gastrin (13-115)	Pepsinogen I (>25)
1	0	***1/320***	19	1.36	*260*	37.4
2	0	***1/320***	7	3.58	***877***	***13.4***
3	0	Negative	14	1.56	***2856***	***3.3***
4	0	***1/320***	4	1.5	***425***	***2.8***
5	0	***1/320***	12	2.65	*256*	161.7
6	2	***1/80***	*21*	1.32	***2043***	***6.8***
7	0	Negative	*22*	1.04	51	74.0
8	0	Negative	7	2.46	*196*	105.1
9	0	Negative	8	2.43	***1327***	***2.3***
10	5	***1/320***	10	2.1	100	52.3
11				***No Data***		
12	0	***1/320***	7	4.06	***1819***	***2.9***
Mother	0	***1/320***	13	2.38	***996***	***3.1***

Pepsinogen I (cases 7 and 10).

As for antibody levels, we found negative levels for antimicrosomal and only a discrete positive in patient 10, and normal levels of anti-intrinsic factor antibodies in all cases except in patients 6 and 7 with a weak positive. The data showed anti-parietal cells antibodies more representative, being positive in 7 siblings and the mother (titers between 1/80 and 1/320).

Finally we conducted a HLA study to assess their involvement as determining genetic factor in the CAG (Table 4). The extended maternal haplotypes were defined (A*03 -B*07 -C *07 -DRB1*15 -DQB1*06:02 -DQA1*01:02 [DQ6.2] and A*02 -B*08 - C*07 -DRB1*03 -DQB1*02:01 -DQA1*05:01 [DQ2.5]) and paternal origin (A*02 -B*07 -C*07 - DRB1*15 -DQB1*06: 02-DQA1*01:02 [DQ6.2] and A*02 -B*44 -C*05 -DRB1*12 - DQB1*03:01 -DQA1*05:05 [DQ7.5]). We emphasize as a fundamental fact the transmission of maternal DQ6.2 to almost all family members except case number 8 and the occurrence of the same HLA by paternal transmission in half of the studied cases [3, 6, 7, 8, 9 and 12].

Table 4: HLA study.

	Maternal HLA	Paternal HLA
1	DQ6.2	DQ7.5
2	DQ6.2	DQ7.5
3	DQ6.2	DQ6.2
4	DQ6.2	DQ7.5
5	DQ6.2	DQ7.5
6	DQ6.2	DQ6.2
7	DQ6.2	DQ6.2
8	DQ2.5	DQ6.2
9	DQ6.2	DQ6.2
10	DQ6.2	DQ7.5
11	No data	
12	DQ6.2	DQ6.2
Mother	DQ2.5	DQ6.2

Discussion

In this work we have studied a large family with high incidence of a pathological process with a genetic basis not yet well established. We do not have verified in the consulted literature a first-degree familial aggregation as important as in the present study, which we believe offers a significant potential for understanding genetic etiology of CAG.

Six out of 11 siblings had CAG diagnostic criteria, i.e, positive gastroscopy and biopsy of the process, (around 50%), although the gender distribution shows that 100% of women are affected (3 of 3) and only a third of the men (3 out of 8) were affected. Mean age of the participant siblings was somewhat over 54, although the positive diagnosis of CAG was between one and three years before the data collection, with a mean of 52.66 years. Some studies state the age at diagnosis around 60-65 years with homogeneous distribution by sexes, or even higher in women, as in this family.[2,4,5] There is no doubt that the appearance of a disease with a possible genetic component in a family promotes the study in the rest of the components of that family so that the diagnosis can be made more prematurely.

We do not have observed any significant laboratory abnormalities in the hemogram, neither the study of iron metabolism nor levels vitamins because we found diagnosis already established and patients had been treated with parenteral cyanocobalamin, being only the patient #9 who was attended in our office, (index case), and who previously had vitamin B12 deficiency and macrocytosis. The other affected siblings presented hematological changes and low B12 levels recorded in their personal histories.

Since the pathogenesis of CAG is an autoimmune disease, we conducted an immunological study in which we found an IgM in the lower limits of normality without any causal relationship with CAG according to consulted literature, but we should consider these data with caution because the studied individuals did not present a clearly altered levels or clinical signs. Moreover we do not observe a clear association between these levels and involvement by CAG.

Detection of autoantibodies is important in the diagnosis of autoimmune processes, and in the case of CAG it is possible to determine the etiology studying specific antibodies. The findings of positive titles of anti-parietal cell antibodies in 8 of the 12 studied cases allow fit the pathology studied with the results, although we note that not all antibody-positive cases have the disease neither all pathological cases have such positive antibodies. One possible explanation of this fact is that we are in presence of the same process affecting all family components with varying degrees of penetrance and diverse biochemical demonstrations, and that some patients without pathology are likely to develop it over time. Anti-intrinsic factor antibodies are more indicative of pernicious anemia than CAG, and in this sense we have only two weak positive cases, although CAG patients are under treatment with vitamin B12 because in some time they were detected with vitamin deficiency.

Gastrin is a hormone secreted by the G cells of the gastric antrum which in turn stimulates parietal cells to fulfill the production of hydrochloric acid through histamine as a mediator.[6] This hormone is able to promote proliferation of gastric epithelial cells and increases the number of parietal and enterochromaffin cells. There are several situations determining an increase in Gastrin production: gastrinomas, gastric neuroendocrine tumors and situations of hypochlorhydria and achlorhydria.

Pepsinogen is a proteolytic enzyme secreted by main gastric cells which becomes Pepsin at acid pH. The Serum levels of this enzyme have been proposed as indicators of the gastric mucosa because depending on the underlying disease they can be raised or lowered. In this sense, levels are increased in superficial gastritis and duodenal ulcer, while in atrophic gastritis and gastric cancer the levels are decreased. Our results on Pepsinogen are consistent with the studied pathology so that some authors suggest the use of these serum markers for diagnosis CAG.[7] In our study, the loss of parietal cells induces an increased pH and hypergastrinemia and decreased Pepsinogen I. As a significant event we point out that affected members with CAG had the highest levels of Gastrin, but three showed Gastrin increases without decreases in Pepsinogen I, which is showing that despite the process itself is not affected, there is some degree of CAG that could be an indication to perform gastroscopy or a close follow up in order to detect early changes in the level of vitamin B12. In this sense, some researchers find abnormalities in Gastric function in first-degree relatives of patients with pernicious anemia.[8]

Several studies have linked CAG with some HLA with different models due to the genetic diversity of the disease and some authors find association with HLA-DRB1*03 or *04, although this correlation occurs in 50% of patients affected with CAG.[9,10] In our case we have observed that most of studied patients show an HLA DQ6.2 via maternal and the same haplotype or DQ7.5 through the father, but we cannot establish a relationship with the accurate diagnosis, or laboratory abnormalities commented previously, although levels of Gastrin correlate with the double dose of DQ6.2 haplotype.

The allele DQB1*06:02 has been associated with several autoimmune diseases as a factor of susceptibility in multiple sclerosis or narcolepsy, and as a protector in type 1 diabetes mellitus.[11-13] With respect to gastric pathology, it has been associated with susceptibility to be a *Helicobacter pylori* carrier, and the development of gastric cancer.[14]

In a subsequent study, our main goal will be evaluate the possible genetic alterations that may be causing this autoimmune process and that other studies are focusing in different directions as the genetic loci MS4A3, CLYBL, FUT6, FUT2, either by increasing the predisposition to infection by *Helicobacter pylori* or by mutations in intrinsic factor which would entail an autoimmune process as the intermediate cause of the CGA.[15,16]

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