Available online at www.sciencedirect.com
ScienceDirect
Digestive and Liver Disease 40 (2008) 248–252
Digestive and Liver Disease
www.elsevier.com/locate/dld

Alimentary Tract

Increased prevalence of celiac disease without gastrointestinal symptoms in adults MICA 5.1 homozygous subjects from the Campania area

N. Tinto [a,b,1], C. Ciacci [c,*,1], G. Calcagno [d], D. Gennarelli [a,b], A. Spampanato [a,b], E. Farinaro [e], R. Tortora [c], L. Sacchetti [a,b]

[a] *Department of Biochemistry and Medical Biotechnology, Federico II University Medical School, Naples, Italy*
[b] *CEINGE Advanced Biotechnology, Naples, Italy*
[c] *Department of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy*
[d] *Department of Health Sciences, University Medical School of Molise, Campobasso, Italy*
[e] *Department of Preventive Medical Sciences, Federico II University Medical School, Naples, Italy*

Received 7 September 2007; accepted 29 November 2007
Available online 20 February 2008

Abstract

Objectives. Polymorphisms in the major histocompatibility complex class I chain-related gene A may influence its binding to the Natural Killer Cell Receptor G2D (NKG2D). We looked for polymorphisms in major histocompatibility complex class I chain-related gene A exon 5 and in Human Leukocyte Antigen (HLA)-DQ/DR in adult coeliac disease patients to determine whether they affected coeliac disease phenotypes.

Methods. Adult coeliac disease patients with ($n=98$) and without ($n=93$) gastrointestinal symptoms (gastrointestinal symptoms+/gastrointestinal symptoms−) and 108 control subjects from Campania (Italy) were characterized by Polymerase Chain Reaction (PCR) sequence specific oligonucleotide followed by PCR sequence specific primer assays for HLA DQ/DR, and by PCR followed by capillary electrophoresis for major histocompatibility complex class I chain-related gene A exon 5 polymorphisms. Immunoglobulin A (IgA) anti-transglutaminase antibodies were also evaluated by immunosorbent assay.

Results. Five different major histocompatibility complex class I chain-related gene A alleles were detected in both coeliac disease patients and control subjects. The major histocompatibility complex class I chain-related gene A 5.1 allele occurred more frequently in patients than in controls ($p<0.05$), and the major histocompatibility complex class I chain-related gene A 5.1/5.1 homozygous genotype increased the risk of gastrointestinal symptoms− coeliac disease (OR = 2.79, 95% CI 1.15–6.79). Gastrointestinal symptoms− coeliac disease patients bearing major histocompatibility complex class I chain-related gene A 5.1/5.1 alleles showed lower anti-transglutaminase levels (18 U/L) than the gastrointestinal symptoms+ coeliac disease patients (35 U/L). HLA-DQ2/DQ8 genotypes did not differ between gastrointestinal symptoms+ and gastrointestinal symptoms− coeliac disease, although DQ8 tended to be more frequent in gastrointestinal symptoms− coeliac disease (11.7%) than in gastrointestinal symptoms+ coeliac disease (6%).

Conclusions. Our study shows that a double dose of the major histocompatibility complex class I chain-related gene A 5.1 allele could predispose to the onset of gastrointestinal symptoms− coeliac disease. We can hypothesize that a lower level of immunological involvement in gastrointestinal symptoms− coeliac disease patients is associated with absence of gastrointestinal symptoms. This test could represent a second step in the genetic typing of high-risk subjects such as first-degree relatives of coeliac disease patients positive for the DQ2/DQ8 molecule.

Keywords: Coeliac disease; Genes; HLA; MICA

* Corresponding author at: Dipartimento di Medicina Clinica e Sperimentale, Università di Napoli "Federico II", via S. Pansini, 5, 80131 Naples, Italy. Tel.: +39 081 7464270; fax: +39 081 7464270.
E-mail address: ciacci@unina.it (C. Ciacci).
[1] These authors contributed equally to the study.

1590-8658/$30
doi:10.1016/j.dld.2007.11.028

1. Introduction

Genes in the HLA region (i.e. HLA class II and MIC genes) and genes in other regions (non HLA genes) are involved in coeliac disease (CD) [1]. The major histocompatibility complex class I chain-related (MIC) gene family is located on chromosome 6 within the MHC region. It is constituted by three pseudogenes, MICC, MICD and MICE, and two functional genes, MICA and MICB [2]. Unlike HLA class I molecules, they do not bind β2 microglobulin, they are conformationally stable independent of conventional class I peptide ligands, and their expression is not regulated by interferon I and II, but by promoter heat shock elements similar to those of HSP70 [3]. MIC expression in the gastrointestinal epithelium could be up-regulated under stress conditions [4]. In fact, stress appears to be required for the enterocyte destruction that leads to overt CD [5]. Furthermore, the α-gliadin peptide p31–49 was shown to directly activate MIC [1]. MICA protein binds to NKG2D, an activating receptor of natural killer cells, CD8+ αβ T cells and γδ T cells [6]. Therefore, MICA may function as a self-antigen that can be stress-induced and recognized by T cells endowed with the TCR variable region $V_\delta 1$, which represent 70–90% of the γδ T cells in the intestinal epithelium [6].

The MICA gene lies 40 kb centromeric to HLA B and is structured in six exons that encode the leader peptide, the extracellular domains α_1, α_2 and α_3, a transmembrane region and a cytoplasmatic tail, respectively [2]. The MICA gene is highly polymorphic and more than 50 alleles have been described, which are mainly localized in extracellular domains (exons 2, 3 and 4) [7]. Exon 5 contains a microsatellite polymorphism consisting in a variable number of GCT repeats. The most frequent are 4, 5, 6, 7, 9, 10 that encode for alanine residues A4, A5, A6, A7, A9 and A10, and the A5.1 allele with a guanine insertion (GCT > GGCT) after the second GCT triplet. The A5.1 allele results in a frameshift mutation that generates a premature stop codon in the transmembrane region that, in turn, truncates the hydrophobic 42-amino-acid-long MICA cytoplasmic tail [8]. Consequently, the truncated soluble protein moves to the apical membrane, unlike the normal protein, which moves to a basolateral location [9]. The MICA 5.1 allele was previously reported to be associated to the form of CD without gastrointestinal symptoms (GIS−) [10].

The aim of our study was to characterize genetically HLA class I MICA gene and HLA class II genes in adult CD patients from the Campania region of Italy to determine whether they affected CD phenotypes.

2. Methods

One-hundred and ninety-one coeliac patients, mean age at diagnosis 32 years, 33 (17.3%) male, were consecutively recruited at the Gastroenterology Department, Medical School, University of Naples Federico II. Coeliac disease was diagnosed according to ESPGAN criteria, namely, anti-transglutaminase (tTG) antibodies in serum and villous intestinal atrophy grade according to Marsh [11]. Patients were classified in GIS+ and GIS− based on presence/absence of gastrointestinal symptoms, namely, diarrhoea, abdominal pain, weight loss, growth delay and cachessia, irrespective of anaemia [12]. Clinical history was collected from each patient to determine whether there was a familial history of CD, allergies, autoimmune disorders, other disorders frequently associated to CD (infertility, skin disease, etc.) or dental enamel dysplasia and hypertransaminasemia. The age of onset of symptoms was recorded to determine the interval from symptom onset to diagnosis. One hundred and eight healthy controls, 38 (36%) males, mean age 32 years, not related to the patients, with no biochemical abnormalities and negative for tTG antibodies were recruited at the Department of Medical and Preventive Sciences of our Medical School. All participants gave their written informed consent to the study and the research was carried out in accordance with the principles of the Helsinki II declaration.

Genomic DNA from patients and controls was extracted from a blood sample + EDTA (Nucleon BACC 2, Amersham Biosciences Europe, Milan, Italy). Serum antibodies were measured in both patients and controls. We used enzyme linked immunosorbent assay (EIA) and human recombinant tTG as antigen (DIA Medix subsidiary of Diagnostics Inc., MIAMI, Florida, USA) to identify IgA tTG. To exclude IgA deficiency, we measured total serum IgA with a nephelometric assay (BN ProSpec System Behring, Marburg, Germany).

We used a three-step procedure for HLA-DQ typing. First, we determined whether the HLA heterodimer DQA1*0501-DQB1*0201 was present using a sequence specific primer (SSP)-PCR procedure previously reported by us [13]. Secondly, we used a sequence specific oligonucleotide (SSO)-PCR based method to type the HLA DQB1* locus (Dynal Biotech Ltd., Bromborough Wirral, UK) to confirm the presence/absence of the DQB1*02 allele and/or to identify the other DQB1*03 risk allele. Lastly, when the DQB1*03 allele was present we used an SSP-PCR technique (Dynal Biotech Ltd. Bromborough, Wirral, UK) to look for the DQB1*0302 allele.

We used an SSO-PCR based method (Dynal Biotech Ltd. Bromborough Wirral, UK) to type HLA-DR and to look for the extended DQ/DR extended haplotype for CD. Microsatellite repeat polymorphisms in the transmembrane region of the MICA exon 5 gene were identified by PCR methodology using primers reported elsewhere [14], the forward primer was labelled at the 5′ end with fluorescent reagent 6-FAM. PCR products were analysed by capillary electrophoresis (CE) on the ABI Prism 3100 system (Applied Biosystem, Foster City, CA, USA) using, for each DNA sample, GENESCAN 400 HD ROX as internal size standard to normalize the variability of the various electrophoretic runs. Allele size was determined with the GeneScan software v3.7