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Alimentary Tract

Allelic frequencies of the hs1.2 enhancer within the immunoglobulin heavy chain region in Dayton, Ohio patients screened for celiac disease with duodenal biopsy

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ABSTRACT

Background: Genetic and environmental factors contribute to the development of celiac disease (CD), but specific genetic predisposing factors remain poorly understood. One candidate is allele 2 of the hs1.2 enhancer within the immunoglobulin heavy chain region. In humans, there are four possible alleles and a previous study of an Italian cohort demonstrated a significantly increased frequency of allele 2 in patients with CD.
Aims: The purpose of the current study was to determine if a similar association between allele 2 and CD exists in an American population from Dayton, OH.
Methods: Subjects were screened for CD via esophagogastroduodenoscopy with duodenal biopsy. All biopsies were microscopically scored using a modified Marsh-Oberhuber classification. DNA was isolated from patients' buccal cells for hs1.2 genotype analysis using PCR.
Results: Unlike the Italian cohort, allele 2 frequency was not significantly different in patients with histopathologic evidence of CD compared to patients without such evidence. However, our patient population as a whole demonstrated a significantly increased allele 2 frequency when compared to that previously reported within diverse ethnic populations.
Conclusions: Since our comparative control patients do not necessarily reflect a healthy control population, an overall increase in allele 2 may reflect an association between allele 2 of the hs1.2 enhancer and a spectrum of gastrointestinal disorders.

1. Introduction

Celiac disease (CD) is an autoimmune disease that occurs in about 1% of the general population, but this prevalence is highly variable according to geographic area [1]. Symptoms arise after the ingestion of gluten, a component of wheat, barley, and rye (reviewed by Ref. [2]). Multiple genetic and environmental factors may influence the development of CD. Through the well-studied connection between CD and HLA-DQ8 and HLA-DQ2.5, it has been established that these HLA alleles are present in almost all individuals with CD (reviewed by Ref. [2]). However, approximately 40% of individuals in Western countries possess these HLA molecules, highlighting the multifactorial nature of CD (reviewed by Ref. [2]). In familial studies, the contribution of HLA in CD heritability was quantified at less than 40% [3,4]. With the addition of 57 SNPs and 5 variants within the MHC region that are independent of the HLA-DQ alleles, the known genetic variations associated with CD amount to approximately 48% of the heritability of CD [5]. To clarify genetically unexplained heritability, genome-wide association studies have been conducted, identifying several non-MHC candidate genes (e.g. IL-2 and IL-21) that may influence the risk of developing CD [6–8].

Besides those identified via genome-wide association analysis, an additional genetic factor within the immunoglobulin heavy chain (*IGH*) region has been implicated in CD and in a number of autoimmune diseases [9–12]. This potential additional factor is

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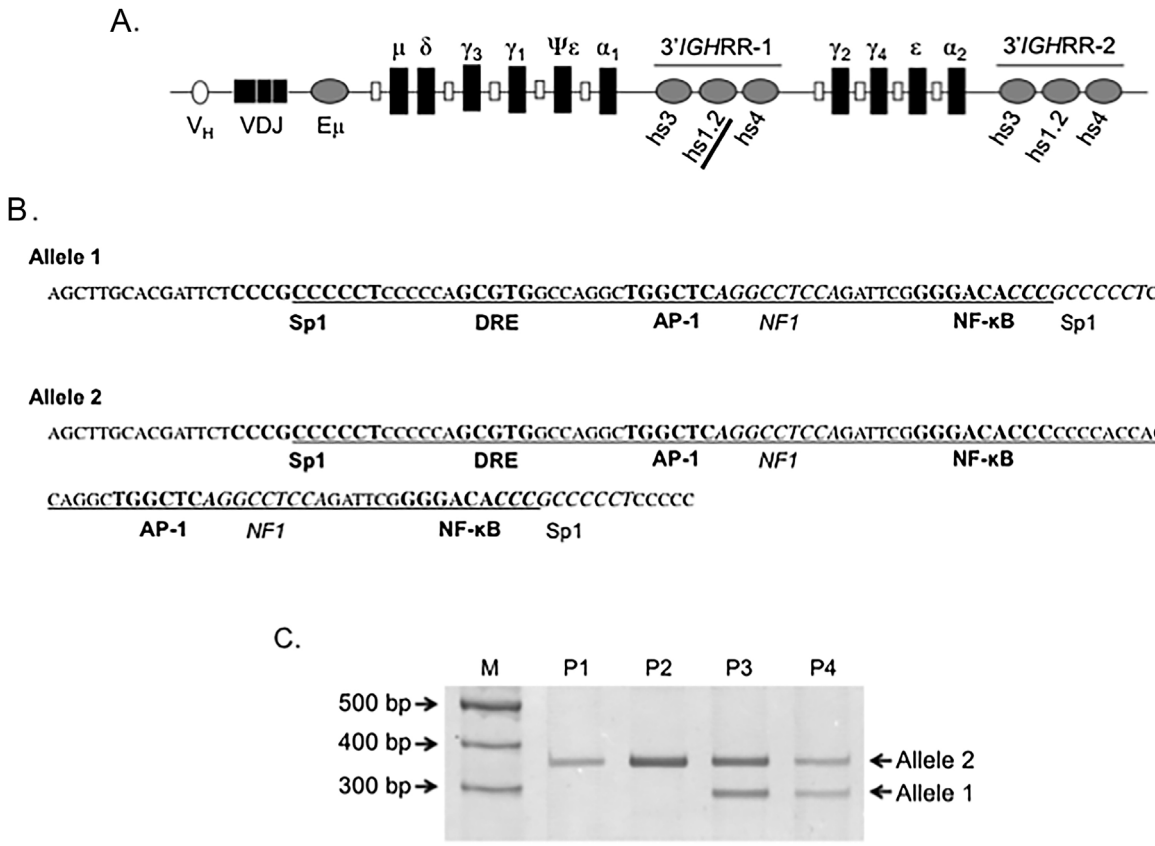

Fig. 1. The human *IGH* locus and polymorphic hs1.2 alleles. (A) Schematic of the rearranged human *IGH* gene, including the variable (VDJ) and constant regions (μ, δ, γ_{1-4}, ε, and $\alpha_{1,2}$) and the known transcriptional regulators, i.e. V_H promoter, intonic enhancer (Eμ), germline promoters upstream of each constant region (open rectangles), and the two 3′*IGH*RRs (3′*IGH*RR-1 and 3′*IGH*RR-2) downstream of each α constant region. In the UCSC Genome Browser Database GRCh38/hg38, the 3′*IGH*RR-1 and 3′*IGH*RR-2 approximately span nucleotides 105,688,954 to 105,704,102 and 105,569,086 to 105,585,123 in Chr14 q32.33, respectively. The hs1.2 enhancer (underlined) within the 3′*IGH*RR-1 has previously been shown to be polymorphic and allele 2 has been associated with CD and several autoimmune diseases [9–12]. (B) Representative sequence for allele 1 and allele 2 based on random sampling of nested PCR products generated from patient samples (buccal cells). Transcription factor binding sites – Sp1, DRE (AhR binding site), AP-1, NF1, and NF-κB – are represented by increased font size with italics and/or bold type and agree with previous sequence analysis (EMBL AJ298015 and AJ298016 for allele 1 and allele 2 respectively) [13,17]. The invariant sequence that can be repeated within the hs1.2 enhancer is underlined. (C) Representative genotyping gel of nested PCR products generated from four patients (P). Patients 1 and 2 are homozygous for allele 2 (350 bp); patients 3 and 4 are heterozygous for alleles 1 (297 bp) and 2 (350 bp). M denotes the 100 bp ladder.

allele 2 of the hs1.2 enhancer within the 3′ *IGH* regulatory region (3′*IGH*RR). In animal models and cell culture, this region is crucial for Ig expression and class switch recombination, which impacts antibody concentrations [13–16]. In humans, the hs1.2 enhancer is polymorphic but the polymorphism is not defined by a single nucleotide polymorphism (SNP). Instead, it is defined by an approximately 53 bp sequence called the invariant sequence that can be tandemly repeated up to four times (Fig. 1) [13,17]. The four alleles of the hs1.2 enhancer are named for the number of invariant sequences they have, i.e. alleles 1, 2, 3, and 4 have 1, 2, 3, and 4 invariant sequences, respectively. In European populations, alleles 1 and 2 are the most prevalent [18]. As the name implies, each repeat of the invariant sequence has high sequence identity and the invariant sequence contains binding sites for several transcription factors known to be involved in B-cell activation and differentiation into antibody-secreting cells [13,15,17]. Additionally, secretion of tissue transglutaminase antibodies, endomysial antibodies, and deamidated gliadin peptide antibodies is a characteristic of CD [19]. However, the relationship of these antibodies with clinical manifestations of CD is poorly understood. Two existing hypotheses are that the antibodies (1) promote damage of small intestinal mucosa or (2) might simply be an epiphenomenon occurring alongside CD pathology (reviewed by Refs. [20,21]).

In a previous study by Frezza et al., the authors reported an increased frequency of allele 2 in patients with CD and an increased risk of developing CD in patients with allele 2 [10]. Their patient population included individuals diagnosed with CD using an intestinal biopsy and the presence of anti-endomysial antibodies. The control population was comprised of Italian blood donors; a negative upper gastrointestinal duodenal biopsy was not a criterion for control individuals [10]. According to the American College of Gastroenterology Clinical Guideline for the diagnosis and management of CD, a small bowel biopsy should be performed when the suspicion of CD is high, regardless of serum antibody results, and is considered critical in patient evaluation [19]. In our study, all subjects had duodenal biopsies to support or exclude a diagnosis of CD.

Overall, our aim was to analyze the association between CD and allele 2 of the hs1.2 enhancer. To do so, we enrolled 154 patients from two groups: (1) those undergoing esophagogastroduodenoscopy with duodenal biopsy (prospective group); (2) those with a previously established diagnosis of celiac disease confirmed by duodenal biopsy (retrospective group). We analyzed their genotypes for the hs1.2 enhancer and categorized the patients as CD or non-CD based on microscopic findings. In keeping with the diagnostic guidelines, a patient was classified as having CD when histopathologic changes met criteria for categories 3A, 3B, or 3C in the modified Marsh-Oberhuber classification [19,22]. Our results did not confirm an association between histopathologic changes of mucosal atrophy and the presence of allele 2 of the hs1.2 enhancer.