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# Celiac Disease and HLA in a Bedouin Kindred

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**ABSTRACT:** We report the prevalence of celiac disease (CD) and its relationship with other autoimmune diseases and HLA haplotypes in a Bedouin kindred. Of 175 individuals sampled and typed for autoantibodies and HLA class II genotypes, six (3.4%) members had CD, and an additional 10 (5.7%) members tested positive for autoantibodies to transglutaminase (TgAA+). Several CD/TgAA+ relatives also had islet cell antigen or adrenal autoimmunity. Affected relatives are more closely related than expected from the pedigree relationships of all family members and were more often the offspring of consanguineous marriages. Individuals with CD or TgAA+ were enriched for DRB1*0301-DQA1*0501-DQB1*0201, a haplotype previously reported as high risk for CD. There was also an increased frequency of DQB1*0201/DQB1*0201 homozygotes among affected relatives. We found no evidence that DRB1*0701-DQA1*0201-DQB1*0201/DRB1*11-DQA1*0501-DQB1*0301 is a high-risk genotype, consistent with other studies of Arab communities. In addition, a nonparametric linkage analysis of 376 autosomal markers revealed suggestive evidence for linkage on chromosome 12p13 at marker D12S364 (NPL = 2.009, $p = 0.0098$). There were no other significant results, including the HLA region or any other previously reported regions. This could reflect the reduced power of family-based linkage and association analyses in isolated inbred populations. *Human Immunology 67, 940–950 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

**KEYWORDS:** Celiac disease; type 1 diabetes; HLA; Bedouin; linkage

**ABBREVIATIONS**

| | |
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| Ab+ | positive for islet cell antigen autoantibodies (without T1D) |
| CD | celiac disease |
| MHC | major histocompatibility complex |
| NPL | nonparametric linkage |
| T1D | type 1 diabetes |
| TgAA+ | positive for autoantibodies to transglutaminase (without celiac disease) |

## INTRODUCTION

Celiac disease (CD) is an autoimmune gastrointestinal disease caused by intolerance to gluten, dietary proteins present in wheat, rye, and barley. The disease usually manifests in childhood, and symptoms include diarrhea, abdominal pain, and growth failure. Symptoms in adulthood include anemia, fatigue, weight loss, diarrhea, constipation, and neurologic symptoms [1, 2]. Environmental factors and multiple genes, including HLA, are involved in the development of the disease. The genetic effects are evident by the high prevalence rate (10%) among first-degree relatives of CD patients [1]. The relative risk to siblings $\lambda_s$ is 30–60, which is high compared with other multifactorial disorders, such as rheumatoid arthritis, type 1 diabetes (T1D), and multiple sclerosis [1]. Furthermore, the concordance among monozygotic twins is 70%, whereas concordance among dizygotic twins is 11% [3].

Genes within or near the major histocompatibility complex (MHC) play a significant role in the etiology of

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CD. Studies of CD patients from populations in Europe and North America reveal that approximately 90% of CD patients have DQA1*05 and DQB1*02, either in *cis* (as DRB1*0301-DQA1*0501-DQB1*0201 [DR3-DQ2]) or in *trans* (usually as DRB1*11-DQA1*0501-DQB1*0301/DRB1*07-DQA1*0201-DQB1*0201 [DR5-DQ7/DR7-DQ2]), compared with 20%–30% of healthy controls [4]. The vast majority of the remainder of CD patients have DRB1*04-DQA1*0301-DQB1*0302 (DR4-DQ8), suggesting a different genetic determinant than that of the DQ(α1*05, β1*02) heterodimer. Although there are studies that suggest non-HLA genes in the MHC play a role in the etiology of CD (reviewed by Sollid), strong genetic and functional arguments can be made that DQA1 and DQB1 themselves are the primary MHC-linked genes [1]. The DQ(α1*05, β1*02) heterodimer preferentially binds negatively charged amino acids, such as deamidated gluten proteins, at specific anchor positions and presents a larger repertoire of gluten proteins compared with the DQ(α1*02, β1*02) heterodimer [1, 5]. Several studies have presented gene dose effects where risk, severity of symptoms, or age of onset depends on the number of possible DQ(α1*05, β1*02) heterodimers that are encoded in *cis* or in *trans* [6]. The causal effects of the DQ(α1*03, β1*0302)(DQ8) molecule are less clear, but as with the (α1*05, β1*02) heterodimer, DQ(α1*03, β1*0302) has a preference for negatively charged residues at several anchor positions [7].

The fact that HLA-specific relative risk to siblings is 2.3–5.5 compared with the overall relative risk to siblings of 30–60 suggests that non-HLA genes play a large role in the etiology of CD [1]. Several linkage studies, either of candidate genes or whole genome screens, to find non-HLA genes have been performed but few regions have been replicated, and replication has occurred only in studies of European or European-derived populations [2, 8–18]. The exceptions are the HLA region, which consistently reveal extremely strong evidence of linkage to CD, and three regions, 2q33, 11p11, and 5q31-33. Chromosome region 2q33, which contains a cluster of immune system–related genes including CTLA-4, was originally implicated by Holopainen and colleagues and confirmed in a large linkage study of European families and in subsequent association studies [9, 19–21]. With 11p11, the initial finding occurred in a study of Irish CD patients and was confirmed in two subsequent studies of multiplex families from the United Kingdom [10, 11, 22]. Evidence for the 5q31-33 region is suggestive in two studies of Italians and of Scandinavians, but when a meta-analysis was performed of data pooled from four linkage studies, the evidence for 5q31-33 reached significance [8, 16, 12].

In this paper, we describe the relationship of CD, T1D, and HLA DRB1-DQA1-DQB1 haplotypes and present the results of a whole-genome screen for CD in a Bedouin kindred living in Israel. This extended family, which has been tracked since 1992, consists of approximately 200 individuals and is characterized by a high prevalence of CD and T1D [23]. The family may be considered a population in itself, and in comparison with other populations, this kindred is characterized by genetic and environmental homogeneity as the result of genetic isolation, inbreeding, and founder effects.

## MATERIALS AND METHODS

### CD, T1D, and HLA Phenotypes

Samples from 182 family members were available for disease diagnosis, measurement of autoantibodies associated with autoimmune diseases, such as CD, T1D, and Addison's disease, and genotyping at the HLA region. All known CD-affected relatives and nearly all the remaining family members from this closed population were sampled. CD and T1D were clinically diagnosed in Israel. Diagnosis of CD was done by serologic testing, and in most subjects was accompanied by a gastrointestinal biopsy. Six subjects displayed clinical symptoms of CD, such as abdominal pain, diarrhea, and anemia. All individuals with serologic negative tests and with normal IgA level were considered unaffected.

A battery of autoantibodies, including antibodies to the CD-associated transglutaminase (Tg), antibodies to T1D-associated glutamic acid decarboxylase (GAD65), insulin, and IA-2/phogrin protein (ICA512), as well as antibodies to the Addison's disease–associated 21-hydroxylase (21OH), were measured by radioassay at the Barbara Davis Center for Childhood Diabetes in Denver, Colorado, USA [20, 21]. Age of subjects at last blood draw ranged from 1–73 years of age, with a median age of 25 years. HLA-DRB1, -DQA1, and -DQB1 alleles were amplified by polymerase chain reaction and typed with sequence-specific oligonucleotide probes [23–25].

### Microsatellite Genotyping

We genotyped 376 autosomal microsatellite markers in 45 family members by using the 10-cM density ABI PRISM Linkage Mapping Set (Applied Biosystems, Foster City, CA, USA) (LMS-MD10). In addition, 16 MHC microsatellite markers were genotyped in all sampled family members to infer the ancestral relationships of haplotypes with identical HLA class II alleles. Genotyping was performed by amplifying genomic DNA by using fluorescently labeled primers and separating the products by electrophoresis in 5% polyacrylamide gels by using an Applied Biosystems 377 semiautomated sequencer. Allele sizing was carried out using GeneScan