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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 ten (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 celiac disease. 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.

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List of abbreviations:

CD	celiac disease
TgAA+	positive for autoantibodies to transglutaminase (without celiac disease)
T1D	type 1 diabetes
Ab+	positive for islet cell antigen autoantibodies (without T1D)
21OH	21-hydroxylase
cM	centiMorgan
NPL	nonparametric linkage

Keywords

Celiac disease; type 1 diabetes; HLA; Bedouin; linkage

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 while symptoms in adulthood include anemia, fatigue, weight loss, diarrhea, constipation and neurological symptoms [1,2]. Both 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 λ_s is 30-60, which is high compared to other multifactorial disorders such as rheumatoid arthritis, type 1 diabetes, and multiple sclerosis [1]. Furthermore, the concordance among monozygotic twins is 70% while concordance among dizygotic twins is 11% [3].

Genes within or near the major histocompatibility complex (MHC) play a significant role in the etiology of 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 to 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(α 1*05, β 1*02) heterodimer. While there are studies that suggest non-HLA genes in the MHC play a role in the etiology of celiac disease (reviewed in [1]), strong genetic and functional arguments can be made that DQA1 and DQB1 themselves are the primary MHC-linked genes. The DQ(α 1*05, β 1*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 to the DQ(α 1*02, β 1*02) heterodimer [1,5]. Several studies (discussed in [6]) have found gene dose effects where risk, severity of symptoms or age of onset depends on the number of possible DQ(α 1*05, β 1*02) heterodimers that are encoded in *cis* or in *trans*. The causal effects of the DQ(α 1*03, β 1*0302) (DQ8) molecule are less clear, but as with the (α 1*05, β 1*02) heterodimer, DQ(α 1*03, β 1*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 [1] compared to the overall relative risk to siblings of 30-60 suggests that non-HLA genes play a large role in the etiology of CD. Several linkage studies, either of candidate genes or whole genome screens, to find non-HLA genes have been performed (e.g., [2,8-18]), but few regions have been replicated, and replication has occurred only in studies of European or European-derived populations. The exceptions are the HLA region, which consistently shows 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 [9] and confirmed in a large linkage study of European families [19] and subsequent association studies [20,21]. With 11p11, the initial finding occurred in a study of Irish CD patients [22] and was confirmed in two subsequent studies of multiplex families from the UK [10,11]. Evidence for the 5q31-33 region is suggestive in two studies of Italians and of Scandinavians [8,12], but when a meta-analysis was performed of data pooled from four linkage studies the evidence for 5q31-33 reached significance [16].

In this paper we describe the relationship of celiac disease, type 1 diabetes (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 celiac disease and type 1 diabetes [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 a total of 182 family members were available for disease diagnosis, measurement of autoantibodies associated with autoimmune diseases such as celiac disease, type 1 diabetes (T1D), and Addisons 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. Celiac disease and type 1 diabetes were clinically diagnosed in Israel. Diagnosis of CD was done by serological testing and in most subjects accompanied by a gastrointestinal biopsy. Six subjects displayed clinical symptoms of CD such as abdominal pain, diarrhea and anemia. All individuals with serological negative tests and with normal IgA level were considered unaffected.

A battery of autoantibodies including antibodies to the celiac disease-associated transglutaminase (Tg); antibodies to type 1 diabetes-associated glutamic acid decarboxylase (GAD65), insulin, and IA-2/phogrin protein (ICA512); as well as antibodies to the Addisons 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 to 73 years with a median age of 25 years. HLA-DRB1, -DQA1 and -DQB1 alleles were amplified by PCR and typed with sequence-specific oligonucleotide probes [23-25].

Microsatellite genotyping

We genotyped 376 autosomal microsatellite markers in 45 family members using the 10 cM density ABI Prism Linkage Mapping Set (LMS-MD10). In addition, sixteen 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 using fluorescently labeled primers and separating the products by electrophoresis in 5% polyacrylamide gels using an Applied Biosystems 377 semiautomated sequencer. Allele sizing was carried out using Applied Biosystems GENESCAN 3.1, and individual genotypes were assigned using Applied Biosystems GENOTYPER 2.5 manually checked to minimize data errors.

Statistical analyses

Statistical tests included Fisher 2x2 and 2xk exact tests to test for frequency differences among phenotype groups. The exact tests treat this extended family as a closed population and ignore family relationships. Odds ratios and confidence intervals were calculated using the CASECONT executable in PEPI (Computer Programs for Epidemiologists) written by J. H. Abramson and Paul M. Gahlinger [26].

Kinship coefficients were used to measure and compare the genetic relatedness between all pairs of relatives, pairs of affected relatives, and pairs of unaffected relatives. The kinship coefficient is the probability that an allele drawn at random from one relative is identical by descent to an allele at the same locus drawn at random from another relative. The KIN (version 1.0.5 [27]) software package was used to calculate kinship coefficients from the genealogical information, which was extended to seven generations after including deceased ancestors.

Inbreeding coefficients were determined for each relative as the kinship coefficient of their parents.

Mean kinship coefficients and mean inbreeding coefficients were calculated for each phenotypic subgroup. In order to determine the statistical significance of differences between subgroups, we compared the observed results with the mean kinship coefficients and mean inbreeding coefficients obtained after assigning the disease phenotypes at random to family members in each of 30,000 permutations of the phenotype data.

We used another computer simulation procedure to test whether specific high-risk DRB1-DQA1-DQB1 haplotypes were transmitted preferentially to affected offspring. Because of the complex pedigree structure of this family (inbreeding loops, multiple affected offspring), standard transmission tests such as the transmission disequilibrium test and the pedigree disequilibrium test could not be used, and thus we used computer simulations to obtain p-values empirically. For these analyses, DRB1-DQA1-DQB1 haplotypes were randomly assigned to 34 founders according to the relative frequencies of 43 founder DRB1-DQA1-DQB1 haplotypes that were identified directly for living founders using the known genotypes of the founders and their offspring, or indirectly for deceased ancestors using a combination of HLA and microsatellite genotypes to infer the ancestral relationships of the HLA haplotypes found in their descendents. Following random assignment of haplotypes to founders, the simulations randomly transmitted these haplotypes through the pedigree to CD-affected and TgAA+ family members. Empirical significance levels for preferential transmission were determined by counting the number of 30,000 replicate simulations in which the number of affected relatives who inherited a given DRB1-DQA1-DQB1 haplotype or genotype was as extreme or more extreme than the observed number of affected relatives with that haplotype or genotype. We tested three haplotypes and four genotypes that the current literature suggests are biologically relevant in terms of the DQ(α 1*05, β 1*02) heterodimer. The three haplotypes included DRB1*0301-DQA1*0501-DQB1*0201, which has the DQ(α 1*05, β 1*02) molecule in *cis*, as well as DRB1*0701-DQA1*0201-DQB1*0201 and DQB1*11-DQA1*0501-DRQ1*0301, which together in a genotype create the DQ(α 1*05, β 1*02) molecule in *trans* but individually should not increase risk of CD. The four genotypes included DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0701-DQA1*0201-DQB1*0201, DRB1*11-DQA1*0501-DQB1*0301/DRB1*0701-DQA1*0201-DQB1*0201, and DQB1*0201/DQB1*0201, which some studies suggest is associated with a more severe form of CD [6].

We performed genome-wide linkage analysis on the 376 autosomal microsatellites by the same method that was described by Babu and colleagues [28]. This sample included four of the six CD and five of the ten TgAA+ family members, comprising all of the CD-affected or TgAA+ relatives who had been diagnosed at the time the genotyping was performed. We ran a nonparametric linkage (NPL) analysis with Simwalk2 [29,30] on two phenotypes: (1) CD or TgAA+ (CD/TgAA+) and (2) CD only.

Results

Clinical characteristics

Of the 182 sampled family members, 175 family members from 57 sibships were surveyed for both CD and Tg autoantibodies and were genotyped for HLA class II alleles. Six family members (6/175=0.034) have or had celiac disease, and two of these six family members (an uncle-nephew pair: F29 and F42 in Table 1) died of intestinal lymphoma. Ten members (10/175=0.057) are positive for autoantibodies to transglutaminase but without documented CD (TgAA+) (Table 1). The sixteen CD-affected and TgAA+ family members represent ten sibships and three generations of descendents of two brothers (D13 and D14), who themselves

are the offspring of a first-cousin marriage. In addition, 25 family members have or had T1D. Of these 25 relatives, 21 were included in our analyses because two of these family members are deceased and unsampled and two did not have serum available for analysis of Tg autoantibodies. Nine additional family members have one or more of GAD65, ICA512, or insulin autoantibodies without T1D (Ab+).

Several family members have more than one autoimmune phenotype. One individual with CD and four TgAA+ individuals also have T1D (Table 1, Figure 1). Three individuals have autoantibodies for 21OH: the one individual with both celiac disease and type 1 diabetes (E41), another individual with celiac disease but with neither T1D nor T1D-associated autoantibodies (F58), and a TgAA+ individual with T1D (G34). Although this family is characterized by high prevalences of celiac disease and type 1 diabetes, we do not observe a higher frequency of T1D among individuals with CD versus family members without celiac disease ($1/6=0.167$ versus $20/169=0.118$, $p=0.541$, Table 2). Similarly, when associated autoantibodies are included into the phenotypes (i.e., CD/TgAA+ and T1D/Ab+), there are no significant frequency differences ($p=0.114$).

Inheritance of autoimmune phenotypes

Family members with celiac autoimmunity or islet autoimmunity are more closely related than expected, as seen in Table 3, which reports mean kinship coefficients. Pairs of relatives with celiac disease or autoantibodies to Tg are more closely related (mean kinship coefficient 0.166) than are pairs of relatives with islet autoimmunity (mean kinship coefficient 0.128) or pairs of relatives with neither celiac or islet autoimmunity (mean kinship coefficient 0.056). The mean kinship coefficient for different subphenotypes of celiac (CD or TgAA+) or islet autoimmunity (T1D or Ab+) are very similar (0.155 - 0.173 for celiac autoimmunity; 0.122 - 0.133 for islet autoimmunity; data not shown). The mean kinship coefficient between pairs of relatives with different autoimmunity phenotypes (one relative with CD or TgAA+ and the other with T1D or Ab+) is also higher (0.144) than the average kinship coefficient for all relative pairs (0.070) or for unaffected relative pairs (0.056). In all cases, the increased genetic relatedness of pairs of affected relatives is highly significant, based on the results of 30,000 random permutations of the phenotype data. The number of replicates for which the mean kinship coefficient exceeded the observed mean kinship coefficient was zero for pairs of celiac/TgAA+ relatives ($p<0.00003$), one for pairs of T1D/Ab+ relatives ($p=0.00003$), and zero ($p<0.00003$) for pairs of relatives in which one relative has celiac autoimmunity and other other has islet autoimmunity. These results indicate that allele sharing among affected relatives contributes to the risk for celiac and islet autoimmunity in the family and that one or more susceptibility alleles influences susceptibility to both celiac and islet autoimmunity.

Consanguinity also contributes to the risk for autoimmune disease in the family. The mean inbreeding coefficient among CD/TgAA+ pairs is 0.072, varying from 0.070 among TgAA+ relatives to 0.075 among CD relatives. The mean inbreeding coefficient for T1D relatives and for islet autoantibody-positive (Ab+) relatives is 0.076. By comparison, the mean inbreeding for all relatives is 0.036 and for unaffected relatives is 0.025. Based on the computer simulations, affected relatives are significantly more inbred than other family members ($p=0.0015$ for CD/TgAA+; $p<0.00003$ for T1D/Ab+). These results indicate that homozygosity for one or more susceptibility alleles contributes to the risk for autoimmune disease in family members.

HLA phenotypes among CD/TgAA+ family members

We found no evidence for a difference between CD and TgAA+ in terms of prevalences of common HLA haplotypes (Figure 2). Both CD relatives and TgAA+ relatives are enriched for DRB1*0301-DQA1*0501-DQB1*0201 compared to unaffected family members ($p<0.0001$,

2-tailed Fisher 2x3 exact test). Otherwise, there are no differences in prevalence among the three phenotypes (CD, TgAA+ and unaffected). Because there appear to be no significant differences between CD and TgAA+ individuals in terms of HLA haplotypes, T1D prevalences, or mean kinship and inbreeding coefficients, our analyses will focus on the combined phenotype of CD or TgAA+ (CD/TgAA+).

All CD/TgAA+ family members have HLA genotypes consisting of a DRB1*0301-DQA1*0501-DQB1*0201 that is often paired with another DRB1*0301-DQA1*0501-DQB1*0201 (n=2), an unusual DR3 haplotype DRB1*0301-DQA1*0102-DQB1*0502 (n=3) that was described in our initial report of this family [23], or DR7 (DRB1*0701-DQA1*0201-DQB1*0201, n=6) (see Table 1). There is one affected family member with DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0405-DQA1*0301-DQB1*0302. Consistent with many other studies, there is an elevated frequency of genotypes that include DRB1*0301-DQA1*0501-DQB1*0201 among CD/TgAA+ individuals versus unaffected individuals (16/16=1.0 versus 65/159=0.409, $p=0.000002$, odds ratio = 22.79) (Table 4).

DRB1*0301-DQA1*0501-DQB1*0201 was paired with eight different haplotypes among CD/TgAA+ relatives, indicating that the presence of at least one copy of this haplotype is the primary determinant of disease risk ($p=0.615$, exact test of frequency differences in the haplotypes paired with DRB1*0301-DQA1*0501-DQB1*0201). Nonetheless, the frequency of DQB1*0201/DQB1*0201 homozygotes was significantly increased in affected relatives (50% vs. 18%; $p=0.01$). Of 38 family members who are DQB1*0201/DQB1*0201 homozygotes, 12 (32%) were homozygous for DRB1*0301, 25 (66%) were heterozygous for DRB1*0301 and DRB1*0701, and 1 (2%) was homozygous for DRB1*0701. Although affected relatives showed a higher frequency of both DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0701-DQA1*0201-DQB1*0201 (2/16=0.125 vs. 10/159=0.063 and 6/16=0.375 vs. 19/159=0.120, respectively), the increased frequency was statistically significant for DRB1*0301/DRB1*0701 heterozygotes ($p=0.01$) but not for DRB1*0301/DRB1*0301 homozygotes ($p=0.301$) by Fisher exact tests (Table 4). Thus, the increased frequency of DQB1*0201/DQB1*0201 among affected relatives reflects an increase in both of the two most common DQB1*0201/DQB1*0201 genotypes, and the differences in the magnitude and statistical significance of the effects of the two genotypes may reflect differences in sample size.

While these results are consistent with a dosage effect of the DRB1*0201 allele, we found no evidence for an increased frequency of DRB1*11-DQA1*0501-DQB1*0201/DRB1*0701-DQA1*0201-DQB1*0201 in which the DQ(α 1*05, β 1*02) heterodimer is encoded in *trans*. In our study, all seven family members with this genotype are *unaffected*, but the difference in the genotype frequencies between affected and unaffected relatives is not statistically significant (0% vs. 4%; $p=0.505$). Based on the upper boundary of the 95% confidence limits, we can comfortably exclude the possibility that the DRB1*11-DQA1*0501-DQB1*0201/DRB1*0701-DQA1*0201-DQB1*0201 has a strong (OR > 7.5) effect on the risk for celiac autoimmunity among family members.

We used computer simulations to implement a family-based test of linkage and association of celiac autoimmunity with DRB1*0301-DQA1*0501-DQB1*0201, DRB1*0701-DQA1*0201-DQB1*0201, and DRB1*11-DQA1*0501-DQB1*0201 haplotypes and genotypes (Table 4). The computer simulation accounts for the frequency and diversity of haplotypes among the pedigree founders and for the complex pedigree structure in which three generations of living relatives are connected through four generations of deceased ancestors and about one-third of marriages are between close relatives such as first cousins. In order to estimate haplotype frequencies in the pedigree founders, many of whom are deceased, we used 16 microsatellite

markers spanning approximately 20 Mb to infer the ancestral relationships of haplotypes with identical class II alleles. HLA-identical haplotypes were considered to be derived from different founders if the region of microsatellite identity surrounding the class II loci was less than 10 Mb and the oldest family members carrying the haplotypes were separated by three or more meioses. Using these criteria, we identified 43 founder haplotypes of which the most common are DRB1*0301-DQA1*0501-DQB1*0201 (6/43, 14%), DRB1*0701-DQA1*0201-DQB1*0201 (9/43, 21%), and DRB1*11-DQA1*0501-DQB1*0301 (7/43, 16%). The remaining 21 ancestral haplotypes consist of twelve different DRB1-DQA1-DQB1 haplotypes each representing less than 10% of all ancestral haplotypes. These frequencies were used to randomly assign haplotypes to pedigree founders in our computer simulation of normal inheritance of HLA haplotypes by affected family members.

We found significant evidence for preferential inheritance of DRB1*0301-DQA1*0501-DQB1*0201 by CD/TgAA+ relatives (Table 4), consistent with a greater than expected transmission frequency of 76% (16/21 transmissions from a heterozygous parent to an affected offspring). The DQB1*0201/DQB1*0201 homozygous genotype was also inherited by affected relatives more often than expected, but the difference was not statistically significant ($p=0.084$). Consistent with the results from the exact tests (Table 4), we found no evidence for an association between celiac autoimmunity and DRB1*11-DQA1*0501-DQB1*0201/DRB1*0701-DQA1*0201-DQB1*0201.

Linkage analysis

Based on the results of genome-wide linkage analysis, we found no evidence that CD/TgAA+ is linked to microsatellite markers that flank the MHC at 6p21.3 (maximum at D6S1610 at 6p21.2: NPL=0.5187, $p=0.0681$) (Figure 3). This is most likely explained by the high frequency of the CD/TgAA+ associated haplotypes, the resultant ambiguities in the distinction of alleles that are identical by descent from alleles that are identical by state, and the low density of the markers used in the genome screen. Based on sixteen MHC microsatellite markers, the sixteen CD/TgAA+ relatives inherited one or both of two founder DRB1*0301-DQA1*0501-DQB1*0201 haplotypes (haplotypes B and C, Figure 4). The region of identity surrounding the class II loci is 5-7 Mb, large enough to be uncertain whether the two founder haplotypes are identical by descent from a recent common ancestor but small enough to cause a significant reduction in the power to detect linkage to the MHC in a 10 cM microsatellite screen.

Table 5 shows that if we assume that founder haplotypes B and C are derived from a recent common ancestor, then there is marginally significant evidence for haplotype sharing at the MHC among affected siblings ($p=0.059$). However, if haplotypes B and C are not derived from a recent common ancestor, then there is no evidence of linkage ($p=0.607$).

The highest NPL score for the CD/TgAA+ linkage analysis was at 12p12-13 (maximum at D12S364 at 12p13: NPL=2.009, $p=0.0098$) with two smaller peaks at 1p13.3-q21.3 (maximum at D1S252 at 1p13.1: NPL=0.9035, $p=0.0326$) and at 4q35 (maximum at D4S426 at 4q35.2: NPL=0.8482, $p=0.0422$) (Figure 3).

In a linkage analysis of the CD-only phenotype, the peak at 12p12-13 disappeared, and there were no other peaks with p -values less than 0.01. This most likely reflects the small number (four) of affected relatives included in the CD-only genome screen, although we cannot rule out the possibility that different genes contribute to the initiation of celiac autoimmunity and the progression to clinical disease. There were three peaks with p -values between 0.01 and 0.05: 7p22.2-21.3 (maximum at D7S531 & D7S517: NPL=0.8930, $p=0.0277$); 2p25 (maximum at D2S319: NPL=0.8928, $p=0.0284$); and 13q32.2-33.3 (maximum at D13S158: NPL=0.8930, $p=0.0288$). Again, there was no overlap with any previously reported results or with our CD/TgAA+ results.

Discussion

We have studied the association of CD, T1D and HLA DRB1-DQA1-DQB1 haplotypes in a large Bedouin kindred. Prevalences of celiac disease and of CD/TgAA+ in this extended family are higher than those reported in studies of European or European-American families but consistent with those reported in Saharawi Arabs [31], who are more similar to Bedouins in their cultural history and genetic background. It has been suggested that the high prevalence of celiac disease in nomadic Arabs may reflect a selective advantage related to a reduced risk for intestinal infections in individuals with celiac enteropathy at a time when the negative effects of the disease were minimized by low gluten intake in the traditional diet [31].

Ours is the first study to examine the association between celiac and islet autoimmunity in an Arab community with a high prevalence of CD/TgAA+. To our knowledge, this is also the first study to examine the co-inheritance of celiac and islet autoimmunity and associations with inbreeding in any population, as these analyses are dependent on data from extended families. Our analyses of mean kinship coefficients confirmed a stronger contribution of genetic factors to the risk for CD/TgAA+ compared with T1D by demonstrating that relatives with CD/TgAA+ + relatives are more closely related (mean kinship coefficient 0.166) than are pairs of relatives with T1D/Ab+ (mean kinship coefficient 0.128). We also found that pairs of relatives with different autoimmunity phenotypes are more closely related (mean kinship coefficient 0.144) than are pairs of relatives without celiac or islet autoimmunity (0.056) and that both phenotypes are strongly associated with consanguinity. The prevalence of CD/TgAA+ is also higher in family members with T1D/Ab+ (16.7%) compared with family members without T1D/Ab+ (7.6%), but the difference is not statistically significant. Williams and colleagues [32] found similar evidence for shared genetic susceptibility factors but with limited phenotypic overlap between islet and celiac autoimmunity in unaffected first degree relatives of patients with T1D. Overall, our findings suggest that the comorbidity of islet and celiac disease is mediated primarily through a common association with DRB1*0301-DQA1*0501-DQB1*0201, while other HLA haplotypes and the genetic background may have more disease-specific effects.

The HLA haplotypes associated with celiac autoimmunity in this family are similar to those found in other Bedouin families and European or European-derived populations [1,33]. We do not see a difference in the distribution of HLA haplotypes between CD patients and TgAA+ individuals without CD, but we may not have sufficient power to distinguish the two groups. Consistent with other studies, the high-risk DRB1*0301-DQA1*0501-DQB1*0201 haplotype is a primary determinant of celiac disease risk: there is a significantly higher frequency of genotypes with DRB1*0301-DQA1*0501-DQB1*0201 among CD/TgAA+-affected individuals. In fact, all affected family members have DRB1*0301-DQA1*0501-DQB1*0201, which encodes the DQ(α 1*05, β 1*02) molecule in *cis*. There was no evidence for a disease association with any other DRB1-DQA1-DQB1 haplotype, including DRB1*0405-DQA1*0301-DQB1*0302, which, although present in combination with DRB1*0301-DQA1*0501-DQB1*0201 in one affected relative, was in very low frequency in the kindred as a whole (1 of 43 founder haplotypes).

Our data support other studies indicating DQB1*0201/DQB1*0201 homozygotes are at increased risk for celiac autoimmunity. Although the sample size is too small to determine if their higher risk reflects a dosage effect of DQ(α 1*05, β 1*02), the fact that DRB1*03-DQA1*05-DQB1*02/DRB1*07-DQA1*02-DQB1*02 heterozygotes contribute disproportionately to the increased risk supports this hypothesis. In contrast, none of seven relatives with the DRB1*11-DQA1*05-DQB1*03/DRB1*07-DQA1*02-DQB1*02 genotype have developed celiac autoimmunity, which conflicts with consistent evidence favoring an effect of this genotype in which the DQ(α 1*05, β 1*02) molecule is encoded in *trans*. While this genotype is a high-risk genotype in southern European populations [34] and among Ashkenazi

Jews [35,36], it did not appear to be a common high-risk genotype in a study of ten Bedouin families [33], or in Saharawi Arabs [37]. Thus, it is not clear if the apparent absence of an effect of this genotype in the Bedouin kindred reflects our small sample size or population-specific effects as reported in other studies [34].

Several studies have reported the DQB1*0201/DQB1*0201 genotype is associated with celiac disease progression, severity, and/or complications [6,38]. Although we confirmed the diagnosis of CD in all affected relatives by serological testing and in some cases by biopsy, additional clinical data for these relatives are limited. However, it is noteworthy that one of the two CD-affected relatives who developed intestinal lymphoma is homozygous for DQB1*0201 while the other is not (Table 1).

To our knowledge, our whole genome screen of this Bedouin kindred is the first linkage analysis of celiac disease in a non-European-derived population. We do not find any evidence of linkage - even suggestive evidence - at locations corresponding to any previously reported peaks, including the MHC region at 6p21.3. The only suggestive evidence for linkage ($p=0.01$) occurred at 12p12-13 ($p=0.0098$), a region spanning approximately 9.5 megabases with over 100 known or predicted genes. This region has never before been linked with celiac disease, and obviously replication is necessary. The apparent absence of linkage with the MHC may be explained by low marker density, ambiguities in distinguishing DRB1*0301-DQA1*0501-DQB1*0201 haplotypes that are identical-by-descent from HLA-identical haplotypes that are identical-by-state, or both. In particular, we have shown that the linkage results are critically dependent on the identity-by-descent status of two DRB1*0301-DQA1*0501-DQB1*0201 haplotypes that have identical alleles at four microsatellite markers extending 6-7 cM on the centromeric side of DQB1. Due to the difficulty in distinguishing the ancestral relationships of these haplotypes, it is not possible to determine how much of the increased risk for celiac autoimmunity among close relatives is explained by identity-by-descent haplotype sharing for the MHC among affected relatives. However, based on the diversity of haplotypes found in combination with DRB1*0301-DQA1*0501-DQB1*0201 in affected relatives, it is unlikely that autozygosity for this haplotype contributes to the increased risk for celiac autoimmunity in the offspring of consanguineous matings.

Sheffield and colleagues have discussed the power of linkage and association studies in extended Bedouin kindreds in the context of rare disorders that display extensive heterogeneity [39,40]. Bardet-Biedl syndrome is a specific example in which a rare mutation at any one of at least ten different loci causes obesity, learning disabilities, retinopathy, polydactyly and other disease phenotypes. Several of these loci were localized by linkage analyses in individual Bedouin families. Given the extensive locus and allelic heterogeneity, it is unlikely any of these loci would have been identified if the investigators had pooled data from different families to increase sample size. Although it is reasonable to suppose that the genetic homogeneity of extended Bedouin kindreds will have the same advantages in reducing the complexity of multigenic disorders, novel analyses using computer simulations are likely to be required to account for increased complexities of the family structures, examples of which are described herein. Other limitations are the reduced informativeness of genetic markers, reduced power of family-based linkage and association analyses, and difficulties in distinguishing disease-causing mutations from polymorphisms that are unique to the kindred under study [39]. The current and previous studies [23] of a Bedouin kindred with a high prevalence and celiac autoimmunity are the first steps toward determining if the benefit of studies of complex phenotypes in isolated inbred populations will be sufficient to overcome the inherent limitations.

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Figure 1. Summary of autoimmune phenotypes in Bedouin kindred. Note that four of the T1D-affected family members have unknown autoantibody statuses with respect to celiac disease and Addison's disease while 25 T1D-affected relatives are negative for celiac and Addison's autoantibodies.

Figure 2.
Prevalences of common HLA DRB1-DQA1-DQB1 haplotypes by CD phenotype. Prevalences are significantly different (indicated by an asterisk) only for the DRB1*0301-DQA1*0501-DQB1*0201 haplotype ($p < 0.0001$, 2-tailed Fisher 2x3 exact test).

Figure 3.

NPL results for the CD/TgAA+ phenotype (9 affected) in Bedouin kindred. Left y-axis: NPL score; right y-axis: $-\log(p\text{-value})$. Long dashed horizontal lines indicate $p=0.05$ and $p=0.01$. Light gray dashed vertical lines divide the 22 autosomes. The one peak significant at the $p=0.01$ level is at D12S364 at 12p13 with $NPL=2.8688$ and $p=0.0098$ ($-\log(p)=2.009$).

Figure 4.
Comparison of the two DRB1*0301-DQA1*0501-DQB1*0201 haplotypes present in CD/
TgAA+ family members

Table 1
Autoimmune phenotypes and HLA genotypes of 45 affected family members arranged by sibship

ID	Sibship	Celiac	Islet	Adrenal	DRB1-DQA1-DQB1 genotype
E33 ^a	D6xD7	no CD, TgAA unk ^b	T1D	Unk	0402-0301-0302 / ???-???-???
E20	D13xD12		GAA+		0301-0102-0502 / 0701-0201-0201
E6	D14xD12		IAA+		0301-0102-0502 / 0701-0201-0201
E12	D14xD15		GAA+		0301-0501-0201/0301-0102-0502
E22 ^c	D14xD15	TgAA+		Unk	0301-0501-0201 / 0301-0102-0502
E41 ^c	D14xD15	CD	T1D	210H+	0301-0501-0201 / 0301-0501-0201
E42 ^c	D14xD15	TgAA+	T1D		0301-0501-0201 / 0301-0102-0502
E43 ^a	D14xD15	no CD, TgAA unk	T1D	Unk	0301-0501-0201 / 0301-0501-0201
F29, ^{cd}	D14xD15	CD			0301-0501-0201 / 1302-0102-0604
E46	D18xD11		T1D		0301-0501-0201 / 0701-0201-0201
E47	D18xD11		T1D		0301-0501-0201 / 0701-0201-0201
F7	E8xE7		IAA+		0102-0101-0501 / 1302-0102-0604
F11	E10xE9		GAA+		0301-0102-0502 / 0701-0201-0201
F15	E10xE9	CD			0301-0501-0201 / 0701-0201-0201
F16	E10xE9	TgAA+			0301-0501-0201 / 0101-0101-0501
F24	E12xE13	TgAA+			0301-0501-0201 / 0701-0201-0201
F42, ^{cd}	E21xE22	CD			0301-0501-0201 / 0701-0201-0201
F43	E21xE22		T1D		0301-0102-0502 / 0701-0201-0201
F45	E21xE22		T1D		0301-0501-0201 / 1503-0102-0603
F47	E21xE22		GAA+		0301-0102-0502 / 0701-0201-0201
F49 ^c	E21xE22	TgAA+			0301-0501-0201 / 1503-0102-0603
F51	E21xE22		T1D		0301-0501-0201 / 0701-0201-0201
F96	E21xE22		T1D		0301-0501-0201 / 0701-0201-0201
F97	E21xE22		GAA+, ICA512 +		0301-0102-0502 / 0701-0201-0201
F61	E35xE34		T1D		0301-0501-0201 / 0301-0501-0201
F80 ^a	E36xE44	no CD, TgAA unk	T1D	Unk	0301-0501-0201 / 0701-0201-0201
F52	E43xE23		T1D		0301-0501-0201 / 0405-0301-0302
F53	E43xE23		T1D		0301-0501-0201 / 0405-0301-0302
F54 ^a	E43xE23	no CD, TgAA unk	T1D	Unk	0301-0501-0201 / 0405-0301-0302
F58 ^c	E43xE23	CD		210H+	0301-0501-0201 / 0405-0301-0302
F64 ^c	E43xE23	TgAA+	T1D		0301-0501-0201 / 0701-0201-0201
F98	E43xE23		T1D		0301-0501-0201 / 0405-0301-0302
G71	F20xF21		GAA+		0301-0501-0201 / 0101-0101-0501
G72	F20xF21		T1D		0301-0501-0201 / 0701-0201-0201
G121	F24xF113		T1D		0301-0501-0201 / 0701-0201-0201
G7	F29xF28	TgAA+			0301-0501-0201 / 1101-0501-0301
G26	F47xF48	TgAA+			0301-0501-0201 / 0301-0102-0502
G29	F47xF48	CD	IAA+		0301-0501-0201 / 0301-0102-0502
G30	F50xE49		T1D		0301-0501-0201 / 0301-0501-0201
G33	F50xF49	TgAA+			0301-0501-0201 / 0701-0201-0201
G34 ^c	F51xF52	TgAA+	T1D	210H+	0301-0501-0201 / 0301-0501-0201
G53	F51xF52		T1D		0301-0501-0201 / 0701-0201-0201
G59	F51xF52		T1D		0301-0501-0201 / 0701-0201-0201
G85	F54xF55		T1D		0301-0501-0201 / 0301-0501-0201
G43	F61xF60		T1D		0301-0501-0201 / 0701-0201-0201

^aNot included in the analyses. E33 and E43 died before the study was initiated and thus were never sampled. They had T1D and were not known to have CD. HLA haplotypes were inferred from their offspring. F54 and F80 were not successfully typed for autoantibodies, but have T1D and are not known to have CD.

^b Unk = unknown autoantibody status

^c CD/TgAA+ individuals who were included in the whole-genome screen

^d CD patients who died of intestinal lymphoma

Table 2

Cross-tabulation of celiac disease and type 1 diabetes phenotypes. Marginal frequencies are listed.

(a) for phenotypes restricted to disease only			
	T1D	no T1D	
CD	1	5	0.167
no CD	20	149	0.118
	0.048	0.032	
Exact test p-value: 0.541			
Odds ratio and 95% confidence interval: 1.49, (0.03, 14.30)			
(b) for phenotypes restricted to disease or associated autoantibodies			
	T1D/Ab+	Unaffected	
CD/TgAA+	5	11	0.313
Unaffected	25	134	0.157
	0.167	0.076	
Exact test p-value: 0.114			
Odds ratio and 95% confidence interval: 2.44, (0.61, 11.03)			

Table 3
Average kinship coefficients for different pairs of relatives

Phenotype of relative 1	Phenotype of relative 2	Average kinship coefficient
Any	Any	0.070
CD/TgAA+	CD/TgAA+	0.166
T1D/Ab+	T1D/Ab+	0.128
CD/TgAA+	T1D/Ab+	0.144
Unaffected	Unaffected	0.056

Table 4
Simulation results for transmission of key DRB1-DQA1-DQB1 haplotypes and genotypes to CD/TgAA+ family members

DRB1-DQA1-DQB1 haplotype or genotype	N ^a	p-value ^b	Fisher p-value ^c	Odds ratio ^d	95 CI
(a) haplotype results					
0301-0501-0201	16	0.022	0.000002	22.79	5.28, ∞
0701-0201-0201	6	0.412	0.117	0.80	0.25, 2.87
11xx-0501-0301	1	0.344	0.527	0.22	0.005, 1.65
(b) genotype results					
0301-0501-0201/0301-0501-0201	2	0.078	0.301	1.81	0.21, 11.51
0301-0501-0201/0701-0201-0201	6	0.114	0.014	3.82	1.17, 15.15
11xx-0501-0301/0701-0201-0201	0	0.512	0.505	0.00	0.00, 7.24
xxxx-xxxx-0201/xxxx-xxxx-0201	8	0.084	0.010	3.56	1.23, 13.61

^aNumber of CD/TgAA+ affected individuals with this haplotype or genotype.
^bEmpirical p-values obtained from 30,000 simulation replicates. See methods section for details.
^cOne-sided Fisher 2x2 exact test p-value
^dLow-bias indicator of odds ratio in the population

Table 5
Effect of DRB1*0301-DQA1*0501-DQB1*0201 founder haplotypes B and C being identical by descent or identical by state on evidence for linkage

Ancestry of haplotypes B and C	Shared haplotypes among siblings		Chi-square test for linkage	
	0	1	2	χ^2_a p-value ^a
Identical by descent	0 (0.00)	8 (0.89)	1 (0.11)	5.67 0.059
Identical by state	3 (0.33)	5 (0.56)	1 (0.11)	1.00 0.607

^aChi-square test of linkage with expected frequencies of 0.25, 0.50 and 0.25 for sharing 0,1 and 2 haplotypes, respectively. P-values were calculated from chi-square statistics with two degrees of freedom.