Brief Genetics Report

A Genome-Wide Search for Linkage-Disequilibrium With Type 1 Diabetes in a Recent Genetically Isolated Population From the Netherlands

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Type 1 diabetes has a substantial genetic component, with consistent evidence for a susceptibility locus in the HLA-DR/DQ region (chromosome 6p) and the insulin gene region (chromosome 11p). Genome scans have identified >18 other genomic regions that may harbor putative type 1 diabetes genes. However, evidence for most regions varies in different data sets. Given the genetic heterogeneity of type 1 diabetes, studies in homogeneous genetically isolated populations may be more successful in mapping susceptibility loci than in complex outbred populations. We describe a genome-wide search in a recently Dutch isolated population. We identified 43 patients that could be traced back to a common ancestor within 15 generations and performed a genome-wide scan using a combined linkage- and association-based approach. In addition to the HLA locus, evidence for type 1 diabetes loci was observed on chromosome 8q24 (marker D8S1128) and on chromosome 17q24 (marker D17S2059). Both the 8q and 17q localization are supported by allele-sharing at adjacent markers in affected individuals. Statistical evidence for a conserved ancestral haplotype was found for chromosome 8q24. *Diabetes* 51:856–859, 2002

Type 1 diabetes is characterized by an absolute insulin deficiency caused by autoimmune destruction of insulin-producing β-cells in the pancreas. Both genetic and environmental factors appear to be involved (1). Several haplotypes at the HLA class II region on chromosome 6p21 are associated with an increased risk to develop type 1 diabetes (2). Also, >90% of type 1 diabetes patients of European ancestry have at least one copy of the HLA-DR3 or DR4 allele, as compared with 45% in the general population. Almost 40% of type 1 diabetic patients have both alleles, although 3% is expected based on the frequency of the general population (3,4). Numerous studies have found several other genotypes within the HLA region to be associated with type 1 diabetes (referred to as IDDM1). In addition, the insulin gene on chromosome 11p15 (IDDM2) has been implicated in the pathogenesis of type 1 diabetes (5).

The HLA class II region and insulin region, identified using association analysis of candidate genes, account for ~50% of the total familial clustering of type 1 diabetes (6). Several genome-wide linkage studies have identified >18 other genomic regions that may harbor susceptibility loci for type 1 diabetes; however, many findings could not be reproduced in other studies (7,8). Although this may suggest that some of the initial linkage findings have been false-positive results, it may also reflect genetic heterogeneity of type 1 diabetes in diverse ethnic groups.

Mapping disease genes in a genetically isolated population rather than a general population of case subjects or families received considerable attention (9,10). A benefit of this strategy is that complex traits are expected to be more homogeneous in isolated populations because of the small number of founders and genetic drift, increasing the power of linkage analysis. In the isolated population, chances are higher that patients have inherited a disease gene from a common ancestor. Since adjacent markers on a chromosome are often transmitted together, patients from recently (since 5–15 generations) isolated populations with a common ancestor are likely to share considerable stretches of DNA around disease gene(s) (11). Until now, the focus has been on populations of prolonged isolation, such as the Finnish and Icelandic populations (12,13).

We ascertained 46 patients with type 1 diabetes in an isolated village in the southwestern region of the Netherlands. Genealogical information was collected for all 46 patients and revealed that 43 patients (93%) could be traced back to a common ancestor within 15 generations. In Fig. 1, the genealogical lineages of these patients are shown, based on the shortest number of meioses separating them from a common ancestor. This figure represents a simplified example of the genealogical structure of this population. In reality, most patients are related with each other in several ways via multiple common ancestors. To ensure a homogeneous study sample, the analyses in this

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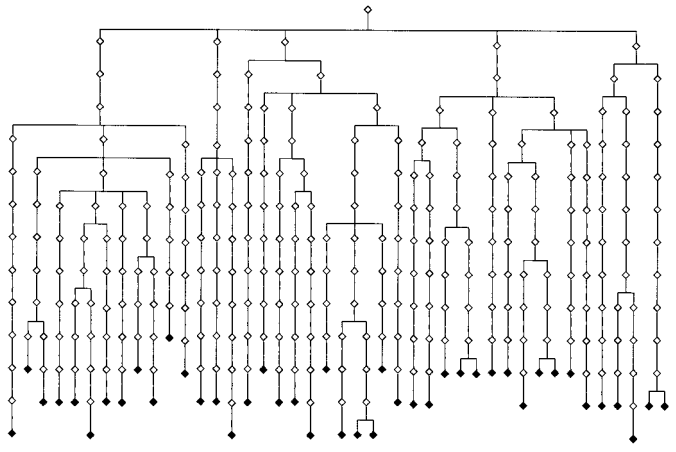

FIG. 1. Genealogical lineages of 43 type 1 diabetic patients that could be traced back to a common ancestor within 15 generations. The figure is based on the shortest number of meioses separating each person from this common ancestor.

study are restricted to these 43 patients who are related within 15 generations.

Characteristics of the patients are given in Table 1. Only 33 patients (73.3%) carried the HLA-DR3 or DR4 allele, and only 8 (17.8%) were heterozygous for the DR3/DR4 allele. These frequencies were lower than expected frequencies of 90% ($P < 0.00006$) and 40% ($P = 0.017$), which are found in most Caucasian populations (3,5), suggesting that other type 1 diabetes genes play a more important role in this population. The lower frequency of the high-risk HLA alleles makes this population very suitable for mapping of new type 1 diabetes genes.

We performed a genome scan using the 43 patients and 86 first-degree relatives. We found evidence for combined linkage and association with three markers: marker D6S1014 in the HLA-region on chromosome 6p ($P = 0.009$, $\lambda = 0.46$), marker D8S1128 on chromosome 8q24 ($P = 0.003$, $\lambda = 0.45$), and marker D17S2059 on chromosome 17q24 ($P = 0.012$, $\lambda = 0.65$). To confirm that the non-HLA regions represent true-positive findings, three additional analyses were performed. First, the risk for type 1 diabetes was determined for the associated alleles of markers D8S1128 and D17S2059 as well as for flanking markers in these regions. We hypothesized that if the initial findings represent true positive findings, flanking markers in these regions should also be associated with increased risk for diabetes. Although the flanking markers in the 8q region did not show a P value <0.016 in the initial genome scan, the ancestral alleles of markers D8S592, D8S1179, D8S1128, and D8S1100 were all associated with an increased risk for type 1 diabetes (Table 2). Also, in the 17q region, allele-sharing at additional markers by affected individuals was observed. The ancestral alleles of the adjacent markers D17S809, D17S1290, D17S2059, and D17S1301 were all associated with a highly increased risk for type 1 diabetes (Table 3). Second, after this observation, a trend analysis was performed for each of these markers. We hypothesized that if the 8q24 and 17q24 localizations represent true susceptibility loci, the risk for diabetes should increase with the number of ancestral alleles present for each marker. For chromosome 8, the odds ratio (OR) for trend reached a maximum of 6.4 at D8S1128 ($P = 0.0005$), whereas the lowest OR was observed for marker D8S592 (OR 1.4, $P = 0.16$) and D8S1100 (OR 1.7, $P = 0.08$), located at ~18 cM on either side of D8S1128 (Table 2). This suggests that a susceptibility gene is probably located around D8S1128. For chromosome 17, a significant trend was observed for all four markers, in particular for D17S2059 (OR 3.1, $P = 0.0005$) (Table 3). Finally, we investigated whether affected individuals showed evidence for an ancestral haplotype surrounding the markers identified in the initial screen. Haplotypes within the 8q region were associated with a highly increased risk for type 1 diabetes, supporting the presence of a true susceptibility locus inherited from a common ancestor (Table 4). In the 17q region, the distribution of two-marker haplotypes was not significantly different between case and control subjects (data not shown).

To study a possible interaction between the 8q or 17q

TABLE 1
Characteristics of 46 type 1 diabetic patients

Male sex	44
Mean age at time of diagnosis (years)	15 (1–30)
Presence of detectable GAD antibodies	39
Presence of detectable IA2 antibodies	24
Presence of both antibodies	15
Frequency of high-risk HLA genotypes	
HLA-DR3 and/or HLA-DR4 allele	73.3
Heterozygous HLA-DR3/DR4	17.8

Data are % or means (range).

TABLE 2
Case-control analysis of flanking markers on chromosome 8q

Marker and genotype	Frequency in control subjects (%)	Frequency in case subjects (%)	Risk (95% CI)	*P*
D8S592 (125 cM, allele 150 bp)				
0	69.7	48.6	1	
1	15.2	40.0	3.8 (1.1–12.6)	0.03
2	15.2	11.4	1.1 (0.3–4.7)	0.91
		Trend	1.4 (0.7–2.8)	0.16
D8S1179 (136 cM, allele 181 bp)				
0	46.9	20.6	1	
1	50.0	58.8	2.7 (0.9–8.1)	0.08
2	3.1	20.6	14.9 (1.5–146.2)	0.02
		Trend	3.3 (1.4–146.2)	0.008
D8S1128 (142 cM, allele 243 bp)				
0	84.4	43.8	1	
1	15.6	46.9	5.8 (1.7–19.2)	0.004
2	0	9.4	—	
		Trend	6.4 (2.0–19.7)	0.0005*
D8S1100 (160 cM, allele 192 bp)				
0	43.8	29.4	1	
1	43.8	47.1	1.6 (0.5–4.7)	0.40
2	12.5	23.5	2.8 (0.7–11.9)	0.16
		Trend	1.7 (0.8–3.3)	0.08

Results of the case-control analysis for markers on chromosome 8q. Results are based on the ancestral allele as identified in the initial screen. **P* value calculated by Fisher's exact test.

TABLE 3
Case-control analysis of flanking markers on chromosome 17q

Marker and genotype	Frequency in control subjects (%)	Frequency in case subjects (%)	Risk (95% CI)	*P*
D17S809 (85 cM, allele 242 bp)				
0	75.0	54.5	1	
1	21.9	30.3	1.9 (0.6–6.0)	0.27
2	3.1	15.2	6.7 (0.7–62.1)	0.09
		Trend	2.2 (1.0–5.1)	0.03
D17S1290 (91, allele 172 bp)				
0	100	83.3	1	
1	0	13.9	—	
2	0	2.8	—	
		Trend	—	0.01*
D17S2059 (106 cM, allele 253 bp)				
0	48.6	13.2	1	
1	37.1	52.6	5.2 (1.5–17.7)	0.008
2	14.3	34.2	8.8 (2.1–37.1)	0.003
		Trend	3.1 (1.5–6.3)	0.0005
D17S1301 (116 cM, allele 154 bp)				
0	64.5	30.3	1	
1	25.8	51.5	4.3 (1.4–13.2)	0.01
2	9.7	18.2	4.0 (0.8–19.4)	0.09
		Trend	2.5 (1.2–5.4)	0.01

Results of the case-control analysis for markers on chromosome 17q. Results are based on the ancestral allele as identified in the initial screen. **P* value calculated by Fisher's exact test. The risk for marker D17S1290 could not be calculated because the ancestral allele was not present in control subjects.

region and the high-risk HLA alleles, a stratified analysis was performed. In this analysis, the distribution of ancestral alleles, as identified in the initial genome scan, was assessed in individuals with or without the HLA-DR3 or DR4 allele. The ancestral alleles of marker D8S1179 ($P = 0.05$) and D8S1128 ($P = 0.0005$) were more frequently observed in patients with the high-risk HLA-DR4 allele than in patients without the HLA-DR4 allele, suggesting an interaction between the susceptibility gene on chromosome 8q24 and the HLA-DR locus. No difference in distribution of the ancestral alleles in the 17q region was observed between the different HLA-subgroups (data not shown).

Both the 8q24 and 17q24 localization are supported by statistical trend analysis and by allele-sharing at additional markers in affected individuals. Very weak evidence of linkage of chromosome 8 to type 1 diabetes, ~5–25 cM centromeric to our location, has been reported previously (8,14). Although linkage to this region could not be replicated in a follow-up analysis by Cucca et al. (15), our study in a homogeneous population supports the initial findings. No evidence for linkage to chromosome 17 has been reported before. Therefore, this locus still remains to be confirmed.

Although it has recently been argued that old genetic isolates will not prove to be more valuable than outbred populations for linkage-disequilibrium mapping of common variants underlying complex disease (16,17), our study in a recently isolated population has yielded promising findings.

RESEARCH DESIGN AND METHODS

Patients with type 1 diabetes were ascertained from a genetically isolated village in the southwest region of the Netherlands. This village was founded by ~150 people in the middle of the 18th century, and up until the last decades, descendants of these founders have lived in social isolation, with minimal immigration (<5%). From the year 1848 on, the population has expanded from 700 up to 20,000 inhabitants. With help from the local health care centers, we were able to recruit 60 individuals diagnosed with type 1 diabetes via local physicians. Medical records covering the last 20 years were available for all patients. The overall participation rate was 77% ($n = 46$). All 46 participants completed a questionnaire on family and medical history and gave blood samples for DNA extraction and detection of autoantibodies. Plasma samples were stored at −70°C within 12 h after venepuncture. GAD and IA2 antibodies were tested by immunoprecipitation as previously described (18). We applied the American Diabetes Association criteria for the diagnosis of diabetes to confirm the diagnosis. A diagnosis of type 1 diabetes was made if diabetes was diagnosed before the age of 30 years and patients were insulin-dependent within 1 year after diagnosis. For each patient, at least two first-degree relatives were asked to give a blood sample to be able to reconstruct haplotypes.

Collection of genealogical information. To determine which of the subjects were descended primarily from the original founders of the isolated village, a genealogical search was completed for each patient, using church and municipal records of births, marriages, and deaths. Genealogical lineages for each patient were traced back 15 generations.

Genotyping. DNA samples were obtained from peripheral white blood cells. The genome screen was performed using 391 markers covering the whole

TABLE 4
Haplotype analysis chromosome 8q

Marker	cM	Allele (bp)	Haplotype	Frequency in control subjects (%)	Frequency in case subjects (%)	Risk (95% CI)	*P*
D8S592	125	150					
			Heterozygous	4.8	15.3	3.5 (0.9–13.4)	0.06
			Homozygous	0	0	—	
					Trend	—	
D8S1179	136	181					
			Heterozygous	1.6	12.1	9.0 (1.1–74.0)	0.04
			Homozygous	0	3.0	—	
					Trend	9.5 (1.2–73.4)	0.004*
D8S1128	142	243					
			Heterozygous	3.2	10.6	3.7 (0.7–18.4)	0.07
			Homozygous	0	1.5	—	
					Trend	4.0 (1.0–18.5)	0.05*
D8S1100	160	192					

Results of the case-control analysis for haplotypes on chromosome 8q. Haplotypes are based on the ancestral alleles as identified in the initial screen. **P* value calculated by Fisher's exact test.

genome, with an average spacing of 10 cM and an average heterozygosity of 0.75 (version 6 of the Weber lab screening set, see http://research.marshfield clinic.org/genetics/sets/combo.html). PCR products were pooled and loaded on an ABI377 automated sequencer, and data were analyzed using ABI GeneScan3.1 and ABI Genotyper2.1 software.

The HLA-DR3 and -DR4 alleles were determined in all participants. DR3 amplification was performed with forward primer (5′-TTGTCCACCCGGC CCCGCT-3′; DR3, Gibco BRL), and reverse primer (5′-CACGTTTCTTG GAGTACTCTACGTCTGTGT-3′; DR3, Gibco BRL). DR4 amplification was performed with forward primer (5′-GTTTCTTGGAGCAGGTTAAAC-3′; DR4, Gibco BRL), and reverse primer (5′-TTCTCGCCGCTGCACTGTGAA-3′; DR4, Gibco BRL).

Statistical analysis. A whole genome scan was performed to test for combined linkage and association of single markers with type 1 diabetes (19). The method used is based on a modification of Terwilliger (20) and assumes that one a priori unknown ancestral allele will be over-represented on chromosomes that carry the disease mutation. The proportion of disease chromosomes with this ancestral allele is represented by the parameter λ. The procedure was originally applied to unrelated individuals, but it can also be extended to deal with pedigree data (19). To this end, we modified the ILINK option of the LINKAGE package, version 5.04. While maximizing the log likelihood over λ, the recombination fraction was fixed at 0.01, and the disease gene frequency was kept constant at 1%, with a penetrance of 40%. Markers with a P value <0.016 were selected for further analysis.

To confirm the initial findings, the risk for type 1 diabetes was estimated for subjects carrying the ancestral allele, using the untransmitted alleles and spouses as control subjects and the type 1 diabetic patients as case subjects. We hypothesized that flanking markers of the initially significant marker should also be associated with an increased risk for diabetes. ORs are presented with 95% CIs as well as P values for trend. Next, ancestral haplotype were formed, and their association with type 1 diabetes was tested.

Finally, to study a possible interaction between the newly associated markers and the high-risk HLA alleles, a stratified analysis was performed. In this analysis, the risk for type 1 diabetes was assessed in individuals with or without the HLA-DR3 or -DR4 allele.

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