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A genetic explanation for the rising incidence of type 1 diabetes, a polygenic disease

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Abstract

We had earlier hypothesized, if parents originated from previously isolated populations that had selected against different critical susceptibility genes for a polygenic disease, their offspring could have a greater risk of that disease than either parent. We therefore studied parents of patients with type 1 diabetes (T1D). We found that parents who transmitted HLA-DR3 to HLA-DR3/DR4 patients had different HLA-A allele frequencies on the *non*-transmitted HLA haplotype than HLA-DR4-transmitters. HLA-DR3-positive parents also had different insulin (*INS*) gene allele frequencies than HLA-DR4-positive parents. Parent pairs of patients had greater self-reported ethnicity disparity than parent pairs in control families. Although there was an excess of HLA-DR3/DR4 heterozygotes among type 1 diabetes patients, there were significantly fewer HLA-DR3/DR4 heterozygous parents of patients than expected. These findings are consistent with HLA-DR and *INS* VNTR alleles marking both disease susceptibility and separate Caucasian parental subpopulations. Our hypothesis thus explains some seemingly disconnected puzzling phenomena, including (1) the rising world-wide incidence of T1D, (2) the excess of HLA-DR3/DR4 heterozygotes among patients, (3) the changing frequency of HLA-DR3/DR4 heterozygotes and of susceptibility alleles in general in patients over the past several decades, and (4) the association of *INS* alleles with specific HLA-DR alleles in patients with T1D.

Keywords: Type 1 diabetes; Rising incidence of type 1 diabetes; Excess heterozygosity

1. Introduction

Type 1 diabetes (T1D) or insulin-dependent diabetes mellitus (IDDM) is a polygenic autoimmune disease with selective destruction of pancreatic β cells and chronic insulin deficiency [1]. The incidence of T1D varies in different countries and populations [2] with Finland and Sardinia having the highest at 30–40 individuals/100,000 per year. In contrast, the incidence is much lower in Japan, 1/100,000 per year, while it is 12–14/100,000 per year in the United States. Surprisingly for a genetic disease, the incidence of T1D is rising at the approximate rate of 3–5% per year [3] in many countries. In the absence of a genetic explanation, some have argued for environmental causes.

The well-established association of T1D with certain HLA genes [4,5] and haplotypes [6], notably DR3 (*HLA-DRB1*0301, DQB1*0201*) and DR4 (*HLA-DRB1*04,*

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*DQB1*0302*) [7–9], was recognized by their increased frequency in Caucasian T1D patients compared with matched controls. The HLA associations reflect a susceptibility gene in the HLA region of chromosome 6 (*IDDM1*), as demonstrated by the higher concordance rate of T1D in HLA-identical siblings of patients (about 15%) than in siblings in general (5–6%) [10]. Because monozygotic twin disease concordance (40–50%), is even higher than that between HLA-identical siblings [10], other unlinked susceptibility genes besides HLA must be involved in T1D pathogenesis. So far, at least 18 putative T1D susceptibility genes have been described [11], the most important of which is the HLA gene. The best documented of the non-HLA susceptibility genes is the insulin gene (*IDDM2*) on chromosome 11 [12]. Moreover, the *CTLA4* gene contributes to T1D risk at the *IDDM12* susceptibility locus [13] and *PTPN22 lyp* [14] is also strongly associated.

Although the mode of inheritance of the MHC T1D susceptibility genes is not completely established, and some authors have suggested mixed recessive-dominant models [15,16], there is also evidence that it is recessive [17–19]. If such is the case and, if the parents of T1D patients are from a homogeneous population, the proportions of homozygotes for HLA-DR3 and for HLA-DR4 as well as HLA-DR3/DR4 heterozygotes among T1D patients should fit the Hardy–Weinberg equilibrium (HWE). This is true for a few populations [20]. However, many Caucasian T1D patient populations show a statistically significant excess of HLA-DR3/DR4 heterozygotes [21,22], indicating that such individuals in the general population are more susceptible to T1D than those who are homozygous for HLA-DR3 or DR4. Increased heterozygosity for other HLA-DR markers has been reported in other, non-Caucasian populations [23,24].

Many attempts have been made to explain the HLA-DR marker heterozygote excess in patients with T1D, including a mixed HLA recessive/dominant genetic model [15,16] and more efficient antigenic peptide presentation by mixed HLA-DQA1/DQB1 heterodimers [25]. That a few Caucasian populations do not show a significant excess of HLA-DR3/DR4 heterozygotes and different populations have excess heterozygotes for different HLA-DR alleles suggest that this phenomenon could be population-related.

T1D seems to confound even the simplest laws of genetics. Before the introduction of insulin therapy about 70 years ago, T1D was life threatening and the genes that caused it were certainly subject to negative selection. Nonetheless, *IDDM1* (and non-HLA) susceptibility alleles are very common in the genetic pool. HLA T1D susceptibility alleles have been estimated to have a general Caucasian population frequency of 37% [26] to 53% [27]. It is unclear how genes associated with a historically lethal disease could have such high frequencies.

Clearly, the introduction of insulin therapy is too recent to explain such a high prevalence of T1D susceptibility genes or the increasing incidence [28] of T1D. The increased life expectancy of patients could increase the prevalence but not the incidence of the disease. At most, insulin therapy could make the reproduction rate of patients equal to that of the general population, certainly not higher. This is illustrated by the unchanged incidences of monogenic diseases such as hemophilia or X-linked agammaglobulinemia despite life-prolonging therapy over many years, allowing patients to survive to reproductive age. Since definitive evidence for changing environmental factors is currently lacking, the increasing incidence of T1D remains unexplained, as does the high frequency of MHC T1D susceptibility genes.

For any disease where susceptibility is determined by more than one gene, susceptibility could be either additive or multiplicative. In the additive model, not all individual susceptibility genes contribute equally and not all are required for disease development. In the multiplicative model, all susceptibility genes are required for disease development and the product of the susceptibility gene frequencies in any population determines the frequency of susceptible individuals in that population. In view of the unexpectedly large number of T1D susceptibility genes already reported for this disease, most genetic models, with a few notable exceptions [26,27,29], seem to have assumed that the genetic basis for this disease is a simple additive model of unlinked susceptibility genes. The hypothesis that we are presenting here is based on the assumption that T1D is a Mendelian multiplicative trait.

We recently proposed that both simple multiplicative Mendelian inheritance and selection against different susceptibility genes could explain the increasing incidence of many polygenic diseases [29]. In multiplicative inheritance, the frequency of disease-susceptible individuals is the product of the frequencies of susceptibles for each susceptibility locus, regardless of the mode of inheritance of susceptibility alleles. According to this hypothesis, in a polygenic disease, over many millennia, natural selection against T1D or genetic drift may have resulted in the reduction in frequency of one or more, but not necessarily all, susceptibility genes to produce protection from disease. Different subpopulations, even within the same geographic area, may have undergone selection against different susceptibility genes. In families with parents from such different subpopulations with reciprocally disparate (contrasting) frequencies of susceptibility genes at different genetic loci, offspring may have an increased incidence of disease due to unlinked susceptibility gene complementation with an increased risk of the polygenic disease because they may have inherited a more complete set of susceptibility genes than that carried by either parent. Thus, in some, *but not all*, instances, parental population admixture may result in increased disease incidence. A mathematical model for this hypothesis is given in Appendix A.

The current study of the parents of patients with T1D was designed to test our hypothesis. We obtained evidence consistent with a T1D parental population that is significantly genetically heterogeneous, explaining a variety of otherwise puzzling and seemingly unconnected phenomena.

2. Materials and methods

2.1. Subjects

Two groups of Caucasian families were analyzed. The first comprised Boston area nuclear families consisting of a young

proband with T1D, both parents and the proband's siblings. Families with a parent with T1D were excluded from analysis (although conclusions were the same when the few families with a patient-parent were included). Families were ascertained as part of an earlier study of the genetics of diabetes at The Center for Blood Research, Boston, MA in collaboration with the Joslin Diabetes Center, Boston, MA. All patients received insulin therapy. Because of the difficulty of obtaining samples from normal control families with young children, the second group consisted of families identified at the Dana-Farber Cancer Institute, Boston, MA. The great majority of probands had leukemia or other hematologic malignancy. None of these diseases has a known genetic basis or is HLA-associated. However, the families were HLA typed to select histocompatible bone marrow donors (included here without regard to success in finding a donor). T1D patients had a mean age of onset of disease of 10.5 years, hematologic disease controls of 9.6 years. All patients or their parents gave informed consent. Experimental protocols were approved by The Center for Blood Research Institutional Review Board.

2.2. HLA typing

Peripheral blood mononuclear cells of T1D patients, their parents and their siblings were analyzed for HLA-A, -B and -DR genotypes according to the 11th International Histocompatibility Workshop [30] and haplotypes were determined by segregation analysis. In all HLA-DR4-positive families, serologic HLA-DR types were confirmed by *HLA-DRB1* allele DNA typing [31]. Because all reported work dealing with HLA-DR heterozygosity in T1D used serological typing, all results here are reported as generic or low-resolution HLA-DR typing.

2.3. IDDM2 typing

The low resolution method of Bain et al. [32] was used to define insulin (*INS; IDDM2*) gene polymorphism at the 3′ end + 1428 *Fok*I site, as previously described [33], in the parents of the T1D patients. DNA was not available for the parents of the transplant families. Alleles at this locus are designated "+" or "−" depending on whether the cleavage site for the restriction enzyme *Fok*I is present or absent, and are in strong linkage disequilibrium with class I and class III *INS* VNTR alleles, respectively. Bands were visualized by ethidium bromide staining following electrophoresis in 4% agarose gels.

2.4. Parental population homogeneity

We used a modification of a test described earlier to examine population stratification between cases and controls in association studies [34]. In the first test used here, the presence of population inhomogeneity among parents of T1D patients was determined by the comparison between the distributions among parents of unlinked non-transmitted genetic markers. For an unlinked genetic marker, we used the *non*-transmitted HLA-A specificity of each of the parents of the T1D patients. The *non*-transmitted HLA-A specificities could not possibly be linked to the HLA specificities transmitted to the patient since they are on different, although homologous, chromosomes. HLA-A was chosen for analysis because its alleles have highly variable frequencies among European populations. The *non*-transmitted HLA haplotypes thus served as population markers for the HLA-DR3-transmitters to HLA-DR3/DR4 patients and were compared with those of the HLA-DR4-transmitters. The concept is shown in Fig. 1. For the control families, we compared the frequencies of HLA-A alleles on *non*-HLA-DR3 haplotypes in parents who carried HLA-DR3 with the frequencies of HLA-A alleles on *non*-HLA-DR4 haplotypes of those who carried HLA-DR4 because there were too few HLA-DR3/DR4 offspring to study HLA-DR3- or DR4-transmitting parents. The second population stratification test was the comparison between the unlinked T1D susceptibility marker *INS* VNTR allele frequencies present in HLA-DR3- and HLA-DR4-positive parents of T1D patients.

2.5. Self-assessment of ethnic homogeneity of parents of patients

At the time of phlebotomy, parents from both the T1D and control groups of families were asked by a clinical coordinator about the national origin (birthplace and/or ethnic background) of their parents and grandparents [34]. Every family presented with this questionnaire responded. Ethnically mixed individuals were defined as those whose parents had different ethnicities or those with at least one parent of mixed national origin/ethnicity.

2.6. Statistical methods

Chi-squared analysis and Fisher's exact test were used for statistical comparisons among the studied groups. Significance was defined as $P \leq 0.05$.

3. Results

To investigate a possible population-specific basis for the rising incidence of T1D, we tested four independent measures of inhomogeneity among the patients' parents.

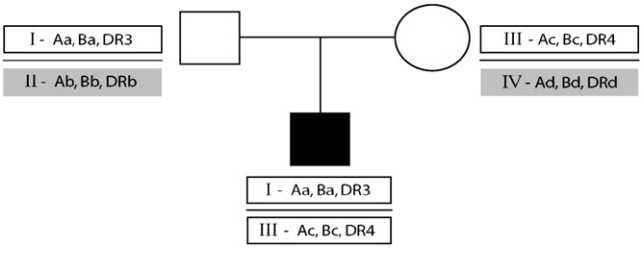

Fig. 1. The non-transmitted stratification test as applied to a family. Haplotypes I and III are transmitted from the parents to the HLA-DR3/DR4 T1D patient. The non-transmitted haplotypes II and IV are non-disease and serve as markers for the genetic backgrounds of the parents. They constitute the normal population of family control haplotypes [6]. In control families, parents who carried HLA-DR3 or DR4 as heterozygotes were analyzed for HLA-A alleles on the other chromosome.

3.1. Frequencies of non-transmitted parental HLA-A specificities

To test for genetic differences between parents who transmitted HLA-DR3 and those who transmitted HLA-DR4 to HLA-DR3/DR4 T1D patients, we compared the frequencies of HLA-A specificities on the non-transmitted (non-disease) HLA haplotypes of the parents in families with unambiguous HLA types. Table 1 summarizes the non-transmitted HLA-A specificities of the parents who transmitted HLA-DR3 or DR4 to their HLA-DR3/DR4 T1D offspring. HLA-A2, a specificity that varies in frequency from 16–40% in different current European populations [35], was found in 5 of the 54 non-transmitted HLA haplotypes (9%) of HLA-DR3-transmitters and 21 of the 54 non-transmitted haplotypes (39%) of HLA-DR4-transmitters (Fisher's exact test, $P < 0.001$). The complete set of non-transmitted HLA-A specificities was significantly different ($P < 0.025$) in the HLA-DR3-transmitters compared with the HLA-DR4-transmitters. In parents of transplant candidate patients, representing a control group with unselected HLA genes from the same geographic area, there was no significant difference in non-DR3- or non-DR4-positive haplotype HLA-A2 (or other HLA-A) frequencies between HLA-DR3- and HLA-DR4-positive parents.

Similarly, HLA-DR3-bearing parents had different HLA-DR specificities ($P < 0.025$) on the opposite haplotypes from those carried by HLA-DR4-bearing parents. Although HLA-B8 was higher in frequency on the opposite haplotypes of DR3-carrying parents ($P < 0.0025$), significance was lost, owing to the very large number of HLA-B specificities, after correction for the number of comparisons. These results are consistent with the parents of patients with T1D not being from a homogeneous population in genetic equilibrium.

Table 1
The non-transmitted HLA-A alleles present in parents of T1D patients compared to parents of control (bone marrow transplant) families

HLA-A alleles	From parents of T1D patients who transmitted		From control parents who are carriers of	
	HLA-DR3 to DR3/DR4 patients	HLA-DR4 to DR3/DR4 patients	HLA-DR3	HLA-DR4
A1	15	7	19	20
A2	5[a]	21[b] ($P < 0.001$[c])	31	32
A3	4	5	14	16
A11	0	2	2	8
A23	2	0	2	2
A24	5	4	8	18
A25	1	1	1	1
A26	8	3	2	5
A28	2	1	4	10
A29	2	0	2	2
A30	2	2	2	2
A31	2	3	4	6
A32	5	1	3	3
A33	1	4	1	1
Total	54	54 ($P < 0.025$[d])	95	126

[a] All transmitted from the mother.
[b] 15 transmitted from the father.
[c] P value for HLA-A2 specificity only, with 0 degrees of freedom.
[d] P value for all HLA-A specificities as a set corrected for 13 degrees of freedom.

3.2. Association of parental HLA-DR and INS alleles

The *HLA* and *INS* loci are located on different chromosomes, 6p21 and 11p15, respectively. In a homogeneous population in genetic equilibrium, there should be no association between *HLA-DR* alleles and *INS* alleles in the parents of T1D patients. In contrast, this may or may not be the case in a heterogeneous or mixed population. To test this assumption, we analyzed 98 families with a T1D patient for alleles at the *INS* and *HLA-DR* loci as genetic population markers. We compared the frequencies of *INS* class I VNTR alleles in HLA-DR3-positive parents of patients with T1D with those in HLA-DR4-positive parents. There were only two parents who were class III homozygous; the other class III carriers were heterozygotes (I/III). In our dataset, 18/33 or 55% of the parents carrying HLA-DR3 carried the *INS* class I VNTR homozygous genotype, compared to 31/40 or 78% of parents who were positive for HLA-DR4 ($P < 0.05$, Table 2). This result is consistent with the parents of patients with T1D not being from a homogeneous population in genetic equilibrium.

3.3. Ethnic admixture in T1D and control families

We attempted to analyze the extent of national or ethnic admixture of families with a T1D patient compared to that of families with a patient tested for bone marrow transplantation. The latter families represented control, i.e., non-HLA-associated disease, families identified in the same geographic area. Patients or parents were considered to be of mixed ethnicity if their parents differed in national origin, or if one of them was of mixed national origin, e.g., British–French or Italian–Spanish. Table 3 presents the results of this analysis. Parents of T1D patients were more ethnically mixed (54%) than parents of patients studied for bone marrow transplantation (27%, $P < 0.001$). These data also show that the increase in ethnicity mixing for both kinds of Boston families was comparable at 25–30% per generation.

Table 2
Association between *IDDM1* and *IDDM2* alleles in T1D family parents who are HLA-DR3-positive, HLA-DR4-negative and parents who are HLA-DR4-positive, HLA-DR3-negative[a]

IDDM2 Genotype	DR3 positive DR4 negative	DR3 negative DR4 positive	DR3 negative DR4 negative
VNTR Class I/I	18 (55%)	31 (78%)	16 (76%)
VNTR Class I/III	15 (45%)	9 (22%)	5 (24%)
Total	33	40	21

[a] 18/33 (55%) of the parents positive for HLA-DR3 also carried the *INS* class I VNTR homozygous genotype, compared to 31/40 (78%) of parents who were positive for HLA-DR4 ($P < 0.05$).

Table 3
Comparison of ethnic admixture in T1D families with that in bone marrow transplant families[a]

	Total no. of parents	Ethnically mixed parents	% Mixed parents	Total no. of patients	Ethnically mixed patients	% Mixed patients
From T1D families	239	129	54%	141	105	74%
From transplant families	128	35	27%	67	37	55%

[a] Parents of T1D patients were more ethnically mixed than parents of patients studied for bone marrow transplantation (54% vs. 27%, $P < 0.001$).

3.4. HLA-DR homozygotes and heterozygotes among parents of T1D patients

Although it is known that in many populations of patients with T1D, HLA-DR genotypes do not fit the HWE, it is not known whether this is also the case for their parents. As shown in Table 4, the distribution of homozygotes and heterozygotes for HLA-DR types in parents of patients with T1D did not fit the HWE. There was a significant increase in HLA-DR4/DRX parents ($P < 0.005$) and a significant *paucity* of HLA-DR3/DR4 heterozygous ($P < 0.01$) and HLA-DRX/DRX (either homozygous or heterozygous) parents ($P < 0.02$). A similar analysis in patients and parents in control families showed no significant deviations from the HWE. This result is consistent with the parents of patients with T1D not being from a homogeneous population in genetic equilibrium.

Table 4
Hardy–Weinberg equilibrium for HLA-DR in T1D and bone marrow transplant control families[a]

	T1D index patients	T1D unaffected parents	Bone marrow transplant parents
DR3 frequency	0.351	0.230	0.170
DR4 frequency	0.346	0.267	0.170
DRX frequency	0.303	0.503	0.660
No. of subjects	198	335	361
Expected DR3/DR3	24.4	17.7	10.4
Observed DR3/DR3	21	19	9
P value	n.s.	n.s.	n.s.
Expected DR4/DR4	23.7	23.9	10.4
Observed DR4/DR4	16	18	7
P value	n.s.	n.s.	n.s.
Expected DRX/DRX	18.2	84.8	157.3
Observed DRX/DRX	22	63	163
P value	n.s.	<0.02	n.s.
Expected DR3/DRX	42.1	77.5	81
Observed DR3/DRX	34	92	76
P value	n.s.	n.s.	n.s.
Expected DR4/DRX	41.5	90	81
Observed DR4/DRX	42	119	77
P value	n.s.	<0.005	n.s.
Expected DR3/DR4	48.1	41.1	20.8
Observed DR3/DR4	63	24	29
P value	<0.05[b]	<0.01	n.s.

n.s., not significant.
[a] HLA-DRX is non-HLA-DR3 and non-HLA-DR4.
[b] *P* values represent significant deviations from HWE expectations.

4. Discussion

Of the many explanations for the HLA-DR3/DR4 excess among some populations of patients with T1D, population differences between parents with gene complementation in their descendants have not been previously considered. Yet excess heterozygosity of different population markers is a cardinal feature of population admixture [36,37].

The HLA-DR genotype frequencies of neither the Boston Caucasian T1D patients nor their parents fit the HWE. The patients had an excess of HLA-DR3/DR4 heterozygotes. In contrast, there was a *paucity* of these heterozygotes in their parents. Neither of these distributions would fit either a recessive or a dominant model if the parent population were ethnically homogeneous. Dominant inheritance of an MHC susceptibility gene would predict even more HLA-DR3/DRX and HLA-DR4/DRX patients and even fewer HLA-DR3/DR4 patients than would recessive inheritance. If one assumes a genetically homogeneous population, *no* genetic model fits the HLA-DR3, DR4 distribution in many Caucasian T1D patient populations.

In the present study, all the signs and consequences of parents of T1D patients deriving from different previously isolated populations were found. Supporting this concept (in addition to deviation from the HWE) was the significantly higher frequency of HLA-A2 on the non-transmitted (non-diabetic) chromosome in the HLA-DR4-transmitting parents compared with the HLA-DR3-transmitting parents as well as the greater self-reported ethnic disparity between the parents of T1D patients compared with that in control families. Finally, the results of *INS* VNTR typing in the parents supported these relationships. Others have observed that the *INS* VNTR class I alleles are transmitted preferentially to HLA-DR4-positive patients from heterozygous parents [38]. Our observation of the co-occurrence of the two markers in parents confirms and extends this finding and our hypothesis provides an alternative explanation.

The present evidence supports the concept that HLA-DR3 and HLA-DR4 broadly mark two subpopulations among Boston Caucasian parents of T1D patients (i.e., HLA-DR3, low HLA-A2, and *INS* VNTR III being common in one subpopulation and HLA-DR4, high HLA-A2, and *INS* VNTR I in the other). Perhaps pertinent are the much higher HLA-DR4 and HLA-A2 frequencies in the Cornish (Celtic) population of southwest England compared with the British (primarily

Anglo-Saxon) population [35]. There appears to be an unusually high prevalence of T1D where these two subpopulations met near Plymouth, England [39]. It is of interest that the HLA-A2 frequency of 0.09 in the Boston HLA-DR3-transmitting parents is lower than that in any reported currently existing European population [35].

The determination of national origin in this study suggests that parents of T1D patients exhibit about twice the ethnic heterogeneity (54%) of control parents (27%). Although the assessment of national origin was based on a simple questionnaire that can only provide a rough estimate of ethnic heterogeneity, the responses from both groups of families showed the same per generation increase in admixture of 25–30%. This result supports the overall validity of this approach. Since the study and control populations showed different levels of heterogeneity but the same generational increase in mixing, this is clearly a general modern phenomenon.

The genes for sickle cell anemia, Tay–Sachs disease and β thalassemia are examples of population-specific genetic markers for susceptibility. In analogy, HLA-DR3 and DR4 (and *INS* class I) mark T1D susceptibility and, at the same time, are markers for previously isolated different populations which, when mixed, result in a higher incidence of T1D because HLA-DR3/DR4 heterozygotes are at greater risk for disease than either HLA-DR3/DR3 or DR4/DR4 homozygotes.

The fact that the incidence of T1D is not highest in countries or regions with a high degree of ethnic admixture, such as the United States or South America, rather than supposedly homogeneous Finland or Sardinia, does not contradict our thesis. It is important to note that it is not the degree of admixture but the contrasting frequencies of susceptibility alleles at different susceptibility loci in the parental populations that determine the rising disease incidence in the offspring. Moreover, recent studies have revealed that Finland is not as ethnically homogeneous [40] as earlier believed. It is clear from Y chromosome markers [41] that the Finnish population derives from at least two distinct ethnic groups. There is a northeastern to southwestern gradient of Y haplotype frequencies. Moreover, the incidence of T1D varies widely from 4 to 245 per 100,000 among municipalities in Finland and is maximal in the middle of the country [42] (perhaps where the two Y-chromosome-marked populations meet). Additional evidence for population heterogeneity is the difference in frequency of the *HLA-DQB1*0302* allele in T1D patients in Turku (southwestern Finland) compared with Oulu (western mid-Finland) [43]. There also were highly significant differences in the positivity rates of *HLA-DQB1*0302*, **0301* and **02*, in newborns in the two cities. Thus, Finland, although previously regarded as highly homogeneous, is not, and Y chromosome and HLA allele distribution in different parts of the country is consistent with the admixture hypothesis.

Similarly, Sardinia has had a long history of invasion by various ethnic groups as reflected in striking differences in the frequency of mitochondrial DNA (mtDNA) polymorphisms [44,45]. Sardinia, like Finland, appears to have several subpopulations. In another telling example, the highest incidence of T1D in the United States is in Hawaii in the descendants of mixed Polynesian parents [46].

The data presented here represent the results from one geographic area (Boston). In other areas, other alleles at other loci may be the genetic markers of admixture. Documentation of the rising incidence of T1D is largely lacking for Boston and most of the Americas. However, where it has been properly examined in the United States (in Allegheny County, Pennsylvania and in Colorado; www.barbaradaviscenter.org), the incidence is clearly rising. What we have shown for Boston are the same features produced by genetic stratification as we predict for populations where the rising incidence is well documented.

The mechanism proposed here to explain the rising incidence of T1D may be operative in other polygenic diseases. Gluten-sensitive enteropathy is an autoimmune disease that is rising in frequency [47]. Susceptibility HLA markers are HLA-DR3 and HLA-DR7 and excesses of HLA-DR3/DR7 and HLA-DR5/DR7 heterozygotes have been reported in some populations [48,49]. It may be that other diseases, such as autism or asthma, of unknown but possibly polygenic nature, are rising in frequency by a mechanism such as that postulated here.

This model also explains the recent unexpected finding that, over the past few decades, the relative frequency of HLA-DR3/DR4 heterozygotes among T1D patients has been falling [50,51]. This is because all the hallmarks of population admixture, including the association of specific HLA alleles with each other and with unlinked genetic markers as well as the lack of fit with the HWE in T1D patients and their parents, will disappear in a few generations as genetic equilibrium is reached. The model also predicts that the rising incidence in T1D will plateau as the offspring of the mixed populations approach genetic equilibrium. The leveling off in the incidence will occur earlier in those populations in which the increasing incidence of disease began earlier since they are the most advanced in the m ixing process.

Yet another prior observation explained by our hypothesis is the excess of HLA-DQ2 or DQ8 alleles on the non-transmitted chromosomes of both mothers and fathers, more pronounced in mothers [52]. Although the authors interpreted this as suggesting a maternal diabetogenic gestational effect on the fetus or imprinting, our explanation that this reflects both maternal and paternal population differences is far more likely (particularly since it includes fathers).

Thus, a single model provides explanations for a number of different, seemingly unconnected reported phenomena that made the genetics of T1D difficult to explain. Consistent with these concepts involving population genetic differences between parents of patients with T1D as the possible basis for the rise in polygenic disease incidence is the much higher incidence of systemic lupus erythematosus in African-Americans than in either Africans or Caucasian-Americans [53,54], in Mestizos compared with the general Mexican population [55], and the fact that NZB and NZW mice do not have systemic lupus erythematosus, but NZB X NZW F1 hybrids do [56].

More definitive evidence for our hypothesis could be obtained from the analysis of highly polymorphic mitochondrial and Y-chromosome genetic population markers, particularly in countries with rapidly rising incidence. Such studies are in progress.

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Appendix A

A mathematical proof for our hypothesis is possible by considering a hypothetical example of a polygenic disease, where, for simplicity, two unlinked genes, *A* and *B*, determine susceptibility. Let us assume that there are two non-interbreeding populations, I and II, where the locus-specific frequencies of susceptible persons in I are a_1 and b_1 and in II are a_2 and b_2. If the expression of *A* or *B* is dominant, at least one copy of each susceptibility gene will render the person susceptible. If it is recessive, two copies are required. Assume that these populations have reciprocally different susceptible person frequencies, so that a_1 is greater than a_2 and b_2 is greater than b_1. The offspring of families with parents where one belongs to population I, and the other to population II, will have a higher incidence of disease than the average incidence of both parental populations. The average incidence of disease in the parental populations is equal to one half the sum of the products of their respective susceptible person frequencies or

$$\frac{a_1 b_1}{2} + \frac{a_2 b_2}{2}.$$

The incidence of disease in the subsequent offspring where one parent is from population I and the other from population II is

$$\frac{a_1 + a_2}{2} \times \frac{b_1 + b_2}{2}.$$

The difference in disease incidence between the parental populations and their mixed offspring, defined here as Δ, will be the difference in incidence in the offspring population minus that in the average of the parental populations:

$$\Delta = \left(\frac{a_1 + a_2}{2} \times \frac{b_1 + b_2}{2}\right) - \left(\frac{a_1 b_1}{2} + \frac{a_2 b_2}{2}\right)$$

$$\Delta = \frac{a_1 b_1}{4} + \frac{a_1 b_2}{4} + \frac{a_2 b_1}{4} + \frac{a_2 b_2}{4} - \frac{a_1 b_1}{2} - \frac{a_2 b_2}{2}$$

$$\Delta = \frac{a_1 b_2}{4} + \frac{a_2 b_1}{4} - \frac{a_1 b_1}{4} - \frac{a_2 b_2}{4}$$

$$\Delta = \left(\frac{a_1 - a_2}{2}\right) \times \left(\frac{b_2 - b_1}{2}\right).$$

So, if $a_1 > a_2$ and $b_2 > b_1$, then Δ is positive. If the inequalities were reversed so that $a_2 > a_1$ and $b_1 > b_2$, then Δ is also positive. Therefore, if the frequencies of susceptibility genes at the two susceptibility loci in different populations are reciprocally different (contrasting), then the offspring of the mixed parental populations will inherit a higher rate of disease susceptibility than either parental average.

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