Type 1 Diabetes in the BB Rat: A Polygenic Disease

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OBJECTIVE—Two type 1 diabetes susceptibility genes have been identified in the spontaneously diabetic biobreeding diabetes-prone (BBDP) rat, the major histocompatibility complex (MHC) (*RT1*) class II *u* haplotype (Iddm1) and *Gimap5* (Iddm2). The strong effects of these have impeded previous efforts to map additional loci. We tested the hypothesis that type 1 diabetes is a polygenic disease in the BBDP rat.

RESEARCH DESIGN AND METHODS—We performed the most comprehensive genome-wide linkage analysis for type 1 diabetes, age of disease onset (AOO), and insulitis subphenotypes in 574 F2 animals from a cross-intercross between BBDP and type 1 diabetes–resistant, double congenic ACI.BBDP-*RT1u,Gimap5* (ACI.BB$^{1u.lyp}$) rats, where both Iddm1 and Iddm2 were fixed as BBDP.

RESULTS—A total of 19% of these F2 animals developed type 1 diabetes, and eight type 1 diabetes susceptibility loci were mapped, six showing significant linkage (chromosomes 1, 3, 6 [two loci], 12, and 14) and two (chromosomes 2 and 17) suggestive linkage. The chromosomes 6, 12, and 14 intervals were also linked to the severity of islet infiltration by immunocytes, while those on chromosomes 1, 6 (two loci), 14, 17, and a type 1 diabetes–unlinked chromosome 8 interval showed significant linkage to the degree of islet atrophy. Four loci exhibited suggestive linkage to AOO on chromosomes 2 (two loci), 7, and 18 but were unlinked to type 1 diabetes. *INS*, *PTPN22*, *IL2/IL21*, *C1QTNF6*, and *C12orf30*, associated with human type 1 diabetes, are contained within the chromosomes 1, 2, 7, and 12 loci.

CONCLUSIONS—This study demonstrates that the BBDP diabetic syndrome is a complex, polygenic disease that may share additional susceptibility genes besides MHC class II with human type 1 diabetes. ***Diabetes*** **58:1007–1017, 2009**

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The biobreeding diabetes-prone (BBDP) rat spontaneously develops type 1 diabetes with high incidence around puberty through a T-cell–mediated autoimmune destruction of pancreatic β-cells (1). Two genes that contribute to disease pathogenesis in this animal have been identified, the *RT1u* allele (Iddm1) and the GTPase immunity-associated protein family member 5 (*Gimap5*, Iddm2) (2–4). Genetic variation in *Gimap5* has been recently associated with humoral anti-pancreatic autoimmunity in human type 1 diabetes as well as with human systemic lupus erythematosus (5,6). Crosses between BBDP rats and type 1 diabetes–resistant strains have shown that homozygosity for the BBDP *Gimap5* allele and at least one BBDP allele of Iddm1 are required for type 1 diabetes development (4,7). These requirements have impeded previous efforts to map additional Iddm loci. However, in an attempt to circumvent these difficulties, two experimental approaches have been used.

First, linkage analyses have been performed in progeny derived from backcrosses (F1N2 or F2N3) between BBDP and type 1 diabetes–resistant strains, specifically designed to enrich for BBDP homozygosity at both Iddm1 and Iddm2. This approach led to the identification of Iddm3 (chromosome 2) in one study, while in others more than one locus was mapped, but they met only suggestive genome-wide statistical criteria (8–12). However, the design of these crosses had two disadvantages. They could not be used to investigate the effects of homozygosity for non-BBDP alleles since they were backcrosses to BBDP. Furthermore, as Iddm1 and Iddm2 were selected to be homozygous, loci in close proximity to them could be missed, as was likely the case for a locus close to Iddm2 (8,13–15).

The second approach examined the genetics of experimentally induced (as opposed to spontaneous) diabetic syndromes following the demonstration that many rat strains carrying the BBDP allele at Iddm1 were highly susceptible to these syndromes (16). This approach had the advantage of eliminating the requirement for BBDP homozygosity at Iddm2, thus increasing the number of informative progeny in crosses. However, only one locus (other than Iddm1) identified by this approach, Iddm14, has been shown to also control spontaneous type 1 diabetes (15). The results of these two approaches strongly suggested that contrary to spontaneous diabetes in humans and nonobese diabetic (NOD) mice, which are both under the control of multiple genes (17–19), type 1 diabetes in the BBDP rat was oligogenic with one or two susceptibility genes other than Iddm1 and 2 depending on the resistant strain used (8,9,12,13).

To determine whether this is the case, we have taken a different approach that overcomes some of the limitations encountered in previous studies. First, we introgressed

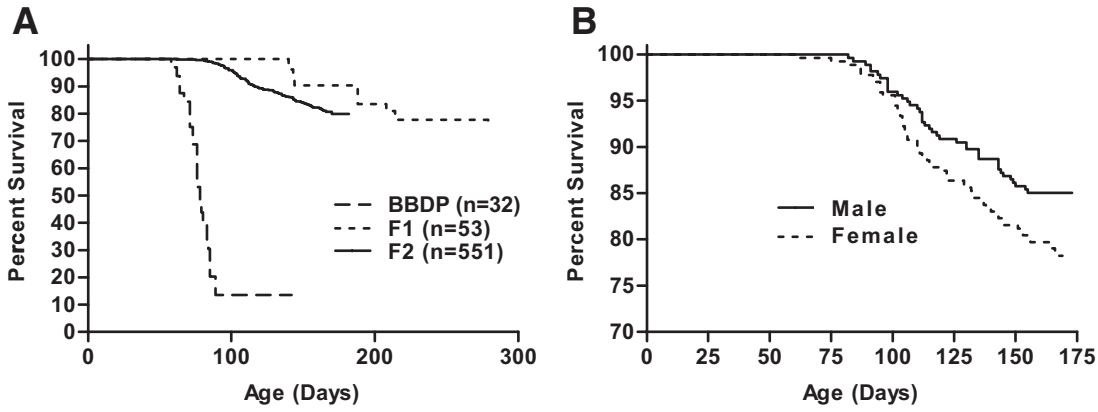


FIG. 1. Analysis of type 1 diabetes–free survival in BBDP, F1 and F2 animals (*A*) and per gender in the F2 cohort (*B*). The percent survival of each cohort is labeled on the vertical axis, and the number of days to onset of diabetes is labeled on the horizontal axis. *A*: Both the F1 and F2 cohorts have significantly increased type 1 diabetes–free survival rate compared with BBDP rats ($P < 0.0001$), whereas the F1 rate is not significantly different from that of the F2 cohort (Kaplan-Meier analysis log-rank statistic). *B*: Females have a lower ($P = 0.048$) type 1 diabetes–free survival rate compared with males.

BBDP-derived Iddm1 and Iddm2 onto the genetic background of the diabetes-resistant August Copenhagen Irish (ACI) inbred strain. The resulting double congenic ACI.BBDP-*RT1*^{u},*Gimap5* (ACI.BB$^{1u.lyp}$) inbred strain exhibits complete resistance to both spontaneous type 1 diabetes and islet inflammation (20), establishing the requirement for at least one other susceptibility locus and allowing us to test the hypothesis that the BBDP diabetic syndrome is also under complex, polygenic control. Specifically, this congenic line allowed us to fix both Iddm1 and Iddm2 loci as BBDP in a cross/intercross with the BBDP strain. Here, using this F2 cohort, we report the results of the most comprehensive genome-wide linkage analysis performed in the BBDP rat.

RESEARCH DESIGN AND METHODS

The genetic analysis consisted of a three-step approach. In the first step, diabetic animals were genotyped across the genome using 229 microsatellite markers at an average of one marker every 12.5 Mb (maximal distance between two markers was 28.7 Mb). Information on the primers and their physical location was obtained from the Rat Genome Database (available at http://rgd.mcw.edu/) and is listed in supplementary Table 1 (available at http://dx.doi.org/10.2337/db08-1215). This was followed by segregation analysis of type 1 diabetes based on the comparison of the observed to the expected genotype distribution at each marker. In the second step, at each peak marker linked to diabetes with a log10 likelihood ratio (logarithm of odds [LOD]) score >1 in the first step, we genotyped all nondiabetic animals. Single-marker analysis was then performed to check that the linkage was type 1 diabetes specific as opposed to the consequence of an overall bias in Mendelian inheritance. In the third step, markers flanking those peaks linked to type 1 diabetes with a LOD >1.5 in step 2 were also genotyped in all nondiabetic subjects to refine each peak through interval mapping analysis (21). Further refinement of linkage peaks was obtained through genotyping of the whole F2 cohort with additional and newly designed markers.

Before linkage analysis, R/QTL (22) was used to identify genotyping errors and to check the marker order by re-estimating the genetic map for each order through calculating the LOD scores relative to the initial order. For QTL mapping, single-marker and QTL × sex interaction analyses were performed using R/QTL with a binary model used for type 1 diabetes and a normal model for other traits. Segregation analysis, interval mapping (21), and composite interval mapping were conducted using Windows QTL Cartographer, version 2.5 (23). For linkage analysis, we calculated LOD scores in both R/QTL and QTL Cartographer using a full model, allowing for both additive and dominance effects. LOD thresholds were determined by permutation testing (n = 1,000 permutations) (24). Suggestive loci were defined as those that exceeded the 37th percentile ($P < 0.63$) of permutation distribution, while significant loci exceeded the 95th percentile ($P < 0.05$). Power calculations showed that for type 1 diabetes there was good power (1-β = 0.8) to detect a relative risk of 2.0 with $\alpha = 1 \times 10^{-4}$, while for quantitative traits we could detect a locus that contributes to 4% of the trait variance at $\alpha = 1 \times 10^{-4}$. The loci identified through the above analyses were designated Iddm25 to Iddm36 by the Rat Genome Database and have been added to the list of previously mapped Iddm loci at http://rgd.mcw.edu/, while details about these 12 loci are available at http://www.t1dbase.org/page/welcome/display/?species = rat. Details of the animals used and phenotypic analysis of the pancreas are provided in the online appendix.

RESULTS

Of 470 F2 animals that remained negative for glycosuria until 165 days, 23 were found postmortem to have glycemia >16.7 mmol/l hence within the diabetic range on the day of killing (25). However, they were excluded from our analysis of cumulative incidence of type 1 diabetes and age of onset (AOO) since we did not have glycemic levels on 2 consecutive days for them. Their pancreata were nevertheless included in the linkage analysis for insulitis. Of 551 F2 animals included for cumulative incidence of type 1 diabetes, 18.9% (n = 104) became diabetic with an AOO of (means ± SD) 120 ± 24 days compared with 84% at 76 ± 8 days in BBDP rats and 20.8% at 180 ± 44 days in the F1 generation, respectively (Fig. 1*A*). There was a small, but significantly higher, cumulative incidence of type 1 diabetes ($P = 0.048$) in female F2 animals compared with males (Fig. 1*B*). This led us to perform linkage analyses for type 1 diabetes, insulitis, and AOO separately in male and female F2 animals. Based on permutation analyses using R/QTL, significant sex difference in linkage (LOD threshold for QTL × sex interaction at 5% level >2.1) was found only for type 1 diabetes at the telomeric locus of chromosome 6 (LOD = 2.3). However, at this locus, although female heterozygotes and ACI homozygotes had higher type 1 diabetes incidence than males, BB homozygote males had a significantly higher incidence than BB females.

Linkage analysis of type 1 diabetes. Through genome-wide segregation analysis performed in diabetic animals only, we identified six loci significantly linked to type 1 diabetes on chromosomes 1, 3, 6 (two loci), 12, and 14 and another suggestive linkage peak on chromosome 2 (Fig. 2). Four of these regions are novel, on chromosomes 3, 6 (centromeric), 12, and 14, while three others overlap with regions previously identified using alternative type 1 diabetes–resistant strains (8,9). When the genetic analysis was extended to the whole F2 cohort, these peaks were all confirmed and an additional chromosome 17 locus reaching suggestive linkage was found. Of note, some regions of

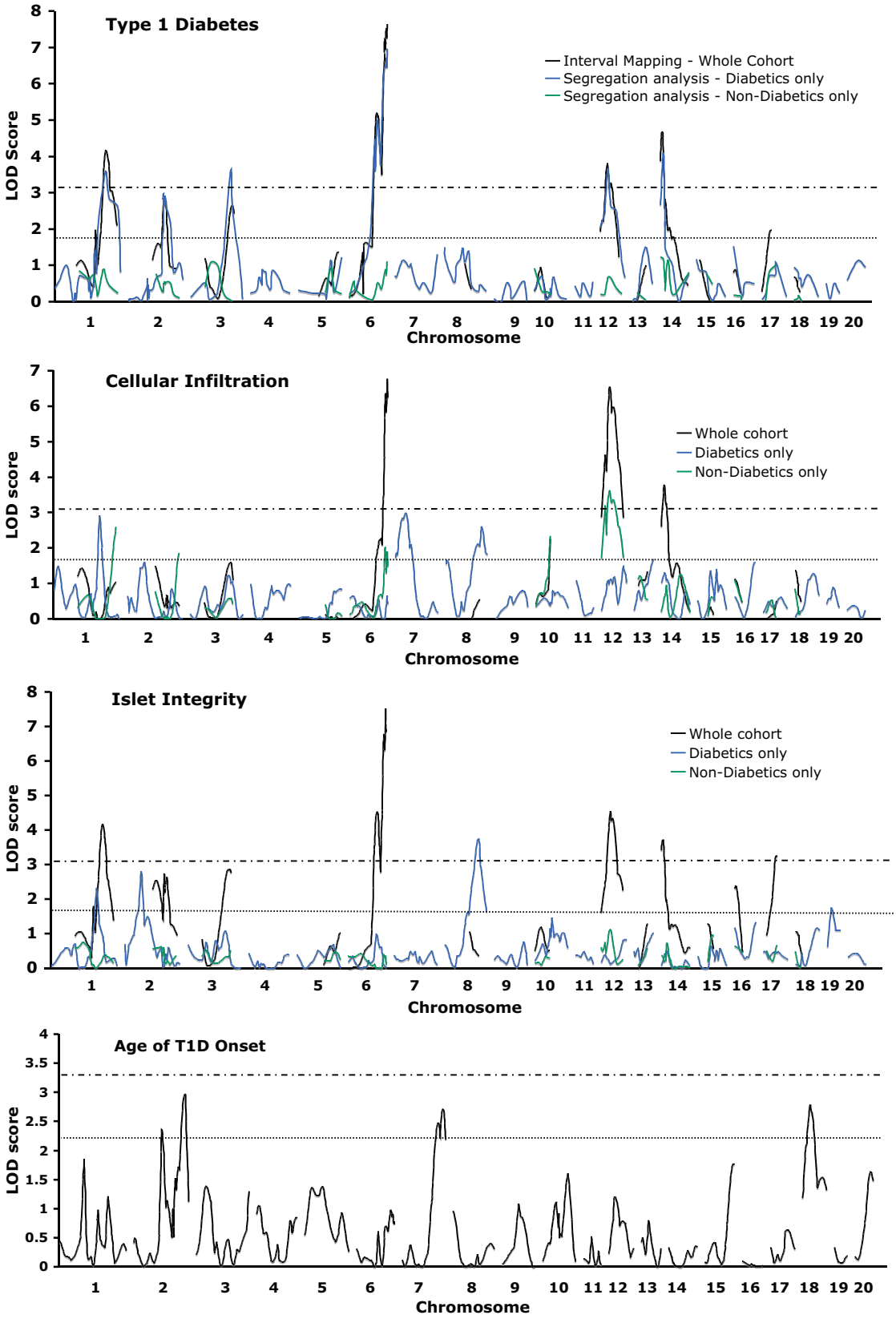


FIG. 2. Mapping of type 1 diabetes and type 1 diabetes subphenotypes in the F2 cohort along chromosomes 1–20. The Y-axis indicates the LOD score and the X-axis the chromosome number and genetic position. Horizontal lines represent the thresholds for suggestive (dotted line) and significant (dashed dotted line) linkage. From the top to bottom are shown the results of linkage analyses for type 1 diabetes, severity of islet infiltration by inflammatory cells, degree of islet integrity, and age of type 1 diabetes onset. Unless otherwise stated in the figure, all linkage analyses were based on interval mapping.

linkage identified at intermediate stages of our study were lost when the whole cohort was genotyped.

BBDP type 1 diabetes susceptibility loci. At five of the eight type 1 diabetes loci (chromosomes 2, 14, 17, and both on 6), the BBDP allele conferred increased susceptibility to the disease (Table 1). The peak of linkage (LOD = 2.90; Iddm26) on chromosome 2 is located at marker D2Arb16 (Table 1), and the 1-LOD interval spans 16 cM (supplementary Table 1 and supplementary Fig. 3*A*). The incidence of type 1 diabetes in F2 animals that were BBDP homozygous and heterozygous at the peak marker was 24 and 22%, respectively, compared with 8% in ACI homozygous animals (Table 1). This locus overlaps with Iddm3, previously identified in a cross between DRlyp/lyp and F344 (8). Interestingly, the peak marker D2Arb16 is only 0.74 Mb away from *Ptpn22* (199.05 Mb), a gene strongly associated with both type 1 diabetes and other autoimmune diseases in humans (26–30) (Table 2).

Two type 1 diabetes loci were located close to each other toward the telomere of chromosome 6 (Table 1, supplementary Table 1, and supplementary Fig. 3*B*). The more proximal locus (LOD = 5.21; Iddm28) had a 1-LOD interval of 20 cM between markers D6Rat23 and D6Got169. The more distal locus is the most significant linkage in this cross (LOD = 7.66; Iddm29), with a 1-LOD interval spanning 10 cM between markers D6Mgh3 and D6Got167. These two loci account for 4.5 and 6.3%, respectively, of the trait variance in this cross. The distal locus overlaps with Iddm8, previously identified in a cross-backcross between the BB/OK and SHR/Mol strains, subsequently confirmed through the development of congenic strains (9). Using composite interval mapping, we confirmed that these two chromosome 6 QTLs are distinct and we better defined their peak location 30 cM apart (supplementary Fig. 4).

Two other susceptibility loci on chromosomes 14 and 17 are also novel (supplementary Table 1 and supplementary Figs. 3*C* and *D*). The former (LOD = 4.7; Iddm31) has a 1-LOD interval spanning 12 cM between markers D14Rat1 and D14Mit6; however, this is possibly an underestimation of the true locus size since the peak is at the most centromeric marker along this chromosome. The latter (LOD = 1.98; Iddm36) has a 1-LOD interval of 30 cM.

BBDP type 1 diabetes resistance loci. There are three loci where the BBDP allele confers resistance (or the ACI allele confers susceptibility) to type 1 diabetes in this cross (supplementary Table 1 and supplementary Figs. 5*A–C*). The strongest BBDP resistance locus (LOD = 4.18; Iddm25) maps to the telomere of chromosome 1 with a 1-LOD interval of 20 cM between markers D1Mit13 and D1Rat76. This locus overlaps with Iddm9/10 previously identified in crosses between the BB/OK and either DA or SHR/Mol strains (9). Strikingly, this locus contains the *Ins-2* gene (202.93 Mb), one of two homologues of the human *INS* gene that is associated with human type 1 diabetes with the second highest odds ratio (31,32) (Table 2).

Two other resistance loci on chromosomes 3 and 12 are novel. Although the former showed significant linkage when diabetic F2 animals were analyzed, linkage was only suggestive (LOD = 2.68; Iddm27) when the whole cohort was included, with a 1-LOD interval spanning 19 cM between D3Rat24 and D3Rat114. The latter locus is the second most significant resistance locus (LOD = 3.82; Iddm30) and has a 1-LOD interval of 24 cM between markers D12Got11 and D12Rat15. *C12orf30*, one of the loci associated with human type 1 diabetes (36.23 Mb), is located within this locus—though outside the 1-LOD interval (18) (Table 2).

Linkage analysis of insulitis. Two subphenotypes of insulitis were assessed in the diabetic and nondiabetic F2 progeny: the intensity of islet infiltration by inflammatory cells and the level of islet integrity. The distribution of these two subphenotypes was very different between the diabetic and nondiabetic F2 animals (Fig. 3). Specifically, while the vast majority of nondiabetic animals had intact islets and no cellular infiltration, all diabetic animals exhibited a mixture of islet atrophy and islet inflammation. Importantly, in both cohorts of diabetic and nondiabetic animals, there was a strong correlation ($P < 0.0001$, Spearman's rank correlation) between islet integrity and inflammation. However, while this correlation was negative in the nondiabetic F2 animals, where islets exhibiting some degree of inflammation tended to have decreased integrity, it was positive in diabetic F2 animals. This observation in diabetic F2 animals most likely reflects the progressive disappearance of inflammatory cells from islets as their β-cells are destroyed (Fig. 3). Of note, though comparison of pancreatic lesions in nondiabetic male and female F2 animals revealed significantly ($P = 0.0013$) more severe islet atrophy in females (data not shown), no evidence for significant QTL × sex interaction was found for either insulitis trait.

It is important to note that while the diabetic F2 animals were genotyped across the whole genome, nondiabetic animals were only genotyped at regions that at some point during the study showed evidence for linkage to type 1 diabetes. Consequently, nondiabetic animals only contributed to linkage analysis at these regions. In most cases, the loci controlling the two insulitis subphenotypes overlap with those described above that influence type 1 diabetes susceptibility.

Cellular infiltration. Three of the loci found to significantly influence cellular infiltration of pancreatic islets overlap with type 1 diabetes loci on chromosomes 6 (LOD = 6.78), 12 (LOD = 6.57), and 14 (LOD = 3.79), as illustrated in supplementary Figs. 3*B*, 5*C*, and 3*C*, respectively, while one novel locus on chromosome 8 (LOD = 2.63) is unlinked to type 1 diabetes (Table 1) and, importantly, regulates cellular infiltration of islets in diabetic F2 animals only (supplementary Table 1, Fig. 2, and supplementary Fig. 6). At the peak of those loci regulating both cellular infiltration and type 1 diabetes, the BBDP allele has consistent susceptibility (chromosomes 6 and 14) or resistance (chromosome 12) effects on the two traits (Table 1). At the novel chromosome 8 locus, found in diabetic F2 animals only, the BBDP allele is associated with a decreased cellular infiltration (Table 1), possibly due to its concomitant deleterious effect on islet integrity hence on the disappearance of β-cells (see below and Fig. 3*E*).

Islet integrity. The vast majority of the loci linked to islet integrity are also linked to type 1 diabetes in this cross (Fig. 2 and Table 1). The seven loci showing significant linkage (supplementary Figs. 3–6) map to chromosomes 1 (LOD = 4.19), 6 (LOD = 4.55 and 7.54 for the centromeric and telomeric loci, respectively), 8 (LOD = 3.75), 12 (LOD = 4.56), 14 (LOD = 3.74), and 17 (LOD = 3.28). Other loci on chromosomes 2, 3, and 16 are suggestively linked to islet integrity in this cross (Table 1 and Fig. 2). Again, at all of these loci, the protective or deleterious effect of the BBDP allele was consistent with its effect on

TABLE 1
Peak markers at each locus linked to type 1 diabetes and related traits

		Position				Genotype and phenotype distribution							
						AA		AB		BB			
Chromosome	Marker	cM	Mb	Trait	Dataset D/ND/W	n/D:ND	Mean ± SD % type 1 diabetes	n/D:ND	Mean ± SD % type 1 diabetes	n/D:ND	Mean ± SD % type 1 diabetes	LOD score	R^2
1	D1Rat159	123.8	198.8	Cellular infiltration	D	35	1.30 ± 0.63	55	1.45 ± 0.85	14	1.81 ± 1.00	2.93	12.73
1	D1Rat159	123.8	198.8	Islet integrity	D	35	1.21 ± 0.83	55	1.51 ± 0.88	14	1.78 ± 0.81	2.34	10.28
1	D1Wox23	132.4	212.1	Islet integrity	W	98	2.44 ± 0.89	219	2.57 ± 0.78	128	2.82 ± 0.50	4.19	3.66
1	D1Wox23	132.4	212.1	Type 1 diabetes	W	36:98	26.9%	56:219	20.4%	11:128	7.9%	4.18	3.73
1	D1Rat76	153.3	230.4	Cellular infiltration	ND	101	0.41 ± 0.64	221	0.58 ± 0.76	123	0.56 ± 0.74	2.61	2.78
2	D2Arb7	41.7	57.2	Islet integrity	D	21	1.30 ± 0.50	49	1.10 ± 0.43	28	0.84 ± 0.41	2.82	13.20
2	D2Rat180	73.3	121.2	AOO	D	19	126 ± 19.9	24	121 ± 22.6	31	107 ± 21.1	2.36	10.37
2	D2Rat180	73.3	121.2	Islet integrity	W	129	2.75 ± 0.59	213	2.59 ± 0.77	104	2.47 ± 0.88	2.55	2.66
2	D2Got121	92.6	193.8	Islet integrity	W	119	2.75 ± 0.57	222	2.61 ± 0.77	102	2.43 ± 0.87	2.74	2.20
2	D2Arb16	94.4	198.3	Type 1 diabetes	W	11:121	8.3%	61:221	21.6%	32:101	24.1%	2.90	2.38
2	D2Rat88	116.1	222.3	AOO	D	18	128 ± 21.3	55	119 ± 23.0	31	110 ± 20.8	2.98	13.92
2	D2Rat88	116.1	222.3	Cellular infiltration	ND	119	0.58 ± 0.74	223	0.51 ± 0.74	104	0.54 ± 0.70	1.88	2.11
3	D3Rat7	112.5	145.6	Islet integrity	W	158	2.42 ± 0.89	259	2.66 ± 0.69	124	2.69 ± 0.72	2.89	2.44
3	D3Rat7	112.5	145.6	Type 1 diabetes	W	45:113	28.5%	41:218	15.8%	18:106	14.5%	2.68	2.21
6	D6Rat88	70.3	100.2	Type 1 diabetes	W	13:117	10%	45:217	17.2%	45:102	30.6%	5.21	4.48
6	D6Rat81	76.3	112.0	Islet integrity	W	107	2.78 ± 0.54	238	2.64 ± 0.72	100	2.38 ± 0.93	4.55	3.91
6	D6Rat94	94.1	131.5	Cellular infiltration	ND	120	0.42 ± 0.66	227	0.52 ± 0.77	94	0.71 ± 0.72	2.04	2.08
6	D6Rat152	98.0	142.5	Cellular infiltration	W	118	0.53 ± 0.75	236	0.67 ± 0.82	92	1.06 ± 0.88	6.78	5.35
6	D6Rat152	98.0	142.5	Islet integrity	W	118	2.81 ± 0.52	236	2.66 ± 0.72	92	2.30 ± 0.94	7.54	5.93
6	D6Rat1	98.4	144.2	Type 1 diabetes	W	8:120	6.3%	50:234	17.6%	46:92	33.3%	7.66	6.32
7	D7Rat30	20.7	30.3	Cellular infiltration	D	26	2.06 ± 0.83	56	1.56 ± 0.62	15	1.36 ± 0.31	3.00	16.78
7	D7Wox48	97.5	129.5	AOO	D	30	121 ± 22.9	52	121 ± 21.8	22	103 ± 20.0	2.71	15.37
8	D8Rat20	69.2	94.9	Islet integrity	D	23	1.24 ± 0.38	44	1.15 ± 0.48	31	0.83 ± 0.42	3.75	18.23
8	D8Rat65	91.3	109.9	Cellular infiltration	D	18	2.12 ± 0.72	51	1.66 ± 0.64	27	1.42 ± 0.63	2.63	12.54
12	D12Rat28	20.3	16.3	Cellular infiltration	ND	96	0.78 ± 0.86	223	0.53 ± 0.72	122	0.37 ± 0.59	3.65	3.69
12	D12Rat28	20.3	16.3	Cellular infiltration	W	96	1.03 ± 0.94	223	0.73 ± 0.81	122	0.47 ± 0.71	6.57	5.19
12	D12Rat28	20.3	16.3	Islet integrity	W	96	2.41 ± 0.87	223	2.59 ± 0.77	122	2.81 ± 0.54	4.56	3.87
12	D12Rat28	20.3	16.3	Type 1 diabetes	W	36:96	27.3%	58:223	20.6%	10:122	7.6%	3.82	3.14
14	D14Rat1	3.4	4.9	Islet integrity	W	127	2.75 ± 0.62	227	2.62 ± 0.76	92	2.40 ± 0.86	3.74	3.30
14	D14Rat1	3.4	4.9	Type 1 diabetes	W	14:127	9.9%	51:227	18.3%	39:92	29.8%	4.70	4.13
14	D14Rat42	8.8	14.3	Cellular infiltration	W	114	0.60 ± 0.78	225	0.68 ± 0.83	86	0.99 ± 0.92	3.79	3.03
17	D17Rat11	19.9	30.9	Islet integrity	W	118	2.70 ± 0.66	229	2.63 ± 0.74	93	2.43 ± 0.89	3.28	3.28
17	D17Rat11	19.9	30.9	Type 1 diabetes	W	20:138	14.5%	51:280	18.2%	33:93	26.2%	1.98	1.95
18	D18Rat116	16.3	40.6	AOO	D	30	107 ± 17.3	50	120 ± 23.0	18	128 ± 24.0	2.79	12.85

For each microsatellite marker, both the genetic (cM) and physical positions (Mb) are shown, and the trait to which linkage was found is indicated. Three different datasets were used: D, diabetic animals only; ND, nondiabetic animals only; W, whole cohort. The distribution of genotypes and phenotypes for each of these datasets is indicated as follows. For the genotypes: AA, ACI.BB$^{Iu.Iyp}$ homozygous, AB, heterozygous, and BB, BBDP homozygous. For each genotype, the first column indicates the number of animals, except for the type 1 diabetes trait for which the number of diabetic and nondiabetic (D:ND) animals is given. The second column shows the mean value for each trait ± SD, except for type 1 diabetes, for which the cumulative incidence is given as a percentage. The final two columns show the LOD score reached and the proportion of trait variance (R^2) accounted for by each locus.

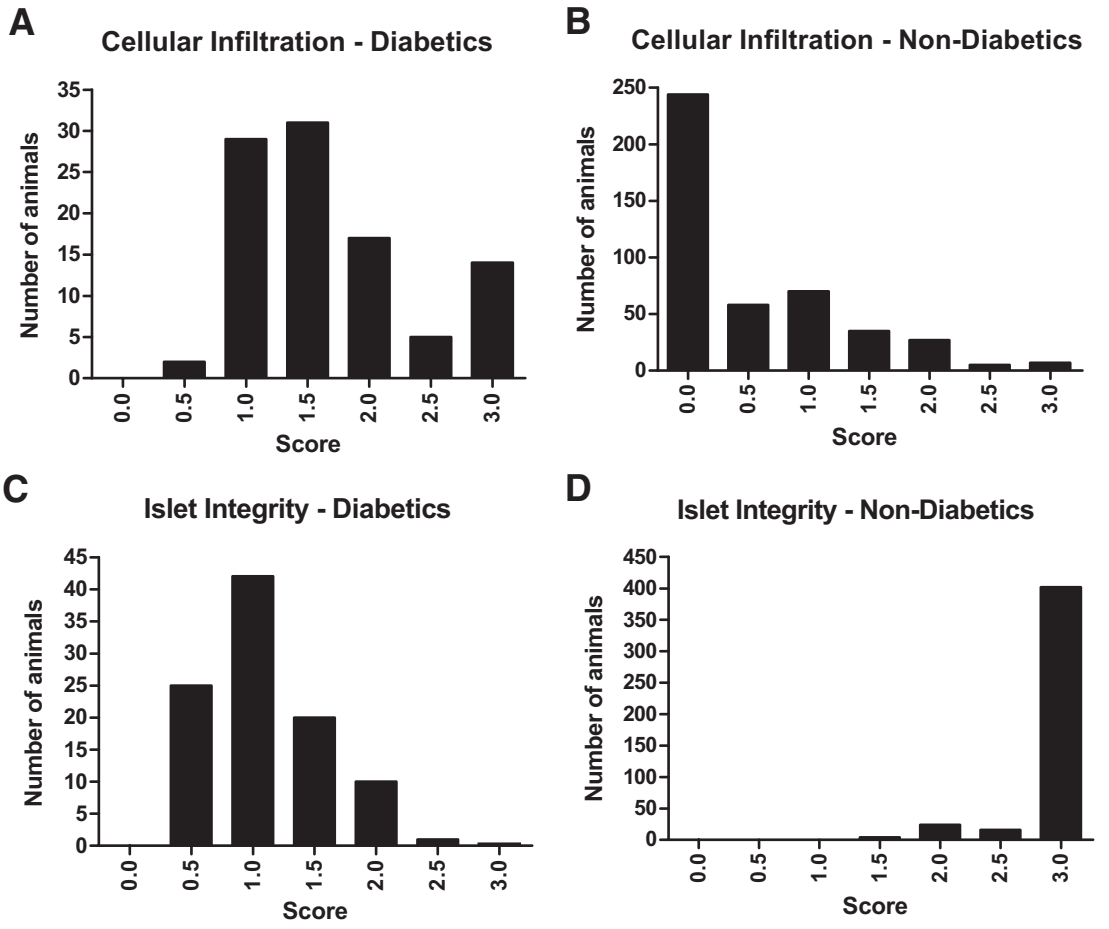
TABLE 2
Murine and human chromosomal regions syntenic to Iddm loci

Rat					Mouse			Human			
Chromosome	Marker (1-LOD interval)	Position (Mb)	Trait	Iddm locus	Chromosome	Position (Mb)	Idd locus (Mb)	Chromosome	Position (Mb)	IDDM locus/GWA (Mb)	Candidate genes
1	D1Mit13 D1Rat76	186.5 230.4	Type 1 diabetes/islet integrity	Iddm25	7	114.7–132.6	Idd27 (86.5–127.0)	16	30.3–31.4		
								10	124.2–135.2		
					19	3.0–24.8		11	6.1–2.4	GWA SNP: rs689 IDDM2 (2.0–2.3)	*INS/IGF2*
								11	58.2–70.8	IDDM4 (59.2–68.5)	*LRP5/GAL/FADD*
								9	69.3–79.4		
2	D2Rat75 D2Mit8	79.8 148.8	AOO/islet integrity	Iddm32	15	31.3–32.2		5	9.7–10.7		
					3	5.6–14.9		8	67.3–86.6		
								3	150.0–181.0	IDDM9 (138.8–170.6)	*CP*
					3	19.4–59.4	Idd3 (36.6–37.3)	4	123.0–130.2	GWA SNP: rs3136534	*ADAD1*, *IL2*, *IL21*, *KIAA1109*
								13	35.2–37.1		
								3	148.5–150.7	IDDM9 (138.8–170.6)	
2	D2Rat44 D2Rat88	181.7 222.3	Type 1 diabetes/islet integrity	Iddm26	3	90.2–147.2	Idd10 (100.1–101.0)	1	143.9–151.4		
							Idd18.2 (101.0–108.2)	1	94.1–120.2	GWA SNP: rs6679677	*PTPN22*
2	D2Rat82 D2Rat69	214.6 247.3	AOO	Iddm33			Idd18.1 (109.9–109.6)	4 1	100.2–120.6 82.0–89.0		*NFKB1/FABP2*
3	D3Rat24 D3Rat114	96.5 149.5	Type 1 diabetes	Iddm27	2	112.4–158.2	Idd13 (114.1–158.3)	15 2 20	30.7–47.2 95.4–113.3 0.3–36.2		*B2m* *IL1A/IL1B*
6	D6Rat23 D6Got169	74.4 123.6	Type 1 diabetes/islet integrity	Iddm28	12	50.6–93.3		14	34.8–88.1	IDDM11 (85.0–92.4)	*PYGL/SEL1*
6	D6Mgh3 D6Got167	127.7 146.7	Type 1 diabetes/cellular infiltration/islet integrity	Iddm29	12	98.3–111.5		14	93.8–105.0		*RAGE*
								7	126.8–158.3		
7	D7Rat21 D7Rat2	96.5 137.7	AOO	Iddm34	15	62.2–99.3		8 22	128.8–146.0 35.0–49.5	GWA SNP: rs229541	*C1QTNF6*, *SSTR3*
								12	38.2–47.7		*VDR*
12	D12Got11 D12Rat15	8.9 29.4	Type 1 diabetes/cellular infiltration/islet integrity	Iddm30	5	126.5–143.9		7	0.7–59.8		*NUDT1*
								7	55.9–101.5		*PON2*
								12	129.9–130.8	GWA SNP: rs3184504	*C12orf30*, *PTPN11*

TABLE 2
Continued

Rat					Mouse			Human			
Chromosome	Marker (1-LOD interval)	Position (Mb)	Trait	Iddm locus	Chromosome	Position (Mb)	Idd locus (Mb)	Chromosome	Position (Mb)	IDDM locus/GWA (Mb)	Candidate genes
14	D14Rat1 D14Mit6	4.9 19.8	Type 1 diabetes/ cellular infiltration/ islet integrity	Iddm31	5	89.3–101.8		4	74.6–89.3		19.8
17	D17Rat6 D17Rat78	16.1 47.6	Type 1 diabetes/islet integrity	Iddm36	13	26.4–53.7	Idd14 (25.4–120.3)	5	174.1–175.2		
								9	89.2–93.0		
								6	2.7–16.3		
								6	22.4–24.5		
18	D18Rat109 D18Arb6	4.3 62.7	AOO	Iddm35	18	15.8–66.5	Idd21.2 (64.6–74.6)	18	22.7–39.1		
								5	110.6–127.6		
								5	137.8–150.1		
								18	52.4–55.2	IDDM6 (34.1–61.2)	*SLC14A1*

In the rat, the chromosome number, the markers flanking the 1-LOD interval, their physical position in Mb, and the trait(s) to which linkage was found are given for each Iddm locus. For murine regions syntenic to each Iddm locus, the chromosome number, the limits of each chromosomal interval (in Mb), and, when present, the Idd loci situated within the syntenic intervals are indicated together with their physical location. For human regions syntenic to each Iddm locus, the chromosome number, the limits of each chromosomal interval (in Mb), and, when present, the IDDM loci situated within the syntenic intervals are indicated together with their physical location. The SNPs recently found to reach significant, genome-wide association (GWA) with type 1 diabetes are also indicated. The last column indicates genes in close linkage disequilibrium with GWA SNPs, as well as the positional candidate genes within the Idd and IDDM loci as listed in www.t1dbase.org.



E

Islet score (3-0) / Cellular Infiltration (0-3)

	3	2.5	2	1.5	1	0.5	0	Total
0								0
0.5						2		2
1				2	8	19		29
1.5		1		5	22	3		31
2			5	5	6	1		17
2.5			2	2	1			5
3			3	6	5			14
Total	0	1	10	20	42	2	0	98

F

Islet score (3-0) / Cellular Infiltration (0-3)

	3	2.5	2	1.5	1	0.5	0	Total
0	244							244
0.5	58							58
1	67	1	1	1				70
1.5	24	7	4					35
2	8	6	12	1				27
2.5		1	4					5
3	1	1	3	2				7
Total	402	16	24	4	0	0	0	446

FIG. 3. Distribution of insulitis subphenotype scores in diabetic and nondiabetic F2 animals. *A*, *C*, and *E*: Data from diabetic animals. *B*, *D*, and *F*: Data from nondiabetic animals. In graphs *A–D*, the vertical axis indicates the number of animals, while scores for cellular infiltration (*A* and *B*) and islet integrity (*C* and *D*) are labeled on the horizontal axis. *E* and *F*: the correlation between cellular infiltration and islet integrity scores in diabetics (*E*) and nondiabetics (*F*).

type 1 diabetes, conferring resistance to type 1 diabetes and relative protection from islet atrophy on chromosomes 1, 3, and 12 as opposed to increased susceptibility to type 1 diabetes and islet atrophy on chromosomes 2, 6, 8, 14, and 17 (Table 1 and supplementary Table 1).

Linkage analysis of AOO of diabetes. Four chromosomal regions on chromosomes 2, 7, and 18 were found to influence the age at which type 1 diabetes develops in F2 animals (Fig. 2 and supplementary Fig. 7). Both chromosome 2 AOO loci (LOD = 2.36, Iddm32; and LOD = 2.98, Iddm33 for the centromeric and telomeric loci, respectively) overlap with two suggestive loci controlling islet integrity (Table 1 and supplementary Table 1). As expected, based on the deleterious effect of the BBDP alleles on islet integrity at both loci, these alleles are also associated with early onset of type 1 diabetes (Table 1). Importantly, *Il-2* and *Il-21*, which are associated with type 1 diabetes in both humans and NOD mice, are located within the Iddm32 locus (Table 2) (18,33). The two AOO loci on chromosomes 7 (LOD = 2.71; Iddm34) and 18 (LOD = 2.79; Iddm35) did not overlap with any type 1 diabetes or insulitis loci (supplementary Table 1). However, the former locus contains *C1qtnf6*, recently found to be associated with human type 1 diabetes by meta-analysis of genome-wide association studies (34). While BBDP homozygosity at the peak of the chromosome 7 locus was associated with early type 1 diabetes development, at the peak of the chromosome 18 locus it delayed onset of the disease (Table 1). Of note, although a type 1 diabetes chromosome 18 locus overlapping with Iddm35 was iden-

tified in a cross between BB/OK and SHR/Mol rats (9), it was not linked to AOO, and the BB/OK allele conferred increased susceptibility to type 1 diabetes (35).

DISCUSSION

This study has identified no less than 12 loci designated Iddm25 to Iddm36, influencing the diabetogenic process in a single cross, thus establishing the polygenic basis of the disease in the BBDP rat. Six of these loci on chromosomes 1, 6 (two loci), 12, 14, and 17 showed significant linkage to type 1 diabetes and/or insulitis, while six on chromosomes 2 (three loci), 3, 7, and 18 reached suggestive linkage to type 1 diabetes or AOO. The BBDP, KDP, and LEW.1AR1 rat strains spontaneously develop type 1 diabetes, and it initially appeared that these syndromes had a relatively simple genetic basis with perhaps few susceptibility genes (36,37). However, the current study demonstrates that this does not apply to an intercross between the BBDP and ACI.BB$^{1u.lyp}$ strains used here. Strikingly, although the type 1 diabetes loci mapped here are numerous, together they only account for <30% of the trait variance observed in the F2 cohort. From the study of Klaff et al. (8), we calculated that Iddm1 and -2 jointly contributed 9.4% of the variance of type 1 diabetes in a cross between the type 1 diabetes–susceptible DRlyp/lyp and type 1 diabetes–resistant F344 strains. Assuming that the contribution of these two genes to type 1 diabetes variance would be similar in a cross-intercross between BBDP and noncongenic ACI rats, our results strongly suggest a genetic complexity for the BBDP diabetic syndrome that is reminiscent of the human disease.

Genetic analyses using the BBDP strain previously performed by others made us aware of the very low number (<1%) of diabetic (hence informative) animals that could be expected in such a classical F2 cohort. Therefore, we reasoned that a large "conditioned" F2 cross with the two major Iddm loci fixed as BBDP would produce more informative animals. Consequently, several loci, including some contributing small effects to the disease, could be mapped. Furthermore, this would allow us to assess the contribution of all three genotypes to the diabetogenic process. However, a limitation of using a conditioned cross is that it could not detect genetic interaction between *Gimap5* or *RT1u* and any of the other loci detected in our linkage analysis.

Having found multiple type 1 diabetes loci, the question follows as to why only three of these on chromosomes 1, 2, and 6 were identified in previous studies (8,9). The first obvious explanation is the extent of genetic polymorphism between BB rats and the type 1 diabetes–resistant strains used. In this regard, using recent information on genome-wide single nucleotide polymorphisms (SNPs) available from the rat genome database (http://rgd.mcw.edu/), we found that the BB genotype differed from the ACI, BN, F344, SHR, and WF strain genotypes at 30, 53, 27, 36, and 23% of the SNPs, respectively. Since the genetic polymorphism distinguishing the ACI from the BB strain is not the most extensive, it is unlikely to be the main explanation for the detection of many type 1 diabetes loci in this cross. Rather, this suggests that the type 1 diabetes–resistant strains used in previous studies had distinct combinations of type 1 diabetes resistance and susceptibility alleles compared with the ACI.BB$^{1u.lyp}$ strain. In support of this, the current F2 cohort could not map two type 1 diabetes loci on chromosomes 4 and 18 that were identified independently using distinct type 1 diabetes–resistant strains, though these loci could segregate in our cross (9,13). Further, it is perhaps surprising that the chromosome 2 (Iddm3) locus was previously identified with a much higher LOD score in a relatively small backcross using the type 1 diabetes–resistant F344 strain (8). It is possible that this high LOD score was due to allelic heterogeneity resulting in a differential effect depending on the resistant strain used and/or the close proximity of two susceptibility loci, only one of which segregates in our cross. This is supported by the slightly more centromeric (~20 cM) location of the peak of linkage to type 1 diabetes on chromosome 2 compared with Iddm3 (8).

A second likely explanation for the detection of multiple type 1 diabetes loci in this cross is its size and its "conditioned" nature, both resulting in a number of diabetic, hence informative, animals significantly larger than in previous studies (8–11). This size-related increase in the power of our study may also have benefited from the use of an intercross as opposed to a backcross, since it allowed the identification of three ACI-susceptible (or BBDP resistance) loci.

The observation of a higher cumulative incidence of type 1 diabetes in female F2 animals was unexpected since contrary to the NOD mouse, the BBDP rat does not exhibit a sex bias in disease susceptibility. This differential, sex-related type 1 diabetes risk in the F2 cohort was, however, minimal, and this could explain our inability to detect loci responsible for the higher disease incidence in females. This raises the possibility of an environmental risk factor for females. Paradoxically, the BBDP susceptibility allele at the only locus showing interaction with sex (chromosome 6) confers higher risk for the disease in males.

A successful approach to map type 1 diabetes loci has taken advantage of the widespread susceptibility to experimentally induced diabetic syndromes among nonlymphopenic, *RT1u* strains of rats (13–16). Linkage analyses of crosses between these and *RT1u*, but type 1 diabetes–resistant, strains identified some type 1 diabetes loci including Iddm14 on chromosome 4 that influences both spontaneous and experimentally induced type 1 diabetes (14,15). Of two other loci on chromosomes 13 and 17 previously shown to control experimentally induced type 1 diabetes, only the latter segregates with spontaneous type 1 diabetes in our cross (13,38). Again, this could reflect genetic polymorphism between the type 1 diabetes–resistant WF and ACI.BB$^{1u.lyp}$ strains used in these studies. Alternatively, it could indicate that some of the loci influencing spontaneous and experimentally induced diabetic syndromes (hence, some of the pathogenic mechanisms involved in these syndromes) are distinct.

Recent genome-wide association studies have successfully identified multiple novel, small-effect genes associated with type 1 diabetes in humans (17–19,34). This identification should facilitate our understanding of antipancreatic autoimmunity at the molecular level and, subsequently, the design of therapeutic approaches interfering with these mechanisms. However, the small effect of these genes has highlighted the importance of type 1 diabetes–prone animals sharing disease susceptibility genes or signaling pathways with their human counterparts. Specifically, they could identify additional genetic risk factors, reveal pathways of previously unknown relevance to the disease, and provide insights into pathogenic mechanisms controlled by shared susceptibility genes. The relevance of type 1 diabetes–prone animals to the

human disease has been illustrated by the NOD mouse, which shares several type 1 diabetes genes and signaling pathways with type 1 diabetes in humans (33,39,40). Similarly, the presence of five genes associated with the human disease within the Iddm loci identified in this cross (Table 2) may facilitate a better understanding of their contribution to antipancreatic autoimmunity.

Another contribution of experimental models is that they are accessible to the dissection of disease-related subphenotypes that are difficult to assess in humans. For example, the linkage analysis of insulitis subphenotypes leads to both expected and unexpected results. It is generally accepted that the outcome of chronic islet inflammation is the destruction of β-cells and the subsequent development of type 1 diabetes. It is therefore not surprising that insulitis and type 1 diabetes loci overlap and that genotypes increasing type 1 diabetes susceptibility are associated with both intense inflammatory infiltrates in islets and severe islet atrophy (e.g., chromosomes 6 [telomere], 12, and 14 [Table 1]). More surprising is the observation that some type 1 diabetes loci (e.g., on chromosomes 1, 2, 6 [centromere], and 17) influence islet integrity but do not seem to control islet infiltration by immune cells. At the moment, we can speculate that these loci may control very early steps of the autoimmune response (e.g., they may influence the immunogenicity of β-cells). Alternatively, they may regulate an immune mechanism that destroys islets independently of the intensity of the local cellular inflammation or the intrinsic ability of β-cells to succumb to the inflammatory response.

The incidence of type 1 diabetes is increasing in the Western world, most rapidly in individuals with onset before the age of 5 years (41). Importantly, early-onset type 1 diabetes is associated with an aggressive course characterized by rapid disappearance of residual β-cell function and a higher risk for preclinical (as assessed by autoantibodies) and overt disease in siblings and parents (42,43). There is strong evidence that AOO is under genetic control (44). Some of the genes associated with AOO have been identified in humans, which are all MHC class I and II genes (17,42,45–47). However, genome-wide association studies have not identified novel AOO genes, possibly due to the fact that many cohorts restricted inclusion to pediatric onset (18,19,48). Our study identifies four loci that appear to influence AOO (though unlinked to type 1 diabetes itself) suggesting that some of these allelic variations may act upstream of those affecting insulitis and type 1 diabetes susceptibility, possibly at the level of the differentiation of the target tissue and/or components of the immune system.

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