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The Rat Diabetes Susceptibility Locus *Iddm4* And At Least One Additional Gene Are Required For Autoimmune Diabetes Induced By Viral Infection

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Abstract

BBDR rats develop autoimmune diabetes mellitus only after challenge with environmental perturbants. These include polyinosinic:polycytidylic acid (poly I:C, a ligand of toll-like receptor 3), agents that deplete regulatory T cell populations (Tregs), and a non-beta-cell-cytopathic parvovirus (Kilham rat virus, KRV). The dominant diabetes susceptibility locus *Iddm4* is required for diabetes induced by treatment with poly I:C plus Treg depletion. *Iddm4* is penetrant in congenic heterozygote rats on the resistant WF background, and is 79% sensitive and 80% specific as a predictor of induced diabetes. Surprisingly, an analysis of 190 (BBDR × WF)F2 rats treated with KRV after brief exposure to poly I:C revealed that the BBDR origin allele of *Iddm4* is necessary but not entirely sufficient for diabetes expression. A genome scan identified a locus on chromosome 17, designated *Iddm20*, that is also required for susceptibility to diabetes after exposure to KRV and poly I:C (LOD score 3.7). These data suggest that the expression of autoimmune diabetes is a complex process that requires both major histocompatibility complex (MHC) genes that confer susceptibility and additional genes like *Iddm4* and *Iddm20* that operate only in the context of specific environmental perturbants, amplifying the immune response and the rate of disease progression.

Introduction

Type 1 diabetes results from inflammatory infiltration of pancreatic islets and selective beta cell destruction. It is thought to be caused by environmental factors operating in a genetically susceptible host (1,2). Susceptibility loci include the MHC, a promoter polymorphism of the insulin gene, and an allelic variant of CTLA4 (3). Among candidate environmental perturbants, viral infection is among the most likely (4). How genes interact with the environment to transform diabetes susceptibility into overt disease is unknown.

BBDR rats model virus-induced autoimmune diabetes remarkably well. (5). They are phenotypically normal and, in clean housing, never develop diabetes. They do, however, become diabetic when challenged with environmental perturbants, among them poly I:C in combination with depletion of regulatory T cells (Tregs) (6). Diabetes can also be induced in BBDR rats with KRV, a non-beta-cell cytopathic parvovirus (7). Naturally occurring KRV infection induces diabetes in ~1% of animals; intentional infection with 10^7 plaque forming units (PFU) induces diabetes in ~30% of BBDR rats (7). Infection with KRV following brief

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pre-treatment with a low, sub-diabetogenic dose of poly I:C (1 μg/gm daily ×3) leads to diabetes in 100% of animals (8). The effect is virus-specific; H-1, which is 98% sequence identical, uniformly fails to induce diabetes (8).

In analyses of (BBDP × WF) × WF rats, we used poly I:C plus Treg depletion to map a locus on chromosome 4 (*Iddm4*) with significant linkage to diabetes (6,9), and we recently positioned *Iddm4* in a 2.8 cM region (10). The BB-origin allele of *Iddm4* is dominant and 79% sensitive and 80% specific as a predictor of diabetes induced by Treg depletion and poly I:C. A radiation hybrid map has assigned *Iddm4* to a 6.3-Mb segment between *PTN* and *ZYX* at 7q32 in the human genome, and to a 5.7-Mb segment between *Ptn* and *Zyx* in the mouse genome (11).

We now report a linkage analysis of 190 (BBDR × WF)F2 rats. It reveals that the BBDR-origin allele of *Iddm4* is necessary but not entirely sufficient for diabetes expression in response to KRV infection. An additional gene or genes on chromosome 17 are necessary.

Methods

Animals

BBDR/Wor, WF.*Iddm4*, and WF.*ART2a* rats (all $RT1^{u/u}$, $ART2^{a}$) were obtained from colonies maintained by us. WF.*Iddm4* congenic rats were generated by repetitive (BBDR/Wor × WF) × WF backcrosses using a marker-assisted selection protocol as described (10). They were studied at the N6 generation. WF.*ART2a* congenic rats were also developed by us and differ from ordinary WF animals in that they express the “a” rather than the “b” allotype of the ART2 T cell alloantigen on chromosome 1 (10). For simplicity, we refer to them here as WF rats. An F2 intercross was bred from (BBDR × WF)F1 hybrids. Animals were housed in viral antibody free conditions, confirmed monthly to be serologically free of rat pathogens (10), and maintained in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996).

Microsatellite and Mapping Analyses

Genomic DNA was prepared as described (10 and Appendix, Figure 3). Microsatellite markers were placed evenly throughout the 20 autosomes. The source of primers and positions of markers on the genetic map are given in Appendix Figure 3. Primers were end-labeled using $^{32}P\gamma$-ATP, used in a PCR reaction, and resolved by polyacrylamide gel electrophoresis as described (6). The position of markers on the genetic map was established by inspection of the dataset and conventional calculation methods to establish meiotic map distances, which are expressed in cM or megabases (Mb) according to the rat genome sequence, June 2003 build (genome.ucsu.edu).

Linkage of diabetes with segregation of BBDR-origin alleles was evaluated by composite interval mapping (CIM) using model 6 of the Zmapqtl program in Windows QTL Cartographer v1.30 (statgen.ncsu.edu/qtlcart/cartographer.html). CIM combines classical interval mapping with multiple regression analysis, allowing for more precise QTL localization than classical interval mapping (12).

Treatment Protocols

KRV-UMass was propagated in NRK cells grown in Dulbecco's minimal essential medium. Poly I:C (Sigma, St. Louis, MO) was dissolved in Dulbecco's PBS, sterile filtered, and stored at -20°C until used. Contaminating endotoxin concentration was <50 units/mg (Charles River Endosafe, Charleston, SC). In studies of KRV alone, rats of either sex 22-28 days old were injected intraperitoneally with 10^7 PFU in a volume of 1 ml. In other experiments, rats 21-25 days of age of either sex were injected intraperitoneally with poly I:C 1 (μg/gm body weight

on three consecutive days) and either not treated further or injected on the following day with KRV. Pretreatment with poly I:C was used because it increases the frequency of diabetes in KRV-treated BBDR rats from ~30% to 100% (8). Animals were screened three times weekly for glycosuria (Tes-Tape® Eli Lilly, Indianapolis, IN). Diabetes was diagnosed on the basis of a plasma glucose concentration >11.1 mM (OneTouch Ultra Glucometer, LifeScan, Milpitas, CA). For study of insulitis, pancreata were removed, fixed in formalin, and stained with hematoxylin and eosin. Pancreata were graded by a qualified pathologist on a scale of increasing intensity 0 to 4+ as described (10).

Results

Autoimmune Diabetes Induced by KRV and TLR3 Ligation

We first confirmed (8) that a significant fraction (41%) of parental BBDR rats become diabetic after infection with KRV alone, that none become diabetic in response to a 3 day course of poly I:C alone, and that 100% become diabetic in response to KRV after poly I:C (Table 1). We also confirmed (13) that WF rats resist diabetes induction in response to either KRV or KRV plus poly I:C (Table 1).

We next tested N6 generation WF.*Iddm4* congenic rats (10) for disease susceptibility. To our surprise, we observed WF.*Iddm4*^{d} rats to be uniformly resistant to diabetes in response to KRV infection either alone or after poly I:C, despite maintaining the expected degree of susceptibility to diabetes after treatment with poly I:C and Treg depletion (Table 1).

Autoimmune Diabetes Induced by KRV and TLR Ligation Segregates with Iddm4 and a Second Locus in (BBDR × WF)F2 rats

To determine if *Iddm4* acts only in the presence of additional BBDR origin genes, we generated (BBDR × WF)F1 progeny. We observed that all F1 rats were resistant to KRV alone, but 38% were susceptible to treatment with KRV plus poly I:C. To identify susceptibility genes, we then generated (BBDR × WF) F2 progeny and treated them with KRV plus poly I:C. Diabetes occurred in 59 of 190 animals (31%) in this segregating population and affected both males and females (Table 1). Looking first at the *Iddm4* interval, we observed that diabetes occurred almost exclusively in animals with at least one BBDR-origin allele of *Iddm4* (58 of 59 diabetic animals, Table 2). The presence of the BBDR allele of *Iddm4* was 98% sensitive but only 31% specific in predicting susceptibility to diabetes, implying that at least one gene of BBDR origin is required for the expression of diabetes in response to infection. We therefore performed a genome-wide scan on this F2 cohort. The remaining genome was assessed for linkage using 144 markers on the 20 autosomes (Appendix, Figure 3).

Composite interval analysis of the linkage data is shown in Figure 1. *Iddm4*, as expected, showed strong linkage to diabetes (LOD >6.0 at ~36 cM on chromosome 4) A second locus on chromosome 17 was linked to the diabetes in the F2 population with a LOD score of 3.7. This locus has been designated *Iddm20* by the curators of the Rat Genome Database (www.rgd.mcw.edu).

To determine the mode of inheritance and the interaction between these two loci, we analyzed the dataset using a life-table analysis. As shown in Figure 2, the highest likelihood of diabetes onset occurs in animals in which the BBDR-origin allele of *Iddm4* is homozygous or heterozygous, and *Iddm20* is homozygous.

Candidate Gene Analysis

The genome-wide scan positioned the *Iddm20* locus in a 1 LOD interval bounded by *D17Rat61* (at 19.7 Mb) and *D17Rat115* (at 27.7 Mb, http://genome.ucsc.edu). To identify

candidate genes within this interval, we constructed a preliminary map using the databases at UCSC and Ensembl (http://genome.ucsc.edu/; http://www.ensembl.org/). The genes in the *Iddm20* region have their human orthologues on human chromosome 5, 6, and 9 and on mouse chromosome 13. Of interest is a confirmed mouse diabetes QTL (*Idd14*) in this homologous region (14,15). Candidate genes in the *Iddm20* interval are listed in Appendix Table 4

Histology

Histologic analysis of islets revealed nearly complete concordance of insulitis scores with diabetes phenotype. Among the 59 diabetic F2 animals, the mean insulitis score was 3.7 with 49 scored 4+ or end stage insulitis. In contrast, among 127 non-diabetic rats with technically satisfactory specimens, the mean insulitis score was 0.3 with 111 (87%) being entirely normal and 8 of the remaining 16 exhibiting only 1+ insulitis. Exocrine pancreatitis was absent.

Discussion

These data establish linkage of rat genotype to a form of environmental perturbation—infection—that is potentially important in the pathogenesis of autoimmunity. They confirm that *Iddm4* is an exceptionally strong non-MHC determinant of susceptibility to autoimmune diabetes in the rat (6,9-11). In previous studies of *Iddm4*, diabetes was induced by chronic treatment with poly I:C plus Treg depletion. The present data now extend the role of *Iddm4* in diabetes pathogenesis to virus-induced disease expression. They also illuminate the complexity of environmental interaction with genetic susceptibility. The diabetogenic potential of *Iddm4* is readily discernable in congenic rats treated with poly I:C and Treg depletion, but is far less apparent in animals treated with KRV plus poly I:C unless additional BBDR genes are present. We have discovered at least one of these genes, designated *Iddm20*, on chromosome 17.

The *Iddm20* interval (Appendix, Table 3) contains at least one gene of particular interest: *Syk*. This gene is involved in TCR-dependent signaling pathways and interacts with *Cblb* (16). This could be important because *Cblb* is a known rat diabetes susceptibility gene (17). Loss of function mutations in *Cblb* lead to activation of autoreactive diabetogenic T cells in the absence of full costimulation (17).

Iddm20, like *Iddm4*, appears to act as a genetic dominant with incomplete penetrance. There is clear disease-promoting activity in the *Iddm20* heterozygote. In the poly I:C plus Treg system, ~70% of both WF.*Iddm4*$^{d/w}$ heterozygotes and WF.*Iddm4*$^{d/d}$ homozygotes become diabetic (10). In the KRV plus poly I:C model, homozygosity for diabetogenic alleles at the *Iddm20* locus increases the penetrance of diabetes in *Iddm4*$^{d/d}$ rats from 25% (in *Iddm20*$^{w/w}$ animals) to 56%, and it increases penetrance in *Iddm4*$^{d/w}$ rats from 11% to 59%. We therefore regard *Iddm20* as a modifier of the *Iddm4* locus.

The mechanisms by which poly I:C and KRV infection act to induce diabetes in genetically susceptible rats are not yet known. We speculate that *Iddm4*, *Iddm20*, or both define a strain-specific response of BBDR rats to KRV infection. KRV is known to infect lymphocytes in the pancreatic lymph nodes (but not the islets) of BBDR rats (18). More recent studies have revealed that KRV also causes a decrease in splenic $CD4^+CD25^+$ Treg cells in both BBDR and normal WF rats (8). In adult LEW rats, KRV-UMass infection is associated with several potentially important effects on both $CD4^+$ and $CD8^+$ T cell populations (19), but whether allelic variations in *Iddm4* or *Iddm20* regulate those responses is not yet known. By itself, KRV infection typically induces diabetes in about 30-40% of BBDR/Wor rats (8). Pre-treatment poly I:C, at a dose that is itself incapable of inducing disease, dramatically increases the penetrance of diabetes (8). The mechanism of this synergy is not clear, but is likely to relate to innate immunity because poly I:C is a ligand of TLR-3 and a potent inducer of type I interferon production by various cells (20) and IL-1 production by monocytes (21). It also

activates NK (22) and B cells (23). In rats, it has been shown that interferon production in response to poly I:C varies substantially in different inbred strains (24), an effect that is presumably genetically determined. It will be of interest to determine if *Iddm4* and/or *Iddm20* is a determinant of the magnitude of the immune response to poly I:C.

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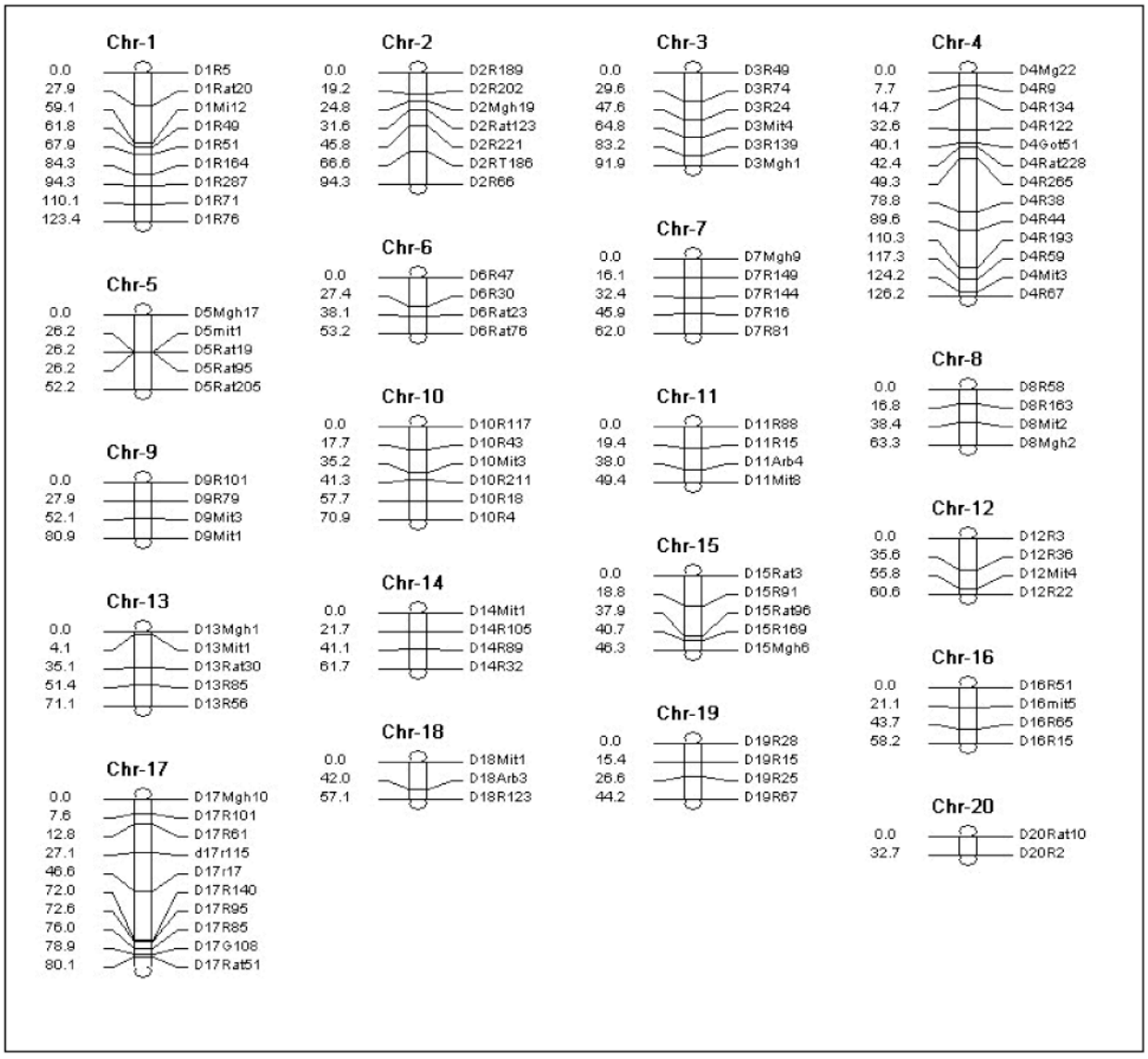
Appendix



Appendix: Figure 3.

Polymorphic markers used in this study. DNA samples and microsatellite markers. Genomic DNA was prepared using one of two protocols. Snap-frozen livers were ground on dry ice and the dispersed tissue was treated with Proteinase K in the presence of 10% sarkosyl and 0.5M

EDTA (pH 8.0). DNA was purified from these digests by phenol-chloroform extraction and dialysis against Tris EDTA (0.01M Tris/0.001M EDTA, pH 7.4). Alternatively, genomic DNA was extracted from rat tail snips using the QIAamp Tissue kit (Qiagen, Stanford, CA) according to the manufacturer's instructions. Most microsatellite primers used in this study are available from Research Genetics, Inc. (Huntsville, AL). The general map location of these microsatellites was taken from our own segregating backcrosses, and from maps published by the Rat Genome Database (http://www.rgd.mcw.edu) and by Dr. R. Wilder and Dr. E. Remmers (www.nih.gov/niams/scientific/ratgbase/index.htm). Additional primer pairs were developed from unique sequences flanking short sequence repeats discovered by inspection of repeat regions in the *Iddm4* and *ART2* intervals (UCSC genome database). Primers found to be polymorphic between parental strains were used. The position of markers on the genetic map was established by inspection of the data set and conventional calculation methods to establish meiotic map distances, which are expressed in megabases (Mb) according to the rat genome sequence, June 2003 build (http://genome.ucsu.edu). Chr: chromosome.

Appendix Table 3

Frequency of Diabetes as a Function of *Iddm4* and *Iddm20* Genotype

As documented by the low frequency of diabetes in the shaded cells, diabetes susceptibility in KRV-infected rats requires *Iddm20*, *Iddm4*, or both. “d” homozygous for the BBDR-origin allele; “”w” homozygous for the WF-origin alleles; “h” heterozygous.

***Iddm4*→ *Iddm20*↓**	**d *sick***	**h *sick***	**w *sick***	**d *well***	**h *well***	**w *well***
d	5	13	1	4	9	6
h	16	19	0	16	32	24
w	2	3	0	6	24	10

Appendix Table 4

Candidate Genes in the *Iddm20* Interval

Genes in the *Iddm20* Interval Abbreviations: *idd14*-NON is the interval determined in the NOD × NON cross (14) and *idd14*B6 is the interval determined in the NOD × B6 crosses and congenic (15). The rat *Iddm20* supported interval ± 1 LOD is shown in gray shading. Chr: chromosome; bp: base pairs

Rat Chr	**Rat Chr Start (bp)**	**Description**	**Mouse Chr**	**Mouse Chr Start (bp)**	**Mouse *Idd14* region**	**Rat *Iddm20***
17	29713540	PAK/PLC-interacting protein 1	13	40443433	idd14-NON	
17	28451133	Zinc finger protein 40 (Alpha A-crystallin-binding 1)	13	41502581	Idd14-NON	
17	28306727	Endothelin-1 precursor (ET-1).	13	41748723	Idd14-NON	
17	27138814	NAD-dependent deacetylase sirtuin 5	13	42822790	Idd14-NON	*Iddm20*
17	27046741	RAN BP9; B cell antigen receptor Ig beta associated p1	13	42855480	Idd14-NON	*Iddm20*
17	26709913	CD83 antigen.	13	43237577	Idd14-NON	*Iddm20*
17		Jumonji protein	13	44287373	Idd14-NON	*Iddm20*
17		Adenylyl cyclase-associated protein 2 (CAP 2).	13	46018959	Idd14-NON	*Iddm20*
17	23821596	Kinesin-like protein KIF13A.	13	46244160	Idd14-NON	*Iddm20*
17	23728906	Malin	13	46507913	Idd14-NON	*Iddm20*
17	23701239	Thiopurine S-methyltransferase	13	46519516	Idd14-NON	*Iddm20*

Rat Chr	Rat Chr Start (bp)	Description	Mouse Chr	Mouse Chr Start (bp)	Mouse *Idd14* region	Rat *Iddm20*
17	23634062	DEK oncogene (DNA binding).	13	46579123	Idd14-NON	*Iddm20*
17		RNP particle component (Fragment).	13	47794113	Idd14-NON	*Iddm20*
17	21994289	Homeobox protein BarH-like 1.	13	48158986	idd14-NON	*Iddm20*
17	21806043	PHD finger protein 2 (GRC5).	13	48298466	idd14B6	*Iddm20*
17	21410701	Ninjurin 1 (Nerve injury-induced protein 1).	13	48685927	idd14B6	*Iddm20*
17	21015178	Osteomodulin precursor (Osteoadherin) (OSAD)	13	49088364	idd14B6	*Iddm20*
17	19659967	Sphingosine 1-phosphate receptor Edg-3	13	50434260	idd14B6	*Iddm20*
17	19520647	SHC transforming protein 3 (SH2 domain protein C3)	13	50457472	idd14B6	*Iddm20*
17	19423770	Cyclin-dependent kinases regulatory subunit 2 (CKS-2).	13	50672083	idd14B6	*Iddm20*
17	19352632	Semaphorin 4D precursor	13	50728006	idd14B6	*Iddm20*
17	19230896	GADD45 gamma (Cytokine responsive protein)	13	50873436	idd14B6	*Iddm20*
17	18443785	Tyrosine-protein kinase SYK (Spleen)	13	51630629	idd14B6	*Iddm20*
17	18106038	nuclear factor, interleukin 3, regulated.	13	52003799	idd14B6	
17	17736908	Tyrosine-protein kinase ROR2 precursor 2.	13	52145865	idd14B6	
17	17243262	Homeobox protein MSX-2 (Hox-8.1).	13	52506491	idd14B6	
17	16655985	D(1A) dopamine receptor.	13	53096324	idd14B6	
17	16497967	Histamine H2 receptor (H2R)	13	53258774	idd14B6	
17	16094098	ADP-ribosylation factor-like 10A	13	53665747	idd14B6	
17	15595893	Retinoid × receptor interacting protein 110.	13	54118171	idd14B6	
17	15512144	Fibroblast growth factor receptor 4 precursor	13	54244110	idd14B6	
17	15353521	Ras-related protein Rab-24 (Rab-16).	13	54409106	idd14B6	
17	15350379	P×19-like protein.	13	54409802	idd14B6	
17	15346165	Max dimerization protein 3.	13	54414783	idd14B6	
17	15313303	Vesicular integral-membrane protein VIP36 precursor	13	54432976	idd14B6	
17	15222027	Regulator of G-protein signaling 14 (RGS14)	13	54459599	idd14B6	
17	15261698	Profilin III.	13	54504680	idd14B6	
17	15251796	coagulation factor XII (Hageman factor)	13	54507774	idd14B6	
17	15194740	Drebrin (Developmentally regulated brain protein).	13	54563349	idd14B6	
17	15147111	DEAD (Asp-Glu-Ala-Asp) box polypeptide 41	13	54627408	idd14B6	
17	15033873	Calcium-signal modulating cyclophilin ligand (CAML).	13	54719996	idd14B6	
17	14834123	Pituitary homeobox 1 (Pt×1).	13	54922627	idd14B6	
17	14286947	Small inducible cytokine B14 precursor (CXCL14)	13	55389282	idd14B6	
17	14068757	Interleukin-9 precursor (IL-9) (T-cell growth factor P40)	13	55582311	idd14B6	
17	13995183	Leukocyte cell-derived chemotaxin 2 precursor	13	55645800	idd14B6	
17	13905503	transforming growth factor, beta induced 68kd	13	55712518	idd14B6	
17	13814377	Mothers against decapentaplegic homolog 5 (SMAD5]	13	55808625	idd14B6	
17	13661202	Short transient receptor potential channel 7 (TrpC7)	13	55879544	idd14B6	

References

1. Hawa MI, Beyan H, Buckley LR, Leslie RDG. Impact of genetic and non-genetic factors in type 1 diabetes. Am J Med Genet 2002;115:8–17. [PubMed: 12116172]

2. Åkerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M. Environmental factors in the etiology of type 1 diabetes. Am J Med Genet 2002;115:18–29. [PubMed: 12116173]
3. Anjos S, Polychronakos C. Mechanisms of genetic susceptibility to type I diabetes: beyond HLA. Mol Genet Metab 2004;81:187–195. [PubMed: 14972324]
4. Yoon, JW.; Jun, HS. Role of viruses in the pathogenesis of type 1 diabees mellitus. In: LeRoith, D.; Taylor, SI.; Olefsky, JM., editors. Diabetes mellitus. A fundamental and clinical text. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 575-590.
5. Mordes JP, Bortell R, Blankenhorn EP, Rossini AA, Greiner DL. Rat models of type 1 diabetes. Genetics, environment, and autoimmunity. ILAR Journal 2004;45:278–291. [PubMed: 15229375]
6. Martin AM, Blankenhorn EP, Maxson MN, Zhao M, Leif J, Mordes JP, Greiner DL. Non-major histocompatibility complex-linked diabetes susceptibility loci on chromosomes 4 and 13 in a backcross of the DP BB/Wor rat to the WF rat. Diabetes 1999;48:50–58. [PubMed: 9892222]
7. Guberski DL, Thomas VA, Shek WR, Like AA, Handler ES, Rossini AA, Wallace JE, Welsh RM. Induction of type 1 diabetes by Kilham's rat virus in diabetes resistant BB/Wor rats. Science 1991;254:1010–1013. [PubMed: 1658938]
8. Zipris D, Hillebrands JL, Welsh RM, Rozing J, Xie JX, Mordes JP, Greiner DL, Rossini AA. Infections that induce autoimmune diabetes in BBDR rats modulate $CD4^{+}CD25^{+}$ T cell populations. J Immunol 2003;170:3592–3602. [PubMed: 12646622]
9. Martin AM, Maxson MN, Leif J, Mordes JP, Greiner DL, Blankenhorn EP. Diabetes-prone and diabetes-resistant BB rats share a common major diabetes susceptibility locus, *iddm4*: Additional evidence for a “universal autoimmunity locus” on rat chromosome 4. Diabetes 1999;48:2138–2144. [PubMed: 10535446]
10. Mordes JP, Leif J, Novak S, DeScipio C, Greiner DL, Blankenhorn EP. The *iddm4* locus segregates with diabetes susceptibility in congenic WF.*iddm4* rats. Diabetes 2002;51:3254–3262. [PubMed: 12401717]
11. Hornum L, DeScipio C, Markholst H, Troutman SA, Novak S, Leif J, Greiner D, Mordes JP, Blankenhorn EP. Comparative mapping of rat Iddm4 to segments on HSA7 and MMU6. Mamm Genome 2004;15:53–61. [PubMed: 14727142]
12. Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Teuscher C. Identification of genetic loci controlling the characteristics and severity of brain and spinal cord lesions in experimental allergic encephalomyelitis. Am J Pathol 2000;157:637–645. [PubMed: 10934166]
13. Ellerman KE, Richards CA, Guberski DL, Shek WR, Like AA. Kilham rat virus triggers T-cell-dependent autoimmune diabetes in multiple strains of rat. Diabetes 1996;45:557–562. [PubMed: 8621003]
14. Brodnicki TC, Quirk F, Morahan G. A susceptibility allele from a non-diabetes-prone mouse strain accelerates diabetes in NOD congenic mice. Diabetes 2003;52:218–222. [PubMed: 12502517]
15. McAleer MA, Reifsnyder P, Palmer SM, Prochazka M, Love JM, Copeman JB, Powell EE, Rodrigues NR, Prins JB, Serreze DV, DeLarato NH, Wicker LS, Peterson LB, Schork NJ, Todd JA, Leiter EH. Crosses of NOD mice with the related NON strain - A polygenic model for IDDM. Diabetes 1995;44:1186–1195. [PubMed: 7556956]
16. Elly C, Witte S, Zhang Z, Rosnet O, Lipkowitz S, Altman A, Liu YC. Tyrosine phosphorylation and complex formation of Cbl-b upon T cell receptor stimulation. Oncogene 1999;18:1147–1156. [PubMed: 10022120]
17. Yokoi N, Komeda K, Wang HY, Yano H, Kitada K, Saitoh Y, Seino Y, Yasuda K, Serikawa T, Seino S. *Cblb* is a major susceptibility gene for rat type 1 diabetes mellitus. Nature Genet 2002;31:391–394. [PubMed: 12118252]
18. Brown DW, Welsh RM, Like AA. Infection of peripancreatic lymph nodes but not islets precedes Kilham rat virus-induced diabetes in BB/Wor rats. J Virol 1993;67:5873–5878. [PubMed: 8371347]
19. McKisic MD, Paturzo FX, Gaertner DJ, Jacoby RO, Smith AL. A nonlethal rat parvovirus infection suppresses rat T lymphocyte effector functions. J Immunol 1995;155:3979–3986. [PubMed: 7561106]
20. DeClercq E. Interferon induction by polynucleotides, modified polynucleotides, and polycarboxylates. Methods Enzymol 1981;78:227–235. [PubMed: 6173595]

21. Akiyama Y, Stevenson GW, Schlick E, Matsushima K, Miller PJ. Differential ability of human blood monocyte subsets to release various cytokines. J Leukocyte Biol 1985;37:519–530. [PubMed: 2984302]

22. Fresa KL, Korngold R, Murasko DM. Induction of natural killer cell activity of thoracic duct lymphocytes by polyinosinic-polycytidylic acid (poly(I:C)) or interferon. Cell Immunol 1985;91:336–343. [PubMed: 2581698]

23. Turner W, Chan SP, Chirigos MA. Stimulation of humoral and cellular antibody formation in mice by poly I:C. Proc Soc Exp Biol Med 1970;133:334–338. [PubMed: 4904563]

24. Davis CT, Blankenhorn EP, Murasko DM. Genetic variation in the ability of several strains of rats to produce interferon in response to polyriboinosinic-polyribocytodilic acid. Infect Immun 1984;43:580–583. [PubMed: 6198280]

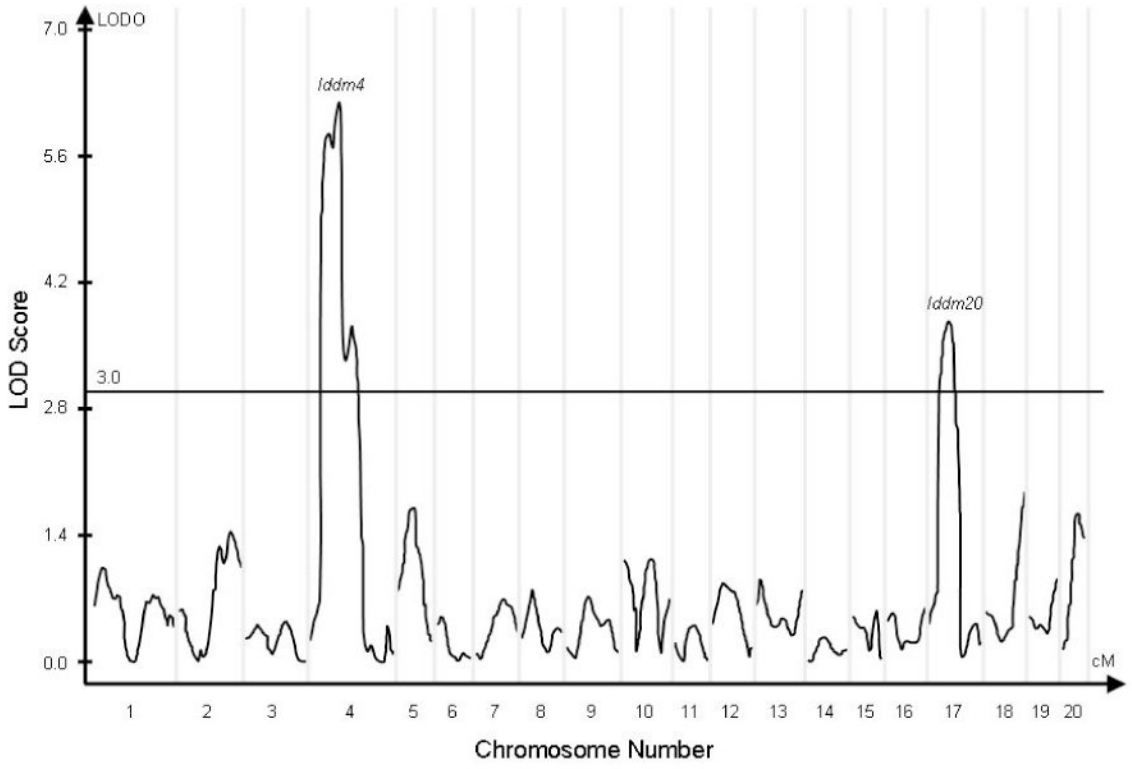


Figure 1.

Composite interval analysis of linkage to the diabetes phenotype in 190 (WF × BBDR)F2 rats. Significant markers were first chosen using a linear regression model with a forward/backward selection procedure in the SRmapqtl module of QTL Cartographer. Markers flanking the test interval were added to the regression model to control for the presence of linked QTL. LOD scores are displayed as peaks over the entire genome, with chromosomes 1 through 20 displayed on the X axis. The curves are discontinuous, and the vertical gray lines delimit each individual chromosome and its associated curve. The horizontal reference line identifies the standard LOD=3 cutoff. The only peaks that significantly exceed this standard are on chromosome 4 (*Iddm4*) and on chromosome 17 (*Iddm20)*.

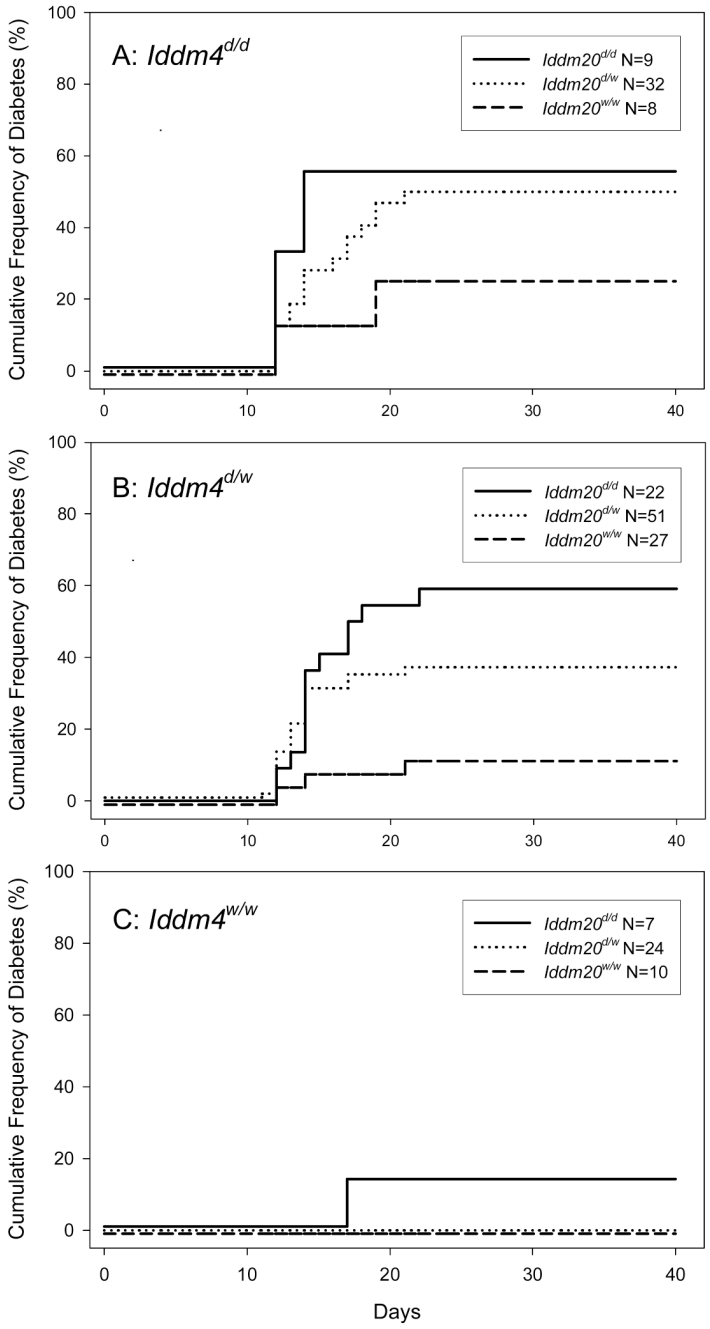


Figure 2.

Kaplan Meier analysis of the cumulative frequency of autoimmune diabetes by inheritance of BBDR-origin diabetes susceptibility loci on chromosomes 4 and 17 in 190 (WF × BBDR)F2 rats. Time on the horizontal axis is in days after infection with KRV. Panel A presents data for F2 rats homozygous for the BBDR-origin allele of *Iddm4*; panel C presents data for F2 rats homozygous for the WF-origin allele of *Iddm4*; panel B present data for the heterozygotes. Within each panel, individual curves show data for subgroups expressing the indicated BBDR- and WF-origin alleles of *Iddm20*. The number of rats in each subgroup is indicated in the figure. Overall, 59 of 190 F2 animals (31%) became diabetic. Among the diabetic rats, 98% (58 of 59) expressed at least one BBDR-origin allele of *Iddm4*, and of these 91% (53 of 58) expressed

at least one BBDR-origin allele of *Iddm20*. Among all rats homozygous for the WF-origin allele of *Iddm20*, 11% (5 of 45) became diabetic and all 5 expressed at least one BBDR-origin allele of *Iddm4*. Overall statistical analysis of the entire dataset for the effect of *Iddm4* genotype stratified by *Iddm20* genotype was statistically significant (log rank = 20.22, df=2, $p<0.0001$). Similarly, analysis for the effect of *Iddm20* genotype stratified by *Iddm4* genotype was statistically significant (log rank = 14.03, df=2, $p<0.001$). These data are shown in tabular form in Appendix, Table 3.

Table 1

Frequency of Diabetes in Rats Treated with KRV, Poly I:C, and Treg Depletion

Frequency of induced diabetes. Male and female rats were entered into the indicated treatment protocols when 21-28 days old. In groups 1 and 2, KRV-UMass was given intraperitoneally at a dose of 10^7 PFU. In group 2, poly I:C was given at a dose of 1 μg/g body weight intraperitoneally on days -3, -2, and -1 relative to KRV. In group 3 poly I:C (1 μg/g) was given 3 times/week for 40 days and anti-ART2.1 mAb (25 μg) was given 5 times/week for 40 days as described (9). Rats in group 4 received only poly I:C (1 μg/g) for 3 consecutive days. Diabetes was defined as a plasma glucose concentration > 11.1 mM (250 mg/dl). The WF.*Iddm4*^{d} congenic rats were from the N6 generation and bear ~2.8 cM of the genetically dominant BBDR rat-derived *Iddm4* region on chromosome 4 and at least one BBDR-origin "a" allele of the ART2 T cell alloantigen on chromosome 1 (10). Diabetic rats in all groups had severe insulitis or "end-stage" islets.

Groups ***Protocols*** ***Rat Strains***	**1** **KRV**	**2** **KRV + poly I:C**	**3** **Anti-ART2.1 mAb + poly I:C**	**4** **Poly I:C alone**
BBDR	11/27	27/27	12/12	0/6
WF	0/3	0/8	3/53	—
WF.*Iddm4*^{d} (N6)	0/4	0/18	7/12	—
(BBDR × WF)F1	0/6	5/13	11/11	—
(WF × BBDR)F2	—	59/190	—	—

Table 2

Frequency of Diabetes in (BBDR × WF)F2 Rats as a Function of *Iddm4* Genotype

All (BBDR × WF)F2 rats in Table 1 were genotyped as described in Methods. They are grouped according to the presence of the WF and BBDR alleles of the microsatellite marker *D4Arb9* used as the genotype for *Iddm4* as described (10). Overall $chi^2 = 22.2$, df=2, $p<0.001$.

***Iddm4* Genotype**	**N**	**Number Diabetic (%)**	
BBDR/BBDR	49	23	(47%)
BBDR/WF	100	35	(35%)
WF/WF	41	1	(2%)