Brief Genetics Report

A Susceptibility Allele From a Non–Diabetes-Prone Mouse Strain Accelerates Diabetes in NOD Congenic Mice

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The nonobese diabetic (NOD) mouse is genetically predisposed for the spontaneous development of type 1 diabetes. Linkage analyses have identified at least 19 susceptibility loci (*Idd1– Idd19*) that contribute to disease pathogenesis in which lymphocytes mediate the specific destruction of insulin-producing β-cells. Interestingly, nondiabetic mouse strains have been shown to confer susceptibility alleles to affected progeny in NOD outcrosses for some of the *Idd* loci. In particular, we noted that diabetic backcross progeny, derived from NOD and C57BL/6 (B6) mouse strains, demonstrated increased heterozygousity for an interval encompassing *Idd14* on chromosome 13. This result suggested that B6 mice harbor a more diabetogenic allele(s) than NOD mice for this locus. To confirm this observation, a NOD congenic mouse strain, containing a B6-derived interval covering the majority of chromosome 13, was generated. Adding to the combination of already potent susceptibility alleles elsewhere in the NOD genome, the chromosome 13 B6-derived interval was able to increase the overall risk of developing type 1 diabetes, which resulted in an earlier onset and increased incidence of type 1 diabetes in congenic mice as compared with NOD mice. Furthermore, this B6-derived interval, in combination with the NOD genetic background, was able to overcome environmental conditions that typically suppressed type 1 diabetes in the NOD mouse strain. *Diabetes* 52:218–222, 2003

The nonobese diabetic (NOD) mouse spontaneously develops glycosuria and hyperglycemia due to increasing hypoinsulinemia, which leads to weight loss and increased urination. These symptoms result from an autoimmune response that selectively targets insulin-producing β-cells for destruction (reviewed in 1). Due to the similarity of disease pathogenesis between this mouse strain and human patients with type 1 diabetes, the NOD mouse has become one of the principal animal models for type 1 diabetes. In parallel to human genetic studies for type 1 diabetes, linkage analyses have been performed using selective mating between NOD mice and nondiabetic mouse strains (2–4). To date, at least 19 different susceptibility loci (termed *Idd1–Idd19*) found on 14 different chromosomes have been linked to diabetes in the mouse (reviewed in 5).

Confirmation of linkage is best achieved using congenic mouse strains that also allow further characterization and the eventual identification of the actual gene contributing to type 1 diabetes susceptibility. Congenic mouse strains are typically generated by controlled mating of resistant and susceptible mouse strains to introduce donor-derived chromosomal intervals that carry the resistance gene onto the susceptible genetic background of the recipient strain. Individual NOD congenic mouse strains have been generated for intervals within chromosomes 1, 2, 3, 4, 6, 11, and 17, confirming a number of the loci previously linked to type 1 diabetes (reviewed in 5). At least five of these loci have now been dissected into separate contributing intervals using recombinant congenic mouse strains, demonstrating that preliminary linkage is often due to combined effects of closely linked genes with partial contribution to disease progression (6–12).

It has become apparent from genetic studies that the NOD genome has not accumulated a set of rare mutations that predispose these mice to type 1 diabetes. More likely, the NOD mouse strain maintains an unfavorable combination of some rare diabetogenic alleles (e.g., *H2-Ab^{g7}*) along with common alleles at a number of other genes (e.g., *H2-E$_\alpha$* and *β2m*) that increase the risk for developing type 1 diabetes (13–15). This model is supported by outcrosses generated with the NOD mouse strain and nondiabetic mouse strains. Typically, the nondiabetic strain contributes resistance alleles for *Idd* loci, and these loci are detected by segregation analysis of affected outcross progeny (2–4). However, certain mouse strains may share a susceptibility allele with the NOD mouse for an *Idd* locus and, therefore, segregation of that locus may not be identified in an outcross with a particular strain, but instead may be identified in outcrosses with other strains. For example, genetic analysis of backcross or F2 mice, using NOD and nonobese normal (NON).*H2^{g7}* mouse strains, did not detect linkage to *Idd4*, *Idd5*, *Idd8*, or *Idd12* (previously found in NOD backcrosses with C57BL/10.*H2^{g7}* or C57BL/6) (2–4). Perhaps more intriguingly, a

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SPF, specific pathogen free.

TABLE 1
Increased heterozygosity in diabetic backcross mice

Marker	cM*	Diabetic mice Ho:He
D13Mit139	32	2:8
D13Mit65	36	1:9
D13Mit11	40	2:8

*Distances were obtained from the Mouse Genome Database (www.informatics.jax.org/).

nondiabetic mouse strain may contribute an *Idd* allele that is more diabetogenic than the NOD allele. At least three loci (*Idd6*, *Idd7*, and *Idd8*) have been mapped in which the allele from the nondiabetic mouse strain is associated with diabetes susceptibility in outcross analyses. In particular, C57BL/10 and NON mouse strains were shown to contribute susceptibility alleles for *Idd7* based on linkage analysis of backcross or F2 mice, whereas homozygosity for the NOD allele at this locus appeared to be associated with decreased risk of disease (2,4). Another backcross study using the BDC2.5 T-cell receptor transgenic mouse model of diabetes also demonstrated linkage to chromosome 7 (overlapping the *Idd7* locus), as well as three other chromosomes (chromosome 1, 2, and 15), and indicated that C57BL/6 (B6) parental mice conferred susceptibility alleles to affected progeny (16). Taken together, these various genetic studies suggest the presence within the *Mus* species of common susceptibility alleles for genes outside the "NOD subset," which can form different allelic combinations that still cause and possibly increase the risk for type 1 diabetes. However, no congenic mouse strain has been used to directly confirm a type 1 diabetes susceptibility allele derived from a non–diabetes-prone mouse strain.

With this in mind, we initially observed increased heterozygosity for an interval on chromosome 13 in a previously generated cohort of diabetic backcross mice generated from selective mating of NOD mice with nondiabetic B6 mice (Table 1) (3). This observation suggested to us that the NOD allele was associated with a decreased risk for type 1 diabetes, whereas B6 mice harbor a susceptibility allele that appeared dominant. However, this result was not statistically significant due to the small number of mice analyzed; therefore, the genotypes were not reported in the earlier study (3). Given this observation, we inspected the previously reported genotypes from cohorts of diabetic backcross mice, generated from NOD and B10.$H2^{g7}$, and noted the increased heterozygosity for chromosome 13 in these mice, but again, this was not statistically significant (2,17). Coincidentally, Gonzalez et al. (16) also detected linkage to chromosome 13 in their backcross analysis mentioned above, but this linkage was due to the integration of the BDC2.5 T-cell receptor transgene on chromosome 13 and would have masked the detection of a B6 susceptibility allele(s). In support of this region, McAleer et al. (4) did obtain linkage to mouse chromosome 13 between, and including, *D13Mit61* (~22 cM) and *D13Mit9* (~45 cM) and designated this susceptibility locus *Idd14*. Linkage to type 1 diabetes was found in this region by genetic analysis of a cohort of diabetic mice generated from an intercross using NOD and NON mouse strains. In contrast to the backcrosses described above, the genotypes from these diabetic F2 mice demonstrated a significant increase in NOD homozygosity and a decrease in NON homozygosity for markers on chromosome 13. This result suggested that the NOD chromosome was associated with an increased risk for type 1 diabetes. While localization of the *Idd14* locus is still defined within a relatively large interval, it appeared that B6 and B10 mouse strains contributed susceptibility alleles for this locus compared with NON and NOD mouse strains, and these alleles could prove to be more diabetogenic in combination with the NOD genome.

TABLE 2
Genetic interval for chromosome 13 congenic mouse strain

Marker	cM*	NOD.B6*Idd14*
D13Mit55	6.0	NOD
D13Mit3	10.0	NOD
D13Mit163	11.0	NOD
D13Mit116	13.0	B6
D13Mit61	22.0	B6
D13Mit139	32.0	B6
D13Mit65	36.0	B6 *Idd14*†
D13Mit11	40.0	B6
D13Mit9	45.0	B6
D13Mit202	47.0	B6
D13Mit36	53.0	B6
D13Mit290	59.0	B6
D13Mit76	61.0	B6
D13Mit151	71.0	B6

*Distances were obtained from the Mouse Genome Database (www.informatics.jax.org/). †Predicted *Idd14* interval (Table 1).

To begin testing the above hypothesis, we generated a NOD congenic mouse strain (NOD.B6*Idd14*) that contained a B6-derived interval for chromosome 13 on the NOD genetic background (Table 2). An interval covering a large portion of the chromosome was maintained to ensure that the disease locus was encompassed, because it was not clear where *Idd14* localized (2–4). After 10 backcross generations, mice that were heterozygous for the chromosome 13 B6-derived interval were intercrossed to generate congenic mouse strains that were B6 homozygous for markers encompassing *Idd14* (Table 2). A cohort of NOD.B6*Idd14* females was monitored for spontaneous diabetes in a conventional animal facility. These congenic female mice demonstrated an earlier onset of disease and a significant increase ($P < 0.002$) in the incidence of diabetes compared with NOD females (Fig. 1). This result confirmed the relatively weak linkage of chromosome 13 to type 1 diabetes in the previous backcross (3) and that B6 mice carry a susceptibility allele(s) within the interval encompassing *Idd14*. As the congenic interval covers most of chromosome 13, the *Idd14* effect may be due to more than one susceptibility gene. To address this possibility and localize *Idd14* more precisely, additional congenic mouse strains selected for smaller B6-derived subintervals will be generated and tested. If the magnitude of the *Idd14* effect is maintained in subsequent recombinant congenic mouse strains, then it should become feasible to fine map this locus.

It might be noted that the NOD control females in this experiment achieved a relatively low cumulative incidence of diabetes (~65%) (Fig. 2) compared with the typical range (70–80%) reported by other labs (18). While this reduction was not large, it reflects the fact that mice in this

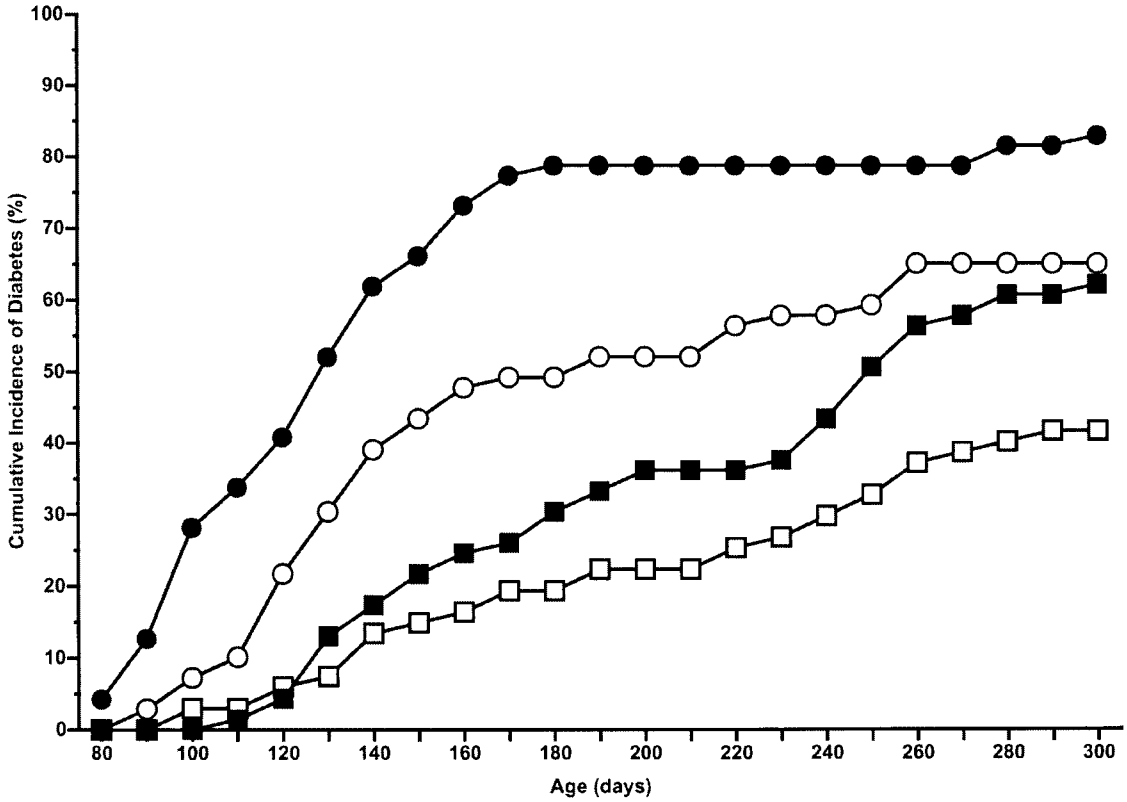

FIG. 1. Increased frequency of diabetes in male and female NOD.B6*Idd14* congenic mice compared with male and female NOD/Lt mice in a conventional mouse room facility. The cumulative incidence of diabetes was observed in age-matched female NOD.B6*Idd14* (●; $n = 72$) and NOD/Lt mice (○; $n = 65$) and male NOD.B6*Idd14* (■; $n = 69$) and NOD/Lt mice (□; $n = 67$). Male and female congenic mice were homozygous for the B6-derived chromosome 13 interval (Table 2). The incidence of diabetes was significantly more ($P < 0.002$) in NOD.B6*Idd14* females than in NOD/Lt females. The incidence of diabetes was significantly more ($P < 0.03$) in NOD.B6*Idd14* males than in NOD/Lt males, but did not achieve a similar diabetes incidence as that observed in NOD.B6*Idd14* or NOD/Lt females.

experiment were monitored in a conventional animal facility. Similar to others, we have observed (over the past 4 years) a consistent difference for diabetes incidence between NOD females housed in conventional and specific pathogen-free (SPF) animal facilities (18,19) (Fig. 2). This decreased incidence observed in conventional animal facilities has been mainly attributed to higher levels of microbial pathogens not present in SPF conditions that may infect NOD mice before disease pathogenesis begins and suppress the onset of disease. A number of different bacterial and viral pathogens have been shown to prevent or delay the onset of type 1 diabetes in NOD mice (reviewed in 20). It has been suggested that this protective effect is due to general immunostimulation in prediabetic mice, which reduces the penetrance of NOD susceptibility alleles. These susceptibility alleles may be responsible for defects observed in the NOD mouse for antigen-presenting cells and/or development of regulatory cells necessary for peripheral tolerance of autoreactive T-cells (5,21,22). Therefore, it was somewhat unexpected that NOD.B6*Idd14* female mice, housed in a conventional animal facility, demonstrated an earlier disease onset and a significant increase ($P < 0.002$) in cumulative diabetes incidence, even compared with NOD mice housed in SPF conditions (Fig. 2), although other conditions (e.g., diet, temperature) were the same. While the actual *Idd14* gene(s) on chromosome 13 is not known, it may be that the B6 allele(s) allows for increased penetrance of the NOD susceptibility alleles regardless of environmental factors and/or the B6 allele contributes to a further defect in peripheral tolerance in combination with the NOD genome.

It has also been well noted that there is a sex bias for the onset of type 1 diabetes, as NOD females have a higher incidence of spontaneous disease than NOD males (18). Similar to other NOD mouse colonies around the world, we also observed a significantly higher incidence of diabetes ($P < 0.002$) in female NOD mice than in age-matched NOD males (Fig. 1). To determine whether the B6-derived chromosome 13 interval could affect this sex bias for type 1 diabetes between NOD males and females, we also monitored the incidence of diabetes in a cohort of age-matched NOD.B6*Idd14* males. Unlike NOD.B6*Idd14* females, NOD.B6*Idd14* males did not exhibit an earlier onset of disease but did have a significant increase ($P < 0.03$) in the incidence of diabetes compared with age-matched NOD males (Fig. 1), and their type 1 diabetes cumulative incidence was almost equivalent to that of NOD females. However, the B6-derived interval did not overcome the sex bias for type 1 diabetes incidence because NOD.B6*Idd14* females had a significant increase ($P < 0.001$) in type 1 diabetes incidence compared with NOD.B6Idd14 males (Fig. 1). Regardless of this sex bias, the NOD.B6*Idd14* strain demonstrated that an allele(s) from a nondiabetic mouse strain can be added to the NOD complement of diabetogenic alleles to increase the overall risk of developing type 1 diabetes.

In general, the NOD genetic background provides a

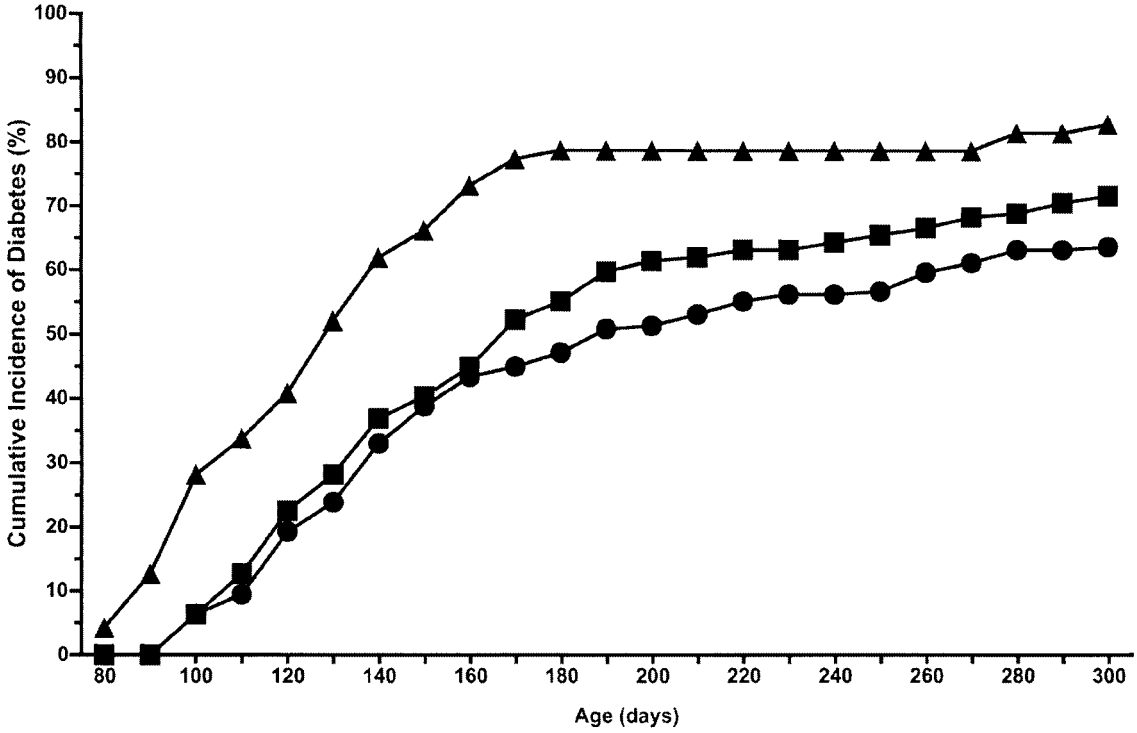

FIG. 2. Comparison of diabetes frequency between SPF and conventional mouse room facilities. From 1998 to 2002, we monitored three separate cohorts of NOD/Lt females in the Institute's SPF facility (■) and three separate cohorts of NOD/Lt females in the Institute's conventional mouse room facility (●). The cumulative diabetes incidence curves represent the combined data from each of these three separate experiments in either the SPF (n = 175) or conventional animal facility (n = 190). The diabetes incidence curve from Fig. 1 for NOD.B6*Idd14* females (▲; n = 71) is placed on this graph for comparison purposes. NOD.B6*Idd14* females have a significantly higher ($P < 0.002$) incidence of diabetes than historical NOD/Lt female control mice from either SPF or conventional animal facilities.

genomic standard for assessing alleles derived from non–diabetes-prone mouse strains with regards to their potential for contributing to type 1 diabetes onset. Outcrosses and congenic mouse strains allow for identification of allelic subsets that confer resistance or susceptibility. Initially, the NON mouse strain was identified as having a resistance allele compared with NOD mice for the locus designated *Idd14* on chromosome 13 (4). In contrast, our congenic mouse strain confirmed that a B6-derived interval encompassing the *Idd14* locus could actually contribute a more diabetogenic allele than the equivalent NOD interval. Unlike the B6 mouse strain, it would have been disadvantageous for the original selection and survival of the NOD mouse strain if it did harbor the same susceptibility allele for this locus in addition to its other diabetes susceptibility alleles (note: successful generation and maintenance of the NOD.B6*Idd14* congenic mouse strain required mating of females at an early breeding age). Although the evidence is circumstantial, it appears that the NOD allele for the *Idd14* locus provides significant protection when compared with the B6 allele, but is more susceptible than the NON allele. If these NON and B6 effects are attributable to the same gene, then *Idd14* may provide the basis for studying an allelic series (NON, NOD, B6) underlying a susceptibility gradient for a type 1 diabetes gene. *Idd6*, located on chromosome 6, presents similar evidence for another susceptibility gradient. Based on linkage analysis of outcrosses, the NOD allele appears to be more susceptible than the B10 allele, but more resistant than the NON allele (2,4). In both cases, the actual type 1 diabetes gene(s) needs to be identified to verify these potential susceptibility gradients. If such diabetes susceptibility gradients exist in mice, then similar allelic variation for type 1 diabetes loci within the human population may be likely.

RESEARCH DESIGN AND METHODS

NOD/Lt (NOD) and C57BL/6 (B6) mouse strains were obtained from The Walter & Eliza Hall Institute SPF facilities. NOD females in our conventional facilities typically have a cumulative diabetes incidence between 60 and 65% and, in our SPF facilities, between 70 and 75% by 300 days of age; B6 females do not develop diabetes. To generate the congenic mouse strain described in this study, B6 females were crossed with NOD males. (NOD × B6)F1 females were then backcrossed to NOD males to generate backcross one generation. Ten subsequent backcrosses were then performed using the appropriate backcross progeny based on genotyping results and using NOD males or females to ensure that the Y chromosome and mitochondrial genome were NOD derived.

DNA samples were extracted from tail biopsies by standard methods and typed with polymorphic markers from chromosome 13 encompassing the *Idd14* region by PCR (4) (Tables 1 and 2). In addition, polymorphic markers flanking previously described susceptibility loci were also screened (*Idd1–19*; rev. in 23). Primers for polymorphic markers were obtained from Research Genetics (Huntsville, AL) and were as previously described (http://www.genome.wi.mit.edu). In general, PCR amplification was performed using ^{32}P radiolabeled primers as described (24). All marker positions and approximate centimorgan distances from the top of chromosome 13 were obtained from the Mouse Genome Database (www.informatics.jax.org/).

Cohorts of female mice were housed in either a conventional mouse facility or an SPF facility and tested once a week for elevated urinary glucose (>110 mmol/l) using Diastix reagent strips for urinalysis (Bayer Australia, New South Wales, Australia) over a 300-day time course. Three consecutive elevated readings indicated the onset of diabetes, at which time the diseased mouse was killed. Pairwise comparisons of diabetes incidence for congenic and NOD mouse strains were done using the log-rank test.

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