NOD × 129. H2g7 Backcross Delineates 129S1/SvImJ-Derived Genomic Regions Modulating Type 1 Diabetes Development in Mice

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OBJECTIVE—Introduction of genes targeted in 129/Sv embryonic stem (ES) cells into NOD mice brings about linked genes that may modulate type 1 diabetes. Our objective was to identify 129S1/SvJ non-MHC regions contributing type 1 diabetes resistance or susceptibility in backcross to NOD/LtJ.

RESEARCH DESIGN AND METHODS—After congenic transfer of the NOD H2g7 haplotype onto 129S1/Sv, 310 females were produced by NOD × (NOD × 129. H2g7)F1 backcross (N2). A genome scan for quantitative trait locus (QTL) affecting clinical diabetes, age of diabetes onset, and insulin sensitivity was performed using subphenotype characteristics to improve power and resolution for detection of diabetes susceptibility loci.

RESULTS—Thirty-six of 310 (11.6%) N2 females developed type 1 diabetes between 14 and 40 weeks. Significant evidence of linkage for only a single previously reported Idd complex locus (Idd10/17/18, chromosome [Chr] 3) was indicated for clinical diabetes. The quantitative traits of insulin resistance either alone or combined with age at type 1 diabetes onset were significantly linked to known Idd regions on Chr 1 (Idd5 region), Chr 4 (Idd9 region), Chr 8 (Idd22), Chr 11 (Idd4.3), and proximal Chr 17 (Idd16 region). Significant 129S1/Sv resistance contributions were identified on Chr 1, 15 (two loci), and 19, with suggestive evidence for additional novel 129/Sv resistance QTL on Chr 5 and 17 and susceptibility on Chr 2.

CONCLUSIONS—The 129S1/SvJ genome harbors collections of both known and potentially novel non-MHC Idd loci. Investigators targeting 129/Sv genes mapping within chromosomal regions reported herein or elsewhere in the genome need to exclude potential contributions from linked Idd loci by generating a NOD.129 control strain expressing the nontargeted allele.

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Gene targeting in embryonic stem (ES) cells represents a powerful molecular genetic tool for interrogating the role of specific genes in the development of type 1 diabetes in the NOD mouse. Although pure ES cell lines have been generated, most show no to negligible germ-line competency (1–4). Thus, almost all targeted mutations reported have been generated in fully germ-line competent ES cell lines derived from various 129/Sv substrains. This presents a problem. Whenever a gene is targeted in 129/Sv ES cells and then introduced into the NOD mouse genetic background, variable numbers of linked genes are inadvertently introduced as well. In the Type 1 Diabetes Resource at The Jackson Laboratory (http://type1diabetes.jax.org/index.html), almost one-third of the NOD stocks in this repository represent congenics carrying introduced 129/Sv genome proximal and distal to the targeted allele. When an alteration in type 1 diabetes incidence is observed in such a congenic stock, the formal possibility exists that some or all of the effect is contributed not by the loss-of-function allele but, rather, by a linked gene or genes. Previous outcrosses between NOD and stocks of C57BL/10J or NON/LtJ mice for which genomes were previously “conditioned” by prior congenic introduction of the NOD’s diabetogenic H2g7 MHC haplotype have permitted identification of major non-MHC Idd diabetes modifier loci from these resistant strains (5). Despite the large numbers of NOD stocks congenic for 129/Sv genomic segments, no comparable search for 129/Sv-contributed non-MHC Idd loci has been undertaken. Our objective was to identify such chromosomal regions derived from 129S1/SvJ, a substrain for which ES cells are commonly used for gene targeting, that contribute type 1 diabetes resistance or susceptibility in a dominant or additive fashion in backcross to NOD/LtJ.

RESEARCH DESIGN AND METHODS

Pedigreed NOD/ShiLtJ and 129S1/SvImJ mice were obtained from The Jackson Laboratory. All mice were maintained at high specific pathogen-free conditions on a 6% fat diet (irradiated Lab Diet 5LG4; PMI, Brentwood, MO) and acidified water. In outcrosses of NOD to MHC-disparate strains, homozygosity at 142 polymorphic non-MHC SNP markers (supplementary Table A1, available in an online-only appendix at http://diabetes.diabetesjournals.org/cgi/content/full/dc09-0120/DC1) were selected as breeders. At N7, the Chr 17 congenic interval spanned NOD markers at D17Mit175 (32 Mb) proximal and D17Mit33 (34.8 Mb) distal, with all non-MHC markers confirmed as homozygous. This stock is currently at N10 and designated 129.NOD- (D17Mit175-H2g7)F1. MHC homozygous sib matings generated 129.H2g7 homozygous male outcross to NOD. Small cohorts of (NOD × 129. H2g7)F1 and F2 mice were aged to 28 and 40 weeks, respectively. The complete absence of type 1 diabetes and insulin in F1 mice and the absence of type I diabetes and rarity of insulin in F2 mice necessitated a backcross strategy. A total of 310 females were produced by NOD × (NOD × 129.H2g7)F1 backcross (N2) and quantitative trait locus (QTL) analysis completed for the phenotypes of clinical diabetes, age of diabetes onset, and insulin severity/extent in nondiabetic animals aged to 40 weeks. Clinical diabetes was assessed by two strongly positive glycosuria readings (Diastix, Bayer) 1 week after congenic transfer of the NOD's H2g7 haplotype onto 129S1/Sv.
apart. Insulitis severity/extent in nondiabetic females surviving to 40 weeks was assessed semiquantitatively in three nonoverlapping sections of aldehyde fuchsin/hematoxylin-eosin–stained pancreata. A scale of 0 (no insulitis, normal β-cell mass) to 4 (only small islet residua with no surviving aldehyde fuchsin staining β-cells, a score characterizing all clinically diabetic females) in 0.5 increments was used. We have previously shown that the information content of another quantifiable phenotype limited to the diabetic probands, their age at diabetes onset, could be combined with the insulitis severity/extent phenotypic score to increase the power of QTL analysis (6). Week of diabetes onset was converted to a similar 0–4 scale covering 27 weekly sampling intervals from 14 weeks (first diabetic mouse) to 40 weeks (last diabetic mouse), with each interval weighted at 0.148 units. Thus, earliest onset time (14 weeks) received maximum score (27 × 0.148 = 4.0), whereas latest onset time (40 weeks) received only one 0.148 increment. When diabetes onset score is combined with the diabetic 4.0 insulitis score, the two variables provided a continuous range of values between 4.148 and 8, reflecting diabetes progression over time.

DNA from tail and spleen was typed using 146 single nucleotide polymorphisms (SNPs) giving 30–30 Mb coverage. An additional 9–24 SNPs were added within each QTL region to increase coverage to 2–5 Mb, for a final total of 313 SNPs genotyped (supplementary Table A2). All SNP typing was performed by KBioscience (Hoddesdon, U.K.). A three-step QTL analysis was performed in R/qtl (7) to search for main effects and pair-wise epistatic interactions followed by fitting a multiple regression model, as described in detail previously (6). Those QTLs meeting or exceeding a 95% genome-wide adjusted threshold assessed by 1,000 permutation analysis (logarithm of odds [LOD] ≥ 2.8) were called significant; those meeting or exceeding a 37% genome-wide adjusted threshold (LOD ≥ 1.8) were called suggestive linkages. Then, simultaneous genome scans for all pairs of markers were implemented to detect epistatic interactions by fitting a two-way ANOVA model with an interaction item as previously described (6). An LOD score contrasting the full model to a null model (with no genomic effects) is computed, and genome-wide significance is established by permutation analysis. A secondary test for the significance of the interaction term is computed only for those pairs that pass the genome-wide screening. A stringent nominal significance level (0.001) is used for interaction test, and only those locus pairs passing both tests are deemed to be interacting. Finally, all the detected main effect and interacting QTLs were used to fit multiple regression models. The ratio of the adjusted R² to its standard error is used for interaction test, and only those locus pairs passing both tests are deemed to be interacting. Finally, all the detected main effect and interacting QTLs were used to fit multiple regression models. The ratio of the adjusted R² to its standard error is used for interaction test, and only those locus pairs passing both tests are deemed to be interacting.

RESULTS AND DISCUSSION

The raw data for this backcross have been deposited online at http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=projects/details&symb=QTL-Leiter1. Of the 310 N2 females aged to 40 weeks, 36 (11.6%) developed clinical diabetes between 14 and 40 weeks of age, with variable insulitis prevalent in females remaining diabetes free out to 40 weeks. For this complex dichotomous trait, suggestive evidence for linkage at two Idd regions previously identified in outcross with the NOD congenic segment. This linkage falls within the Idd3 region at 146 Mb, whereas Idd4.3 region at 142 Mb within the NOD-related AβB/Ut and CTS/Shi strains (8).

Quantitative subphenotypes of a binary disease trait can improve power for mapping common genetic determinants (9). Indeed, of the two quantitative traits analyzed, insulitis score alone or combined with age at diabetes onset (designated “combination trait”), the latter was more robust for identification of significant and suggestive QTL (Fig. 1B and C and summarized in Table 1). On Chr 1, significant evidence for linkage was found at the more distal end (Idd5.4 region) of the previously described Idd5 complex for both traits (10). Genetic complexity across this region was indicated by three 129-derived linkage peaks at 85, 105, and 121 Mb. Suggestive evidence for a novel 129S1/Sv-derived susceptibility Idd region on Chr 2 clearly proximal to the previously described Idd13 region (11) was obtained using the combination trait. This QTL at 77 Mb is near loci encoding two candidate autoantigens: IGRP (69 Mb) and GAD65 (70.4 Mb). The combination trait, but not insulitis alone, showed suggestive linkage at 103.6 Mb on Chr 3. None of the phenotypes screened showed linkage to the Idd9.3/12 region sited more proximally on Chr 3, an expected absence, since this region is shared (identical by descent) between the two strains (12). On Chr 4, both quantitative measures showed significant linkage of markers within the Idd9.2 region at 146 Mb, with the combination trait also showing significant linkage to a marker at 126 Mb (Idd9.17). A 129S1/Sv marker at 31 Mb on Chr 5 just missed the 1.8 LOD cutoff for suggestive linkage to the combination trait. The combination trait showed significant linkage with a marker on Chr 8 overlapping the Idd22 resistance region previously described in outcross with the ALR/Ut strain (13). Both traits showed significant linkage in the Idd4.2 region on Chr 11 as well as to a more proximal region (Idd4.3), where C57L resistance was reported (14). This finding, coupled with the absence of any linkage for the trait of clinical diabetes, might indicate that 129S1/Sv alleles at these loci were primarily involved in retarding leukocyte infiltration rather than affecting rates at which they killed β-cells. Genome scan for both subphenotypes identified two novel 129S1/Sv-derived resistance QTL on Chr 15, with peaks at 31 and 55 Mb. On Chr 17, only the combination trait allowed identification of a significant QTL at 24 Mb, sited above the congeneric MHC region whose upper boundary was marked by the NOD allele at D17Mit175 at 32 Mb. A C57BL/6j-
H1.5

is shown. *Megabase (Mb) positions from NCBI Ensembl Build 37.

53.9Mb) was detected (Fig. 2). Epistasis was indicated by interaction between Chr 15 (at 55.2 Mb) and Chr 17 (at 50 Mb on Chr 19 for both insulitis alone and the combination trait. The interaction between an SNP marker on Chr 17; the inset marks NOD homozygosity (N) or heterozygosity (H) at Chr 17. 129S1/Sv heterozygosity on Chr 17 abrogates NOD homozygosity-conferred susceptibility on Chr 15.

Significant linkage requires LOD ≥2.8; suggestive linkage requires LOD ≥1.8. Percent variance contributed by each locus to the phenotype is shown. *Megabase (Mb) positions from NCBI Ensembl Build 37.

derived resistance allele in this region has also been called "Idd16" (15). A novel 129S1/Sv resistance locus was identified at 50 Mb on Chr 19 for both insulitis alone and the combination trait. Interestingly, an NOD stock congenic for a targeted mutation in the closely linked 129S2/Sv Ins1 gene (52.3 Mb) exhibited a markedly suppressed diabetes incidence (16). Although the protection was attributed to loss of preproinsulin 1 itself because a control congenic carrying the wild-type 129S1/Sv allele did not protect (16), importation of this stock to the Type 1 Diabetes Resource at The Jackson Laboratory revealed a markedly attenuated type 1 diabetes onset in females and almost complete suppression in males (see http://type1diabetes.jax.org/incidence_studies/005352.html). Hence, an additional 129S1/Sv passenger gene near Ins1 or an expression variant of the Ins1 gene itself may also contribute to the resistance observed. We propose that one such recessive locus maps on Chr 17. 129S1/Sv-DERIVED Idd4.3?

-114 NOD 1.72 1.3
-31 NOD 2.90 3.1
-108 NOD 2.09 2.21

Table 1

Summary of genome-wide main effects scan for QTL controlling the combination trait (insulitis with age of diabetes onset) and insulitis only

| Chr | Locus | Peak at Mb* | Susceptibility donor | Combination trait % Variance | Insulitis only % Variance |
|-----|-------|-------------|----------------------|-----------------------------|--------------------------|
| 1   | Idd5.4 region | 85          | NOD                  | 3.62                        | 3.69                     |
| 1   | Idd5.4 region | 105         | NOD                  | 3.42                        | 2.8                      | 2.75                     | 5.4                     |
| 1   | Idd5.4 region | 121         | NOD                  | 3.41                        |                          |                          | 3.14                     |
| 2   | Novel      | 77          | 129                  | 2.17                        |                          |                          | 2.8                      |
| 3   | Idd10/17/18 | 104         | NOD                  | 2.24                        |                          |                          | 4.6                      |
| 4   | Idd9.1?    | 126         | NOD                  | 2.95                        |                          |                          |                          |
| 4   | Idd9.2     | 146         | NOD                  | 4.28                        |                          |                          | 4.8                      | 2.76                     | 4.7                     |
| 5   | Novel      | 31          | NOD                  | 1.72                        |                          |                          | 1.3                      |
| 6   | Idd22      | 108         | NOD                  | 2.90                        |                          |                          | 3.1                      |
| 11  | Idd4.3?    | 42          | NOD                  | 2.09                        |                          |                          | 2.21                     |
| 11  | Idd4.2?    | 114         | NOD                  | 3.13                        |                          |                          | 1.7                      | 3.96                     | 3.5                     |
| 15  | Novel      | 31          | NOD                  | 4.11                        |                          |                          | 3.52                     |
| 15  | Novel      | 55          | NOD                  | 3.83                        |                          |                          | 6.2                      | 2.75                     | 3.5                     |
| 17  | Idd16?     | 24          | NOD                  | 2.51                        |                          |                          | 1.1                      |
| 19  | Novel      | 50          | NOD                  | 3.07                        |                          |                          | 1.4                      | 3.84                     | 4.0                     |

Significant linkage requires LOD ≥2.8; suggestive linkage requires LOD ≥1.8. Percent variance contributed by each locus to the phenotype is shown. *Megabase (Mb) positions from NCBI Ensembl Build 37.

Preliminary results from IngenuityPathway analysis indicate nine pathways containing potentially interacting proteins encoded within the two genetic regions, including a direct protein-protein interaction between Ddef1 (Chr 15), a GTPase activator, and Ralbp1 (Chr 17), associated with oxidative stress. Elucidation of such gene × gene interactions in the control of type 1 diabetes is critical toward understanding how an individual’s "genetic architecture" contributes to the development of a complex disease. In summary, we have identified both known and potentially novel Idd loci with additive or dominant effects in the 129S1/Sv genome. These loci in aggregate only account for ~30% of the variance in the combination trait. Because a backcross analysis can only detect additive or dominant contributions, any recessive contributions would be missed. One such recessive locus maps on Chr 10 near D11M187, close to the interferon γ receptor gene (17). Investigators targeting 129S1/Sv resistance QTL on Chr 17 was detected for clinical diabetes at 41 Mb and for the combination trait at 24 Mb, a distinct Chr 17 locus at 54 Mb showed significant interaction with the Chr 15 QTL. This more distal Chr 17 interactive QTL is novel, since it mapped well below either of the two regions in the literature referred to as "Idd16."

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FIG. 2. Significant interaction between an SNP marker on Chr 17 (17-050794277-N) and Chr 15 (15-055111506-N) for the combination trait. The x-axis shows NOD homozygosity (N) or heterozygosity (H) at Chr 17; the inset marks NOD homozygosity (●) or heterozygosity (○) at Chr 15. 129S1/Sv heterozygosity on Chr 17 abrogates NOD homozygosity-conferred susceptibility on Chr 15.
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