Type 1 Diabetes Genes in Rats: Few or Many?

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That type 1 diabetes in humans and in animal models represents a complex genetic disease is universally accepted. Genetic dissection of type 1 diabetes in humans has typically entailed the collection of large numbers of individuals scored on the basis of the presence or absence of clinical disease (case/control subjects). Statistically significant differences in allele frequencies distinguishing the affected subjects from control subjects are used to demonstrate associations. Human major histocompatibility complex (MHC) alleles, primarily but not exclusively within the class II sets of genes, are estimated to contribute up to 50% of genetic risk. Because of the strength of the MHC contribution and the genetic heterogeneity extant in humans, identification of non-MHC susceptibility loci by genome-wide association has required the accumulation of large numbers of case/control populations. Recent studies have identified up to 10 of such non-MHC susceptibility regions under conditions where multiple HLA haplotypes are segregating (1).

In the NOD mouse, the paramount role of the MHC was confirmed by the finding that almost all diabetic mice generated by outcross with diabetes-resistant strains were homozygous for the NOD's susceptibility-conferring $H2^{g7}$ MHC haplotype. Because inbred strains were used, linkage was ascertained by treating type 1 diabetes as a dichotomous trait, with diabetes susceptibility (*Idd*) genes identified by significant deviations from expected Mendelian ratios in the diabetic segregants. Initial segregation analyses in crosses in which $H2^{g7}$ was also segregating only suggested effects of several non-MHC *Idd* genes. However, the true polygenic nature of type 1 diabetes in this model followed the generation of outcross partner strains congenic for NOD's susceptibility-conferring $H2^{g7}$, such that only the non-MHC susceptibility modifiers segregated in a cross (2,3). By now, over 30 non-MHC murine loci have been identified, some of which (e.g., *Ctla4*, *Il2*) represent likely orthologs for certain human non-MHC loci identified by genome-wide association (4). Because extensive genomic sequence information is available for NOD and outcross partner strains, geneticists can subdivide loci identified by linkage analysis across a broader genetic region and use high-density single nucleotide polymorphisms and polymorphic microsatellite markers to facilitate fine-mapping and candidate gene analysis.

Where does the rat figure into our understanding of type 1 diabetes genetic susceptibility? Consistent with the pivotal contributions of MHC in humans and NOD mice, only rat strains expressing $RT1^u$ MHC class II alleles on chromosome 20 are susceptible to the development of spontaneous diabetes. The most widely distributed (and thus most studied) are Biobreeding (BB) rats, a Wistar Furth–related strain (5). The DP-BB (spontaneous diabetes prone) substrain carries a recessive mutation on chromosome 4 (*Gimap5* [GTPase, IMAP family member 5]) producing severe peripheral T-lymphopenia, a phenotype not found in at-risk humans or NOD mice. The nonlymphopenic DR-BB (spontaneous diabetes resistant) strain carries a wild-type *Gimap5* allele and does not develop type 1 diabetes when protected by virus antibody–free high-barrier facilities (5). However, type 1 diabetes can be induced by an antibody that depletes naïve and regulatory T-cells combined with either poly I:C treatment to simulate a virus infection or by infection with a rat parvovirus (5). This inducible DR-BB model stands in sharp contrast to the NOD mouse model, in which such immunostimulatory challenge typically prevents rather than induces type 1 diabetes. In addition to the two BB rat models, spontaneous type 1 diabetes also develops in three other nonlymphopenic rat strains: the Komeda Diabetes Prone (KDP) rat, the LEW.1AR1 rat, and the LEW.1WR1 rat (6,7). In all of these strains, segregation analyses following outcross to type 1 diabetes–resistant strains indicated oligogenic rather than polygenic control, seemingly distinguishing these rat models from both at-risk humans and the NOD mice in terms of genetic complexity.

This conclusion, based upon these earlier studies, can now be rejected. In this issue of *Diabetes*, Wallis et al. (8) have adopted the tactic used in NOD genetic analyses of "preconditioning" the genome of the resistant strain, in this case, the ACI rat. Before outcross to DP-BB rats, a stock of ACI rats doubly congenic for the BB rat $RT1^u$ locus (*Iddm1*) and the T-lymphopenia–inducing mutation in the *Gimap5* gene (*Iddm2*) had been generated. The hypothesis was that segregation of resistance alleles at either of these two loci masked contributions from weaker polygenes. This bicongenic stock remained type 1 diabetes free, demonstrating the presence of additional *Iddm* alleles conferring resistance (9). However, F1 hybrids exhibited a 21% incidence of type 1 diabetes by 180 days (compared with a control DP-BB incidence of 84% by 76 days), showing the introduction of additional additive or dominant alleles from the BB genome. Indeed, without fixing susceptibility at *Iddm1* and *Iddm2*, an F1 incidence of zero would have been likely. Given the high incidence in F1, the authors generated 574 F2 rats, 19% of which became diabetic. A first-pass screen for linkage to the dichotomous (qualitative) trait of clinical diabetes was done using 224 polymorphic microsatellite markers. For those markers producing a log10 likelihood ratio (LOD) score >1, a follow-up analysis of the whole cohort (diabetics and nondiabetics) was performed to confirm type 1 diabetes specificity of the linkage. Additional genetic information was gained by statistical analysis of scores for three semiquantitative traits. Two (extent of cellular infil-

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tration and degree of islet integrity) were phenotypes allowing identification of quantitative trait loci that control insulitis developing in many nondiabetic rats as well as diabetic probands. The third, age of type 1 diabetes onset, allowed identification of quantitative trait loci contributing to the rate at which insulitis-mediated β-cell destruction developed.

The results of the study by Wallis et al. (8) leave little doubt that the complexity of type 1 diabetes development in the lymphopenic BB rat model is no less than that of NOD mice or at-risk humans. For the multiple traits analyzed, 12 *Iddm* loci (designated *Iddm25-Iddm36*) were indicated: seven with significant LOD scores and five with suggestive scores. Interestingly, with the contributions of *Iddm1* and *Iddm2* factored in, the authors estimated that the newly identified linkages could account for not more than 40% of the phenotypic variance. Thus, even more loci, each with small effects (3% of the variance or less), are likely contributing. Further, existence of strain-unique combinations of these non-MHC *Iddm* genes is indicated by reports of different linkages in the initial KDP and LEW.1.AR1 analyses. The Wallis et al. linkages show regions of synteny with known NOD *Idd* and human type 1 diabetes linkages. There are some notable overlays, such as with the *Il2* gene in NOD mice and the *PTPN22* gene in humans. What Wallis et al. clearly demonstrate is that geneticists now have the tools for robust genome-wide analysis for complex traits in the rat. The rat models of type 1 diabetes have not previously received attention comparable with that given to the NOD mouse. Although the immune mechanisms underlying type 1 diabetes development in the mouse and rat are similar in broad terms, the precise pathways underlying the immune dysregulation are strain dependent, as reflected by the KDP rat with a *Cblb* mutation not common to the other rat models (7). Those rat models in which viral triggering of type 1 diabetes by action on the immune system rather than directly on the pancreatic β-cells deserve much more attention than they are currently receiving. In such an environmentally triggered type 1 diabetes model, it will be of great interest to see how many of the loci identified by Wallis et al. for development of spontaneous type 1 diabetes will be identified and how many new ones will emerge. Collectively, the combined results from mouse and rat genetics show that studies in humans have many more non-MHC loci yet to uncover beyond the 10 now confirmed.

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