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Diabetes quantitative trait locus research: from physiology to genetics and back

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Abbreviations QTL: quantitative trait locus ·
RI: recombinant inbred

The last decade has seen remarkable advances in mammalian genome annotations and has delivered improved genetic resources and genotyping technologies that should prove crucial for tackling the genetic determinants of type 1 and type 2 diabetes, obesity and essential hypertension. Features common to all four conditions are a complex pathogenesis and aetiology involving both genes and environment, and non-Mendelian inheritance. These are believed to be the main confounding factors that hamper the discovery of mutations or sequence variants accounting for the most common forms of type 2 diabetes.

Building on decades of biochemical, physiological and pharmacological investigation, geneticists have concentrated on inbred rodent strains, because these are genetically homogenous, environmental factors can be controlled or at least monitored, and large cohorts of hybrids can be derived. Previously focused on rodent models of Mendelian disease inheritance (*fa/fa*, *ob/ob*, *db/db*), research has progressed towards more complex situations in which several genes collectively contribute to spontaneous or experimentally induced syndromes resembling those seen in diabetes. This increasingly active field of research builds on models that carry either natural or experimentally induced sequence variants that for the most part modulate gene function rather than inactivate gene expression [1]. A wide range of inbred strains, each carrying a specific combination of disease-susceptible and -resistant alleles, is

available, and novel models have emerged, including ethyl-*N*-nitrosourea-mutagenised models and congenic panels [1]. Genetic studies require the production of hybrid animals, generally F₂ or backcross cohorts or recombinant inbred (RI) strains, which are used to follow the cosegregation of genotypes and disease-related phenotypes. In this issue of *Diabetologia*, Kobayashi and colleagues have addressed the genetic basis of spontaneous and high-fat-diet-induced diabetes and obesity in mice of the RI strain SMXA-5 [2].

As in all SMXA RI strains, SMXA-5 mice are homozygous at all loci and their genotype structure is determined equally by the parental strains SM/J and A/J, as illustrated in Fig. 1. The mapping of genes controlling variables related to diabetes and obesity was carried out in a large panel of hybrid mice of a two-generation cross derived from SM/J and SMXA-5 strains (Fig. 1). In this context, in all hybrids, A/J genotypes only segregate across half of the genome, including at diabetes susceptibility alleles, whereas the other half remain fixed for the SM/J homozygous genotype. Each hybrid mouse was characterised for a relatively thorough spectrum of diabetes and obesity phenotypes that exhibit obvious biological relationships but are not necessarily controlled by the same genes. Quantitative values of the traits were used to test for evidence of genetic linkage. A total of nine regions of linkage ranging from suggestive to highly significant were identified, demonstrating complex and probably polygenic control of these phenotypes in the cross [2]. The strongest statistical evidence of linkage was found between a locus in chromosome 2 and variations in BMI, glycaemia, insulinaemia and glucose tolerance.

The use of quantitative values of continuous biological traits is now widely used in genetic studies of complex traits. It has led to the concept of quantitative trait locus (QTL) mapping, which remains an important methodological development because it prevents the arbitrary classification of individuals in affected and unaffected groups [3]. QTL mapping has been extensively used in rodents to

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Fig. 1 Strains and crosses used to map and validate diabetes and obesity QTL. Phenotype and genotype investigations were carried out in fat fed (SMXA-5×SM/J) F₂ hybrids (a) and mice of a congenic strain (b) designed to contain A/J alleles at the major locus linked to diabetes and obesity in the cross.

dissect the genetic control of blood pressure [4], which, like blood glucose, behaves as a normally distributed phenotype in the general population and in experimental crosses. QTL research typically requires multidisciplinary strategies that integrate phenotype information, genetic methods, genotyping technologies and statistical tools. The development of statistical methods designed to use quantitative phenotype variables in genetic linkage studies has had a profound impact upon genetic investigations of complex traits. Statistical, technical and theoretical issues still largely dominate QTL research, and sometimes overshadow the poor pathophysiological relevance of limited phenotype screenings in some studies. Guidelines proposed for QTL studies focus on statistical and validation issues [5], but do not address the impact of physiological studies in experimental crosses and mapping panels, which would favour integrative strategies.

A striking result from the study of Kobayashi et al. is the consistent linkage of almost all phenotypes quantified in the cross to chromosome 2 loci, such that A/J alleles in the region contribute significantly to glucose intolerance, hy-

perinsulinaemia and high BMI [2]. The involvement of a single gene in all these variables (which could reflect a phenomenon termed pleiotropy, where one gene controls several variables) is a reasonable hypothesis. The issue here is whether these phenotypes should be considered as logical partners of a single pathophysiological process or independent variables, thus requiring different adjustments in the statistical model to correct for independent or correlated phenotypes.

Another important finding in the study is the apparent replication of the diabetes QTL mapped to chromosomes 2 and 12 in other crosses derived from genetically distinct mouse strains. Evidence of replicated linkage or association is a strong criterion when assessing the robustness of genetic findings in humans, and should also apply in rodent QTL. QTL comparisons might cause problems because of differences in strain combinations and experimental conditions (e.g. age at phenotyping, diet), but data from genealogies of mice suggest that the pool of alleles [6], including naturally occurring variants contributing to a disease trait, is relatively limited in commonly used inbred

strains [7]. Thus, colocalised QTL detected in different strains can actually be caused by one or several shared variants.

The statistical and apparent biological significance of the QTL mapped to chromosome 2 makes it an attractive target for positional cloning of the causative gene(s). Congenic strains carrying chromosomal segments harbouring a QTL introgressed onto a permissive background currently provide the most reliable and widely used tools for progressing from QTL to disease susceptibility genes [8]. They provide tools for fine QTL mapping and, more important, for translating statistical estimates characterising a QTL into a pathophysiological description of the biological function of underlying genes. The congenic strain described in this paper carries a region of over 110 Mb covering the QTL mapped to chromosome 2, which was transferred by repeated backcross breeding and inbreeding stages from the A/J strain onto the genetic background of the SM/J strain (Fig. 1). Overall, results from physiological investigations in fat-fed congenic mice are consistent with data obtained in mice of the SMXA-5 RI strain and the SMXA-5×SM/J intercross [2]. They show that when the genetic background is fixed for SM/J genotypes, the A/J haplotype at the QTL is associated with hyperglycaemia, hyperinsulinaemia, glucose intolerance and obesity. One fundamental question remains unaddressed: are genes underlying susceptibility to spontaneous diabetes in SMXA-5 mice also involved in high-fat-diet-induced diabetes in the cross and in the congenics?

What is the impact of these findings and how can they be translated in human diabetes genetics? The relevance of QTL findings in animal models to human disorders remains a topic of debate and continues to raise some scepticism. However, some reasons for optimism come from the recent demonstration that sequence variation in the gene encoding type II SH2 domain-containing inositol 5-phosphatase accounts for both a diabetes QTL in a polygenic rat model and susceptibility to major components of the metabolic syndrome in humans [9]. Given the high degree of synteny (preservation of gene content and gene mapping order) between rodent and human genomes [10], and extensive cDNA sequence similarities between species, identifying human chromosomal segments homologous to the A/J haplotype in the congenic strain derived by Kobayashi et al. is straightforward, and can be used to prioritise genetic association and linkage studies in humans. The fundamental importance of comparative genomics in biomedical research lies in the fact that gene and functional annotations derived in different species can be exploited. This is particularly important when genes are specifically expressed in organs that cannot be accessed in patients [11]. With these resources, data from congenics can highlight positional and functional candidate genes because their biological effects can be refined by physiological and pharmacological studies in these strains.

The initial screening of the congenic strain carried out here provides a solid foundation for further, more extensive

phenotyping to test the possible involvement of attractive positional candidate genes mapped to the congenic interval, including the gene encoding liver X receptor- α (LXR- α). It is likely there are sequence variations between the A/J and SM/J strains in terms of the gene for LXR- α , but proving that they cause the QTL effects is the greatest challenge ahead, one that can be addressed by in vivo and in vitro studies in novel congenic strains with further dissection of the QTL. Ongoing progress in mouse genome resequencing and projects aimed at identifying single-nucleotide polymorphisms [7] could identify shared haplotypes between the A/J and SM/J strains at the QTL—which are unlikely to carry the gene for diabetes—thus facilitating the selection of candidate genes at the locus. Finding putative disease-causing sequence variants may eventually prove more straightforward than establishing the causal relationship between genetic variation and phenotype.

This study highlights the importance of phenotype screens for accurate assessment of the inheritance and biological impact of multiple pathophysiological components participating in the onset and progression of complex diseases in crosses and congenic strains. Broad phenotype datasets will undoubtedly play an increasingly prominent role in quantitative genetics to characterise the impact of naturally occurring variants upon the modulation of biological processes, including those regulating glucose homeostasis and body weight.

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