

assortative mating.

In spite of these difficulties, significant progress has been made towards cloning of the gene responsible for X-linked progressive mixed deafness with peri-lymphatic gusher (DFN 3). DFN 3 may account for half of all X-linked hearing loss. DFN 3 was initially mapped to Xq13-Xq21 and this localization was supported by the identification of patients with cytogenetically visible deletions encompassing all or part of Xq21. These patients manifest DFN 3 in the context of a contiguous deletion syndrome comprised of variable features which include mental retardation, hypogonadism and choroideremia. Subsequent molecular characterization of a large number of deletions, including submicroscopic ones, in Xq21 has resulted in the assignment of DFN 3 to Xq21.1 (ref. 17).

The recent mapping of the gene for Monge's Deafness (low frequency hearing loss I LFHL 1) to 5q31 represents a significant milestone on the road towards cloning other genes responsible for non-syndromic hearing loss¹⁸. This is a fully penetrant, dominant disorder characterized by progressive sensorineural hearing loss. The authors were able to identify a large pedigree living in relative isolation near San Jose, Costa Rica,

the members of which could all trace their ancestry to a common founding ancestor of Spanish descent named Felix Monge. This successful mapping of LFHL 1 was possible as most of the confounding variables complicating the study of large families in which non-syndromic hearing loss is segregating were eliminated. The simplifying factors included the dominant mode of inheritance of the disorder, founder effect, isolation of the population and assortative mating. A directed search for dominant hearing loss in similar populations would greatly increase the likelihood of mapping additional loci.

To analyse families with probable autosomal recessive non-syndromic hearing loss, the alternative approach of homozygosity mapping of affected individuals in consanguineous pedigrees could be utilized in conjunction with conventional linkage analysis of nuclear families with multiple affected siblings^{19,20}. The use of homozygosity mapping with affected offspring of first cousins is a much more efficient approach than reliance on small nuclear non-consanguineous pedigrees, as fewer families are needed to have the same likelihood of demonstrating linkage. This strategy involves looking for homozygosity of DNA variants in

affected children from consanguineous unions on the premise that the region adjacent to the disease locus will be homozygous by descent. Such approaches will identify candidate regions within the genome likely to encode a gene for non-syndromic hearing loss. The feasibility and power of such approaches will dramatically improve with the increasing density of the human genetic map and the availability of highly heterozygous PCR based short tandem repeat polymorphisms (STRPs).

It is unlikely that localization of genes for hearing loss by these approaches will be of sufficiently high resolution to allow for positional cloning of the targeted genes, but the integration of candidate genes into the genetic map should facilitate their recovery. The list of potential candidate genes will grow as we begin to understand the signal transduction pathways required for the development of the inner ear and define at a molecular level the components of the auditory sensory transduction system²¹. The progress that continues to be made in mapping and identifying genes responsible for hearing loss holds great promise for advancing our understanding of pathological mechanisms, and will lead to improvements in our ability to diagnose and treat all forms of auditory sensory impairment. □

Bouncing off microsatellites

Lee M. Silver

Department of
Molecular Biology,
Princeton
University,
Princeton, New
Jersey 08544-1014,
USA

Since the publication of Garrod's *Inborn Errors of Metabolism* in 1909¹, there has been an ever-increasing recognition that heredity can play at least a facilitating role in most, if not all, non-infectious diseases that affect human beings. Nevertheless, for the greater part of the twentieth century, the mapping of genes in mammals was relegated to backwater institutions where progress was steady but very, very slow. The problem was the nature of the enterprise. Even for the mouse — with its rapid generation time — mapping could only be performed on a handful of loci at a time. As a consequence, linkage maps were built-up in piecemeal fashion over many years. But if the mapping of simple mendelian or unifactorial

traits was considered difficult by this approach, dissecting more complex multifactorial and quantitative traits — which include most common variations among individuals within a species — was downright impossible.

The situation improved considerably in the 1980s with the utilization of restriction fragment length polymorphisms (RFLPs) to follow the segregation of an unlimited number of loci in any one cross or pedigree². But although RFLP analysis has changed the face of genetics, it has not been a panacea — finding RFLPs within a cloned region is often not easy; when they are found, their polymorphic content is often limited and di-allelic; finally, typing large

numbers of RFLP loci is extremely labour-intensive. Thus, while RFLPs allowed the mapping and cloning of genes responsible for numerous unifactorial traits, the characterization of multifactorial traits remained, for the most part, beyond reach. Now with the dawn of the 1990s has come what may indeed be the magic bullet that geneticists have been waiting for — a genomic element with unusually high polymorphic content, which is present at high density throughout all mammalian genomes examined, and is easily uncovered and quickly typed: the microsatellite.

Two recent papers from Lander and colleagues³ illustrate the potential of these markers. In the June issue of

Genetics, they present a dense genetic linkage map of the mouse containing over 300 microsatellite markers. And in this issue of *Nature Genetics*, microsatellites are applied to map some of the loci underlying autoimmune type I diabetes mellitus in a rat model of the disease⁴.

A microsatellite — also known as a simple sequence repeat (SSR) — is a genomic sequence that consists of a mono-, di-, tri- or tetranucleotide repeated in multiple tandem copies. Although microsatellites containing all nucleotide combinations have been identified, the class found most commonly in mammalian genomes contains a (CA)_n•(GT)_n dimer, and is often referred to as a CA-repeat. The existence of CA-repeats, their presence at high copy number, and their dispersion throughout the genomes of a variety of higher eukaryotic species was first demonstrated a decade ago by Hamada and his colleagues⁵. Although independent examples of CA-repeat polymorphisms surfaced over the following decade, it was not until 1989 that Weber and May⁶ investigated this phenomenon in a more systematic way through a computer-aided comparison of microsatellite-containing human sequences with two or more independent entries in GenBank. Surprisingly, they found variation in CA-repeat number among alleles at seven of the eight loci examined. This finding led to the development by Weber and May of a general method for typing polymorphic microsatellites by polymerase chain reaction (PCR). Amplification between two unique oligonucleotides that flank and define the locus was followed by separation of alleles having different repeat numbers by polyacrylamide gel electrophoresis. In the three years since the publication of the Weber and May results, numerous reports have used this method to confirm the

extreme polymorphism associated with many microsatellite loci in a variety of vertebrate species.

The uniqueness of microsatellites as tools for genetic mappings lies within the ease with which they can be uncovered, the ease with which they can be disseminated, and the ease with which they can be typed. To develop a panel of microsatellite loci for analysis of the mouse genome, Todd and his colleagues simply searched through the EMBL and GenBank databases⁷. To increase the size of this panel for higher resolution analysis, Todd⁸, Lander and colleagues³ screened a genomic libraries containing short 250–500 base pair inserts with a CA-repeat probes, and sequenced positive clones. Using a combined panel of 317 microsatellite loci, Lander's group developed a whole genome, mouse linkage map with an average spacing of 4.3 centiMorgans. With the publication of all 317 pairs of oligonucleotide sequences that define and allow the typing of each locus, the markers become available to everyone in a democratic fashion. (Research Genetics Inc. has made life even easier for the mouse genetics community by offering each primer pair in this panel at a greatly reduced cost relative to custom DNA synthesis.) As microsatellite typing is PCR-based, and there is no need for blotting or probing, results can be obtained rapidly with a minimal expenditure of often-precious material and time. Lander's group report that two scientists can "genotype new crosses for the entire genome in a few weeks per cross"³ which represents an order of magnitude improvement over RFLP-based approaches.

Microsatellites can serve not only as tags for anonymous loci but for functional genes as well. In three species examined to date — human, mouse and rat — the density of CA-repeats alone (found on average every

18–28 kilobases⁹) is high enough to provide a good likelihood that any cloned gene will have a microsatellite nearby that can be harnessed for use as a tag for segregation analysis in experimental crosses and human pedigrees. Thus, microsatellites can be viewed as universal genetic mapping reagents.

A fantasy of those who practice formal genetics is having the ability to define all of the genetic parameters involved in any form of inherited variation, no matter how complex. The problem is that many multifactorial and quantitative traits can only be unravelled by following the segregation of every block of genomic material from both parents to every offspring in a cross. With microsatellites as tools, comprehensive studies of this type will become feasible on a routine basis for the first time, and like so many other past objects of desire in the world of molecular genetics, fantasy will soon turn into reality. Already, hypertension in the rat^{10,11}, autoimmune type I diabetes mellitus in both mouse¹² and rat⁴, and mouse epilepsy¹³ have been tackled with some success, and on the horizon lie the genes that play a role in behaviour. For the first time, one can say without exaggeration that the possibilities for the coming generation of geneticists appear truly unbounded. □

References

1. Garrod, A.E. *Inborn Errors of Metabolism* (Oxford University Press, London, 1909).
2. Botstein, D., White, R.L., Skolnick, M. & Davis, R.W. *Am. J. hum. Genet.* **32**, 314–331 (1980).
3. Dietrich, W. et al. *Genet.* **131**, 423–447 (1992).
4. Jacob, H.J. et al. *Nature Genet.* **2**, 56–60 (1992).
5. Hamada, H., Petrino, M.G. & Kakunaga, T. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6465–6469 (1982).
6. Weber, J.L. & May, P.E. *Am. J. hum. Genet.* **44**, 388–396 (1989).
7. Love, J.M., Knight, A.M., McAleer, M.A. & Todd, J.A. *Nuc. Acids Res.* **18**, 4123–4130 (1990).
8. Cornall, R.J. et al. *Genomics* **10**, 874–881 (1991).
9. Stallings, R.L. et al. *Genomics* **10**, 807–815 (1991).
10. Hilbert, P. et al. *Nature* **353**, 521–529 (1991).
11. Jacob, H.J. et al. *Cell* **67**, 213–224 (1991).
12. Todd, J.A. et al. *Nature* **351**, 542–547 (1991).
13. Rise, M.L., Frankel, W.N., Coffin, J.M. & Seyfried, T.N. *Science* **253**, 669–673 (1991).