nature genetics
volume 13 no. 4 august 1996

Haemochromatosis ... definite maybe!

The cornerstone of the human genome project was in many respects laid 18 years ago at a small scientific retreat in the Wasatch Mountains near Salt Lake City, Utah. Two of Mark Skolnick's graduate students were discussing the association of a disease gene with the highly polymorphic human lymphocyte antigen (HLA) region on the short arm of chromosome 6, while at the same time bemoaning their inability to apply a similar strategy for other traits that mapped elsewhere in the genome. Two of the invited guests, David Botstein and Ron Davis, quickly divined that other traits could be mapped by tracking their segregation patterns with random DNA polymorphisms identified with restriction enzymes, as they had routinely in model organisms. After months of further discussion, Botstein, Davis, Skolnick and Ray White proposed the framework for a comprehensive genetic map, culminating in 1980 in their classic publication[1].

But what of the disease that was occupying so much of the attention of Skolnick's group nearly 20 years ago? That was haemochromatosis — from the Greek words *haima* for blood, *chromatos* for colour — which although a mere footnote to the Utah story, is now recognized as being one of the most common single-gene hereditary disorders. Among whites, haemochromatosis occurs in about 1 in 400 people, with a remarkable carrier frequency of 1 in 10. According to the study on page 399 by Roger Wolff and colleagues at Mercator Genetics, the tortuous wait for the haemochromatosis gene may well be over[2]. Haemochromatosis develops in mid-adulthood, but despite its prevalence is widely underdiagnosed. As excessive iron accumulates in various organs including the pancreas, liver and heart, patients suffer from conditions that include diabetes, cirrhosis, liver cancer and cardiac dysfunction, often culminating in early death. Ironically, there is a simple and effective treatment — regular episodes of bleeding, or phlebotomy, as practiced for hundreds of years, to deplete the surplus iron. Indeed, if the disease is diagnosed early enough, life expectancy is normal. In light of its frequency and the potential for safe and effective diagnosis, haemochromatosis has been dubbed 'the genetic disorder of the twenty-first century'[3].

Even though it has been 20 years since French researchers first mapped hereditary haemochromatosis to the vicinity of the HLA region, further progress has been hampered largely because of extensive linkage disequilibrium around the area. Earlier this year, an Australian group presented a 4.5-megabase (Mb) yeast artificial chromosome

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Blue is the colour: Liver biopsy in hereditary haemochromatosis. Iron, stained with Perls' prussian blue, is visibly deposited primarily in hepatocytes.

contig of the region immediately telomeric of HLA (where recombination events have tended to place the haemochromatosis gene). Two markers strongly linked to the disease, *D6S265* and *D6S1260*, some 700 kilobases apart, were thought to represent a possible 'critical region'[4]. But in the new study[2], the Mercator group extended the search for the gene well beyond *D6S1260*. Using a group of 101 haemochromatosis patients, Wolff and colleagues sought to narrow the critical region by defining the segment of the ancestral chromosome that showed the greatest degree of conservation. Wolff's team cloned a total of 6 Mb of DNA, concentrating on the segment distal to HLA. Several complementary approaches drew them to a key marker, *D6S2241*, which lies about 1.5 Mb beyond *D6S1260*: this locus lies in the region of peak linkage disequilibrium and Hardy–Weinberg disequilibrium. Additional evidence came from somatic cell hybrid studies of disease chromosomes. *D6S2241* represents the distal boundary of a 250-kb interval conserved on six separated chromosomes, the most likely candidate region.

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The Mercator group sequenced the entire 250-kb region and began isolating genes from it as well. A total of 15 genes were identified: one of them, termed *HLA-H*, encodes a 343-amino-acid protein with significant similarity to MHC class-I proteins. This novel protein is predicted to traverse the cell membrane, and features several extracellular cysteine residues required for proper conformation. Interestingly, one of these residues is altered (a Cys282Tyr substitution) in 85% of 356 chromosomes from 178 patients, of whom 147 were homozygotes, and nine heterozygotes. The variant was also found on 3.2% control chromosomes, about what would be expected for such a common disorder. A second variant, His63Asp, was found on the remaining allele in 8 of the 9 heterozygotes, but its potential role in the disease is less certain. In all, 87% haemochromatosis patients are homozygous for Cys282Tyr or compound (Tyr 282/Asp 63) heterozygotes.

Pumping irony: Despite this wealth of genetic data, there remains uncertainty surrounding the role of *HLA-H* in haemochromatosis, which may not be dispelled until functional studies have been made. The significance of the alteration of Cys 282 is strengthened by the possibility that HLA-H interacts with β2-microglobulin, given that mice deficient in β2-microglobulin exhibit signs of iron overload[5,6]. The formal possibility remains that *HLA-H* merely sits in linkage disequilibrium with the true gene, but assuming that the general location of the haemochromatosis gene deduced by Wolff's group is correct, all of the genes within it seem to have been accounted for. What about the 13% of patients studied by Mercator who possess neither *HLA-H* variant? The patients were selected on the basis of severity of iron overload regardless of family history, so it is not so unreasonable to invoke environmental or other genetic causes in these patients. (There is at least one other hereditary disorder of elevated iron absorption, not linked to HLA, prevalent in patients from sub-Saharan Africa[3].)

For Mercator, the cloning of *HLA-H* marks the single most important achievement in the company's young life. Chief executive officer Elliott Sigal says that haemochromatosis has been its primary goal, with 20–25 people working on the project. Sigal says that haemochromatosis "will be a paradigm for a genetic test. Intervention is available, and it's been established that intervention helps patients." Mercator is holding discussions with various reference laboratories to explore, Sigal says, a number of "ethical, regulatory, clinical and cost issues". Insurance companies, for example, might find the cost of offering routine genetic screening for haemochromatosis far more economical than paying for a liver transplant. It would be ironic if the Mercator findings pave the way towards more widespread treatment of the disease of the twenty-first century by bleeding, the medicine of the eighteenth.

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