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The Cloning of the Gene for Hereditary Hemochromatosis

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Featured Article: Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13: 399–408.[4]

The early 1990s represented the beginning of modern-day human genomics. Methodologies for genetic linkage analysis of human disease genes had become well established, and a new class of polymorphic microsatellite genetic markers had recently been discovered. The ability to manipulate human genomic DNA as yeast and bacterial artificial chromosomes, coupled with these new tools, provided an unprecedented way to construct detailed physical and genetic maps. Combining these maps with emerging technologies to identify expressed sequences, as well as the introduction of high-throughput capillary-based DNA sequencing, made gene finding more tractable than ever. Collectively, the application of these methods to the isolation of human disease genes was known as "positional cloning," and the early 1990s saw the emergence of several biotechnology companies founded to capitalize on the confluence of these technologies.

One of these companies was Mercator Genetics of Menlo Park, California. The company's plan was simple: establish a positional cloning platform and "rapidly" clone the gene responsible for the iron overload disorder known as hereditary hemochromatosis (HH) *(1)*. Hemochromatosis presents with symptoms indistinguishable from other afflictions and hence is often underdiagnosed *(2)*. Although effectively treated with phlebotomy, HH could previously be diagnosed accurately only by liver biopsy. From a commercial and public health perspective, this disease was the perfect proof-of-concept project, because identification of the disease-causing mutation(s) could lead to a diagnostic test to replace the invasive biopsy procedure.

There was a pragmatic reason as well, and that was that the gene had been linked in 1976 to the MHC region on chromosome 6p, where HH had been shown to cosegregate with the HLA-A3 allele. This finding suggested the existence of a strong founder effect *(3)*. Studies in the early 1990s had also positioned the HH gene to <1 centimorgan from the HLA-A locus *(4)*. In terms of the predicted physical distance, this result translated to approximately 1×10^6 bases of DNA, a distance that should have certainly lent itself to the rapid identification of the gene.

In 1993, Mercator Genetics entered the race to clone the HH gene by using an identity-by-descent cloning approach. The first step was to establish the haplotype of the ancestral chromosome upon which the disease-causing mutation had occurred. This goal was accomplished by creating somatic cell hybrids containing a single copy of chromosome 6. Because individual phased chromosomes were analyzed, we could accurately recreate the haplotype of the ancestral chromosome. The first surprise the team encountered was that a majority of HH patients appeared to maintain the ancestral haplotype over a physical distance of $>6 \times 10^6$ bases. This lack of recombination explained why the gene had been recalcitrant to previous cloning efforts that had relied on studies of families for identifying chromosomal breakpoints and disease segregation. Nevertheless, Mercator's efforts focused on 2 parallel approaches: (*a*) additional genotyping with newly discovered microsatellite markers to define the smallest region of identity by descent and (*b*) assembling a physical map of the region. The pivotal moment arrived when the team met in January of 1996 to review the data and determine the most likely location of the gene. The interpretation was clear: The gene must reside between 2 markers known as D6S2238 and D6S2241, a physical distance of 250 kb of DNA. We had previously cloned the genes from this region by using yeast and bacterial artificial chromosomal DNA as probes and substrates for direct selection *(5)* and exon trapping *(6)*, but we also embarked on a genomic DNA–sequencing effort of the region to ensure that no gene was missed. There were 3 candidate genes within the minimal region, and the HH-causing mutation had to reside on one of them. The "Eureka!" moment came as one of us ran through the hallways waving an autoradiograph with a G-to-A transition that was consistent with it being the disease-causing mutation at position 845 of cDNA24, the HH gene.

Ultimately, Mercator cloned the gene for HH because it pursued the right strategy at the right time and

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with the right team. Although the company's accomplishments during its brief tenure did not translate into long-term commercial success, the pride of every scientist involved in this combined and committed effort is evoked in knowing that the identification of the disease-causing mutations gave rise to a diagnostic tool that continues to make a difference in the lives of HH patients by providing for early diagnosis and treatment.

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