Collaborations on the Isolation of Disease Genes on Chromosome 16

Polycystic Kidney Disease (PKD1). Polycystic kidney disease is a common dominant single-gene disorder (affecting at least 1 in 1000 Caucasians) that is responsible for cystic kidneys, accompanied by hypertension and renal failure. The principal locus for the genetic defect, PKD1, has been assigned to chromosome band 16p13.3 by genetic linkage with polymorphic DNA markers shown to reside in that band.

Steve Reeders (Yale University School of Medicine), Anna-Maria Frischauf (Imperial Cancer Research Fund), and collaborators have constructed both a long-range restriction map (covering 1 million base pairs) and an ordered contig map (covering 75,000 base pairs) that span the entire PKD1 region. Construction of the contig map by cosmid walking from multiple start sites within the region was greatly aided by the use of two chromosome 16-specific cosmid libraries constructed at Los Alamos. A gene-by-gene search is now being carried out in the region to identify candidate disease genes (genes that are expressed in the kidney and that have alleles that are specific to affected individuals). This effort will probably soon lead to the identification of the gene that is responsible for the disease.

Batten's Disease (CLN3). Batten's disease is a juvenile-onset neurodegenerative disease with incidence rates of up to 1 in 25,000 live births. It is characterized by the accumulation of autofluorescent fatty pigments in neurons. The responsible locus (CLN3) is inherited in an autosomal recessive pattern. That is, the defective allele must be present on both chromosomes in order for the disease to be manifested. The gene responsible for this disease has been mapped to the region between two polymorphic markers in the chromosomal band 16p12.

We have found thirteen cosmid contigs and one YAC clone from our physical map that lie in this same interval, and in collaboration with groups in London (Mark Gardiner), the Netherlands (Martijn Breuning), and Australia (David Callen), we are developing new polymorphic DNA markers from these contigs in an attempt to find markers that are closer to the disease locus. We have used prior knowledge of the repetitive-sequence fingerprint of four of these cosmid clones to develop STSs containing GT-repeat sequences present on these clones. Since GT repeats tend to be variable in length, we expect these STSs to be polymorphic and therefore useful for linkage analysis. We are now evaluating their informativeness in linkage studies. (Genetic-linkage markers for the remaining cosmids are being developed by the other laboratories with the aid of the fingerprint data.) The development of these new genetic-linkage markers in the Batten's-disease region will allow the disease gene to be localized to a manageable region (approximately 1 million bases). Then construction of a detailed physical map starting from the existing contigs and YACs in the region can be completed. The availability of the Los Alamos clones in the Batten's region has substantially reduced the extensive work that would have been required to find genetic-linkage markers from this region and to construct a complete map of the region.

Familial Mediterranean Fever (FMF). FMF is an autosomal recessive form of arthritis that is characterized by acute attacks of fever with inflammation of the lining of the abdominal cavity (peritonitis), pleural cavity (pleurisy), and joints (synovia). The gene frequency among non-Ashkenazic Jews, Armenians, Turks, and Middle Eastern Arabs is comparable to the gene frequency for cystic-fibrosis defects among Caucasians (1 in 25). As with Batten's disease, genetic-linkage markers flanking

the disease locus have been identified by researchers led by Dan Kastner at the National Institutes of Health. We are working with that group to identify contigs and YACs that lie within this region so that additional genetic-linkage markers can be developed.

Rubenstein-Taybi Syndrome (RTS). RTS is characterized by abnormal facial features, broad thumbs and big toes, and mental retardation. RTS is a rare disorder that accounts for an estimated 1 in 500 institutionalized cases of mental retardation. Almost all cases seem to arise from spontaneous mutations. Three patients with RTS have been found to have translocations involving the short arm of chromosome 16. Using fluorescence in-situ hybridization, Martijn Breuning (Leiden University) was able to pinpoint the location of breakpoints in two of these patients relative to cosmids that he had ordered in the region in his group's effort to map breakpoints associated with ANLL M4. One of these cosmids, RT1, appeared to be very close to the breakpoints and was found to be deleted in 6 out of 24 patients. By screening our gridded arrays of chromosome 16 cosmids with RT1, Breuning identified one cosmid, 316H7, that overlapped RT1 by 10 kilobases. This overlapping cosmid was also hybridized to metaphase chromosomes from the two patients with RTS. In both cases, Breuning found three signals, one on the normal chromosome 16, a second signal on the aberrant chromosome 16, and a third on the chromosome that the p arm of 16 had translocated to. These results indicated that cosmid 316H7 spanned both translocation breakpoints in these RTS patients. Since the gene(s) responsible for RTS is likely to be disrupted by these breakpoints, the identification of cosmid 316H7, which spans the breakpoints, opens the door for identification of the gene(s) that causes this syndrome.

Acute Nonlymphocytic Leukemia (ANLL). In contrast to PKD1, CLN3, and FMF, which follow a Mendelian pattern of inheritance, acute nonlymphocytic leukemia is a polygenic trait, that is, it involves the interaction of several genes. A high frequency of rearrangements (inversions and translocations involving both the p and q arms) of chromosome 16 is associated with a specific subtype of acute nonlymphocytic leukemia known as ANLL subtype M4 (see "What's Different about Chromosome 16?"). This association suggests that chromosome 16 may contain at least one of the genes involved in the progression of the disease state and that the chromosomal rearrangements disrupt the functioning of that gene. We are collaborating with groups in the United States, Australia, and the Netherlands to isolate the chromosomal breakpoint regions associated with ANLL. Our prior identification of chromosome 16-specific repeats that map near these regions is aiding the search for the breakpoint regions. Genes that are disrupted as a result of the chromosomal rearrangements will be candidates for having a role in ANLL.

Breast Cancer. Like ANLL, breast cancer appears to be a polygenic trait involving specific alterations of chromosome 16 in addition to alterations in other genes. Deletions in the q22 region of chromosome 16 that are not always detectable at the gross microscopic level occur at a relatively high frequency in the malignant cells of breast tumors. These deletions are readily detectable using fluorescence in-situ hybridization by noting the absence of a positive hybridization signal from a probe that usually hybridizes to the deleted region and the presence of a signal from a second probe that hybridizes to the centromere. We have sent cosmid clones from the q arm of chromosome 16 to Joe Gray (UCSF), who is attempting to pinpoint the region of deletion associated with breast cancers. A gene-by-gene search through the deleted region will presumably lead to the identification of a gene whose function suppresses the development of cancer (tumor-suppressor gene). ■