A HIGH-RESOLUTION GENETIC MAP OF HUMAN CHROMOSOME 16
AND LOCALIZATION OF THE MEF GENE

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by

YANG SHEN  M.S.

Department of Paediatrics, Women's and Children's Hospital,
The University of Adelaide

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SUMMARY

Genetic linkage maps of human chromosomes are important tools for the localization of disease genes. One of the first five year goals of the Human Genome project is the construction of genetic maps of all human chromosomes with highly polymorphic markers spaced an average of 2-5 centimorgans apart (Jordan, 1992). A number of genetic maps have been constructed for human chromosome 16 (Donis-Keller et al., 1987; Keith et al., 1990; Julier et al., 1990; NIH/CEPH collaborative mapping group, 1992; Kozman et al., 1993). These maps were based mainly on RFLP markers which are less efficient for localization of disease genes and for refining linkage distance for positional cloning than highly polymorphic PCR based markers. Therefore, there was a need for more highly informative markers to be placed on high density cytogenetic-based physical and genetic linkage maps of chromosome 16.

Two approaches were used to isolate simple tandem repeat (STR) markers. Initially the random isolation approach was exploited to generate STR markers from the chromosome 16 cosmid library (Stallings et al., 1990). After (AC)n repeats isolated from the random approach were mapped to 9 intervals of chromosome 16 defined by breakpoints in somatic cell hybrids, a second targetted approach was used to generate additional repeat markers with which to fill in the deficient intervals or to isolate more repeat markers in the regions of particular interest [fragile sites FRA16A and FRA16B, disease gene regions of adult autosomal polycystic kidney disease (PKD1), familial...
Mediterranean fever (FMF) and Batten disease (CLN3)]. This second approach was involved the isolation of STR markers from cosmid clones which have been previously mapped to specific regions of chromosome 16 (Callen et al., 1992; Stallings et al., 1992).

A total of 32 (AC)n repeat markers were isolated from these two approaches. 22 (AC)n repeat markers had heterozygosity greater than 0.68. The 32 (AC)n repeat markers and 32 (AC)n repeat markers from other laboratories were physically mapped on the high-resolution cytogenetic map of chromosome 16 using the hybrid panel. 79 STR markers were used for construction of a genetic linkage map of human chromosome 16 (Shen et al., submitted). Of these 27 markers were generated by the candidate, 20 other STR markers were isolated by other members of our laboratory and 32 STR markers were from other laboratories

This PCR-based genetic linkage map of human chromosome 16 was constructed from 79 STR markers, 1 VNTR marker and 1 RFLP marker. These 2 non-STR markers were chosen because they extend the map towards the telomeres. This map covers the entire length of chromosome 16. The length of the map is in remarkable agreement with those published genetic and chiasma maps of chromosome 16 (Shen et al, submitted). The median distance and the average distance between markers on the framework map is 2.7 and 3.2 cM, respectively. In comparison with the averaged resolutions of other STR-based linkage maps of human chromosome 1, 4, 11, 12, 13, 18, 20 and 22, the resolution of this map is much higher.
The framework and comprehensive maps were anchored to the high-resolution cytogenetic map, which was divided into 66 breakpoint intervals (on average 1.5 Mb per interval) by a panel of 67 hybrids. The cytogenetic map is one of the most detailed maps available for any of the autosomes (Callen et al., 1992). It is apparent that the combination of genetic linkage analysis and physical mapping can be extremely helpful in resolving locus order at the resolution of the comprehensive map.

These integrated genetic and physical maps of human chromosome 16 (Shen et al. submitted) provided an efficient means for regional localization of genetic disorders located on chromosome 16, for detection of loss of heterozygosity in cancers and imprinting of chromosomes in inherited disorders, for evaluation of linkage disequilibrium and disease causing mutations, and for analysis of multifactorial diseases.

To localize the gene \(\textit{MEF}\) responsible for familial Mediterranean fever, which is an autosomal recessive disorder characterized by attacks of fever and serosal inflammation, 4 (AC)n repeat markers \((D16S291, D16S94, D16S523\) and \(D16S453\)) were genotyped on 62 FMF families. The linkage analysis was carried out using the computer program MLINK of \textsc{Linkage} (version 5.1). The lod scores showed these 4 markers were significantly linked to \textit{MEF}. The observed recombination events, homozygosity mapping and multipoint linkage analysis defined the centromeric boundary at \(D16S523\) and the telomeric boundary at \(D16S246\). The \textit{MEF} gene was localized between these two markers in a genetic interval of less than 1.6 cM.