[S3-4] [10/19/2001(Fri) 16:10-16:50 / Hall C]

Host Genetic Epidemiology by SNP Study

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The completion of the whole human genome sequences is now a reality. Emphasis on the analysis of genetic variation on both an individual level and a population level is more important than ever. The identification and characterization of single nucleotide polymorphisms (SNP) in target genes or candidate genes plays a crucial role in identifications of disease genes and in expediting drug discovery/development. Identification of known SNPs can be easily accomplished by searching the ever-expanding public domain databases. However, discovery of new SNPs is best accomplished by sequencing a reasonable subset of the applicable population groups. Particular SNPs of interest will be the ones that effect coding changes and regulatory functions of genes. Once SNPs have been identified, the next step is to examine their frequency in disease models by accurate, cost-effective and high-throughput SNP genotyping methods. This presentation will cover the overview of principal techniques of SNP genotyping which have been developed so far. And also will be discussed the importance of well-defined disease model and error-free high-throughput SNP genotyping in candidate genes and genome-wide SNP screening in the near future.

What are the effects of SNPs?

Where	Result		Effect
In coding region	May be silent, e.g.,UUG→CUG, leu in both cases	Silent	Usually no change in phenotype
In coding region	May change amino acid sequence, e.g., UUC→UUA, phe to leu, Some characterize these as the least common and most valuable SNPs, Many being patented	Missense	Phenotype change (may be subtle depending on amino acid replacement and position)
In coding region	May create a "Stop"codon, e. g., UCA→UGA, ser to stop	Nonsense	Phenotype change
In regulatory region	May affect the rate of transcription (up-or down-regulate)		Possible phenotype Change
Other regions	No affect on gene products. May act as genetic markers for multi-component diseases. These are sometimes called anonymous SNPs and are the most common.		

Method	Key of detection	Principle	dye/detection
SSCP	Conformation	Mobility	Isotope, Silver staining, fluorescence
PCR-RFLP	Restriction enzyme	Mobility	EtBr
AS-PCR	Allele specific primer	PCR product	EtBr
GC-tail primer	Allele specific primer	Tm	Syber green I
DASH	Hybridization	Tm	Syber green I
TaqMan	Hybridization	Tm	Fluorescence
Molecular Beacons	Hybridization	Tm	Fluorescence
OLA	Ligation	Mobility	Fluorescence
Invader	Hybridization	Cleavage	Fluorescence
Pyrosequencing	Nucleotide incorporation	PPi	Light
Single Base Extension	Single Base Extension	Dye terminator	Fluorescence
FP	Single Base Extension	Dye terminator	Fluorescence polarization
MALDI-TOF	Time-of-Flight	Weight of nt	-

Table 1. Comparison of **SNP** analysis methods

The development of human genetic markers

Type of marker	No. of loci	Features
Blood groups 1910-1960	~ 20	May need fresh blood, rare antisera Genotype cannot always be inferred from phenotype because of dominance No easy physical localization
Electrophoretic mobility variants of serum proteins 1960-1975	~ 30	May need fresh serum, specialized assays No easy physical localization Often limited polymorphism
HLA tissue types 1970-	1 (haplotype)	One linked set Highly informative Can only test for linkage to 6p21.3
DNA RFLPs 1975-	$>10^5$ (potentially)	Two allele markers, maximum heterozygosity 0.5 Initially required Southern blotting, now PCR Easy physical localization
DNA VNTRs (minisatellites) 1985-	$>10^4$ (potentially)	Many alleles, highly informative Type by Southern blotting Easy physical localization Tend to cluster near ends of chromosomes
DNA VNTRs (microsatellites) (di-, tri- and tetranucleotide repeats)	$>10^5$ (potentially)	Many alleles, highly informative Can type by automated multiplex PCR Easy physical localization Distributed throughout genome
DNA SNPs (single uncleotide polymorphisms) 1998-	$>10^6$ (potentially)	Less informative than microsatellites Can be typed on a very large scale by automated equipment without gel electrophoresis