workshop c2: saturday, march 11

New Advances in Laboratory Diagnostics

Human telomere probes in clinical cytogenetics: probe characteristics, methods, applications and limitations. D.H. Ledbetter, Department of Human Genetics, University of Chicago, Chicago, IL.

Increasing evidence supports the notion that cryptic deletions or translocations involving the telomeric regions of human chromosomes may be a significant cause of unexplained mental retardation. Recently, Flint and co-workers (Lancet 354:1676, 1999) demonstrated a 7.4% frequency of abnormalities in patients with moderate to severe mental retardation. A high proportion of these proved to be associated with familial translocations, further increasing their clinical significance. Transition of this technology to routine clinical use will depend on a number of considerations: 1) Probe characteristics – there are 41 different human telomeres, excluding the acrocentric short arms. Probes should be well-characterized, unique sequence probes that are at a known distance from the end of the chromosome. 2) Methods available for analysis include single probe FISH analysis, multi-color FISH, multi-probe FISH assays, and comparative genomic hybridization (CGH) arrays (i.e., "telomere chips"). 3) Applications of telomere FISH range from targeted FISH analysis using individual telomere probes in cases in which a candidate chromosome is suspected, to whole genome scans in cases of unexplained mental retardation, multiple miscarriage and pregnancy loss. 4) There are limitations to telomere analysis at present, including the cost of current technologies and an incomplete knowledge of genotype-phenotype correlations for monosomies and trisomies of each telomere region. For example, several patients have been identified with telomere imbalance segregating in families without abnormal phenotypes. This fact makes it imperative that parental studies be done in all cases of abnormal telomere findings in order to draw any conclusions regarding phenotype and causal relationships.

Application of Tandem Mass Spectrometry to Newborn Screening. S.I. Goodman. Univ. of Colorado Health Sciences Center, Denver

The mass spectrometer is a device to separate and quantify ions based on their mass/charge (m/z) ratios. In gc-ms, the analytes are separated by gas chromatography and then identified by ms as they exit the column, but in ms-ms the analytes are first separated by molecular weight in one mass spectrometer, and then identified on the basis of their fragmentation patterns by a second mass spectrometer. Analysis is done by computer, either by asking for the molecular weights of all compounds that produce a particular ion (*parent ion mode*), or the molecular weight of all compounds that have lost a particular neutral fragment (*neutral loss mode*). For example, the butyl esters of all acylcarnitines produce an ion of m/z 85, and butyl esters of all α-amino acids lose a neutral fragment of mass 102. Also, scan functions can be changed many times during an analysis, so one can detect and measure acylcarnitines and α-aminoacids in the same sample. While separation and identification by gc-ms is limited by gc run time, analysis by ms-ms takes only seconds and is thus capable of the high throughput needed in newborn screening.

PKU, maple syrup urine disease and hypermethioninemias, which are now screened for in many programs, can be diagnosed by ms-ms with better sensitivity and specificity than in most current programs. Ms-ms also allows the screening menu to be expanded to include many organic acidemias and disorders of fatty acid oxidation. In particular, glutaric acidemia type I and medium-chain acyl-CoA dehydrogenase (MCAD) deficiency fit current requirements for newborn screening. Severe penalties are paid for late diagnosis in the form of striatal degeneration and/or death, and there is evidence that presymptomatic treatment can prevent the late complications.

There are, however, difficulties. Substantial costs include the price of the instrumentation, and its maintenance and service, as well as the additional costs of medical foods and medications, and the physicians, nurses, and nutritionists that would be needed to treat and monitor the additional patients. Nonetheless, if instrument cost is amortized over several years, ms-ms can probably be added to existing programs for an incremental cost of less than $10 per sample. The unknown sensitivity for many diseases in the newborn period is also an issue, as is the fact that some of the disorders that would be detected cannot be treated. It should also be noted that ms-ms cannot totally replace existing programs, as biotinidase deficiency, virilizing adrenal hyperplasia, galactosemia, hypothyroisism and hemoglobinopathies cannot be detected by ms-ms at this time.