0497 High throughput mutation scanning for *LDLR* by meltMADGE

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We have developed and applied to *LDLR*, a mutation scanning approach suitable for whole population screening for unknown mutations. The method, meltMADGE, is a reconfiguration of DGGE enabling combination with microplate array diagonal gels (MADGE). Throughput per day per person is 4×10×96well gels in 2×2l tanks, representing 4,000 amplicons. Scanning cost: 50Eu/Mb; rate 10Mb/week.
Assays of *LDLR* exons 3 and 8 were validated in 460 familial hypercholesterolaemics with known mutations. We then applied the exon 3 assay in several DNA banks representing ~8,000 subjects with known cholesterol values and applied both assays in one DNA bank (n=3,600). In exon 3 we identified one known moderate mutation, P84S (n=1), also associated with moderate hypercholesterolaemia in this subject; an unknown silent variant, N76N (n=1); and known severe hypercholesterolemia splice mutation 313+1G>A (n=2). Around exon 8 we identified a paucimorphism (n=35) at splice site 1061-8T>C (known to be in complete linkage disequilibrium with T705I); and unknown mutations 1186+11G>A (n=1) and D335N G>A (n=1). D335N and a significant fraction of T705I subjects displayed cholesterol values above the 95th centile. In contrast with case collections, CpG mutations predominated.
MeltMADGE will permit definition of population-based 'reference ranges' for rarer sequence variation; characterisation of 'paucimorphisms' (arbitrarily defined here as variants of rarer allele frequency, $0.05\% < q < 5\%$) and identification of severe mutations at the population level.