

**0457** High throughput mutation scanning for *MC4R* by meltMADGE  
<sup>1</sup>Khalid K. Alharbi and <sup>2</sup>Ian N. Day  
*1*King Saud University, college of applied medical sciences,  
P.O.Box 10219 Riyadh 11433 email: [kharbi@hotmail.com](mailto:kharbi@hotmail.com).  
*1,2*Human Genetics Division, Southampton General Hospital,  
MP808, Southampton SO16 6YD

Identification of mutations has remained laborious, expensive, and only viable for small numbers of subjects or cases. Population based 'reference ranges' of rarer sequence diversity have not been available. However, the research and diagnostic interpretation of sequence variant scan be crucially dependent on such information. We have developed a high-throughput system, meltMADGE, which reduces scanning cost to a fraction of PCR cost (1/7) rather than a multiple of it (10-100x). MeltMADGE combines the properties of Microplate Array Diagonally compatible PAGE gels with are configuration of denaturing gradient gel electrophoresis, such that the independent (denaturing) variable is a DNA melting thermal ramp in time instead of a chemical (urea) gradient in space. The temporal dimension of the melt then permits use of high-density 2D arrays of electrophoresis tracks, such as used in MADGE. Two heteroduplexes and two homoduplexes bands should resolve from a heterozygote amplicon. Throughput per worker per day is 40x96 well gels=4,000 amplicons, using two 2l tank staking 10-gels each for 2hr runs. We developed nine assays representing the *MC4R* gene and examined a population sample of 1,100 subjects. Two 'paucimorphisms' were identified (V103I in 27 subjects and -178A>C in 22 subjects). Anthropometric studies of these variants have the power to detect, for example, BMI effects as little as 0.5 units. Two rare variants were also identified, one previously described (T112M), one unknown (A87D) BMI of 31.5 in the latter might point to mild functional effect, although high birth weight (4763g) argues against postnatal hyperphagia. Approximately 3 million bases were scanned in a total time of 1 week at a total cost around 500 Euros. Expansion to much larger survey sizes would be straightforward.